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# The environmental behaviour and toxicological effect of propetamphos in an estuarine environment.

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*Award date:*
2002

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University of Wales, Bangor

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# THE ENVIRONMENTAL BEHAVIOUR AND TOXICOLOGICAL EFFECT OF PROPETAMPHOS IN AN ESTUARINE ENVIRONMENT

• PRIFYSGOL CYMRU •
UNIVERSITY OF WALES
BANGOR


A thesis submitted to the

University of Wales

by

**Susana García-Ortega**

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In candidature for the degree of

***Philosophiae Doctor***

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**October 2002**

## Summary

Inorganic and organic characterisation of water and sediment from five sites along the Conwy River and Estuary (CRE) was carried out. Sequential extraction showed 90 to 100% of metal content in sediment and suspended particulate matter (SPM) to be bound to organic and mineral fractions. High concentrations of Zn and Fe were found in pore water throughout CRE (Pb was found in river sediment pore water). Zn and Fe exhibited conservative behaviour indicating that the estuary was a sink for them. Mn and Al presented non conservative trends; a potential source for Al was identified as Dolgarrog Aluminium. Trace levels of selected metals from along the Estuary were analysed using a pre-concentration procedure and ICP-OES analysis validated as part of this work.

Dissolved natural organic matter (NOM) was characterised by total phenol, DOC, "weight-averaged molecular weight" (MW) and UV-visible spectroscopy. One MW distribution was generally found (<1100); two in Autumn. Positive, linear correlations were found between MW and total phenol, the humification ratio ($E_4/E_6$) and DOC and negatively with molar absorptivity ($\varepsilon$). DOC and $\varepsilon$ correlated following an exponential decay model. Correlations between metals and Suwannee River NOM showed Ca and Na preferentially in the high MW fraction, Mg and K with medium MW fractions and Zn with in low MW fraction.

The behaviour and fate of propetamphos (PPT) was tested in the presence of NOM, salinity and metals in sediment from the CRE. Initially PPT was rapidly sorbed onto sediment, then more slowly (<50% sorbed after 8 hrs). Freundlich coefficients ($K_f$) correlated with sediment organic matter. PPT was readily biodegraded by the microbial sediment population from the CRE. Biodegradation was reduced considerably with higher salinity or metal ions. PPT reduced sediment microbial glucose respiration. Toxic effects of equimolar binary and ternary combinations of PPT with metals (Zn, Pb) were additive. NOM decreased toxic effects but only slightly. The toxic effect of commercial PPT was 162 times greater than pure PPT based on $EC_{10}$ values.

***To Enrique***

*Because if not were for you I wouldn't have put myself in this position, and you know what I mean. Thanks for being there always even in the worst moments.*

*"If knowledge can create problems, it is not through ignorance that we can solve them"*

**Isaac Asimov (1920-1992)**

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## Abbreviations and Acronyms

| | |
|---|---|
| ε | Molar Absorptivity |
| AA | Annual Average concentration |
| ACHe | Acetylcholinesterase |
| AI | Additive Index |
| ASS | Artificial Sea Salts |
| ASW | Artificial Sea Water |
| ANOVA | Analysis of Variance |
| BC | Betws y Coed |
| BS | Bird Sanctuary |
| BSA | Bovine Serum Albumin |
| CC | Conwy Castle |
| CIS | Critical Ionic Strenght |
| CRE | Conwy River and Estuary |
| DDW | Doubly distilled water |
| DOC | Dissolved Organic Carbon |
| DOM | Dissolved Organic Matter |
| $DT_{50}$ or t½ | Half Life |
| $E_4/E_6$ | Indicator of Humification |
| EA | Environment Agency |
| $EC_{50}$ | Chemical concentration at which 50% of the population is affected |
| $EC_{10}$ | Chemical concentration at which 10% of the population is affected |
| EPA | Environmental Protection Agency |
| EQS | Environmental Quality Standard |
| ERASM | Environmental Risk Assessment Steering Committee |
| FA | Fulvic Acid |
| GLM | General Lineal Model |
| HA | Humic Acid |
| HMW | High Molecular Weight |
| HOC | Hydrophobic Organic Compounds |
| HPLC | High Performance Liquid Chromatography |
| HPSEC | High Performance Size ExclusionChromatography |
| HS | Humic Substance |

| | |
|---|---|
| ICP-OES | Inductive coupled Plasma-Optical Emission Spectroscopy |
| IHSS | International Humic Substance Society |
| IR | Infra Red |
| $K_f$ | Freunlich sorption coefficient |
| $K_{ow}$ | Partitioning Coefficient n-octanol-water |
| LL | Llanrwst |
| LMW | Low Molecular Weight |
| LOD | Limit of Detection |
| LOEC | Lowest Observed Effect Concentration |
| M | Molar |
| MAC | Maximum Allowable Concentration |
| MAFF | Ministry of Agriculture, Fisheries and Food |
| MATC | Maximum Toxicant Concentration |
| MW | Molecular Weight and Weight Average Molecular Weight |
| Na-PSS | Sodium Polystyrensulfonate |
| NOEC | No Observed Effect Concentration |
| NOM | Natural Organic Matter |
| NOM-SR | Natural Organic Matter from Suwannee River |
| NRA | National Rivers Authority |
| NPOC | Non purgeable Organic Carbon |
| OM | Organic Matter |
| OP | Organophosphate pesticides |
| PAH | Polycyclic Aromatic Hydrocarbon |
| POM | Particulate Organic Matter |
| PPT | Propetamphos |
| PPT-Ind | Propetamphos commercial formulation |
| QC | Quality Control |
| Rt | Retention Time |
| S | Sum of toxic contribution for a chemical mixture |
| SEC | Size Exclusion Chromatography |
| SEM | Standard Error of the Mean |
| $\sigma$ | standard deviation |
| SP | Synthetic Pyrethroid |
| TC | Tal y Cafn |

| | |
|---|---|
| TMDL | Total Maximum Daily Load |
| TOC | Total Organic Carbon |
| TU | Toxic Unit |
| Vr | Retention Volume |
| Vt | Total Permeation Volume |
| Vo | Void Volume |

## Acknowledgements

Dr. P. J. Holliman and Dr. D. Jones for their supervision and encouragement over the course of this investigation.

Consejo Nacional de Ciencia y Tecnologia (CONACyT), Mexico for providing the scholarship that allowed me to undertake this study.

Vericore Ltd., that provided samples of radiolabel propetamphos and the industrial formulation Ectomort centenary to perform the experiments in this investigation.

Dr. E. Olivares, Dr. V. Thoss and Dr. S. Jones for their invaluable advice, help and support.

Dr. C. Freeman and Dr. D. Thomas for the usage of their equipment.

The Society of Chemistry and Industry and the Society of Ecology, Toxicology and Chemistry for providing partial funding to participate in the SETAC conference in Baltimore USA (November 2001).

The technical and secretarial staff at the Department of Chemistry, The School of Agricultural and Forest Sciences and the Institute of Environmental Sciences for providing help and equipment whenever required.

My family, for their support and belief in me, they were there when I most need them.

Friends that I have come across this path along this time, just for bringing sanity into my life.

# CHAPTER 1. INTRODUCTION

## 1.1. Area of study.

In recent decades, public concern about the environment has being growing. One reason for this is that the environment is a complex balance of many interdependent factors, which, if disrupted with chemical spills or the misuse of chemicals, can lead to disturbance of entire ecosystems.

An example of this concern is the effort that the Environmental Protection Agency (EPA) in the USA and the Environment Agency (EA) in the UK had put in to regulate or give guidelines to control the quality of different ecosystems in their regions. Most countries around the world have followed this initiative and have also signed treaties at international levels to regulate and diminish pollution in the environment.

In the USA, Environmental State Agencies have to give regulations called TMDL or Total Maximum Daily Load, which is defined by EPA as "a calculation of the maximum amount of a pollutant that a water body can receive and still meet water quality standards, and an allocation of that amount to the pollutant's sources" (EPA, 1999). Some of these TMDL values are defined as acute and chronic criteria, some examples are shown in Table 1.1. These TMDL values differ from state to state depending on the sources and intrinsic nature of the water bodies. The values will also depend to a large extent on water hardness and pH values.

**Table 1.1**. Examples of Total Maximum Daily Load, (TMDL) of inorganic compounds for different USA Rivers (EPA, 1999).

Criteria for Eagle River Alaska, USA final TMDL ($\mu g\ l^{-1}$)

| Pollutant | Summer | Winter |
|---|---|---|
| **Copper** | 11 | 12 |
| **Lead** | 2.7 | 3.1 |
| **Silver** | 3.3 | 3.9 |
| **Ammonia** | 1800 | 2000 |
| **Chlorine** | 2.0 | 2.0 |

Water quality criteria Ten Mile Creek

| Pollutant | Acute criteria | Chronic |
|---|---|---|
| **Al, Total ($mg\ l^{-1}$)** | 0.75 | None |
| **Fe, Total ($mg\ l^{-1}$)** | none | 0.5 |

Acute criteria are evaluated as instantaneous values.
Chronic criteria are evaluated using a four-day average

Estuaries are important ecosystems, because they support a great diversity of organisms and can be highly productive fishery grounds (Prohic and Kniewald, 1987). They are also areas where large amounts of dissolved and particulate organic matter are produced (Head, 1976). The combination of these factors along with the mixing of fresh and salt water gives estuaries unique chemical and biological properties (Burton, 1976; Manahan, 1994). For humans, estuaries represent a source of food, leisure and jobs but also a place to release domestic and industrial waste (Prohic and Kniewald, 1987; Birch, 1996). Estuaries can

also play an important role in the movement of contaminants (Turner and Millward, 1994). For instance, a down-estuary metal gradient concentration was found in the two main estuaries in Sydney by Birch (1996) from which he concluded that this trend was source related. Furthermore estuaries can act as traps for sediment derived from coastal erosion (Bryan, 1980; Kinne, 1980; Turner *et al.*, 1994). Dissolved chemicals can react within the estuary to be removed from the dissolved phase. Geochemical processes such as adsorption, flocculation and precipitation along with biochemical processes aid the removal of dissolved chemicals with the estuary effectively acting as a filter (Sharp *et al*, 1984).

Speciation is increasingly being considered to be a key element in understanding the mobility, toxicology and bioavailability of inorganic and organic substances (Caroli, 1995; Tack and Verloo, 1995). For instance, sorbed species may be less bioavailable and may exhibit different photoreactivity (Gustafsson and Gschwend, 1997). Speciation has been defined by the IUPAC as the process yielding evidence of the atomic or molecular form of an analyte (Caroli, 1995). Chemical speciation and the reactions involved in the transformation of species are often the main factor by which pollutants interact in the aquatic environment (Forstner and Wittmann, 1983; Gustafsson and Gschwend, 1997). It has been said that the chemical form of a metal determines it's reactivity (sorption/desorption, precipitation/dissolution) (Tack and Verloo, 1995). Organic contaminants in estuaries, in the same way as metals, may exist in several forms including, dissolved, bound to organic matter, adsorbed to particulate matter or associated with surface sediments (Sharp *et al*, 1984; van Loon and Duffy, 2000).

Commonly, both organic and inorganic pollutants are present in the environment. On this basis the behaviour of xenobiotics in estuaries is an important area of study. The interactions between metals, organic contaminants and natural organic compounds in a river estuary environment, are the main interest in the present work. Most of the studies to date have focused on the toxicological effects of a single chemical or mixtures of chemicals from the same group. One major question to address is therefore whether the toxicant effect of organic xenobiotics and metals is additive, antagonistic or synergistic? And whether physicochemical properties of the aqueous solution, such as natural organic matter and salinity affect the environmental fate and behaviour of organic xenobiotics.

## 1.2. Aims

### 1.2.1. General aim:

This thesis reports an investigation into the behaviour of organic and inorganic compounds found in a river-estuarine environment. This study has also assessed natural organic matter - metal interactions. The environmental fate and toxicological effect of the organophosphate pesticide known as propetamphos has been studied, assessing the effects of solution chemistry.

### 1.2.2. Hypotheses

- The inorganic and organic characteristics of water and sediment samples of the Conwy River-estuary will play an important part in the sorption and transport of chemicals and hence bioavailability.

- The sorption and desorption behaviour of propetamphos in river and estuarine sediments, will be affected by salinity, natural organic matter and metal ions.

- The biodegradation of propetamphos by sediment microorganisms, (measured by the half-life), will be affected by salinity, dissolved metals and natural organic matter.

- The toxicological effects of propetamphos measured as the respiration rate of sediment microorganisms, will be affected by the presence of dissolved metals and natural organic matter.

**1. 3. Field site.**

The Conwy estuary is located in North Wales (Figure 1.1). It drains a predominantly upland catchment (590 $km^2$), and discharges to Liverpool Bay at Conwy after flowing a distance of 56 km (NRA, 1995). The river is subject to a wide ranging flow regime (ranging between 0.3 $m^3s^{-1}$ and 486.6 $m^3s^{-1}$ during the period 1982 to 1992), with an average flow of 24 $m^3s^{-1}$ (NRA, 1995).

The Conwy estuary is a macro tidal estuary. The upper limit of tidal influence of the river occurs approximately 10 km upstream from the main body of estuarine water at Conwy. The maximum spring tidal range in the estuary is about 7-8 m (Elderfield *et al.* 1971).

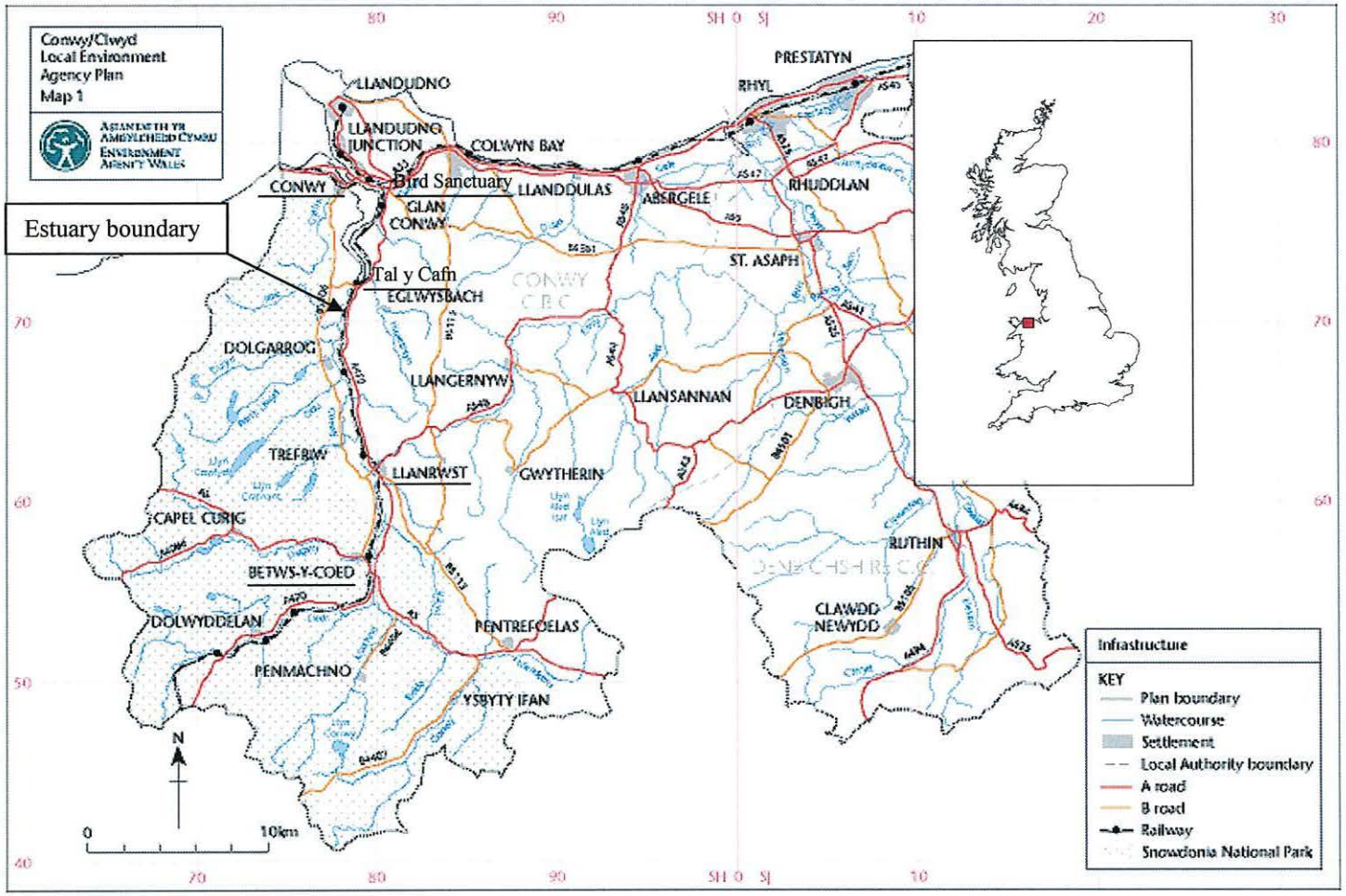


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**Figure 1.1.** Conwy River and Estuary, North Wales. Sampling sites are underline and the estuary boundary is shown. Environment Agency, (2001).

The level of metal pollution through the past years has been a big concern. The metal pollution, source and toxicant effect in the Conwy River and Estuary have been reported by several authors (Elderfield *et al.* 1971; Elderfield *et al.*, 1979, Gao and Bradshaw, 1995). On the western bank of the river, the area has a number of disused quarries with a legacy of dereliction and some water pollution. Nant Gwydyr, a tributary of the River Conwy has been affected by metal wastes from a lead and zinc mine (Parc Mine), through contaminated mine drainage waters and episodic erosion of an unstable tailings heap. Gao and Bradshaw (1995) reported that 14 years after stabilisation work on the Nant Gwydyr the stream was still polluted. Under normal discharge conditions, it contributed approximately 1 tonne of Zn, 0.2 tonne of Pb and 0.05 tonne of Cd per year to the River Conwy. It was speculated by Elderfield *et al.* (1971) that the presence of zinc and lead in ionic or weakly complexed forms could be responsible for the poor oyster larvae performance (*Ostrea edulis*) at the White Fish Authority's Hatchery at Conwy. On the other hand, most of the catchment provides low-intensity grazing, thus sheep farming is the main agricultural activity (see Figure 3.19). The industrial development is limited to the Llandudno/Llandudno Junction area and Dolgarrog (NRA, 1995). There are several private sewage treatments outflows (Figure 3.17), including an industrial discharge from Dolgarrog Aluminium Ltd (see Figure 3.18). The NRA (1995) had specified that the water quality of the Conwy River is generally very good throughout the catchment. Nevertheless, parts of the upland catchment including Llyn Conwy are acidified to an extent that significantly affects river ecology, while a 5 km length of the estuary fails to achieve the highest quality designation.

On this basis, the Conwy River and Estuary (CRE) is a good site to investigate the behaviour and relationship between natural organic compounds and metals in river and estuarine sediments. Furthermore the environmental fate and behaviour of propetamphos, an organophosphate pesticide typically used in sheep dipping, will be assessed on sediment from the CRE.

### 1.4. Metal speciation

A key aim of elemental speciation is to more accurately to identify and quantify the risks posed to human health and the environment by the various forms under which an analyte may occur (Caroli, 1995). For instance the mode of binding to the solid phase is related to the extent of metal release to the liquid phase and hence the likelihood of remobilization and bioavailability (Bubb and Lester, 1991; Bryan and Langston, 1992).

Metal mobility can be estimated by comparing sequential extraction results before and after treatment of the solid material by controlled intensification of relevant release parameters like pH, redox-potential and temperature (Tack and Verloo, 1995). Metal speciation in metal toxicity studies may help in explaining the observed toxicity of different media. However, interpretation of data is often difficult because, besides metal speciation, other factors influence the toxicity of metals (Bryan, 1980). For instance, many of the most interesting chemical species in toxicology and ecotoxicology have a small relative molecular mass, whereas in biological systems the essential effects of trace elements are often regulated by their interactions with macromolecules (Behne, 1992).

Fractionation of total metal content may suggest the origin of the metal pollution. For instance high levels in the exchangeable, acid soluble and easily reducible fractions may indicate pollution from anthropogenic origin (Hung *et al.*, 1993; Mesuere *et al.*, 1991). Further, high Cu and Zn levels have been found in the exchangeable and acid soluble fractions of coastal sediments as compared to ocean sediments, suggesting a pollution problem arising from discharges (Hung *et al.*, 1993). Even high contents in the more resistant fractions, except the residual, may be a threat in the long term (Clevenger, 1990).

On the other hand, colloidal phases in natural waters may be important to various environmental questions, especially those concerning the cycling of vital and toxic trace chemicals (Gustafsson and Gschwend, 1997). To complete this section, it is known that is possible to track the fate of pollutants in sediments and overlying waters in estuaries, through analysis and speciation of the metal content (Abdel-Moati, 1990; Battiston *et al.*, 1993).

### 1. 5. Metal pollution in Estuaries.

Forstner and Wittmann (1983) distinguished five different sources of environmental metal pollution; geological watering; the industrial processing of ores and metals; the use of metals and metal components; the leaching of metals from landfill sites and solid waste dumps, and finally animal and human excretions which contain heavy metals. These sources of metal input into receiving water bodies can be described as being diffuse, nonpoint and point

source.

Interactions between dissolved and particulate forms may occur, modifying the nature and fluxes of riverborne material to the sea (Harrison and Mora, 1996). Most of the heavy metals transported by river waters occur by translocation on particulate material under normal physicochemical conditions. However, heavy metals adsorbed in bottom sediments may be released because of chemical changes in the aquatic system (Forstner and Witmann, 1983). The mobility of contaminants in an aquatic environment is additionally determined by the pH, redox conditions and the presence of complexing agents like dissolved organic matter and inorganic anions (Salomons, 1995). Interstitial or pore waters are an important phase in the sediment complex and, on average, interstitial waters often appear enriched in certain trace elements relative to the overlying sea water (Aston and Chester, 1976). For instance, Elderfield *et al.* (1979) reported that dissolved Zn concentrations are enriched in interstitial water (average ~ 110 μg $l^{-1}$) as compared with Conwy Estuary water (~10 μg $l^{-1}$) and river water (~ 50 μg $l^{-1}$).

Sediments have a high storage capacity for contaminants with the precise capacity being dependent on composition. The important components for retaining contaminants in sediments are the organic matter content, the clay mineral content and nature of the clay minerals, the iron and manganese content and the carbonate content as a buffer against pH changes (Salomons, 1995). For instance, heavy metals could be incorporated in the crystal lattice of the minerals which make up the sediment, other fractions are distributed between the organic matter, the

hydrous manganese and iron oxides, and any discrete minerals formed by the metals (de Groot *et al*, 1976). As an example, there are clay minerals which are known to possess a high adsorbing capacity due to their coating such as hydrous manganese, iron oxides, and organic substances (Salomons, 1995). Adsorption is a selective process, which accounts for preferential adsorption of specific cations and the release of equivalent charges associated with other species. Furthermore, sorption is a phenomenon in which fine-grained materials with large surface areas are capable of accumulating heavy metal ions at the solid liquid interface as a result of electrostatic attraction (Forstner and Wittmann, 1983). Surface phenomena of this kind can be explained by the electric double layer model. The adsorption processes of heavy metals onto clay minerals may be controlled to a large extent by pH. In the case of organic pollutants, a strong relationship with natural organic matter has been observed in sediments (Salomons, 1995).

Typically metals in their free ionic form, e.g. $Cd^{2+}$, $Cu^{2+}$ and $Zn^{2+}$, have been found to be the most toxic chemical species for living organisms because of their high bioavailability (Forstner and Wittmann, 1983; Kelly and Allison, 1988). The dissolved concentration of these metals varies with pH and salinity (Phillips, 1995). Coonley *et al.* (1971) have described an example of metal behaviour in an estuarine mixing zone of the Mullica River in New Jersey. When the river water enters Great Bay, New Jersey, a sudden rapid decrease in the iron concentration with increasing chloride content was present, suggesting that iron was removed from the mixture of the river water and seawater (Figure 1.2). This order of precipitation was probably connected with a charge reversal from positively charged sediments in freshwater to negatively charged sediments in seawater,

which occurred between 2% and 6% salinity (Pravdic, 1970).

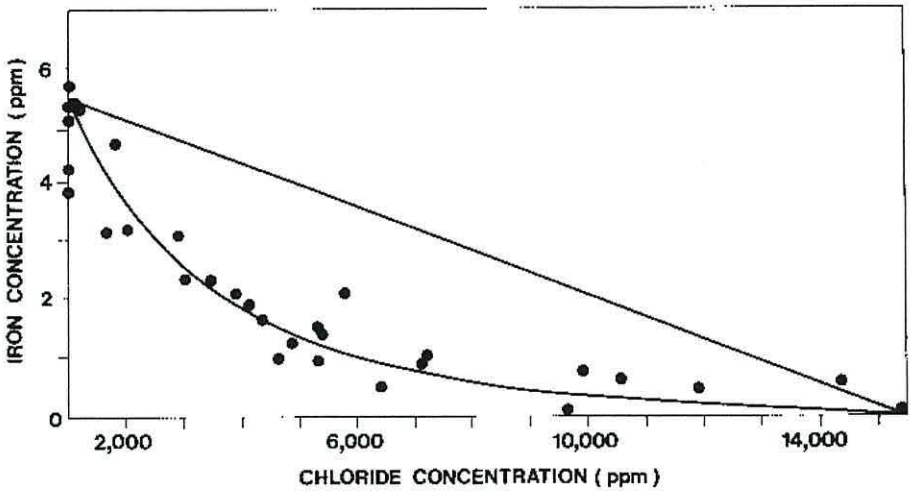



**Figure 1.2.** Geochemical estuarine sink behaviour of dissolved iron in the freshwater/sea water mixing zone of Mullica River, Great Bay New Jersey. (Coonley *et al.*, 1971).

### 1.6 The role and importance of natural organic matter.

The organic matter of aquatic systems consists of the remains of biologically produced compounds as well as the remains of synthetic organic substances. Decomposition of higher-molecular weight organic substances is mostly due to microbiological action leading to the formation of smaller and more soluble fragments (Forstner and Wittmann, 1983). The most important products formed during the decomposition of organic substances are humic acids. These can be found/produced in soils, in limnic and marine sediments, as well as in the corresponding aqueous solutions (Forstner and Wittmann, 1983). Humic acid describes a group of compounds which are extremely varied natural polymeric materials. They are characterised by the wide variability of their molecular mass

which ranges from less than 700 to more than 2,000,000 (Choudhry, 1984). In much of this work, the general term "dissolved or colloidal organic matter" (DOM) will be used to identify organic components of natural systems (river and estuarine). This term includes both aquatic humic and fulvic substances, and often it will also be referred as "natural organic matter" (NOM).

Dissolved organic matter DOM has a role in food chains. It is believed that particulate organic matter (POM) produced by plants may be consumed directly by herbivores or by protozoa and bacteria (Head, 1976). Therefore, POM is broken down and re-synthesized into cellular material or respired, releasing simpler organic compounds (e.g. proteins, carbohydrates, tannins, lignins, polyphenols and quinones). Besides, DOM not only mobilises heavy metals but also can affect their bio availability. From an environmental point of view, the study of metal-humic interactions is often aimed at predicting the effect of aquatic HS on the bioavailability of heavy metal ions in the environment (Rocha *et al*, 2000). Kelly and Allison (1988) expressed that if a toxic species (metal) associates itself with either the suspended matter or dissolved ligands the toxicity of the water or groundwater decreases.

Humic substances also are capable of reacting with many specific anthropogenically derived organic compounds found in water and in soil. Organic pesticides are a prime example (van Loon and Duffy, 2000). Gaffney *et al.* (1996) states that the ability of humic substances to bind hydrophobic organics can affect not only their mobility, by decreasing the sorption to sediment, but also the rate of chemical degradation, photolysis, volatilization and biological uptake

of these organics. This interaction can serve to lengthen the lifetimes and transport distances of these contaminants in the environment. It is said that the sorption mechanisms of uncharged organic compounds to natural aquatic sorbents is dominated by hydrophobic interactions and organic matter is the primary sorbing constituent known (Karickhoff, 1984; Zhou *et al.*, 1997).

Ionic strength and pH factors also influence the interactions between small neutral organic molecules and humic material in either dissolved or particulate forms (van Loon and Duffy, 2000). The reasoning here is that humate can become insoluble when riverine sources encounter sea water in estuaries, then the aggregated material can co-precipitate organic solutes with it further, by direct association with solid HS, such solutes can be "immobilized". Hence, the bioavailability and therefore the toxicology of organic compounds such as pesticides can be influenced by their interactions with NOM and metals.

The need for studies regarding the nature of interactions between organic chemicals and aquatic HS, as well as a better knowledge of metal-aquatic HS interactions in different molecular size fractions had been emphasised before by Rocha *et al.* (2000). This is one of the objectives of the current study.

### 1.7. Organic pollution

With respect to organic pollutants, thousands of synthetic chemicals are in daily use. Some of these have been quantified and monitored in environmental samples. Many compounds are continuously introduced into the environment in

large quantities (e.g. solvents, components of detergents, dyes and varnishes, additives in plastics and textiles, chemicals used for construction, antifouling agents, herbicides, insecticides, fungicides and pesticides) (Schwarzenbach *et al.*, 1993).

It would be unwise to dismiss the importance of pesticides in the development of the world economy. For example Fest and Schmidt (1982) reported how the use of pesticides in crops helped to increase the national production of cocoa in Ghana and also the production of rice in Japan in the 1950's. Besides the great advantages of using pesticides there can also be deleterious environmental effects. For instance, by long persistence in soil (e.g. DDT and dieldrin) (Fest and Schmidt, 1982), or aquatic pollution by pesticides that enter the aquatic system either by direct application, or indirectly through surface run-off of contaminated soils or spray drift, which is the case for pyrethroids (Zhou *et al.*, 1997). Organophosphorus pesticides (diazinon and propetamphos) are known to reach the aquatic environment either by run-off or accidental overspill or incorrect disposal of spent dip directly to a drain or watercourse (Virtue and Clayton, 1997).

The processes which an organic chemical is subjected to when it is introduced into a freshwater system are represented in Figure 1.3. These include physical, chemical and biological processes which are promoted because of transport and mixing phenomena, transfer processes, chemical, photochemical, and/or biological transformation reactions. These processes can leave the structure of a chemical unchanged or transform it into one or several products of different

environmental behaviour and effects (Schwarzenbach *et al.*, 1993).

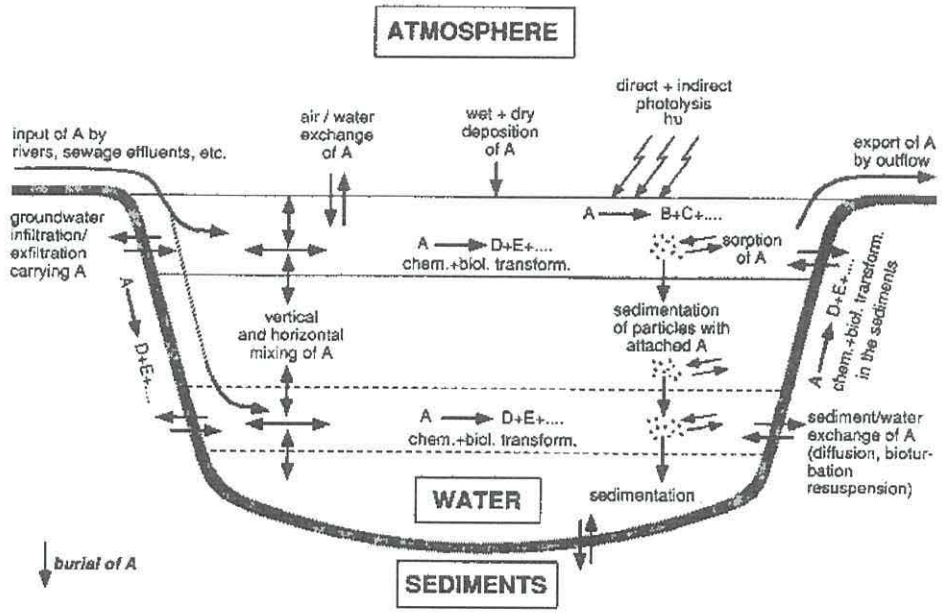



**Figure 1.3.** Processes that determine the distribution, residence time, and sinks of an anthropogenic organic compound in freshwater, after Schwarzenbach *et al.*, (1993).

Organic contaminants in estuaries may be found in several forms including dissolved, bound to dissolved organic matter, adsorbed to suspended particulate matter (SPM), and associated with surface sediments (Readman *et al*, 1984). The form in which an organic pollutant tends to be in the environment will influence their fate, mobility and bioavailability and therefore their toxicology, which in ecological terms is the most important factor to regulate usage and allowable pollution levels.

The environmental fate of a chemical is described by their transport and transformation parameters. Transport will be influenced by the thermodynamic descriptors of the chemical, such as vapour pressure, water solubility, and the

equilibrium between organic phase and water, (distribution coefficient in n-octanol-water). Also the environmental equilibrium which involves the physical phases is important. For instance in a water-solid phase equilibrium (sediments, suspended sediments or soils), sorption and desorption kinetics influence the chemical transport in the environment. The transformation of a chemical includes purely chemical means (e. g. hydrolysis, photolysis or Redox reactions), and/or biological means. A chemical can be degraded by living organisms through enzymatic reactions and metabolic mechanisms. Bioaccumulation and biomagnification from the uptake of food, sediment and water is the ultimate fate of a chemical. In conclusion, fate is determined by a substance's intrinsic chemical and physical properties, by the chemical, physical and biological properties of the environment into which it is released and by the amounts and rate at which it enters to an ecosystem (Forbes and Forbes, 1994).

## 1.8 Toxicology

Among the many pollutants that are recognised threats to human health and the environment are various heavy metals, organic chemicals like pesticides and oil (and various substances associated with its extraction), excess nutrients and various types of plastics (Forbes and Forbes, 1994). All these compounds are said to be hazardous when they affect the normal physiological behaviour of living organisms. To measure these effects, the science of toxicology was developed.

Toxicology has been defined as "the study of the adverse effects of chemicals on living organisms" (Klaassen and Eaton, 1991). Toxicological chemistry centres

on the relationship between the speciation of toxicants and their toxicological effects (Manahan, 1994).

Typically the toxicology of a chemical for a specific organism is measured in terms of its acute, subchronic and chronic effect. The length of the exposure period is defined relative to the length of the test organisms' life cycle (Rand, 1995).

Though, in nature there is always more than one compound in the environment that could affect the living organisms. When two or more poisons are present in an effluent they may exert a combined effect on an organism in and additive fashion. Alternatively, they may interfere with one another (antagonism), or their overall effect on an organism may be greater than when acting alone (synergism) (Nirmalakhandan *et al*, 1994; Mason, 1996).

The assessment of effects of pollutants in sediment has become a well-accepted branch in ecotoxicology. Sediment biology is characterised by a broad spectrum of interacting species and communities. Bacteria represent the lowest trophic level present in sediment, and they are responsible for the mineralization of dead biomass and organic matter and for the degradation of pollutants in the environment. A change in microbial community structure in sediment will have consequences for the higher trophic levels and for the environmental status of the overlying water column as well (Eismann and Montuelle, 1999). Due to their function and ubiquitous presence micro-organisms can act as a relevant environmental indicator of pollution (van Beelen and Doelman, 1997). In the

case of microbial studies metabolic parameters such as growth, enzyme activity, respiration and uptake or conversion of substrates are the best to measure the toxic effects (Eismann and Montuelle, 1999). In this thesis, the ecotoxicological assessment of a pesticide (propetamphos) and metals will be assessed using the natural microbial population of sediment by measuring respiration through the uptake of an organic substrate (glucose).

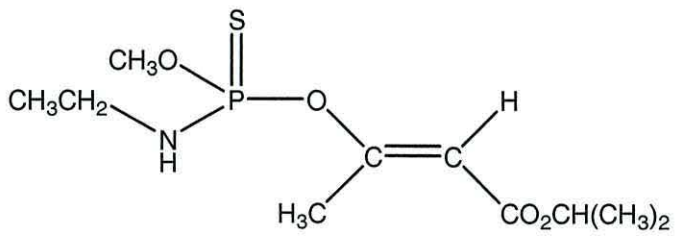
### 1.9 The pesticide.

The first known reaction involving phosphoric acid and sulphuric acid was done by J. L. Lassaigne in 1820. The elucidation of the fundamental reactions of organophosphorus chemistry was carried on by C. A. A. Michaelis and A. E. Arbusov later between 1898 and 1905 (Fest and Schmidt, 1982). The potential insecticidal application of organophosphorus compounds was later understood by the German scientist Dr Gerhard Schrader after the Second World War. Parathion was the first major pesticide of this form to be marketed and others soon followed (Hough, 1998).

It is recognized that even after many years after the discovery of parathion and diazinon their insecticidal action has been difficult to surpass. Due to their ester nature, living organisms can metabolize them to inorganic phosphates. However, the acute toxicity of the phosphoric acid esters does not discriminate between mammals or insects (Fest and Schmidt, 1982). Therefore they represent a potential toxicant for organisms that are not the prime target of the pesticide.

Organophosphate pesticides (OP) have been found to be less resilient in the environment in comparison with the organochlorine pesticides (e.g. DDT and Dieldrin) (Fest and Schmidt, 1982). In consequence OP pesticides have become more popular and in some countries have completely replaced organochlorine pesticides. For instance in the UK, OP pesticides replaced organochlorines in sheep dips during the 1980s (Hough, 1998).

Propetamphos is an organophosphate pesticide used by the sheep farming industry in the UK against insect pests (Virtue and Clayton, 1997). In Figure 1.4 some of the thermodynamic descriptors of propetamphos and its chemical structure are presented.




Mol. Wt. 281.3; B. P. 87-89 °C/0.005 mmHg; V. p. 1.9 mPa (20 °C); $K_{ow}$ 6600; Solubility in water 110 mg $l^{-1}$ (24 °C); pKa 13.67 (23 °C)

**Figure 1.4.** Propetamphos chemical structure and physico-chemical properties (Tomlin, 1994).

Sheep are easily infested by ectoparasites (*Psoroptes ovis* or *Sarcoptes scabiei*)

which can cause discomfort and even death (Hutchings, 1999). In Wales the most accepted treatment to control sheep scab is the immersion of sheep in an insecticide solution, better known as sheep dip. Other than propetamphos, the most common pesticides used are the OPs diazinon and the synthetic pyrethroids cypermethrin and flumethrin (Dunstone, 2000). For reasons discussed later in this section, other application methods have grown in popularity over recent years, such as spraying dip directly onto sheep, and pour-on treatments (Virtue and Clayton, 1997).

Pollution by organophosphates still occurs and thus has been monitored in the UK, for instance by the Scottish Environment Protection Agency (Virtue and Clayton, 1997) and the Environment Agency (Hutchings, 1999; Dusntone, 2000). Reports of pollution incidents involving sheep dip in Scotland between 1974 and 1989 were reported by Virtue and Clayton (1997) (Table 1.2). The Environmental Quality Standard (EQS) maximum allowable concentration (MAC) and the annual average concentration (AA) proposed by the Water Research Centre in the UK (Table 1.3) for key organophosphates have been breached recently in Wales (Hutchings, 1999; Dusntone, 2000).

Traditionally sheep dipping sites have been located close to watercourses, which then served as a ready supply of dilution water for the dip concentrate. An investigation led by the Tweed River Purification Board in 1989 indicated that about 40% of the dipping sites were a pollution risk due to: (a) the sitting; (b) the sheep dipping practice; and (c) the method of disposal of sheep dip (Virtue and Clayton, 1997). Thanks to the work of the UK EA in co-operation with UK

farmers, the traditional methods of sheep dipping have been modified reducing considerably extreme cases of pollution. Nevertheless, Hutchings (1999) still reported that a 1998 survey confirmed that pollution by sheep dip pesticides remains widespread in upland Wales. Positive results for sheep dip chemicals were recorded at 75 % of sheep dip sites, and levels were environmentally significant at 29 % of sites. Biological surveys (invertebrates' composition amongst stream gravels at key locations) suggested that up to 9 % of rivers and streams could have been affected by sheep dip in 1998 in Wales. UK (Dunstone, 2000).

**Table 1.2.** Pollution incidents involving sheep dip between 1974 and 1989 in Scotland as reported by Virtue and Clayton (1997).

| Catchment | Date | Active Ingredient | Extent of damage |
|---|---|---|---|
| Eddleston Water | February 1987 | Unknown | Depressed invertebrate numbers |
| Leader Water | November 1988 | Unknown | Severe impoverishment of invertebrates |
| Tarth Water | August 1989 | Unknown | Severe impoverishment of invertebrates for several kilometres |
| Oxnam Water | November 1987 | Diazinon | Depressed invertebrate numbers |
| Quair Water | April 1978 | Unknown | 11 fish killed |
| Leader Water | August 1980 | Unknown | Approximately 700 fish killed over 1.5km |
| River Tweed | August 1980 | Unknown | 70 fish killed over 3 km |
| Blackadder Water | July 1984 | Unknown | 1500 fish killed over 3 km |
| Ettrick Water | July 1984 | Unknown | Approximately 500 fish killed |

Despite the fact that in 1993 the UK Veterinary Products Committee concluded that there was no environmental evidence to justify banning the use of

organophosphate sheep dips these were taken off the market temporarily at the beginning of 2000 (Chemistry in Britain, February 2000). Following concerns that the pesticide containers posed a risk of farmers coming into contact with dip concentrate. The Ministry of Agriculture, Fisheries and Food (MAFF) recommended that by the end of August 2001, OP products should return to the market with vented taps and revised labels as a temporary measure until new containers with "closed delivery systems" are designed and approved (Chemistry in Britain, October 2000)

**Table 1.3.** Environmental quality standards for sheep dip chemical in water. Environmental Agency

| Sheep dip chemical | Freshwater ($ngl^{-1}$) | | Saltwater ($ngl^{-1}$) | |
|---|---|---|---|---|
| | MAC | AA | MAC | AA |
| Diazinon (OP) | 100 | 10 | 150 | 15 |
| Propetamphos (OP) | 100 | 10 | nr | nr |
| Clorfenvinphos (OP) | 100 | 10 | nr | nr |
| Cypermethrin (SP) draft | 1.0 | 0.1 | nr | nr |
| Flumethrin (SP) | nga | nga | nr | nr |

MAC = Maximum allowable concentration.
AA = Annual average concentration
nga = no agreed standard
nr = not reported

In humans and farmers in particular, organophophate sheep dip has been linked with health problems such as nerve damage (nausea and headaches) and the so-called "dipper's flu", after treating sheep with organophosphate pesticides (Hough, 1998). Organophosphates act by blocking the normal function of the enzyme acetylcholinesterase (AChe) at neuronal or neuromuscular junctions thereby inhibiting the normal transmission of nerve signals (Mason, 1998).

However, organophosphates have been reported to be capable of inhibiting other enzyme systems such as neurotoxic esterases known to cause a persistent delayed neuropathy, that affect the lower limbs some 14 days after exposure (Nutley and Cocker, 1993; Mason, 1998).

In summary, up to now there is enough evidence that propetamphos is highly toxic to fish ($LC_{50}$ from 0.13 mg $l^{-1}$ in bluegill and 0.36 mg $l^{-1}$ in rainbow trout and to aquatic invertebrates ($LC_{50}$ 0.68 to 14.5 µg $l^{-1}$ in *Daphnia magna*) (EXTOXNET, 1996). Further, it is widely used in sheep dipping in the UK and regardless of the efforts of the Environment Agency, Wales it is still an environmental hazard to aquatic environments. On this basis, studies to evaluate the behaviour, environmental fate and toxicology of propetamphos in ecotoxicological terms are essential and this is a primary goal of this investigation.

# Chapter 2. Experimental

## 2.1. Sampling program

A sampling technique was developed following the guidelines of Krajca (1989) and Keith (1988). Throughout this research several samplings were performed, for inorganic and organic characterisation of water and sediment samples along with specific sorption-desorption, biodegradation and toxicology experiments.

### 2.1.1. Location of sampling sites

Five sites in the Conwy River and estuary, North Wales, were chosen to include a range of salinities from fresh water through to almost sea water (see Figure 1.1). Betws y Coed (BC) and Llanrwst (LL) were considered to be freshwater sites and were located in the river. Tal y Cafn (TC) was an intermediate salinity site in the upper estuary, while in the lower estuary Bird Sanctuary (BS) and Castle Conwy (CC) sites were located in the low estuary close to the estuary mouth. Figure 2.1 shows photographs of LL, TC and BS sampling sites. The coding system used to label the samples followed the pattern shown in Table 2.1. For instance, a sediment sample from Tal y Cafn used for inorganic characterisation had the code TCSI.

(a)

(b)

**Figure 2.1** Sampling sites in the Conwy River and Estuary. Two views of Llanrwst, (a) Down the River; (b) Up the River.

(c)

(d)

**Figure 2.1** Sampling sites in the Conwy River and Estuary (c) Tal y Cafn, under the bridge; (d) Bird sanctuary shore, looking towards Conwy.

**Table 2.1.** Code used for water and sediment samples collected in the Conwy River and Estuary.

| Site | Code | Phase | Characterisation |
|---|---|---|---|
| Betws y Coed | **BC** | Water (**W**) | Inorganic (**I**) |
| | | | Organic (**O**) |
| | | Sediment (**S**) | Inorganic (**I**) |
| | | | Organic (**O**) |
| Llanrwst | **LL** | Water (**W**) | Inorganic (**I**) |
| | | | Organic (**O**) |
| | | Sediment (**S**) | Inorganic (**I**) |
| | | | Organic (**O**) |
| Tal y Cafn | **TC** | Water (**W**) | Inorganic (**I**) |
| | | | Organic (**O**) |
| | | Sediment (**S**) | Inorganic (**I**) |
| | | | Organic (**O**) |
| Bird Sanctuary | **BS** | Water (**W**) | Inorganic (**I**) |
| | | | Organic (**O**) |
| | | Sediment (**S**) | Inorganic (**I**) |
| | | | Organic (**O**) |
| Castle Conwy | **CC** | Water (**W**) | Inorganic (**I**) |
| | | | Organic (**O**) |
| | | Sediment (**S**) | Inorganic (**I**) |
| | | | Organic (**O**) |

### 2.1.2. Inorganic and organic characterisation

Physicochemical parameters such as pH and conductivity were determined in selected water and sediment samples. Determination of dissolved Ca, Mg, Na, K, Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in river water and sediment pore water was carried out using inductively coupled plasma-optical emission spectroscopy

(ICP-OES). Also a partial speciation of metals in sediments was carried out using sequential extraction. Inorganic nutrients ($NH_4^+$-N, $NO_3^-$-N, and $PO_4^{3-}$-P) were analysed in sediment samples. X-ray powder diffraction, infra red spectroscopy (IR), size grain fractionation and moisture content were performed on selected sediments.

Selected organic parameters were measured, including total organic carbon (TOC), total phenol, UV-visible (UV-VIS) spectroscopic scans and fixed wavelength readings. In addition, high performance size exclusion chromatography (HPSEC) using HPLC with a UV detector for water and sediment pore water samples. Total C, H and N of sediments was also determined.

### 2.1.3. Behaviour of propetamphos (PPT) experiments

Selected sediments were used to measure the degradation, sorption, desorption and toxicology of the organophosphate pesticide propetamphos (1-methylethyl (e)-3-{{(ethylamino)methoxyphosphinothioyl}oxy}-2=butenoate).

## 2.2. Pre-treatment of samples

All glassware and plastic containers that came in contact with water or pore water samples were cleaned following the procedure described by Newman (1996). Glassware and plastic ware were soaked overnight in 10 % Decon® 90 diluted in

water and then rinsed thoroughly with tap water, and then with doubly distilled water (DDW). Finally equipment was soaked or rinsed with 10 % $HNO_3$ (Analar grade; Fisher) and rinsed again at least four times with DDW. Plastic ware was allowed to dry and where appropriate glassware was dried in an electrical oven at 110 °C. After the cleaning procedure, Pyrex® glass bottles used to contain samples for organic characterisation were heated in a muffle furnace at 550 °C for approximately seven hours.

Prior to used, glass fibre filters (GF/F, Whatman®) to filter water and pore water for organic characterisation, were wrapped in aluminium foil and pre-ashed in a muffle furnace at 550 °C for *ca.* six hours.

Water samples were collected with a glass or plastic bottle (2.5 l). In each case, the bottle was first rinsed three times with river or estuarine water, avoiding the superficial micro layer. The bottle was then unscrewed about 20 cm below the surface, facing the direction of the current. Bottles were filled completely to avoid head space as much as possible and the top screwed in place below the water surface. Then bottles were clearly marked and stored in a cool box pre-filled with cool blocks for immediate transport back to the laboratory. Each sample was marked with the code previously determined for each site, see Table 2.1. The code included information about the site, the nature of the sample (water or sediment), the purpose of the analysis (inorganic or organic characterisation) and the date of sampling.

In the estuary, sediment samples were taken from recently exposed sediment,

always when the tide was outgoing. In the river, sediment samples were taken from the river bed. Areas with abundant stones or pebbles were avoided.

In each case, a high density polyethylene plastic tube (diameter 10 cm), pre-rinsed with water from the site was used to collect the sediment with the help of a plastic spatula. Each time the plastic tube, spatula and bottles were rinsed with water from the site to avoid cross contamination. The core was pushed to approximately 10 cm depth. The sediment was then removed from the tube and put in a zipped plastic bag with the spatula. Approximately 1 kg of sample was collected from each site. If necessary, additional material was taken from nearby undisturbed areas. Once the sediment was placed in the bag, air was expelled as much as possible and the bag closed. Each bag was then placed into a second plastic bag clearly marked using the codes in Table 2.1. The final sample was then placed in a cool box, to delay any microbiological or biochemical changes, and then immediately transported to the laboratory for storage or analysis.

Selected physicochemical parameters were measured on site (temperature, electrical conductivity, pH and redox potential). The latter was measured using a portable Water Test instrument (Hanna Instruments). Prior to each sampling visit, the instrument was calibrated in the laboratory, following the instructions in the operator's manual. On site, and for each measurement, the water test container was rinsed with the site water three times prior to any measurements. The measurements were carried out in triplicate and recorded in a field notebook. Standard solutions were also measured in the field to ensure that the calibration had not drifted. If standard readings were out of range by ± 5 % then the

instrument was re-calibrated *in situ*.

## 2.3. General treatment of the samples arriving to the laboratory

### 2.3.1. Inorganic characterisation, water samples

Water samples for inorganic characterisation and metal analysis were filtered using 0.45 μm pore size cellulose acetate membrane filters (Whatman®), acidified with nitric acid (1 ml $l^{-1}$) to *ca.* pH ≤ 2 then place in acid cleaned bottles, and stored at 5 or -5 °C, depending on how immediate the following analyses were performed.

Water samples for organic characterisation were filtered through pre-ashed glass fibre filters (GF/F Whatman®), acidified with nitric acid (1 ml $l^{-1}$) to pH ≤ 2 then transferred to pre-ashed Pyrex® glass bottles and stored at 5 or -5 °C, again following the same criteria mentioned above.

pH and electrical conductivity were measured in the laboratory with a bench pH/conductivity analyser A-S 507 (Whatman). Buffer solutions (BDH) pH 4 and 7 were used to calibrate the pH meter and a solution of 0.01 M KCl equivalent to 1413 μSiemens $cm^{-1}$ was used to calibrate the electrical conductivity meter.

Quantitative analysis of total Ca, Mg, Na, K, Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and

Zn was carried out using inductive coupled plasma-optical emission spectroscopy (ICP-OES) using a Jobin Yvon Instruments Emission, JY 138 Ultra-Trace Spectrometer with JY ICP 5.01 software. The peristaltic pump was a PERIMAX 12 model, working at a speed of 547 rpm, using PVC tubing (code black/black). The auto sampler was a Gilson 222 and the nebulizer was a Meinhard with a cyclonic spray chamber. Typical plasma temperatures were between 8000 and 10000 K. Analyses were performed in different aqueous matrices including water, pore water, metal extraction solutions (DDW, 0.1M KCl, 0.1M HCl), preconcentration (final matrix was 0.1M $HNO_{3(aq)}$), and high performance size exclusion chromatography (HPSEC) mobile phase (HPLC grade water, Riedel-de Haen). When high salinity matrices were analysed (0.1 M KCl or estuarine water) a Burgener nebulizer (0.7-1.2 l $min^{-1}$; nominal pressure for 1 l $min^{-1}$ was 45 psi and normal operating pressure 35- 50 psi), and a Scott spray chamber was used.

The emission lines measured in these analyses are shown in Table 2.2. All calibration curves included at least five points, and the regression coefficients, calculated by the ICP-OES software, were typically $r^2 > 0.998$. Multi-element standards were prepared from single element atomic absorption grade stock solutions (1000 mg $l^{-1}$; Fisher Co.), by appropriate dilution. The concentration range of the standards depended on the nature and predicted metal concentrations in the samples. For instance in order to analyse samples in $\mu g\ l^{-1}$ range, the standards ranged from 125 to 3000 $\mu g\ l^{-1}$, including a blank and the instrument was optimised to achieve the lowest possible practical limit of detection (LOD).

**Table 2.2.** ICP-OES emission lines used throughout this investigation.

| Element | ICP-OES line (nm) |
|---|---|
| Ca | 315.887 or 393.366 |
| Na | 588.995 or 589.592 |
| Mg | 279.553 or 280.270 |
| K | 766.940 |
| Al | 396.152 |
| Cd | 228.802 or 226.502 |
| Cr | 205.552 |
| Cu | 324.754 |
| Fe | 259.940 |
| Mn | 257.610 |
| Ni | 221.647 or 231.604 |
| Pb | 220.353 |
| Zn | 213.856 |

Quality control (QC) and reagent blanks were run every 10 or fewer samples to monitor the ICP-OES performance. If the QCs were within 10 % then the analysis was carried out but, if the QCs exceeded this, then the analysis was stopped and continued on another day because calibration had to start again from the beginning looking for the best emission line for each element. This occurred frequently during the ICP-OES analysis and reliable results could only be assured by the operation of a rigorous QC procedure.

To validate the data produced, a series of DDW blanks were analysed to calculate the LODs for each element as LOD = blank concentration + 3 $\sigma^2$. In each case

the blank concentration was calculated from the average value of 10 consecutive readings of DDW and $\sigma^2$ was the standard deviation of these values (Miller and Miller, 1993). Any sample value below LOD has been quoted as < LOD.

**Method for trace metal preconcentration.**

A method of metal preconcentration was developed, based on a combination of two methods, Nojiri *et al.* (1985) and Watanabe *et al.* (1981). The present method was developed to enable the detection of trace metals at concentrations near or below the limit of detection (LOD) of the ICP-OES. Samples had a wide range of salinity. The protocol is divided here into three sections for clarity.

The solutions were prepared as follows; 100 ml of 1 % w/v of Analar grade 8-hydroxyquinoline (BDH) acidified to *ca.* 2 pH with Analar grade HCl (Fisher Co.), 100 ml of 2M Analar grade ammonium hydroxide (Fisher Co.) and 100 ml of 0.1M Analar grade $HNO_3$ (Fisher Co.). Doubly distilled water (DDW) was prepared fresh every day and HPLC grade methanol (Rathburn Co.) was used. Finally the solid phase extraction cartridges used were Sep-Pak $C_{18}$ (non-endcapped) cartridges (1g / 6ml; Waters®).

Natural water samples were treated as mentioned in the Section 2.3.1. 8-hydroxyquinoline was then added (2.5 ml of 1 %) to 500 ml of sample. The pH was adjusted to 8.9 by the addition of 2M ammonium hydroxide and the samples left to stand overnight.

A commercially available solid phase extraction vacuum manifold, (Visiprep

Manifold Supelco) was used along with the Sep-Pak cartridges. Methanol (5 ml) was passed through the cartridge to condition it followed by two portions of DDW (10 ml each). Then the sample (*ca.* 500 ml) was passed through the cartridge at a rate of 10-20 ml $min^{-1}$. The column was washed three times with DDW and the analytes eluted with 5 ml of methanol. The Sep-Pak was not allowed to dry out at any point during this procedure.

The methanol eluted was collected in a 50 ml conical flask containing 0.5 ml of 10 M hydrochloric acid and then evaporated to near dryness. 0.5 ml of nitric acid was added to the samples and evaporation continued nearly to dryness. After repeating the addition of nitric acid and evaporation three times, the residue was diluted to 10 ml with 0.1 M nitric acid. To avoid sample loss through boiling, all the evaporation procedures were carried out very slowly. The final extracts were then analysed by ICP-OES.

Method validation and quality control was achieved as follows. Three series of internally produced reference samples (Table 2.3) were tested using the pre-concentration procedure described above. Seven replicates were analysed for each reference and nine elements were measured simultaneously (Al, Cd, Cu, Fe, Mn, Ni, Pb, Sr and Zn). For each of the seven samples all the metals were added at the concentrations listed in Table 2.3. These groups of samples were prepared in three matrices across the salinity range as follows; doubly distilled water (DDW), and *ca* 15 and 30 mSiemens $cm^{-1}$ conductivity solutions prepared using $NaCl_{(aq)}$.

**Table 2.3.** Arrangement of spiked samples to test metal preconcentration in three matrices. Spiked metals were Al, Cd, Cu, Fe, Mn, Ni, Pb, Sr and Zn.

| Sample No. | Concentration of each metal ($\mu g\ l^{-1}$) | DDW | 15 µSiemens $cm^{-1}$ (NaCl) | 30 µSiemens $cm^{-1}$ (NaCl) |
|---|---|---|---|---|
| 1 | 0 | ✔ | ✔ | ✔ |
| 2 | 1 | ✔ | ✔ | ✔ |
| 3 | 5 | ✔ | ✔ | ✔ |
| 4 | 10 | ✔ | ✔ | ✔ |
| 5 | 20 | ✔ | ✔ | ✔ |
| 6 | 50 | ✔ | ✔ | ✔ |
| 7 | 75 | ✔ | ✔ | ✔ |

In addition to these internally produced reference materials, two samples of Certified Reference Material; Estuarine Water LGC6016 and Trace Metal Fortified Water TM-26.2, (LGC), were tested in the same way. The fresh water reference material (500 ml) was tested using three replicates and the estuarine water reference material (50 ml) was tested in duplicate.

### 2.3.2. Inorganic characterisation, sediment samples

Sediment samples were stored in a refrigerator overnight as soon as possible after returning to the laboratory. Usually the following morning, wet sieving was carried out using a 2 mm mesh plastic sieve. The sample was then divided for organic and inorganic characterisation if necessary, which meant that half was stored in a glass jar (0.5 l) at 5 °C for organic or -5 °C in a plastic bag for inorganic characterisation.

$pH_w$ and electrical conductivity of sediments were measured according to the procedure described in Page *et al.* (1982). Ammonium ($NH_4^+$-N) was measured by the indophenol blue reaction (Page *et al.*, 1982). Nitrate ($NO_3^-$-N), was measured by the improved hydrazine reduction method (Downes, 1978). Phosphate ($PO_4^{3-}$-P), was measured with the molybdenum blue method (Murphy and Riley, 1962).

**Minerals**

Dried sediment samples, separated into three fractions by sieving as follows (2mm to 630 μm, 200 to 63 μm, and less than 63 μm), were analysed for 1 hour in a Philips X-ray powder diffractometer (5-75° 2θ); the software used was the XRD analyzer version 1.0 (Philips). Sub-samples of the same sieved fractions were prepared as KBr disks and scanned using a Perkin Elmer 1600 Series FTIR, with 4 $cm^{-1}$ resolution between 450 and 4400 $cm^{-1}$.

Sediment samples, previously sieved to pass a 2mm mesh, were dried at 110 °C for 8 hours, and then successive wet sieving was carried out and the following fractions collected (> 630, > 200, > 63 and < 63 μm). Each fraction was dried and weighed. The finest fraction (< 63 μm) was then analysed using a particle size analyzer (SediGraph 5000ET Micromeritics) equipped with an X-ray detector. Standardisation and analysis were carried out as instructed in the instrument manual. The results of the wet sieving were plotted on a "Triangular Diagram" according to the Soil Survey of England and Wales (Hodgson, 1976). The finest fraction results were plotted in a "cumulative curve" (Day, 1967).

Sediment samples, previously sieved to pass a 2mm mesh were weighed in triplicate and dried at 110 °C for over 12 hours and then weighed again to calculate % moisture as described by Page *et al.* (1982). This value was considered as the oven-dry basis for all the subsequent measurements of metals, minerals or organic analysis and calculations.

Sequential extraction (SE) of metal ions was performed in the following order; DDW, 0.1M KCl and 0.1M HCl. A mixture of sediment and solution (1:10; w:v) was shaken for over 1 hour on a Heidolph Unimax 2010 LabPlant shaker and then centrifuged at 15,000 rpm (≈ 20,000 *g*) for 10 min. The supernatant solution was collected and filtered through 0.45 μm pore size cellulose acetate membrane filters (Whatman®). Filtrates were acidified if necessary with $HNO_3$ to *ca.* 2 pH and stored at 5 °C until further analysis by ICP-OES. Extractions were performed in triplicate.

### 2.3.3. Inorganic characterisation, pore water samples

Pore water was obtained by centrifugation of the sediment samples following the procedure of Jones and Edwards (1993). This procedure was based on the original experimental design proposed by Elkhatib *et al.* (1987) and Soon and Miller (1977) and was modified to suit the samples as follows. The device used consisted of two centrifuge tubes; one was a 50 ml polypropylene tube and the second, was a 31 ml polypropylene tube. A hole was cut in the bottom of the smaller tube for drainage. The top of this smaller tube was cut *ca.* 2.5 cm (Figure 2.2) and this piece was used as a support. The actual positions of the tubes were

as follows the top of the small tube was placed inside the larger tube, followed by the perforated tube with a small portion of glass wool and a GF/F (Whatman) filter in the bottom. Approximately 20 g of naturally wet sediment was placed in the inner tube. The combined tubes were then centrifuged at 5,000 rpm (≈ 3,000 *g*) for 30 min at a temperature of 5 °C in a MSE-Europa 24M centrifuge with a 8 x 50 ml rotor (43114-143). The pore water passing through the hole was collected in the bottom of the larger tube.

### 2.3.4. Inorganic characterisation, particulate matter

Water samples were filtered as mentioned before (2.3.1). The particulate material captured on the filter was subjected to the same sequential extraction for sediments mentioned in 2.3.2. The fractions were analysed in the ICP following the same procedure and quality control program explained in previous sections.

### 2.3.5. Organic characterisation, water samples

To avoid chemical changes for the pore waters, after the collection, extraction and filtration the sediment pore water samples from the anoxic sites were purged with $N_{2(g)}$ and stored in syringes as recommended by Chin and Gschwend (1991).

#### Total phenol

The Folin-Ciocalteau method (Lowe, 1993; Swain and Hillis, 1959) was used to calculate the total phenol content in water and pore water.

**Total organic carbon (TOC)**

Total organic carbon (TOC) and dissolved organic carbon (DOC) were measured using three different instruments; TOC-500 Total Organic Carbon Analyser (Shimadzu); TOC-5000 MQ 1001 (Shimadzu); or TOC-V CSH/CSN (Shimadzu) analyser as non-purgeable organic carbon (NPOC). The instrument used each time depended on availability. Calibration was performed following the specific instructions for each instrument. The calibration curve for TOC was performed using an aqueous solution of Analar grade potassium hydrogen phthalate (BDH) (e.g. 5 to 100 mg C $l^{-1}$ for NPOC) dissolved in doubly distilled water (DDW) against a blank of DDW. During analysis, QCs and blanks were run every 10 samples. If QCs were within 5 % of the expected value, the analysis was continued, if not, it was stopped and the instrument re-calibrated.

For NPOC, after acidifying the sample to pH 2 to 3, sparge gas ($N_{2(g)}$) was bubbled through the sample to remove the inorganic carbon (IC) component as $CO_2$. The carbon remaining was then measured to determine total organic carbon, and this result is reported as NPOC (non-volatile organic carbon).

**Spectroscopic characteristics**

Two measurements were made using UV-visible (UV-VIS) spectroscopy. Water, pore water and natural organic matter (NOM) solutions were scanned in a UNICAM UV/VIS Spectrometer UV4 using the UNICAM software VISION. Scans were made and recorded from 200-600 nm along with fixed wavelength readings at 224, 254, 280, 465 and 665 nm. 280 nm was chosen because $\pi$-$\pi^*$ electron transitions tend to occur in this region for phenolic substances (Chin *et*

*al.*, 1994). 665 and 465 nm were selected to determine $E_4/E_6$ ratio (an indicator of humification) (Chin *et al.*, 1994). At the beginning of the measurements, the instrument was zeroed twice, first with double distilled water DDW followed by HPLC grade water (Riedel-de Haen). In the reference cell DDW or HPLC grade water was used depending on the sample matrix.

**Molecular weight of natural organic matter.**

Natural organic matter (NOM) in water, pore water and natural organic matter from the Suwannee River (NOM-SR; International Humic Substances Society) was characterised by molecular weight fractionation using high performance size exclusion chromatography (HPSEC).

The Suwannee River is located in South Georgia, U.S.A. (Figure 2.3). The NOM-SR sample was produced by the IHSS by reverse osmosis, then $H^+$-saturation, freeze drying and homogenisation and it contains 92.9 % of the organic carbon of untreated Suwannee River water. The elemental composition of the sample, found by the IHSS is shown in Table 3.31. The NOM-SR has previously been used as a standard reference material by Chin *et al.* (1994) and Chin and Gschwend (1991). It will therefore provide a reference for the studies reported here.

Calibration for molecular weight was carried out using a series of Na-polystyrenesulfonate standards Na-PSS (Polysciences, Inc.). Their molecular weight specifications are provided in Table 2.4. HPLC grade acetone (Riedel-de Haen) was used to set the total permeation volume (Vt) and blue dextran (Sigma)

to determine the void volume (Vo) of the column. Standards solutions were diluted with 0.1M NaCl or in a buffer solution as illustrated in Table 2.5. The standard concentrations used were chose after preliminary tests to obtain the best chromatographic conditions see Table 2.5.

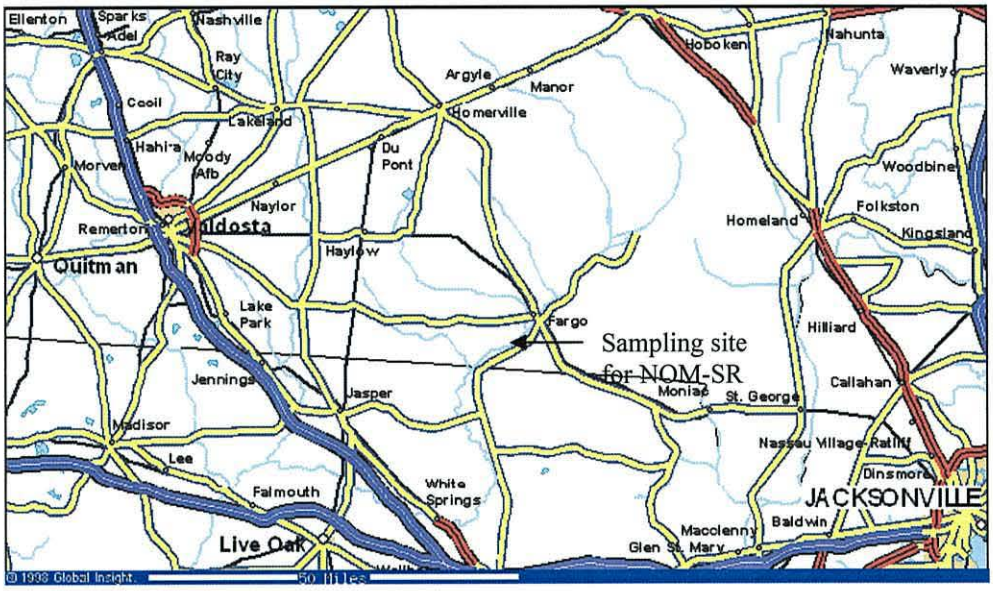



**Figure 2.3.** Part of the Suwannee River, near Fargo, South Georgia USA, showing approximately the sampling site for the natural organic matter acquired from the IHSS.

Standards were run in duplicate using a mobile phase either comprised of 0.002 M $KH_2PO_4$; 0.002 M $Na_2HPO_4$; 0.1 M NaCl and 5 % methanol or in 0.1 M NaCl all prepared using HPLC grade water. The correlation to determine the molecular weight was semi-logarithmically linear with standardized retention volumes given by Vr/Vo, where [Vr = Rt x flow rate] and Vo was the column void volume defined as the elution volume of a very large molecule. Samples were run using the following Kontron HPLC system; HPLC 560 auto sampler; HPLC 535

detector; pump 525 system, Kontron Instruments, with KromaSystem 2000 software.

**Table 2.4.** Standards solutions for Molecular Weight calibration by size exclusion chromatoraphy.

| Standard | Nominal MW Daltons | Weight Average MW | Peak Molecular $M_p$ | Standard concentration |
|---|---|---|---|---|
| **Acetone** | 58 | NA | NA | 50 % v/v |
| **Na-polystyrenesulfonate*** | 4600 | 4300 | 3800 | 100 mg $l^{-1}$ |
| **Na-polystyrenesulfonate*** | 8000 | 6780 | 6710 | 100 mg $l^{-1}$ |
| **Na-polystyrenesulfonate*** | 35000 | 32000 | 30900 | 100 mg $l^{-1}$ |
| **Blue dextran†** | 2000000 | NA | NA | 500 mg $l^{-1}$ |

* Data provided by the manufacturer (Polysciences, Inc.), analysis by GPC.
† Data provided by supplier Sigma
NA = Not applicable

Column performance was determined using a Bio-Rad gel filtration standard. This contained the following series of vitamins and proteins; thyroglobulin (670000 MW), bovine gamma globulin IgG (158000 MW), chicken ovalbumin (44000 MW), equine myoglobin (17500 MW), and vitamin B12 (1350 MW). The standards were run in all three mobile phases (0.1M NaCl, buffer solution and HPLC grade water), at the same time as the Na-PSS standards and the results were compared with the columns performance report, provided by Bio-Rad at the time of purchase (see Table 2.6).

To find the critical ionic strength (CIS) of the column, acetone (50 % v/v) and four solutions of sodium benzoate of varying ionic strength (0.0001; 0.001; 0.01;

0.1 M) were analysed using the SEC column using HPLC grade water as mobile phase (Chin and Gschwend, 1991) and with the chromatographic conditions shown in Table 2.5.

**Table 2.5.** Standard chromatographic conditions used for MW determination of dissolved natural organic matter.

| | |
|---|---|
| **Column** | Bio-Sil®SEC 125-5, 300 mm x 7.8 mm (Bio-Rad) |
| **Injection volume** | 100 µl |
| **Mobile phase:** | 0.1M NaCl or Buffer solution or HPLC grade water |
| **Flow rate:** | 0.6 ml $min^{-1}$ |
| **Detection:** | UV @ 224 and 254 nm |
| **Detector sensitivity:** | 0.05 AUFS (Abs units full scale) |

**Method validation**

To validate the HPSEC method described above, ultra-filtration was carried out on solutions containing compounds with known molecular weights using Centricon® centrifugal filter devices with a 3,000 MW cut-off. TOC and UV-vis data before and after ultra-filtration were compared with those from HPSEC.

The Centricon® centrifugal filter devices were pre-rinsed using two methods to remove any glycerol which might interfere with the TOC and UV-VIS analysis. The pre-rinsing method of Chin and Gschwend (1991) was modified slightly, as follows. The filter devices were left to soak overnight in a methanol: water solution (30:70 v/v), followed by thorough rinsing with doubly distilled water (DDW). Water was then removed by centrifugation (6500 g) for 1 hour. The

final water residue was then removed by inverting the device and centrifuging at 2000 *g* for 2 to 4 min. This procedure was repeated three times. The filters were then left to soak in DDW for approximately 48 hours. At the end of this period, the devices were again rinsed with HPLC grade water, which was then removed by centrifugation in the same way as before, as many times as was necessary until the rinse water gave TOC and UV-VIS fixed wavelength and scan readings that were similar to the blank. In this case the blank was HPLC grade water.

**Table 2.6**. Performance report Bio-Rad Gel Filtration Std.

| | |
|---|---|
| Sample Name: | Bio-Rad Gel Filtration Std (Catalog #151-1901) |
| Injection Volume: | 20 µl |
| Mobile Phase: | 0.10 M sodium phosphate, 0.15 M NaCl<br>0.02 M sodium azide, pH 6.8 |
| Flow Rate: | 1.0 ml $min^{-1}$ |
| Back Pressure/ Temp: | 64 kg $cm^{-2}$ (908 psi) / 23 °C |

| Peak Name | Retention Time Rt (min) | Plates | Half-Width | Area |
|---|---|---|---|---|
| Thyroglobulin | 5.27 | 4604 | 0.18 | 3165402 |
| IgG | 5.89 | 1500 | 0.36 | 3635181 |
| Ovalbumin | 6.84 | 3368 | 0.28 | 2089055 |
| Myoglobin | 8.03 | 9177 | 0.20 | 3072768 |
| Vitamin B12 | 9.96 | 22350 | 0.16 | 3743497 |

The second method used was the one recommended by the filter manufacturer. DDW was passed through the filter at least three times in both directions, and then a solution of 0.1 M NaOH was filtered in the same way. The membrane was then washed with DDW at least three times before a final rinse with HPLC grade water. The filtrate was collected and checked for TOC and UV-VIS fixed

wavelength and scan readings as before.

Two solutions of natural organic matter from the Suwannee River (NOM-SR) were prepared (*ca.* 50 mg C $l^{-1}$); one dissolved in DDW, and the other in 0.1M NaCl. A standard containing Na-PSS (4,600 MW) was also prepared in 0.1M NaCl as explained earlier. These samples were subjected to the ultra-filtration method explained below.

2 ml of sample (accurately measured) was added to the top section of the ultrafiltration device Centricon® (AMICON) (Figure 2.4) and then filtered at 6500 *g* for two hours. This procedure was repeated twice using the same filter device aiming to collect a total of 4 ml of filtrate, enough samples for subsequent analyses. The filtrate was collected in a pre-ashed glass vial and stored at 5 °C waiting for analysis.

### 2.3.6. Organic-metal complexes fractionation of dissolved organic matter (DOM)

#### Qualitative analysis of natural organic matter (NOM)

This method was similar to the one described before (2.3.5) with the difference that the Na-PSS standards, acetone, and blue dextran were dissolved in HPLC grade water in the same concentrations as for the calibration (Table 2.4). These standards were run with HPLC grade water as mobile phase using the same chromatographic conditions as used for the calibration. Samples were run in duplicate.

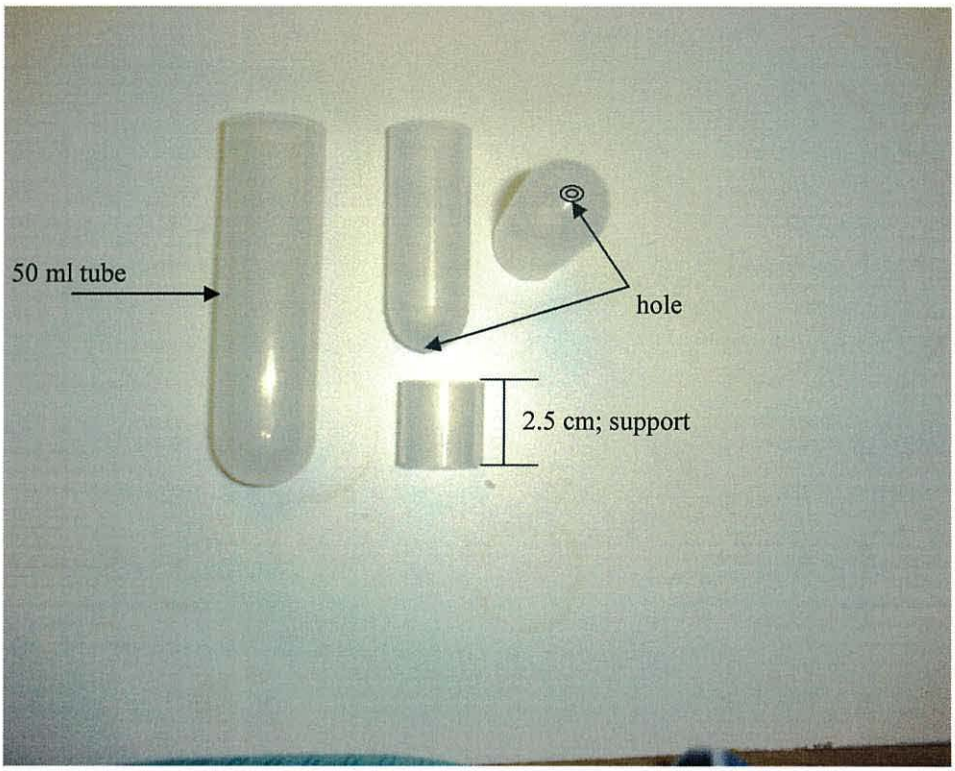



**Figure 2.2**. Centrifugation device for sediment samples, to get pore water. The support and the small tube are placed inside the 50 ml tube in the shown position.

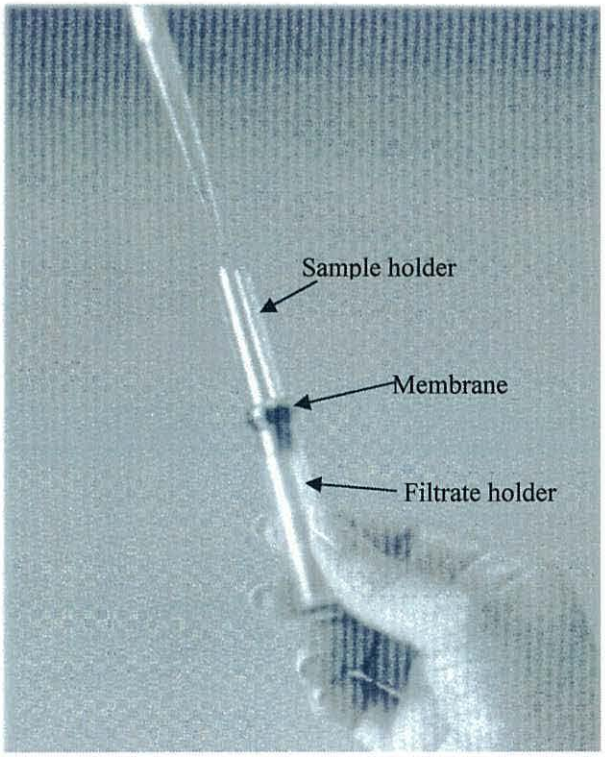



**Figure 2.4** Centricon centrifulgal ultrafiltration devices (Amicon). Showing sample holder, membrane and filtrate holder.

Solutions of NOM-SR were prepared with HPLC grade water for chromatographic analysis with a nominal dissolved organic carbon (DOC) concentration of 40 mg $l^{-1}$ with calculations based on the data in Table 2.7. Five subsequent solutions were prepared with or without spiking with metal ion solutions as described in Table 2.7. Each solution was adjusted to pH 7 with 0.1 M NaOH, and left shaking overnight. A sixth sample was prepared in the same way as sample five but the pH was adjusted in this case to 4.5. On the following day, all the samples were filtered through pre-ashed GF/F glass filters (Whatman). Aliquots of these solutions were taken for further analysis (pH, conductivity, TOC, UV-VIS fixed wavelength and scan readings, and metal content by ICP-OES). Samples were also run through the HPLC using a gel Bio-Sil®SEC 125-5 column (300 mm x 7.8 mm; Bio-Rad).

**Table 2.7.** Specifications of NOM-SR solutions used for NOM-metal complex fractionation

| | **Sample specification** | |
|---|---|---|
| **Code** | **pH** | **Metals** |
| **NOM-SR-1** | 7.0 | None |
| **NOM-SR-2** | 7.0 | 10 mg Zn $l^{-1}$ |
| **NOM-SR-3** | 7.0 | 100 mg Zn $l^{-1}$ |
| **NOM-SR-4** | 7.0 | 10 mg Zn $l^{-1}$; 10 mg Pb $l^{-1}$; 20 mg Mg $l^{-1}$; 20 mg Ca $l^{-1}$; 20 mg K $l^{-1}$ 20 mg Na $l^{-1}$ |
| **NOM-SR-5** | 7.0 | 100 mg Zn $l^{-1}$; 50 mg Pb $l^{-1}$; 100 mg Mg $l^{-1}$; 100 mg Ca $l^{-1}$; 100 mg K $l^{-1}$; 100 mg Na $l^{-1}$; |
| **NOM-SR-6** | 4.5 | 100 mg Zn $l^{-1}$; 50 mg Pb $l^{-1}$; 100 mg Mg $l^{-1}$; 100 mg Ca $l^{-1}$; 100 mg K $l^{-1}$; 100 mg Na $l^{-1}$ |

A solution of EDTA 20 μg $l^{-1}$ was prepared and run through the HPLC column as the mobile phase for five minutes followed by HPLC grade water for 15 minutes after each sample with the aim of removing any metal ions remaining on the column and thus preventing sample carry over. Samples of these wash solutions were collected for metal analysis to quantify the possible metal-binding of the gel column.

**Table 2.8.** Optimised times for fractions of NOM-SR run on the HPSEC. Key # is the sample code as in Table 2.7. e.g. For NOM-SR-1 fraction 1 was labelled F1-1, fraction 2 F1-2, etc.

| Fraction Num. | Time period of eluent collection (mins) |
|---|---|
| **NOM-SR-F#-1** | 1.3 to 10.0 |
| **NOM-SR-F#-2** | 10.0 to 19.0 |
| **NOM-SR-F#-3** | 19.0 to 39.0 |
| **NOM-SR-F#-4** | 39.0 to 60.0 |

The conditions for the HPSEC were the same those described in Table 2.5 for the calibration, but only one detector channel was used (254 nm). Samples were run in duplicate and four fractions were taken as explained in Table 2.8. Every fraction was acidified with 2M HCl and stored at 5 °C before analysis by ICP-OES. Two analytical methods were used to analyze samples, blanks and fractions. These two methods were set up for different ranges of metal content. Method A was a multi-element standard calibration comprised by Ca, K, Mg, Na Pb and Zn from 0 to 3000 μg $l^{-1}$, while method B was a calibration between 0 and

110 mg $l^{-1}$. These were used depending on the metal content expected in each sample. For instance the spiked samples NOM-SR were analysed using method B, because it was assumed that they had the metal content specified in Table 2.7.

### 2.3.7. Organic characterisation, sediment samples

Sediments previously sieved and dried were analysed for their carbon, hydrogen and nitrogen content in an Elemental analyzer CHN-2000 (Leco), following the manual instructions. Instrument was calibrated with Analar grade EDTA. In principle the sample was combusted in a chamber, the $CO_2$, $H_2O$, $N_2$ and $NO_x$, as products of the combustion process in gas form, passed through an infrared cell to determine the carbon and hydrogen content and a thermal conductivity cell to determine $N_2$. The content of organic matter by loss on ignition (LOI) at 550 °C in sediments was carried out using the standard method as described in Page *et al.* (1982). Three replicates per samples were run each time.

## 2.4. Propetamphos (PPT) fate and mobility

### 2.4.1. Propetamphos sorption and desorption

The sites chosen for these experiments were Llanrwst (LL), Tal y Cafn (TC) and Bird Sanctuary (BS). Sediments were subjected to % moisture, elemental content of carbon, nitrogen and hydrogen, and particle size fractionation analysis as described previously.

Experiments were carried out in Pyrex® glass bottles (25 ml) with Teflon® liners to avoid any adsorption of PPT by the container. The sediment: aqueous solution ratio was 1:10 by weight (2 g to 20 ml) and $NaN_3$ 0.25 % v/w was added to minimize any microbial activity. Bottles were shaken at *ca.* 150 rpm in the dark and at room temperature. Dilutions of propetamphos (PPT) were made using the commercial formulation of PESTANAL grade propetamphos® (Sigma). 50 µl of a 1 µCi (37 mBq) solution of $^{14}$C-propetamphos was added to all the test samples.

Sample solutions were removed immediately after spiking (5 minutes), and then at 2, 4, 8, and 24 hours. Each well-mixed supernatant solution (1.5 ml) was transferred to a 1.5 ml Eppendorff® tube. This solution was then centrifuged for 5 minutes at 12,000 rpm (*ca* 10,000 *g*) in a Denver Instrument Force 16, rotor 12 x 1.5 ml centrifuge. The centrifuged supernatant (1 ml) was mixed with scintillation cocktail and the $^{14}$C activity in solution from the $^{14}$C-PPT non-sorbed was measured in a scintillation counter (EG & G WALLAC 1409) following the default program for the $^{14}$C isotope. The instrument was tested approximately once a month with a standard solution of known radioactivity, and the expected readings were always within 5 %. The default program quantified the radioactivity of the $^{14}$C isotope by measuring the light emission 60 times over a 1 minute period with the final reading being the average of these. The adsorbed pesticide was calculated as the difference between the initial pesticide concentration and the supernatant concentration at equilibrium. This data was fed into the FITFUNC.BAS computer program to determine the sorption isotherms equations according to the particular model, Langmuir and Freundlich. The

amount of sorption was expressed as μg of propetamphos per g of dry sediment.

#### 2.4.1.1. Propetamphos concentration effect

Duplicate sediments from LL, TC and BS were tested using PPT concentrations of 1, 5, 10, 25 and 75 μg $ml^{-1}$. Duplicate controls were also included at each PPT concentration, which had the solution but no sediment. The ratio of sediment: aqueous solution for these experiments was 1:10 (e.g. 2g of sediment dry weight base to 20 ml aqueous solution). The sorption kinetics was measured at 5 minutes, 2, 4, 8 and 24 hours.

#### 2.4.1.2. Effect of salinity on PPT sorption

To test the effect of ionic strength on propetamphos sorption, two experiments were carried out. The first one used sediments from TC and BS. The aqueous matrix used was 40 g $kg^{-1}$ of ASS and, as before, PPT concentrations were 1, 5, 10, 25 and 75 μg $ml^{-1}$. A blank was also included. The kinetics were measured at five points in time; the beginning (5 minutes), 2, 4, 8 and 24 hours. Both, controls and samples were performed in triplicate. There were controls for each concentration (no sediment).

In the second experiment the effect of salinity gradient was tested (ASS = 0, 10, 20, 30 and 40 g $kg^{-1}$). The propetamphos concentrations used were 0.5 and 10 μg $ml^{-1}$ and the kinetics was measured at the beginning, and at 12 and 24 hrs. Controls (no sediment) for each PPT concentration and the test samples were

performed in triplicate.

### 2.4.1.3. Effect of NOM on PPT sorption

To assess the effect of NOM on PPT sorption three experiments using humic acid as NOM were performed. The experiments were designed to measure firstly if a high content of NOM affected the sorption kinetics in sediments from three different sites. Secondly, experiments were carried out to test if the concentration of NOM affected the sorption in one sediment sample. The last experiment was used to test if the origin of natural organic matter made any difference to the sorption of propetamphos.

The ratio of sediment: aqueous solution was the same as used previously (1:10). The conditions of incubation, extraction of sub-samples and the measurement of the $^{14}C$ activity were also carried out as described previously. The kinetics of the three experiments were all measured at the beginning (5 minutes), and at 2, 4, 8 and 24 hours. Moreover, all three experiments were carried out in triplicate and the controls (no sediment) for each concentration in duplicate. The details of the experiments are described below.

In the first experiment, sediments from LL, TC, and BS were tested using *ca.* 40 mg DOC $l^{-1}$ aqueous solutions comprised of humic acid (Aldrich). PPT concentrations were 0.5, 1, 10, 25 and 75 mg $l^{-1}$. A blank was also included. For the second experiment, LL sediments were tested at a range of humic acid concentrations (0, 5, 10, 30 and 50 mg C $l^{-1}$). The PPT concentration in each case

was 1.0 μg $g^{-1}$. In the third experiment, four sediment sub-samples from LL were spiked with 1.0 μg $g^{-1}$ PPT. Natural organic matter (*ca.* 40 mg C $l^{-1}$) from four sources (humic acid (Aldrich), fulvic acid and humic acid (samples from Norway), and NOM-SR (IHSS)) were then added to each sediment sub-sample.

The humic and fulvic acid from Norway were extracted from the lake Skervatjern in Western Norway. This lake has been the subject of a project called HUMEX (Humic lake, acidification experiment). The humic substances used here corresponded to the background soil (not acidified). The elemental composition of these humic substances can be seen in section 3.2.3.1, (Table 3.31).

#### 2.4.1.4. Effect of metal solutions on PPT sorption

Sediment from LL was used to measure the effect of metal ions on PPT sorption. Metals (Al, Fe, Pb, and Zn) at a concentration of 1mM, were added to the sediments separately with 1.0 μg $g^{-1}$ PPT, and then in an equimolar mixture (1mM of each metal ion or 269 μg $g^{-1}$ Al; 558 μg $g^{-1}$ Fe; 2072 μg $g^{-1}$ Pb and 653 μg $g^{-1}$ Zn) with 0.5 μg $ml^{-1}$ PPT. The parameters such as the sediment: aqueous solution ratio, incubation characteristics, sub-sampling and kinetics readings were kept the same as for the earlier experiments.

#### 2.4.1.5. Industrial grade propetamphos sorption (PPT-Ind)

The sorption kinetics of the industrial propetamphos (Ectomort centenary; Vericore Ltd.) were measured using sediments from LL, and BS. Sediment

samples were spiked with a range of Ind. PPT solutions; 0.5, 1.0, 10, 25 and 75 $\mu g\ ml^{-1}$ (PPT-Ind), along with a blank. The kinetics were measured at the beginning (5 minutes) and after 2, 4, 8 and 24 hours. Four replicates were used for each PPT concentration, and the control (no sediment) for the lowest and the highest PPT concentrations were tested in duplicate.

Finally the effect of ionic strength on the sorption kinetics of Ind. PPT was tested with a range of ionic strength solutions (0, 10, 20, 30, and 40 $g\ kg^{-1}$ ASS). The PPT concentration was 0.5 $\mu g\ ml^{-1}$, and the other experiment parameters were the same as used previously.

The sorption kinetics were calculated by subtracting the control in each case and standardised to percentile concentrations of mass as $\mu g\ g^{-1}$. The sorption was estimated using the linear, Freundlich isotherm expression (Equation 3, section 4.1.1) (Fytianos *et al.*, 2000),

**2.4.1.6. Desorption experimental procedure**

For every sorption experiment, after the last sample for the sorption experiment had been removed, the remaining sediment solution was transferred to a 50 ml centrifuge tube. Solutions were centrifuged at 10,000 rpm (*ca* 9,000 *g*) for 10 minutes and a sub-sample was removed (1 ml) for $^{14}C$ radioactivity measurements, the rest of the solution was safely discharged. Finally, respective solutions of DDW, ASW, or NOM with 0.25 % $NaN_3$, (20 ml) were thoroughly mixed with the remaining sediment. Bottles were shaken in the dark and

solutions were sampled for $^{14}C$ radioactivity at 1, 24 and 48 hours, after centrifugation of an aliquot. The concentration of propetamphos desorbed was determined in the supernatant.

### 2.4.2. Propetamphos biodegradation

**Physico-chemical characterisation of sediments**

Three sediments from different locations along the River Conwy (Llanrwst, Tal y Cafn and Bird Sanctuary), were analysed to determine moisture content, conductivity, % C, % N, pH, N-$NH_4^+$, K, P-$PO_4^{3-}$, and particle size fractionation following the standard methods described earlier.

Biological activity was measured as respiration, using a CIRAS-SC $CO_2/H_2O$ gas analysis respirometer (PP Systems Ltd.). For each sample, 30 g of wet sediment was weighed into a plastic tube and placed on the respirometer. Measurements (three replicate sediment samples) were taken every minute, for 1 hour to stabilise the instrument readings.

Experiments were sub-divided into four categories, depending on their purpose, as described below.

#### 2.4.2.1. Propetamphos (PPT) biodegradation kinetics in aerobic and anaerobic conditions.

Wet sediment samples (*ca.* 2g, dry weight) from LL, TC and BS were spiked with

propetamphos (PPT) (0.685 μg $g^{-1}$), supplied in its commercial formulation PESTANAL® (Sigma), and 8.46 nCi (313 Bq) $^{14}$C-propetamphos (Huntingdon Life Sciences Ltd., specific activity 19.8 μCi $mg^{-1}$ (733 Bq $mg^{-1}$), radiochemical purity 97.9 %). The ratio of solid: aqueous solution in bottles, was 1:1 (w:v) for all the experiments, e.g. 2g of sediment by dry weight: 2 ml aqueous solution including the intrinsic water. Four control replicates (no sediment) were run simultaneously.

The experiment under aerobic conditions was as follows. Propetamphos spiked sediments were left to incubate in 25 ml Pyrex® glass bottles with 1ml of 1M NaOH in 1.5ml Eppendorff® tubes held in the neck of the bottle to trap the $^{14}$C-$CO_2$ evolved during the incubation period (Figure 2.5). The bottles were placed into 100 ml centrifuge tubes with an air-tight rubber stopper. At the end of the incubation period the 1ml of 1M NaOH was mixed with scintillation cocktail (OptiPhase "Hisafe" 3 Wallac®) and the activity in solution was measured using the scintillation counter described previously (EG & G WALLAC 1409). Experiments were run at room temperature (20 ± 3 °C) and laboratory light conditions (50 to 100 μmol $m^{-1}$ $s^{-1}$). The total incubation period lasted 32 days, but samples were taken at regular intervals (1 day) during the first week, and then every three and five days. Biodegradation was expressed as a percentage of $^{14}$C-$CO_2$ recovered from the initial amount of $^{14}$C pesticide added.

To maintain an anaerobic atmosphere during the incubation period samples were purged with $N_2$ gas for ½ hour using a system of tubing and syringes (Figure 2.6),

after inoculation and on each occasion $^{14}C\text{-}CO_2$ samples were collected. A loop system tubing adaptation was used to ensure air tightness. Apart from this modification, experimental conditions were kept the same as for aerobic incubations. Extractions were also carried out at the beginning and at the end of incubation in the same manner as before.

#### 2.4.2.2. $^{14}CO_2$ evolution kinetics

The evolution of $^{14}C\text{-}CO_2$ was measured daily during the first week after $^{14}C$ addition, then on every other day during week 2, then once every three days in week 3 and finally every five days until the 32nd day after addition. The 1M NaOH trap was replaced with a fresh solution on each occasion. Four ml of liquid scintillation cocktail was added to the NaOH and mixed thoroughly in a Vortex-2 (Gebue Scientific Industries). The activity of $^{14}C\text{-}CO_2$ was measured in a liquid scintillation counter (EG & G WALLAC 1409). This procedure was followed for all experiments.

#### 2.4.2.3. PPT biodegradation kinetics when in the presence of added organic nutrients.

Wet sediment samples (*ca.* 2g, dry weight) from TC and BS were spiked with propetamphos (0.685 $\mu g\ g^{-1}$), and 8.46 nCi (313 Bq) $^{14}C$-propetamphos. Organic nutrients (D-glucose and Bovine serum albumin or BSA) were spiked into

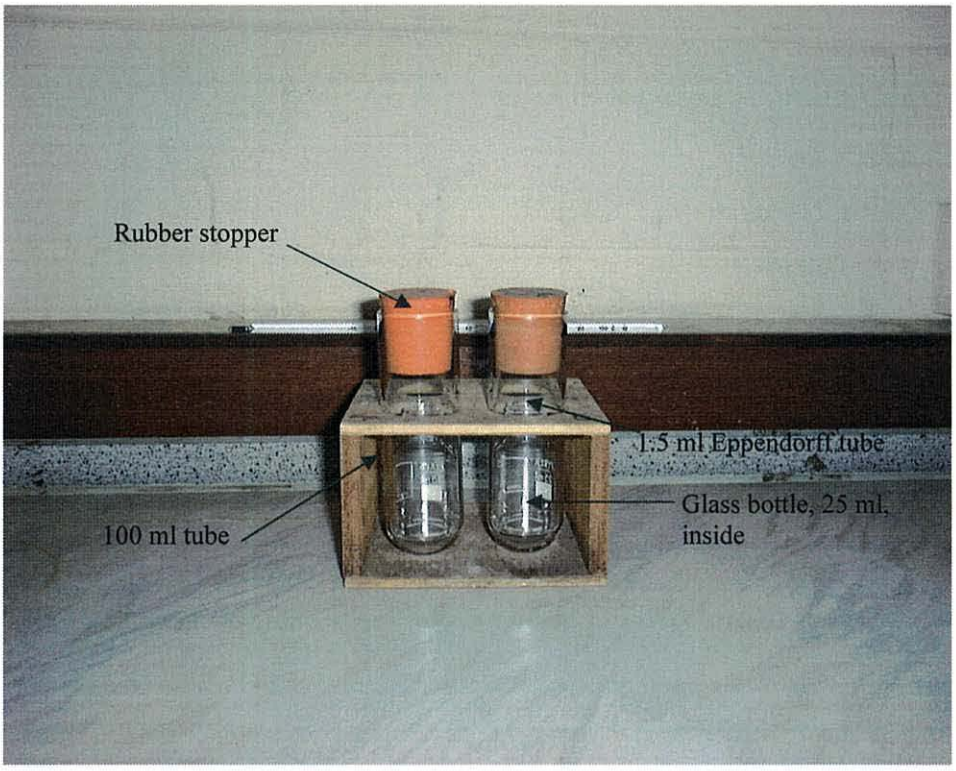



**Figure 2.5** Experimental set up for biodegradation in aerobic conditions. A glass bottle (25 ml) is place inside a 100 ml tube with a 1.5 ml Eppendorff tube with 0.1M NaOH solution and air tight sealed with a rubber stopper.

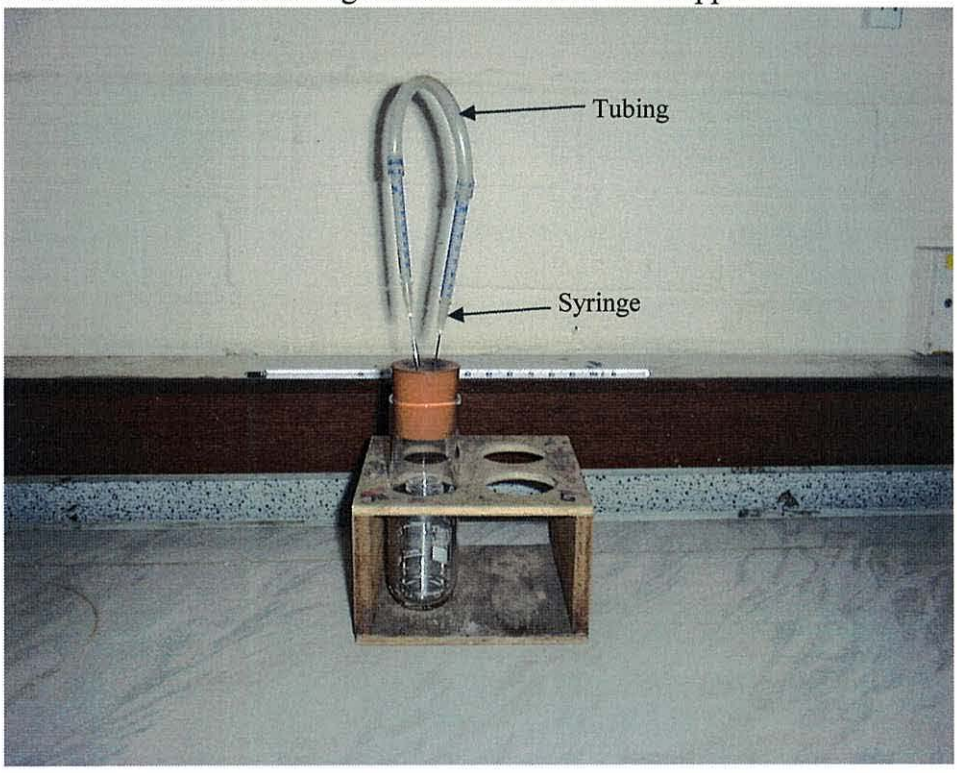



**Figure 2.6** Experimental set up for biodegradation in anaerobic conditions. The syringe an tubing systems was used to purge $N_{2(g)}$ after sampling and were kept in this position during incubation.

sediments in the appropriate dilution to have 0.5 mM D-glucose and 90 μg $l^{-1}$ BSA concentrations (or 90 μg $g^{-1}$ each). Spiked sediments were left to incubate as described before.

Three replicates were run per sample along with control I (no sediment), and control II (no nutrients), which were run at the same time. The evolution of $^{14}C$-$CO_2$ was measured as described previously.

#### 2.4.2.4. Effect of concentration on propetamphos biodegradation

To find out which concentration was biodegradable without affecting the microbial activity an experiment was set up as follows. Wet, natural sediment samples (*ca.* 2g, dry weight) from LL were spiked with propetamphos (0.07; 0.15, 1.5, 3.0, 7.0, 15 30, 60 and 75.6 μg $g^{-1}$) and 6.0 nCi (222 Bq) $^{14}C$-propetamphos. Control samples were also spiked with D-glucose-UL-$^{14}C$, (Sigma, specific activity 300 mCi $mmol^{-1}$ ($11.1\times10^9$ Bq $mmol^{-1}$) purity ≥ 98 %) 0.25 mM, or 5.3 nCi (196 Bq) as an organic substrate, which is more available source of carbon. This control indicated any effect that the PPT had on the microbial population.

#### 2.4.2.5. Matrix effect (DDW, ASW, and NOM).

The effect of the aqueous matrix on PPT degradation kinetics was tested by preparing the propetamphos solution (1.0 μg $g^{-1}$) in three different matrices; doubly distilled water (DDW), artificial sea water (ASW) at 3.3, 6.7, 13.4 and

26.8 g $kg^{-1}$ of artificial sea salt (ASS) (Sigma) and NOM-SR (*ca.* 130 mg C $l^{-1}$). Wet sediment samples from LL (sand) were spiked with these solutions, and the $^{14}C$-$CO_2$ evolved during the incubations time was sampled and measure as previously described.

### 2.4.2.6. Effect of Zn and Pb on propetamphos biodegradation

To test how the presence of metals affected the biodegradation of propetamphos the next experiments were carried out. Natural, wet sediment samples from LL (*ca.* 2g, dry weight) were spiked with, 6.0 nCi (222 Bq) $^{14}C$-propetamphos. $^{14}C$-glucose (0.25 mM; 5.3 nCi, (196 Bq)) was used as a control substrate. Sediments (LL sand) with propetamphos or glucose as organic substrates, were spiked with zinc (2.2, 4.4, 21.9, 43.8 and 219.1 μg $g^{-1}$), or with lead (5.5, 11.1, 54.9, 110.6 μg $g^{-1}$). The fact that the sediment was characterised as sand had advantages for these experiments because sorption was limited, so there should have been at least in theory more propetamphos bioavailable for degradation. The results have to consider the different intrinsic microbial load.

Spiked sediments were left to incubate in the same manner as described previously. Three replicates per sample and 3 controls were run at the same time. The control experiment consisted of the same concentrations and combinations of metals but with glucose as an organic substrate instead of PPT. The incubation conditions were the same as described previously. The incubation period lasted 10 days. The evolution of $^{14}C$-$CO_2$ as result of the biodegradation of $^{14}C$-PPT by the indigenous microbial population was measured twice, the first time at the 5$^{th}$

day and the second time at the end of the 10th day, and the results were analysed statistically using the ANOVA-one way test and if $P<0.01$ the Tukey's pairwaise comparison was considered.

### 2.4.2.7. Biodegradation of industrial grade propetamphos (PPT-Ind)

The biodegradation of PPT-Ind (Vericore Ltd) was tested with different aqueous matrices. Experiments were run at controlled temperature (10 ± 3 °C) and in darkness. The incubation period lasted 10 days. The aqueous solution was centrifuged and $^{14}C$ activity measured as described previously. Sub-samplings were carried out on the 2nd, 5th, 8th and 10th day.

**Table 2.9.** Matrix of experiment permutations for biodegradation experiments at 10 ± 3 °C, darkness and during 10 days incubation period.

| Compounds | DDW | ASW (3.3 g $kg^{-1}$ ASS) | NOM (130 mg C $l^{-1}$) | NOM & ASW |
|---|---|---|---|---|
| Glucose (OS1) | ✓ | ✓ | ✓ | ✓ |
| PPT (OS2) | | | | |
| Ind PPT (OS3) | | | | |
| OS1 and Zn (2.2 µg $g^{-1}$) | ✓ | ✓ | ✓ | ✓ |
| OS2 and Zn (2.2 µg $g^{-1}$) | | | | |
| OS3 and Zn (2.2 µg $g^{-1}$) | | | | |
| OS1 and Pb (5.5 µg $g^{-1}$) | ✓ | ✓ | ✓ | ✓ |
| OS2 and Pb (5.5 µg $g^{-1}$) | | | | |
| OS3 and Pb (5.5 µg $g^{-1}$) | | | | |
| OS1, Zn and Pb | ✓ | ✓ | ✓ | ✓ |
| OS2, Zn and Pb | | | | |
| OS3, Zn and Pb | | | | |

Organic substrate; OS1 glucose (0.25 mM); OS2 PPT (1 µg $g^{-1}$); OS3 Ind. PPT (1 µg $g^{-1}$)

The permutations for this last experiment are shown in Table 2.9. In summary the effect of simple or complex matrices on PPT-Ind biodegradation was measured and these were compared with the controls (glucose) and the experiments with Pestanal grade PPT.

## 2.5. Propetamphos Toxicology

The indigenous sediment microbial population was subjected to stress through the addition of propetamphos, metals (zinc and lead) diluted in DDW and NOM-SR dissolved in DDW added either individually or in combination. One hypothesis of this experiment was that the indigenous microbes would respond to ecotoxicological stress by consuming the added organic substrate at a lower rate than without stress.

Sediments were collected from LL (River Conwy) for the toxicology experiments. Moisture content, size grain fractionation, elemental analysis (C %, N %, and H %), $pH_w$, and background respiration to measure the indigenous biological activity, were carried out on this sediment. Sediments were also submitted to sequential extraction as it was explained in section 2.3.2 to determine a broad metal speciation. All these methods have been described in previous sections.

### 2.5.1. Effective Concentration ($EC_{50}$), ($EC_{10}$) and Maximum Acceptable Toxic Concentration (MATC)

The effective concentration ($EC_{50}$) was calculated as the concentration of propetamphos that caused a 50 % reduction in respiration in comparison with the control (unstressed sample) with the $EC_{10}$ corresponding to a 10 % reduction. The percentage inhibition (%) at different concentrations of the toxicant was taken as the reduction in $^{14}C$-$CO_2$ production of spiked bottles compared to the control. The % inhibition values were then plotted against the respective toxicant concentrations ($Log_{10}$; mg $l^{-1}$), and the data were fitted to the best empirically derived equation and from these plots, the concentration causing 50 % inhibition ($EC_{50}$) was determined. (Nirmalakhandan *et al.*, 1994). $EC_{10}$ was determined in the same way but at 10 % inhibition.

The maximum acceptable toxicant concentration (MATC) is defined as the estimated toxic threshold concentration falling between the highest concentration revealing no effect (NOEC) and the next highest concentration indicating a toxic effect (LOEC), or the geometric mean, when compared to the controls (Sanchez *et al.*, 2000). This MATC was found using the ANOVA one way statistical test.

The ratio of sediments and aqueous solution was 1:1 (e.g. 2 g of sediment dry weight: 2 ml aqueous solution). $^{14}C$-glucose was added as an organic substrate. Radio labelled $^{14}C$-$CO_2$, the product of glucose respiration was trapped using 1 ml of 1.0 M NaOH. This NaOH trap solution was then measured for $^{14}C$ radioactivity using a scintillation counter (EG & G WALLAC 1409) as described

previously. The incubation period was only 1 hour, and was performed at controlled temperature (10 ± 3 °C).

Propetamphos was tested three times under the same conditions, to determine the variability of the proposed method. The PPT concentrations were 0.1, 1, 10, 60, 110 and 140 μg $g^{-1}$. Zinc and lead were also tested individually at concentrations of 10, 50, 100, 500, 1000, and 2000 μg $g^{-1}$. A blank was tested for each compound.

The experiment with Ind. propetamphos was run at room temperature in a fume cupboard, for safety reasons, at 0.1, 1, 10, 60, 110, 140, 500 and 1000 μg $g^{-1}$. The safety reasons were that highly concentrated solutions were used and the recommendation of the manufacturer were that the experiment should be kept in a well ventilated area. Blanks were included for each experiment. This series of experiments was carried out in DDW.

To test the effect that NOM had on PPT, lead and zinc's $EC_{50}$ and MATC, these compounds were tested again using the same conditions and concentrations mentioned earlier, the only variable was the matrix used included NOM-SR (*ca.* 40 mg C $l^{-1}$).

### 2.5.2. Binary combinations effect

The ecotoxicological effect of binary combinations of PPT and metals were performed at an equitoxic ratio 1:1 calculated from the $EC_{50}$ calculated before,

and testing a range of toxic units TU (0.25, 0.5, 0.75, 1.0, 1.25, 1.5 and 1.75). The TU of a mixture was calculated by adding the ratios of the concentrations of each metal in the mixture divided by its $EC_{50}$ when present alone. Thus a mixture of two compounds where the concentration of each metal was equal to one half of its $EC_{50}$ yields a sum TU = 1 (Sprague and Ramsay 1965). Blanks were included for each experiment. The compounds involved were propetamphos, zinc and lead. This series of experiments was carried out in DDW.

**Table 2.10.** Ternary combination experiments to measure toxicity.

| Mixture | Matrix | Equitoxic ratio |
|---|---|---|
| PPT : Zn : Pb | DDW | 1:1:1 |
| | NOM-SR | |
| Ind. PPT : Zn : Pb | DDW | 1:1:1 |
| | NOM-SR | |
| PPT : Zn : Pb | DDW | 1:100:10 |

### 2.5.3. Ternary combinations effect

Ternary combination experiments were carried out mostly in an equitoxic ratio 1:1:1. Experiments were carried out in DDW and NOM-SR (40 mg C $l^{-1}$), and

both pure and industrial grade propetamphos were included. The last experiment was done at a different toxic ratio (1:100:10) just to mimic the ratio in which these compounds are roughly found in the environment. The TU were the same as for the binary combinations experiment mentioned earlier. Combinations of these experiments are presented in Table 2.10.

# Chapter 3. Inorganic and organic characterisation of water and sediment from the Conwy River and estuary

## 3.1. Introduction

Chapter one outlined the important role that estuaries play in determining the movement and fate of inorganic and organic contaminants within the environment. There are an extensive number of studies about inorganic contaminants in estuaries, but fewer examples on the behaviour of organics. This thesis focuses on the behaviour and fate of the pesticide propetamphos (PPT), a study not previously reported in the literature. It is hoped that through this work the environmental fate and toxicology of organophosphate compounds such as PPT in estuaries will be better understood. The present chapter details the inorganic and organic characterisation of water and sediment samples used throughout the later study. This will ultimately provide a better knowledge of the fate and behaviour of PPT in the river and estuary in question.

### 3.1.1. Inorganic characterization

Physical separation to differentiate between "soluble" and "particulate" matter, in aqueous solutions was by filtration through 0.45 μm pore size membranes. This procedure is reported to work equally for metals (Ross, 1994), and dissolved

organic carbon (Head, 1976). This initial characterisation is rather crude but will primarily help to assess the reactivity of any soluble material.

The reactivity of chemicals in estuaries has been assessed by applying the concept of conservative and non-conservative behaviour (Sharp *et al.*, 1984). In this approach, the concentration of any material is plotted against salinity. A straight line with a negative slope is interpreted as conservative mixing behaviour indicating an estuarine sink e.g. filtration whilst an upward curve indicates non-conservative behaviour, which in turn can indicate an estuarine source. This method will help to understand the role that the estuary plays in the movement and fate of contaminants, and the possible interactions between natural organic matter and inorganic compounds.

In sediments as in soils the most important factors influencing metal speciation are pH, organic matter (quality and quantity), Fe and Mn oxides and clay content (Ross, 1994). These factors will control the forms in which metal species are found in the environment (e.g. particulate, colloidal and soluble fractions).

Metal availability is often directly correlated with how mobile and mobilisable the metals are. Berrow and Burridge, (1980) summarised the principal forms of mobile and mobilisable toxic metals in soil as follows:

(i) in the soil solution (ionic, molecular, chelated and colloidal forms), removed by water extraction

(ii) at the exchange interface (readily exchangeable ions in inorganic or organic fractions), extracted by ion exchange with neutral salts.

(iii) in adsorption complexes (more firmly bound ions), extracted by $H^+$ ions from dilute acids.

(iv) incorporated in precipitated sesquioxides and insoluble salts, extracted by strong acids.

(v) fixed in the crystal lattice of secondary clay minerals

Though that these concepts have mainly been applied to soil, they are also readily applicable to sediments. Further, soil solution can be viewed as being analogous to sediment pore water. The main difference with sediments, however, is that their water content is typically much higher and thus the potential metal mobility is usually greater.

The general approach to determine the metal content in these different fractions is to extract the metals using a sequence of solvents, starting with the least aggressive to remove the most weakly bound metal and ending with the most aggressive to remove the most strongly bound. Despite the range of sequential chemical extractions that have been published, the general agreement is clear that, for the soluble and readily exchangeable metal fractions, dilute salt solutions capable of replacing cations are the most appropriate solvents. Organic bound complexes are released using oxidising agents, whilst concentrated or boiling nitric acid has been used to assess incorporated or fixed metals (Ross, 1994).

Generally the speciation of trace metals in aquatic environments will depend on their interaction with organic ligands. Davis and Leckie (1978) reported that the distribution of trace metals in natural aqueous systems may be controlled by

surface binding on colloidal particles coated with humic compounds rather than reactions with simple oxide surface sites. The importance of these processes is illustrated by the following examples. If, for instance, organic ligands are not present in sea water then the copper ion concentration can be toxic to many organisms, (see section 3.1.3). In addition, Cu, Fe, Zn, Cd and Mn uptake by marine phytoplankton and bacteria depends on the concentration of free metal ion in solution rather than the total concentration (Mackey and O'Sullivan, 1990). It is therefore proposed that humic compounds can have an impact on the bioavailability of organic pesticides (e.g. PPT) through direct interactions, or through interactions with metals.

One of the most important groups of organic ligands in rivers and estuaries is natural organic matter which is the decomposition product of leaves, detritus, and natural organic products. Unsurprisingly natural organic matter is a complex mix of large and small MW organic molecules. However, it has been divided into two main sub-groups of compound, namely humic and fulvic acids. Fulvic acids are materials that are soluble in water at all pH values. Humic acids are materials that are insoluble at pH values less than 2 but are soluble at higher pH values (Gaffney *et al.*, 1996). It is well known that these substances have a substantial capacity to form soluble complexes with metal ions and or cationic organic molecules and to interact with mineral surfaces (Zhang *et al.*, 1996), though the precise nature of the metal-humic interaction remains poorly understood. However, due to their large number of carboxyl groups it is known that humic macromolecules have a large negative charge in solution. It is therefore to be expected that cationic metal ions will interact with humics. For instance, Zhang *et al.* (1996) found that, for

copper and lanthanum, a significant increase in weight-averaged molecular weight occurs as the concentration of these metal ions increases. They also used gel permeation chromatography technique to fractionate metal-humic complexes. They clearly showed that metal ion-humic interactions are selective, suggesting that both the chemistry of the metal ions and of the humic ligands are critical factors controlling organic matter-metal ion behaviour in natural waters. NOM-metal complex is the generic term used throughout this work to explain these interactions.

Humic and fulvic acids are also known to form aggregates of colloidal dimensions. Guetzloff and Rice (1996) showed that humic and fulvic acids are able to solubilize hydrophobic organic compounds (HOC) in the same way that micelle-forming surfactants do, but not at concentrations that are environmentally relevant. Moreover, they found that some HOCs are not solubilized to the same extent as others.

### 3.1.2. Dissolved organic matter (DOM) characterization

Information regarding the chemical reactivity and mobility of humic substances or dissolved organic matter (DOM) as a whole can be gained by measuring bulk properties such as molecular weight and light absorption. At the same time, characterisation of DOM in "free" and pore water is essential to understand their role in the fate, reactivity and transport of inorganic and organic pollutants (Chin and Gschwend, 1991; Chin *et al.*, 1994). For instance Backhus and Gschwend

(1990), suggested that pore water humic colloids may play an important role in binding hydrophobic organic compounds in interstitial waters, affecting the speciation of transition metals by coating mineral surfaces and, in that way, changing sorbent properties.

Previously, Head (1976) defined DOM and DOC as follows: the fraction passing through a filter (0.45 μm) is termed "dissolved organic matter" (DOM) and its carbon content is known as "dissolved organic carbon" (DOC), even though it will contain colloidal and fine particulate material, in addition to truly dissolved species. These definitions will be used throughout this work. The term "natural organic matter" (NOM) refers to humic substances (HS), humic acid (HA) and fulvic acid (FA) (see earlier).

The humification process describes the conversion of plant debris into high molecular weight humic substances via either environmental conditions or microbial transformations. The most favoured mechanisms are those involving condensation reactions of polyphenols and quinones. Polyphenols derived from lignin or synthesized by microorganisms are enzymatically converted to quinones, which undergo self condensation or combine with amino compounds to form N-containing polymers (Stevenson, 1994).

In general, HS yield uncharacteristic spectra in the visible (400-800 nm) and ultraviolet (200-400 nm) regions. Their absorption spectra are featureless, the optical density usually decreases as the wavelength increases, and normally an absorption maximum can be found in the 260-300 nm region (Choudhry, 1981).

It has also been reported that the ratio of optical densities (OD) or the absorbance of dilute aqueous HA and FA solutions at 465 and 665 nm, (defined as the $E_4/E_6$ ratio), can be used in the characterization of HSs as an indicator of humification. For instance, Banerjee (1979) reported that a low molecular weight fraction has higher $E_4/E_6$ values whilst Chin *et al.* (1994) found a weak relationship between $E_4/E_6$ and aromaticity. By comparison, the same workers found a stronger relationship between the absorbance at 280 nm and organic carbon content which allowed determination of the molar absorptivity ($\epsilon$), which then provided a good estimate of DOM aromaticity and the contribution of terrestrial materials. Electron transitions of the type $\pi$-$\pi^*$ occur in the region of the UV range (270 to 280 nm) for phenolic substances, aniline derivatives, benzoic acids, polyenes, and polycyclic aromatic hydrocarbons. Humic substances from terrestrial sources have many of these components, thus the molar absorptivitiy $\epsilon$ at 280 nm is an important characteristic of dissolved NOM. Chin *et al.* (1994) stated that this measurement may also be subject to sample matrix effects.

Chin *et al.* (1994) also stated that the molecular weight of HS is an important characteristic as it has been found that this is directly related to other HS characteristics such as molar absorptivity at 280 nm ($\epsilon$). This information can be used to speculate upon the chemical reactivity of HS. However, before determining the molecular weight of HS it is necessary to understand changes to HS structure due to environmental characteristics. For instance, it has been found that HSs may form coiled structures as the pH is reduced or as the ionic strength is increased. Also, the molecular size of HS increases with hydrogen ion and metal

ion concentrations. Carter and Suffet (1982) also reported that the humic polymer becomes less hydrophilic as its charge is neutralized or as the ionic strength is increased. Therefore, it seems reasonable that a less hydrophilic form of the polymer would bind hydrophobic compounds more effectively and that hydrophobic organic compounds would be more likely to associate with uncharged portions of the humic polymer. In summary, Carter and Suffet (1982) reported that the extent of binding depends on the source of the humic material, the pH, the calcium concentration, the ionic strength, and the concentration of humic materials.

Chiou *et al.* (1987) concluded that the difference in water solubility enhancement of scarcely soluble organic solutes, (p,p'-DDT, 2,4,5,2',5'-PCB and 2,4,4'-PCB), by dissolved aquatic humic materials is closely related to the molecular composition of the aquatic humic material. For example, they detected a small solubility enhancement effect with a natural humic extract in accordance with increased oxygen content and a decreased carbon content of the sample in comparison with commercial humic acids. Also they concluded that the effect of the molecular size of these humic materials on solubility enhancement appeared to be secondary to the molecular composition, presumably because the molecular weights of the organic solutes are large.

Backhus and Gschwend (1990) indicated that the presence of Aldrich humic acids doubled the mobile load of hydrophobic pollutants such as perylene, a polycyclic aromatic hydrocarbon (PAH), but had little effect on the mobility of less hydrophobic pollutants like the PAH phenanthrene. Later Schlautman and

Morgan (1993) proposed a possible mechanism that the binding of HS to hydrophobic organic compounds was dependent on their structure. They pictured the humic materials as an open structure with hydrophobic cavities, the dimensions and hydrophobicity being sensitive to variations in pH, salt concentration and valence of cations.

Having established the importance HS molecular weight it is time to introduce the analytical technique that was used in this work. Size exclusion chromatography (SEC), also known as gel filtration, is a method of compound separation based on molecular size by elution through beds of porous beads (Pryde and Gilbert, 1979). The principle of SEC is that small species move with the eluant both within and out with the gel particle whilst bigger species only move outside the gel particles. Therefore larger species are eluted first, followed in order by the smaller species; although this doesn't follow a linear correlation (Stevenson, 1994). Figure 3.1 shows the idealized calibration curve and all the terms applicable to this technique. In this context, it is important to minimize the propensity of the colloids to change their degree of coiling in order to achieve consistent size exclusion. Because the micro-pores in the gel essentially separate molecules by size, the system is usually calibrated against molecules with nominal molecular weights. It is therefore necessary to add electrolytes to the mobile phase which mimic the ionic strength of the injected solution (Chin and Gschwend, 1991) to minimize unwanted coiling and uncoiling.

Ion exclusion, ion exchange and adsorption are other potential artefacts that can affect SEC performance. Stationary phases used in SEC are composed of

modified silica which can possess residual negatively charged sites at mobile phase pH values from 2 to 4. Therefore, large polyelectrolytes, such as HS may be prevented from diffusing into the stationary pores by electrostatic repulsion. To avoid this phenomenon, the addition of an indifferent electrolyte (NaCl) to the mobile phase tends to shrink the thickness of the surface double layer to sizes which are small relative to pore openings, in this way allowing the diffusion of charged analytes into the small pores of the stationary phase. In fact, it is necessary to use a mobile phase buffer which exceeds the critical ionic strength (CIS) to minimize the charge exclusion effect (Chin and Gschwend, 1991). The CIS was identified by Chin and Gschwend (1991) as the ionic strength above which the normalized retention volume (Vr/Vo) of benzoate approached that of an uncharged probe which was acetone.

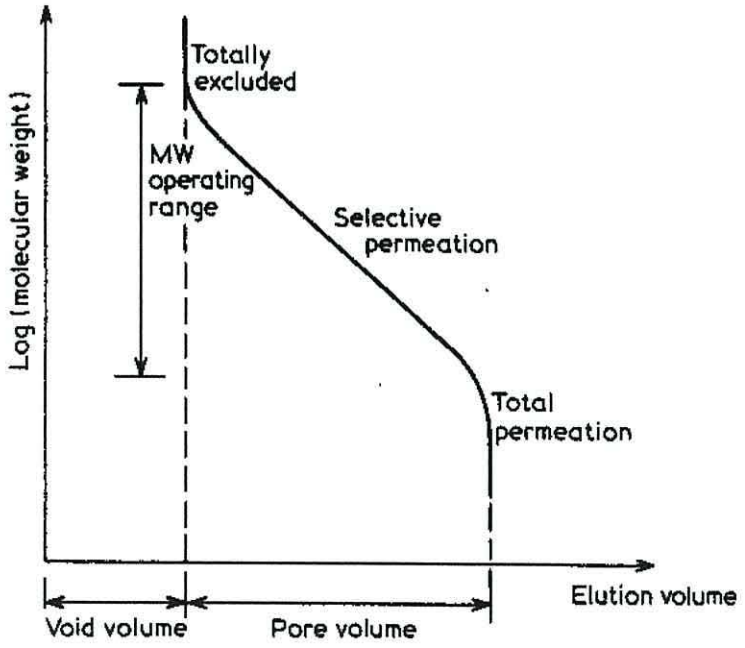



**Figure 3.1.** Idealized calibration curve for exclusion chromatography. (Pryde and Gilbert, 1979).

A number of investigators have advocated the use of random coil standards such as polystyrene sulfonates (PSS) instead of globular proteins (Chin and Gschwend, 1991; Chin *et al.*, 1994; Chin *et al.*, 1998), because the latter tend to over predict the molecular weights of humic substances by a factor of 5 or more. Chin and Gschwend (1991) found that the coiled configuration of PSS and that of Suwannee River fulvic acid appeared to be nearly identical.

High-performance size exclusion chromatography (HPSEC) has been confirmed by Conte and Piccolo (1999) to be a precise method to evaluate the relative molecular-weight distribution of dissolved HS. For instance, when molecular weight values obtained with HPSEC were compared with values determined by vapour pressure osmometry (VPO), field flow fractionation (FFF), and ultracentrifugation (Chin and Gschwend, 1991; Chin *et al.*, 1994), the results were within the expected values (see Table 3.38).

The scope of this part of the work was therefore to characterise, as much as possible, the natural organic matter of water, pore-water and sediment from the CRE. The primary aim was to establish links and relationships with the fate, reactivity and mobility of the trace metals present in solution and those of the organophosphate pesticide propetamphos.

### 3.1.3. Natural organic matter-metal complex fractionation in aqueous samples

The importance of natural organic matter (NOM) in the environment is due in part to its interaction with other organic (Carter and Suffet, 1982; Chiou *et al.*, 1987; Schlautman and Morgan, 1993) and inorganic components (Cabaniss and Shuman, 1988; Rottmann and Heumann, 1994; Wells *et al.*, 1998; Rocha *et al.*, 2000). In aquatic systems NOM often alters the behaviour of chemical compounds that otherwise would be in solution as free metals. For instance, Lores and Pennock (1998) concluded that the binding of Cu to DOM under estuarine conditions will probably reduce the uptake or bioavailability to most estuarine organisms, protecting them from potential Cu toxicity. Ultimately a clear understanding of the bioavailability of specific compounds is necessary in order to make better predictions of their possible environmental risks (Lores and Pennock, 1998).

There have been numerous efforts to characterise NOM. However, as stated earlier, despite all these efforts we still have a poor understanding of this highly complex and variable material. In reality, only some parts of the NOM structure have been confirmed either theoretically or practically. Infrared (IR) and carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) spectroscopy have identified some major groups within HS that were suspected from the elemental content of NOM. These are aliphatic, alkoxyl, carbohydrate type, aromatic components and carboxylic groups. Also the NMR spectra of humic materials have shown the presence of paramagnetic species such as $Fe^{3+}$ and stable organic radicals (van

Loon and Duffy, 2000). Using this information some authors have presented theoretical structures of humic substances. For example in Figure 3.2 van Loon and Duffy (2000) presented a portion of hypothetical structure of a generic humate molecule (*ca.* 1000 MW). This molecule features aliphatic and aromatic character, varied functionality and a polymeric nature.

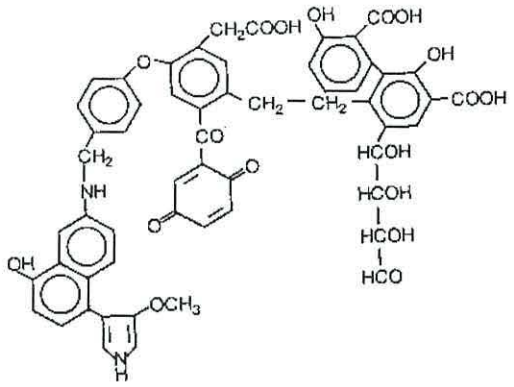



**Figure 3.2.** Structure of a generic molecule of a humic substance, van Loon and Duffy (2000).

This HS has hydrophobic as well as polyfunctional groups, thus it can interact simultaneously with many organic (e.g. PCB, PAH, and pesticides) and inorganic (e.g. heavy metals) pollutants in an aquatic system (Rottmann and Heumann, 1994). Usually the NOM-metal interaction occurs through a cationic-anionic route, while the NOM-organic interaction occurs through hydrophobic-hydrophilic process. Further, the HS structure also allows it to function as a surfactant (Gaffney *et al.*, 1996)

In aqueous systems, HS have been found to play a role as proton donor/acceptors

and so contribute to charge balance and pH buffering. In addition to this, HS also react with metals in solution through the formation of ionic or covalent bonds. For instance Cu(II), Pb(II) and the trivalent metals Al(III) and Fe(III) have large stability constants (van Loon and Duffy, 2000).

The metal binding of humic macromolecules can occur at "specific" binding sites; that is particular functional groups or combinations of functional groups which may co-ordinate metal ions. Carboxylate groups are primarily responsible for binding metals under most natural conditions (Gaffney *et al.*, 1996). Alternatively metal binding may be "non-specific", the electrostatic interaction between the negatively charged surface of a humic macromolecule and positively charged cations (Zhang *et al.*, 1996).

There have been some efforts in the past to speciate NOM-metal complex compounds. Some of these efforts have been focused on the relationship between NOM molecular weight and metal species (Morita *et al.*, 1980; Hausler and Taylor, 1981; Gardner *et al.*, 1982; Rottmann and Heumann, 1994). These authors have made important advances in this field using high performance size exclusion chromatography (HPSEC) to separate by size the organic compounds and the inductively coupled plasma (ICP) technique either by optical emission spectroscopy (OES) or by mass spectrometry (MS) to determine the metal content of the eluent fractions from the chromatographic system.

In this thesis, the NOM-metal complex interactions of natural water and sediment pore water samples from the Conwy River and estuary were studied to understand

the bioavailability and toxicity of metals and subsequently to understand how these interactions could affect NOM-pesticide behaviour and toxicity.

## 3.2. Results

This section is sub-divided into three main parts; the first will cover the characterisation of the inorganic compounds found in water and sediments. The second covers the characterisation of the dissolved natural organic matter and the third studies the interaction between selected metals and natural organic matter.

### 3.2.1. Inorganic characterization

#### 3.2.1.1. Water samples

Water from the Conwy River and estuary is the aqueous phase characterised in this work. In general the water and sediment samples were collected with the outgoing tide. This did not have any relevance to the collection of samples in the river sites (BC and LL sites), but it was important to the remaining sites that were in the upper and lower estuary (TC, BS and CC sites). In this way both the sediments and water samples were easy to collect and also turbulence was not a factor.

Table 3.1 shows the averaged physicochemical measurements carried out *in situ* for all seasons. The data show that, as expected, there was a trend towards increasing pH for the sites closer to the open sea. However, for LL the pH tended to be slightly

more acidic than at BC. One explanation for this might be the nearby input of one of the Conwy river tributaries which drains from the Gwydyr Forest which contains a number of disused mines which are known to release acid mine drainage.

**Table 3.1.** pH, salinity and Redox of water samples, calculated as the average values of all samples collected throughout all seasons ± standard error with n = 7 unless stated.

| Sample | pH | Salinity (g $kg^{-1}$) | Redox (mV) |
|---|---|---|---|
| **BCWI** | 7.1 ± 0.1 | 0.5 ± 0.1 | 174 ± 6* |
| **LLWI** | 6.9 ± 0.1 | 0.6 ± 0.1++ | 158 ± 36* |
| **TCWI** | 7.4 ± 0.3 | 10.1 ± 5.0++ | 159 ± 18** |
| **BSWI** | 7.8 ± 0.1++ | 29.7 ± 3.4# | 166 ± 13+ |
| **CCWI** | 8.1 ± 0.1 | 25.6 ± 5.2 | 149 ± 24+ |

* n=4; ** n=5; + n=6; ++ n=8; # n=9

As expected, salinity increases down the estuary, the variability of which increases along the estuary probably being due to river discharge rates at the different sampling dates. Although the discharge rate was not measured significant seasonal differences were observed. The redox values presented in Table 3.1 show that the sites in the estuary are slightly less oxic than those in the river. Further, the variability is high e.g. 11 % in TC and 16 % in CC. The high variability in redox observed in the estuary indicates that the estuary is a highly dynamic system.

Filtered and acidified (pH ≤ 2) water samples from each site were characterized for their dissolved metal content. The results (Table 3.2.) are expressed both as a range

and as an average ± standard error because of the high seasonal variability. In general, the alkaline earth metal concentrations tended to increase in the Estuary. Cd and Cu were below the LOD, while Al, Fe, Mn and Zn were found in some of the analyzed samples. The exception was for the CC sample, in which only the earth metals were detected. This was probably because the high salinity was a source of interference for metal analysis by ICP-OES. Assuming that these elements are in solution, it was decided to focus on Al, Fe, Mn and Zn because these metals could play a role in the interaction, chemistry and behaviour of organic compounds.

Water samples were also subjected to a preconcentration method, developed as part of this study specifically for these samples. The method used proved effective in determining the metal concentration of Cd, Cu, Mn, and Ni with a range of recoveries from 54 to 96 %. Also, Fe presented a good recovery (103 %) but with a high variability (9.6 % s. e.). Salinity samples included all the range presented before in Table 3.1.

The results obtained show that water samples have a low concentration of dissolved heavy metals (Cd, Cu and Ni), but that these are present in all samples (Table 3.3). Fe is present in the highest salinity concentration sites in the lower estuary (BS and CC).

Following the definition of Sharp *et al.* (1984) given in the introduction, the conservative and non-conservative behaviour of some of the metals in the estuary are shown in Figure 3.3. Zn and Mn presented typical conservative and non-conservative behaviour respectively, implying that the estuary functions as a sink or

**Table 3.2.** Dissolved metal content in water samples (μg $l^{-1}$). Range (top row) and average ± standard error (lower row) in brackets, Values of all samples collected throughout all seasons, n = 4 minimum.

| Metal | BCWI | LLWI | TCWI | BSWI | CCWI | LOD |
|---|---|---|---|---|---|---|
| **Ca** | 822 – 10294<br>(4187 ± 3059) | 5305 - 14980<br>(10142 ± 4837) | 2207 – 141626<br>(58264 ± 30131) | 81087 – 265287<br>(180948 ± 44675) | 10480 – 270545<br>(147107 ± 54049) | 0.0 |
| **Mg** | 1464 – 3127<br>(2295 ± 832) | NA | 1103 – 342575<br>(121592 ± 148566) | 237390 – 425313<br>(307313 ± 42951) | 21920 – 660370<br>(341304 ± 135379) | 3.0 |
| **K** | NA | NA | 200 – 448343<br>(151225 ± 148566) | 278645 – 581709<br>(430177 ± 151532) | 17570 – 581709<br>(299639 ± 282069) | 9.0 |
| **Al** | < LOD | 18.0 – 63.0<br>(43.4 ± 13.2) | 22.7 – 129.3<br>(54.4 ± 20.0) | 27 – 108.9<br>(67.9 ± 40.9) | NA | 15.0 |
| **Cd** | < LOD | NA | < LOD | < LOD | < LOD | 7.0 |
| **Cu** | < LOD | < LOD | NA | NA | < LOD | 13.0 |
| **Fe** | 35.2 – 78.5<br>(56.9 ± 21.7) | 34.1 – 125.5<br>(88.6 ± 19.7) | 31.4 – 585.7<br>(174.7 ± 104.7) | NA | NA | 24.0 |
| **Mn** | NA | 7.0 – 13.1<br>(10.0 ± 3.1) | 33.7 – 92.7<br>(58.6 ± 17.6) | 53.7 – 137.1<br>(95.4 ± 41.7) | NA | 5.0 |
| **Zn** | 45.1 – 165.8<br>(100.1 ± 26.8) | 11.0 – 80.0<br>(49.2 ± 15.0) | 7.9 – 108.0<br>(61.8 ± 29.1) | NA | < LOD | 5.0 |

NA: non applicable, less than 4 readings.

filter for Zn but that there are sources of Mn throughout the estuary. Cadmium and copper showed no tendency to either a conservative or non-conservative behaviour, thus the speculation here is that the estuary buffers the total concentration of these two metals due to particulate matter or dissolved organic matter. Fe possessed a non-conservative behaviour with one single reading off the linear plot (TC site) which means that there may be a source of Fe from some of the tributaries near TC. The Al data presents a line with a gentle positive slope. It would be expected that Al might present conservative behaviour but the trend shown here may indicate that Dolgarrog Aluminium Ltd. does contribute to the concentration of Al in the estuary.

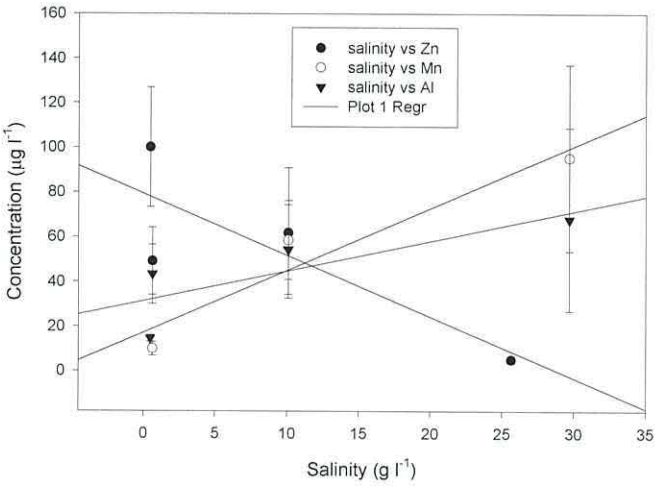
**Table 3.3.** Preconcentration method results for water samples from five sites down the Conwy River. Range (top row) and average ± standard error (lower row) in brackets of dissolved metal content in water samples ($\mu g\ l^{-1}$), n = 3.

| Metal | BCWI | LLWI | TCWI | BSWI | CCWI |
|---|---|---|---|---|---|
| **Cd** | 0.5 – 1.9<br>(1.2 ± 0.7) | 0.1 – 0.4<br>(0.3 ± 0.1) | 0.2 – 0.6<br>(0.4 ± 0.2) | 0.2 – 0.4<br>(0.3 ± 0.0) | 0.2 – 1.5<br>(0.6 ± 0.4) |
| **Cu** | 2.5 – 3.2<br>(2.8 ± 0.4) | 0.5 – 4.8<br>(2.9 ± 1.3) | 2.4 – 4.4<br>(3.4 ± 1.0) | 1.4 – 4.8<br>(3.0 ± 0.6) | 0.8 – 6.2<br>(3.3 ± 1.6) |
| **Fe** | 39.3 – 153.9<br>(96.6 ± 57.3) | 54.1 – 132.1<br>(97.9 ± 23.0) | 111.9 – 514.7<br>(313.3 ± 201.4) | 6.2 – 325.4<br>(69.9 ± 51.5) | 5.5 – 41.7<br>(17.6 ± 12.1) |
| **Ni** | 0.1 – 3.4<br>(2.0 ± 1.4) | 0.6 – 1.4<br>(0.9 ± 0.3) | 0.8 – 1.0<br>(0.9 ± 0.10) | 0.3 – 1.8<br>(0.8 ± 0.3) | 0.0 – 0.7<br>(0.4 ± 0.2) |

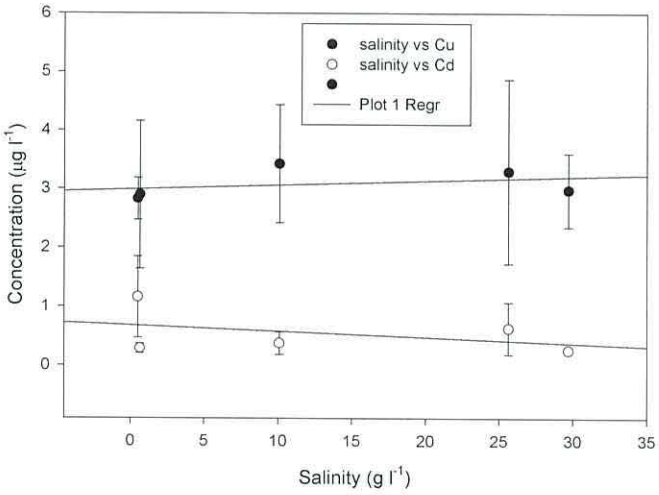
### 3.2.1.2. Sediment samples

Sediment characterization involved examination of the two main phases; the "so called" pore water liquid phase and the sediment solid phase. Pore water was extracted as explained in Chapter 2. The pH values obtained from the pore water

(a)




(b)




(c)

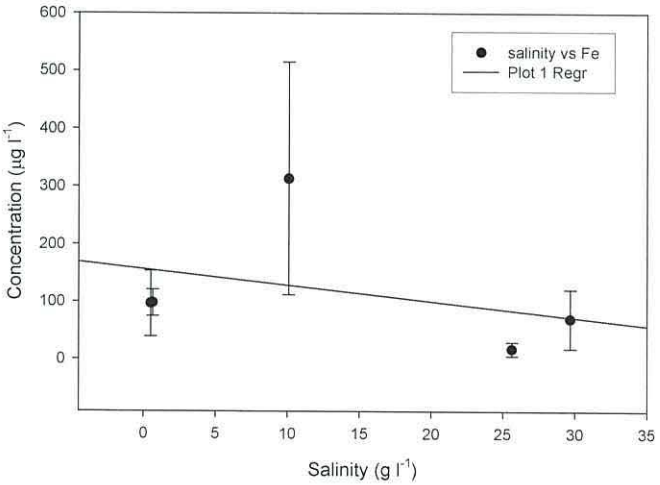



**Figure 3.3.** Plots of conservative and non conservative behaviour of metals in the Conwy estuary, concentration vs salinity (a) aluminium, manganese and zinc; (b) cadmium and copper; and (c) iron. Points represent the averaged experimental data and lines represent the behaviour trend.

show values close to neutrality and with a lower variance than water samples, see Table 3.4. Martin and Bullock (1994) reported that an increasing mobility of heavy metals is strongly associated with increasing soil acidification and there is no reason to doubt that the same could apply to sediment. Again pore water from the LL site is slightly more acidic than the other sites in agreement with earlier data for the main column water.

**Table 3.4.** pH, salinity and Redox of pore water samples, calculated as the average values of all samples collected throughout all seasons ± standard error with n = 4 unless stated.

| Sample | pH | Salinity (g $kg^{-1}$) | Redox (mV) |
|---|---|---|---|
| **BCSI** | 7.4 ± 0.3 | 1.5 ± 0.2 | 180 ± 30 |
| **LLSI** | 6.5 ± 0.3*** | 1.1 ± 0.2** | 175 ± 28 |
| **TCSI** | 7.8 ± 0.1* | 17.7 ± 2.9** | 163 ± 28 |
| **BSSI** | 7.7 ± 0.2 | 28.3 ± 2.9*** | 172 ± 26 |
| **CCSI** | 7.7 ± 0.1 | 31.3 ± 2.5* | 183 ± 16 |

*n=5; **n=6; ***n=7

Salinity increases considerably in contrast with the analogous water samples (Table 3.1), in accordance with the idea that pore water dissolves the salts, ions, and nutrients that are available from the solid phase. Finally, in general, the redox values are slightly higher than those for the water samples, where we could expect the contrary. The reason for this could be due to the fact that these readings were made after the sediments were manipulated in the laboratory (see Chapter 2) and the original redox values may have changed during this treatment. However as

expected, the redox value for TC is the least oxic which is in agreement with the nature of the site, where the sediment showed an anoxic natural condition (black and sulphurous smell), possibly indicating presence of FeS from $SO_4^{2-}$ reducing bacteria.

The dissolved metal content of pore water is generally speaking higher than that present in the water samples (Table 3.5). One possible reason may be that the water phase is in direct contact with the solids working actively to dissolve ions and easily exchangeable metals. On the other hand it also reflects the partitioning behaviour between the two phases. This behaviour will also be affected by mixing metal loading, pH and content of organic matter in the sediments.

The data presented in Table 3.5 show that Al, Fe and Zn, in addition to being present in all samples, also decrease in concentration by approximately one order of magnitude down the estuary. By comparison Cd, Ni, and Pb were all below the LOD in the estuarine samples (TC, BS and CC) despite being found in the water samples. However, Cd and Ni were detected using the preconcentration method in the water samples (See section 3.2.1.1.). On this basis it is not possible to rule out the possibility that these metals are not present in the estuary, but if they are, then their concentrations are extremely low. However, the high salinity of these samples could have been a source of interference. Unfortunately, it was not possible to apply the preconcentration method to the pore water samples mainly because the difficulty on obtaining sufficient sample volume. For example, to obtain 5 to 10 ml of pore water, approximately 10 centrifuge tubes had to be prepared and this was a very consuming time process.

**Table 3.5.** Metal content in pore water (μg $l^{-1}$). Range (top row) and average ± standard error (lower row) in brackets. Values of all samples collected throughout all seasons of, n = 4 minimum.

| Metal | BCSI | LLSI | TCSI | BSSI | CCSI |
|---|---|---|---|---|---|
| **Ca** | 11860 - 495348<br>(149566 ± 115556) | 3710 – 214143<br>(65964 ± 49651) | 78382 – 173220<br>(128004 ± 64002) | 111613 – 441370<br>(251711 ± 76477) | 130523 – 177399<br>(153961 ± 23438) |
| **Mg** | 12392 – 15493<br>(13943 ± 1550) | 3348 – 19036<br>(10697 ± 4556) | 150500 – 280030<br>(219432± 109716) | 150500 – 602634<br>(357828 ± 99914) | 252630 – 701610<br>(477120 ± 224490) |
| **K** | NA | NA | 116805 – 402534<br>(259670±142865) | 523310 – 551090<br>(537200 ± 13890) | NA |
| **Al** | 377 – 7542<br>(3960 ± 3583) | 20.5 – 31617<br>(5955 ± 5147) | 131.8 – 840.3<br>(486.1 ± 250.5) | 205.5 – 564.0<br>(368.2 ± 74.1) | 97.6 – 411.5<br>(254.6 ± 157.0) |
| **Cd** | < LOD | 15.8 – 51.1<br>(33.5 ± 17.6) | < LOD | < LOD | < LOD |
| **Cu** | < LOD | NA | < LOD | < LOD | 168.3 – 2475<br>(1322± 1153) |
| **Fe** | 48.3 – 13256<br>(4575 ± 4342) | 68.9 – 31019<br>(4694 ± 4389) | 69.9 – 1285<br>(446.0 ± 283.0) | 28.1 – 219.9<br>(252.9 ± 116.5) | 362.1 – 1022<br>(662.0 ± 192.9) |
| **Ni** | NA | NA | < LOD | < LOD | < LOD |
| **Pb** | 65.2 – 314.7<br>(154.9 ± 80.1) | 98.8 – 611.7<br>(355.3 ± 256.5) | NA | < LOD | < LOD |
| **Zn** | 169.7 – 5020<br>(1305 ± 942) | 49.7 – 6288<br>(1195 ± 862) | 29.2 – 267.1<br>(94.6 ± 57.6) | 19.4 – 492.7<br>(143.4 ± 116.5) | 34.0 – 736.7<br>(289.9 ± 224.2) |

NA: non applicable, less than 4 readings. < LOD below limit of detection.

Having identified metal loading in river and sediment pore water, a sequential extraction (SE) procedure was carried out to separate the metals into soluble, exchangeable, organic and mineral bound fractions. This was of interest because the first two fractions could be potentially leachable into water and thus bioavailable to the microbial population and also sequential extraction gives some indication of the speciation of metals. In Table 3.6 fractions of Al, Fe and Zn are shown; these samples were collected on the same day and belong to five different sites. Typically only small percentages of Al and Fe were soluble in water or potentially leachable, while for Zn and the rest of the metals analysed (not shown) the only fraction detected was the organically bound. The implication of this is that the majority of the chemical interactions are between metals and organic matter in the sediments.

The sediment samples collected from the LL site see Table 3.7 on different dates show some differences worth noting. This particular site had two distinctive sediment samples that were classified as sand and soil due to their size grain fractionation (Figure 3.6). The "soil" sample showed a very different pattern for Al and Zn. For instance, 6 % of the Al extracted by the sequential extraction (SE) procedure was in the water soluble fraction in contrast with < 1 % in the rest of the LL sediment samples. On the other hand, Zn presented the most diverse speciation pattern, 4.5 % was in the water soluble fraction and 23.9 % was in the readily exchangeable fraction.

The TC sediment fractionation, see Table 3.8 is also worth mentioning. As mentioned previously, the sediment at this location showed anoxic conditions prevailed. Even though attempts were made to maintain anoxic conditions during

**Table 3.6.** Total and percentage of aluminium, iron and zinc by sequential extraction of sediments from the Conwy River collected on the 14-12-98. Samples by triplicate, standard errors were less than 5%.

| | BCSI | | | | LLSI | | | | TCSI | | | | BSSI | | | | CCSI | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Element** | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % |
| **Al** | 327 | 0.3 | < LOD | 99.7 | 277 | < LOD | < LOD | 100 | 256 | 3.9 | < LOD | 96.1 | 179 | 3.8 | < LOD | 96.2 | 144 | 1.7 | < LOD | 98.3 |
| **Fe** | 254 | 0.5 | < LOD | 99.5 | 117 | 1.2 | < LOD | 98.8 | 814 | 0.8 | < LOD | 99.2 | 418 | 1.1 | < LOD | 98.9 | 364 | 0.4 | < LOD | 99.6 |
| **Zn** | 108 | < LOD | < LOD | 100 | 25 | < LOD | < LOD | 100 | 119 | < LOD | < LOD | 100 | 40 | < LOD | < LOD | 100 | 44 | < LOD | < LOD | 100 |

< LOD below limit of detection.

**Table 3.7.** Total and percentage of aluminium, iron and zinc by sequential extraction of sediments from Llanrwst (LLSI), collected on various dates. Samples by triplicate, standard errors were less than 5%.

| **Date** | **14-12-98** | | | | **18-01-00 (soil)** | | | | **18-01-00 (sand)** | | | | **30-05-01** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Element** | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % |
| **Al** | 277 | < LOD | < LOD | 100 | 462 | 6.0 | 0.2 | 93.8 | 235 | < LOD | 0.4 | 99.6 | 176 | < LOD | < LOD | 100 |
| **Fe** | 117 | 1.2 | < LOD | 98.8 | 263 | < LOD | < LOD | 100 | 224 | < LOD | < LOD | 100 | 200 | 1.9 | < LOD | 98.1 |
| **Zn** | 25 | < LOD | < LOD | 100 | 197 | 4.5 | 23.9 | 71.5 | 65.4 | 0.5 | 12.7 | 86.8 | 42 | < LOD | 5.5 | 94.5 |

< LOD below limit of detection.

**Table 3.8.** Total and percentage of metals by sequential extraction of sediments from Tal y Cafn (TCSI), collected on various dates. Samples by triplicate, standard errors were less than 5%.

| **Date** | **14-12-98** | | | | **18-01-00** | | | | **1-08-00** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Element** | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % |
| **Al** | 255 | 3.9 | < LOD | 96.1 | 233 | < LOD | < LOD | 100 | 212 | < LOD | < LOD | 100 |
| **Cd** | 0.6 | < LOD | < LOD | 100 | 0.4 | < LOD | < LOD | 100 | 0.5 | < LOD | < LOD | 100 |
| **Cr** | 0.5 | < LOD | < LOD | 100 | 0.5 | < LOD | < LOD | 100 | 0.3 | < LOD | < LOD | 100 |
| **Cu** | 2.5 | < LOD | < LOD | 100 | 1.2 | 14.2 | < LOD | 85.8 | 3.9 | 5.2 | < LOD | 94.8 |
| **Fe** | 814 | 0.8 | < LOD | 99.2 | 1084 | 0.5 | < LOD | 99.5 | 522 | 0.7 | < LOD | 99.3 |
| **Ni** | 1.0 | < LOD | < LOD | 100 | 1.7 | < LOD | < LOD | 100 | 1.6 | < LOD | < LOD | 100 |
| **Pb** | 13.5 | < LOD | < LOD | 100 | 7.4 | 3.6 | < LOD | 96.4 | 9.0 | 3.4 | < LOD | 96.6 |
| **Zn** | 119 | < LOD | < LOD | 100 | 109 | < LOD | < LOD | 100 | 108.8 | < LOD | < LOD | 100 |

< LOD below limit of detection.

sample collection and storage, this was not possible during the sequential extraction procedures. Furthermore, it was noticed that, after the first extraction, the sediment had changed from a black colour to a predominantly brownish coloration. This may indicate a change in the redox potential though no measurements were made. Fe is known to be highly susceptible to reoxidation under these conditions. However, the soluble Fe fraction presented in these samples is consistent with samples from other sites (*ca.* 0.8 %), thus it was concluded that the extra manipulation of this procedure did not significantly affect the original speciation of metals in the sediments. In the TC sediment, larger amounts of Cu and Pb were present in the water soluble fraction; up to 14.2 % for Cu, and up to 3.6 % for Pb. It was speculated that much of this copper and lead may have been in colloidal forms, although this was not quantified. At pH 7, the main copper ions species in both oxidizing and reducing aqueous environment could be likely to be $Cu^{2+}$, $CuOH^{+}$ and $CuHCO_3^{+}{}_{(s)}$; the lead ions species are $Pb^{2+}$, $PbOH^{+}$, $PbHCO_3^{+}{}_{(s)}$ (van Loon and Duffy, 2000) and these species could also be expected to contribute to the soluble fractions. Results presented in Table 3.9 show that apart from Al, Fe and Zn the rest of the heavy metals analysed were not present in large quantities within the sediments. Suspended particulate matter (SPM) was separated from the water samples by filtration and this fraction was also subjected to SE (SPM is considered to be a solid phase). This was performed to evaluate the importance of SPM in the fate and behaviour of metals in the Conwy River. Since SPM is a very important vector for mobility of metals, being subjected to daily mixing processes in the estuary, the information obtained would help to gain a more complete picture of the metal partitioning process between the two aqueous and solid phases.

**Table 3.9.** Total content of metals ($\mu g\ g^{-1}$) obtained by sequential extraction. The values represent mean values ± standard error with n=3

| Metal | BCSI | LLSI | TCSI | BSSI | CCSI |
|---|---|---|---|---|---|
| **Al** | 327 ± 31 | 288 ± 62 | 234 ± 13 | 203 ± 18 | 144 ± 31 |
| **Cd** | 1.1 ± 0.1 | 0.5 ± 0.2 | 0.5 ± 0.1 | < LOD | < LOD |
| **Cr** | < LOD | < LOD | 0.4 ± 0.1 | 0.4 ± 0.0 | < LOD |
| **Cu** | 3.3 ± 0.0 | 3.7 ± 0.9 | 2.5 ± 0.8 | 4.5 ± 0.5 | 2.2 ± 0.7 |
| **Fe** | 254 ± 81 | 201 ± 31 | 807 ± 162 | 360 ± 50 | 364 ± 81 |
| **Ni** | 1.9 ± 0.5 | 1.9 ± 0.9 | 1.4 ± 0.2 | 1.1 ± 0.4 | 0.6 ± 0.5 |
| **Pb** | 53.8 ± 4.1 | 19.4 ± 7.6 | 10.0 ± 1.8 | 9.0 ± 3.0 | 9.5 ± 4.1 |
| **Zn** | 108 ± 15 | 82.1 ± 39.1 | 112 ± 4 | 38.3 ± 2.4 | 44.1 ± 15.0 |

Table 3.10 presents the results for Al, Fe and Zn and indicate that SPM is an important transport medium for these elements. SPM contained the highest concentration per unit (ppm) of these metals, and they are almost entirely bound to the organic (organic suspended matter) and mineral (clays) fractions with the exception of Al where a small percentage is in the water soluble fraction (colloids) in BS and CC samples. Also Fe in CC sample has a small presence in the readily exchangeable fraction.

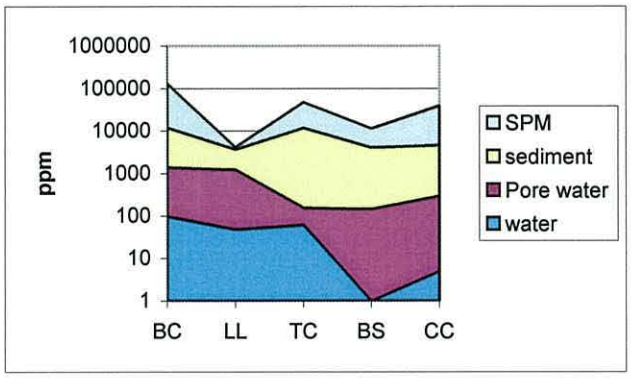
Examples of the distribution of metals in and within the different phases, (aqueous = water and pore water; solid = sediment and SPM) and also throughout the river and estuary from BC to CC are shown in Figure 3.4. The concentrations are presented on a $Log_{10}$ scale to emphasize the differences between the four phases. Metal concentrations in water vary over a two orders of magnitude range with metals in

**Table 3.10.** Total and percentage of aluminium, iron and zinc by sequential extraction of suspended particulate matter from the Conwy River collected on the 14-12-98. Samples by triplicate, standard errors were less than 5%.

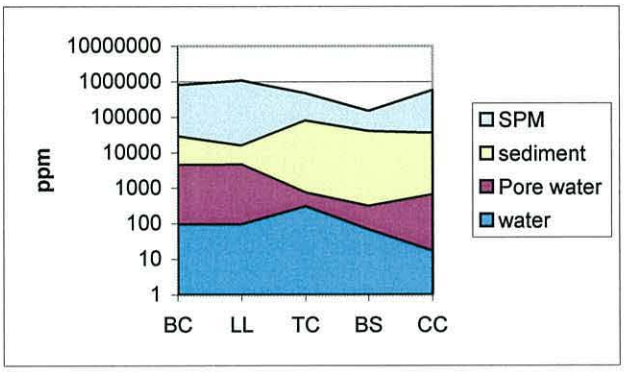
| | BCWI | | | | LLWI | | | | TCWI | | | | BSWI | | | | CCWI | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Element** | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % | Total $\mu g\ g^{-1}$ | $H_2O$ % | KCl % | HCl % |
| **Al** | 3049 | < LOD | < LOD | 100 | 2749 | < LOD | < LOD | 100 | 305 | < LOD | < LOD | 100 | 309 | 1.4 | < LOD | 98.6 | 1515 | 2.2 | < LOD | 97.3 |
| **Fe** | 7991 | < LOD | < LOD | 100 | 10919 | < LOD | < LOD | 100 | 4063 | < LOD | < LOD | 100 | 1110 | < LOD | < LOD | 100 | 5567 | < LOD | 1.3 | 98.7 |
| **Zn** | 1218 | < LOD | < LOD | 100 | <LOD | < LOD | < LOD | < LOD | 357 | < LOD | < LOD | 100 | 73.5 | < LOD | < LOD | 100 | 348 | < LOD | < LOD | 100 |

< LOD below limit of detection.

(a)




(b)




(c)

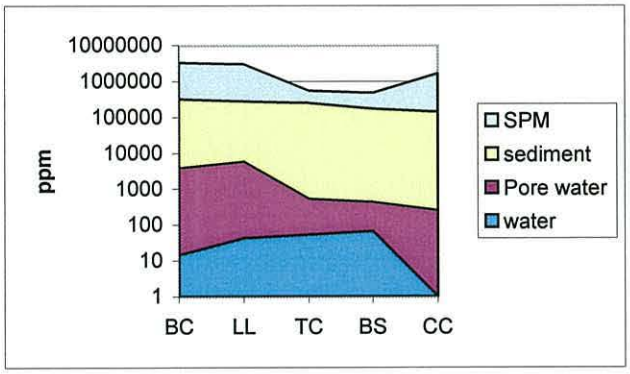



**Figure 3.4**. Concentration of metals (ppm) in the water-column and in the sediment at the five sites of the Conwy River and Estuary. (a) zinc; (b) iron and (c) aluminium. Suspended particulate matter (SPM) and sediment values as $\mu g\ g^{-1}$. Water and pore water values are represented as $\mu g\ ml^{-1}$.

pore water approximately 10 fold less than in sediment which are 10 fold less than in the SPM phase. Zn, Fe and Al were concentrated mainly bound to organic and inorganic material in the SPM and in the sediments (Figure 3.4 a, b and c). With respect to metal ion transport the importance of these phases follows the following pattern: SPM > sediment > pore water > water.

The load of inorganic nutrients was also characterised at each of the five location sites (Figure 3.5). Generally, the phosphate ($PO_4^{3-}$-P) content increased towards the low estuary (non conservative behaviour), while nitrates had the opposite trend (conservative behaviour) and ammonium increased slightly towards the estuary.

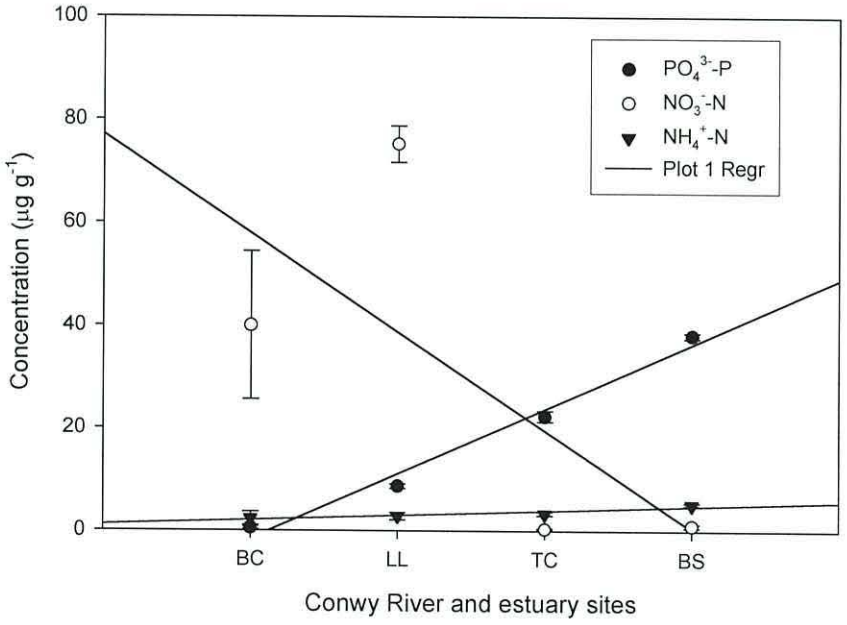



**Figure 3.5.** Trends of inorganic nutrients throughout the Conwy River and estuary. Values are means ± standard error (n = 3). Points represent the averaged experimental data and lines represent the behaviour trend.

Using IR spectroscopy, some peaks were identified as being of mineral origin (Table 3.11). The X-ray diffraction patterns were compared with the computer software library, and also an internet library (Barthelmy, 2002). The only positive identification was for quartz, aluminium phosphate (berlinite) and graphite, which partially agreed with the IR information. The intensity of the quartz XRD pattern was very strong and it is possible that other minerals present were partially masked preventing their detection. The main three lines of aluminium phosphate (berlinite) were identified in some of the spectra, however, because this is a rare high-temperature mineral, largely confined to Sweden, this requires further confirmation. Silicates seem to dominate the mineral content of the sediments of the Conwy River, regardless of site and size grain fraction.

**Table 3.11.** Infra red (IR) spectroscopy most common peaks ($cm^{-1}$) detected in the sediment samples of different size grain fractions. Stretch vibration (γ) and bend vibration (δ).

| Sediment | 3450 γ (OH) | 1638 δ (OH) | 1400 γ ($CO_3^{2-}$) | 1030 γ (SiO) | 796 Quartz |
|---|---|---|---|---|---|
| TC > 360 µm | ✓ | ND | ✓ | ✓ | ✓ |
| TC < 63 µm | ✓ | ✓ | ND | ✓ | ND |
| BS > 360 µm | ✓ | ✓ | ND | ✓ | ✓ |
| CC > 63 µm | ✓ | ✓ | ND | ✓ | ND |
| CC < 63 µm | ✓ | ✓ | ✓ | ✓ | ✓ |

ND not detected

The particle size distribution of the sediment was also determined and found to vary greatly between sites and sampling date (Figure 3.6). For example, at one

sampling date the LL sediment composed approximately 75 % sand, 15 % silt and 10 % clay while at another the sediment was mainly composed of sand (95 %). Sediment samples from TC had in general less sand (*ca.* 60 %) more silt (*ca.* 20 %) and some clay (*ca.* 20 %).

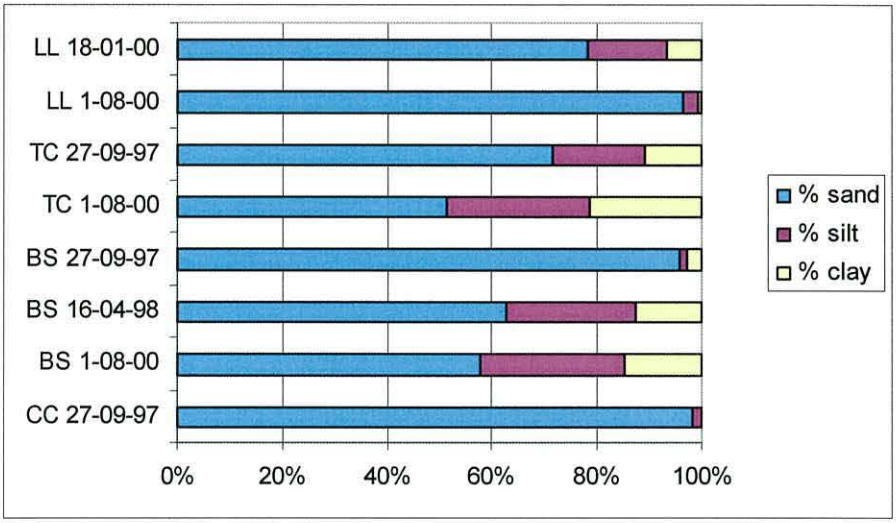



**Figure 3.6.** Particle size distribution of sediments from the Conwy River. Data by duplicate, standard errors were less than 5 %.

**Table 3.12.** Moisture and loss of ignition (LOI) of the Conwy River sediments. Values represent means ± standard error with n > 4.

| Samples | Moisture (g $100g^{-1}$) | LOI (g $100\ g^{-1}$) |
|---|---|---|
| BC | 24.0 ± 2.2 | 2.5 ± 0.3 |
| LL | 25.3 ± 1.1 | 3.2 ± 0.2 |
| TC | 35.8 ± 2.2 | 3.2 ± 0.4 |
| BS | 27.5 ± 2.9 | 2.9 ± 1.3 |
| CC | 23.0 ± 1.6 | 1.4 ± 0.9 |

The average moisture content and of loss on ignition (LOI) data for the five sediments are shown in Table 3.12. The loss on ignition values provides estimates of organic matter content. The results presented in Table 3.12 indicate that all samples were low in organic matter, especially sediment from CC which may be related to the content of sand in each sample. Even though that the content of organic matter is low, through the SE results it is clear that the organic fraction in sediments is quite important for metal partitioning in the River and estuary.

### 3.2.2. Dissolved organic matter (DOM) characterization

#### 3.2.2.1. Water and sediment pore water samples

Water and sediment pore water samples were obtained as described previously (Chapter 2), and the following analysis performed; total phenol content, dissolved organic carbon content (DOC), fixed and scan UV-vis absorbance and molecular weight determination of dissolved natural organic matter. Using the data obtained from the fixed UV wavelength readings and the DOC, other parameters were calculated such as the humification index ($E_4/E_6$ ratio), and the molar absorptivity of the dissolved organic carbon (DOC) ($mol^{-1}$ DOC l $cm^{-1}$).

The total phenol content of the water samples was approximately one order of magnitude lower than found in the sediment pore water samples (Table 3.13). Low concentrations in the sediment pore water (< 1.0 mg $l^{-1}$) correlated with sediments containing predominantly sand (e.g. $LL^a$ and CC). By comparison the

sediment sample that was defined as soil ($LL^b$) was one order of magnitude higher (*ca.* 25 mg $l^{-1}$) than the rest of the sediment samples (*ca.* 5.0 mg $l^{-1}$).

The DOC content of the water (Table 3.14) followed a similar pattern to that found for total phenol (Table 3.13), meaning that lower DOC and total phenol were found in water samples compared to pore water. The DOC content of the pore waters was one to two orders of magnitude higher than in the water samples. The water DOC concentrations reported here are slightly greater than reported previously; Head (1976) reported that water DOC values in estuaries range between 1 and 5 mg $l^{-1}$, with concentrations exceeding 5 mg $l^{-1}$ usually associated with polluted situations. Under these criteria the TC water would be considered contaminated. Chin and Gschwend (1991) reported that sediment pore water of the Upper Mystic Lake (Arlington MA, USA) had a maximum DOC of 45 mg $l^{-1}$ and Burdige (2001) reported a maximum concentration of 21 mg $l^{-1}$ in pore water from estuarine sediments from the Chesapeake Bay (USA).

**Table 3.13.** Total phenol content of water and pore water from the Conwy River (mg $l^{-1}$). Values represent mean ± standard error (n > 3).

| Sites | Water | Pore water |
|---|---|---|
| **BC** | <LOD | $5.18 \pm 4.58^{**}$ |
| **LL** | <LOD | $0.82 \pm 0.51^{a**}$<br>$25.58^{b}$ |
| **TC** | $0.80 \pm 0.42^{**}$ | $5.83 \pm 4.12^{\dagger}$ |
| **BS** | $0.71 \pm 0.18^{\dagger}$ | $5.65 \pm 0.88^{\dagger}$ |
| **CC** | $0.68 \pm 0.52^{**}$ | $0.96 \pm 0.52^{**}$ |

a) Pore water extracted from sediment characterised as sand; b) Pore water extracted from sediment characterised as soil, single data (no replicates); ** n=3; † n=4

**Table 3.14**. Dissolved organic carbon (DOC) of water and pore water (mg $l^{-1}$). Values represent mean ± standard error (n>2).

| Site | Water | Pore water |
|---|---|---|
| **BC** | 4.2 ± 2.0$^{\dagger}$ | 82.7 ± 46.1$^{\dagger}$ |
| **LL** | 3.4 ± 0.9$^{\dagger}$ | 52.4 ± 13.6$^{a\dagger}$<br>138.9 ± 44.6$^{b*}$ |
| **TC** | 6.2 ± 1.3$^{\ddagger}$ | 109.2 ± 35.0$^{**}$ |
| **BS** | 4.0 ± 0.7$^{\dagger\dagger}$ | 150.4 ± 44.6$^{\ddagger}$ |
| **CC** | 4.3 ± 1.2$^{\ddagger}$ | 110.6 ± 37.0$^{\ddagger}$ |

a) Pore water extracted from sediment characterised as sand; b) Pore water extracted from sediment characterised as soil.; * n=2; ** n=4; † n=5; ‡ n=6; †† n=7

In general, the DOC content of the sediment pore water was greater than in the water with concentration site dependent. The sediment from LL$^{b}$ was an exception compared to the other sites. Here the DOC was higher, and this sediment sample had a strong terrestrial influence. Sediment from LL$^{a}$ had the lowest concentration of DOC and this may be related with the size grain classification (sand).

High performance size exclusion chromatography (HPSEC) was used to characterise the molecular weight distribution of the dissolved natural organic matter (DNOM). Initially the chromatographic conditions were optimized and validated against another method (ultrafiltration). These results are summarized in the following section.

To find the critical ionic strength (CIS) of the column, a series of sodium benzoate and acetone standards were run using water as the eluent. Table 3.15 shows that, as the ionic strength increases, the retention time of the peaks approach that of

acetone (which is defined as the total permeation volume (Vt)). Higher ionic strengths were not tested due to peak broadening and the risk of overloading the column. Consequently 0.1 M was the CIS used for this analysis.

**Table 3.15.** Sodium benzoate retention times and standardise retention volume (Vr/Vo) as determined by HPSEC. Values are mean, standard errors were less than 5 %, (n = 2).

| Solution ionic strength | Ret. Time (min) | Vr/Vo |
|---|---|---|
| 0.0001 M Na-benzoate | 10.7 | 0.45 |
| 0.001 M Na-benzoate | 11.2 | 0.47 |
| 0.01 M Na-benzoate | 13.4 | 0.56 |
| 0.1 M Na-benzoate | 18.2 | 0.77 |
| Acetone 1:1 v/v | 23.7 | 1.00 |

**Table 3.16.** HPSEC standardise retention volumes (Vr/Vo) for molecular weight (MW) globular protein standards. Value represent mean, standard errors were less than 5 %, (n = 2).

| Globular protein | Nominal MW | Vr/Vo |
|---|---|---|
| Thyroglobulin | 670000 | 1.02 |
| Bovine gamma globulin (IgG) | 158000 | 1.25 |
| Ovalbumin | 44000 | 1.41 |
| Myoglobin | 17500 | 1.77 |
| Vitamin B12 | 1350 | 2.16 |

The Bio-Rad gel standard, described previously in Chapter 2, was used as a measure of column efficiency, and was run several times during analysis of the samples. A typical example of the standardised retention volumes Vr/Vo for gel standard run in a 0.1M NaCl ionic strength mobile phase are shown in Table 3.16.

The standard was run in water or with a 0.1M NaCl mobile phase. Regression analysis, ($Log_{10}$ MW *vs.* Vr/Vo) indicates that the column performs better with a 0.1M NaCl mobile phase Table 3.17.

**Table 3.17** Regression analyses of the HPSEC gel standards (Bio-Rad) performed either in a water or NaCl mobile phase.

| Mobile phase | Equation | $r^2$ | s |
|---|---|---|---|
| Water | $Log_{10}$ MW = 7.51 - 1.51 Vr/Vo | 0.866 | 0.37 |
| 0.1M NaCl | $Log_{10}$ MW = 8.05 - 2.28 Vr/Vo | 0.981 | 0.15 |

**Table 3.18.** Spectroscopic and dissolved organic carbon results of ultra-filtration of natural organic matter, Suwannee River (NOM-SR) solutions, using Centricon® centrifugal filter devices (3000 MW cut-off), comparing two pre-rinsing methods (I and II). Experiment 1. Values are means, standard errors were less than 5 %, (n = 2).

| Sample | AU at 224 nm | AU at 254 nm | AU at 280 nm | NPOC mg $l^{-1}$ |
|---|---|---|---|---|
| **NOM-SR[1]** | 1.828 | 1.253 | 0.972 | 21.7 |
| **NOM-SR; GF/F filtrate[2]** | 1.725 | 1.173 | 0.909 | 19.3 |
| **NOM-SR Filtrate; method I[3]** | 0.932 | 0.613 | 0.456 | 20.3 |
| **NOM-SR Filtrate; method II[3]** | 0.904 | 0.596 | 0.443 | 23.6 |

AU Absorbance units. NPOC non purgeable organic carbon. [1] Before GF/F filtration. [2] Before ultra-filtration. [3] After ultrafiltration.

The five peaks observed in the chromatogram were visually compared with the original performance report supplied by Bio-Rad (Figure 3.7). The official Bio-Rad data was obtained at a higher flow rate (1.0 ml $min^{-1}$) with a different mobile phase (0.10 M sodium phosphate, 0.15 M NaCl, 0.01 M sodium azide) and

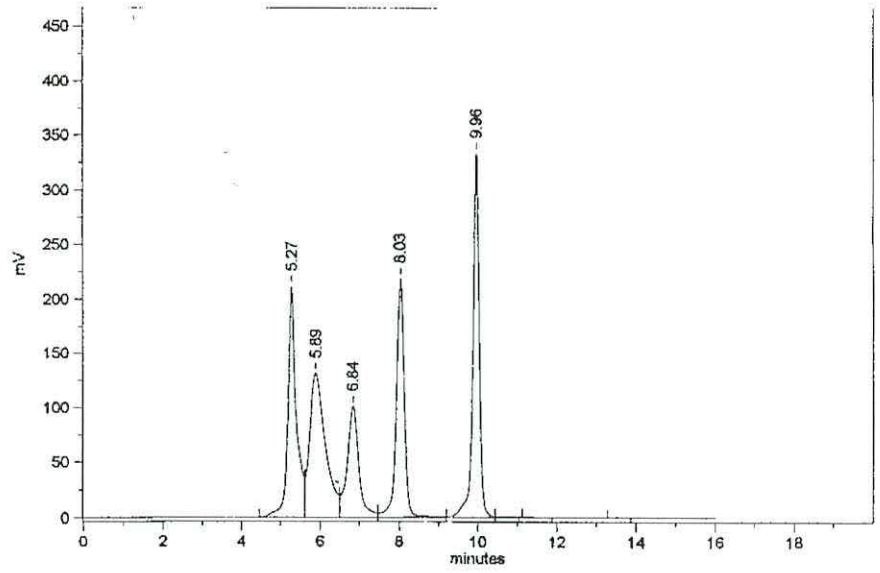



**Figure 3.7.** Bio-Rad gel filtrations standard chromatogram as provided in the Bio-Rad performance report. Numbers represent the retention times (Rt). From left to right: Thyroglobulin, IgG, Ovalbumin, Myoglobin and Vitamin B12.

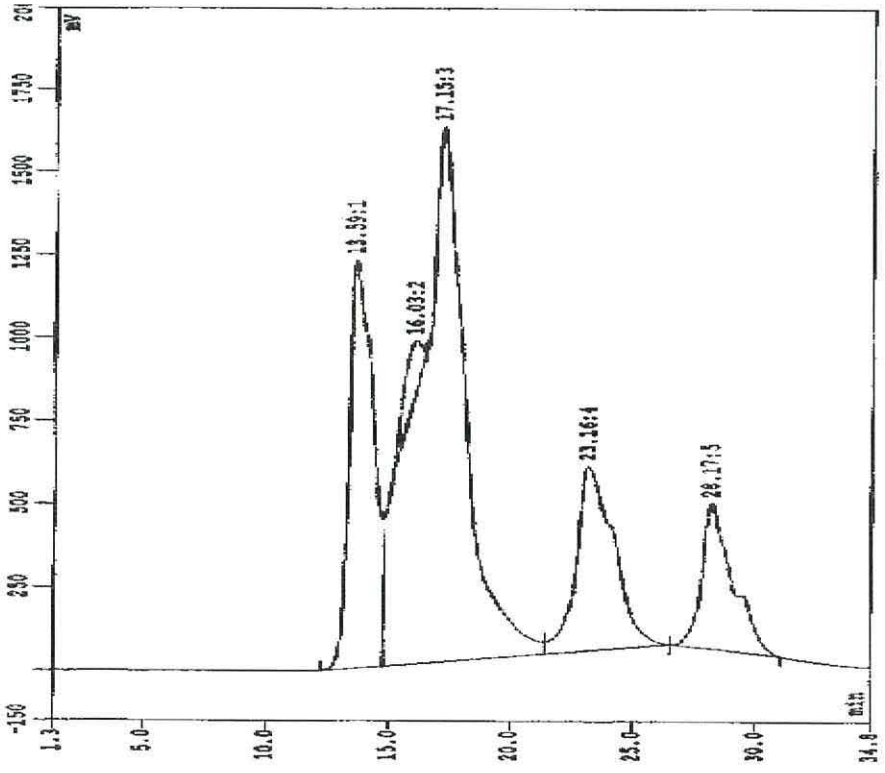



**Figure 3.8** Typical Bio-Rad gel standard chromatogram obtained in this work. Numbers represent the retention times (Rt).

consequently the retention times were generally shorter. By comparison in Figure 3.8 is shown a typical chromatogram of the same gel standard run in the system used here (see Table 2.5).

The ultra-filtration devices Centricon® (3000 MW cut off) were pre-rinsed before use, due to their glycerine coating. Two pre-rinsing methods described in Chapter 2 were evaluated to see which one was better. HPLC grade water was therefore ultra-filtered and the filtrates analysed by UV-vis spectroscopy and HPSEC. No DOC contamination was observed in either washing procedures load (data not shown).

A sample of NOM-SR was dissolved in HPLC grade water and then ultra-filtered. This initial experiment was done to test the efficiency of the pre-rinsing methods and also to see if there were any artefacts introduced in MW analysis from the filter organic coating (glycerine) or by the uncoiling of the compounds. Original samples; before filtration and GF/F filtered together with filtrates of both pre-rinsing methods (I and II) were analysed using UV-vis spectroscopy and DOC analysis in the form of non purgeable organic carbon (NPOC) (Table 3.18). The filtrates were also analysed by HPSEC with water as mobile phase to check if the NOM-SR had aggregates with MW < 3000 Da (Table 3.19). The results show that both ultra filtrates (methods I and II) seem to be similar in their spectroscopic characteristics. All the filtered samples produced multiple HPSEC peaks (Figure 3.9). The height and area of peak No. 1 was greater using the method II for pre-rinsing (Table 3.19). This indicated possible organic carbon enrichment; and was supported by DOC measurements of the filtrates which were higher in method II (23.6 mg $l^{-1}$) compared to method I (20.3 mg $l^{-1}$). This indicated incomplete

removal of glycerine by pre-rinsing method II. Alternatively the NOM-SR compounds may have uncoiled in a solution with low ionic strength.

**Table 3.19.** HPSEC results of ultra-filtration of natural organic matter, Suwannee River (NOM-SR) solutions, using Centricon® centrifugal filter devices (3000 MW cut-off), comparing two pre-rinsing methods (I and II). Experiment 1. Values are means, standard errors were less than 5%, (n = 2).

| Peak No. | Parameters | NOM-SR GF/F filtrated | Filtrate, (Method I) | Filtrate, (Method II) |
|---|---|---|---|---|
| 1 | **Ret. Time (min)** | 10.4 | 11.1 | 10.4 |
| | **Height (mV)** | 32.8 | 15.9 | 15.4 |
| | **Area (mV x min)** | 55.2 | 22.2 | 27.9 |
| 2 | **Ret. Time (min)** | 12.1 | 11.8 | 12.0 |
| | **Height (mV)** | 22.9 | 15.6 | 13.3 |
| | **Area (mV x min)** | 25.9 | 18.6 | 13.9 |
| 3 | **Ret. Time (min)** | 13.5 | 13.4 | 13.4 |
| | **Height (mV)** | 20.0 | 15.4 | 16.3 |
| | **Area (mV x min)** | 8.7 | 7.4 | 7.2 |

A second series of experiments were carried out to compare the HPSEC method against the ultra-filtration method using samples containing sodium polystyrenesulfonate (Na-PSS) of 4600 MW (100 mg $l^{-1}$) and a dilute NOM-SR solution. Samples were run with an aqueous mobile phase and ionic strength of 0.1M NaCl and filter devices pre-rinsed using method I. It is important to note that the mobile phase used for HPSEC was the same as the solvent for the samples, to avoid any artefacts arising from coiling or uncoiling of compounds. From the results presented in Table 3.20 it is clear that the ultrafiltration devices retained almost the 100% of the Na-PSS standard (4600 MW), which should be

expected due to the cut off of the ultrafilters being 3000 MW. Some of the NOM-SR components were also retained, and NPOC analysis indicated that *ca.* 84% of the DOC from the NOM-SR sample was less than 3000 molecular weight. In conclusion, pre-rinsing method I was superior to method II, and NOM-SR, was largely composed of low MW DOC.

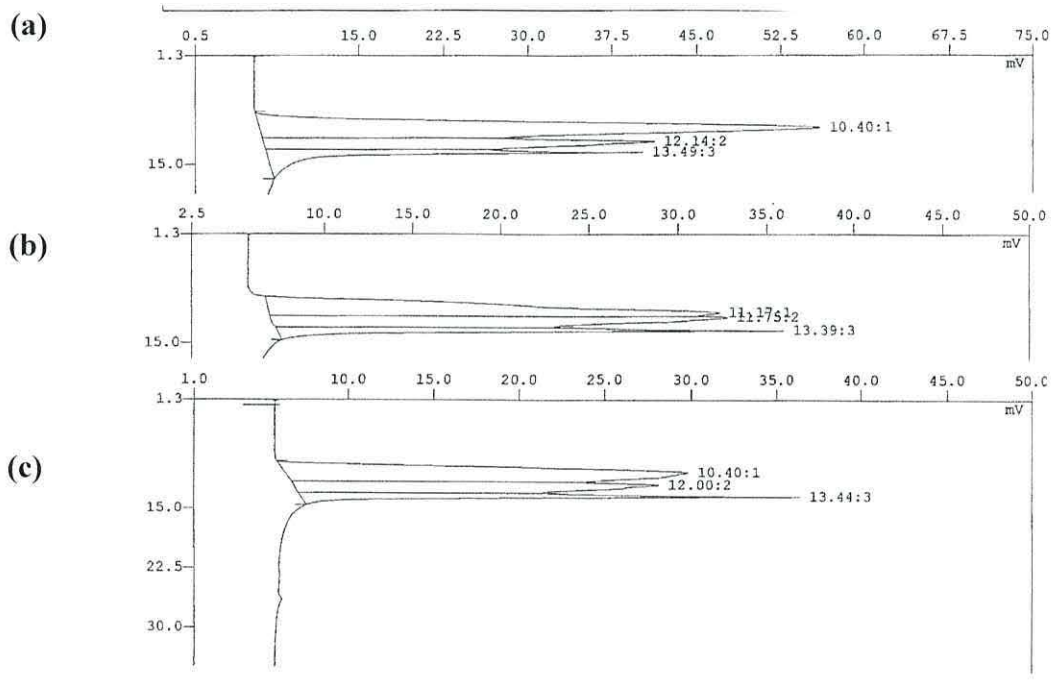



**Figure 3.9** HPSEC chromatograms of natural organic matter from Suwannee River (NOM-SR) solutions. From top to bottom, (a) before ultrafiltration, (b) after ultrafiltration, method I and (c) after ultrafiltration, method II. Note the difference in mV scales.

The HPSEC results (Table 3.21) of the NOM-SR filtrate, do not show significant changes in retention times. On the other hand the height, base width and area are reduced considerably by the ultra-filtration process. The results of the Na-PSS filtrate are presented as non applicable (NA), because the chromatogram showed

no peaks (Table 3.21), confirming the almost complete retention by the 3000 MW cut-off filter.

**Table 3.20.** Spectroscopic and dissolved organic carbon results of ultra-filtration of natural organic matter from Suwannee River (NOM-SR) solutions and Na-PSS samples, using Centricon® centrifugal filter devices (3000 MW cut-off), comparing before and after ultra-filtration. Experiment 2. Values are means, standard errors were less than 5%, (n = 2).

| Sample | Before or after ultra-filtration | A.U. at 224 nm | A.U. at 254 nm | A.U. at 280 nm | NPOC mg $l^{-1}$ |
|---|---|---|---|---|---|
| **NOM-SR** | Before | 2.064 | 1.406 | 1.077 | 32.3 |
| | After | 1.237 | 0.811 | 0.595 | 26.9 |
| **Na-PSS, 4600 MW** | Before | 3.154 | 0.556 | 0.395 | 694.4 |
| | After | 0.280 | 0.012 | 0.003 | 12.4 |

AU Absorbance units. NPOC non purgeable organic carbon.

**Table 3.21.** HPSEC parameters, (retention time, height, base width and area of peaks) of natural organic matter from Suwannee River (NOM-SR) solutions and Na-PSS samples before and after ultra-filtration. Values are means, standard errors were less than 5 %.

| Sample | Before or after ultra-filtration | Ret. time (min) | Height (mV) | Base-width (min) | Area (mV x min) |
|---|---|---|---|---|---|
| **NOM-SR** | Before | 19.3 | 25.08 | 6.3 | 87.68 |
| | After | 19.6 | 19.18 | 4.8 | 50.26 |
| **Na-PSS 4600 MW** | Before | 16.5 | 6.49 | 2.6 | 9.73 |
| | After | NA | NA | NA | NA |

NA non applicable (no peak detected)

The molecular weight calculated by HPSEC is defined as "weight-averaged

molecular weight" (MW, units Daltons (Da)). The values presented in Table 3.22 were estimated using a calibration with polystyrene sulfonate standards (PSS) with calibrations carried out every sample was run. A typical example of the semi-logarithmic equation produced by the calibration was as follows;

$$Log_{10}MW = 7.3 - 2.50 \times \left( \frac{Vr}{Vo} \right) \qquad (r^2 = 0.998, (n=4))$$

Where Vr is the retention volume of the peak, Vo is the retention volume that represented the void volume, as determined with Dextran blue (2 x $10^6$ MW).

The results in Tables 3.22 and 3.23 for water and sediment pore water samples respectively show that the "weight-averaged molecular weight" (MW) of the DOM was below 1000 with a minimum of 130 and a maximum of 1100 MW. The MW of water and pore water dissolved NOM throughout the sites were similar. There is a slight increase in the average of MW in sediment pore water samples, in comparison with the water samples LLSO, BSSO and CCSO (*ca.* 40, 100 and 20% higher). The highest recorded value in pore water corresponded to the sediment characterised as soil samples or from terrestrial origin (576 MW). These results indicated that the DOM in the Conwy River was comprised mainly of relatively small molecules, with slightly higher MW in the pore water.

During the sampling and analysis of the DOM in the water samples there was one set of data that were out of the ordinary; these were the water samples collected on the autumn of 1999. The most significant difference compared with other seasons was that the chromatograms showed bimodal peaks, (e.g. Figure 3.10 and Table

3.24). The HPSEC chromatograms were expressed as time (min) versus mV, where mV is the signal intensity at 224 nm. These samples were collected on the same date (5th October) with the exception of the second samples from TC collected one month later (11th November). The rest of the water samples collected on the 11th of November 1999 did not present bimodal peaks. Characteristically the water samples collected in the 5th of October 1999 had a brownish coloration.

**Table 3.22.** Range and average of calculated "weight-average molecular weight" MW of water samples from the Conwy River and Estuary, (Da) ± SEM, (n>3).

| Sample | Weight-average molecular weight MW Range | Weight-average Molecular weight MW Average ± SEM |
|---|---|---|
| **BC** | 237 - 878 | 497 ± 136 ** |
| **LL** | 248 - 440 | 365 ± 59 * |
| **TC** | 237 - 1019 | 499 ± 225 * |
| **BS** | 134 - 1007 | 242 ± 200 ** |
| **CC** | 132 - 1111 | 453 ± 226 ** |

* n=3; ** n=4

The spectroscopic characteristics of DOM in water and sediment pore water samples were studied at a range of wavelengths. An example of a typical scan is shown in Figure 3.11. Fixed wavelength readings were used to calculate the molar absorptivity at 280 nm ($mol^{-1}$ OC 1 $cm^{-1}$) and the $E_4/E_6$ ratio which is defined as the light absorbance ratio at 465 and 665 nm. These parameters were used in the following section to determine the possible correlations between the NOM characteristics.

**Table 3.23.** Range and average of calculated "weight-average molecular weight" MW of sediment pore water samples from the Conwy River and Estuary, (Da) ± SEM, (n>3).

| Sample | Weight-average molecular weight MW Range | Weight-average Molecular weight MW Average ± SEM |
|---|---|---|
| **BC** | 299 - 694 | 447 ± 86† |
| **LL**[a] | 211 - 941 | 535 ± 215** |
| **LL**[b] | NA | 576* |
| **TC** | 182-868 | 489 ± 202** |
| **BS** | 188 - 937 | 494 ± 227** |
| **CC** | 214 - 1032 | 542 ± 250** |

a) sand samples; b) Pore water extracted from sediment characterised as soil, single data (no replicates); NA non applicable; ** n=3; † n=4

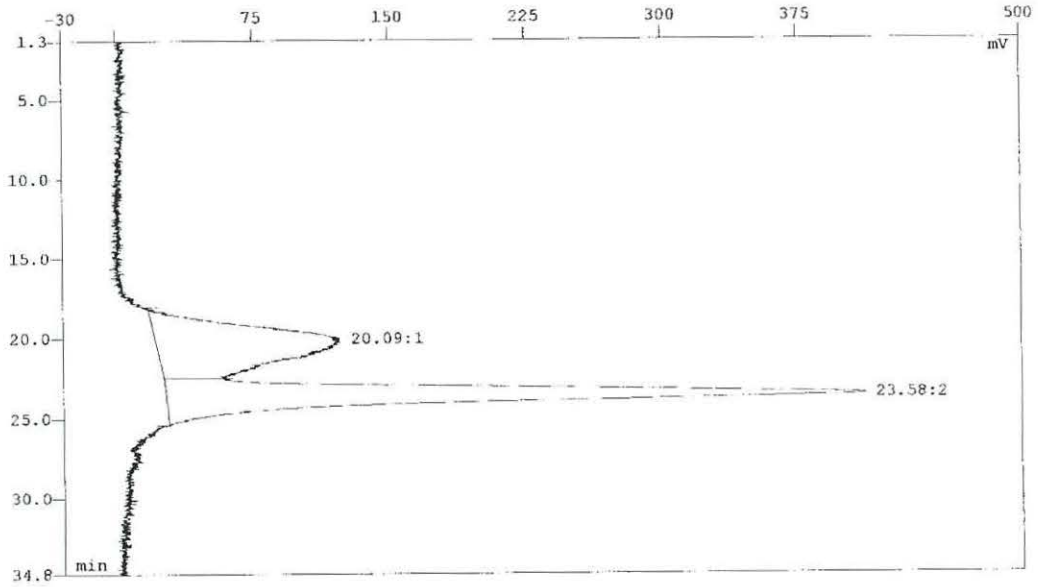



**Figure 3.10.** HPSEC chromatogram of the BS water sample, showing bimodal "weight-average molecular weight" MW peaks distribution. Sample collected on 5th October 1999.

The correlation statistics between the NOM characteristics of the water samples

from the Conwy River are summarised in Table 3.25 and the corresponding plots are shown in Figure 3. 12. A brief description is given shortly and the discussion of this is presented in the following section.

**Table 3.24.** Calculated "weight-average molecular weight" MW of bimodal peaks of specific water samples (Da), standard errors were less than 5%. (n =2)

| Date of sampling | Sample | MW Peak 1 | MW Peak 2 |
|---|---|---|---|
| 5th October 99 | BC | 878 | 163 |
| | TC | 1019 | 174 |
| | BS | 1007 | 153 |
| | CC | 1111 | 131 |
| 11th November 99 | TC | 242 | 157 |

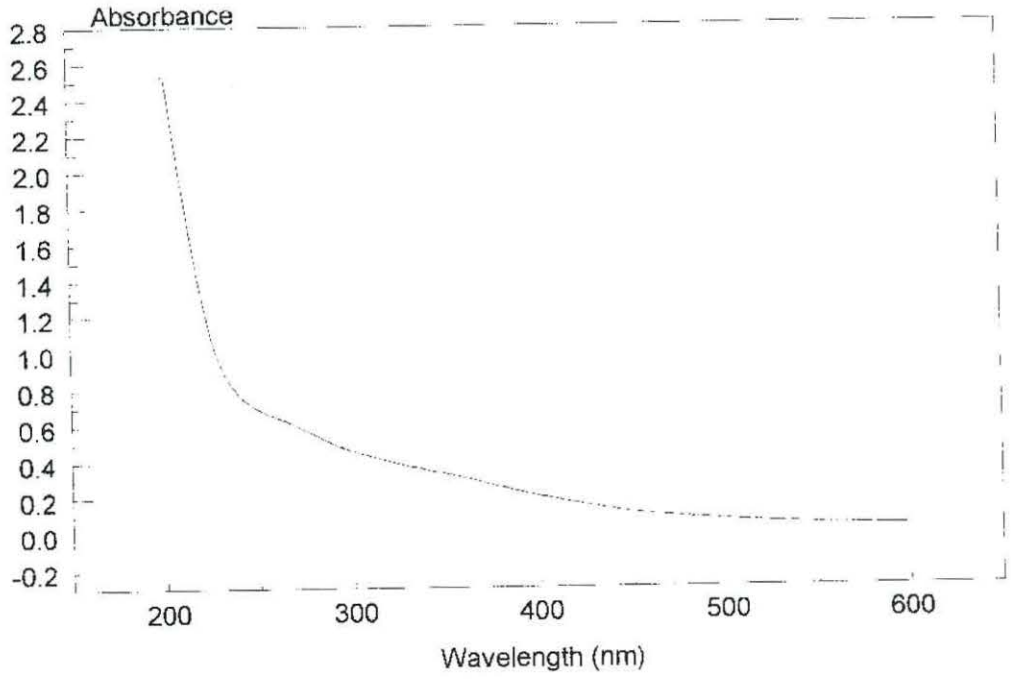



**Figure 3.11** Typical UV-vis scan from 200 to 600 nm of the dissolved natural organic matter (NOM) of water and sediment pore water samples from the Conwy River.

The correlation between the MW and total phenol content (mg $l^{-1}$) (Figure 3.12.a) gave a positive-linear correlation, indicating that increasing NOM MW also indicated increased phenolic moieties.

The MW and the molar absorptivity ($\epsilon$) at 280 nm ($mol^{-1}$ OC 1 $cm^{-1}$) presented a negative linear correlation (Figure 3.12.b) whilst MW and the $E_4/E_6$ ratio (humification indicator) gave a positive linear correlation (Figure 3.12.c). This explains the high MW found in sediments with terrestrial origin; these samples had a major degree of humification. The MW and the content of DOC gave a positive linear correlation (Figure 3.12.d) as expected; an increment in MW would be expect to have an increment in DOC, since carbon is the base of HS Both the total phenol content (mg $l^{-1}$) and the ratio of humification $E_4/E_6$ and the total content of phenol and the content of DOC had a positive linear relationship (Figure 3.12.e and f).

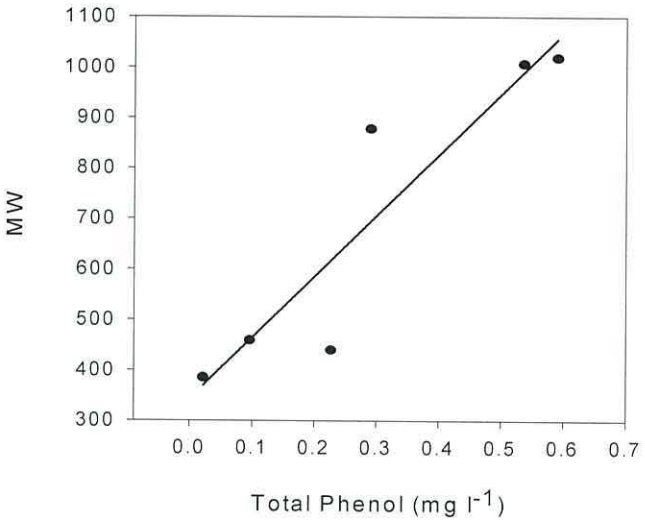
The correlation between the total content of phenol and the absorbance at 280 nm in water samples (Figure 3.12.g) gave a positive linear correlation. This correlation indicates that phenol content of water samples could be inferred by the absorbance at 280 nm.

The molar absorptivity ($\epsilon$) at 280 nm and the DOC content correlation was best represented as an exponential decay (Figure 3.12.h). The content of DOC in relation with the spectrophotometer absorbance at 280 nm in absorbance units (AU), also gave a positive linear correlation (Figure 3.12.i).

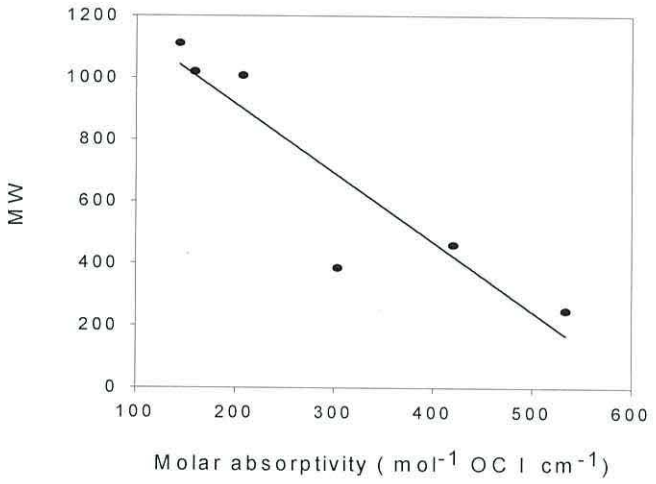
**Table 3.25** Correlations between NOM characteristics of water samples from the Conwy River and estuary.

| Plot | Correlation | | Equation | No. of points | $r^2$ |
|---|---|---|---|---|---|
| Mw vs. Total phenol (mg $l^{-1}$) | positive | linear | Mw = 344 + 1207 × Total phenol | n = 6 | 0.851 |
| Mw vs. ε (Molar absorptivity) | negative | linear | Mw = 1365 – 2.24 × ε | n = 6 | 0.844 |
| Mw vs. $E_4/E_6$ | positive | linear | Mw = 81.3 + 140 × $E_4/E_6$ | n = 9 | 0.802 |
| Mw vs. DOC (mg $l^{-1}$) | positive | linear | Mw = 356 + 55.6 × DOC | n = 7 | 0.788 |
| Total phenol vs. $E_4/E_6$ | positive | linear | Total phenol = -0.025 + 0.075 × $E_4/E_6$ | n = 6 | 0.870 |
| DOC vs. (mg $l^{-1}$) Total phenol | positive | linear | DOC = 0.87 + 4.01 × Total phenol | n = 7 | 0.947 |
| Total phenol vs. AU @ 280 nm | positive | linear | Total phenol = -0.060 + 4.506 × AU @ 280 nm | n = 8 | 0.905 |
| ε (Molar absorptivity) vs. DOC | negative | exponential | $\varepsilon = 122 \times e^{(0.0002 / DOC + 0.0001)}$ | n = 9 | 0.850 |
| DOC vs. AU @ 280 nm | positive | linear | DOC = -2.64 + 86.4 × AU @ 280 nm | n = 8 | 0.832 |

(a)




(b)




(c)

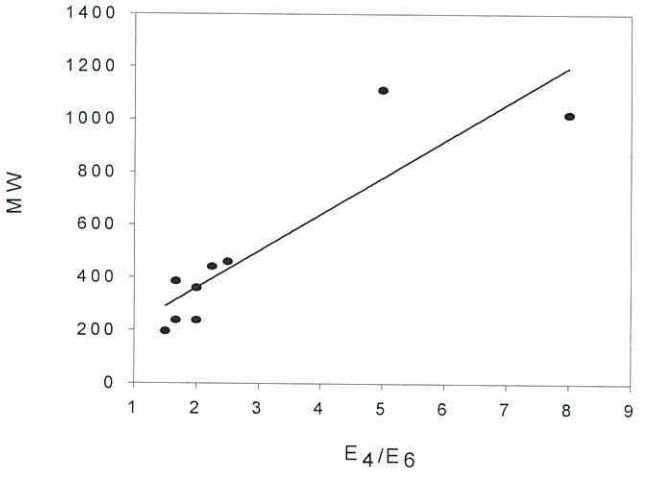
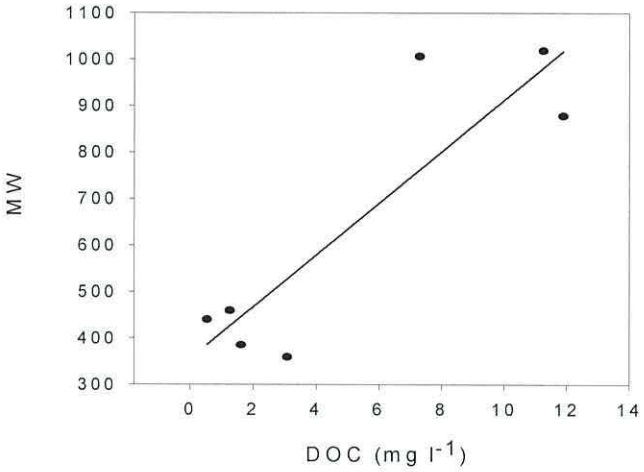
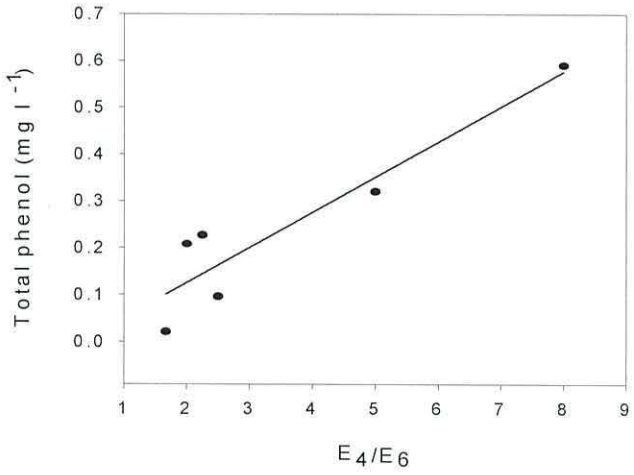



**Figure 3.12** Correlation of organic properties of NOM of water samples from the Conwy River. (a) Mw vs. Total phenol; (b) Mw vs. Molar absorptivity; (c) Mw vs. $E_4/E_6$.

(d)




(e)




(f)

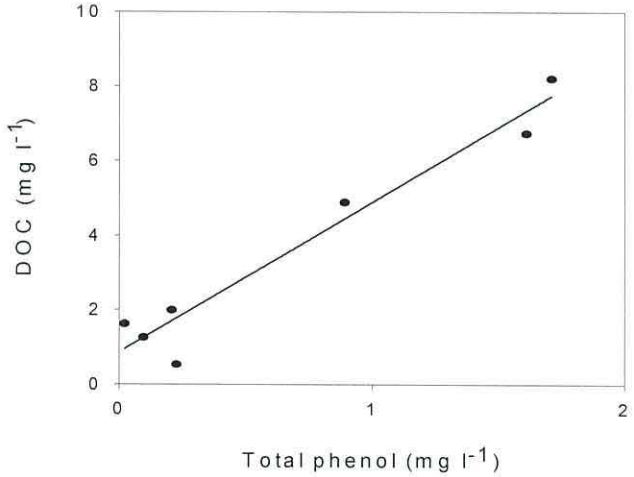
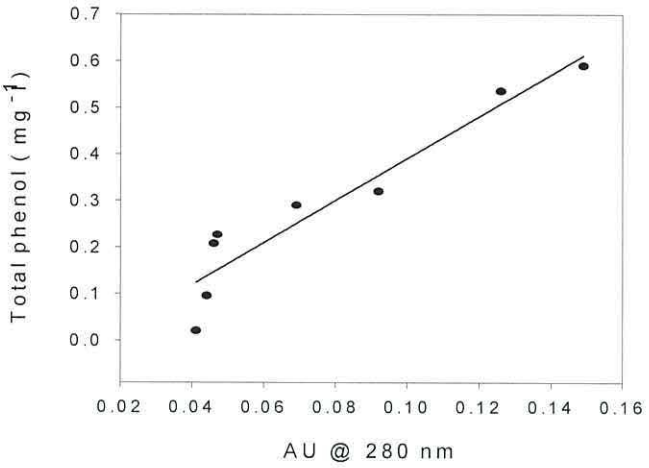
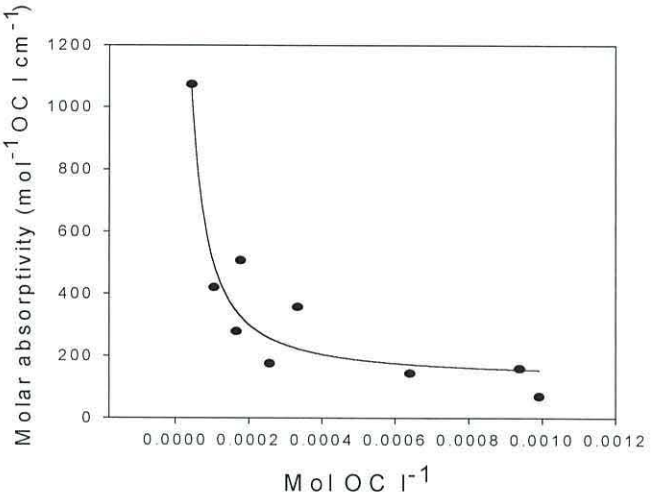



**Figure 3.12** Correlation of organic properties of NOM of water samples from the Conwy River. (d) Mw vs. DOC; (e) Total phenol vs. $E_4/E_6$; (f) DOC vs. Total phenol.

(g)




(h)




(i)

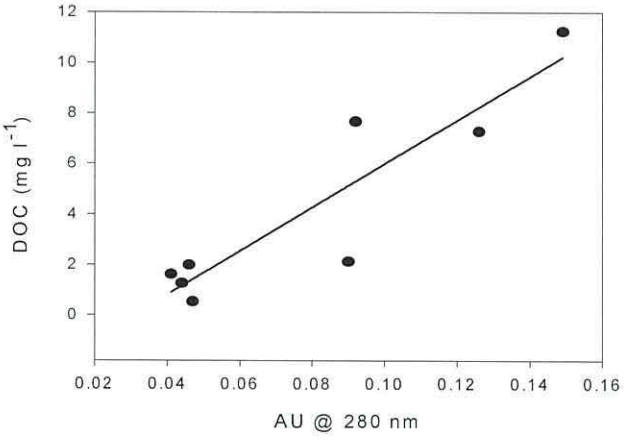



**Figure 3.12** Correlation of organic properties of NOM of water samples from the Conwy River. (g) Total phenol vs. Absorbance @ 280 nm; (h) Molar absorptivity vs. DOC; (i) DOC vs. Absorbance @ 280 nm.

The correlations between organic characteristics of the sediment pore water samples are presented in Table 3.26, and the plots shown in Figure 3.13. It was observed that some correlations that were well described in water were not present in pore water. These will be described here and discussed in a later section. Those pore water correlations that presented the same tendencies as in the water samples will not be described.

The MW and the content of total phenol in pore water did not correlate well (Figure 3.13.a). Also the total phenol and the absorbance at 280 nm did not show any significant correlation (Figure 3.13.g). These two examples involved the total content of phenol. The last relationship that did not correlated significantly for the pore water samples was DOC and the absorbance readings at 280 nm (Figure 3.13.i).

### 3.2.2.2. Organic characteristics of sediment samples

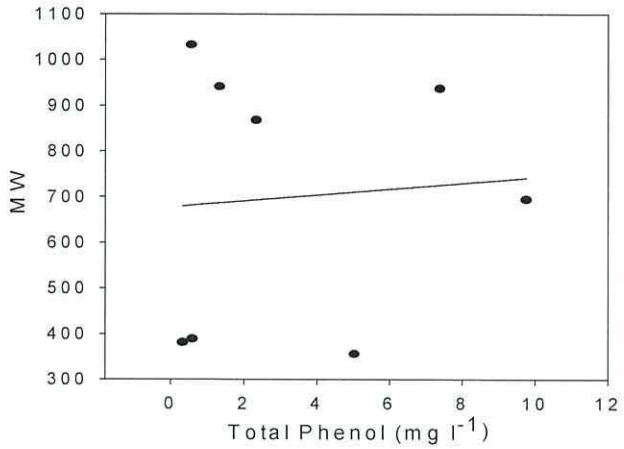
The organic carbon content of the sediments was characterised using; loss on ignition at 550 °C (LOI), see Table 3.27 and elemental analysis (CHN), see Table 3.28. The results were in agreement. Sediment samples that had higher sand contents (e.g. BC, BS and CC) were those with the lowest carbon content. Those samples that had higher content of silt or that were considered as having terrestrial source (e.g. $LL^b$ and TC), also had higher carbon content. The C/N ratio in Table 3.28 gives corroboration of the source of the sediments, higher values like $LL^b$ (25.8) may be from terrestrial origin as has been assumed from the beginning and therefore with a high organic fraction; lower values like CC (9.0) indicate a

**Table 3.26** Correlations between NOM characteristics of pore water samples from the Conwy River and estuary.

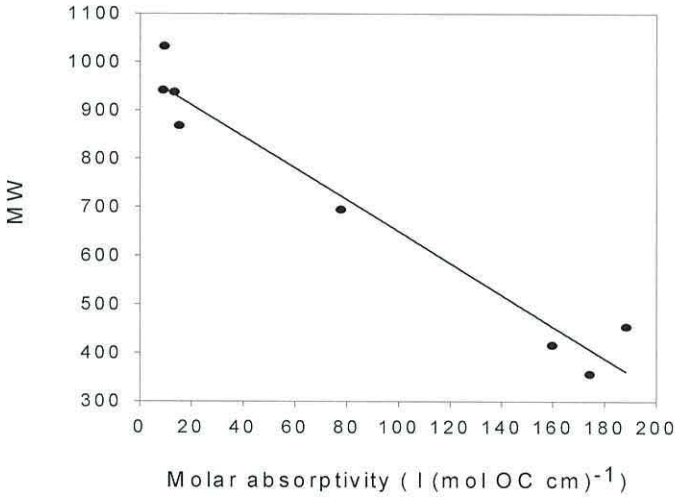
| Plot | Correlation | | Equation | No. of points | $r^2$ |
|---|---|---|---|---|---|
| Mw vs. Total phenol (mg $l^{-1}$) | NA | NA | Mw = 678 + 6.43 × Total phenol | n = 9 | 0.006 |
| Mw vs. ε (Molar absorptivity) | negative | linear | Mw = 976 – 3.27 × ε | n = 8 | 0.951 |
| Mw vs. $E_4/E_6$ | positive | linear | Mw = -181 + 245 × $E_4/E_6$ | n = 9 | 0.880 |
| Mw vs. DOC (mg $l^{-1}$) | positive | linear | Mw = 377 + 2.41 × DOC | n = 9 | 0.886 |
| Total phenol vs. $E_4/E_6$ | positive | linear | Total phenol = -3.91 + 2.30 × $E_4/E_6$ | n = 7 | 0.855 |
| DOC vs. (mg $l^{-1}$) Total phenol | positive | linear | DOC = 5.9 + 41.2 × Total phenol | n = 7 | 0.886 |
| Total phenol vs. AU @ 280 nm | NA | NA | Total phenol = -0.700 + 11.23 × AU @ 280 nm | n = 9 | 0.099 |
| ε (Molar absorptivity) vs. DOC | negative | exponential | $\varepsilon = 15.48 \times e^{(0.01 / DOC + 0.002)}$ | n = 9 | 0.919 |
| DOC vs. AU @ 280 nm | NA | NA | DOC = 107 + 78 × AU @ 280 nm | n = 9 | 0.025 |

NA not applied

(a)




(b)




(c)

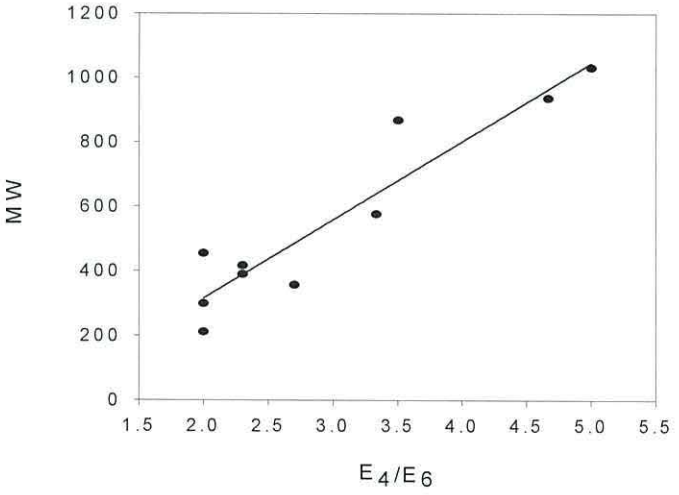
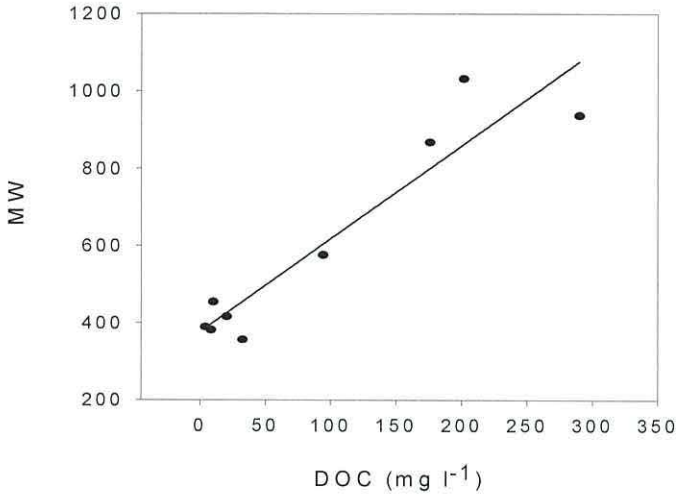
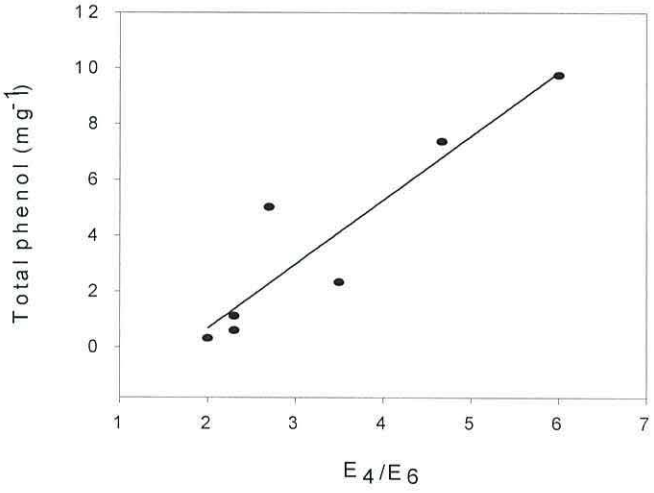



**Figure 3.13** Correlation between organic properties of sediment pore water samples from the Conwy River. (a) Mw vs. Total phenol; (b) Mw vs. Molar absorptivity; (c) Mw vs. $E_4/E_6$.

(d)




(e)




(f)

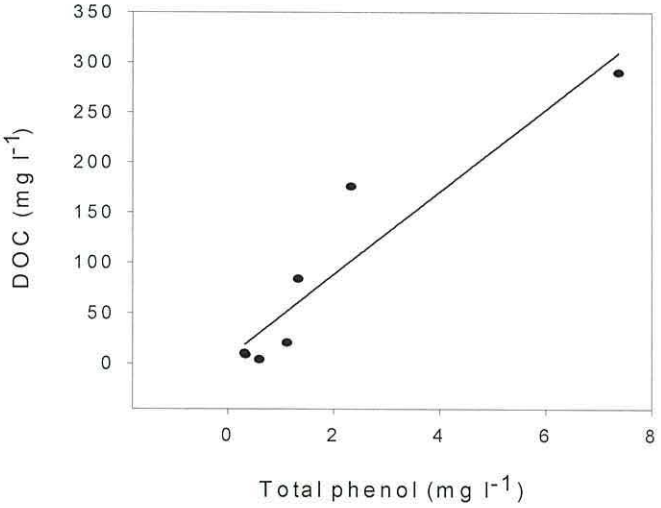
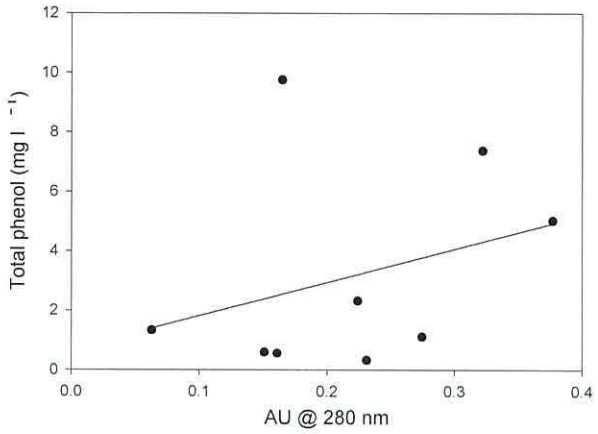
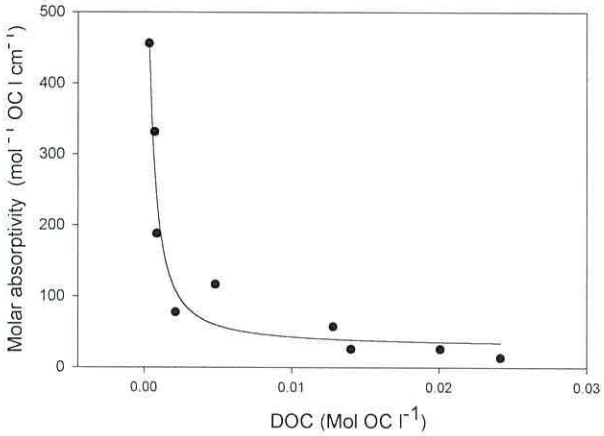



**Figure 3.13** Correlation between organic properties of sediment pore water samples from the Conwy River. (d) Mw vs. DOC; (e) Total phenol vs. $E_4/E_6$; (f) DOC vs. Total phenol.

(g)




(h)




(i)

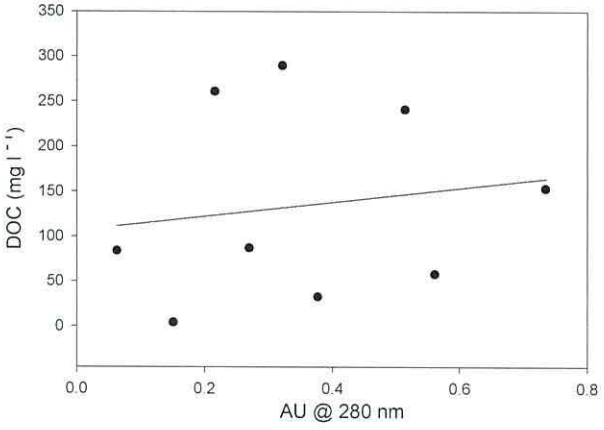



**Figure 3.13** Correlation between organic properties of sediment pore water samples from the Conwy River. (g) Total phenol vs. Absorbance at 280 nm; (h) Molar absorptivity vs. DOC; (i) DOC vs. Absorbance at 280 nm.

greater inorganic fraction.

**Table 3.27**. Loss of ignition (LOI) in percentages of sediments from the Conwy River and Estuary. Calculated as average of all values ± SEM (n>2).

| Samples | % LOI |
|---|---|
| **BCSI** | 2.5 ± 0.3* |
| **LLSI** | 3.2 ± 0.2** |
| **TCSI** | 3.2 ± 0.4** |
| **BSSI** | 2.9 ± 1.3† |
| **CCSI** | 1.4 ± 0.9* |

n=2; ** n=3; † n=4

**Table 3.28.** Elemental content (C, H and N) in percentages and C/N ratio of sediment samples from the Conwy River and Estuary. Calculated as average of all values ± SEM, (n>3).

| | **BCSO**** | **LLSO**a** | **LLSO**b*** | **TCSO**† | **BSSO**‡** | **CCSO*** |
|---|---|---|---|---|---|---|
| **%C** | 0.55 ± 0.11 | 0.42 ± 0.05 | 2.32 ± 0.57 | 1.58 ± 0.26 | 0.89 ± 0.13 | 0.45 ± 0.37 |
| **%H** | 0.42 ± 0.01 | 0.48± 0.02 | 0.67 ± 0.04 | 0.40 ± 0.03 | 0.36 ± 0.12 | 0.11 ± 0.07 |
| **%N** | 0.03 ± 0.00 | 0.05 ± 0.00 | 0.09 ± 0.03 | 0.10 ± 0.03 | 0.07 ± 0.01 | 0.05 ± 0.03 |
| **C/N** | 18.3 | 8.4 | 25.8 | 15.8 | 12.7 | 9.0 |

a) sediment characterised as sand; b) sediment characterised as soil; * n=3; ** n=6; † n=8; ‡ n=9.

### 3.2.3. Fractionation of natural organic matter-metal complexes in aqueous samples

This section of results is divided into three parts, the first one explains how the

separation method (high performance size exclusion chromatography, HPSEC) was optimised, the second one presents the results of the actual methods (HPSEC and ICP-OES), including the physicochemical and spectroscopic characteristics of the natural organic matter from the Suwannee River (NOM-SR) and metal ion solutions, and the third includes the mass balance of the fractions resulted from the separation of the NOM-metal complex solutions, using NOM-SR.

### 3.2.3.1. Optimisation of the method

One of the main practical problems associated with this work was the low metal content of the river waters which were generally in the 1 to 100 $\mu g\ l^{-1}$ range (e.g. Cu, Pb, Mn) although metal content in sediment pore water were in the 100 to 5000 $\mu g\ l^{-1}$ range (e.g. Al, Fe and Zn). Besides this limitation, the volume of sample injected in to the HPSEC was also a considerable restriction; the volume injected during the preliminary experiments was 20 µl which, at a mobile phase flow rate of 0.6 ml $min^{-1}$ caused the original sample to be diluted approximately 900 fold during a 30 minute run. It was predicted, however, that some of the metal ions would be associated with specific molecular weight fractions, thus, they would become concentrated reducing this dilution problem.

Preliminary experiments were run with the same mobile phase as used previously for the determination of NOM molecular weight (buffered mobile phase, see Table 2.5). However, methanol in the mobile phase (5 % v/v), (used as a bactericide for the column) interfered with the ICP-OES signal. Further as the HPSEC mobile phase had an ionic strength of 0.1 M samples had to be analysed

using a Burgener nebulizer and a Scott spray chamber for high ionic strength matrices.

To try to overcome the problem with the ICP's LOD the fractions eluted from the chromatographic system were spiked with an appropriate multi-standard at a metal concentration of 1 mg $l^{-1}$. It was thought that the initial metal concentrations could be calculated by subtraction of the spiked concentration. However, this did not prove possible due to the very small amounts of metal in the eluent stream in comparison to the added spike. Therefore experiments were undertaken in which the water and pore water samples were spiked with a multi-element-standard solution (1 mg $l^{-1}$) prior to HPSEC fractionation. In this case, however, the resulting chromatograms were drastically altered such that the peaks were too broad and intense, indicating saturation of the UV detector. However, the added metals were not detected by the ICP in all the fractions. The next option investigated involved spiking the samples with individual metal. Unfortunately, albeit to a lesser degree, the same phenomenon was observed in the chromatograms, but still not enough to detect significant metal quantities in fractions after HPSEC separation.

Next, fresh Conwy river pore water NOM samples were tested. However, this time the level of metal spiking was increased to exceed the LODs of the ICP-OES. For instance, Pb, Zn and Cd were spiked to have 100 μg $l^{-1}$ and K, Ca and Mg were spiked to have 500 μg $l^{-1}$ in solution. The main problem with this experiment was that analysis of the ICP-OES quality controls (QC) showed that for example Pb had a very high error; ICP value of 300 μg $l^{-1}$ compared with a

QC value was 100 $\mu g\ l^{-1}$. This kind of problem was not isolated. However, in those cases where it occurred, the results were discarded and the analysis repeated with fresh samples. Samples for this experiment were analysed on three separate occasions. The small sample volume was analyzed manually and the rest of the samples had to be analysed in two different dates. However when QC data was used to correlate between samples run the data did not match QC protocol (i.e. > 20 % error) therefore data was discarded.

Up until this point the injection volume was 20 µl and the run time was 30 min. Therefore, a higher volume injection loop (100 µl) was used hoping that increasing sample volume and two different levels of spiking were the best way to overcome the problems experienced. For instance it was observed that the concentration of Ca in the first HPSEC fraction of some samples was high. At first, it was thought that this could be an indication that Ca could be associated with macromolecules that were not detected by the UV-vis. However, this behaviour was not observed all the time and it was noticed that sometimes it happened when a previous injection had a higher natural content of Ca. To test if this effect was genuine, samples were run in duplicate and blanks were injected at certain intervals and this strategy helped to notice that Ca apparently eluted after 30 minutes. On the basis of this work the run time was increased to 40 min to prevent the first fraction of some samples being contaminated with the previous injection.

In the final stages of the preliminary experiments, recently collected water and sediment pore water were fractionated by HPSEC either un-spiked or spiked with

a multi-element-standard, or spiked with specific elements. Spiked Ca and Mg concentrations were 20 mg $l^{-1}$, while Al, Fe and Zn concentrations were 10 mg $l^{-1}$, with runs lasting 40 min and four fractions taken; one before any peak, then one fraction for each of the 2 peaks observed in the chromatograms and the last one after the final peak. The sensitivity of the UV detector had to be adjusted to prevent over-saturation because a higher volume of sample was injected (100 µl). Despite the changes and the continuous repetition of this experiment with fresh samples taking three or even six fractions, the results did not give consistent trends (data not shown). Because of the possible interaction between the mobile phase ($PO_4^{3-}$) with the cation metals in the column, this scheme was abandoned and a different approach was taken.

Firstly, the determination of molecular weight was given a lower priority and was replaced by a theoretical fractionation of NOM. Therefore the mobile phase used for the following experiments was HPLC grade water, but first of all the chromatographic system had to be tested.

A similar procedure to the one used to determine NOM molecular weight in section 3.2.2.1 was used. Standards were run in water and a calibration curve was generated. The correlation values for the molecular weight calibration are shown in Table 3.29. In both mobile phases, the calibration data were good, ($r^2$ are over 0.93), though calibrations in 0.1M NaCl were better ($r^2 > 0.99$) (Table 3.29). In addition the standard deviations were lower for the $NaCl_{(aq)}$ solution and the calculated MW values for the NOM-SR solutions for 0.1 M NaCl mobile phases was within values calculated by other authors, (see Table 3.38). The MW

calculated using water as mobile phase was four times greater than the value obtained by Chin *et al.* (1994) for Suwannee River water (MW = 2190).

**Table 3.29.** Semi-logarithmic correlations to determine "weight averaged molecular weight" MW, based on a Na-PSS standards calibration, using water and 0.1 M NaCl mobile phases. MW of natural organic matter from Suwannee River. Data by duplicate, standard errors were less than 5 %.

| Mobile phase | Equation | $r^2$ | s | NOM-SR MW (calculated value) |
|---|---|---|---|---|
| Water | $Log_{10}$ MW = 5.66 – 1.47 Vr/Vo | 0.926 | 0.33 | 9180 |
| 0.1M NaCl | $Log_{10}$ MW = 7.39 – 2.53 Vr/Vo | 0.991 | 0.11 | 1280 |

Using either water or 0.1 M NaCl as mobile phase, the highest MW fraction eluted first followed by decreasing MW fractions. However, precise quantification of MW based on HPSEC retention time was not considered to be absolute, but qualitative. Therefore the results presented in this section should be considered in the light of the chromatographic system used here.

### 3.2.3.2. Fractionation of natural organic matter

The use of natural samples from the River Conwy and estuary was abandoned because of the difficulties of obtaining enough sample volume, in the case of sediment pore water, and also the variability of metal concentrations and DOC between the different sampling sites. Instead, natural organic matter from the Suwannee River (NOM-SR) was used in all subsequent experiments. It was assumed that the NOM-SR solution would have the advantage of having a more

consistent metal and DOC content. The concentration of the NOM-SR solutions (40 mg $L^{-1}$) was chosen because the natural River Conwy and estuary sediment pore water solutions had DOC concentrations in this range. Metal spiking (Table 2.7) was based on two criteria; firstly the concentrations in the River Conwy and secondly their ability to be detected by ICP-OES. The combinations and ratio of multi-elements was selected again to mimic the natural samples, keeping in mind that one of the main goals of this work was to test the effects and behaviour of combinations or "cocktails" of chemicals as found in the environment.

After an overnight equilibration period, samples were tested for conductivity, pH, TOC and metal loading. NOM-SR-5 had the highest loading of metals. However, a precipitate was observed when the pH was neutralized to pH 7 and, after filtration, the resulting solution was almost clear. As can be seen in Table 3.30, the NPOC value was also considerably reduced. In addition, the content of Zn and Pb of sample NOM-SR-5 was depleted in comparison with sample NOM-SR-6 which contained the same initial metal loading. By comparison for NOM-SR-6, the pH was initially raised only to 4.5 to avoid precipitation of the metals.

During sample treatment it was noted that the pH values of the solutions did not remain stable (see Table 3.30). In addition, conductivity values increased with metal concentration as expected. The mean NPOC value was 27 mg $l^{-1}$ ranging from 14-33 mg $l^{-1}$ which was below the anticipated NPOC concentration of 40 mg $l^{-1}$. There was no apparent cause for this because the solutions were prepared using the values for carbon content reported by the IHSS, (Table 3.31.). On this basis, the C and N content of the NOM-SR was examined and shown to be similar to that reported by the IHSS. One possibility could be the solubility of the NOM-

SR at pH values from 3.2 to 6.8 (see Table 3.30). Though, the 50 % reduction of NPOC occurred only in the NOM-SR-5 the sample that presented precipitation. As an alternative option, the NOM could have interacted with the metals and the colloids suffered aggregation and precipitation, and became trapped during filtration in the GF/F membrane.

The UV-visible spectroscopic characteristics of the solutions shown in Table 3.30 were also measured and the data are presented in Table 3.32. The data were recorded between 200 and 600 nm and showed that the addition of metals affected the absorbance of the lower spectrum (200 – 250 nm). For instance the gentle slope in the NOM-SR-1 absorbance between 200 and 400 nm, (Figure 3.14) was transformed to a clear absorbance edge occurring between 220 and 250 nm, producing a "broad peak" with a maximum absorbance of 3.44 at 214 nm, in the solution with the highest metal loading (Figure 3.15). The UV detector was saturated below absorption edge.

**Table 3.30.** Physicochemical characteristics (pH and conductivity) and dissolved organic carbon (NPOC) of natural organic matter from Suwannee River (NOM-SR) solutions. Data by duplicate, standard errors were less than 5 %.

| Sample code | Final pH | Conductivity (μSiemens $cm^{-1}$) | NPOC (mg $l^{-1}$) |
|---|---|---|---|
| **NOM-SR-1** | 6.8 | 180 | 30.5 |
| **NOM-SR-2** | 6.6 | 85 | 25.6 |
| **NOM-SR-3** | 3.2 | 760 | 32.7 |
| **NOM-SR-4** | 3.5 | 670 | 28.1 |
| **NOM-SR-5** | 5.7 | 2140 | 14.2 |
| **NOM-SR-6** | 4.7 | 2270 | 22.8 |

**Table 3.31.** Micro-elemental composition of humic substances including humic acid (HA) Aldrich, humic acid from Norway, fulvic acid from Norway and natural organic matter, Suwannee River (NOM-SR) and C/N ratio.

| | Humic substance, elemental content (%) | | | | |
|---|---|---|---|---|---|
| **Element** | **H A (Aldrich)** | **HA (Norway)** | **FA (Norway)** | **NOM-SR** | **NOM-SR*** |
| **C** | 40.5 | 48.6 | 52.1 | 47.5 | 48.8 |
| **H** | 4.2 | 4.1 | 4.3 | 4.2 | 3.9 |
| **N** | 0.4 | 0.8 | 0.5 | 1.0 | 1.0 |
| **C/N** | 96.4 | 60.7 | 104.2 | 47.5 | 48.8 |

* IHSS reported values.

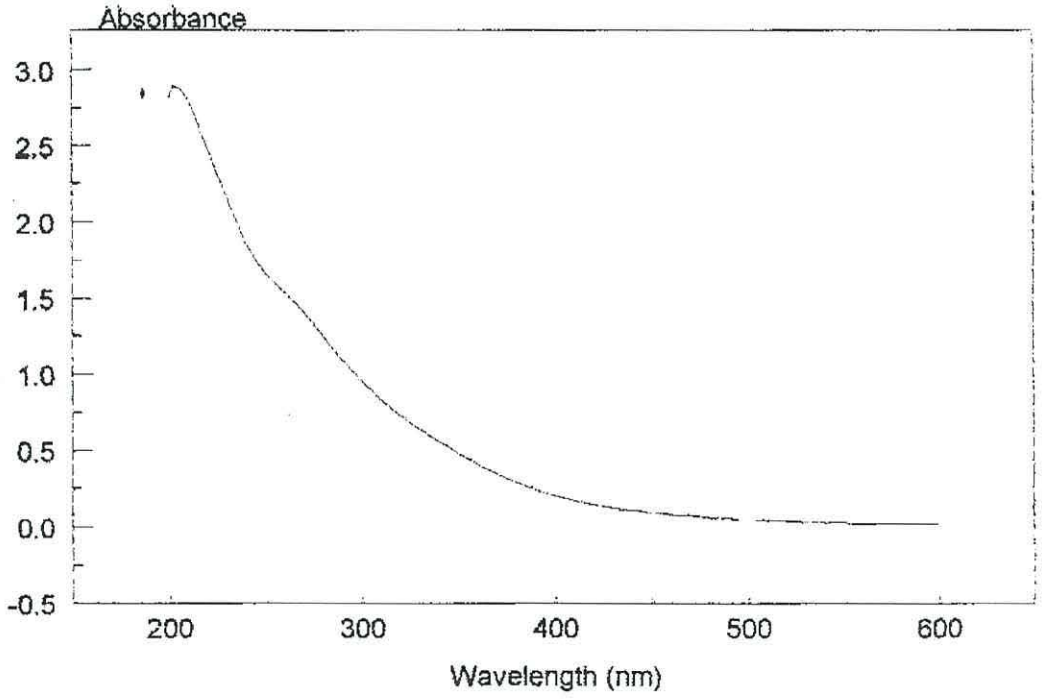



**Figure 3.14.** UV-vis scan (200 to 600 nm) of an aqueous solution of natural organic matter from Suwannee River (NOM-SR-1), (pH=7.0).

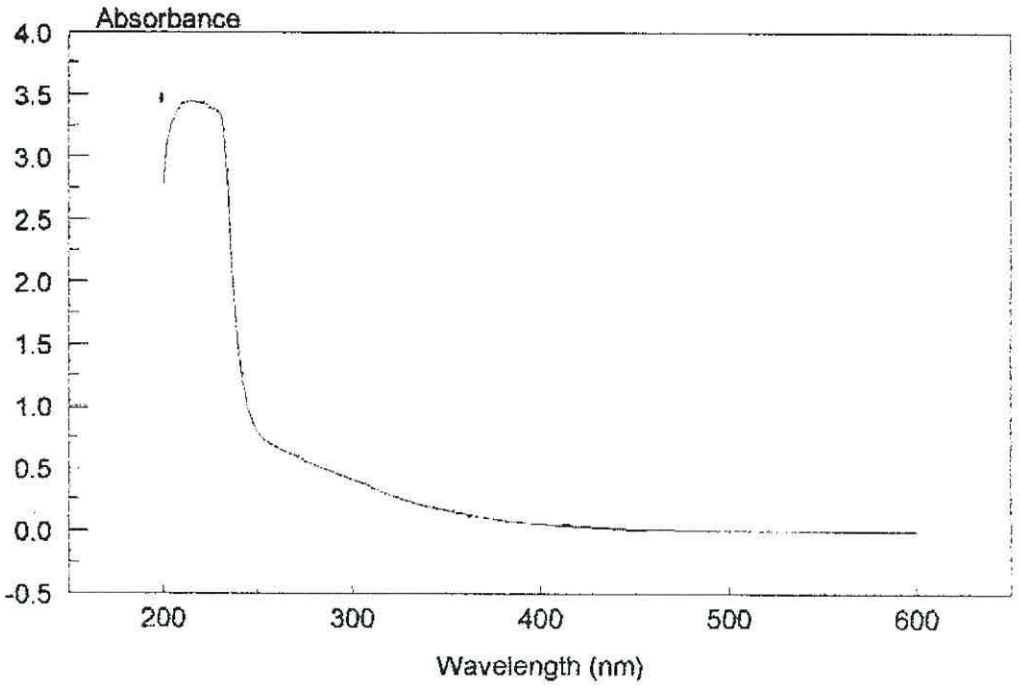



**Figure3.15.** UV-vis scan (200 to 600 nm) of metal spiked aqueous solution of natural organic matter from Suwannee River (NOM-SR-6), (pH= 4.5).

**Table 3.32.** Spectroscopic characteristics (absorbance unit, AU) of natural organic matter from Suwannee River (NOM-SR) solutions used for HPSEC fractionation. Data by duplicate, standard errors were less than 5 %.

| | **A. U. at λ** | | | | |
|---|---|---|---|---|---|
| **Sample code** | **224 nm** | **254 nm** | **280 nm** | **465nm** | **665 nm** |
| **NOM-SR-1** | 2.399 | 1.637 | 1.269 | 0.086 | 0.017 |
| **NOM-SR-2** | 1.863 | 1.254 | 0.971 | 0.065 | 0.008 |
| **NOM-SR-3** | 2.287 | 1.387 | 1.005 | 0.053 | 0.008 |
| **NOM-SR-4** | 3.628 | 1.255 | 0.932 | 0.046 | 0.006 |
| **NOM-SR-5** | 2.998 | 0.297 | 0.215 | 0.004 | 0.000 |
| **NOM-SR-6** | 3.407 | 0.735 | 0.537 | 0.017 | 0.002 |

To determine the effect of the metal alone on the absorbance readings, single metal solutions were prepared in the same concentrations as for the NOM-SR

solutions. The results (Table 3.33) show that most of the solutions prepared in this way had high absorbance at 224 nm, but that there was very little influence from the metals. Therefore the absorbance edge observed in Figure 3.15 does seem to indicate a metal ligand interaction between the NOM and the metals ions added to the solution.

The solutions mentioned before (Tables 3.30 and 3.32) were then analyzed to establish the metal loading left after the precipitation observed in NOM-SR-5. The metal content of each solution is reported in Table 3.34. HPLC grade water was also analyzed to establish the blank metal concentration or background because this matrix was used both as the HPLC mobile phase and also as the sample matrix. The results indicated that the NOM-SR solution does have a natural background metal loading. The Pb concentrations of the spiked samples NOM-SR-5 and 6 were lower than expected (Table 3.34). This could be explained by the partial precipitation or filter retention as mentioned earlier for NOM-SR-5.

**Table 3.33.** Spectroscopic characteristics (absorbance unit, AU) of aqueous metal solutions. Data by duplicate, standard errors were less than 5 %.

| | **A. U. @ λ** | | | | |
|---|---|---|---|---|---|
| **Concentration** | **224 nm** | **254 nm** | **280 nm** | **465nm** | **665 nm** |
| **10 mg Zn $l^{-1}$** | 0.022 | 0.001 | 0.001 | 0.0004 | 0.0003 |
| **100 mg Zn $l^{-1}$** | 0.201 | 0.001 | 0.001 | 0.0003 | 0.000 |
| **10 mg Pb $l^{-1}$** | 0.283 | 0.004 | 0.002 | 0.001 | 0.001 |
| **50 mg Pb $l^{-1}$** | 1.315 | 0.006 | 0.002 | 0.000 | 0.000 |
| **Mixture low** | 2.199 | 0.008 | 0.005 | 0.000 | 0.000 |
| **Mixture high** | 2.761 | 0.026 | 0.021 | 0.001 | 0.0003 |

Mixture low = 10 mg $l^{-1}$ (Zn and Pb); 20 mg $l^{-1}$ (Mg, Ca, K and Na):
Mixture high = 50 mg $l^{-1}$ (Pb); 100 mg $l^{-1}$ (Zn, Mg, Ca, K, and Na)

**Table 3.34.** Metal content of natural organic matter from Suwannee River (NOM-SR) solutions dissolved in HPLC grade water. Obtained by ICP-OES, and expected concentrations. Data by duplicate, standard errors were less than 5 %.

| Sample | | Metal concentration (mg $l^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Ca** | **K** | **Mg** | **Na** | **Pb** | **Zn** |
| **NOM-SR-1** | Obtained | 0.5 | 139.8 | 0.1 | 6.0 | 0.03 | 0.2 |
| **NOM-SR-2** | Obtained | 0.4 | 9.6 | ND | 2.3 | ND | 9.2 |
| | Expected | NA | NA | NA | NA | NA | 10 |
| **NOM-SR-3** | Obtained | 0.5 | 19.0 | ND | 3.3 | ND | 98.3 |
| | Expected | NA | NA | NA | NA | NA | 100 |
| **NOM-SR-4** | Obtained | 20.6 | 30.3 | 21.0 | 19.5 | 7.8 | 9.4 |
| | Expected | 20 | 20 | 20 | 20 | 10 | 10 |
| **NOM-SR-5** | Obtained | 96.3 | 126.5 | 104.7 | 100.5 | 7.5 | 83.8 |
| | Expected | 100 | 100 | 100 | 100 | 50 | 100 |
| **NOM-SR-6** | Obtained | 99.2 | 112.8 | 104.7 | 103.9 | 23.8 | 94.1 |
| | Expected | 100 | 100 | 100 | 100 | 50 | 100 |
| **HPLC grade water** | Obtained | 0.02 | 0.1 | 0.01 | 0.3 | 0.0 | 0.04 |

ND= not detected; NA = not applicable

The discrepancies found in the K loading for samples NOM-SR-1 to 3, could not be fully explained, because NOM-SR samples were prepared from the same stock solution and then spiked individually. Thus, they would be expected to have the same load of K in all samples, but instead the concentration detected in samples NOM-SR 2 and 3 was considerably reduced. It is possible that the ICP-OES analysis had experienced ionization interferences because of the high level of K present in samples. Mg and Pb in samples NOM-SR-2 and 3 were not detected in the ICP analytical method B (section 2.3.6), but they could be present at the same

level as the stock sample NOM-SR-1 (0.1 mg $l^{-1}$), considering that this was the same matrix and that the concentrations were low. The rest of the elements concentrations were well within the expected values.

### 3.2.3.3. Mass balance calculations.

For mass balance calculations, the initial value for the elements was that reported in Table 3.34. Due to the extremely low loading of metals in the fractions obtained by HPSEC, it was necessary to explore all the possible sources of contamination or intrinsic metal load during the experimentation procedure. For instance, the background values provided by the HPLC grade water (Table 3.34) were considered during calculations.

An example of a mass balance calculation is explained below. To simplify matters all calculations were carried out using the calculated mass of every analyte injected. On this basis, because 100 µl of sample was injected into the column, all calculations were carried out to find the mass of metal in this volume of sample. For instance, NOM-SR-1; had 241.0 µg Zn $l^{-1}$ (see Table 3.34), therefore in 100 µl (0.0001 l) of sample, a mass of $2.4 \times 10^{-2}$ µg of Zn. The background content of Zn in the HPLC grade water used as mobile phase was 40.9 µg $l^{-1}$. The total volume of elution was $3.6 \times 10^{-2}$ l therefore the mass of Zn in this volume was 1.463 µg. This added to the mass of Zn in the sample, gives a total of 1.5 µg of "expected" Zn. The four fractions collected were treated in the same way to calculate the mass of each one in µg, as follows in Table 3.35. In this particular case the percent recovery of Zn was 105.9 %, from the total

"expected" Zn in 100 µl (1.5 µg).

**Table 3.35.** Zn content (µg) in the four NOM-SR-1 fractions by HPSEC and Total. Data by triplicate, standard errors were less than 5 %.

| Fraction 1 | Fraction 2 | Fraction 3 | Fraction 4 | Total (µg) Zn |
|---|---|---|---|---|
| 0.3 | 0.2 | 0.5 | 0.5 | 1.6 |

**Table 3.36.** Percent recovery of metals after fractionation by HPSEC of natural organic matter from Suwannee River solutions (NOM-SR).

| Sample code | Ca | K | Mg | Na | Pb | Zn |
|---|---|---|---|---|---|---|
| **NOM-SR-1** | 43.6 | 22.0 | 76.1 | 466 | 5023 | 106 |
| **NOM-SR-2** | 62.7 | 73.5 | 86.9 | 392 | 2592 | 69.3 |
| **NOM-SR-3** | 105 | 83.9 | 84.6 | 489 | 0.0 | 14.1 |
| **NOM-SR-4** | 271 | 56.0 | 15.6 | 447 | 14.0 | 90.9 |
| **NOM-SR-5** | 12.8 | 26.2 | 3.8 | 498 | 0.0 | 17.6 |
| **NOM-SR-6** | 7.7 | 31.2 | 2.9 | 387 | 18.0 | 20.0 |

The percentage recoveries of each element per sample are presented in Table 3.36. From these results it can be seen that in general the recovery tends to decrease as metal concentration increases and is lowest in those samples with the highest concentrations of metals (samples NOM-SR-5 and 6). Another general observation was that Ca, K Mg and Zn had reasonably good recovery in samples with low conductivity or ionic strength (NOM-SR 1 to 4), which indirectly indicated the metal loading. On the other hand, pH does not seem to play an important role for these metals, because the best recovery of K, Mg and Zn occurs at both ends of the pH recorded (3.2 – 3.5 and 6.6 – 6.8), and the worst recovery

occurs in the middle of this ranges (4.7 – 5.7). Besides, $K^+$ and $Mg^{2+}$ solubility should be independent of these pHs.

Na presented a particular problem. There seemed to be some contamination of Na in the chromatographic process and this observation was reinforced by the results obtained from the ICP analysis of the mobile phase collected at the outflow of the column (Table 3.37). There was an extra source of Na that could not be identified. By comparison the percentage recoveries of K and calcium in spiked samples with Zn (NOM-SR 2 and 3) were reasonably good (62.7 to 105.15 for Ca and 73.5 to 83.8 for K). However, in the rest of the samples these recoveries tended to decrease. This could be due to the fact that these elements accumulated in the column as seen in the next experiment.

**Table 3.37.** Metal concentration of mobile phase (HPLC grade water), after chromatographic process. Number of sample indicates order of injection. And metal background of HPLC grade water. Data by duplicate, standard errors were less than 5 %.

| Element | Concentration ($\mu g\ l^{-1}$) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | HPLC grade water |
| Ca | 98.2 | 8.2 | 8.6 | < LOD | 15.1 |
| K | 2624 | 1565 | 1908 | 1444 | 119.0 |
| Mg | 18.0 | 3.9 | 3.6 | 3.1 | 12.1 |
| Na | 1553 | 1063 | 1411 | 1454 | 334.6 |
| Pb | < LOD | < LOD | < LOD | < LOD | < LOD |
| Zn | < LOD | < LOD | 4.2 | < LOD | 40.9 |

< LOD below limit of detection

For Pb, the excessive recovery calculated for Pb in NOM-SR 1 and 2 could be due to the very poor reproducibility of the ICP at levels near the limit of detection (9.0 $\mu g\ l^{-1}$), which had been observed throughout this study. On the other hand for the lack of recovery in NOM-SR 3 to 6, pH could be involved in this response; the NOM-Pb complexes could become unstable at pHs higher than 4 and precipitate.

It was also noticed that there was a large increase in the concentration of K in solution and to a lesser extent of calcium in the HPLC grade water collected after HPSEC was completed (raw data not shown). Therefore to test this observation a new experiment was carried out. In this case, only mobile phase was run in the chromatographic system and it was collected at the end, exclusively to test if the system contributed to the metal loading in the fractions. On this occasion, four samples of mobile phase (HPLC grade water) were injected and the eluent collected without fractionation. The samples were numbered in the sequential order they were injected (1 to 4). The results (Table 3.37) had a considerable variability between them. This result could imply that the column had stored some of the elements from the last run of samples and that with the application of water only as the mobile phase these elements were subsequently eluted. The accumulation was most significant for K decreasing to Na and then to calcium.

The study of the fractionation of natural organic matter by molecular weight and the metals associated with these fractions (Figure 3.16) showed that there may be a trend between some metals and the molecular weight fractions of the dissolved NOM. For instance approximately 36.4 % of calcium in samples NOM-SR-1, 2

and 3 (Figure 3.16.a) was related to the first fraction, which corresponded to the highest molecular weight NOM. However, the chromatogram only showed a peak for the second fraction (there is *ca.* 37.2 % of calcium correlated with this fraction), and the rest was distributed between the two subsequent lower MW fractions. By comparison, in samples NOM-SR 5 and 6 *ca.* 80 % of Ca was found in the first fraction, and the 20 % left was approximately evenly divided between the three MW lower fractions. The sample NOM-SR-4 clearly had an odd behaviour, more than 90 % of the Ca was associated with the second MW fraction, the complexity of the solution chemistry (20 mg $l^{-1}$ Ca, K, Mg and Na; and 10 mg $l^{-1}$ Pb and Zn; pH 3.5 and 28 mg $l^{-1}$ NPOC) may have affected the interaction of Ca with NOM MW fractions. In general Ca seems to associate preferably with high molecular weight NOM ($2 \times 10^6$ to $10 \times 10^6$ MW)

K on the other hand, (Figure 3.16.b) had more or less the same pattern distribution pattern throughout all the samples. Less K was seen in the first fraction, (*ca.* 15 %) (highest MW) and increasing in percentage as the MW decreased (*ca.* 26 % second fraction; 30 % third fraction and 33 % fourth fraction). There were small differences but they were not significantly different. Broadly, K seems to relate to fractions of 1000 to $2 \times 10^6$ and 54 to 1000 MW.

Generally Mg (Figure 3.16.c) had an even distribution between the first two high MW fractions and the lower MW last two fractions (*ca.* 45:55 %). There were small differences in the high MW fractions (*ca.* 25 %), in samples NOM-SR 1 to 3, compared to samples NOM-SR 4 to 6, in which the Mg percentage dropped to 17 %, consequently changing the distribution in the second fraction. The general

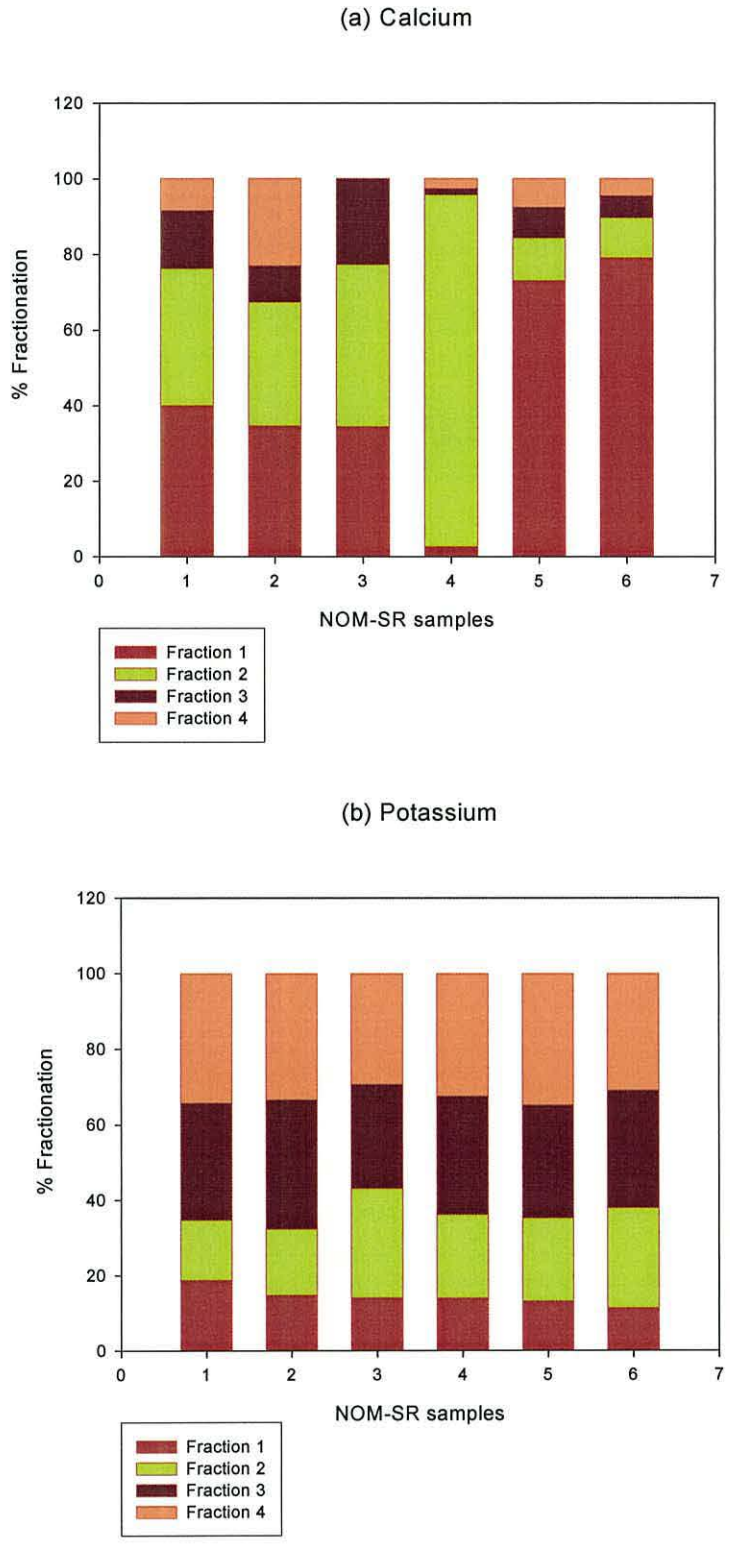
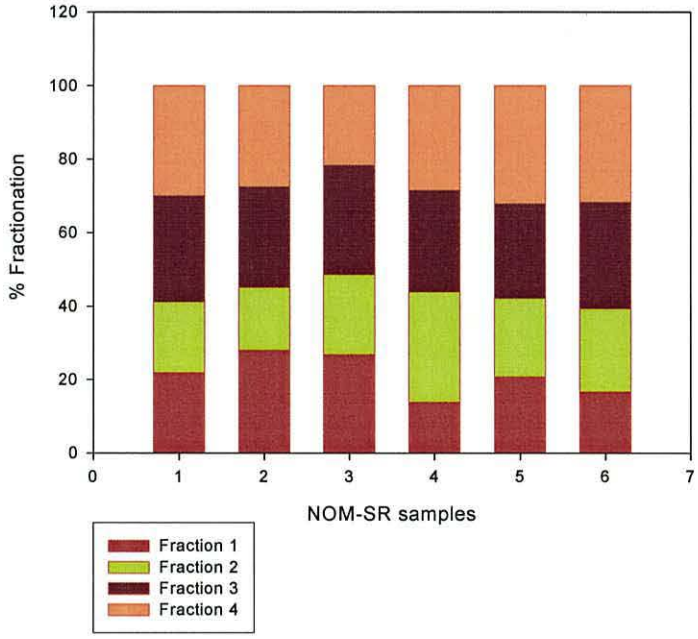



**Figure 3.16.** Percent fractionation of NOM-SR-metal ion in aqueous samples by HPSEC and ICP-OES. (a) Ca, (b) K. Fraction 1 (2,000,000 to 10,000,000 MW); fraction 2 (1,000 to 2,000,000 MW); fraction 3 (54 to 1,000 MW) and fraction 4 (< 54 MW).

(c) Magnessium




(d) Sodium

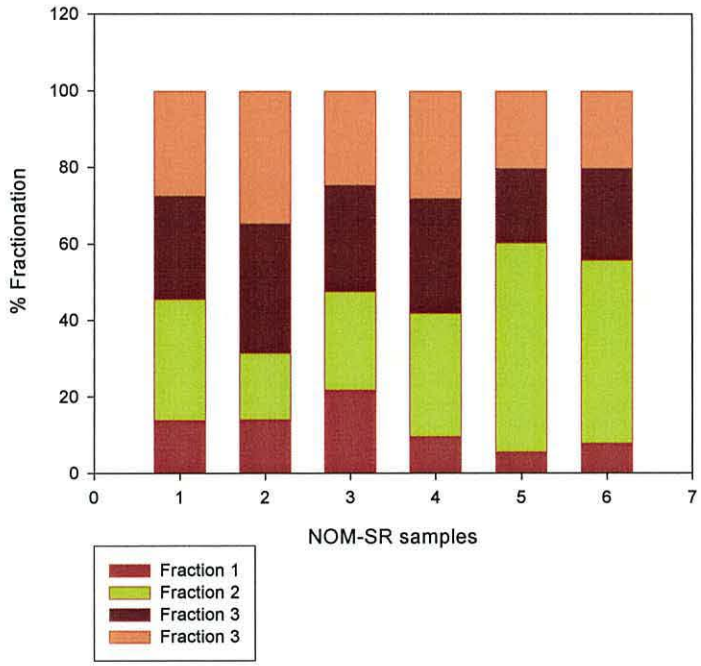



**Figure 3.16.** Percent fractionation of NOM-SR-metal ion in aqueous samples by HPSEC and ICP-OES. (c) Mg (d) Na. Fraction 1 (2,000,000 to 10,000,000 MW); fraction 2 (1,000 to 2,000,000 MW); fraction 3 (54 to 1,000 MW) and fraction 4 (< 54 MW). (Continuation).

observation was that Mg also favoured the fractions 1000 to 2 × $10^6$ and 54 to 1000 MW.

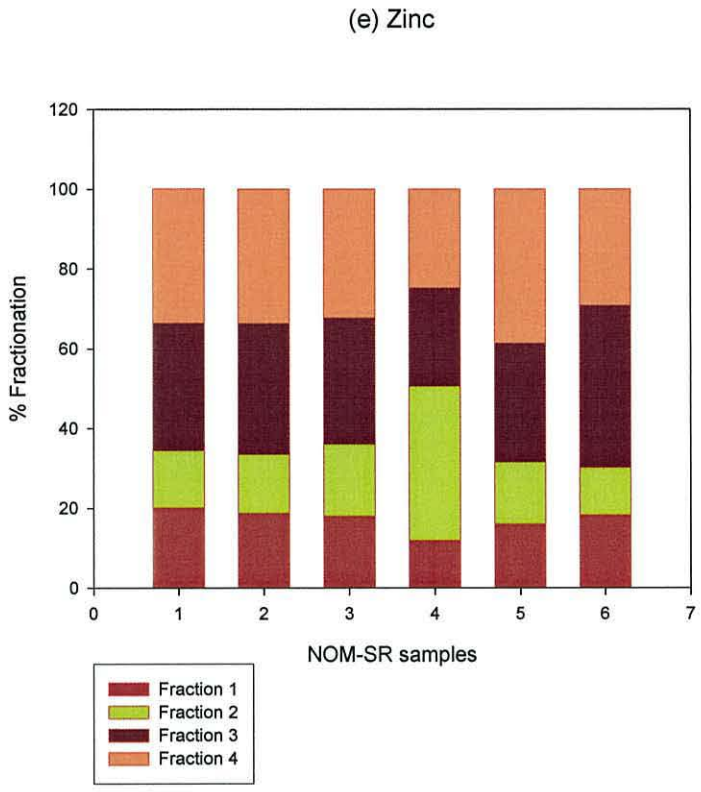



**Figure 3.16.** Percent fractionation of NOM-SR-metal complex in aqueous samples by HPSEC and ICP-OES. (e) Zn. Fraction 1 (2,000,000 to 10,000,000 MW); fraction 2 (1,000 to 2,000,000 MW); fraction 3 (54 to 1,000 MW) and fraction 4 (< 54 MW). (Continuation)

The interaction of Na with different MW NOM fractions (Figure 3.16.d) seemed to be affected by the chemistry of the aqueous solution. Comparing the percentage of Na related to the first and second fraction, the differences were more drastic. For instance, Na in the first fraction of samples NOM-SR 1 and 2 was 14 %, while for samples NOM-SR 4 to 6 it reduced to *ca.* 9 %, while sample NOM-SR-3 had 21 %. In the second fraction the average distribution of Na was

lower for samples NOM-SR 1, 3 and 4 (*ca.* 30 %), than for samples NOM-SR 5 and 6 (*ca.* 50 %). This indicated that Na tended to correlate better with NOM of 1000 to 2 $\times 10^6$ MW.

In the case of Zn (Figure 3.16.e), the distribution over most of the fractions was similar. In average samples NOM-SR 1, 2, 3, 5 and 6 presented the following distribution; 18 % first fraction, 14 % second fraction, 33 % third fraction and 33 % fourth fraction. In sample NOM-SR-4, over 38 % was found in the second fraction. This showed that Zn preferred to associate with low MW fractions (54 to 1000) and even dissolved species.

Pb data was not considered for NOM-metal complex fractionation, because Pb was not detected in most of the fractions, and also because of the artefacts previously described.

In summary, the MW NOM-metal complex distribution of some of the metals studied here showed different patterns when dissolved in aqueous solution with higher ionic strength (*ca.* 2000 μSiemens $cm^{-1}$). Calcium for instance was found almost entirely (*ca.* 76 %) in the first fraction. Na also shown some differences, approximately 50 % was related to the second fraction (UV visible NOM).

Considering only the first three samples, since these showed fewer artefacts in the UV-vis characteristics, it was found that, calcium was the only metal that associated more with the high MW NOM fractions (74 %) (first and second fraction). The order of association with high MW NOM fractions was; Mg (45

%) > Na (41 %) > K (37 %) > Zn (34 %) at low ionic strength solutions (<760 μSiemens $cm^{-1}$). By default, the remaining metal percentages are associated with low MW NOM or in inorganic state as ions.

## 3.3. Discussion

One of the main objectives of this thesis section was to characterize the water and sediments of the Conwy River and to identify any contaminated areas and potential inorganic pollutants. This was done specifically to identify compounds that could interact and affect the fate and behaviour of propetamphos under river and estuarine conditions with an aim to critically assessing the impact of these interactions upon its ecotoxicity.

### 3.3.1. Inorganic characterization

The results presented here indicate that most metals in the water of the Conwy River and estuary are present at background levels, with some exceptions (e.g. Fe). There are two industrial discharges into the Conwy River, one at Dolgarrog and other between Llanrwst and Dolgarrog (Figure 3.17), which could be the source of Fe. In contrast, the sediment pore water, particularly at the LL site, contained significant quantities of Cd, Pb and Zn (Table 3.5). It is speculated that these metals are a result of direct inputs from acid mine drainage coming from the many former mines in the area (Gao and Bradshaw, 1995).

Another specific objective of this work was to investigate the conservative and non-conservative behaviour of elements within the estuary and to identify possible sources of pollutants. Specifically, the aim was to assess whether the estuary functions as a sink or filter for metals. The results presented here indicate that Fe behaves non-conservatively with inputs of Fe into the river between Llanrwst and Tal y Cafn, possibly due to one of the industrial discharges near Dolgarrog.

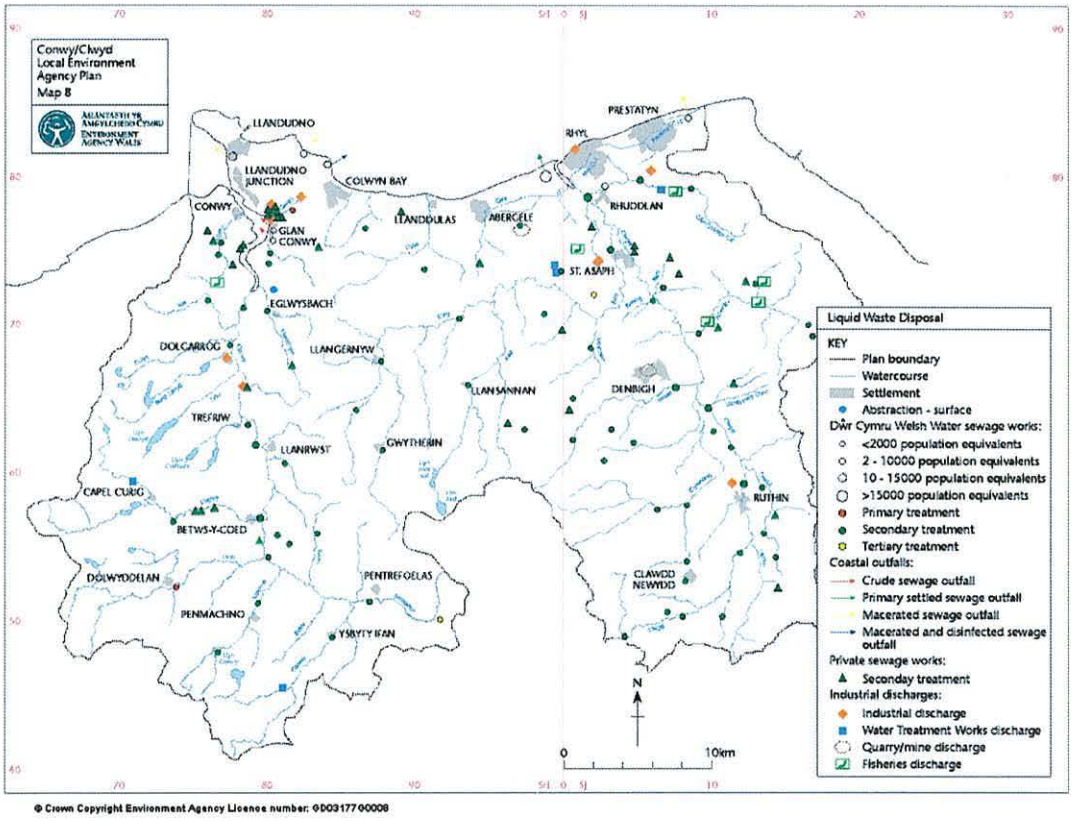



**Figure 3.17.** Sources of liquid waste discharge into the Conwy River. Source: Environment Agency (2001).

By comparison, Zn exhibited a conservative behaviour despite the known point source mine inputs within the Conwy River catchment area. In the case of Zn, the estuary appears to be operating as a filter as the concentration in sediment pore

water decreased towards the mouth of the estuary (Table 3.5). This is supported by estuarine sediment samples which have enhanced Zn contents compared to those upstream (Tables 3.9 and 3.10). In contrast, Cd and Cu concentrations remain reasonably stable or showed no clear trends in behaviour.

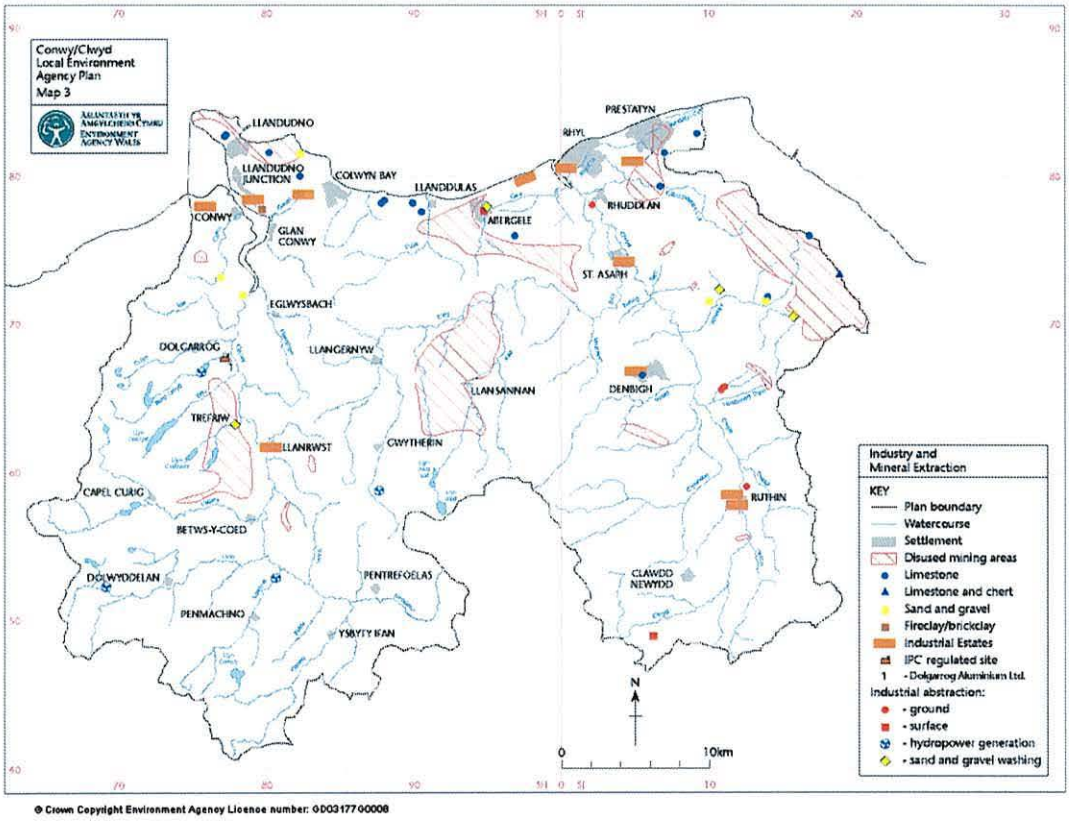



**Figure 3.18.** Areas of industrial activity and mineral extraction within the Conwy River catchment area. Source: Environment agency (2001).

During 2001, the Environment Agency Wales reported that there was a marginal failure of water quality with respect to $Cu^{2+}$ in the Lledir tributary however, this was not detected in the main body of the river. It may be possible that the dissolved copper is probably interacting with suspended particulate matter (SPM)

in the estuary removing it from the main water column (20 μg $g^{-1}$ of copper in the SPM at TC and BS) rather than just a simple dilution effect of the tributary water.

Correlations of Al and Mn concentrations with salinity indicated a non-conservative behaviour possibly indicating that there are sources of these metals throughout the estuary. It has been pointed out in the previous section that Dolgarrog Aluminium Ltd. may be responsible for this (see Figure 3.3). Additionally, Al inputs could arise from acid mine drainage from the former mining areas near Trefriw. Other potential inputs of metals could include direct discharges from Welsh Water (Dŵr Cymru) sewage treatment works located at Betws y Coed, Llanrwst and Trefriw (Figure 3.17)

The final objective of this section of work was to assess the speciation of metals in samples and particularly their association with organic compounds (e.g. polyphenols, pesticides) held in the main water column and in the sediment pore water. The hypothesis is that these interactions with metals may influence the bioavailability of these organic compounds. Within the main water column, the metals appeared to be largely associated with the SPM rather than free in solution. In contrast, significant amounts of metals were generally found in both the solid and pore water phases of the sediments although proportionally more were associated with the solid phase. Taken together, the results suggest that SPM is responsible for carrying large quantities of metals from the Conwy catchment area, however, it is speculated that only a small proportions of these bound metals are bioavailable.

Finally, the inorganic nutrient load in sediments has been investigated to identify any further possible sources of pollution in the river and estuary. The increase in $PO_4^{3-}$ concentrations in the estuary area (Figure 3.5.) may be related with human input of detergents and other house holds products possibly present in the sewage discharges from the secondary water treatment site along the River and estuary (Figure 3.17). Another potential source of phosphates could be the crude sewage outfall in Glan Conwy and finally from bird inputs.

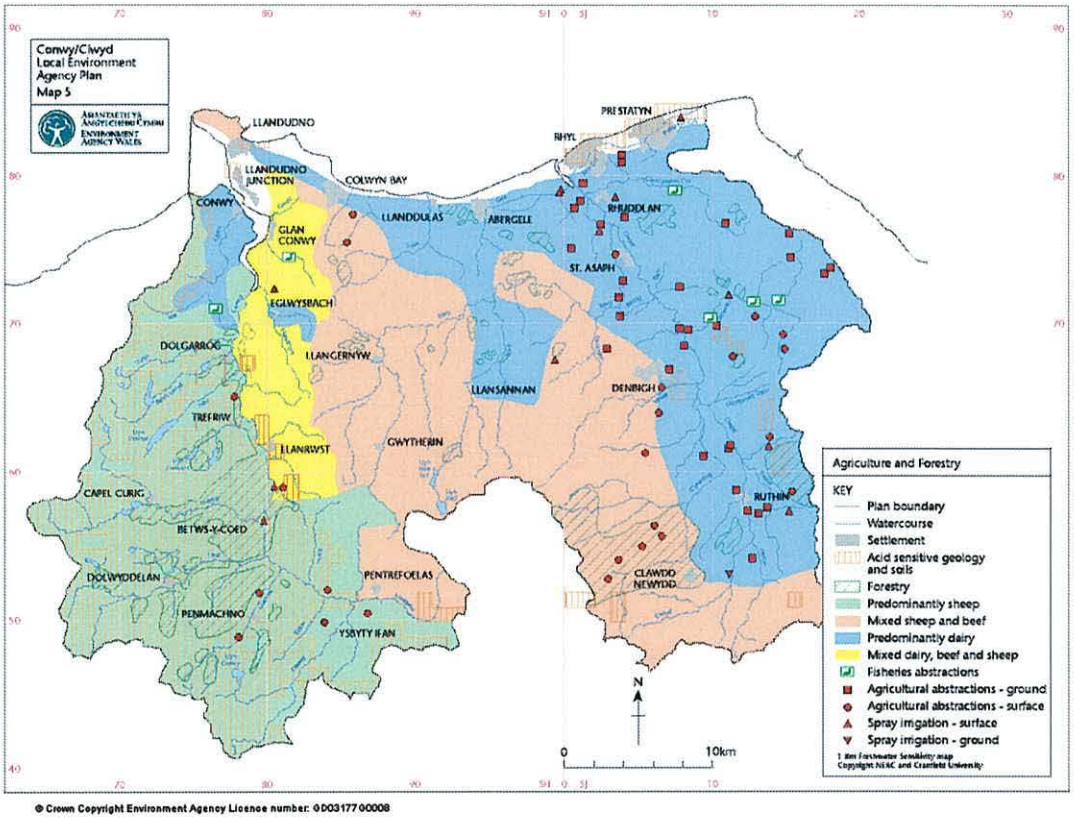



**Figure 3.19.** Conwy River bank agro-forestry activities. Source: Environmental Agency (2002).

In contrast to $PO_4^{3-}$, nitrates were found in higher concentrations in the river in comparison to the estuary which could be due in part to the agricultural activities

in the upper estuary and river. For example, around Llanrwst there is extensive sheep farming on the south bank of the river, and dairy, beef and sheep farming on the north bank (Figure 3.19). By comparison, the $NH_4^+$-N concentration increases only slightly in the estuary possibly due to inputs from bird faecal material which is urea/uric acid rich and readily mineralized to $NH_4^+$. The release of inorganic nitrogen could also be the result of *in situ* mineralization of dissolved organic N compounds and SPM both as a result of biotic (microbial) and abiotic (UV oxidation) processes. Unfortunately, a detailed investigation into the sources of N and its fate within the river system were beyond the scope of this project.

The chemical and physical characterisation of the sediments has revealed some of the groups that will be most likely to interact with inorganic and organic compounds. Analysis has revealed that the particle size of the sediments varies greatly between location and also sampling time (Figure 3.6), reflecting the dynamic nature of river systems. As expected, all the sediments contained significant quantities of quartz and variable amounts of sulphide and hydrous Al/Fe oxides.

### 3.3.2. Organic matter characterization

In general, the organic characteristics (e.g. total phenols and DOC) displayed an order of magnitude greater concentration in the sediment pore water in comparison to the main water column. The total phenol parameter can be considered as the total pool of monomeric and polymeric phenols in their various degrees of polymerization. Natural sources of polyphenols include plants (e.g.

lignin and tannin) and microorganisms. Lignin is the most important natural source of aromatic compounds in river systems while microorganisms tend to synthesize polyphenols enzymatically and convert these to quinones (Stevenson, 1994). Although, the phenolic nature of the organic compounds within the samples was not explicitly characterised, it was speculated that the phenolic compounds may be unique to the Conwy River and estuary. This is supported by Thoss (1999) who in a North Wales river water survey indicate that the phenolic "fingerprint" of the Conwy River was different trend from other freshwaters sites. Further, Thoss results showed that the production of phenol is higher in summer and lower in winter, and the concentration of polyphenolics was *ca.* one order of magnitude higher than the monophenolics.

The highest concentration of DOC in sediment pore water was found in samples that were considered to have anoxic conditions (TC). Similar findings have been reported by Otsuki and Hanya (1972) and Burdige (2001). In the latter study, the higher DOC contents were linked with periods of bioturbation and low rates of mineralization of the low molecular weight fraction. In the present study, bioturbation was largely confined to the TC sediments where abundant macro fauna was present. Another explanation for the higher DOC concentrations was given by Burdige and Zheng (1998) in which they suggested that elevated levels could be due to higher inputs of organic matter. Again, a detailed assessment of the fluxes of C within the main water column and the sediments is required to decide which of these hypotheses is correct however, this was beyond the scope of this project.

A method for fractionating the DOC using high performance size exclusion chromatography (HPSEC) was developed. The results indicated that careful choice of the critical ionic strength (CIS) is required to minimize charge exclusion effects between the colloids and the column packing material. In agreement with the recommendation of Chin and Gschwend (1991) the CIS for the SEC system was found to be 0.1M with sufficient resolution of standard solution (polystyrene sulfonate and Bio-Rad globular proteins standard)

For validation purposes, the HPSEC method was directly compared with ultracentrifuge MW filtration based upon the work of Chin and Gschwend (1991). Careful attention was made to thoroughly wash the ultracentrifuge filters to remove contaminants and also to normalize ionic strength in the two methods (0.1M NaCl). In summary, the HPSEC and ultra-filtration devices yielded similar results. Further validation was also undertaken with some solutions of humic substances and natural organic matter from the Suwannee River (NOM-SR) and compared with the results of other authors such as Chin and Gschwend (1991), and Chin *et al.* (1994) (Table 3.38). The HPSEC results obtained here for the commercial humic acid (Aldrich) were in accordance with the results obtained by the field flow fractionation technique (Beckett *et al.*, 1987) but were over one order of magnitude higher compared to values obtained by Chin and Gschwend, (1991). The latter workers argued that Beckett overestimated the molecular weight of the commercial HA because they did not clean up the solution and it contained some ash material, which increased the apparent MW. In this work the HA (Aldrich) solutions tested included cleaned and also non-cleaned HA solutions of different concentrations. These samples were run by HPSEC and the

standardize retention volumes (Vr/Vo) were always the same. It is possible that the differences found between the different workers using HPSEC as the analytical method are due to differences in the chromatographic columns as found by Conte and Piccolo (1999). The average molecular weight (MW) of the NOM-SR was in accordance with the MW of the fulvic acid from the Suwannee River tested by different techniques and authors (see Table 3.38). Considering that the specific composition of the humic compounds has only been partially deduced, and currently it is accepted that humic compounds are organic molecules aggregated by different mechanisms, then the values found in the present work are well within the range reported previously. In view of these results, the HPSEC method was considered to be a satisfactory system to estimate the MW of dissolved natural organic matter. It should be stressed that, as suggested previously (Conte and Piccolo, 1999), the size MW values should be regarded as relative to this particular system rather than absolute.

The molecular weight determination showed a slight difference, as mentioned before (Tables 3.22 and 3.23), between water and sediment pore water. The results could indicate that the origin and composition of the sediment could influence the MW of the NOM of pore water. In all cases, the NOM appeared to be of a low molecular weight nature (1000-2000).

The samples that presented bimodal peaks apparently had two distinct MW NOM groups with a size difference of about one order of magnitude. Table 3.24 shows that the samples which presented bimodal peaks were from both the tidal and non-tidal stretches of the river and that the bimodality was not due to salinity induced

changes in conformation (Table 3.1).

**Table 3.38**. Comparison of the average molecular weight (MW) of humic substance standards and NOM-SR determined by various methods. Calibration was undertaken with random coil polystyrene sulfonate sodium salt standards. 'HPSEC' indicates high pressure size exclusion chromatography and 'FFF' indicates field flow fractionation

| Humic substance | Method | MW |
|---|---|---|
| Humic Acid (Aldrich) | HPSEC[1] | 24840 |
| | HPSEC[2] | 4300 |
| | FFF[3] | 14500 |
| Fulvic acid (Suwannee River) | HPSEC[2] | 1700 |
| | FFF[3] | 1910 |
| | HPSEC[4] | 2310 |
| | HPSEC[5] | 1340 |
| Suwannee River water | HPSEC[4] | 2190 |
| NOM-SR (IHSS) | HPSEC[1] | 1280 |
| Humic acid (Norway) | HPSEC[1] | 8280 |
| Fulvic acid (Norway) | HPSEC[1] | 4292 |

[1] This work;
[2] Chin and Gschwend, 1991;
[3] Beckett *et al.*, 1987;
[4] Chin *et al.*, 1994;
[5] Leenher *et al.*, 1989.

The UV-vis spectra of the DOM were largely featureless similarly to humic substances previously described by different authors (Choudhry, 1981). In river and sediment pore water the readings of absorbance at 665 nm ($E_6$), were unreliable making $E_4/E_6$ calculations variable. Similar problems were reported by Chin *et al.* (1994) in pore waters of a freshwater wetland. The first studies to employ this humification index ($E_4/E_6$; Kononova, 1966) were carried out on

solutions obtained from highly humified soil samples in which the 665 nm absorbance readings were more intense.

The correlations found between the different organic characteristics of the water and sediment pore water samples showed positive linear correlations between MW and total phenol content, the $E_4/E_6$ ratio, and the DOC content. All these data seem to be logical, since the humification level of a sample is directly related to its polyphenol content, and the molecular size of the organic compounds. The observed correlation between MW and the humification index ($E_4/E_6$) in this work was positive. In contrast Banerjee (1979), found an opposite relationship with humic acids extracted from Indian soils. These differences may due to the composition, nature, origin and chemical process occurring in aquatic environments compared to those in soils. In support of this (see Table 3.14), the content of DOC for the soil sample was greater than the average of the rest of the samples. Another option could be that Banerjee worked with highly humified samples, and intrinsically the NOM in water and pore water would have lower degree of humification. Burdige (2001) presented a model for pore water NOM in which the high molecular weight organic compounds are further hydrolyzed and fermented to monomeric low molecular weight DOM compounds and it is possible that these processes occurred in the Conwy estuary and this explains the positive correlation between MW and $E_4/E_6$.

The MW of DOM present in the pore water samples presented good correlations with the humification index and DOC concentration but not with total phenol content. This could possibly arise by two routes: firstly, the analytical method

used to determine total phenol (Ciocalteou's method) (Swain and Hillis, 1959) may suffer from interference effects in sediment pore water samples. In particular, during the assay pore water samples turned cloudy due to the high carbonate content, and these solutions had to be centrifuged in order to obtain a clear supernatant and absorbance reading. This may have introduced some artefacts. Secondly, the sediment samples sometimes had greater or lesser terrestrial origin, and thus a different level of humification. On this basis, the content and forms of phenolic compounds could have been highly variable. This can be seen in Table 3.13, where it can be seen that the total phenol concentration of the sediment with the highest terrestrial influence was much greater than for the aquatic samples. More sampling times and points would be required to confirm this as the limited number of samples studied here are insufficient to draw conclusions.

In both water and pore water samples the MW and the molar absorptivity presented a negative linear correlation. This contrasts with Kononova (1966) and Chin *et al.* (1994) who have reported the opposite (positive) correlation between these parameters. By comparison, Ladd (1969) and Swift *et al.* (1970) reported that optical extinction coefficients increased with decreasing molecular weight and Swift proposed that the degree of aromaticity increased as the molecular weight decreases and that the low molecular weight components represent the end products of the humification process. Swift *et al.* (1970) worked with a wider size range of organic compounds (1360 to 62,000 MW) and he reported an exponential decrease between the extinction coefficient at 400 nm and MW. In this thesis, the MW of the NOM was lower (< 1100 MW), and a linear correlation is observed.

This result could imply that a low degree of condensation of aromatic moieties (AU @ 280 nm / DOC mg $l^{-1}$) can be represented by molecules of high molecular weight that are highly branched (see Figure 3.2). Conversely molecules with a greater proportion of aromatic ring condensation tend to be smaller molecules with no branches. If this is true, then this correlation could help to provide a better idea of the structure of the DOM in these samples. The total phenol content of the samples also presented positive linear correlations with the $E_4/E_6$ humification index and the AU @ 280nm, indicating that if a water or pore water sample has a high indicator of humification the NOM will present a high content of total phenol (monomers or polymers), which is corroborated by the AU @ 280 nm, at which $\pi$-$\pi^*$ electron transitions of phenolic substances occur (Chin *et al.*, 1994). The content of DOC in water and pore water correlated adequately well with the content of total phenol. This indicated that higher contents of DOC could be due to high concentration of phenolic substances. The carbon units in the NOM will increase as the plant origin polyphenolic compounds suffer biodegradation and transform to branched compounds. This also agrees with the findings of Thoss (1999) about the relative concentration of poly and mono-phenols in the Conwy.

A negative exponential correlation was found between the molar absorptivity at 280 nm and the DOC as mol OC $l^{-1}$ in water and pore water samples suggesting that the degree of condensation of aromatic moieties in water and pore water decreases exponentially with the content of DOC. Considering the correlation between MW and molar absorptivity (Figure 3.12.b) this suggests that a lower degree of condensation (more branches) correlates with a higher content of DOC.

Loss on ignition (LOI) provides an indication of total organic matter, while the elemental analysis provides the proportion of carbon (organic and inorganic) in the samples. In general, the sediment from the Conwy River and estuary were not highly organic (1.4 to 3.2 % LOI). With some exceptions ($LL^b$; 2.3 % C) the total C values were also correspondingly low (<1.6 %; Table 3.28). It was suspected that differences between sites were due to the origin of the sediment, the C: N ratio helped to corroborate this. The C:N ratio could indicate the source of the sediments, as we can see the value for the $LLSO^b$ (soil) sample is high compared with the rest, possibly indicating a terrestrial origin (i.e. nitrogen-poor material). Burdige, (2001) reported a similar site in his work, with these results differing from the two other sites from the Chesapeake Bay.

### 3.3.3. Natural organic matter-metal complex fractionation in aqueous samples

The HPSEC method used to investigate the interaction of metal ions with natural organic matter presented many difficulties. This discussion will be limited to the difficulties found in the final analytical method and the final results, but not the preliminary results. One major logistical problem was the recovery of only small volumes of sediment pore water necessitating the use of large quantities of sediment. In addition, some fresh water samples had very low metal concentrations, which after HPSEC fractionation meant that the concentrations were close to or below the LOD of the ICP-OES. Another problem was the nature of NOM varied significantly between both sampling sites and times. As a result,

inter laboratory comparisons with earlier published work could be complicated. This problem was recognised by Cabaniss and Shuman (1988) and they advocated the use of a standard reference material to circumvent this problem.

The critical ionic strength (CIS) of the mobile phase was an important factor affecting the structure of NOM in the HPSEC method (Chin and Gschwend, 1991) and therefore any NOM interactions with metal ions (Hering and Morel, 1989; Lores and Pennock, 1998). Thus changing the CIS of the mobile phase had a strong influence on the appearance and interpretation of the chromatograms. It was decided to use water as the mobile phase as advocated by Gardner *et al.* (1982) and used by Rottmann and Heumann (1994). Gardner *et al.* (1982) also recognised that the apparent MW of ionic dissolved components appeared to increase due to the formation of a hydration layer and/or other hydrogen bonding or ionic repulsion mechanisms due to sample-column interactions in the absence of salts or buffers in the mobile phase. Despite this implication, water based size exclusion chromatography was the best option for this work, because it was considered likely to fractionate dissolved metals.

The main problem experienced with the ICP-OES during this work, was the lack of reproducibility between runs performed on separate days, although precision and reproducibility was good within each run. The use of spiked samples as quality controls (QC) also indicated that the data though precise could on occasion be inaccurate, sometimes by more than 200 %. These errors made the data difficult to correlate between separate analytical sessions.

It was hoped, early on in the work, to couple the HPSEC and the ICP-OES techniques together to establish a continual fractionation of metal in the eluent of HPSEC. This was not possible due to the technical difficulties involved, and besides the ICP-OES software did not allow continual multi-element measurements. Instead the option of measuring mass budgets per fraction was chosen, to indicate percentiles of MW NOM-metal complex fractions in aqueous solutions. The disadvantage of this decision was that some of the fractions were only about 2 ml volume and these samples had to be analysed manually in the ICP because the auto-sampler needed at least 5 ml to analyse five elements per sample. This made the analysis very time consuming. Another disadvantage was that the resolution on the chromatogram was not sufficient to resolve all the peaks of organic metal ion species. In addition, the calibration carried out in HPLC grade water could not be used to calculate absolute MW of the NOM because of the artefacts mentioned above.

With regard to the NOM-SR solution chemistry and characteristics, the concentration of metals in the NOM-SR-1 solution was within values reported by other authors. For instance, van Loon and Duffy (2000) reported that a sample of water containing a dissolved humic material (8 mg $l^{-1}$) from the Canadian Shield had the following concentration of associated ions: $H^+$ (pH=5.88); $Na^+$ = 76 µg $l^{-1}$; $K^+$ = 51 µg $l^{-1}$; $Mg^{2+}$ = 124 µg $l^{-1}$, $Ca^{2+}$ = 569 µg $l^{-1}$.

The loss of both NPOC and metal ions by precipitation after pH neutralisation and after the GF/F filtration can be explained in different ways. For instance, the metal analysis of the solution NOM-SR-5 indicated a lost of *ca.* 92 % Pb and 17

% Zn in the same solution (Table 3.35) with precipitation occurring during the pH neutralisation stage. One explanation is that it is possible that the formation of NOM-based macro-micelles was favoured by the initial ionic strength of the solutions. Changing the pH and the ionic strength may have changed the state of aggregation and the solubility of the humic ligands as suggested by Zhang *et al.* (1996). These macro-micelles could have aggregated to the point that the colloids collapsed and suffered sedimentation along with the bound metals. This is supported by the loss of DOC (*ca.* 35 % in NOM-SR-5 sample) after filtration which would be directly related to the aggregation of humic substances at low pH, high ionic strength and high concentration. However, the most favoured possibility was the change in pH, since the precipitation was observed during the process of neutralisation.

The UV-vis spectrum and fixed readings of the NOM-SR solutions and their counterparts in water samples could indicate some possible metal ion-NOM interaction. The absorbance edge shift observed from samples NOM-SR-1 to 6 (Figures 3.14 and 3.15) could be due to a charge transfer transition process. The influence of metal spiking on the UV-vis spectra seems to increase as the concentration and the complexity of the mixture also increases. It has been reported that the binding of metals to DOM is ligand specific and dependent on the number and type of ligands present in the DOC. It is also dependent on the concentration of all metal ions and the competition between them (Lores and Pennock, 1998). $Ca^{2+}$ $K^{+}$, $Mg^{2+}$ and $Na^{+}$ were classified as type A metals or oxygen seeking by Nieboer and Richardson (1980). They demonstrated that type A metals tend to seek functional groups such a carboxylate, carbonyl and

phosphate. $Pb^{2+}$ and $Zn^{2+}$ were classified as borderline type metals or intermediate, which means that their preference is ambivalent between oxygen and nitrogen/sulphur. However, they stated that $Zn^{2+}$ behaves more like a type A or oxygen seeking metal whilst $Pb^{2+}$ character is closer to type B or nitrogen/sulphur seeking. This could imply in this work that the metals spiked in NOM-SR solutions were competing for specific functional groups in the NOM.

The total recoveries of metals in the experiment showed some of the problems with the system. For instance the low recoveries observed in the "cocktail" spiked samples (NOM-SR-5 and 6 in Table 3.36) could have been because the solutions were too complex and had not reached equilibrium by the time the fractionation was carried out. It is known that the kinetic reactivity of metals in metal complex formation reactions is governed by the rate of water-loss from the metal. Much longer equilibration times would be predicted for slow-reacting metals such as $Cr^{3+}$ and, to a lesser extent, $Fe^{3+}$ and $Ni^{2+}$ (Lavigne *et al.*, 1987). It has also been reported by Hering and Morel (1989) that when $Cu^{2+}$ is added to mixtures of natural and synthetic ligands the equilibrium distribution of metal species may be established only after hours, days, or even months. The presence of $Ca^{2+}$ could also be affecting the initial reaction of a stronger ligand, retarding the complexing process as Hering and Morel (1989) had suggested in the case of $Cu^{2+}$, which initially formed complexes with the weaker ligands in solution. Thus it is thought that the type A metals ($Ca^{2+}$ $K^{+}$, $Mg^{2+}$ and $Na^{+}$) had formed weak complexes with oxygen-containing functional groups (e.g. –COOH) of the NOM ligands and, during fractionation, the column dissociated those complexes and probably bonded the metals in question, eluting them at a later time. Also the low metal

recovery could be linked with ionic repulsion mechanisms, attraction or repulsion of ions by the column, due to sample-column interactions in the absence of salts or buffers in the mobile phase as Gardner *et al.* (1982) had pointed it out. These workers reported a similar phenomenon, where the stationary phase (Sephadex G-10) had a strong interaction with metals in a distilled water matrix. They suggested that weak complexes could be dissociated during competition for these metals. In turn, the improved recoveries of Ca, K, Mg and Zn in some of the lower ionic strength solutions (NOM-SR-1 to 4 in Table 3.37) may be explained by the opposite phenomenon of ionic repulsion mechanisms occurring in the high ionic strength solutions. However, these successful recoveries could also be linked with the equilibrium of the aqueous solutions, since these solutions had less metals added in lower concentrations, it would be expected that these solutions would reach equilibrium sooner than the more complex and concentrated solutions. As a result, they experienced fewer disturbances in the chromatographic system.

The role of pH in the recovery of metals during size fractionation may be connected with the solubility of the metal species. For example, it is known that Zn has amphoteric solubility behaviour in water, which means that its solubility increases above and below the minimum pH value. The same behaviour could be occurring in the NOM-SR spiked solutions for K, Mg and Zn, but this needs further study.

The obvious contamination of Na during the chromatographic process could indicate that the column was effectively "desalting" from previous samples. The

manufacturers of the column (Bio-Rad) claimed that the $Na^+$ would be eluted within one or two bed volumes, but in this case the elution of Na was constant and continued for more than two bed volumes, thus the source of Na remains un-explained. The experiment with water further confirmed that the earth metals had interacted with the column and that the metals were dissolved in the mobile phase (HPLC grade water) as Gardner *et al.* (1982) had also mentioned.

The Pb contamination in samples NOM-SR-1 and 2 (low ionic strength) and the almost complete loss of Pb from the rest of the samples could have been due to a similar phenomenon to that reported by Rottmann and Heumann (1994). They found that their system had Pb contamination and explained it as the lead interacting with the stationary phase. Possibly the weak complexes of lead with DOM dissociated and the free lead ions were bound to the stationary phase or the lead concentration of the double-distilled water used as eluent was very low. Lead from this water could then be concentrated on the column as the eluent passes. When a natural water sample is injected, free specific binding sites of DOM can act as a complexing agent elute to the lead bound on the stationary phase. To "clean up" the column from unwanted lead a stronger ligand may be necessary. The EDTA solution (20 $\mu g\ l^{-1}$) used either as mobile phase or *via* injection after the fractionation of each sample would have been enough to solubilise lead if it was present in the column, but the results did not show a higher load of lead than either water or sample fractions. An alternative explanation for the lead recovery results could be that the content of lead in the HPSEC fractions was so low that the ICP-OES sensitivity for lead was not sufficient.

The four fractions collected approximately corresponded to the following nominal molecular weights: Fraction 1 (2,000,000 to 10,000,000) including suspended particles such as algae or bacteria; Fraction 2 (1,000 to 2,000,000) the colloidal range including NOM-SR, humic and fulvic acids within this range; Fraction 3 (54 to 1,000) the dissolved species such as aqueous salts or even low molecular weight fulvic acid; Fraction 4 dissolved species that could belong to the NOM-SR solutions or could have washed off the column.

The most important fractions from the environmental point of view are the second and the third because metal bioavailability is generally believed to depend on whether the metals are complexed to NOM of high or low molecular weight or dissolved in solution. Bioavailability is generally believed to increase for low MW NOM.

Ca showed different behaviour in the six samples. The low ionic strength and Zn only spiked solutions had shown that a considerable proportion (*ca.* 37.2 % of the Ca recovered) could be complex to the second MW NOM-SR fraction (1,000 to 2,000,000 MW). In the NOM-SR-4 solution the proportions changed considerably, almost all the Ca recovered was in the humic substances fraction (1,000 to 2,000,000 MW). This behaviour changed again with the highest ionic strength samples (NOM-SR-5 and 6). In these samples only a small part of Ca was found in the second fraction. By comparison, Cabaniss and Shuman (1988) found that the effect of ionic strength on copper speciation in Suwannee FA solution is greater than previously reported for river water. The erratic behaviour

of Ca could indicate that Ca also speciates differently depending on ionic strength and complexity of matrix. Gardner *et al.* (1982) identified three peaks for Ca in natural water samples. He suggested that they belonged to at least three forms of this metal. They explained the last peak as $CaCO_3$ crystals which were common in their natural water samples.

In the case of K and Mg the relative ratios of the fractions were quite similar in all the samples regardless of the matrix ionic strength, metal loading or pH, indicating that K and Mg speciate in similar ways. For both, Mg and K, the third fraction (soluble salts) is slightly greater relative to the second fraction (organic metal ion complexes). These findings correlate with those of Gardner *et al.* (1982) for the speciation of Mg in natural waters where these workers suggested the occurrence of at least three forms of this metal with metal carbonates making up the third fraction.

The speciation of Zn in the NOM-SR samples seemed to indicate that a large percentage of Zn (*ca.* 66 %) is found associated with low MW NOM (< 1,000 MW), these species being the most bioavailable for living organisms suggesting a potential toxicity for those aquatic organisms exposed. A smaller proportion of Zn was found in the fraction where humic substances reside, these Zn complexes being potentially less bioavailable. Approximately 34 % of chelated Zn was colloidal but *ca.* half of these ligands were in the larger colloidal size range. This findings correlate well with those of Wells *et al.* (1998), which working with water samples from the Narragansett Bay found that the majority (~ 90 %) of chelated Zn and Cd, along with their respective unbound ligands, were found in

the operationally-defined soluble < 1 kDa (< 1000 MW) by cross flow filtration. Through the results shown here, it is possible to say that $Zn^{2+}$ tends to correlate with low MW fractions of NOM-SR (< 1000 MW) in solutions of low ionic strength and that increasing IS does not seems to change the fractionation.

The effects on fractionation of MW NOM-Ca, Na and K in high ionic strength solution could be explained by the morphological changes of the humic colloids and their correlations with metals. For instance Lores and Pennock (1998) demonstrated that the binding of Cu to DOM under estuarine conditions probably reduces the uptake or bioavailability for most estuarine organisms, and that Cd, Cr and Zn bioavailability in marine environments will be less affected by DOM. In addition, Hering and Morel (1989) observed a slow equilibration on the addition of Cu to a mixture of humic and synthetic ligands in the presence of Ca at seawater concentrations. Therefore when natural systems undergo perturbations of metal speciation slow equilibration could result in short-term non–equilibrium effects, for example, increased metal toxicity or chemical reactivity (Hering and Morel, 1988).

One aspect that was not studied here was the effect of DOC concentration. Cabaniss and Shuman (1987) found that the binding of copper by Suwannee FA at low DOC had a first order correlation with respect to DOC concentration in the FA fraction between 500 to 2000 MW.

Wells *et al.* (1998) concluded that the organic molecules which complex bioactive metals have different size distributions. The findings here agree with this

statement, under the restrictions that the speciation of metals can be affected by matrix ionic strength, degree of equilibrium and aqueous solution chemistry. The analytical method applied to discern the NOM-metal ion correlation has proved to be much more difficult to achieve without affecting the natural matrix or adding bias to the final result, as has been explained previously. However, we can hint at some propositions. It may be that to obtain a more reliable answer, a different separation technique should be used, and more robust and sensitive analytical method. These could be ultra-filtration for molecular weight fractionation and inductively coupled plasma mass spectrometry (ICP-MS) for the speciation of metals and organic compounds.

# Chapter 4. Environmental behaviour of propetamphos

## 4.1. Introduction

Since the discovery of agrochemical compounds in supposedly clean ecosystems, the factors that control their transport and persistence in the environment have been studied. It has been hypothesised that the interactions between sorption and biodegradation processes, rather than these processes alone actually determine persistence and mobility (Sims *et al.*, 1991). Further, from an understanding of desorption, the bioavailability in both the water and the sediment phase can be predicted (Fytianos *et al.*, 2000). For instance, Lawrence *et al.* (2000) found that adsorption isotherms can predict the sediment bioavailability of 2, 4-dichlorophenol and pentachlorophenol.

This chapter aims to investigate the possible factors controlling the transport and fate of PPT in a river and estuary environment. In particular PPT sorption, desorption and biodegradation and the interactions between these processes will be discussed.

### 4.1.1. Sorption and desorption of propetamphos

Pesticide sorption and desorption data are imperative in the prediction of xenobiotic attenuation and bioavailability in ecosystems (Gao *et al.*, 1998; Lawrence *et al.*, 2000). The kinetics and mechanisms of sorption and desorption

will largely control the mobility, volatilization and degradation of the pesticide (Farrell and Reinhard, 1994).

Theoretical descriptions of sorption mechanisms have been divided into two categories, namely adsorption and partitioning. Adsorption occurs when the condensation of vapour or solutes is brought about by physical or chemical bonding forces, whilst partitioning takes place when an organic chemical permeates into a network of an organic medium by van der Waals or similar forces (Sims *et al.*, 1991).

There are several types of adsorption phenomena with the most important probably being electrostatic attraction to charged surfaces. This occurs with some types of colloids such as clay minerals which possess a surface charge (usually negative). For instance, iron or aluminium oxides surfaces can be protonated or deprotonated depending on the pH but at most environmental pHs they are deprotonated and so possess a negative surface charge. Humic material can also carry variable charge, deprotonation of carboxyl groups resulting in negative charge with protonation of amino groups generating a positive charge.

The electrical double layer theory can explain the association of ionic species in solution with a colloid which has charged groups. The counter ions form a diffuse layer surrounding the colloid surface, but this is not a clearly defined set of ions; but one which changes depending on the chemistry of the surrounding solution (van Loon and Duffy, 2000). A second, largely irreversible adsorption

phenomena known as specific adsorption, involves the formation of covalent chemical bonds between solute species and surface atoms of a colloid.

Historically the process of sorption and desorption has been represented by mathematical models regardless of the mechanism involved. The most frequent models used are the Langmuir and Freundlich equations. The Langmuir relation assumes a surface with a finite number of sorption sites with each one capable of binding a solute molecule. This model therefore implies monolayer coverage (Fytianos *et al.*, 2000) and, as a result, a maximum sorption capacity can be calculated using this model. One form of the mathematical expression is shown in Equation 1;

$$C_s = \frac{b \times C_{aq} \times C_{sm}}{(1 + b \times C_{aq})} \quad \ldots\ldots\ldots 1$$

Where, $C_s$ is the quantity of solute adsorbed ($\mu g\ g^{-1}$); b is the binding constant (ml $\mu g^{-1}$), which depends on the physical and chemical nature of the solid material; $C_{aq}$ is the equilibrium solution concentration ($\mu g\ ml^{-1}$); and $C_{sm}$ is the maximum sorption capacity ($\mu g\ g^{-1}$). The units used here are the ones used throughout this work. The Langmuir relation gives no indication of the adsorption mechanism. However, it has been extensively used to model the adsorption of ions in soils and sediment (e.g. phosphate; van Loon and Duffy, 2000).

The Freundlich relation (Equation 2) is an empirical equation also used to describe adsorption on environmental colloids (Fytianos *et al.*, 2000). Like the

Langmuir relation, this equation does not imply any particular mechanism of adsorption.

$$Cs = K_f \times C_{aq}^{\ 1/n} \quad \ldots\ldots\ldots 2$$

Here $C_s$ is the quantity of solute adsorbed per mass unit (μg $g^{-1}$); $C_{aq}$ is the equilibrium solution concentration (μg $ml^{-1}$); and $K_f$ (ml $g^{-1}$) and 1/n (no units) are the empirical Freundlich constants. By treating Equation 2 with $Log_{10}$ functions the data can be linearised into equation 3.

$$Log_{10}\, C_s = \mathrm{Log}_{10}K_f + \frac{1}{n} \times Log_{10} C_{aq} \quad \ldots\ldots\ldots 3$$

By comparison with the Langmuir relationship, this model does not have a maximum adsorption limit, instead it assumes that, when the surface is covered, additional adsorbed species can be accommodated but this becomes progressively more difficult as more and more adsorbate accumulates (van Loon and Duffy, 2000).

The sorption of organic compounds by natural solids has been described either by means of a linear isotherm model (e.g. 2,4-dichlorophenol by Fytianos *et al.*, 2000) or using a Freundlich isotherm (e.g. organophosphorus pesticides by Fröbe *et al.*, 1989; Sujatha and Chacko, 1992; phthalates and phenol by Changming *et al.*, 1997; selected pesticides by Gao *et al.*, 1998). The so-called linear isotherm

relationship (Equation 4) indicates a partitioning process of the solute onto the sediment or soil organic matter (Fytianos *et al.*, 2000).

$$C_s = K_L \times C_{aq} \ldots\ldots\ldots\ldots 4$$

$K_L$ is the simplified distribution coefficient describing the partitioning of the solute between the solid phase and water (van Loon and Duffy, 2000).

In the environment, the more active species with respect to neutrally charged organics are natural organic macromolecules which are either suspended in the water column or present in sediment. Humic acids, a major component of the organic fraction, have been reported to be a good scavenger of various pollutants (Carter and Suffet, 1982). Organic matter can have polar properties because of its oxygen-containing functionalities, but also contains important hydrocarbon regions where non-polar solutes have little competition from surface-bound water molecules. Therefore, this allows small hydrophobic solutes to "dissolve" within the non-aqueous interior. The term sorption is more appropriate because this process is more similar to absorption than to adsorption, the retentive forces going beyond surface forces (van Loon and Duffy, 2000). Also it is likely that a variety of mechanisms are responsible for the retention of any particular organic solute in a given system (Sims *et al.*, 1991). The term sorption will be used throughout this work, being a general term describing retention with no distinction between the specific processes of adsorption or partitioning.

It has been reported (Brunk *et al.*, 1997) that estuaries operate as sinks for hydrophobic pollutants, and that sorption is responsible for the observed pollutant trapping. The physicochemical factors that regulate solute sorption in these aquatic systems include sediment texture, mineral surface condition, chemical nature as well as properties and composition of the aquatic phase. The dramatic changes in sorption behaviour observed in estuaries have been hypothesized to be due to increases in salinity (Brunk *et al.*, 1997). However, the magnitude and influence of salinity has been observed to be a complicated function of pH, divalent ion concentration, and is influenced by the specific organic macromolecule being studied (Carter and Suffet, 1982; Schlautman and Morgan, 1993).

Desorption processes are also of fundamental importance in quantifying the transport of organic compounds and for calculating ecosystem mass balances. Irreversibility of the adsorption process plays a significant role in determining the mobility of any pesticide in the aquatic environment. For instance, the solid phase can either provide a permanent sink or a temporary reservoir releasing the chemical back into solution in response to a decrease in solution concentration (Gao *et al.*, 1998). It has been observed that the sorption and desorption isotherms of organic contaminants often show hysteresis. This term is used for a system where the desorption isotherm is not co-incident with the sorption isotherm (Lawrence *et al.*, 2000).

The desorption kinetics of organic chemicals from soils and sediments has often been observed to occur in two stages: a rapid stage followed by a stage of much

slower release (Farrell and Reinhard, 1994; Cornelissen *et al.*, 1998; Schmitt *et al.*, 1999; Kan *et al.*, 2000). Slow diffusion in sediment aggregates such as organic matter and hydrophobic walls of micropores are thought to explain "slow desorption" (Cornelissen *et al.*, 1998). For example, Farrel and Reinhard (1994) found that desorption behaviour of halogenated organics cannot be explained using a pore diffusion model and that the physical properties of the solids were not useful for making any predictions. Also, it has been thought that "voids" in organic matter can entrap organic chemicals and therefore desorption is retarded. Cornelissen *et al.* (1998) suggested that the limitation in the desorption of a trapped organic compound would not be the release from the void itself but rather the diffusion from the void to the exterior of organic matter or the sediment particle. These workers (1998) also indicated that desorption processes occurring in organic matter dominate over the processes in the mineral matrix, at least for sediment with moderate to high organic matter contents.

In the present work, the influence of factors such as salinity, dissolved organic carbon concentration and quality, and the presence of metal will be tested to see if they play a key role in determining PPT sorption and desorption process. The data will be modelled using theoretical isotherm models and the chemistry of the ecosystem will also be discussed.

### 4.1.2. Biodegradation

Biodegradation is the process whereby microbes convert an organic substrate into inorganic products directly or through their extra cellular enzymes (i.e.

mineralization). The microbial community involved therefore can make use of some of the carbon in the substrate to convert it to cell constituents, producing energy in the process (Alexander, 1981; Matsumura, 1982). By comparison, co-metabolism is a microbial process by which pesticides are degraded with the main difference being that microbial growth does not occur (Alexander, 1981). As such these microbial populations chemically transform the pollutant but obtain no energy benefit from the process (Reineke, 1984). Alexander (1981) proposed a hypothetical model for population changes and metabolism of a chemical modified by mineralizing and co-metabolizing populations (Figure 4.1). Figure 4.1.a represents a mineralizing population and it shows the increment of the microbial population and the disappearance of the compound that has been used as a carbon and energy source for growth. Figure 4.1.b corresponds to a co-metabolizing population, in which the loss of the chemical is very slow. The chemical is co-metabolized by bacteria which use some other compound in the natural ecosystem as a source of carbon and energy, thus no increase in bacterial population size is observed.

For several pesticides in the environment, microbial degradation is the only mechanism for structural change (Sethunathan *et al.*, 1982). Indeed, current evidence suggests that indigenous microbial populations are the main agents of molecular metabolization in water and soils (Alexander, 1981). Importantly, the degradation of pesticides *in situ* is usually believed to be achieved by a consortium of microbes rather than a single species (Aislabie and Lloyd-Jones, 1995).

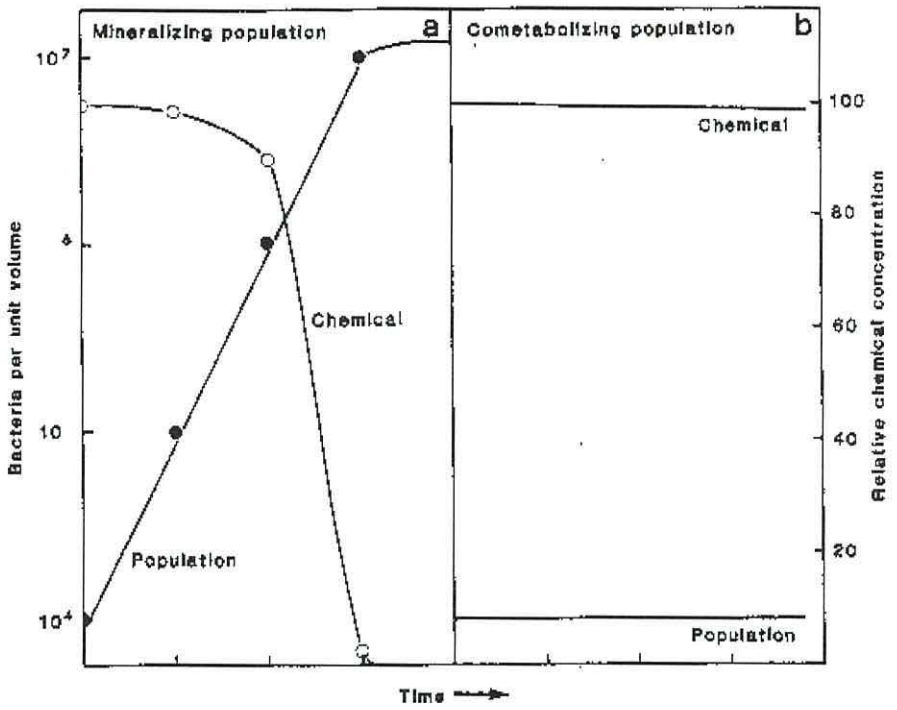



**Figure 4.1**. Hypothetical model for population changes and metabolism of a chemical modified by (a) mineralizing and (b) co-metabolizing populations. (Alexander, 1981).

The adaptability of microorganisms to new environmental conditions through mutation and induction towards chemicals that are, in principle, toxic to them is believed to be very important in removing pollutants from the environment (Matsumura, 1982; Sethunathan *et al.*, 1982). It is for these reasons that the microbial metabolism of pesticides has been studied intensively during the past few years. One favoured method is to grow pure microbial cultures with pesticides as the sole carbon, N, P, or energy source, but as Levanon (1993) noted, the conditions in the field are far from those existing in pure culture conditions. Pure culture experiments, however, have helped to understand and elucidate many

transformation processes. For instance, it is now known that the ability to transform or mineralize pesticides is much more widespread amongst bacteria than among fungi (Levanon, 1993).

In soil or sediment, organic matter content, redox status, temperature, sorption-desorption, and mineral constituents are known to greatly influence microbial activities and therefore the persistence of pesticides in the environment (Sethunathan *et al.*, 1982). Microbial populations are also influenced by the availability of essential nutrients such as carbon, nitrogen, phosphorus and oxygen, and the presence or lack of any of these nutrients has also been found to limit the biodegradation rate (Aislabie and Lloyd-Jones, 1995).

The most important quantitative parameter obtained from abiotic and biotic degradation studies is the half life ($DT_{50}$ or t½). The half life is defined as the time for half of the pesticide to be converted into something else, (National Survey Center, 2002). The calculation of half-life and first-order rate coefficients for pesticide degradation is based on the assumption that the relationship between $Log_{10}$ concentration and time is linear. This equation takes the form of

$$Log_{10} (C/C_o) = -kt + b \quad \ldots\ldots\ldots\ldots 5$$

where $C/C_o$ represents the fraction of chemical remaining at time t, k is the first-order rate coefficient, and b is the intercept, see Figure 4.2 (Cotham and Bidleman, 1989; Green *et al.*, 1993).

It is also well known that microbial communities often require time to acclimatise to new and possibly adverse environmental conditions. In degradation studies this is known as the lag phase a time where no significant decomposition is observed. However, during acclimatisation the pesticide-degrading microbial population may increase to such a level that enhanced degradation rates may subsequently occur (Aislabie and Lloyd-Jones, 1995). For example Freed *et al.* (1979) observed a lag period in the early stages of parathion and phosmet biodegradation (Figure 4.3).

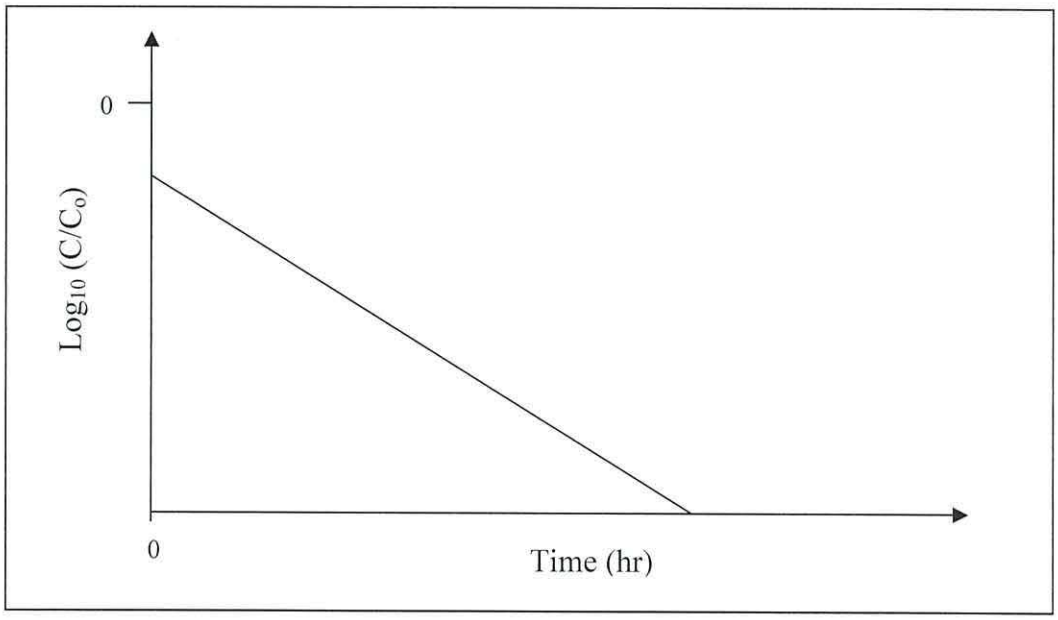



**Figure 4.2.** First order rate degradation plot $Log_{10}$ ($C/C_o$) versus Time (hr).

Organophosphorus pesticides, the subject of these studies, replaced the chlorinated pesticides in the 1970s because the later were found to be very persistent in the environment while organophosphate pesticides were considered to be more readily degradable (Aislabie and Lloyd-Jones, 1995). Nevertheless, it has been shown that organophosphorus pesticides may persist in the environment.

For example, the half life of the pesticide parathion in an estuarine water (pH = 7.8, room temperature) has been reported to be 200 days (Weber, 1976).

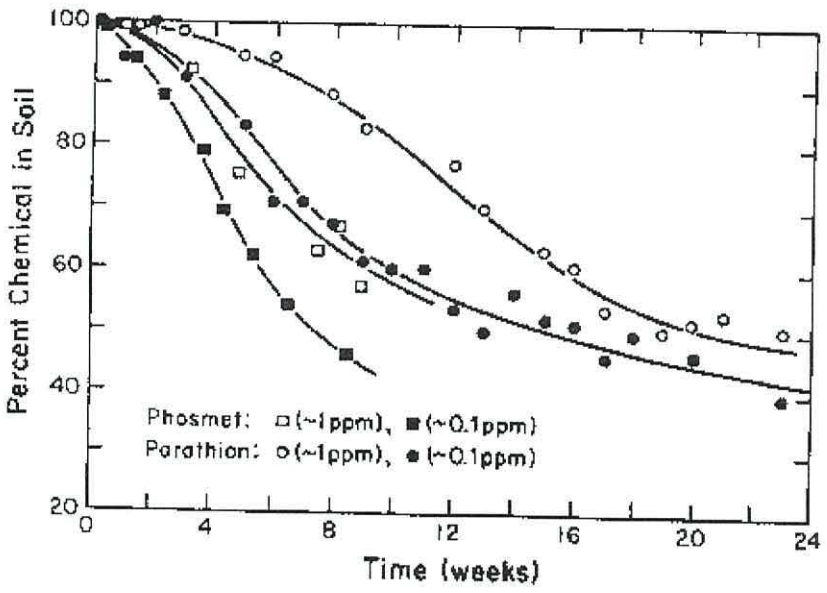



**Figure 4.3** Typical organophosphate degradation in moist soil (Willamette clay loam ~50% field capacity at 20 °C, pH 6.2), showing a lag period for parathion and phosmet (Freed *et al.*, 1979).

Organophosphorus pesticides are known to be degraded through hydrolysis of the ester groups which is described as a phase I (primary) metabolism (Sethunathan *et al.*, 1982; Wyman and Ballard, 1982). This can occur either by enzymatic processes or abiotic chemical degradation (Adhya *et al.*, 1981). The hydrolysis products are either non-toxic or can be detoxified by conjugation in animals (Wyman and Ballard, 1982). Conjugation is also a form of phase II metabolism (secondary); conjugated metabolites are derivatives of the pesticide that have reacted with a natural component of the organism (i.e. glucose in insects) to form a new material (Wyman and Ballard, 1982).

In addition, the degradation of organophosphorus pesticides (methyl parathion, fenitrothion and parathion) can occur by the reduction of the nitro group. This has been reported to be essentially a microbiological process occurring under anaerobic conditions (Adhya *et al.*, 1981). In addition, the biodegradation of parathion is predominant over chemical degradation, whilst for chlorpyrifos it is the contrary (Sharom *et al.*, 1980). This provides indication that generalizations for groups of chemicals should be taken with caution.

Chemical processes in anaerobic ecosystems include the chemical transformation of pesticides and heavy metals usually mediated by the microbial by-products of the anaerobic decomposition of inorganic soil components (Sethunathan *et al.*, 1982). These include the hydrolysis of polymeric substances like proteins or carbohydrates to monomers and the subsequent decomposition to soluble acids, alcohols, and carbon dioxide ($CO_2$). The final steps of anaerobic biodegradation are often performed by denitrifying, sulphate-reducing or methanogenic bacteria (ERASM, 1999). Nitrate-reducing microorganisms as well as many other decomposing bacteria are mostly facultatively anaerobic; i.e. they are able to grow and to degrade organic substances under aerobic as well as anaerobic conditions (ERASM, 1999).

Anaerobic ecosystems, such as lakes, estuarine sediments, flooded soils, sub-surface soil layers are widespread and their contribution to pesticide degradation should also be considered. For instance, depending on the level of eutrophication, water depth and season, freshwater sediments are usually anaerobic below the surface few mm or cm (ERASM, 1999).

It has been found in some specific cases that some intrinsic characteristics of sediment can significantly affect the cellular metabolism and thus pesticide degradation rates. For instance, Visser (1985) found that humic substances can affect processes such as growth, respiration, photosynthesis and nitrogen fixation. The addition of readily available carbon and energy sources (e.g. glucose, protein or NOM) can lead initially to an overall enhancement of microbial activity which may accelerate organic chemical decomposition, particularly if co-metabolic pathways are operative. On the other hand, biodegradation can occur after sorption because toxicity is apparently reduced (Aislabie and Lloyd-Jones, 1995). It has been reported by the same workers that adverse environmental conditions can slow microbial activity and degradation is inhibited. They also report that not all microbial communities possess the ability to degrade a specific pesticide before the first exposure to a pesticide.

In this chapter the biodegradation of PPT under different conditions and factors has been studied to determine its half life and the critical factors affect its biodegradation in river and estuarine sediments.

## 4.2. Results

### 4.2.1. Sorption and desorption

The sorption and desorption experiments were designed to determine the rate and quantity of PPT sorption by the solid phase (sediment). Sorption and desorption

data were evaluated against Langmuir, Freundlich and linear sorption models. Factors affecting sorption and desorption were also tested (salinity, DOC content, nature of humic substances and the influence of metals). Experiments were also undertaken to assess whether the commercial formulation of PPT behaved like the pure form.

#### 4.2.1.1 Sorption kinetics

The sorption kinetics indicate that less than 50% of the PPT was sorbed by the sediments over a 24 hour period, with most of the propetamphos sorbed onto the solid phase within the first 2 to 4 hours (Figure. 4.4); the system appeared to reach a quasi-equilibrium after only 8 hours. The data showed a very fast sorption in the first few minutes, considering that the initial reading was done immediately after PPT addition with the initial measurements showing that between 5 and 28% of the PPT sorbed. A slower sorption rate was then recorded over the next 8 hours. In Figure 4.5, the same data as Figure 4.4 was plotted except that the y-axis was changed to $\mu g\ g^{-1}$ of PPT sorbed onto the LL sediment. Here it can be seen that the quantity of PPT sorbed increases as the PPT concentration increases. Figure 4.4 describes the percentage of PPT sorbed; here the concentration increased as the percentage of PPT uptake decreased. Error bars are not shown because the raw data was converted to percentages.

In Figure 4.6, a comparison of the sorption behaviour of three sediments indicated that the kinetic rate and behaviour was similar for TC and BS, whilst sorption on

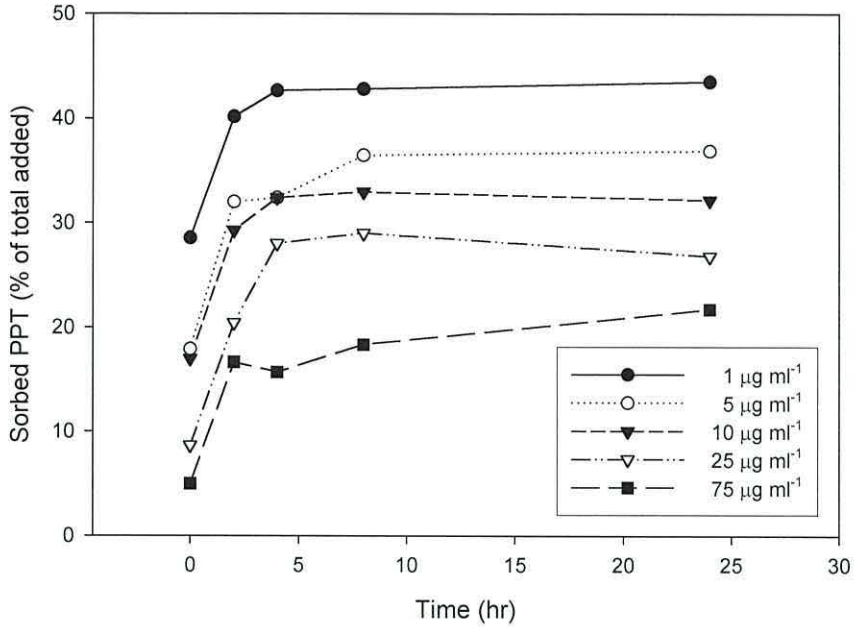



**Figure 4.4.** Sorption kinetics of PPT solution onto the Llanrwst sediment, shown as percentage of the total PPT added. The legend shows the initial solution PPT concentration.

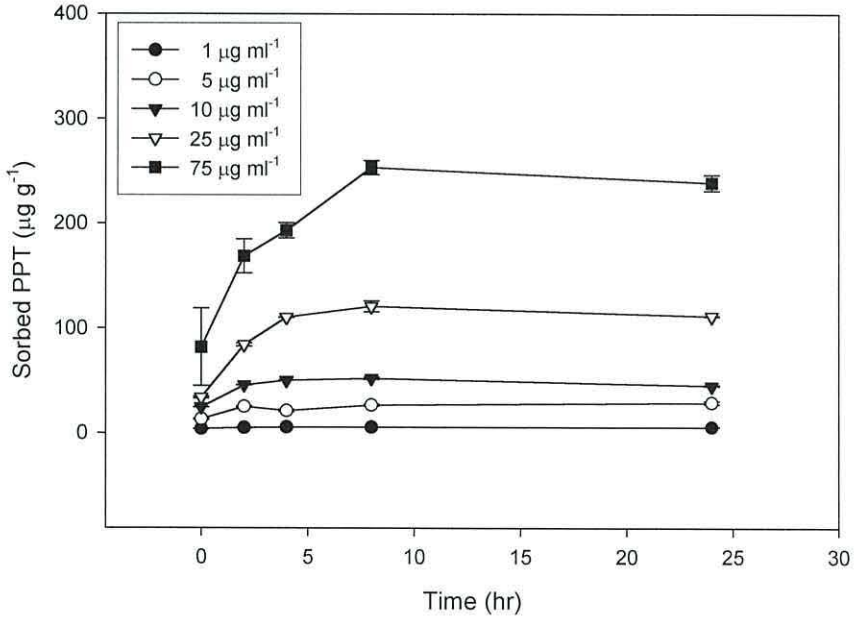



**Figure 4.5.** Sorption kinetics of PPT onto the Llanrwst sediment. Values represent mean ± SEM (n=3). The legend shows the initial solution PPT concentration.

to the LL sediment was significantly faster with *ca.* three times greater sorption of PPT.

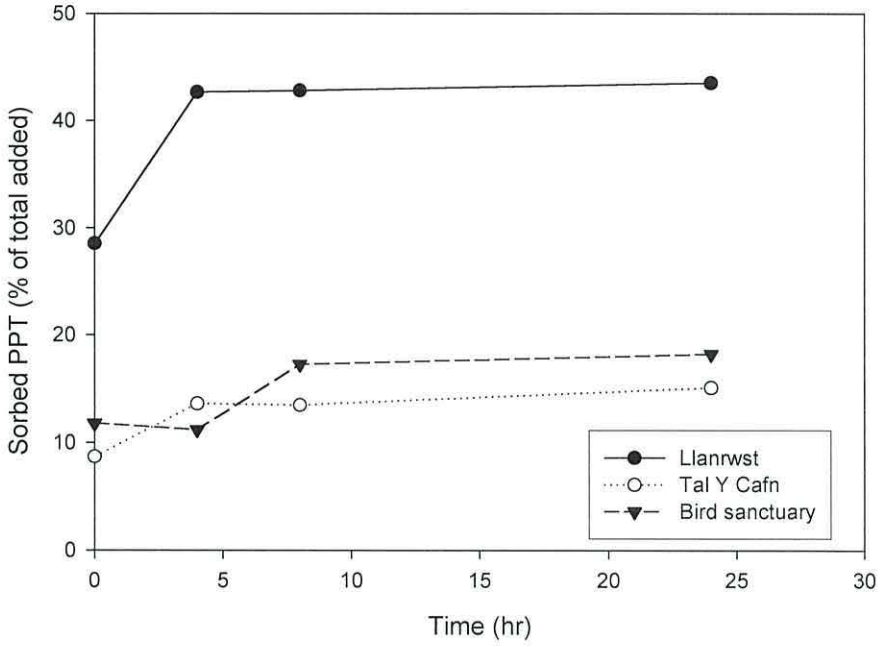



**Figure 4.6**. Sorption kinetics of PPT onto three sediment samples from the Conwy River and estuary. The initial PPT concentration was 1.0 μg $ml^{-1}$.

### 4.2.1.2 Sorption isotherms

The Langmuir and Freundlich equations were fitted to the experimental data using the computer program FITFUNC.BAS. Both models showed a good correlation with the experimental data (Table 4.1). Since the Freundlich model has historically been used to describe the sorption isotherms of organic compounds, the rest of the data is presented here in terms of the Freundlich model.

**Table 4.1**. Correlation coefficient ($r^2$) for the sorption isotherms of PPT in three sediments. The sorption isotherms were determined after a 24 hr equilibration time period.

| Site | Langmuir ($r^2$) | Freundlich ($r^2$) |
|---|---|---|
| Llanrwst | 0.995 | 0.995 |
| Tal y Cafn | 0.999 | 0.999 |
| Bird sanctuary | 0.999 | 0.999 |

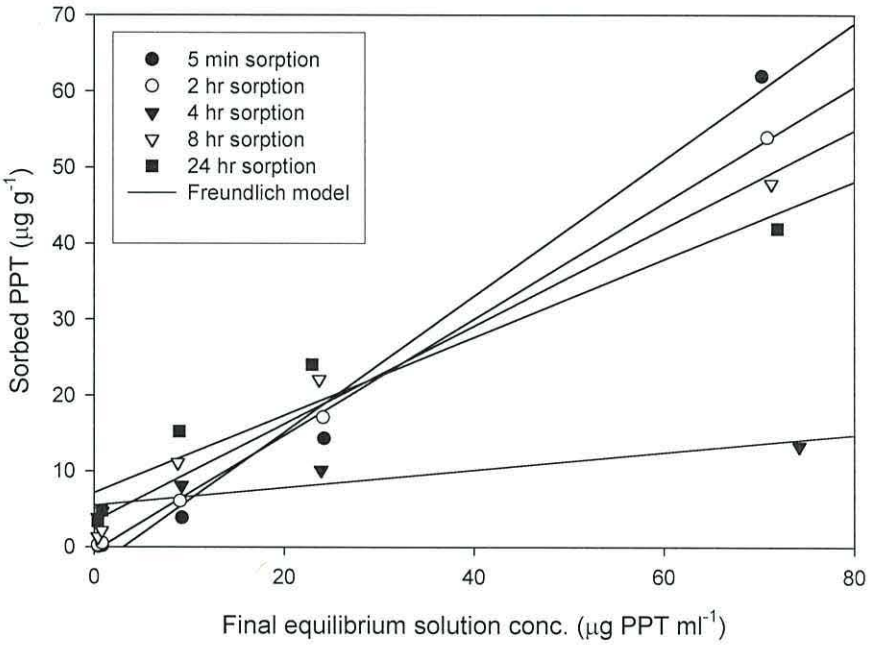



**Figure 4.7**. Freundlich sorption isotherms of PPT onto sediment obtained from the Bird Sanctuary field site. Points represent the averaged experimental data and lines represent regression fits of respective Freundlich isotherm equations.

An example of the typical data obtained with the Freundlich model is presented in Figure 4.7 for PPT sorption on to Bird Sanctuary sediment with the Freundlich coefficient ($K_f$) and the constant (1/n) shown in Table 4.2. In Figure 4.8, the

Freundlich isotherms for the three sediments after a 24 hr equilibration period are shown. Here the sorption capacity of the LL sediment is confirmed as being significantly higher than the TC and BS sediments.

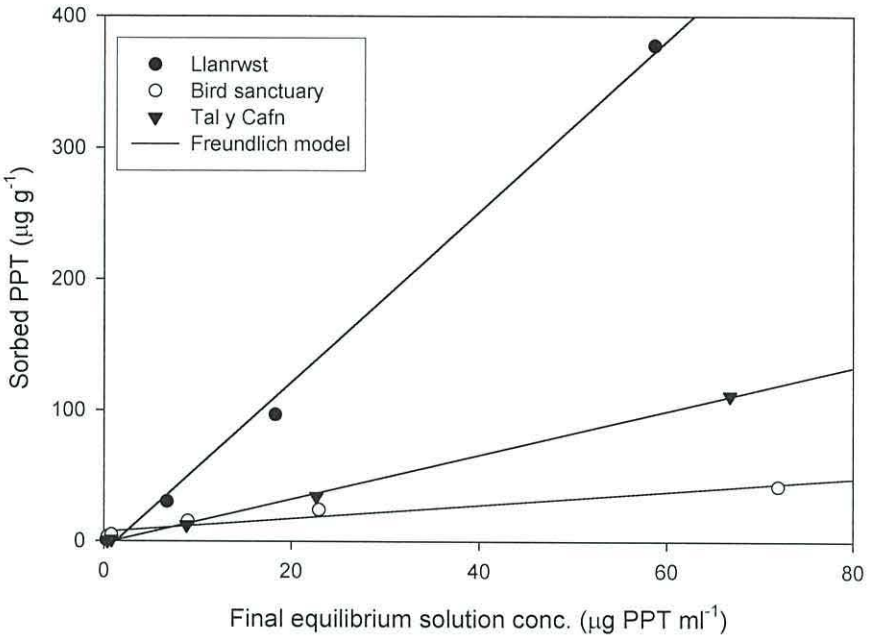



**Figure 4.8** Freundlich sorption isotherms of PPT after 24hr equilibration onto sediment obtained from the field sites Llanrwst, Tal y Cafn and Bird Sanctuary. Points represent the averaged experimental data and lines represent regression fits of respective isotherm equations.

Table 4.3 shows that the carbon content of the LL sediment is *ca.* double than TC and BS sediments indicating that PPT sorption may be dominated by organic matter. In contrast, the LL sediment had approximately the same content of silt and sand as the TC sediment indicating that sorption may not be primarily a mineral phase reaction. However, there was no correlation between $K_f$ and carbon content. To confirm a lack of correlation between $K_f$ and sediment carbon

content, the sorption data was $Log_{10}$ transformed and then the Freundlich model was fitted and the coefficients and constants are presented in Table 4.2. The linearized Freundlich model fits better at lower concentrations. The $Log_{10}$ $K_f$ values differ from those calculated in the original Freundlich model and in addition they correlate well with the carbon content in the sediments ($r^2$ = 0.9999). From this limited data set (three sites only) we hypothesize that the sorption capacity of the sediments is related to their carbon content.

**Table 4.2**. Freundlich sorption isotherms parameters ($K_f$ and 1/n), and correlation coefficients ($r^2$) extracted from the common and linearized models, after an equilibration period of 24 hr between PPT and three sediments from the Conwy River.

| | **Freundlich model** $C_s = K_f C_{aq}^{1/n}$ | | | **Linearized Freundlich model** $Log_{10} C_s = Log_{10} K_f + 1/n\ Log_{10} C_{aq}$ | | |
|---|---|---|---|---|---|---|
| **Sediment** | **$K_f$** | **1/n** | **$r^2$** | **$Log_{10} K_f$** | **1/n** | **$r^2$** |
| LL | 3.213 | 1.170 | 0.995 | 11.47 | 0.833 | 0.999 |
| TC | 1.144 | 1.090 | 0.999 | 2.60 | 0.847 | 0.999 |
| BS | 5.228 | 0.487 | 0.999 | 2.54 | 0.738 | 0.999 |

#### 4.2.1.3 Impact of solution chemistry on PPT sorption

The influence of solution salinity and DOC content on the sorption characteristics of PPT were tested on sediments from TC and BS. From Figure 4.9.b, it is apparent that the increase in salinity had a positive effect on the sorption of PPT after an equilibration time of 24 hr for BS sediment, when compared with distilled

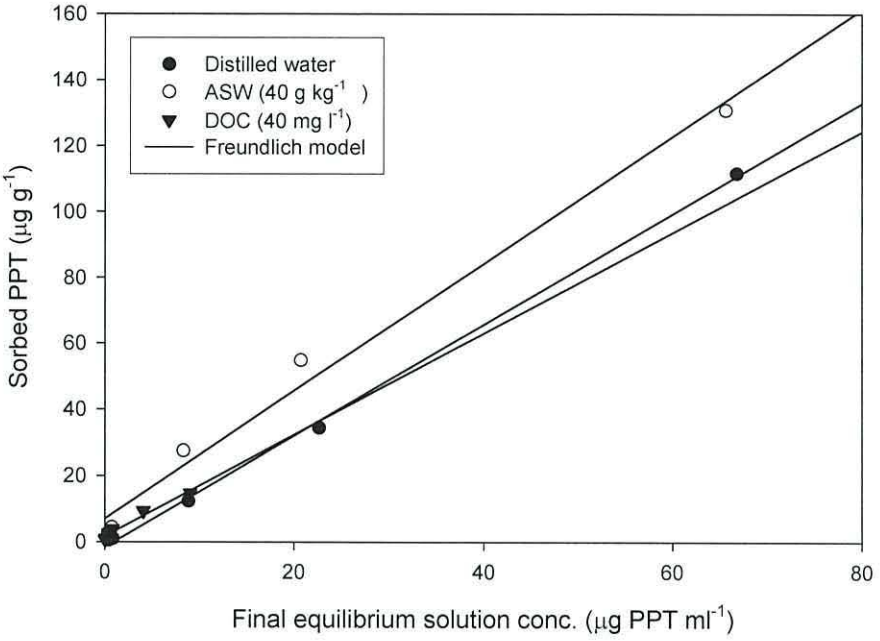
water and DOC matrices. In contrast, this salinity effect was not observed for the sediment from TC (Figure 4.9.a). The isotherms of the experiments performed in the presence of high concentrations of DOC are similar to the results obtained for distilled water, showing no significant effects. Prior to this experiment it was felt that a matrix containing high levels of DOC would have a greater impact on PPT sorption. To confirm this, a new set of experiments was therefore carried out. Here, only the initial uptake (5 min) and the sorption at 12 and 24 hours have been measured and two PPT concentrations were tested. These data have been statistically analysed using a one-way ANOVA test to determine if there is any significant difference between treatments in conjunction with Tukey's pairwise comparison.

**Table 4.3.** Physico-chemical characteristics of the sediments from the Conwy river and Estuary used for the sorption and desorption experiments.

| Site | Water (%) | Size fraction | | | Carbon (%) |
|---|---|---|---|---|---|
| | | Sand (%) | Silt (%) | Clay (%) | |
| Llanrwst | 42.5 | 78.1 | 15.3 | 6.6 | 3.4 |
| Tal y Cafn | 27.2 | 71.5 | 17.7 | 10.8 | 1.2 |
| Bird Sanctuary | 25.5 | 95.7 | 1.3 | 3.0 | 1.2 |

The ANOVA analysis carried out for the sorption experiments using five matrix salinities on TC sediment, gave a value $P < 0.05$ for at least three of the four readings, indicating that at least one of the treatments is significantly different (see Table 4.4). The Tukey's pairwise comparisons show that in the three

**(a)**




**(b)**

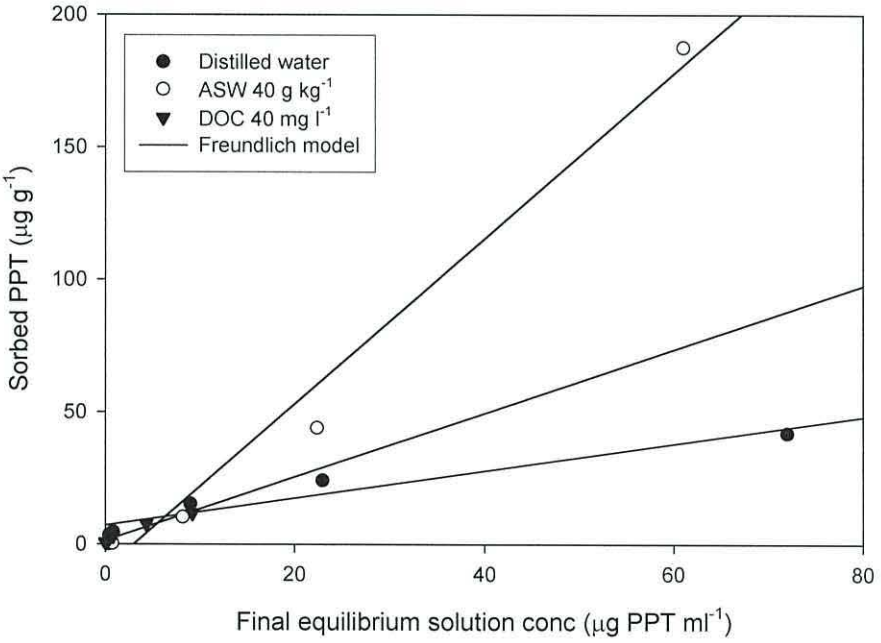



**Figure 4.9** Effect of artificial sea water and a matrix with high DOC concentration on the PPT sorption isotherms after 24 hr using (a) Tal y Cafn sediment and (b) Bird Sanctuary sediment. Points represent the averaged experimental data and the lines represent regression fits of respective isotherm equations.

**Table 4.4.** Results of a statistical comparison between matrix salinity treatments for PPT sorption onto River and Estuary sediments. One way ANOVA (P value) and Tukey's pairwise comparison data are shown when $P < 0.05$ as those treatments that were significantly different.

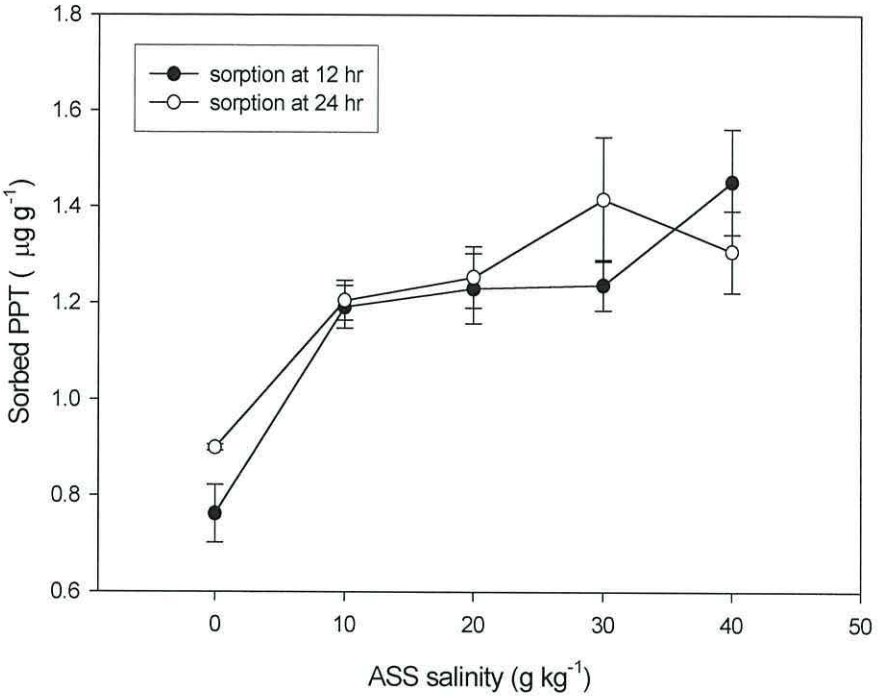
| Treatments | Sediment | PPT ($\mu g\ ml^{-1}$) | Equilibration Time (hr) | Anova (P value) | Tukey |
|---|---|---|---|---|---|
| 0 (Distilled water)<br>10 (10 g $kg^{-1}$ ASS)<br>20 (20 g $kg^{-1}$ ASS)<br>30 (30 g $kg^{-1}$ ASS)<br>40 (40 g $kg^{-1}$ ASS) | Bird Sanctuary (BS) | 0.5 | 12 | 0.158 | None |
| | | | 24 | 0.002 | $0 \neq 20$; $0 \neq 30$; $0 \neq 40$; $10 \neq 20$; $10 \neq 30$; $10 \neq 40$ |
| | | 10 | 12 | 0.045 | $0 \neq 40$ |
| | | | 24 | 0.864 | None |
| | Tal y Cafn (CF) | 0.5 | 12 | 0.016 | $0 \neq 40$ |
| | | | 24 | 0.113 | None |
| | | 10 | 12 | 0.002 | $0 \neq 20$; $0 \neq 30$; $0 \neq 40$ |
| | | | 24 | 0.037 | $0 \neq 40$ |

ASS, artificial sea salts (Sigma)

positive cases, the highest salinity (40 g $kg^{-1}$) is having a significant effect increasing PPT sorption. For PPT sorption (10 μg $ml^{-1}$) after 12 hr of equilibration the 20, 30 and 40 g $kg^{-1}$ salinities were different to the same treatment in distilled water. The sorption of 0.5 μg $ml^{-1}$ of PPT at 24 hr of equilibration showed no difference at all between the five matrices. Graphical representations of these results are shown in Figure 4.10. The results with sediment from BS are less clear (Figures 4.11.a and b). Although $P < 0.05$ values were found in two of the PPT concentrations indicating some differences, the other two treatments had no effect. The experiment with low PPT concentration (0.5 μg $ml^{-1}$) and 24 hr of equilibration showed the greatest differences; the lowest salinity concentrations (0 and 10 g $kg^{-1}$) were significantly different to the higher salinity concentration, favouring sorption at higher salinities.

The effect of varying DOC concentration on PPT sorption was tested in sediment from TC, employing five concentrations of DOC. Figure 4.12 shows that concentrations of more than 10 mg DOC $l^{-1}$ appear to reduce PPT sorption. However, the ANOVA tests carried out with the same data give no indication of significant differences between treatments ($P > 0.05$) (see Table 4.5). This result was not expected, and because the source of DOC was a commercial humic acid (Aldrich), it was thought that the quality of humic material could have had an impact on the sorption results. Therefore a new experiment with three other types of DOC was carried out. These included, two humic materials from Norway (humic and fulvic acid) whose elemental composition is shown in Table 3.31. The last sample was purchased from the International Humic Substance Society (IHSS) and was natural organic matter from the Suwannee River (NOM-SR) in

**(a)**

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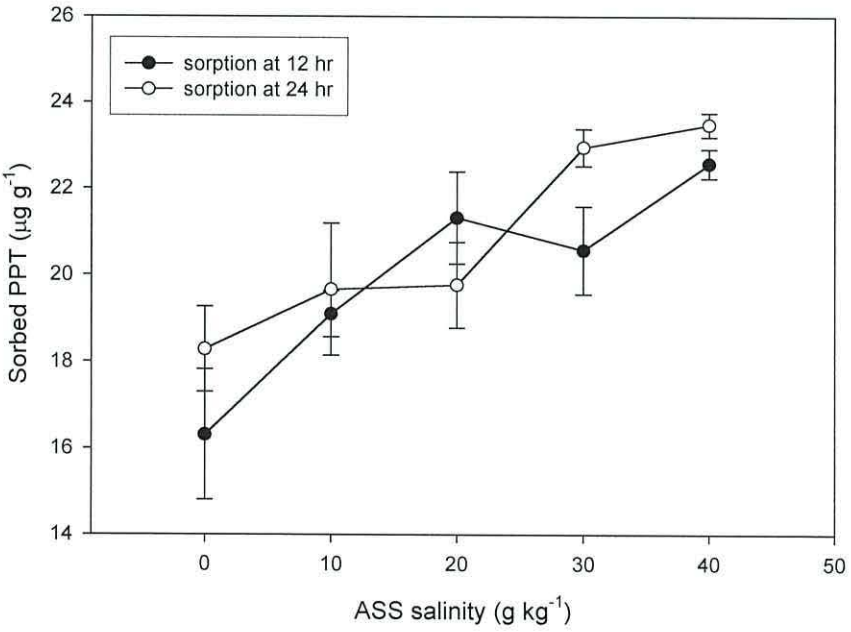
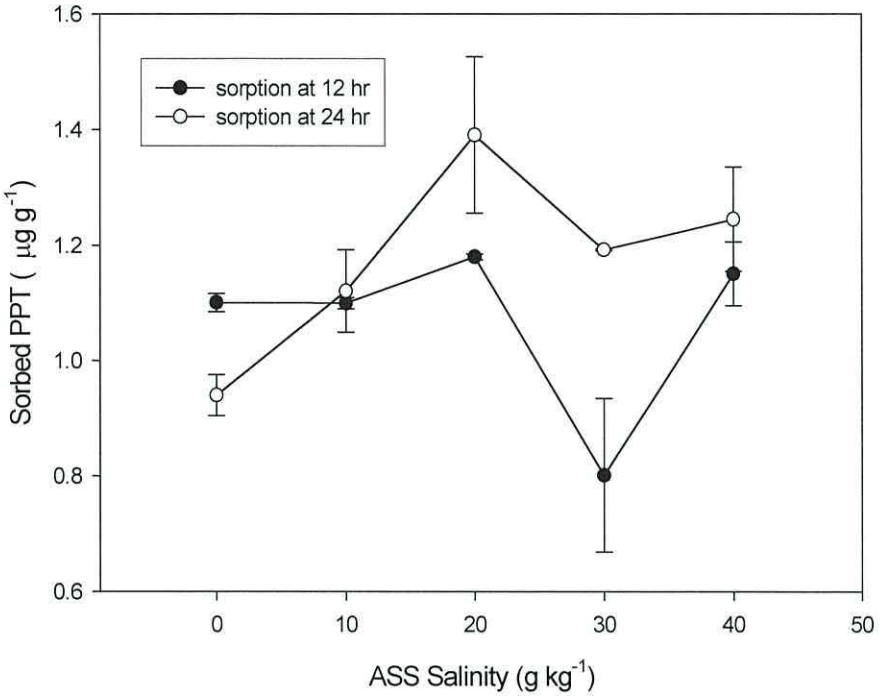



**Figure 4.10.** Effect of varying salinity concentrations artificial sea salts (ASS, g $kg^{-1}$) on PPT sorption onto Tal y Cafn sediment at two initial PPT concentrations. (a) 0.5 µg $ml^{-1}$ PPT (b) 10 µg $ml^{-1}$ PPT. Values represent mean ± standard error of the mean (SEM), n=3.

**(a)**

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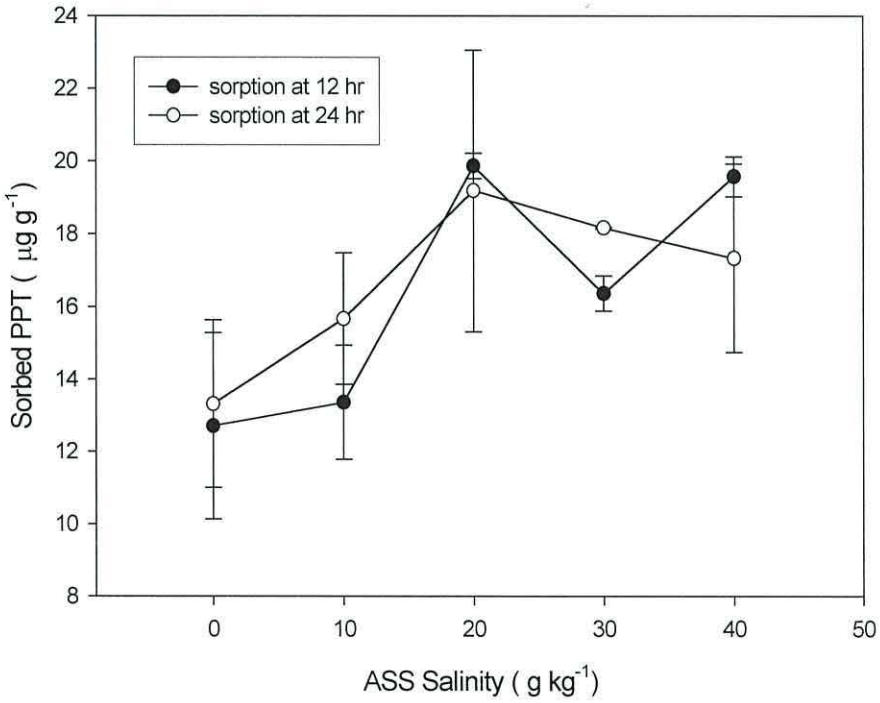



**Figure 4.11.** Effect of varying salinity concentrations artificial sea salts (ASS, g $kg^{-1}$) on PPT sorption onto Bird Sanctuary sediment at two initial PPT concentrations. (a) 0.5 µg $ml^{-1}$ PPT. (b) 10 µg $ml^{-1}$ PPT. Values represent mean ± standard error of the mean (SEM), n=3.

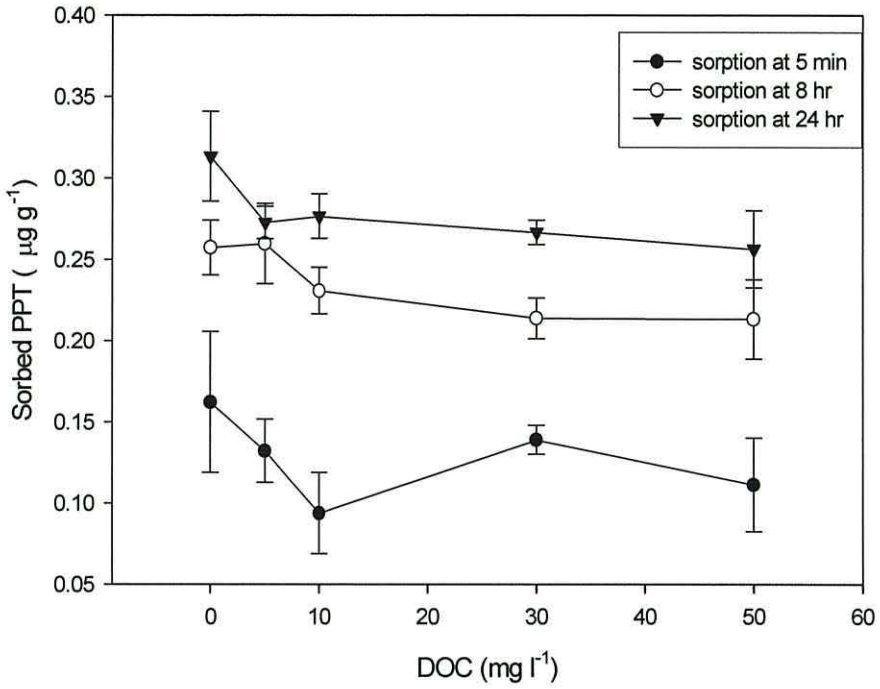



**Figure 4.12**. Effect of DOC concentrations (0, 5, 10, 30 and 40 mg $l^{-1}$, humic acid, Aldrich) on the sorption of PPT (0.1 µg $ml^{-1}$) onto Tal y Cafn sediment. Values represent mean ± standard error of the mean (SEM), n=3.

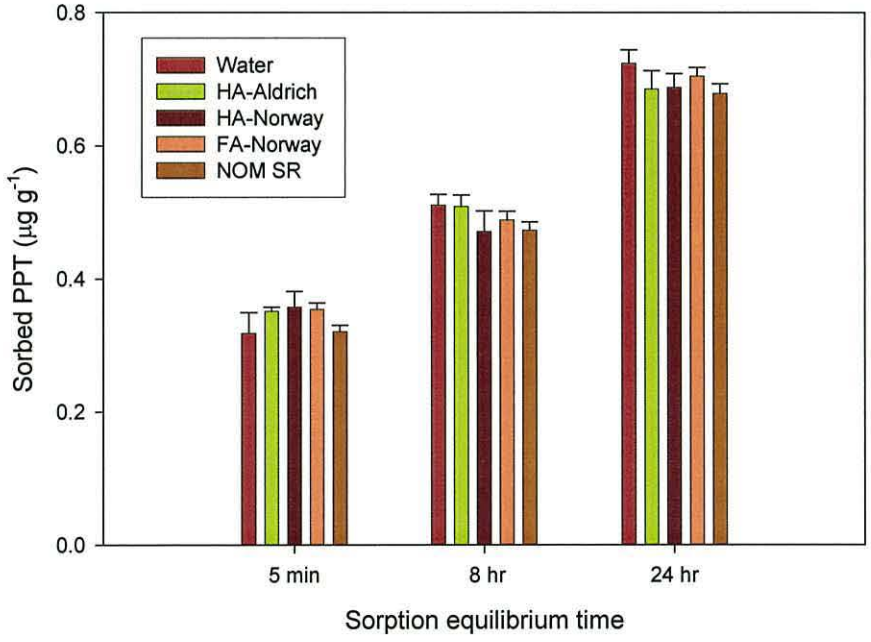



**Figure 4.13.** Effect of varying type and/or source of humic substance (40 mg DOC $l^{-1}$) on the sorption of PPT (0.1 µg $ml^{-1}$) onto Llanrwst sediment at different times of equilibration. Bars represent mean ± standard error of the mean (SEM), n=3.

**Table 4.5.** Results of a statistical comparison between matrix dissolved organic carbon (DOC) concentration and a variety of humic substances for PPT sorption onto River (Llanrwst) and Estuary sediments (Tal y Cafn) at different equilibrium times. One way ANOVA (P value) data are shown.

| | **Treatments** | **Sediment** | **PPT ($\mu g\ ml^{-1}$)** | **Equilibration Time** | **Anova (P)** |
|---|---|---|---|---|---|
| DOC (Humic Acid, Aldrich) | Distilled water<br>5 mg DOC $l^{-1}$<br>10 mg DOC $l^{-1}$<br>30 mg DOC $l^{-1}$<br>50 mg DOC$l^{-1}$ | Tal y Cafn (TC) | 0.1 | 5 min | 0.746 |
| | | | | 24 hr | 0.445 |
| Humic substances (40 mg DOC $l^{-1}$) | Distilled water<br>Humic Acid, Aldrich<br>Humic Acid, Norway<br>Fulvic Acid, Norway<br>Natural Organic matter, Suwannee River (NOM-SR) | Llanrwst (LL) | 0.1 | 5 min | 0.416 |
| | | | | 2 hr | 0.407 |
| | | | | 24 hr | 0.540 |

the USA. The experiment was carried out with sediment from LL, the nominal DOC concentration was 40 mg $l^{-1}$ and the sorption was monitored at five equilibration times. The ANOVA results again confirmed that the DOC content in solution had no significant effect on the sorption rate of PPT regardless of the source or quality of the humic substance, ($P > 0.05$ in the five readings; see Table 4.5). Figure 4.13 shows these results more clearly, the sorption values being almost the same for all the experiments.

A further experiment was carried out to determine if the presence of metals in solution affected the sorption of PPT. The metals tested included Al, Fe, Pb and Zn and a mixture of all of those metals in an equimolar solution (1 mM). The degree of sorption was monitored at four equilibration times ranging from 5 min to 24 hr. The ANOVA test indicated some differences ($P < 0.05$) for the readings taken after 8 hours (see Table 4.6) but the Tukey's pairwise comparisons was inconclusive, since each pair had to present the same positive or negative result to be considered different. Instead there was only an indication of which treatments could be different, which were that lead could be different to that of distilled water and the treatment with Zn (see Figure 4.14). On this basis the Tukey's pairwise comparison is not shown. The statistics for the other treatments indicated no significantly differences from the distilled water treatment.

#### 4.2.1.4 Propetamphos industrial grade (PPT-Ind)

The industrial formulation of the pesticide has been tested using the same general conditions as those used for pure PPT. The formulation indicates that the

**Table 4.6.** Results of statistical comparison between treatments with metals for PPT sorption onto River sediment (Llanrwst). One way ANOVA (P value) and Tukey's pairwise comparison data are shown when $P < 0.05$ as those treatments that could be significantly different.

| Treatments | Sediment | PPT-Ind ($\mu g\ ml^{-1}$) | Equilibration Time (hr) | Anova (P value) | Tukey |
|---|---|---|---|---|---|
| (Distilled water)<br>Al (1 mM)<br>Fe (1 mM)<br>Pb (1 mM)<br>Zn (1 mM)<br>Mixture (1 mM equivalent) | Llanrwst (LL) | 0.5 | 2 | 0.074 | None |
| | | | 4 | 0.222 | None |
| | | | 8 | 0.041 | Pb ≈ DDW; Pb ≈ Zn |
| | | | 24 | 0.705 | None |

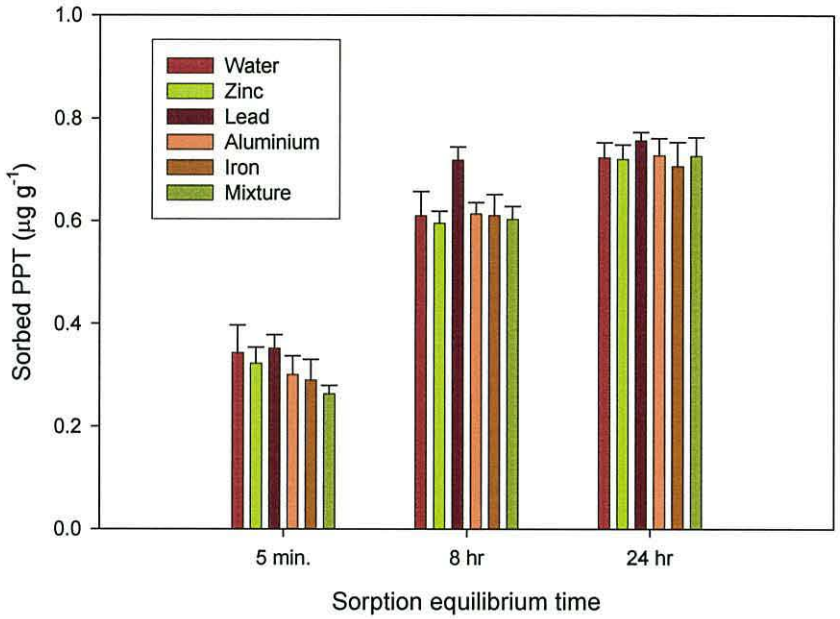



**Figure 4.14.** Effect of equimolar metals ions (1 mM) on the sorption of PPT (0.1 µg $ml^{-1}$) onto the Llanrwst sediment. Bars represent mean ± standard error of the mean (SEM), n=3.

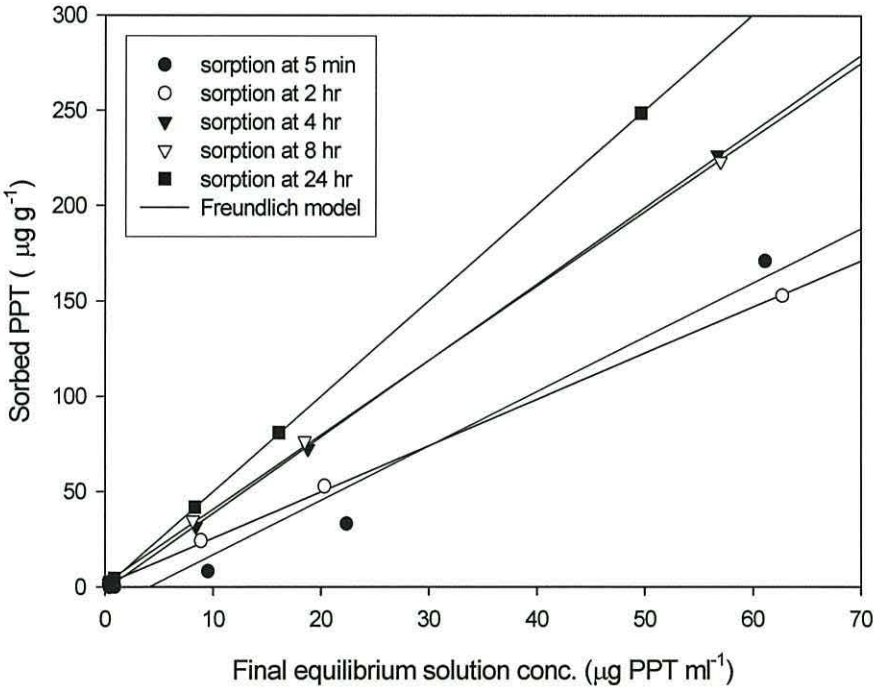



**Figure 4.15.** Freundlich sorption isotherms of PPT-Ind onto sediment obtained from the Bird Sanctuary field site. Points represent the averaged experimental data and lines represent regression fits of the Freundlich isotherm equation.

pesticide is in a concentration of 8% in a solution that includes an aromatic solvent known as Shellsol R ® added to increase PPT solubility. Thus it was possible to test higher PPT concentrations in these sorption experiments. Experiments to test the impact of the aromatic solvent on PPT sorption have not been carried out because Shellsol R was withdrawn from the market at the time of this study (this information was provided by Philippa Connor the global marketing assistant of Shell Chemical UK Ltd).

The sorption data was again been fed into the FITFUNC.BAS computer programme. Again it was found that Freundlich model fitted the data well (Figure 4.15). The sorption kinetics of PPT-Ind present a similar behaviour to their Analar grade counterpart (Figure 4.16) with a fast initial sorption followed by a slower uptake over several hours, reaching quasi-equilibrium after 8 hours.

Figure 4.17 shows the Freundlich isotherms for Analar grade and industrial PPT. The industrial formulation seems to be sorbed to a greater extent onto both sediment (LL and BS). However, the biggest difference is observed in the BS sediment. A further sorption experiment was carried out with PPT-Ind, testing the possible effects of salinity. The results presented in Figure 4.18 and analysis by ANOVA confirmed that changes in salinity significantly affect the sorption of PPT-Ind. The effects varied; a low salinity (10 g $kg^{-1}$) decreased the sorption rate during the first 4 hours. This effect was also detected by the Tukey's pairwise comparison (see Table 4.7). However, at high salinity (40 g $kg^{-1}$), the sorption increased but it seemed that the amount of PPT sorbed was approximately the same at 4 and 8 hours. This could mean that increased salinity increases the

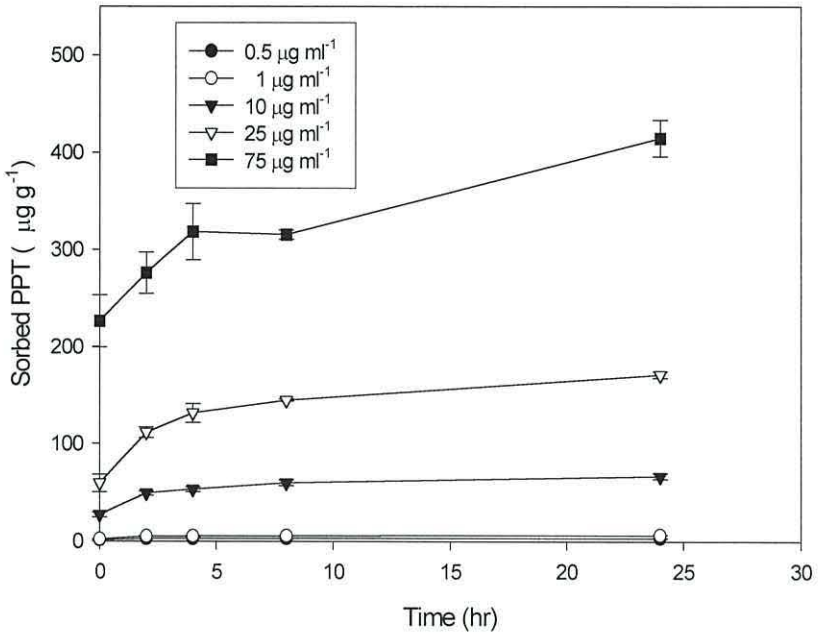



**Figure 4.16** Sorption kinetics of PPT-Ind onto the Llanrwst sediment. Values represent mean ± standard error of the mean (SEM), n=3. The legend shows the initial solution PPT concentration.

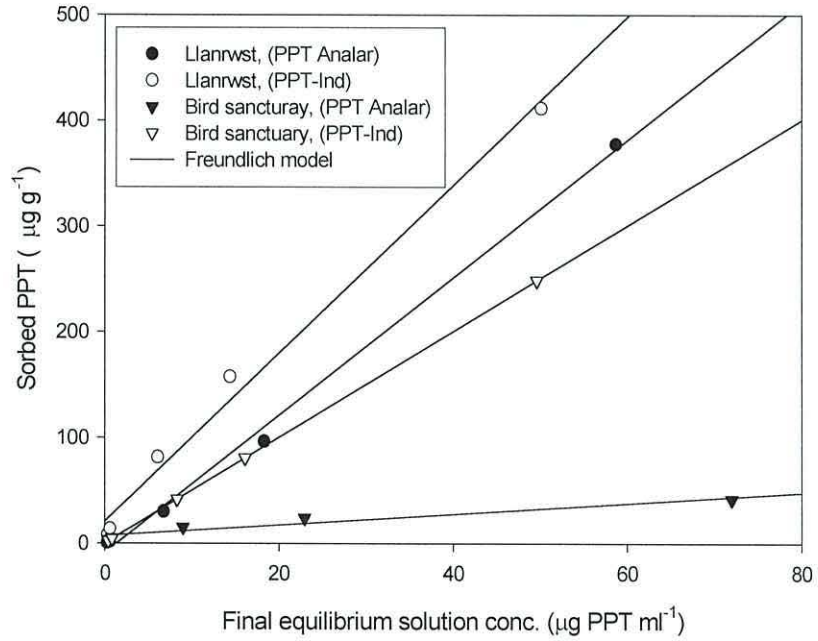



**Figure 4.17**. Comparison of the Freundlich sorption isotherms (24 hr) for Analar and Industrial grade PPT onto sediment obtained from the Llanrwst and Bird sanctuary field sites. Points represent the averaged experimental data and lines represent regression fits of respective isotherm equations.

sorption rate while the system reaches quasi-equilibrium very rapidly. To confirm this, the kinetics was studied (Figure 4.19). The rate of sorption does continue to increase slowly after 8 hours, thus the system had not reached equilibrium. This could mean that in high salinity matrices PPT-Ind could be sorbed more thoroughly, but longer experiments would be necessary to confirm this. However, in natural systems (rivers or estuaries) equilibrium is difficult to reach because of the constant flow of water.

#### 4.2.1.5 Desorption kinetics

A typical example of the desorption kinetics of Pestanal grade PPT are shown in Figure 4.20. It can be seen that a large proportion (*ca.* 38%) of PPT was desorbed within a short period of time (1 hr) and then the rate becomes very slow. After 48 hr the proportion of PPT desorbed was *ca.* 47% in LL sediment. Regardless of the sediment, desorption behaviour was similar (see Figure 4.20) though, the proportion of PPT desorbed in the BS sediment was slightly higher than LL or TC; 53% after one hour and 60 % after 48 hours.

The quantity of PPT desorbed has been measured at different initial concentrations and the data fitted to a linear model. Most of the desorption isotherms fit well to this model and in Figure 4.21 the isotherms for sorption at 48 hr and desorption at 1, 24 and 48 hr are shown for BS sediment. The y-axis represents the PPT concentration sorbed, or for the case of desorption, the concentration of PPT still left on the sediment after desorption. The isotherms after 1, 24 and 48 hours of desorption are close together, showing that the

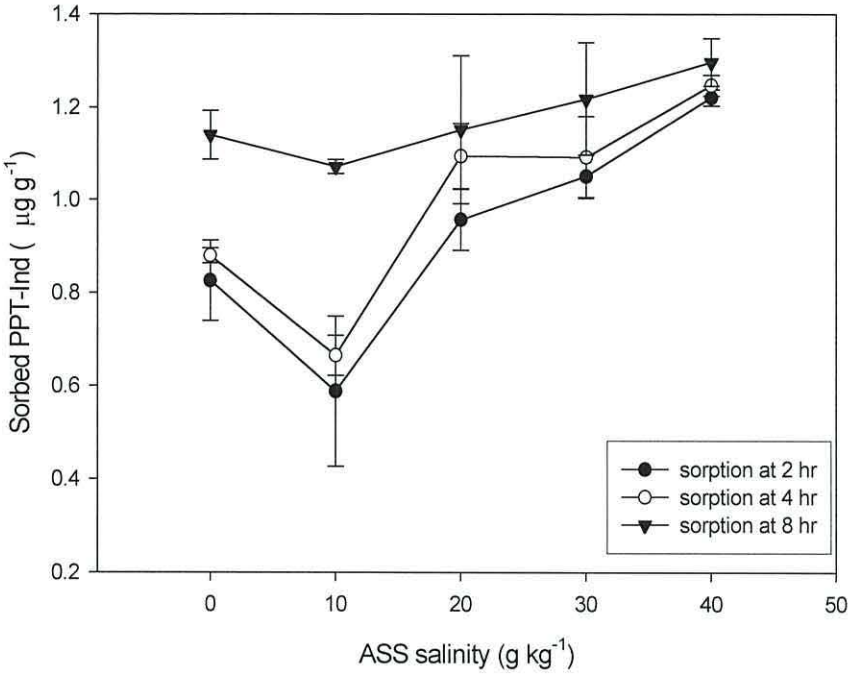



**Figure 4.18.** Effect of salinity concentrations artificial sea salts (ASS, g $kg^{-1}$) on PPT-Ind sorption (0.5 µg $ml^{-1}$) onto sediment obtained from the field site Tal y Cafn at differente equilibration times. Values represent mean ± standard error of the mean (SEM), n=3.

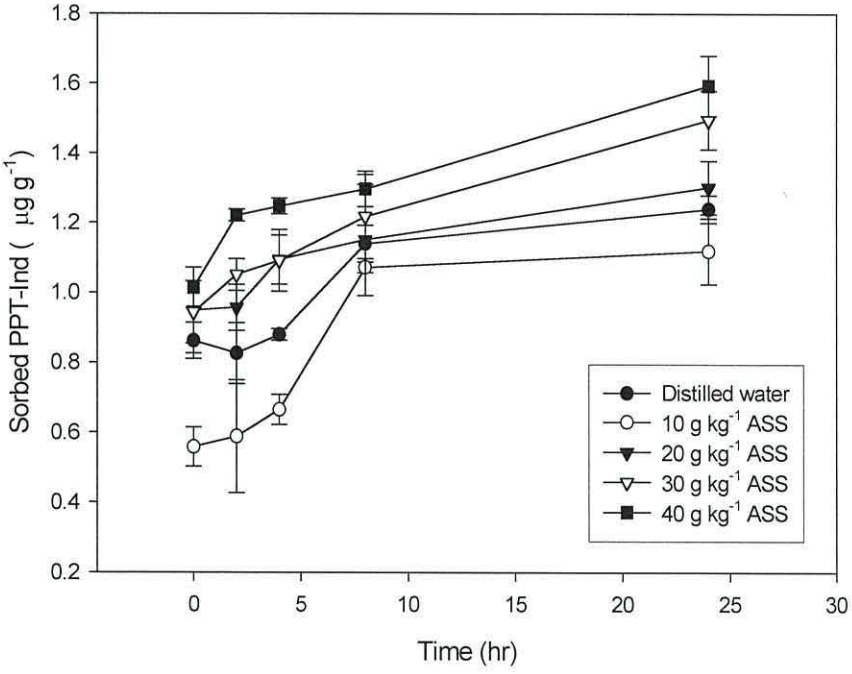



**Figure 4.19.** Sorption kinetics of PPT-Ind (0.5 µg $ml^{-1}$) onto sediment obtained from the field site Tal y Cafn in the presence of a range of salinities (0 to 40 g $kg^{-1}$ ASS). Values represent mean ± standard error of the mean (SEM), n=3.

**Table 4.7.** Results of statistical comparison between salinity treatments for PPT-Ind sorption onto Estuary sediment (Tal y Cafn). One way ANOVA (P value) and Tukey's pairwise comparison data are shown when $P < 0.05$ as those treatments that were significantly different.

| Treatments | Sediment | PPT-Ind ($\mu g\ ml^{-1}$) | Time | Anova (P value) | Tukey |
|---|---|---|---|---|---|
| 0 (Distilled water)<br>10 (10 g $kg^{-1}$ ASS)<br>20 (20 g $kg^{-1}$ ASS)<br>30 (30 g $kg^{-1}$ ASS)<br>40 (40 g $kg^{-1}$ ASS) | Tal y Cafn (CF) | 0.5 | 5 min | 0.036 | $10 \neq 40$ |
| | | | 2 hr | 0.001 | $0 \neq 40$; $10 \neq 20$; $10 \neq 30$; $10 \neq 40$ |
| | | | 4 hr | 0.000 | All different except 20 = 30 |
| | | | 8 hr | 0.361 | None |

ASS= artificial sea salts (Sigma)

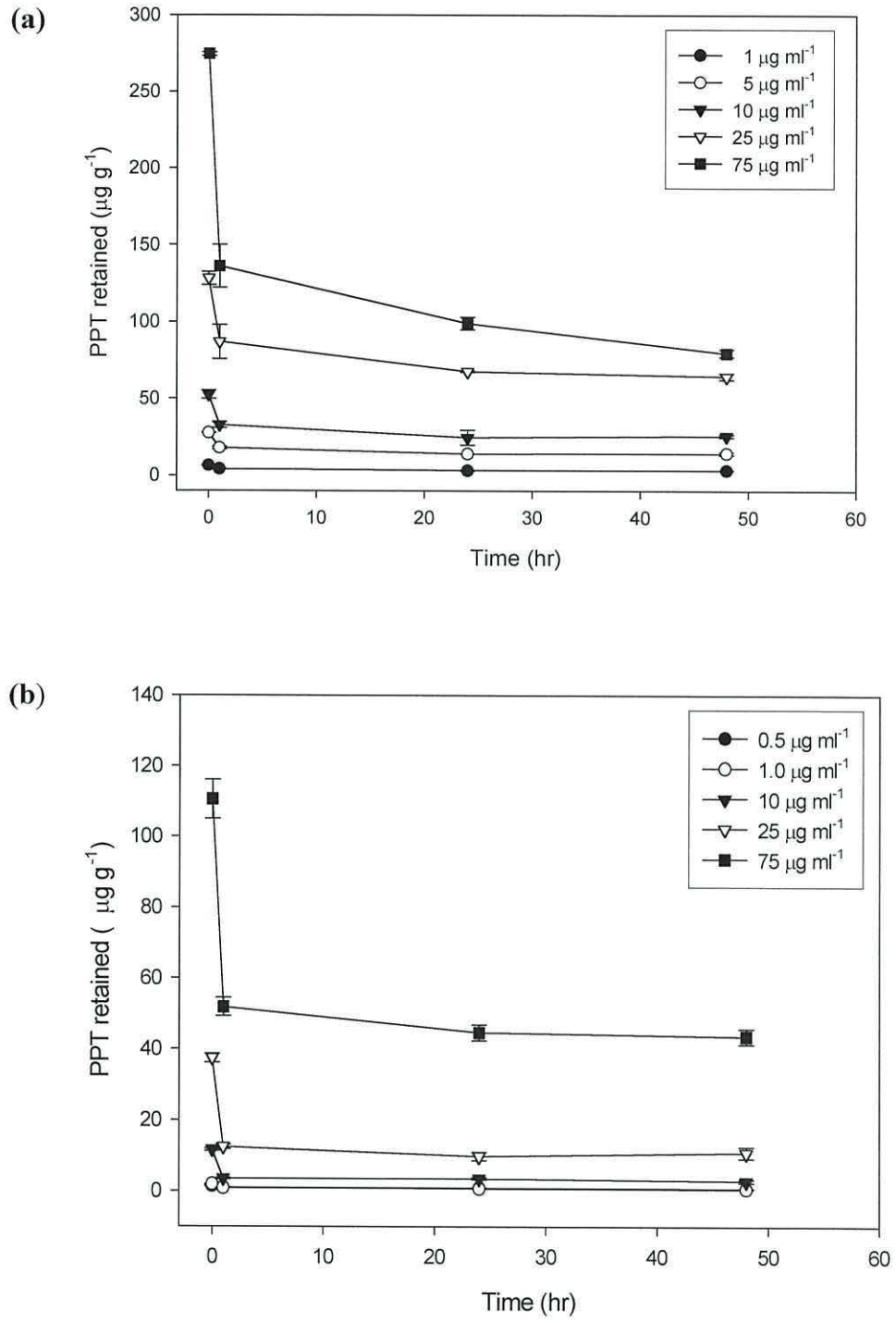



**Figure 4.20.** Desorption kinetics of PPT from sediment obtained from the field sites (a) Llanrwst and (b) Bird Sanctuary. Values represent mean ± standard error of the mean (SEM), (n = 3). The legend shows the initial solution PPT concentration.

desorption coefficients ($K_f$) are similar (Figure 4.21). However, the sorption coefficient at 48 hr was significantly different. This could imply that the desorption mechanism is different to that of sorption.

The linear regression equations and standard deviations (s) for desorption isotherms of the three sites are shown in Table 4.8. The linear coefficient ($r^2$) is not shown because it is not relevant to measure fitness for a linear regression with the origin at zero. In this case the standard deviation (s) gives a degree of fit. Desorption isotherms for the three sites are compared in Figure 4.22, and again there is a significant difference between the sediments from the estuary (TC and BS) compared with the river (LL).

**Table 4.8** Linear regression equations and standard deviation (s) of desorption isotherms after 1 hr desorption.

| Site | Equation | s |
|---|---|---|
| LL | $C_{sorb} = 21.3(C_{aq})$ | 14.06 |
| TC | $C_{sorb} = 2.30(C_{aq})$ | 2.48 |
| BS | $C_{sorb} = 5.92(C_{aq})$ | 2.61 |

#### 4.2.1.6 Impact of salinity and DOC on PPT desorption

Similar parameters to those used to test the effects on PPT sorption have also been used to determine the effects on PPT desorption. The data have also been similarly analysed to determine if any of the parameters had a significant effect on desorption behaviour of PPT. Both low (10g $kg^{-1}$) and high salinities (40 g $kg^{-1}$)

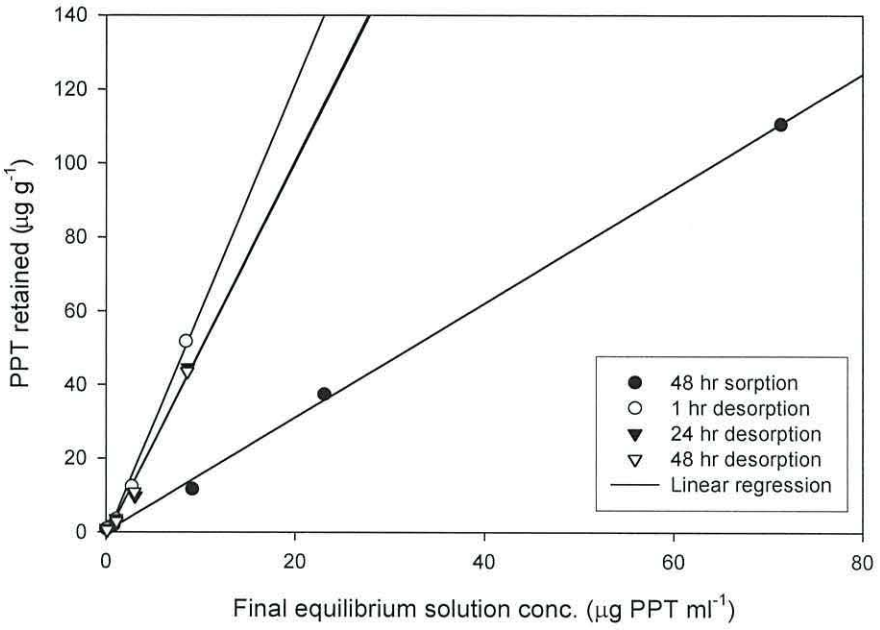



**Figure 4.21.** Linear desorption isotherms for the Bird Sanctuary sediment. Points represent the averaged experimental data and lines represent regression fits for the respective isotherm equations.

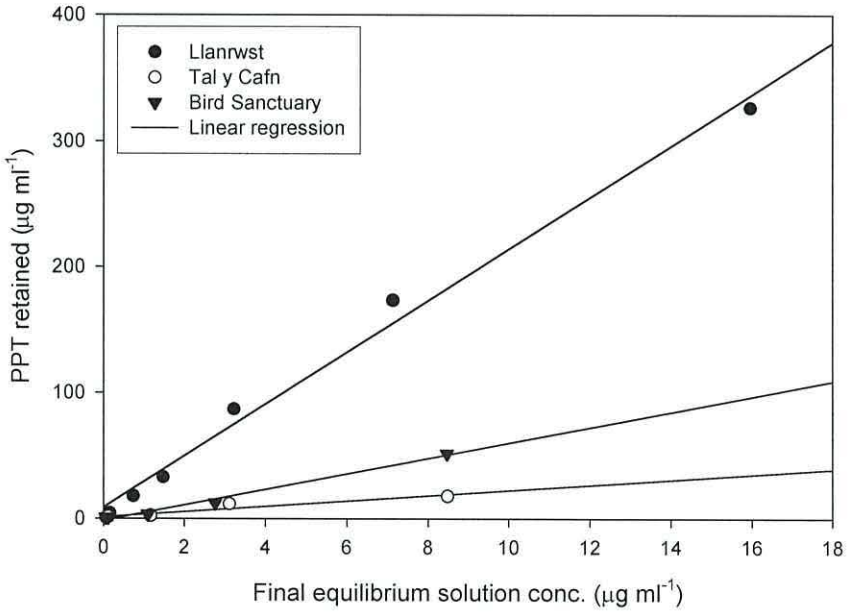



**Figure 4.22.** Desorption linear isotherm after 1 hr, from three sediment samples from the Conwy River and Estuary. Points represent the averaged experimental data and lines represent regression fits for the respective isotherm equations.

had a significant effect on the PPT desorption from BS sediment. In Table 4.9, the statistical results of the one way ANOVA and the Tukey's pairwise comparison are shown. These results showed no consistent trends. Desorption from the BS sediment is affected at PPT concentrations of both 0.5 and 10 μg $ml^{-1}$. Low salinity (10 g $kg^{-1}$) tends to slow desorption rate while the highest salinity (40 g $kg^{-1}$) makes it faster. By comparison, desorption from TC sediment is only affected (slowed) at 0.5 μg $ml^{-1}$ PPT and only at high salinity. It appears that results from these two sediments contradict each other. However, it could mean that the desorption mechanism is different in each case depending on the sediment characteristics.

Changing the DOC concentration in the solution had no significant impact on PPT desorption from LL sediment (Table 4.10), however, varying the source of humic substances did have a small effect, some differences are detected even at 48 hour of equilibration (e.g. LL). For instance, fulvic acid from Norway increased PPT desorption while humic acid (Aldrich) and NOM-SR tended to retard it. It has to be noted that any significant differences were observed in comparison with distilled water.

#### 4.2.1.7 Propetamphos industrial grade (PPT-Ind)

Desorption data for PPT-Ind also fitted well to a linear model. Figure 4.23 shows an example of the linear desorption isotherm for LL sediment. The desorption isotherms are significantly different when compared to the 48 hour

**Table 4.9.** Results of a statistical comparison between matrix salinity treatments for PPT desorption onto Estuary sediment (Tal y Cafn and Bird Sanctuary). One way ANOVA (P value) and Tukey's pairwise comparison data are shown when $P < 0.05$ as those treatments that were significantly different.

| Treatments | Sediment | PPT ($\mu g\ ml^{-1}$) | Desorption Time | Anova (P value) | Tukey |
|---|---|---|---|---|---|
| 0 (Distilled water)<br>10 (10 g $l^{-1}$ ASS)<br>20 (20 g $l^{-1}$ ASS)<br>30 (30 g $l^{-1}$ ASS)<br>40 (40 g $l^{-1}$ ASS) | Bird sanctuary (BS) | 0.5 | Initial | 0.028 | $0 \neq 40$ |
| | | | 24 hr | 0.009 | $10 \neq 30$; $10 \neq 40$ |
| | | 10 | Initial | 0.112 | None |
| | | | 24 hr | 0.002 | $0 \neq 30$; $0 \neq 40$; $20 \neq 40$ |
| | Tal y Cafn (TC) | 0.5 | Initial | 0.001 | $0 \neq 40$; $10 \neq 40$; $20 \neq 40$; $30 \neq 40$ |
| | | | 24 hr | 0.067 | $0 \neq 40$ |
| | | 10 | Initial | 0.000 | $0 \neq 40$; $10 \neq 40$; $30 \neq 40$ |
| | | | 24 hr | 0.019 | None |

ASS= artificial sea salts (sigma)

sorption isotherm.

The desorption kinetics (Figure 4.24) again show an initial fast desorption during the first hour, after which very slow desorption was observed. In the LL sediment, the average desorption during the first hour was *ca.* 41%. Whilst after 48 hr the proportion of PPT-Ind desorbed was only 43%.

The desorption experiment to test the impact of salinity showed that the rate of PPT-Ind desorption is not significantly affected in BS or TC sediment (see Table 4.11) compared with distilled water (DDW).

### 4.2.2 Biodegradation of PPT under various conditions

The biodegradation of $^{14}C$ labelled PPT by the indigenous bacterial population in the sediments of the Conwy River has been assessed by measuring the main product of respiration ($^{14}C$-$CO_2$).

#### 4.2.2.1. PPT biodegradation under aerobic and anaerobic atmospheres

The first condition tested was the impact of an aerobic or anaerobic atmosphere on PPT biodegradation in three sediments. The results have been presented in two ways. The first represents the amount of $^{14}C$-$CO_2$ evolved on a daily basis. The second represents the cumulative percentage degradation per g of sediment, to obtain a semi-logarithmic linear biodegradation model.

**Table 4.10.** Results of a statistical comparison between dissolved organic carbon (DOC) concentrations and humic substances for PPT desorption from River (Llanrwst) and Estuary (Tal y Cafn) sediment. One way ANOVA (P value) and Tukey's pairwise comparison data are shown when $P < 0.05$ as those treatments that were significantly different.

| | **Treatments** | **Sediment** | **PPT ($\mu g\ ml^{-1}$)** | **Desorption Time** | **Anova (P value)** | **Tukey** |
|---|---|---|---|---|---|---|
| DOC | 0 (Distilled water)<br>5 (5 mg DOC $l^{-1}$)<br>10 (10 mg DOC $l^{-1}$)<br>30 (30 mg DOC $l^{-1}$)<br>50 (50 mg DOC$l^{-1}$) | Tal y Cafn (TC) | 0.1 | Initial | 0.908 | None |
| | | | | 24 hr | 0.610 | None |
| Humic substances | DW = Distilled water<br>HA (Ald) = HA Aldrich<br>HA (Nor) = HA Norway<br>FA (Nor) = FA Norway<br>NOM-SR* | Llanrwst (LL) | 0.1 | Initial | 0.019 | FA (Nor) ≠ HA (Ald); FA (Nor) ≠ NOM-SR |
| | | | | 1 hr | 0.445 | None |
| | | | | 24 hr | 0.025 | DW ≠ NOM-SR; HA (Ald) ≠ NOM-SR |

* NOM-SR represents natural organic matter from the Suwaanee River

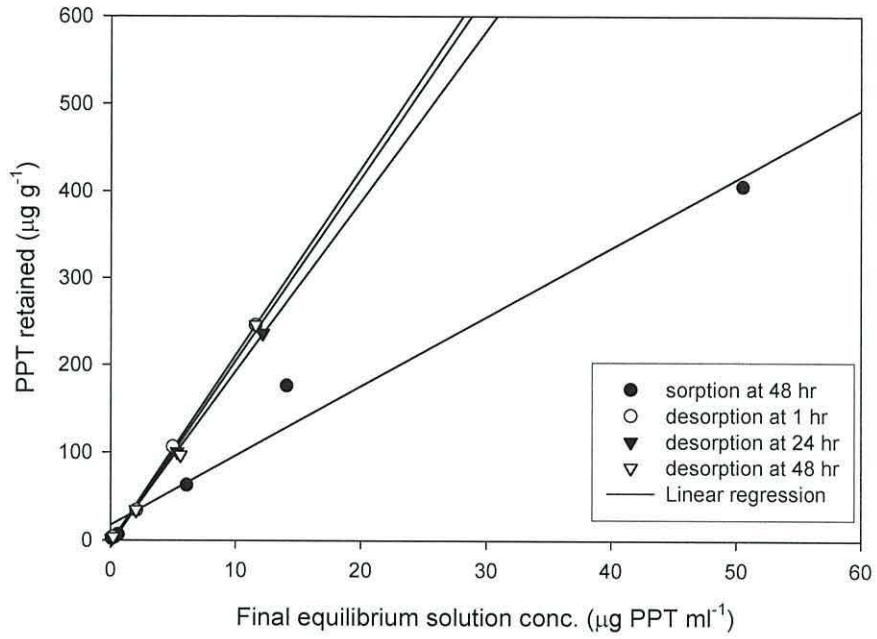



**Figure 4.23.** Linear desorption isotherms of PPT-Ind from Llanrwst sediment. Points represent the averaged experimental data and lines represent regression fits of respective isotherm equations.

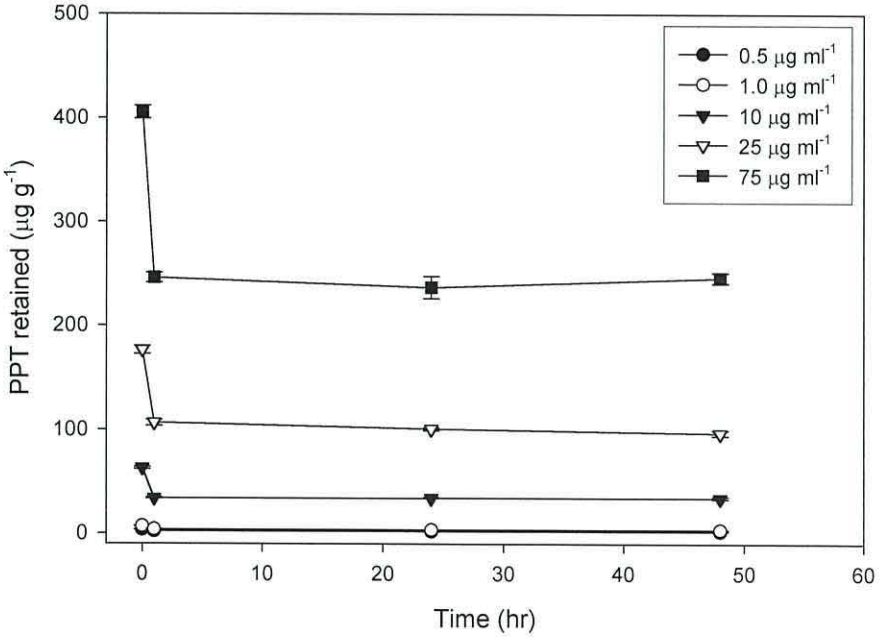



**Figure 4.24.** Desorption kinetics of PPT-Ind from Llanrwst sediment. Values represent the mean ± standard error of the mean (SEM), (n = 3). The legend shows the initial solution PPT concentration.

The results obtained for the aerobic experiments are shown in Figures 4.25 and 4.26. The daily evolution of $^{14}C$-$CO_2$ indicates a different pattern between the three sediments with the fastest rate observed in the BS sediment, followed by LL and then TC sediment. Sediments from LL and TC seem to have a "conditioning" time (lag phase), which was not apparent in the BS sediment. Generally, the highest amount of $^{14}C$-$CO_2$ evolved in the three sediments occurs in the first 10 days after which the rate gradually decreases.

The cumulative biodegradation curves under aerobic conditions (Figure 4.25) show that there are small but significant differences between the three sediments with the control representing data obtained without sediment. The data was analysed against a semi-logarithmic linear model of degradation kinetics (Table 4.12). From this model the theoretical half life ($DT_{50}$) was calculated, and from the graphs the experimental half lives ($t_{½}$) were determined (see Table 4.12). Both data show the degradation rate of PPT under aerobic conditions in the LL and TC sediments is significantly slower than in the BS sediment ($p < 0.001$, GLM). However, in general, the $DT_{50}$ values are over estimated compared with the experimentally derived values. Nonetheless the fits to the model are adequate ($r^2 > 0.84$).

Results from the experiment performed under anaerobic conditions show that the evolution of $^{14}C$-$CO_2$ (Figure 4.27) follows a similar pattern of biodegradation to that observed under aerobic conditions. The main difference found is that the maximum evolution of $^{14}C$-$CO_2$ under an anaerobic atmosphere is *ca.* three quarters of that in oxic conditions but the total degraded after 35 days is similar

**Table 4.11.** Results of a statistical comparison between matrix salinity treatments for PPT-Ind desorption onto Estuary sediment (Tal y Cafn and Bird Sanctuary). One way ANOVA (P value) and Tukey's pairwise comparison data are shown when $P < 0.05$ as those treatments that were significantly different.

| Treatments | Sediment | PPT-Ind ($\mu g\ ml^{-1}$) | Desorption Time | Anova (P value) | Tukey |
|---|---|---|---|---|---|
| 0 (Distilled water)<br>10 (10 g $l^{-1}$ ASS)<br>20 (20 g $l^{-1}$ ASS)<br>30 (30 g $l^{-1}$ ASS)<br>40 (40 g $l^{-1}$ ASS) | Bird Sanctuary (BS) | 0.5 | Initial | 0.206 | None |
| | | | 24 hr | 0.486 | None |
| | Tal y Cafn (TC) | 0.5 | Initial | 0.005 | $10 \neq 30$; $10 \neq 40$ |
| | | | 24 hr | 0.273 | None |

ASS= artificial sea salts (Sigma)

for both aerobic and anaerobic conditions in all sediments. The cumulative graphs are shown in Figure 4.28, the respective equations, the calculated and experimental half life values are presented in Table 4.12. Looking at the position after 22 days the rate of degradation in the BS sediment is faster than the mean of the three sediments and for TC it is slower ($P < 0.001$, GLM). Looking at the data after 13 days all three sediments have degradation rates which are statistically different from the mean calculated by GLM. In summary, the average half life ($DT_{50}$) under aerobic conditions is 359 hours (*ca.* 15 days) compared with 446 hours (*ca.* 19 days) under an anaerobic atmosphere.

PPT degradation rates could have been affected by the physico-chemical sediment characteristics (Table 4.13). For instance, the slower rate of degradation in the TC sediment could be due to its greater sorption behaviour. PPT degradation rates in the BS sediment are the fastest under both aerobic and anaerobic experiments. This could be due to the fact that sediment from this site had the highest microbial activity, measured as respiration (35.3 pmol $CO_2$ $g^{-1}$ $s^{-1}$). At the same time it could be due to it possessing a higher content of inorganic nutrients ($PO_4^{3-}$-P 3.2 μmol $g^{-1}$; $NH_4^+$-N 0.4 μmol $g^{-1}$ and $K^+$ 6.4 μmol $g^{-1}$).

#### 4.2.2.2 Impact of labile carbon compounds on PPT biodegradation

An experiment was carried out to check if alternative carbon substrates in solution were preferred by the microbial population to PPT. These experiments used glucose and bovine serum albumin (BSA) as alternative carbon substrates. The cumulative mineralization graph is presented in Figure 4.29. Apparently the

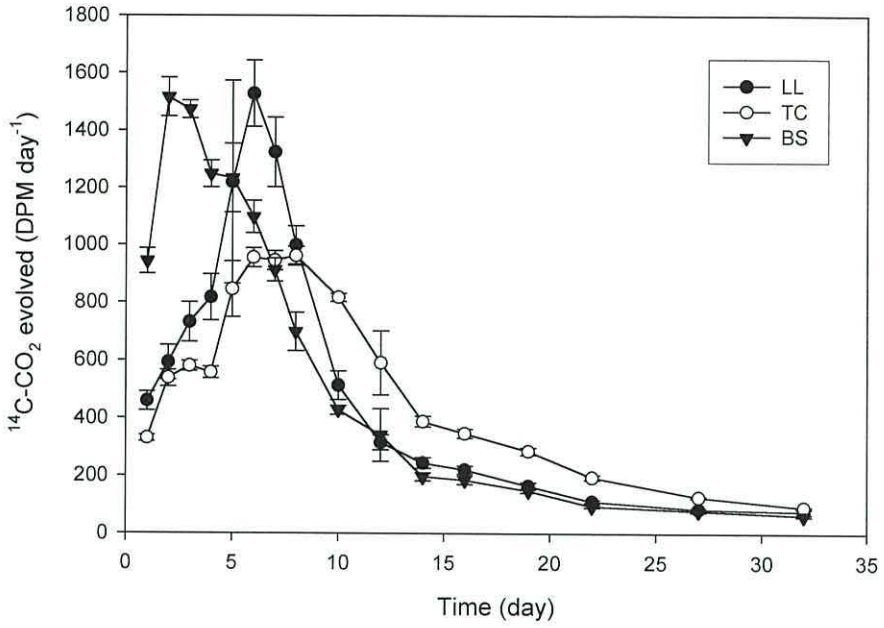



**Figure 4.25.** Evolution of $^{14}C$-$CO_2$ per day from $^{14}C$-PPT in three sediments from the Conwy River under aerobic conditions. The initial PPT concentration was 0.685 μg $g^{-1}$. Values represent mean ± standard error of the mean (SEM), (n=3).

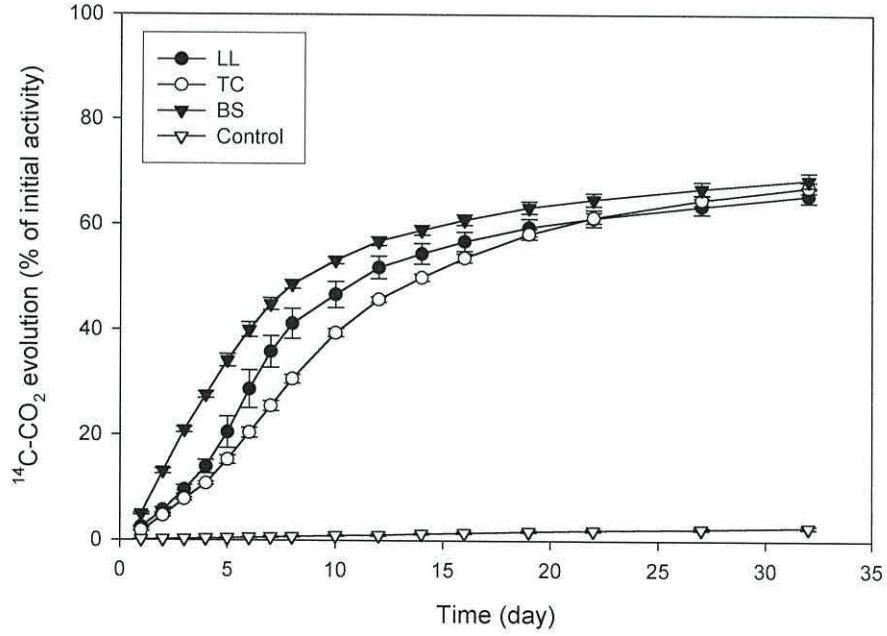



**Figure 4.26.** Cumulative evolution of $^{14}C$-$CO_2$ arising from the degradation of $^{14}C$-PPT in three sediments from the Conwy River under aerobic conditions. The control does not have sediment present. The initial PPT concentration was 0.685 μg $g^{-1}$.Values represent mean ± standard error of the mean (SEM), (n=3).

biodegradation rate in the control samples (without organic nutrients) is faster than in the two sediments tested (TC and BS). However according to the GLM test these are not significant differences ($p > 0.01$), compared with the controls, not even in the early stages of the experiment. This implies that the microbial community responsible for the biodegradation of PPT does not prefer non-toxic sources of carbon to PPT, and co-metabolism may be the biodegradation pathway occurring here because they don't gain energy from PPT degradation. Thus for the following experiments the controls had glucose as the main source of carbon and this will be considered as "normal" behaviour. Also glucose was selected as a substrate because the solute interaction with humic acid was expected to be negligible (Meredith and Radosevich, 1998).

#### 4.2.2.3 Impact of salinity on PPT biodegradation

The effect of salinity on glucose (Figure 4.30.a) and PPT (Figure 4.30.b) degradation has been tested at four salinity concentrations using a DDW control. In both cases, even a slight increase in salinity has a significant effect ($P < 0.05$ ANOVA) reducing the initial activity of $^{14}C\text{-}CO_2$ evolved at the fifth and tenth day. A comparison between the salinity concentrations shows no significant differences were detected using the Tukey's pairwise comparison test. It should be noted that this test has been carried out on the LL sediment from the freshwater part of the Conwy River. The results presented show that the indigenous microbial population at LL had a low tolerance to salinity. Also it should be noted that the degradation of PPT reduced but was not completely inhibited. The following experiments involving salinity as a variable were therefore carried out

**Table 4.12.** Semi-$Log_{10}$ biodegradation kinetic model of PPT in three sediments from the Conwy River under aerobic and anaerobic conditions. Standard deviation of the curve (S) and correlation coefficient ($r^2$). Calculated and experimental half lives. Data collected under laboratory light conditions, 20 °C and pH 5.7 - 8.3.

| Sediment | Aerobic | | | | | Anaerobic | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Equation ($Log_{10}$ $C/C_o$ =) | S | $r^2$ | $DT_{50}$ (hr) | $t_{1/2}$ (hr) | Equation ($Log_{10}$ $C/C_o$ =) | S | $r^2$ | $DT_{50}$ (hr) | $t_{1/2}$ (hr) |
| LL | -0.051 – 0.00066 t | 0.057 | 0.86 | 377 | 270 | -0.024 – 0.00063 t | 0.041 | 0.93 | 442 | 345 |
| TC | -0.011 – 0.00072 t | 0.038 | 0.94 | 404 | 330 | -0.021 – 0.00054 t | 0.013 | 0.99 | 523 | 580 |
| BS | -0.110 – 0.00065 t | 0.063 | 0.84 | 295 | 205 | -0.042 – 0.00069 t | 0.044 | 0.93 | 373 | 290 |
| | | | Average | 359 | 268 | | | Average | 446 | 405 |

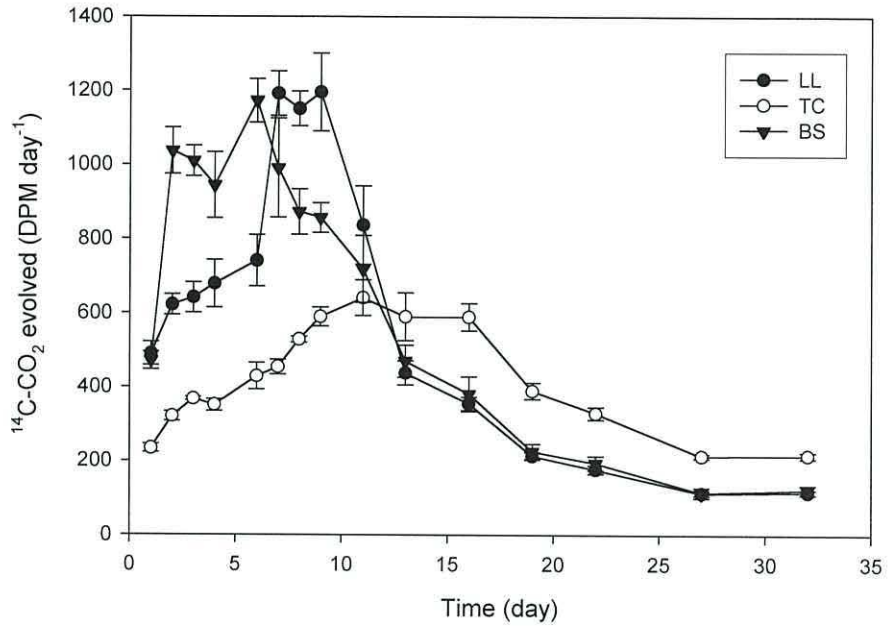



**Figure 4.27.** Evolution of $^{14}C$-$CO_2$ per day from $^{14}C$-PPT in three sediments from the Conwy River incubated under anaerobic conditions. The initial PPT concentration was 0.685 μg $g^{-1}$. Values represent mean ± standard error of the mean (SEM), (n=3).

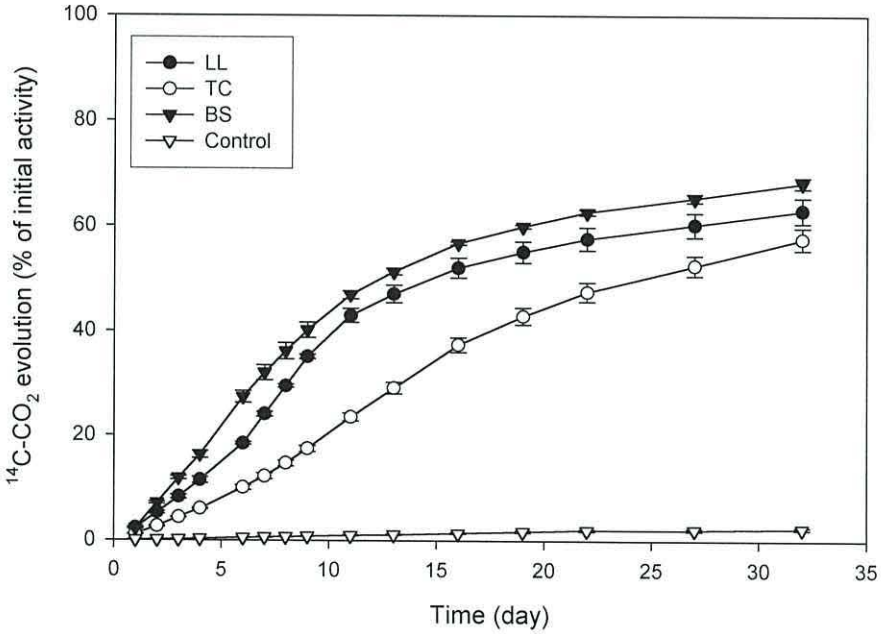



**Figure 4.28.** Cumulative respiration product ($^{14}C$-$CO_2$) in three sediments from the Conwy River incubated under anaerobic conditions. The initial PPT concentration was 0.685 μg $g^{-1}$. Controls do not have sediments. Values represent mean ± standard error of the mean (SEM), (n=3).

using the lowest salinity concentration (3.35 g $kg^{-1}$), because this produced a significant effect.

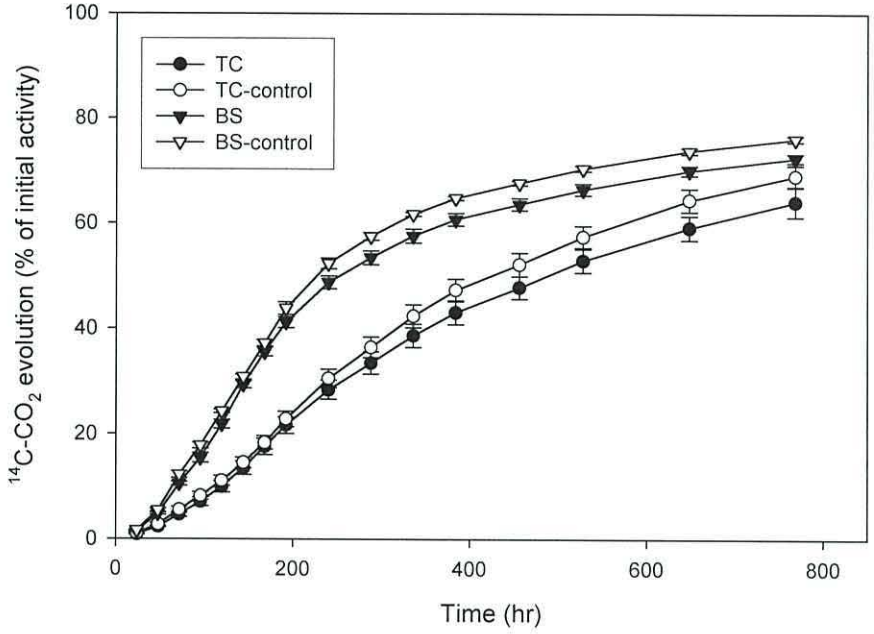



**Figure 4.29.** Cumulative respiration product ($^{14}C$-$CO_2$) from $^{14}C$-PPT in two sediments from the Conwy River in the presence of organic nutrients (glucose and BSA). Controls have no carbon nutrient source added. Values represent mean ± SEM (n = 3).

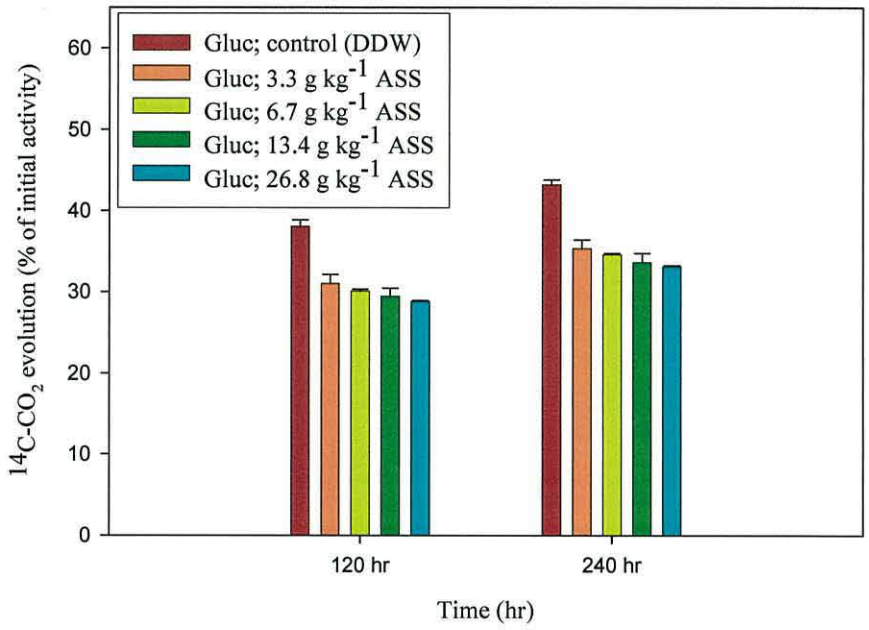
### 4.2.2.4 Impact of NOM on PPT biodegradation

The effect of NOM on PPT degradation was tested in the LL sediment. Only one concentration of NOM was used because sediment NOM did not vary greatly and was generally considered to be high. Biodegradation of glucose in four different aqueous matrices, DDW; ASW (3.3 g $l^{-1}$ ASS); NOM (130 mg C $l^{-1}$) and a combination of ASW and NOM (Figure 4.31.a) shows that varying the aqueous matrix did not have a significant effect ($P > 0.5$ ANOVA) over the time period

**Table 4.13.** Physico-chemical characteristics of the Conwy River sediments used in biodegradation experiments. Elemental composition (% by dry weight). Standard error was less than 5%, (n = 3).

| | Physico-chemical characteristics | | Microbial activity | Elemental content | | Size grain fractionation | | | Inorganic nutrients | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sediment** | **pH** | **Conductivity (mSiemens $cm^{-1}$)** | **$CO_2$ (pmol $g^{-1}s^{-1}$)** | **C (%)** | **N (%)** | **Sand (%)** | **Silt (%)** | **Clay (%)** | **P-$PO_4^{3-}$ (µmol $g^{-1}$)** | **N-$NH_4^+$ (µmol $g^{-1}$)** | **$K^+$ (µmol $g^{-1}$)** |
| LL | 5.73 | 72 | 6.7 | 0.4 | 0.5 | 96.6 | 2.81 | 0.6 | 0.7 | 0.2 | 0.6 |
| TC | 8.06 | 1095 | 32.9 | 1.7 | 0.4 | 51.5 | 27.2 | 21.4 | 1.9 | 0.2 | 5.9 |
| BS | 8.35 | 1690 | 35.3 | 1.3 | 0.3 | 57.7 | 27.3 | 15.0 | 3.2 | 0.4 | 6.4 |

**(a)**




**(b)**

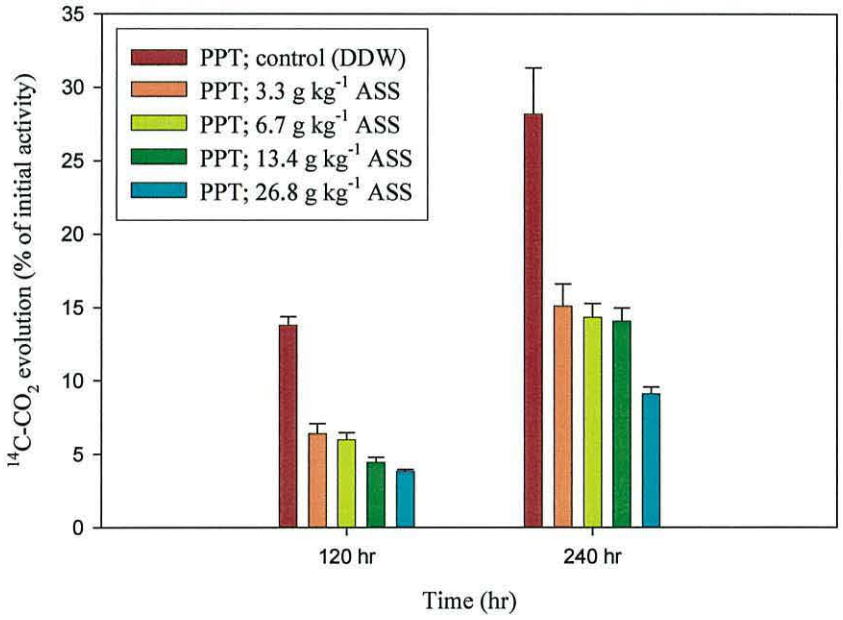



**Figure 4.30.** Salinity effect on degradation of (a) $^{14}C$ labelled glucose and (b) $^{14}C$ labelled PPT on the fifth day (120 hr) and tenth day (240 hr) in Llanrwst sediment. Bars represent mean ± standard error of the mean (SEM). (n =3).

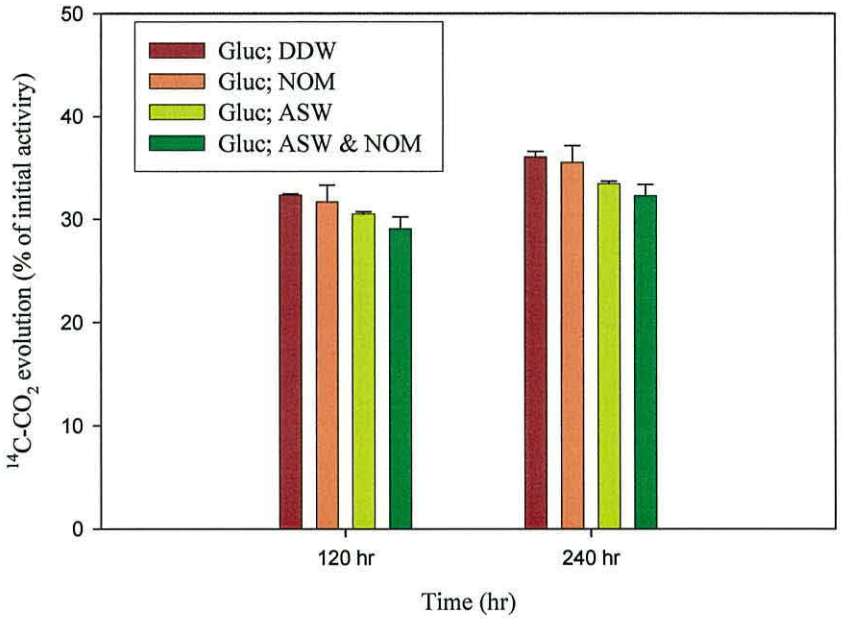
tested. By comparison, PPT degradation (Figure 4.31.b) is considerably reduced ($P < 0.05$ ANOVA) in ASW solutions and in the solution combining ASW and NOM as the Tukey's pairwise comparison also shows. NOM alone did not show any differences either for glucose or PPT degradation.

#### 4.2.2.5 Impact of metals on PPT biodegradation

To test the effects of metals on PPT biodegradation, different concentrations of zinc and lead were added to LL sediment. The biodegradation of glucose in the presence of zinc (Figure 4.32.a) was not significantly affected at the two times monitored ($P > 0.05$ ANOVA). By comparison, the biodegradation of PPT (Figure 4.32.b) was affected even at the lowest concentration of zinc (2.2 $\mu g\ g^{-1}$), and the percentage of $^{14}C\text{-}CO_2$ evolved was significantly reduced again with increasing zinc concentration, ($P < 0.05$ ANOVA and Tukey's pairwise comparison). The results also indicate that the effects are more apparent at the tenth day (240 hours) in comparison to after the fifth day. All the zinc treatments were different to each other and to the DDW control ($P < 0.05$ ANOVA and Tukey's pairwise comparison).

The effect of lead concentration on glucose degradation was minimal ($P > 0.05$ ANOVA) at the two concentrations tested on the second day (Figure 4.33.a). However, on the tenth day an effect was observed at the highest lead concentration ($P < 0.05$ ANOVA and Tukey's pairwise comparison). The lead had a positive impact on the degradation of glucose which was not expected. At the same time for PPT degradation, lead had the opposite effect; reducing the

**(a)**




**(b)**

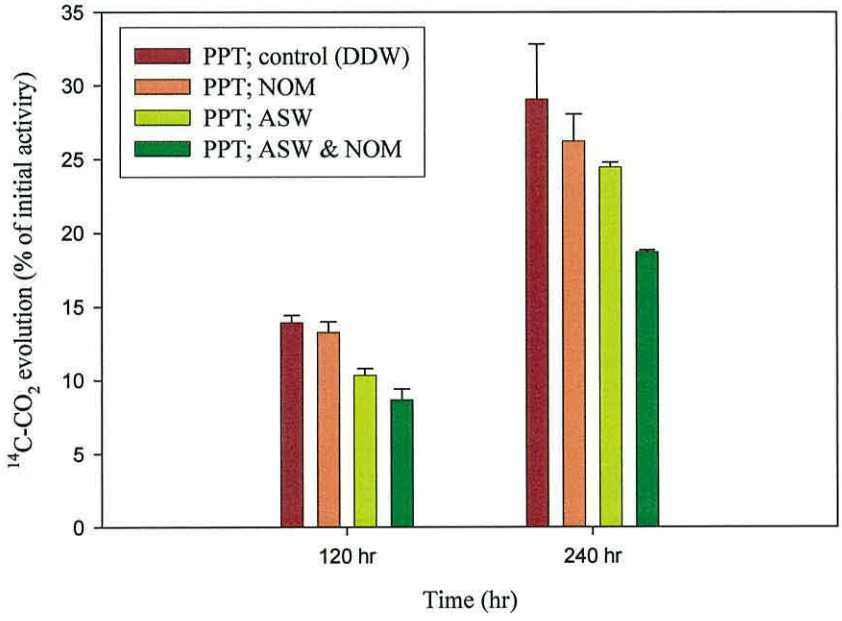
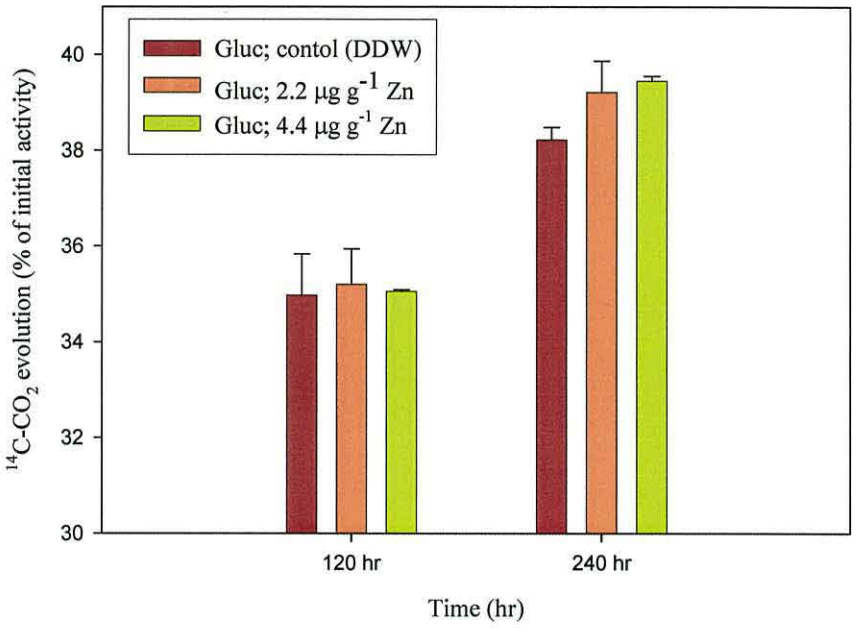



**Figure 4.31.** Matrix nature effect on the degradation of (a) glucose and (b) PPT at the fifth day (120 hr) and tenth day (240 hr). Control with DDW; NOM (130 mg C $l^{-1}$), ASW (3.3 g $kg^{-1}$ ASS), NOM and ASW in the Llanrwst sediment. Values represent mean ± standard error of the mean (SEM), (n=3).

**(a)**




**(b)**

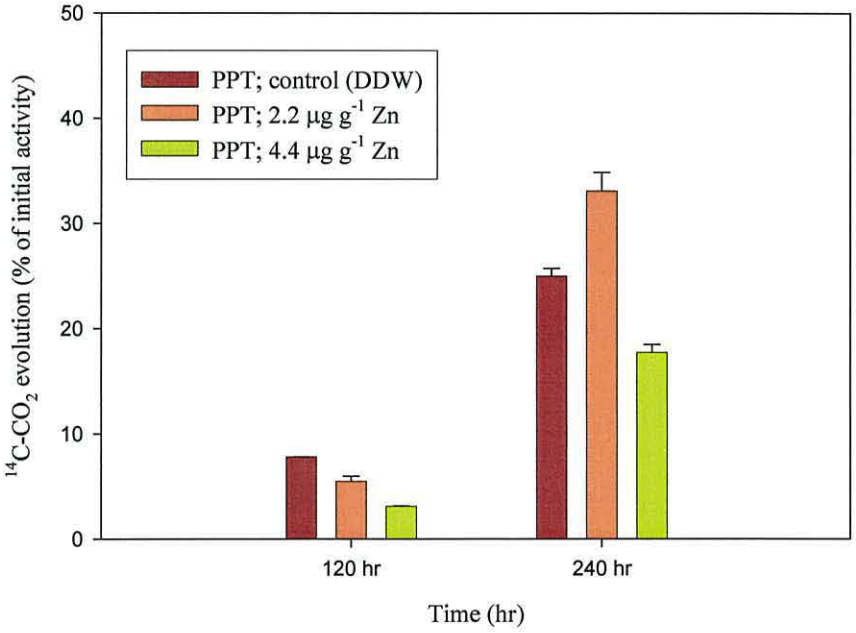



**Figure 4.32.** Zinc concentration effect on the biodegradation of (a) glucose and (b) PPT at the fifth day (120 hr) and the tenth day (240 hr) in the Llanrwst sediment. Values represent mean ± standard error of the mean (SEM), (n=3).

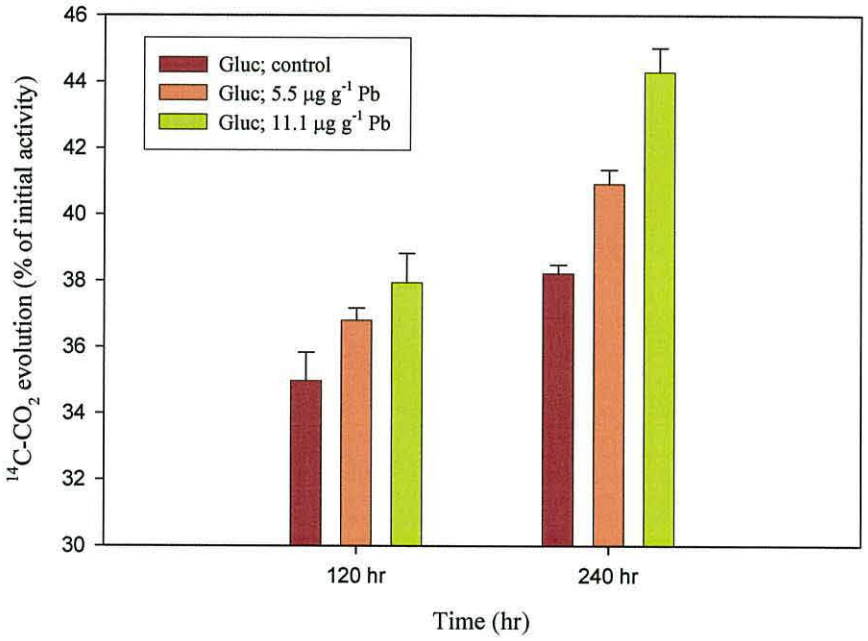
percentage of $^{14}C$-$CO_2$ evolved ($P < 0.05$ ANOVA) at the two concentrations shown in Figure 4.33.b.

#### 4.2.2.6 Impact of metals and NOM on PPT biodegradation

The combined effect of metal concentration and nature of the aqueous matrix on the biodegradation of PPT is shown in Figures 4.34 to 4.37. These experiments were monitored four times between 50 and 240 hours. Having calculated the semi logarithmic linear biodegradation kinetic equation for each data set, any differences between each batch were analyzed, using the GLM test. None of the groups show any significant differences ($P > 0.01$ GLM). Nevertheless, comparing individual time points showed some differences. For instance, comparing the effect at the fifth day shows that PPT degradation follows the pattern DDW = NOM > ASW > NOM & ASW mixture ($P < 0.05$ ANOVA and Tukey's pairwise comparison). Also the presence of lead and the combination of lead and zinc in the four matrices reduced the percentage of $^{14}C$-$CO_2$ evolved ($P < 0.05$ ANOVA and Tukey's pairwise comparison) in the four times compared individually. The degradation follows the pattern PPT = PPT-Zn > PPT-Pb = PPT-Zn-Pb.

Industrial grade propetamphos (PPT-Ind) was also tested using the same conditions and the results are presented in Figures 4.38 to 4.42. Again, the GLM test does not show any significant differences between the treatments presented, whilst analysis of data at set times does show some differences. For instance, the NOM + ASW matrix had a negative effect on PPT-Ind degradation on the 2$^{nd}$ and

**(a)**




**(b)**

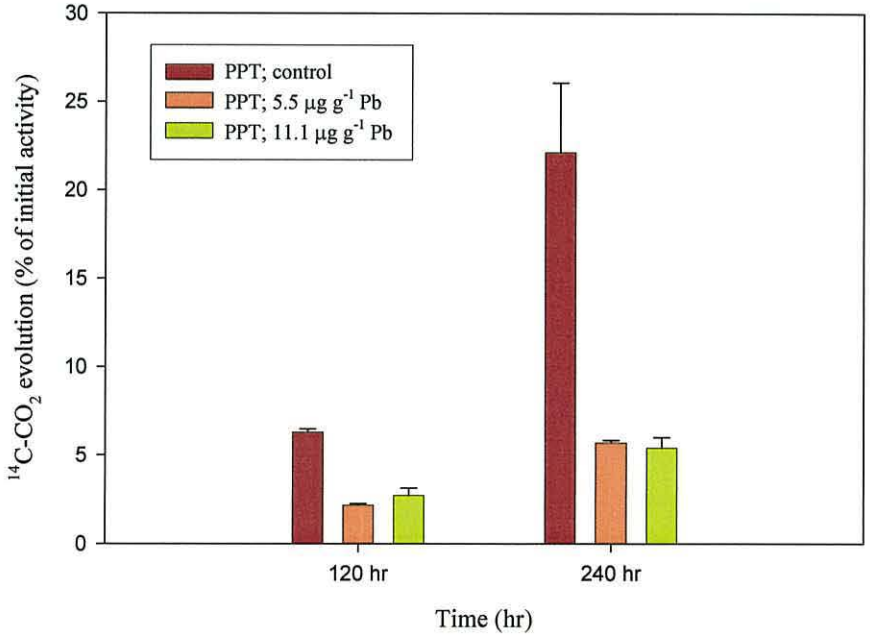



**Figure 4.33.** Lead concentration effect on biodegradation of (a) glucose and (b) PPT at the fifth day (120 hr) and the tenth day (240 hr) in the Llanrwst sediment. Values represent mean ± standard error of the mean (SEM), (n=3).

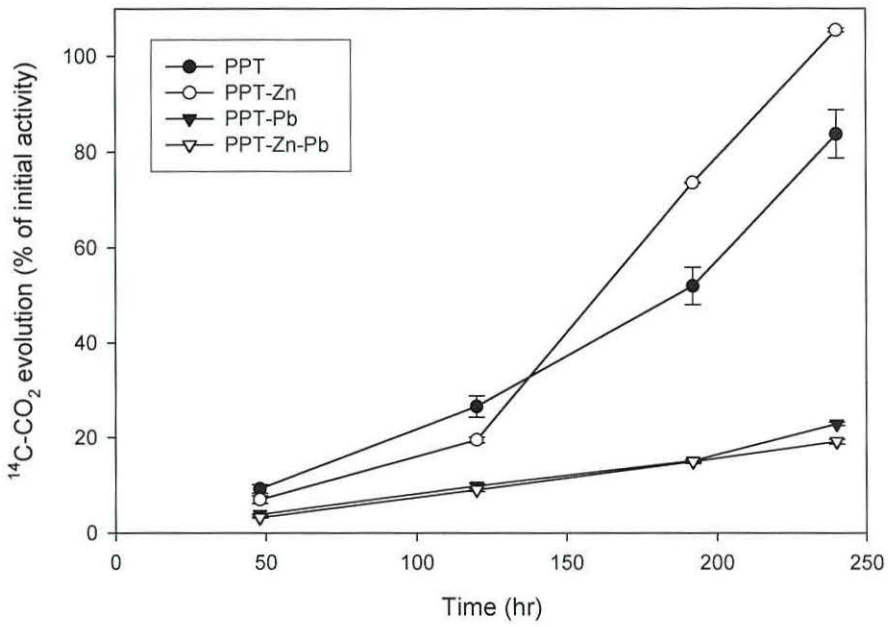



**Figure 4.34**. Biodegradation kinetics of PPT (0.685 μg $g^{-1}$) on its own and/or with zinc (2.24 μg $g^{-1}$) and lead (5.53 μg $g^{-1}$) in a DDW aqueous matrix in the Llanrwst sediment. Values represent mean ± SEM (n=3).

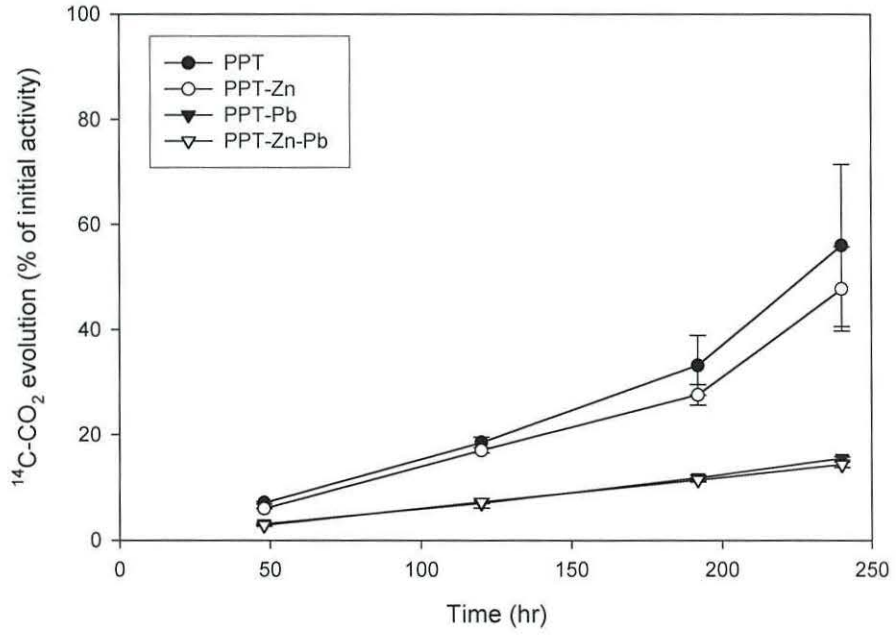



**Figure 4.35.** Biodegradation kinetics of PPT (0.685 μg $g^{-1}$) on its own and/or with zinc (2.24 μg $g^{-1}$) and lead (5.53 μg $g^{-1}$) in an ASW (3.35 g $kg^{-1}$) aqueous matrix in the Llanrwst sediment. Values represent mean ± SEM (n=3).

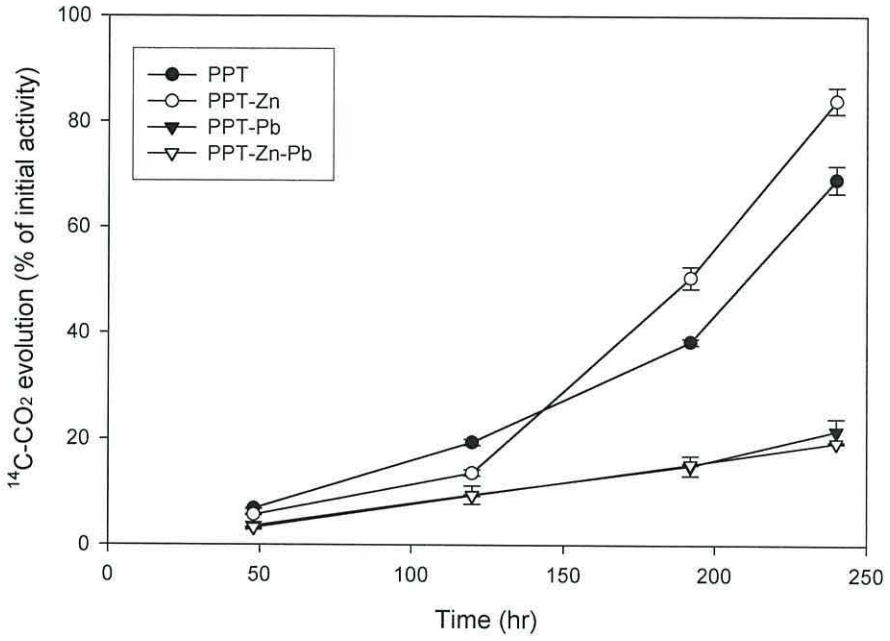



**Figure 4.36.** Biodegradation kinetics of PPT (0.685 μg $g^{-1}$) on its own and/or with zinc (2.24 μg $g^{-1}$) and lead (5.53 μg $g^{-1}$) in NOM (126 mg C $l^{-1}$) aqueous solution in the Llanrwst sediment. Values represent mean ± standard error of the mean (SEM), (n=3).

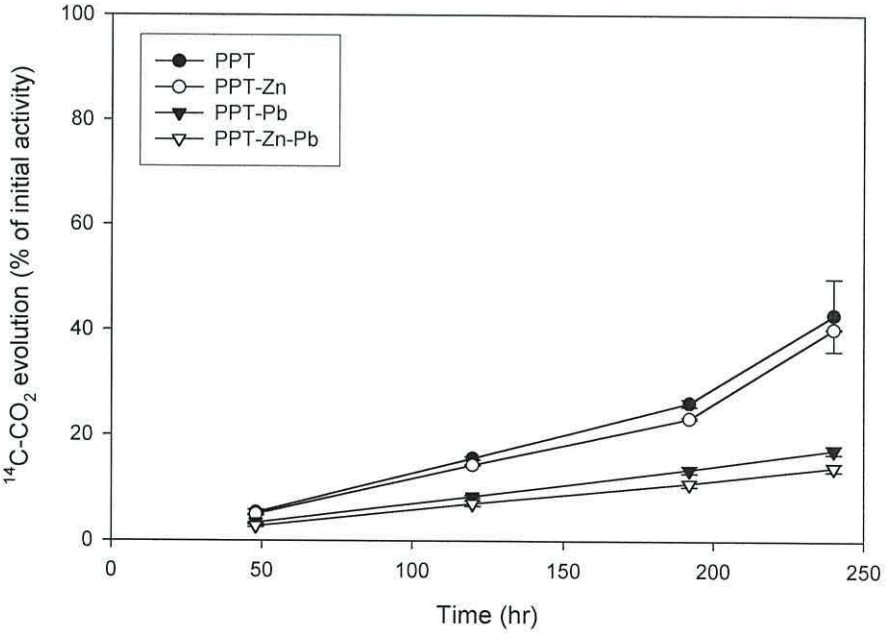



**Figure 4.37.** Biodegradation kinetics of PPT (0.685 μg $g^{-1}$) on its own and/or with zinc (2.24 μg $g^{-1}$) and lead (5.53 μg $g^{-1}$) in an aqueous matrix comprised of ASW (3.3 g $kg^{-1}$) and NOM (126 mg C $l^{-1}$) in the Llanrwst sediment. Values represent mean ± standard error of the mean (SEM), (n=3).

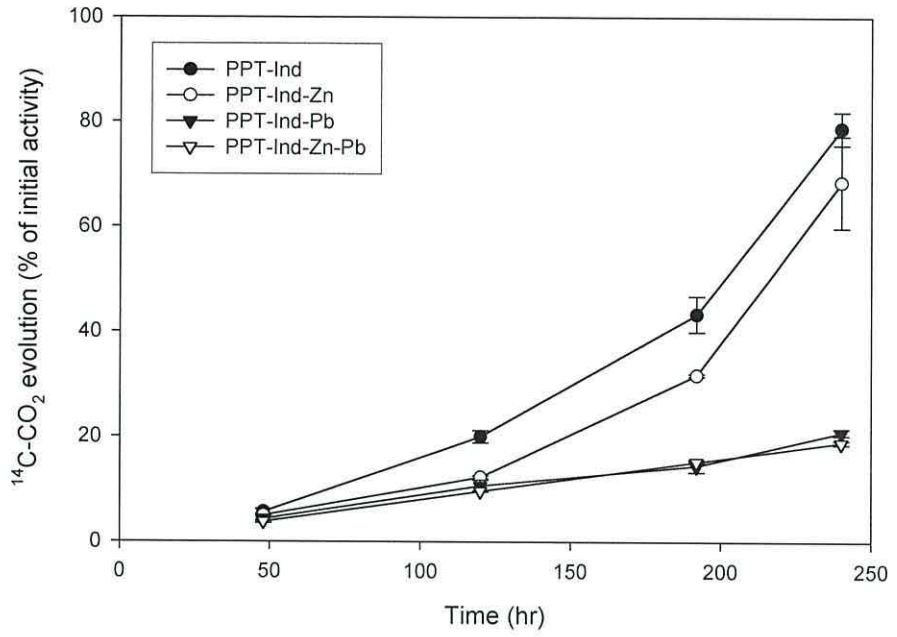



**Figure 4.38.** Biodegradation kinetics of PPT-Ind (0.685 μg $g^{-1}$) on its own and/or with zinc (2.24 μg $g^{-1}$) and lead (5.53 μg $g^{-1}$) in DDW water as aqueous matrix in the Llanrwst sediment. Values represent mean ± standard error of the mean (SEM), (n=3).

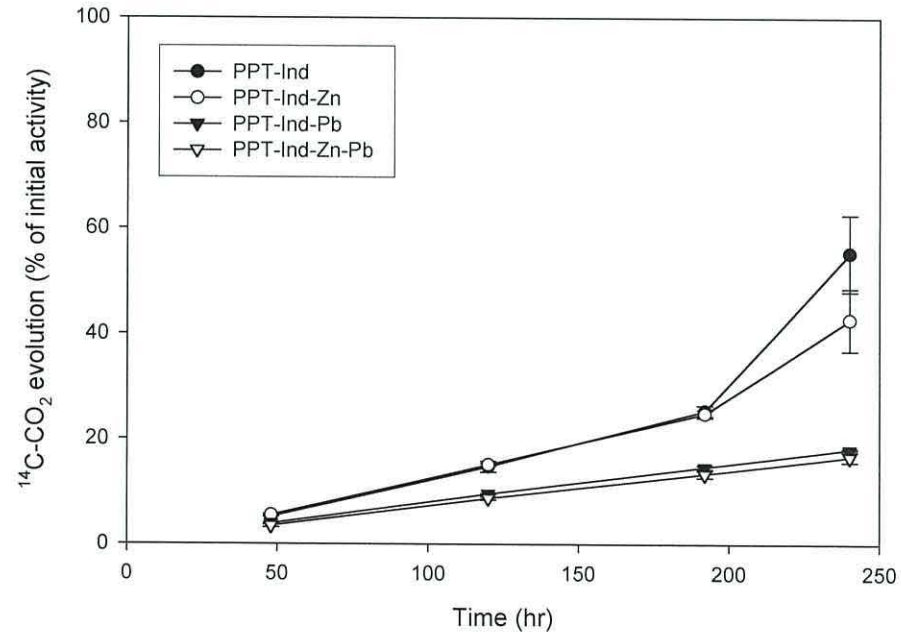



**Figure 4.39.** Biodegradation kinetis of PPT-Ind (0.685 μg $g^{-1}$) on its own and/or with zinc (2.24 μg $g^{-1}$) and lead (5.53 μg $g^{-1}$) in ASW (3.35 g $kg^{-1}$) as aqueous matrix in the Llanrwst sediment. Values represent mean ± standard error of the mean (SEM), (n=3).

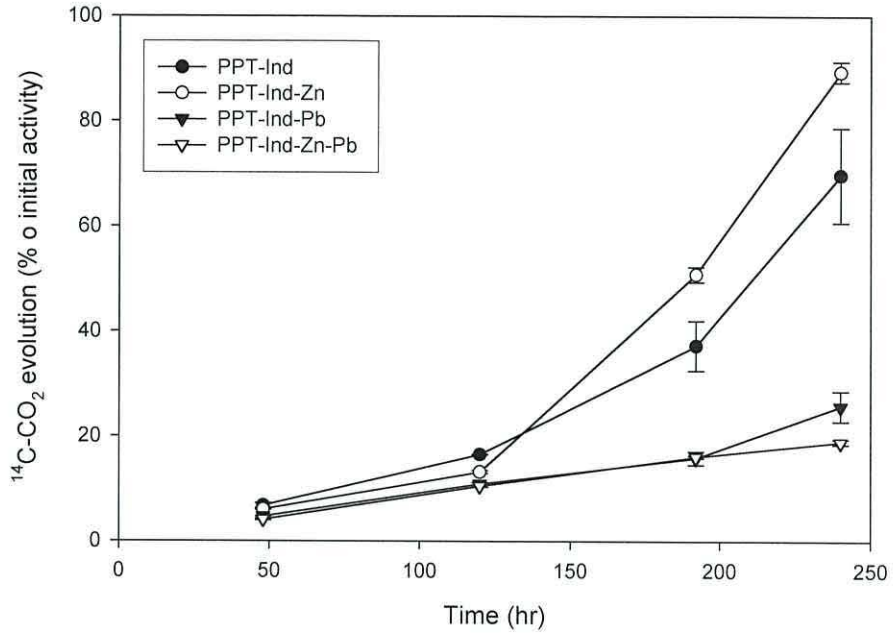



**Figure 4.40.** Biodegradation kinetics of PPT-Ind (1 μg $g^{-1}$) on its own and in presence of Zn (2.24 μg $g^{-1}$) and or Pb (5.53 μg $g^{-1}$) in NOM (130 mg C $l^{-1}$) aqueous solution as matrix in the Llanrwst sediment. Values represent mean ± standard error of the mean (SEM), (n=3).

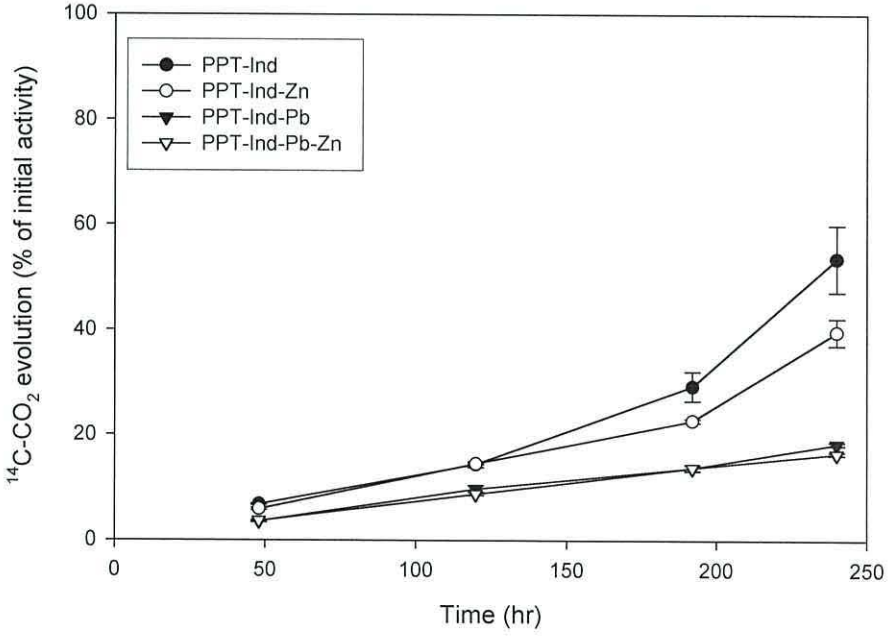



**Figure 4.41.** Biodegradation kinetics of PPT-Ind (1 μg $g^{-1}$) on its own and in presence of Zn (2.24 μg $g^{-1}$) and/or Pb (5.53 μg $g^{-1}$) in an aqueous matrix comprised by NOM (130 mg C $l^{-1}$) and ASW (3.35 g $kg^{-1}$) in the LLanrwst sediment. Values represent mean ± standard error of the mean (SEM), (n=3).

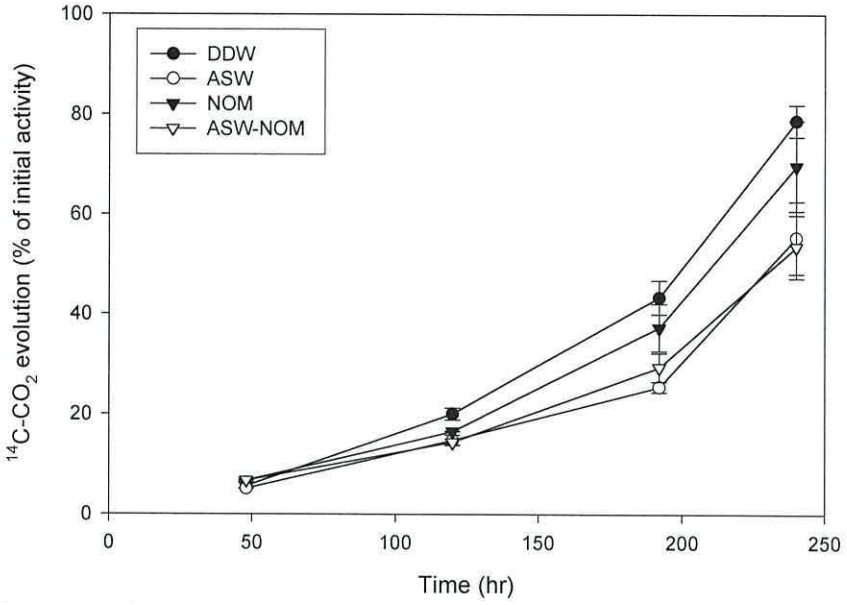



**Figure 4.42.** Matrix effect on the biodegradation kinetics of PPT-Ind in the Llanrwst sediment. NOM (130 mg C $l^{-1}$) and ASW (3.35 g $kg^{-1}$) Values represent mean ± standard error of the mean (SEM), (n=3).

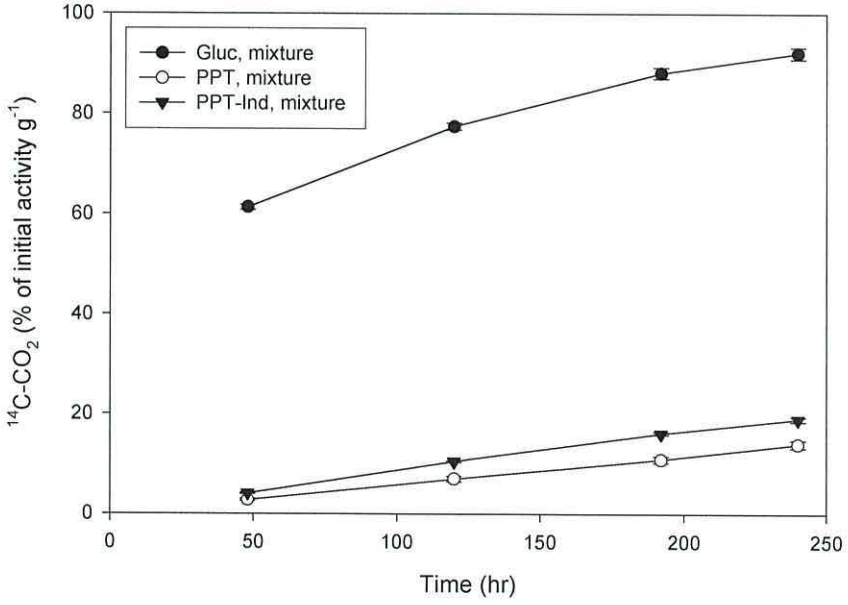



**Figure 4.43.** Biodegradation kinetics of glucose, PPT, and PPT-Ind, in presence of lead and zinc Zn (2.24 μg $g^{-1}$) and Pb (5.53 μg $g^{-1}$) in an aqueous solution comprised by NOM (130 mg C $l^{-1}$) and ASW (3.35 g $kg^{-1}$) in the Llanrwst sediment. Values represent mean ± standard error of the mean (SEM), (n=3).

$5^{th}$ day ($P < 0.05$ ANOVA and Tukey's pairwise comparison). The presence of lead and the combination of lead and zinc in solution had the same effect ($P < 0.05$ ANOVA and Tukey's pairwise comparison). Overall the data followed the same pattern observed for PPT (Pestanal); PPT = PPT-Zn > PPT-Pb = PPT-Zn-Pb.

A comparison of PPT (pestanal) and PPT-Ind degradation in a matrix of zinc, lead NOM and ASW using glucose degradation as a reference is shown in Figure 4.43. The GLM test ($P < 0.01$) indicates that the three semi-logarithmic biodegradation kinetic curves are different to the mean, although there is no significant difference between the Pestanal and industrial grade PPT.

## 4.3. Discussion

### 4.3.1. Sorption and desorption

Sorption of propetamphos (PPT) was found to reach quasi-equilibrium after 8 hours with less than 50% sorbed by the sediments after 24 hours. This is similar to the findings of Fröbe *et al.* (1989) who reported that the organophosphorus pesticides demeton-s-methyl, methidathion, azinphos-methyl and phosalone had completed the sorption process within 5 hours in natural pond sediments. In addition, Sujatha and Chacko (1992) reported that approximately 80% of methyl parathion and 60% of malathion were sorbed onto estuarine sediments after 6 hours. One difference between the method used here and those of Fröbe and

Sujatha was that they air-dried and pulverized their sediments and then stored the samples in a refrigerator prior to performing the sorption experiment. This pre-treatment has been shown to significantly change sediment physicochemical characteristics (Jones and Edwards, 1993). This could, in part, be responsible for the differences observed between these PPT sorption data and those of Fröbe *et al.* (1989) and Sujatha and Chacko (1992). In the present study, the 40 % of PPT remaining in solution was presumably more bioavailable and allowing degradation, bioaccumulation, hydrolysis and/or volatilization reactions to take place (Sims *et al.*, 1991; Lawrence *et al.*, 2000).

The quantity of PPT sorbed onto the sediments is dependent on the initial concentration of PPT in solution. The percentage of sorbed PPT is proportionally higher for low initial PPT concentrations compared with higher initial concentrations. In addition the initial sorption rate (< 1 hr) appears faster at low PPT concentrations. After the initial sorption rate, the rest of the concentration treatments presented similar sorption rates. By comparison, the total amount of PPT sorbed is greater at higher concentrations.

These data can be explained when it is considered that the same amount of sediment adsorption sites were present in all the experiments. For low initial PPT concentrations there were many adsorption sites available but not all the PPT was sorbed. However, at high initial PPT concentrations most of the available sorption sites were occupied relatively quickly and then the rate decreased. It may be possible that, at low concentrations, sorption occurs on the surface of the

solids, whilst at higher concentrations after occupying the surface the sorption occurs in voids or micro pores, or that double or multilayer sorption may occur.

The experimental data fitted well to the three contrasting sorption models tested. The Langmuir equation has been used traditionally to explain the sorption of ions by soils or sediment (Echeverria *et al.*, 1998). This model assumes a monolayer capacity of the sorbent and could cover adsorption based on electrostatic interactions as shown by equation (2) in the introduction. The Freundlich and linear model on the other hand have been used to explain sorption of non-ionic organic molecules. PPT is non-ionic and is stable in aqueous solution (5 mg $l^{-1}$) in sunlight with no chemical degradation (hydrolysis) occurring during 70 hr and half life $t_{½}$ is 1 year at pH 6 (25 °C) (Tomlin, 1994). Considering these facts the Freundlich model was chosen to describe the sorption behaviour of PPT.

The Freundlich model is a purely empirical model that does not explain the sorption mechanism. However some information can be extracted from it, for instance the linear form of the Freundlich isotherm model generally is considered as evidence that the uptake of neutral solute molecules by soil or sediment can be regarded as being a process of partitioning (dissolution) in the sorbent organic matter, rather than physical adsorption (Chiou *et al.*, 1979; 1983). Nonetheless there have been examples of hydrophobic compounds that have presented isotherm nonlinearity (azinphos-methyl and phosalone), indicating that the partition model does not fully reflect the sorption behaviour and that some other mechanisms can participate in the sorption process (Frobe *et al.*, 1989). Even so, Gao *et al.* (1998) suggested that the degree of non-linearity of the isotherm

increases with the increasing hydrophobicity of the pesticides. The PPT octanol/water partition coefficient ($K_{ow}$) is reported to be 6600 ($Log_{10} K_{ow} = 3.82$), showing low solubility (110 μg $ml^{-1}$ at 24 °C) (Tomlin, 1994). The Freundlich constant 1/n in this study was lower than 1 (0.73 – 0.83) (see Table 4.2) and therefore the sorption process may not be entirely due to a single partitioning mechanism, but instead to a combination of different mechanisms depending of the degree of equilibrium in the system. Nevertheless a partitioning mechanism is still considered important in the sorption of PPT.

The fraction of organic matter in the solid sorbent is known to affect the partitioning process (Schlautman and Morgan, 1993). In this work the importance of organic matter was corroborated by the correlation obtained for the carbon content in the sediment and the Freundlich coefficient Log Kf, ($r^2 = 0.99$). In addition the comparison of sorption between three sediment types taken from contrasting stretches of the Conwy River indicates that the degree of sorption is site specific. The non-tidal, LL sediment possessed the highest sorption capacity, while sediments from the tidal estuary (BS and TC) exhibited a similar, lower degree of sorption. The carbon content of the LL sediment was slightly more than double that of the TC and BS sediments indicating again that PPT sorption reaction may be associated with organic matter. In contrast, the LL sediment had approximately the same content of clay as the TC sediment, indicating that sorption on this phase was less important.

The effect of varying matrix DOC had little impact on PPT sorption in any of the sediments examined. Indeed, neither the quantity nor the source of DOM

significantly affected PPT sorption during 24 hours. In contrast, the quality and source of NOM had some minor effects on the sorption after an equilibration time of 48 hours and on desorption after 24 hours. Most of the studies reported in the literature claim that the quality and source of NOM does have an effect on the sorption of hydrophobic organic pollutants (Carter and Suffet, 1982; Schlautman and Morgan, 1993). However, these studies were carried out only in aqueous solution with no sediment or soils. The findings in this work could indicate that the binding forces between the DOM and the PPT aqueous phase are weak while the content of solid OM in the sediment may play a more important role in increasing sorption through stronger PPT-sorbent interactions. The effects detected after 48 hours of sorption may occur because the most favourable PPT sorption sites were already filled and the weak PPT-DOM interactions were more comparable with the weaker interactions from the remaining less favourable PPT-sorbent sites. Considering the data in more detail, fulvic acid (Norway) increased the sorption rate whereas humic acid (Aldrich) and NOM-SR decreased it, compared with each other, but compared to the distilled water treatment, no differences were detected. This effect could be related with the size or the structural properties of the NOM. With fulvic acid (Norway) having a significantly lower MW than humic acid (Aldrich) (see Table 3.38), possibly suggesting HA had more hydrophobic moieties.

The non-consistent effects of salinity on PPT sorption for TC and BS sediment could be due to the differing aqueous matrices. The NOM and ions originally present in the sediment could be dissolved into solution, thus the chemistry solution would inherit the sediment physicochemical characteristics. Schlautman

and Morgan (1993) concluded that the binding of non-ionic hydrophobic organic compounds (anthracene, pyrene and perylene) by Suwannee River humic and fulvic acid was highly dependent upon solution chemistry. Another possible reason is that, when the NOM is mobilized into solution, its structure and chemistry are affected by the ionic composition of the matrix. For instance, the dimensions and hydrophobicity of the NOM voids are sensitive to variations in pH, salt concentration, and cation valence, and consequently the ability to bind hydrophobic organic compounds will change (Schlautman and Morgan, 1993). Also the humic polymer (NOM) becomes less hydrophilic as the ionic strength is increased, becoming more able to bind hydrophobic compounds (Carter and Suffet 1982). Although, NOM has been found to be crucial for PPT sorption, where salinity increases, the mineral fraction may have a more important role to play, since the surface charge in the sediment will change with ionic strength, affecting sorption site availability.

The lack of impact of metals on PPT sorption supports the hypothesis that metals tend to sorb to mineral phases rather than organic matter as for PPT (Echeverria *et al.*, 1998). These authors found that organic matter forms complexes on the surface are more stable for $Pb^{2+}$ and $Cu^{2+}$ than for $Cd^{2+}$, $Ni^{2+}$ and $Zn^{2+}$. However, these data show that PPT sorption is stronger than that with Pb. Though the statistical data was not conclusive and to reach further conclusions more work is needed.

The experiments carried out with industrial grade PPT indicate that the commercial formulation of the pesticide behaves in a similar way to that of the

Analar grade compound. Essentially the only difference was that the Freundlich sorption coefficients were higher for PPT-Ind, than those of the Analar grade compound (see Figure 4.14). It is possible that the aromatic solvent (Shellsol R®) enhances the sorption onto the organic phase in the sediments. This could imply that the industrial formulation is less bioavailable in the estuary.

The sorption kinetics process was also similar in the fact that it had two stages, a fast initial sorption followed by a slower phase until reaching quasi-equilibrium. Only at very high PPT concentration (75 $\mu g\ ml^{-1}$) was a lack of quasi-equilibrium apparent with sorption steadily increasing over time. In TC sediment, and similar to Analar grade PPT, low salinity tended to reduce the sorptivity of PPT-Ind to the solid phase whilst higher salinity produced the opposite effect. As mentioned earlier this mixed behaviour could be due to differences in solution chemistry, changing the properties of NOM, sediment surface charge and PPT. Comparison of these results with other studies was not possible because of an absence of published literature comparing Analar vs. industrial formulation.

The desorption kinetics showed an initial fast desorption phase followed by slow desorption. One theory frequently used to explain this is the slow diffusion of the organic sorbent in sediment aggregates. Wu and Gschwend (1986) hypothesized that desorption rates are consistent with a reversible diffusive exchange mechanism. However, Farrel and Reinhard (1994) showed that none of the physically measurable properties of the solid phase (e.g. size fraction) had any correlation with the fraction of slow desorbing trichloroethylene. Cornelissen *et al.* (1998) concluded that the entrapment process is not the only mechanism that

determines slow desorption, the diffusion through hydrophobic pores should also be considered. These workers all indicate that, with respect to slow desorption, the processes occurring in the OM dominate over processes in the mineral matrix, when OM contents are about 0.1 to 0.5 %. In the present work, desorption also seems to be linked mainly to the OM content of the sediment.

A linear model fitted desorption data well. Further, a considerable difference was observed between the sorption and the desorption isotherms. Consequently, the linear coefficients were quite different. These data indicate that the sorption/desorption processes show hysteresis (Fytianos *et al.*, 2000), meaning that sorption is not completely reversible. The extent of hysteresis has been shown to be related to the physicochemical properties of certain pesticides (Gao *et al.*, 1998). Kan *et al.* (2000) proposed three kinetically different desorption phases. The first phase is the fraction of labile chemical, which readily desorbs. The second and third are fractions of the sorbed chemicals that are entrapped by the soil organic matter. This model predicts that a fraction of the chemical is irreversibly sorbed. The data presented here shows hysteresis and therefore part of the PPT sorbed could be irreversibly sorbed.

Salinity also had a significant effect on desorption of PPT. However, the trends were complex and further work is required to further understand the processes involved.

The concentration of NOM did not show any great effect on desorption of PPT. However, the quality and source of the humic substances did affect the desorption

rate. After a desorption period of 24 hr, the ANOVA results (Table 4.10) indicate that NOM-SR tended to slow down desorption rate in comparison with humic acid (Aldrich) and the distilled water treatment. It was noticed that the quality of NOM could affect the sorption and desorption mechanisms of PPT after some period of equilibration (48 hr for sorption and 24 hr for desorption). This could mean that the possible interactions with humic substances are slower than was earlier thought. This fact enforces the theory that PPT first occupies favourable sites and then slowly starts to fill more weakly sorbing sites. In desorption, the first sites freed are the weak sites, then the more favourable sites start to become vacant.

The PPT-Ind desorption results indicate that desorption also shows hysteresis, similar to Analar grade PPT. The desorption kinetics showed the same fast and slow phases. The percentage of Analar grade PPT desorbed after 48 hrs was 47% and for PPT-Ind it was 43%. Longer equilibration times would be needed to determine if the commercial formulation of PPT is more or less desorbed than the Analar pesticide. Increasing salinity did not affect desorption of PPT-Ind. This could indicate that the solvent (Shelsoll R®) buffers the chemical interactions observed previously in the same experiments with Analar pesticide. More work is needed here to fully understand the desorption processes.

In conclusion, the sorption processes of PPT in Conwy River sediments indicate that the movement of PPT is limited by OM. Most sorption occurs in sediments from the river (fresh water), with the sorption capacity of estuarine sediment considerably lower. Although the influence of salinity was less clear, it does

appear to affect PPT sorption and desorption possibly due to interaction with other components. The effects depend on salt concentration indicating a complex influence on PPT sorption along the Estuary. It seems that the behaviour of PPT-Ind differs from that of Analar grade PPT, possibly due to the presence of solvent in the former. More work is therefore required comparing and using the commercial formulations of pesticides to reach more realistic conclusions that can be used for decision making.

### 4.3.2. Biodegradation

This work has studied the environmental conditions which affect the biodegradation of PPT in sediment-aqueous systems but did not attempt to identify the microbes involved or the degradation mechanisms.

Sharom *et al.* (1980) and Lartiges and Garrigues (1995) have claimed that pesticide persistence in the environment cannot be estimated on the basis of broad chemical groups. However Matsumura (1982) and Diaz *et al.* (1995) have proposed that the chemical characteristics of xenobiotic compound are the most important factor in determining their degree of persistence. Our data show evidence in support of both of these phenomena.

In soils, pesticide molecules may bind tightly to soil organic matter, reducing bioavailability (Aislabie and Lloyd-Jones, 1995). In sediment, the aqueous phase is in contact with the solid phase for most of the time. This may increase

pesticide bioavailability depending on solubility or hydrophobic or hydrophilic properties.

It is known that the organophosphorus pesticides diazinon and parathion, two organophosphorus pesticides, are biologically degraded by the bacterium (ATCC 27551) *Flavobacterium* sp. This is a facultatively anaerobic bacteria which possesses an enzyme capable of hydrolysing the P—O—C phospho ester linkage of diazinon and parathion (Sethunathan and Yoshida, 1973).

The evolution of $^{14}C$-$CO_2$ in the aerobic and anaerobic experiments, which were directly related to PPT biodegradation, suggested that the microbial population of each site was different. LL and TC sediments presented a lag phase which may have indicated that the microbial population was only able to degrade PPT after some "conditioning" to the new aqueous chemistry. In contrast, biodegradation of PPT in the BS sediment was rapid and almost immediate. This information may reflect the "quality" of the three sites (i.e. whether they have a history of pollution). On the other hand Freed *et al.* (1979) indicated that, when microbial and chemical degradations are relatively slow, compounds which are easily hydrolyzed in water may become more persistent when incorporated into soil, if the soil surface is relatively inert. If this possibility is considered for these studies, then PPT may pose a higher risk in TC, where biodegradation of PPT in the sediment from this site presented the slowest biodegradation rate.

The half life of PPT calculated from a first order kinetic equation or derived experimentally indicated that PPT degradation was faster in aerobic than

anaerobic conditions. It is known that in sediments aerobic conditions occur in the top 2 cm with anoxic conditions under that (ERASM, 1999). Therefore if PPT passes below the top 2 cm layer, the biodegradation rate will slow presenting a greater toxic risk to the environment. This is more relevant in sites like LL and TC where the lag phase was observed.

The maximum calculated half life was for the TC sediment (*ca.* 21 days) which is a typical value for an organophosphorus pesticide. For instance Cotham Jr. and Bidleman (1989) reported that the half life of endosulfan I in a seawater/sediment system was 22 days and fenvalerate 12 days (20 °C, pH 7.3-7.7). PPT chemical hydrolysis is extremely slow (Tomlin, 1994) thus it can be concluded that the degradation reported in this work was primarily done by the indigenous microbial population.

Even though degradation was slower under an anaerobic atmosphere, the half life was not drastically different. This may be because the microbial population involved in the biodegradation of PPT was facultative and it had to acclimatise to anoxic conditions. For instance *Flavobacterium* sp is known to hydrolyze diazinon under both aerobic and anaerobic conditions, but has been reported to do so more rapidly under aerobic conditions (Sethunathan and Yoshida, 1973).

Glucose is a well known source of carbon for bacteria, and it has been used in this work as a reference for "normal" biodegradation. It has been considered that the specific bacterial species capable of degrading PPT may not be the only ones

degrading the glucose, thus the degradation of glucose could represent the sum of the degradation activity of all the microbial species in the sediment.

Soluble organic components serve as inducers for the production of enzymes necessary for the mineralization of pesticides. Ou and Thomas (1994) suggested that the addition of glucose was responsible for blocking the enzyme inducers and thus preventing activation of the genes responsible for the mineralization of fenamiphos. However, this did not happen in these studies when glucose and protein were added to the system. The suppressing effect of salinity on glucose and PPT biodegradation could be due to the fact that the indigenous bacteria from LL in the sediment suffered osmotic shock as it is unlikely that bacteria from this freshwater stretch of the river would be expressing a salt tolerance mechanism at the time of substrate addition. It is known that microbes respond readily to stimuli other than to contaminants (Burton Jr., 1991), and therefore these data should be interpreted with caution. For instance the sorption of PPT under high salinity concentrations in some cases is known to increase (see section 4.2.1), and this theoretically would imply that less PPT is bioavailable for biodegradation.

DOC could be considered a major substrate for the microbes inhabiting river sediments. In soils, the microbial activity is considered to be very intimately associated with organic matter content (Sethunathan *et al.*, 1982). It has been reported that the addition of organic material increases microbial activity and in turn accelerates pesticide degradation (Aislabie and Lloyd-Jones, 1995). In the experiments undertaken here the microbial population did not favour DOM instead of glucose or PPT. Sethunathan *et al.* (1982) reported that the stimulatory

effect of added organic matter was less pronounced in soils with high native organic matter content. In the LL sediment tested here organic matter was low (0.4% C) and no stimulatory effect was found which contradicts Sethunathan's report. In addition, the microbial population of LL was also low based on respiration (6.7 pmol $CO_2$ $g^{-1}$ $s^{-1}$). This may not have been sufficient to observe significant effects, since normally a low microbial activity population would be more benefited from the addition of extra substrate. Further, Visser (1985) found that microbes in an organic humus-rich soil were more stimulated by humic substances than organisms from a low organic matter sandy soil, and the LL sediment was characterised as sandy sediment.

It should also be noted that NOM could have acted as a sorption substrate for PPT reducing bioavailability. However, this was not the case as shown in the previous section as NOM did not significantly influence the sorption behaviour of PPT. A similar case was presented by Meredith and Radosevich (1998) in which they found that humic acid had no effect on the degradation of quinoline suggesting that the importance of adsorption to humic acid was insufficient to limit decomposition.

The reduction observed in the early stages of PPT biodegradation ($5^{th}$ day) in ASW and the combined ASW and NOM treatments are most likely due to the environmental stress that an increase in ionic strength had over the microbial population since NOM alone did not have any significant impact. Increasing ionic strength could have affected cell membrane permeability and the microbial population could have been reduced by the environmental change. Polonenko *et*

*al.* (1986) studied the effects of salinity on growth and displacement of soil bacteria and they concluded that salt stress either displaced non-viable bacteria, preferentially, from the soil or that salinity was lethal to certain components of the population. The observations presented here could just be a reflection of the microbial population tolerance. For example Shiaris (1989) studied the effect of varying salinity on phenanthrene mineralization in sediments along a natural salinity gradient in an urban tidal river and suggested that phenanthrene degraders in low salinity estuarine sediments subject to salt water intrusion are tolerant to a wide range of salinities. By comparison, Wang and Lenahan (1989) compared the biological and chemical degradation of fenthion in water subjected to ionic effects and concluded that higher salinity resulted in a shorter half-life.

The absence of any effect of Zn on glucose degradation may be an indication that the sediment microbial community was acclimatised to Zn. This reflects earlier data presented in this thesis which found elevated Zn levels in water and sediment in the Conwy River and Estuary. However, after the biodegradation experiment, the aqueous solutions were analysed and Zn concentrations were below detection limits suggesting that Zn was not readily bioavailable. The same sort of behaviour could have been expected in the PPT degradation experiments, but a significant reduction in $^{14}C$-$CO_2$ evolution was observed. Since it has been considered that Zn was not readily bioavailable at these concentrations, the possibility that PPT in combination with Zn may had become more toxic than on its own will be explored in the following chapter.

In contrast, Pb exhibited a more complex behaviour; the positive impact on glucose degradation registered at the 10th day (see Figure 4.30) suggests that the performance of the microbes is enhanced by Pb in solution. However, PPT degradation was considerably reduced in the presence of Pb, thus again indicating that the combined effect of PPT and Pb may stress the microbial population, (the "cocktail" effect). At these concentrations Pb was sorbed by the sediments after 10 days (results not shown).

The differences between glucose and PPT degradation in the same conditions may be explained by the different ways in which microbes degrade these organic compounds. For instance it is expected that the transformation and mineralization of pesticides is co-metabolic. If the product of the action of one of these enzymes involved in catalysing an initial reaction is not a suitable substrate for any other enzyme in the organism, that compound will accumulate, so that the products of one organism can be utilized by another as a growth substrate (Alexander, 1980).

Degradation of PPT in the presence of Zn or Pb may be affected because the microbes had to deal simultaneously with more than one toxic compound. In this situation, their response had to be divided to be able to cope with both compounds, and the biodegradation of PPT could be reduced because of the physiological response to the extra stress. Additionally, the specific activity of the enzyme responsible for the hydrolysis of PPT could have been directly affected by the presence of Zn and Pb. For example Brown (1979) demonstrated that the specific activity of the phosphotriesterase of *Flavobacterium* sp was not affected by the separate inclusion of $Zn^{2+}$, $Cu^{2+}$, $Co^{2+}$, $Cd^{2+}$, $Mn^{2+}$ and $Mg^{2+}$. However,

this enzyme differs distinctly from phosphotriesterases observed in other micro-organisms with respect to inhibition or activation by metal ions, and in substrate specificity.

The last group of experiments confirmed the results presented previously with PPT degradation related to aqueous solution chemistry following the order DDW = NOM > ASW > ASW & NOM. Once again it has to be stressed that these experiments were carried out on fresh water LL sediment. When the salinity of the aqueous solution increased the cellular membrane of the inherent microbial population could have shrunk (Downing, 1997) ultimately affecting the cellular membrane performance to reducing transport of enzymes and respiration products.

Organophosphorus insecticides undergo hydrolysis by a number of different enzymatic processes, hydrolysis may occur at the ester or acid anhydride bond (Wyman and Ballard, 1982) resulting in phosphate and organic by products, from which $CO_2$ and water will result (the end point of these studies).

The response obtained in the presence of Pb and Zn indicated that the presence of Zn did not affect PPT biodegradation. LL was one of the sites in which the pore water had a high content of total Zn (50 – 6288 $\mu g\ g^{-1}$). Presumably the microbes were adapted to this condition, and hence, their ability to degrade PPT was not affected by additional Zn. On the contrary, the presence of Pb in all the matrices reduced PPT degradation. Pb was also found in LL sediment but in a lesser amount to Zn (99 – 612 $\mu g\ Pb\ g^{-1}$). Pb seems to affect the performance of the

microbial population when PPT is the subject of degradation even if microbes are supposed to be conditioned to Pb. Therefore the possible answer is that the combination of Pb and PPT may be more toxic for the microbial population.

Biodegradation of Industrial grade propetamphos (PPT-Ind) had the same behaviour as pure PPT. Thus the same explanations are applied with the addition that the aromatic solvent (Shell R®) in which PPT is dissolved did not have an extra effect in PPT biodegradation at least at the concentration used for the experiments (1 $\mu g\ g^{-1}$).

# Chapter 5. Ecotoxicology of propetamphos in the presence of selected metals

## 5.1. Introduction

The present study was undertaken to investigate the combined ecotoxicological effects of PPT in combination with selected metals. According to Chapman (1995), three major types of effects can change an ecosystem. The first type and probably the most important are physical effects; in particular habitat change which is the major cause of species extinction (e.g. dredging activities). Secondly, biotic changes which can occur naturally, (e.g. competition and predation among and within communities), or caused by human intervention via either the deliberate or accidental introduction of new species. The third effect results from anthropogenic pollution which implies harm to a living resource or risks to human health. This often takes the form of excessive chemical contaminant release which causes toxicity. Here contamination is defined as being an artificial increase of a chemical above the background level.

Ecotoxicology has been defined in Solomon (1999) as the "study of toxicity-mediated effects on assemblages of two or more species of organisms". He also stressed the ecological importance of populations which are less sensitive than their most sensitive member and likewise, that functions of communities and ecosystems are less sensitive than their most sensitive components.

Chapman (1995) identified four levels of biological organization that can be affected by contaminants: biochemical and cellular (i.e. biomarkers); whole organism; population; and community (a microbial community being the fourth level of biological organization).

The key role that microorganisms play in the ecology of soils and sediments has been acknowledged for some time (van Beelen and Doelman, 1997; Eismann and Montuelle, 1999). For instance, the mineralization of dead biomass and organic matter is performed by bacteria, and bacteria are also responsible for the degradation of many pollutants in the environment. Natural microbial processes in soils and sediments are usually performed by different communities of microbial species, whilst some species might be sensitive to specific pollutants others can be insensitive. However, it has been recognized (Eismann and Montuelle, 1999) that a change in microbial community structure in sediments will have consequences for the higher trophic levels and for the environmental status of the overlying water column as well.

From a practical standpoint the usefulness of monitoring the microbial community is due, in part, to its ability to respond quickly to environmental conditions (e.g. toxicant exposure). In addition, the experiments are simple to perform, have low costs and time requirements, and can indicate effects at the enzymatic, cellular and community levels (Burton Jr., 1991; Eismann and Montuelle, 1999). It has been considered that pollutant effects must be greater in microoganisms than in macrofauna because the former

reproduce and adapt relatively quickly. In addition, tests with microorganisms often measure a process performed by a microbial community (van Beelen and Fleuren-Kemilä, 1999). On the other hand, microbes respond readily to stimuli other than to contaminants, and therefore data need to be interpreted with caution (Burton Jr., 1991).

The permeability of the cells to environmental toxicants is believed to be an important aspect of toxicity studies using bacteria. The cell outer membrane is an effective diffusion barrier against hydrophobic substances and the diffusion of hydrophilic compounds is restricted by specific membrane proteins that form water-filled channels (Bitton and Koopman, 1992).

Metabolic parameters such as growth, enzyme activity, respiration and conversion of substrates are the preferred end points to assess toxicant effects in microbial studies (Eismann and Montuelle, 1999). Specifically, the impact of toxic chemicals on the carbon cycle is conveniently determined by measuring microbial respiration (Bitton and Koopman, 1992) and the addition of easily degradable substrates (e.g. glucose or acetate) enhances respiration. It is said that this higher activity can facilitate the measurement of the effects of pollutants (van Beelen and Doelman, 1997).

The information obtained by microbial methods includes ecological and toxicological aspects. In this context, Eismann and Montuelle (1999) recognised that microbial ecotoxicology can permit a more comprehensive image of polluted ecosystems and historically the assessment of effects of pollutants in sediments has become a well-

accepted branch in ecotoxicology (Eismann and Montuelle, 1999).

In aquatic environments, micro and macro fauna are exposed to many different combinations of toxicants in various environmental conditions (Forget *et al.*, 1999). In view of this situation there have been increasing attempts to evaluate the joint toxic effects of multiple chemical mixtures. The importance of assessing the toxicant effect of mixtures is based on the premise that they may have a greater impact than individual toxicants (Nirmalakhandan *et al.*, 1994).

Quantitatively, toxicology has focussed on population tolerance distributions, which is measured in $LC_{50}$ and $EC_{50}$ tests. The $LC_{50}$ (or $EC_{50}$) is the chemical concentration at which 50% of the population is affected. Virtually any sublethal effect can be used to estimate a population median effect concentration ($EC_{50}$) but for the median lethal concentration ($LC_{50}$), death is used as a measure of effect (Forbes and Forbes, 1994). In environmental terms, $EC_{10}$ plays a major role since it has been found that a 10% inhibition of a process can be accompanied by a 50% inhibition of the most sensitive species in a community (van Beelen and Fleuren-Kemila, 1999). Other parameters that have been frequently used to evaluate the ecotoxicological effect of pollutants are the no observed effect concentrations (NOEC), the lowest observed effect concentration (LOEC) and the maximum toxicant concentration (MATC) which is the geometric mean between NOEC and LOEC. However, these values cannot be compared quantitatively because of their high variability, therefore Chapman (1995) suggested that they should not be used for regulatory purposes. In addition, $EC_{10}$ is the preferred endpoint for

toxicity tests with microbial processes (van Beelen and Doelman, 1997). A description of the calculation of all these parameters is presented in Chapter 2.

The model used to evaluate the joint action of toxicants in this work is the one formulated by Marking and Dawson (1975). It is based upon the toxic unit concept; the sum of action of various components of a mixture is represented by the formula

$$\frac{Am}{Ai} + \frac{Bm}{Bi} = \mathrm{S} \quad \ldots\ldots\ldots \quad (6)$$

where $A$ and $B$ are chemicals, and $i$ and $m$ are the respective toxicities ($EC_{50}$) of $A$ or $B$ individually ($i$) or in a mixture ($m$), and S is the sum of activity. Therefore, if it is assumed that $A$ and $B$ are equitoxic and have similar modes of toxic action, then the fractional combination of two chemicals considered to be equitoxic would have the same effect as one toxic unit of either material. These values can be substituted into the formula (1): for instance, if each chemical contributes ½ toxic units,

$$\frac{Am}{Ai} + \frac{Bm}{Bi} = \frac{1}{2} + \frac{1}{2} = 1.0 \text{ of S}$$

Marking and Dawson (1975) devised a system in which additive, greater than additive, or less than additive effects are represented by zero, positive or negative values, respectively in a linear scale. This is shown in Figure 5.1. If the additive index (AI) is positive then the effect of the mixture is greater than additive and if the AI is negative then the toxic

effect is less than additive. When AI values equals zero, then the toxic effect is only additive.

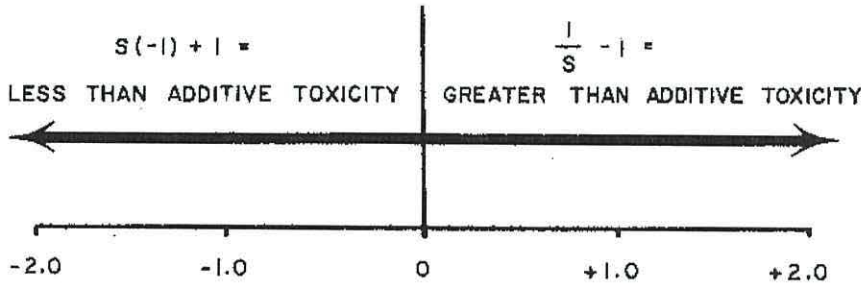



**Figure 5.1**. Sums (S) of toxic contributions for a chemical mixture were corrected into additive index (AI) in a linear system which follows a direction of plus and minus values. Marking and Dawson (1975).

The significance of deviation from zero can be determined by substituting 95% confidence intervals for the $EC_{50}$ values into the additive index formula to establish a range for the additive indices. The lower limits of the individual toxicant (*Ai* and *Bi*) and the upper limits of the mixtures (*Am* and *Bm*) can be substituted for $EC_{50}$ to determine the lower values of the index. Correspondingly, the upper limits of the individual toxicants and the lower limits of the mixtures can be substituted into the formula to determine the upper value of the index. In the past, mixtures that resulted in ranges for the AI that overlapped zero were judged to be only additive in toxicity: ranges that did not overlap zero are either greater or less than additive in toxicity (Marking and Dawson, 1975)

Metals and pesticides are among the major contaminants of the estuarine environment as mentioned throughout this work. The mode of action of metals is through metabolic pathways (Maher, 1999), whereas OP pesticides are direct anticholinesterase (ACHe) inhibitors (Forget *et al.* 1999). Organophosphorous compounds containing a P-S double bond (e.g. parathion and propetamphos) show a significantly lower inhibition of ACHe than the corresponding compounds having a P-O double bond (e.g. paraoxon; DuBois, 1961; Galli *et al.*, 1994). During the degradation of PS-compounds in soil, PO-analogues are the major breakdown products and therefore they can play an important role in the overall toxicity of pesticides towards aquatic organisms (Galli *et al.*, 1994). Other modes of action have been suspected for organophosphorus pesticides such as fenitrothion and thiomethon including possible metabolic activation as the inhibition of ACHe was not directly correlated to their toxicant effect (Galli *et al.*, 1994)

In addition, the potential availability of metals and possible toxicity may be influenced by chemical and physical reactions and factors such as oxygen/redox gradients, pH, temperature, adsorption, sedimentation, complexation, precipitation and sediment grain size (Forstner and Wittmann, 1983). Further, many common sediment bacterial communities can metabolize and alter metal valence states *via* oxidation-reduction reactions, thereby altering their chemical fate and toxicity (Wood, 1987). Finally, the presence of even small amounts of dissolved organic matter in an aqueous solution can significantly enhance the apparent water solubility of a hydrophobic organic compound (Guetzloff and Rice, 1996). Metals can also be scavenged by chelating agents or adsorbed by the sediment matrix, or by high molecular organic matter (Livens, 1991). As

a consequence a pollutant fraction may remain more or less bioavailable and toxic (Eismann and Montuelle, 1999). The present Chapter therefore deals with the toxicant effect of PPT, zinc and lead in binary and ternary combinations. The presence of dissolved organic matter on their toxicological potential has also been assessed.

## 5.2. Results

The following experiments were designed to measure the physiological response of the sediment's indigenous microbial population following the addition of propetamphos, lead and/or zinc. $^{14}$C-glucose was added as an organic substrate at a concentration of 4.5 $\mu g\ g^{-1}$, and the respiration product ($^{14}C$-$CO_2$) was measured 60 minutes after the spike was added. It was expected that the rate of glucose uptake by the sediment microbial community, or at least those that prefer glucose to other organic or inorganic substrates present, would be affected by the increasing PPT or metals concentrations either individually or in combination.

In order to simplify the experimental design, only sediments from LL were tested. The elemental composition of the sediment in question indicated a small content of carbon (Table 5.1.). The microbial activity was also evaluated by measuring the average respiration of the sediment. This was low (0.33 pmol $CO_2\ g^{-1}\ sec^{-1}$), indicating that this sediment in particular had a low microbial activity. The effect of NOM in solution (40 mg C $l^{-1}$) was also tested.

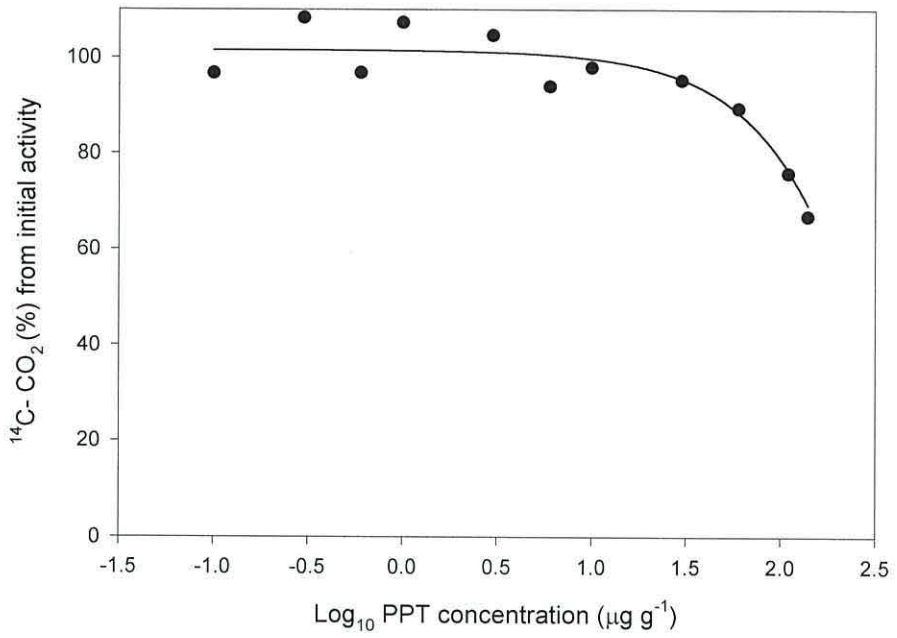
The experiment to determine the effect of PPT on microbial respiration was repeated three times on different occasions. The results were averaged and the error was determined to verify the reproducibility of the experimental procedure. The error (s ± 9.3 μg $g^{-1}$), see Table 5.2, was acceptable considering that a physiological response of a heterogeneous indigenous bacterial community was being measured.

**Table 5.1**. Physico-chemical characteristics of the LL sediment used to test the toxicant effects. Standard errors were less than 5%, (n=3).

| % C | % H | % N | Respiration $CO_2$ (pmol $g^{-1}$ $sec^{-1}$) | pH |
|---|---|---|---|---|
| 0.39 | 0.48 | 0.04 | 0.33 | 5.7 |

The empirical equations fitted to the data typically showed good correlations and the lowest $r^2$ recorded was 0.88. Figure 5.2 and 5.3 show some examples of the regressions applied to the data for the toxic effects of single contaminants. Figure 5.2.a shows that the microbial population has a certain resistance to low PPT concentrations (up to 10 μg $g^{-1}$), but above these concentrations the evolution of $^{14}C$-$CO_2$ drops considerably. In contrast, a toxic zinc effect (Figure 5.2.b) was observed even at low concentrations, and the $^{14}C$-$CO_2$ rate decreased in a clear pattern as Zn concentration increased. On the other hand, lead (Figure 5.3.a) seems to have a similar effect to PPT. The microbial population is more resistant to lead than Zn with a decline in respiration only observed at concentrations ≥ 500 μg $g^{-1}$. The impact of PPT-Ind on glucose mineralization followed a sigmoidal pattern (Figure 5.3.b), with no effect observed up to 1 μg $g^{-1}$,

(a)




(b)

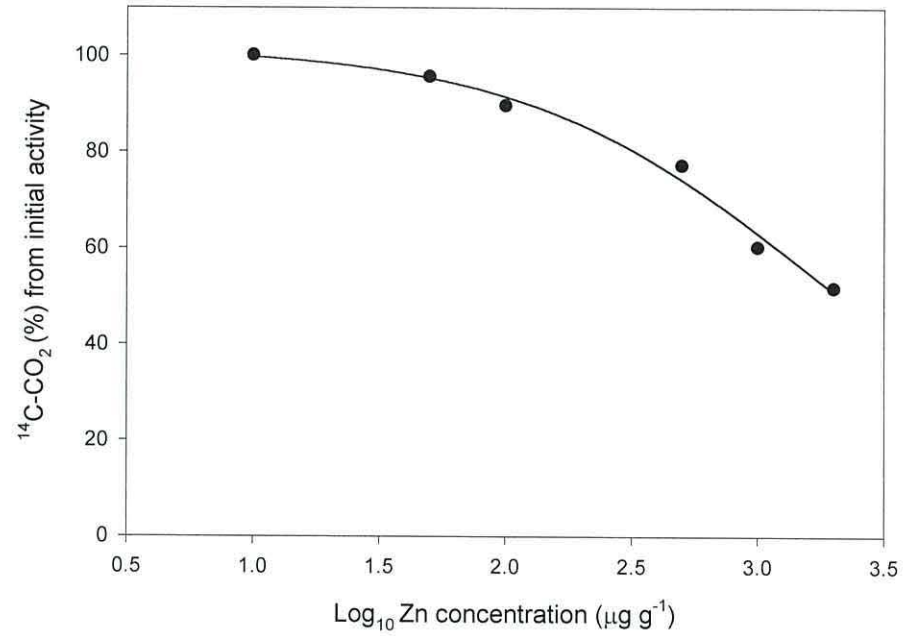
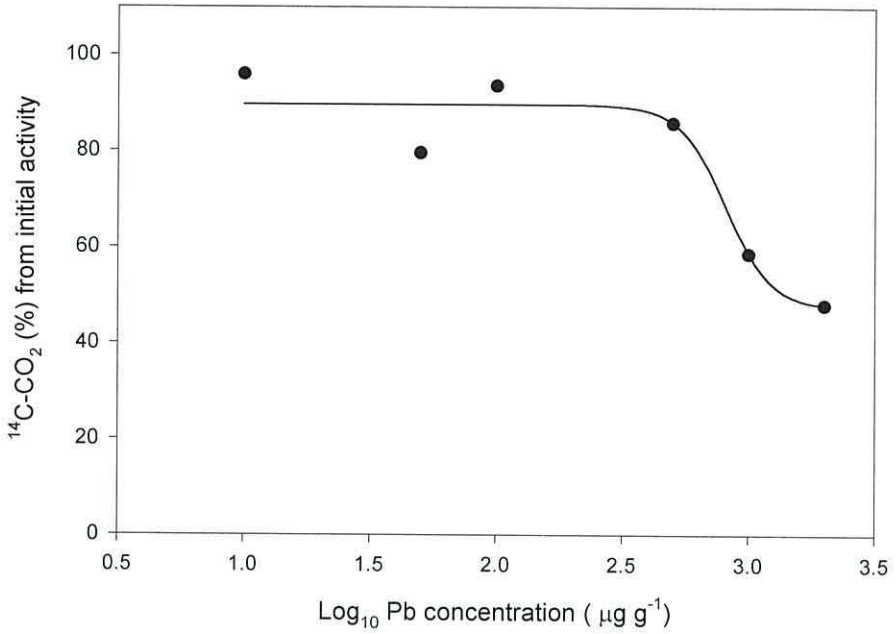



**Figure 5.2**. The effects of (a) PPT and (b) zinc on the mineralization of $^{14}C$-glucose after 60 min in a (1:1) sediment:aqueous solution ratio, in a distilled water (DDW) system. The curves show the best fit to theoretical equations.

(a)




(b)

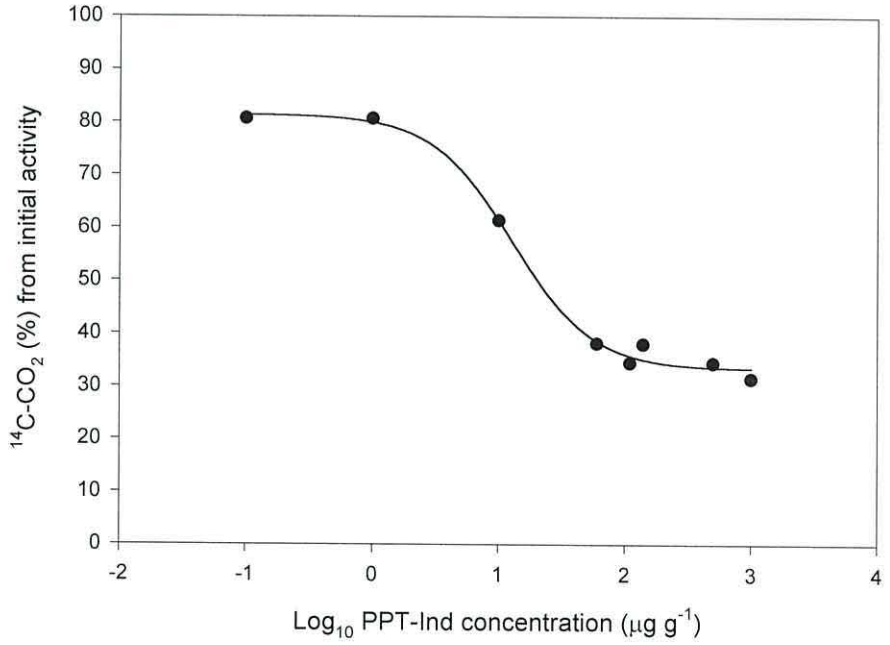



**Figure 5.3**. The effects of (a) lead and (b) PPT-Ind on the mineralization of $^{14}C$-glucose after 60 minutes in a (1:1) sediment:aqueous solution ratio, in a distilled water (DDW) system. The curves show the best fit to theoretical equations.

after which the production of $^{14}C$-$CO_2$ dropped but then seemed to stabilize. In comparison, the $EC_{50}$ of PPT was considerably higher (236 μg $g^{-1}$) than obtained for PPT-Ind (21 μg $g^{-1}$), indicating that the commercial formulation of the pesticide induced a 10 fold stronger toxic effect over the LL sediment microbial population.

**Table 5.2**. $EC_{50}$ in μg $g^{-1}$, in brackets 95% confidence limits. PPT, zinc, lead and PPT-Ind were applied to the LL sediment in a distilled water matrix or one containing dissolved NOM (40 mg C $l^{-1}$). Experiments were run at 15 °C with the exception for PPT-Ind. Values represent means ± SEM with 95% confidence intervals in brackets.

| **Pollutant** | **$EC_{50}$ (μg $g^{-1}$)** | |
|---|---|---|
| | **DDW** | **NOM (40 mg C $l^{-1}$)** |
| **Propetamphos** | 236 ± 9; n=3<br>(187 – 448)* | 404<br>(120 - > 1000) |
| **Propetamphos Ind. grade** | 14.0 @ 22 °C<br>(6.6 – 30.9)<br>20.5 @ 15 °C<br>(16.2 – 35.5) | 23.0 @ 22 °C<br>(16.4 – 38.0) |
| **Zinc** | 2127<br>(1122 - 4467) | 5248<br>(1622 - 10000) |
| **Lead** | 1445<br>(447 -10000) | 2065<br>(371- 10000) |

*average of 3 experiments

The commercial formulation of PPT was tested at 5 and 22 °C (Table 5.2). The

increase in temperature appears to reduce the $EC_{50}$ (14 μg $g^{-1}$) making the PPT – Ind appear more toxic.

The same compounds (PPT, zinc, lead and PPT-Ind) were tested again but this time in the presence of HS in the form of NOM-SR (40 mg C $l^{-1}$) added to the aqueous solution. The $EC_{50}$ values calculated for these experiments are also presented in Table 5.2. The presence of NOM approximately doubles the $EC_{50}$ values of PPT, zinc and lead. The $EC_{50}$ of PPT-Ind was also increased in the presence of NOM but only by *ca.* 60%.

The MATC, LOEC and NOEC were deduced statistically and for comparison the $EC_{10}$ values were also calculated. These results are shown in Tables 5.3 and 5.4. At this low end of toxicological effect, the addition of NOM did not appear to have a positive effect on the MATC of either pure or commercial grade PPT (Table 5.3). The MATC for PPT was 85 μg $g^{-1}$ either in presence or NOM absence, while for the PPT-Ind in both experiments the MATC was 5.5 μg $g^{-1}$. On the other hand, for zinc and lead, the addition of NOM significantly reduced their MATC. Considering the $EC_{10}$ values obtained for the same experiments, in general the $EC_{10}$ values are lower than the MATC values. The addition of NOM to the sediments seems to have a varied effect on the $EC_{10}$ values of these compounds. In the case of lead, $EC_{10}$ could not be calculated because the theoretical equation fitted to this experiment showed an initial response lower 10% inhibition (see Figure 5.3.a). In general, the calculation of $EC_{10}$ values seemed to be more sensitive than MATCs but, at the same time, this depended on how well the data was fitted to a specific model, and also to the sensitivity of the

analytical method at low concentrations.

**Table 5.3**. Values of maximum toxicant concentration (MATC) in $\mu g\ g^{-1}$, for the addition of PPT, PPT-Ind, Zn and Pb to the LL sediment in a distilled water matrix (DDW) or one containing dissolved NOM (40 mg C $l^{-1}$). The values in brackets are the lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC).

| **Compound** | **MATC [NOEC – LOEC]; ($\mu g\ g^{-1}$)** | |
|---|---|---|
| | **DDW** | **NOM (40 mg C $l^{-1}$)** |
| **PPT** | 85 [60 – 110] | 85 [60 – 110] |
| **PPT-Ind** | 5.5 [1 – 10] | 5.5 [1 – 10] |
| **Zinc** | 750 [500 – 1000] | 300 [100 – 500] |
| **Lead** | 750 [500 – 1000] | 300 [100 – 500] |

The Llanrwst (LL) sediment was analysed to determine the metal fractions in various pools and the results are presented in Table 5.5. Aluminium and iron were the most abundant metals in the sediment, while lead and zinc were also present in appreciable concentrations. Zinc was present in each of the three fractions measured, however, the majority was recovered in the HCl extractable fraction (95 %). Lead was almost exclusively in the last fraction.

**Table 5.4**. $EC_{10}$ values in $\mu g\ g^{-1}$ for the addition of PPT, PPT-Ind, Zn and Pb to LL sediments in a distilled water matrix (DDW) or one containing dissolved NOM (40 mg C $l^{-1}$). The values in brackets are the 95% confidence limits.

| Compound | $EC_{10}$ ($\mu g\ g^{-1}$) | |
|---|---|---|
| | DDW | NOM (40 mg C $l^{-1}$) |
| PPT | 53.7 (18.2 – 87.1) | 6.94 (0.1 – 40.73) |
| PPT-Ind | 0.33 (0.10 – 2.00) | 0.13 (0.10 – 1.32) |
| Zinc | 120 (10 – 331) | 138 (0 – 501) |
| Lead | NA | 136 (0 – 676) |

**Table 5.5.** Metal fractionation within the LL sediment used to measure the toxicological effect of organic and inorganic pollutants. ND = below detection limit. Standard errors were less than 5%, (n=3).

| Element | Total ($\mu g\ g^{-1}$) | Water extraction (%) | KCl extraction (%) | HCl extraction (%) |
|---|---|---|---|---|
| Al | 239.6 | 0.1 | 0.03 | 99.9 |
| Cd | 0.6 | ND | 10.8 | 89.2 |
| Cu | 2.4 | ND | ND | 100.0 |
| Fe | 192.9 | 1.1 | ND | 98.9 |
| Ni | 1.1 | ND | ND | 100.0 |
| Pb | 8.7 | ND | ND | 100.0 |
| Zn | 50.5 | 0.2 | 4.6 | 95.1 |

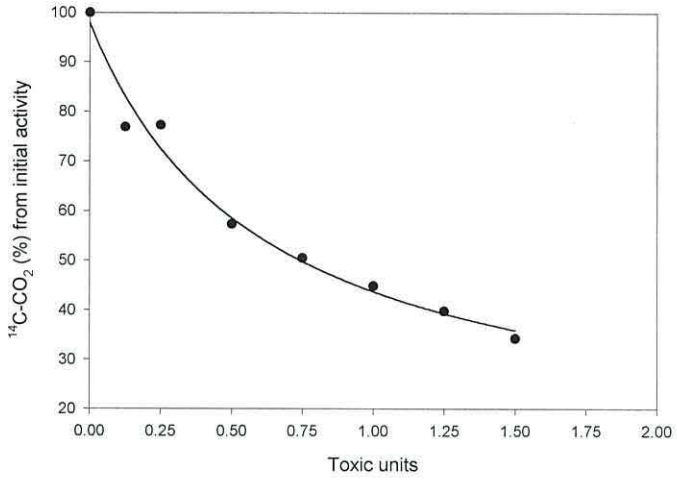
### 5.2.1. Effects of binary combinations

The following experiments were carried out to test the toxicological effect of binary combinations of pollutants in LL sediment. The data was treated in the same way as described previously with the only difference being that the toxic unit (TU) replaced the toxicant concentration as explained in the introduction. Examples of the plots of empirical equations that best fitted these data are shown in Figure 5.4. These binary combinations of toxicants had an additive effect, because the lower end of the additive index overlapped with zero (Table 5.6).

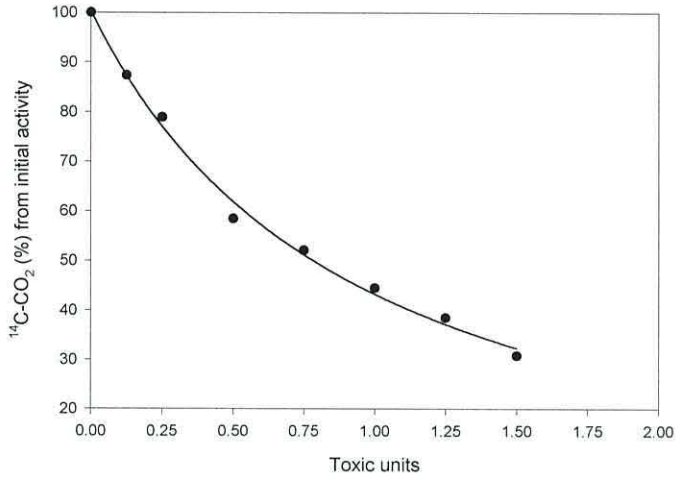
**Table 5.6**. Toxicity of binary combinations of PPT, Zn and Pb, expressed as $EC_{50}$ or *Am* and *Bm* (50 % inhibition in the mixture), sum of activity (S) and additive index (AI). Values in brackets are the 95% confidence interval for $EC_{50}$ and S and the range for AI.

| **Toxicant ratio 1:1** | **$EC_{50}$ ($\mu g\ g^{-1}$)** | **Sum of activity** | **Additive index** |
|---|---|---|---|
| PPT (*Am*)<br>&<br>Zinc (*Bm*) | 87.4; (68.5 – 107.5)<br><br>787.0; (616.8 – 967.8) | 0.74<br>(0.58 – 0.91) | 0.35<br>(-0.44 – 2.40) |
| PPT (*Am*)<br>&<br>Lead (*Bm*) | 93.3; (83.3 – 101.6)<br><br>570.9; (509.5 – 621.5) | 0.79<br>(0.70 – 0.86) | 0.26<br>(-0.93 – 3.22) |
| Zinc (*Am*)<br>&<br>Lead (*Bm*) | 691.3; (489.2 – 989.1)<br><br>469.7; (332.4 – 672.1) | 0.66<br>(0.46 – 0.93) | 0.54<br>(-1.40 – 3.22) |

(a)




(b)




(c)

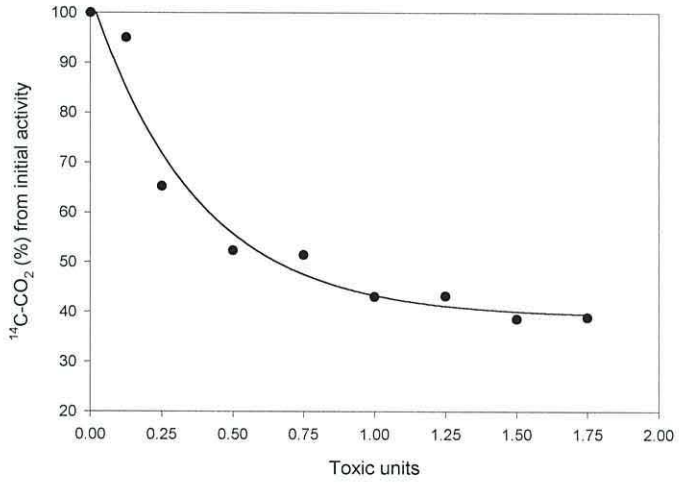


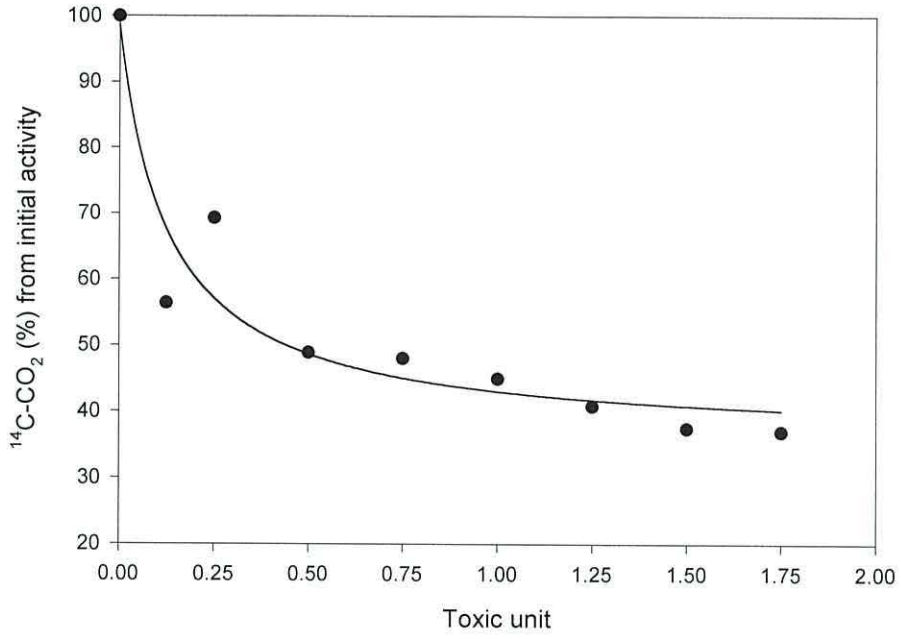

**Figure 5.4**. The effects of equitoxic binary combinations of (a) PPT and zinc, (b) PPT and lead and (c) zinc and lead on the mineralization of $^{14}C$-glucose after 60 minutes in a (1:1) sediment: aqueous solution ratio, in a distilled water (DDW) system. The curves show the best fit to theoretical equations.

### 5.2.2. Effects of ternary combinations

The "cocktail" effect was tested in the presence or absence of NOM (40 mg C $l^{-1}$). The curves for the best fitted empirical equations are shown in Figures 5.5 and 5.6. The toxicant effect of the ternary combinations in these experiments was additive following the criteria given by Markin and Dawson (1975), (Table 5.7), for pure and industrial grade PPT. The addition of NOM had a positive effect by reducing the toxic response from Pestanal PPT in combination with zinc and lead. However for the commercial formulation of the pesticide (PPT-Ind) in combination with zinc and lead, NOM had no appreciable effect on the toxic response from sediment microorganisms.

The last experiment to measure the toxic effects of the ternary combination was carried out using a more realistic ratio, of 1:100:10 (PPT:zinc:lead), i.e. close to the concentrations found in natural samples. The additive index was 0.35 and the calculated range indicated a more than additive effect, with concentrations as low as 0.52; (0.46 – 0.61) μg $g^{-1}$ of PPT; 472.0; (415 – 555) μg $g^{-1}$ of zinc and 32.1; (28.2 – 37.7) μg $g^{-1}$ of lead. The $EC_{10}$ values calculated for this experiment were 0.06; (0.03 – 0.08) μg $g^{-1}$ for PPT; 51.0 (25.0 – 76.0) μg $g^{-1}$ for zinc and 3.5 (1.7 – 5.2) μg $g^{-1}$ for lead.

(a)




(b)

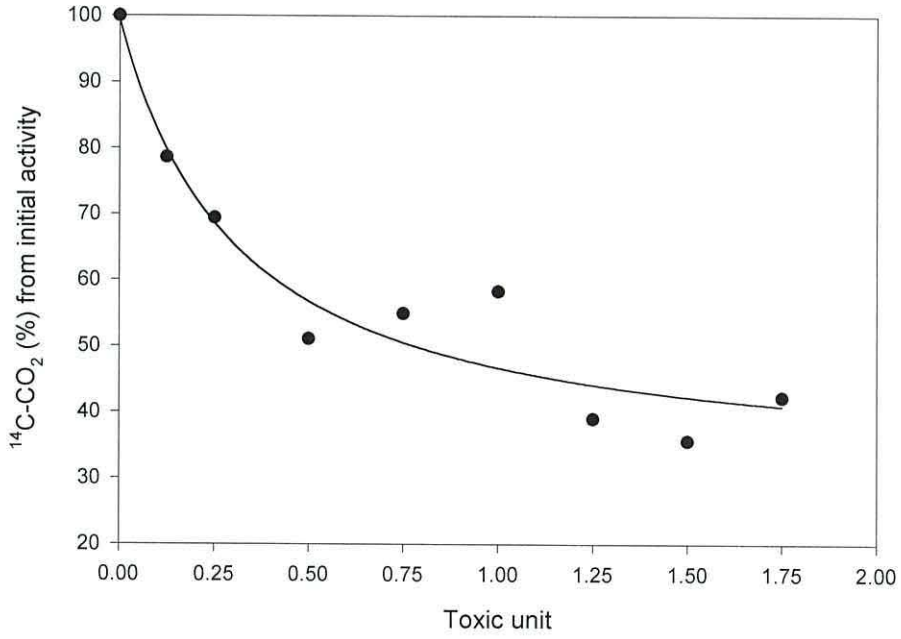
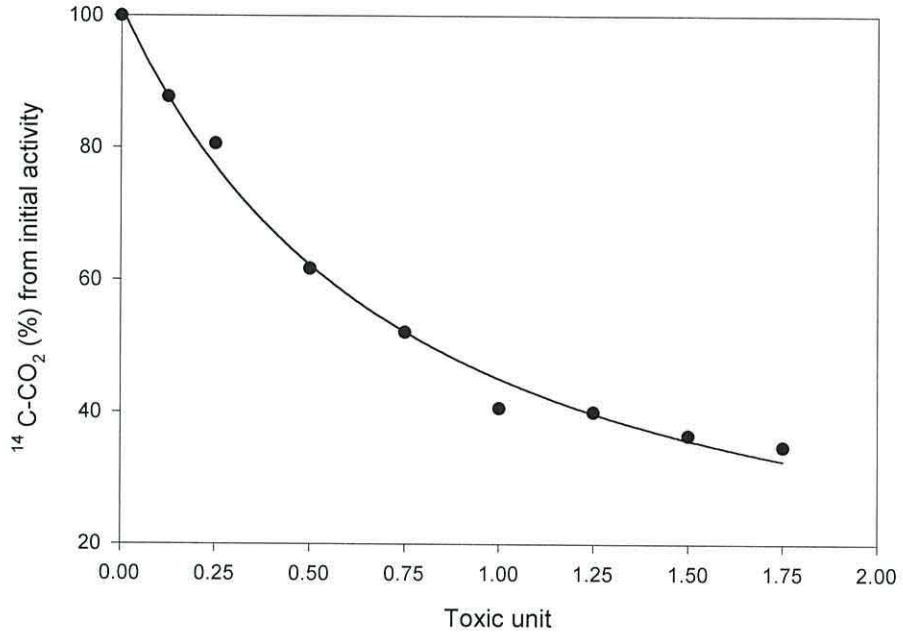



**Figure 5.5**. The toxic effect of equitoxic ternary combinations of PPT, zinc, and lead in (a) DDW and (b) NOM (40 mg C $l^{-1}$) on the mineralization of $^{14}C$-glucose after 60 minutes in a (1:1) sediment: aqueous solution ratio. The curves show the best fit to theoretical equations.

(a)




(b)

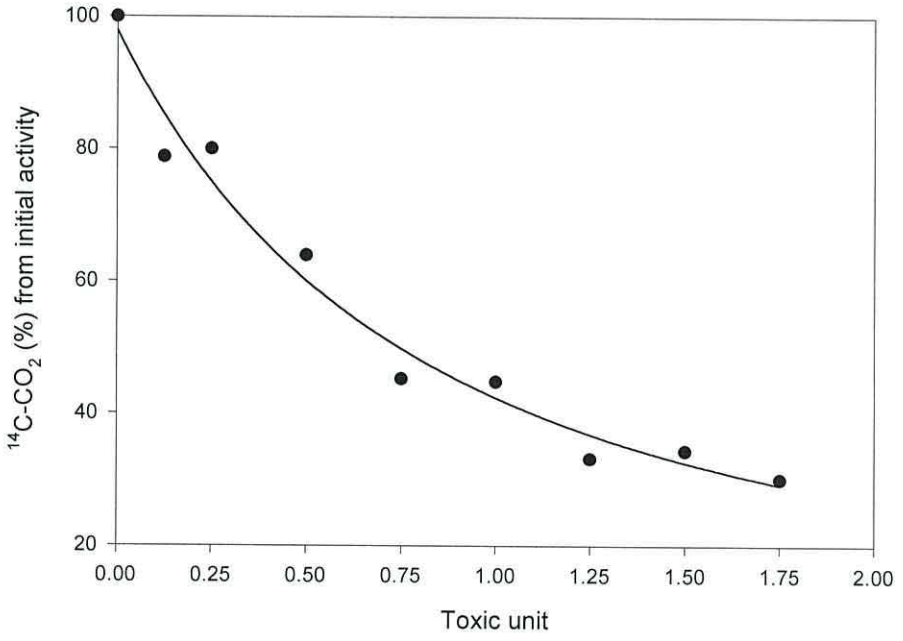



**Figure 5.6**. The effect of equitoxic ternary combinations of industrial grade PPT (PPT-Ind), zinc, and lead in (a) DDW and (b) NOM (40 mg C $l^{-1}$) on the mineralization of $^{14}$C-glucose after 60 minutes in a (1:1) sediment: aqueous solution ratio. The curves show the best fit to theoretical equations.

**Table 5.7.** Toxicity of ternary combinations in equitoxic ratios of PPT, PPT-Ind, Zn and Pb, expressed as $EC_{50}$ or *Am, Bm* and *Cm* (50 % inhibition in the mixture), and additive index (AI). Chemicals were applied to LL sediments in a distilled water matrix (DDW) or one containing dissolved NOM (40 mg C $l^{-1}$). Values in brackets are the 95% confidence interval for $EC_{50}$ and the range for AI.

| **Toxicant** | **DDW** | | **NOM (40 mg C $l^{-1}$)** | |
|---|---|---|---|---|
| **Ratio 1:1:1** | **$EC_{50}$ (µg $g^{-1}$)** | **Additive index** | **$EC_{50}$ (µg $g^{-1}$)** | **Additive index** |
| PPT (*Am*) | 34.6; (28 – 41) | 1.27 (-0.10 – 6.26) | 60.6; (37 – 126) | 0.29 (-2.82 – 9.75) |
| Zn (*Bm*) | 312; (255 – 369) | | 546; (333 – 1134) | |
| Pb (*Cm*) | 212; (173 – 250) | | 371; (226 – 770) | |
| PPT-Ind (*Am*) | 4.7; (4.2 – 5.2) | 0.22 (-1.34 – 2.48) | 4.3; (3.3 – 5.1) | 0.33 (-0.85 – 5.38) |
| Zn (*Bm*) | 581; (518 – 645) | | 532; (411 – 631) | |
| Pb (*Cm*) | 395; (352 – 438) | | 361; (279 – 429) | |

## 5.3. Discussion

The experiments described here were devised to assess the toxic effects of organic and inorganic pollutants on the sediment's microbial population. The procedure, based on the microbial production of $CO_2$ from added glucose, had good reproducibility. Despite the fact that organophosphate pesticides are known to inhibit acetylcholinesterase (ACHe) activity (Gälli *et al.* 1994), these experiments show significant effects on the glucose respiration by the sediment microbial population. Gälli *et al.* (1994) reported that for some organophosphorus

pesticides (e.g. fenitrothion) the toxicity observed was not directly correlated to the inhibition of ACHe activity (Table 5.8) and that metabolic activation of the insecticides as well as different mode of actions could be possible explanations for these observations. These workers also reported a complete inhibition of ACHe at PPT concentrations of 1000 mg $l^{-1}$ (Table 5.8) in blood serum, but the results presented suggest other mode of actions, at least for PPT, should also be considered. Although a detailed investigation of toxic mechanisms was not an objective of this study, the PPT mode of action is thought to be different to that of the two metals assessed here.

The experiments were designed knowing that indigenous microbial populations respond rapidly to environmental change, both in terms of community composition and in metabolic rate (Burton Jr., 1991). Thus the exposure time was limited to 60 minutes following preliminary experiments which showed this was sufficient time to produce accurate measures (data not shown). This time period was also not enough to allow significant microbial acclimatisation to occur, since biodegradation of PPT during the first day of incubation in LL sediment was only *ca.* 2% (see Chapter 4). On the other hand this time would probably not be enough for the system to reach chemical equilibrium and so it represented a compromise. It is certainly known that the duration of contact between the chemical and sediment particles can affect both the partitioning and the bioavailability of the toxicant (Burton Jr., 1991). The data presented in Chapter 4 show that the sorption capacity of the Conwy sediment to PPT was directly related to carbon content. As the sediments used here had low organic carbon content (0.4%) (Table 5.1), it was assumed that sediment sorption was

**Table 5.8.** $EC_{50}$ values of organophosphate pesticides, determined by various methods and authors.

| Pesticide | $EC_{50}$ | Medium | Method |
|---|---|---|---|
| Propetamphos | 21.4 μg $ml^{-1}$ † | Water | Microtox (*Photobacterium phosphoreum*) |
| Propetamphos | 1000 μg $ml^{-1}$ † | Blood serum | ACHe[a] |
| Dichlorvos | 44.6 μg $ml^{-1}$ † | Water | Microtox (*Photobacterium phosphoreum*) |
| Dichlorvos | 30 μg $ml^{-1}$ † | Blood serum | ACHe[a] |
| Fenitrothion | 3.51 μg $ml^{-1}$ † | Water | Microtox (*Photobacterium phosphoreum*) |
| Fenitrotion | > 1000 μg $ml^{-1}$ † | Blood serum | ACHe[a] |
| Fenitrotion | 0.0002 μg $ml^{-1}$ † | Water | Acute immobilization (*Daphnia magna*) |
| Diazinon | 74.58 μg $ml^{-1}$ ‡ | Water | Microtox (*Photobacterium phosphoreum*) |
| Malathion | 33.73 μg $ml^{-1}$ ‡ | Water | Microtox (*Photobacterium phosphoreum*) |
| Malathion | 0.85 μg $ml^{-1}$ * | Water | Microtox (*Photobacterium phosphoreum*) |
| Tetrachlorvinphos | 2.56 μg $ml^{-1}$ ‡ | Water | Microtox (*Photobacterium phosphoreum*) |
| Parathion | 0.72 μg $ml^{-1}$ * | Water | Microtox (*Photobacterium phosphoreum*) |
| Propetamphos | 236 μg $g^{-1}$ ** | Sediment:water | $CO_2$ from glucose (indigenous microbial population) |
| Propetamphos (Industrial grade) | 14 μg $g^{-1}$ ** | Sediment:water | $CO_2$ from glucose (indigenous microbial population) |

a Complete inhibition of ACHe (estimation from dilution experiments).
† Gälli *et al.* 1994
‡ Ruiz *et al.*, 1997
* Johnson and Long, 1998
** This work

relatively low, and therefore PPT bioavailability was high. It is true that the equilibrium and bioavailability interactions are still a point of debate as discussed by Landrum and Robbins (1990). However it can be assumed that the non-sorbed pollutant at this critical point in time was potentially bioavailable and therefore represented a hazard for the environment.

It should also be mentioned that this sandy, low organic sediment was chosen for study because presumably the toxicant response would be clearer due to reduced toxicant partitioning. Through the results obtained in Chapter 4, it was observed that the quantity of PPT sorption depends upon the quantity of PPT in solution. Van Beelen and Doelman, (1997) also stressed that mineralization tests with high substrate concentrations which enable microbial growth, are less sensitive than similar tests with low concentrations of substrate. They concluded that the latter tests were more relevant for natural ecosystems. Considering this statement, microbial growth may have been promoted in the experiments carried out in this work (the glucose concentration was 4.5 $\mu g\ g^{-1}$), but 60 minutes was not long enough for significant microbial growth.

The toxic effects of organophosphorus pesticides have been extensively assessed in the past using different methods (Table 5.8). For PPT some differences were observed with the $EC_{50}$ dependant on the sensitivity of the method and the species assessed. It may be that sediment microbial populations have a certain resistance to PPT, since the $EC_{50}$ for PPT found here was considerably higher than values reported for other living organisms.

It is known that the microbial community of a given soil, or in this instance sediment, consists of many species each with its own sensitivity for toxicants, maximal growth rate and affinity for a given substrate (van Beelen *et al.*, 1991). Thus it is thought that the different empirical equations that were fitted to each set of data for the individual applications of pesticide zinc and lead could indicate either that different microbial communities were affected in each case, or that the same communities were affected but in different ways. For instance, some microorganisms become intoxicated while others may be resistant to a pollutant and can increase their number and biomass because of decreased competition while others specific microorganisms will actually grow on the organic pollutants (van Beelen and Doelman, 1997).

Four different mechanisms of community resistance of microorganisms to pollutants were identified by van Beelen and Doelman (1997). First, they considered those microorganisms that live under specific stressing conditions (pollution). For these species to withstand toxic stress they tend to either limit uptake or maximize excretion or detoxification of the pollutant. Secondly, when some microorganisms become acclimatised to a toxicant over long periods, a phenotypic physiological resistance can be induced. Thirdly, genetic resistance can occur in individuals that gain a competitive advantage and these become dominant after many generations of natural selection. Fourthly, communities can also become "resistant" because the sensitive species are replaced by resistant ones which might or might not perform similar functions. It is likely that one of these mechanisms may have occurred in the indigenous microbial population of

LL, not necessarily in the case of PPT but possibly for zinc and lead where some previous exposure had occurred.

The $EC_{50}$ values obtained for zinc and lead obtained here are summarized in Table 5.9 along with literature values. The values obtained in this work are considerably higher than the rest with the exception of that reported for zinc by van Beelen *et al.* (1991). Apparently the aquatic bacteria *Photobacterium phoshoreum* is a sensitive species to zinc and also to zinc and copper in combination. Van Beelen and Fleuren-Kemilä, (1997) reported that *Pseudomonas putida* is also a sensitive sediment bacteria to zinc with maximum toxicity observed at pH 7. The results in Chapter 3 of this thesis showed that there is a high concentration of zinc in the river water (49 $\mu g\ l^{-1}$), sediment pore water (1305 $\mu g\ l^{-1}$), and in the sediment mineral phase (82 $\mu g\ g^{-1}$) at LL. The indigenous microbial population of the Conwy River has been exposed and adapted to zinc over a long period and it would be expected that these microbes would also have developed resistance. These authors, van Beelen and Doelman (1997) also discussed that an elevated background concentration is a common problem in the risk assessment of naturally occurring chemicals like metals since it is difficult to find a control sample where prior exposure has been absent.

At first, the addition of NOM seems to reduce the toxic effect of the pollutants. However, if the 95% confidence intervals are considered, the effect is very small. It might be expected that the pollutants would be involved in a partitioning processes with the dissolved NOM, thus bioavailability would be reduced.

However, the results presented here indicate that dissolved NOM does not have an important role to play in the partitioning PPT even after 24 hours.

**Table 5.9.** $EC_{50}$ values of zinc and lead, determined by various methods and authors.

| Metal | $EC_{50}$ | Medium | Method |
|---|---|---|---|
| Zn | 70–1000 μg $g^{-1}$ † | Soil | $CO_2$ from acetate, Anaerobic (subsoil microcosms) |
| Zn | 1.13 μg $l^{-1}$ ‡ | Water | Microtox (*Photobacterium phosphoreum*) |
| Zn mixture with Cu | 0.66 μg $l^{-1}$ ‡ | Water | Microtox (*Photobacterium phosphoreum*) |
| Zn (pH = 6) | 11 μg $g^{-1}$ * | Sediment | $CO_2$ from glucose (*Pseudomonas putida*) |
| Zn (pH = 7) | < 1 μg $g^{-1}$ * | Sediment | $CO_2$ from glucose (*Pseudomonas putida*) |
| Zn | 2127 μg $g^{-1}$ ** | Sediment: water | $CO_2$ from glucose, (indigenous microbial population) |
| Pb | 1445 μg $g^{-1}$ ** | Sediment:water | $CO_2$ from glucose, (indigenous microbial population) |

†van Beelen *et al.*, 1991
‡Parrot and Sprague, 1993
*van Beelen and Fleuren-Kemilä, 1997
**This work

$EC_{10}$ and MATC values are considered to be more important for decision making than $EC_{50}$, because these values indicate the levels of pollution that a specific community can tolerate without suffering drastic ecological changes. The results

presented here for $EC_{10}$ values seem to provide a more sensitive method to measure this limit of pollution, because it does not depend on the concentrations used in the actual experiment but rather how well a mathematical model fitted the toxicant response.

In a natural sediment microbial community, some species would be expected to be more sensitive to a certain toxicant. Thus, it might be expected that those species could be totally inhibited at a toxicant concentration that inhibits only a small percentage of the total activity. This may be occurring in the system assessed here. Therefore at the $EC_{10}$ values calculated here some of the microbial species could have been eradicated or at least much damaged. A 10% inhibition of a process can be accompanied by more than 50% inhibition of the most sensitive species (van Beelen and Fleuren-Kemilä, 1999) and it has been suggested by van Beelen and Doelman (1997) that resistant microorganisms often fail to perform specific ecological functions, thus ecological impact can be even greater. At the $EC_{10}$ values reported here, there is still some ecological damage to the microbial community of the tested sediments.

The commercial formulation of PPT was also tested and compared directly with pure PPT. The results show a clear difference. The commercial formulation is considerably more toxic, possibly due to the Shellsol R® solvent either making the pesticide more soluble and bioavailable or that the solvent is toxic itself and the effect observed is actually the combination of two toxic organic chemicals. Another less likely possibility could be that metabolic activation of PPT could be enhanced by the aromatic solvent since it is known that some organophosphorous

compounds must undergo conversion to active metabolites to produce the desired effect of the pesticides (Pape-Lindstrom and Lydy, 1977). Again the higher toxicant effect of the industrial grade pesticide confirms the importance of assessing commercial pesticide formulations, because the use of analytical pesticides grade may mask the real ecological behaviour and fate of pesticides.

The binary and ternary equitoxic combinations of PPT, zinc and lead showed that their combined action could be classified as additive. It would have been expected that the binary combinations that included PPT and one of the metals would have presented either a more or less than additive effect since the mode of action would be different (Parrot and Sprague, 1993). For instance, Chen and Yeh (1996) reported that greater than additive effects were quite frequently observed (18%) among chemicals with different mechanisms of toxicity, and some of them were severely synergistic. On the other hand the binary combination of metals would have been expected to be additive. For example, Parrot and Sprague (1993) found that copper and zinc were additive in their effect to bacterial luminescence.

Marking (1985) proposed that several mechanisms such as increases in the rate of uptake, formation of toxic metabolites, reduction in excretion rates, alteration of distribution, and inhibition of detoxification mechanisms may be responsible for some synergistic toxicity effects. It could also be possible that physicochemical environmental characteristics could influence the toxicity of some toxicants. In the studies presented here, the pH values varied from one solution to another, but in general were between 4.1 and 6.0 with conductivities from 25 to 3000 $\mu$Siemens $cm^{-1}$. Van Beelen and Fleuren-Kemilä (1997) reported that an increase

in pH from 4.5 to 8 reduced the toxic effect of pentachlorophenol on the $CO_2$ production from acetate from *Pseudomonas putida*, whereas zinc and cadmium became more toxic (see Table 5.9).

Pollutants in aquatic environments have previously been found in "cocktail" combinations in different ratios and the effect of these mixtures over microbial communities may be different than those of equitoxic combinations. The last experiment therefore attempted to "mimic" the ratio in which PPT, zinc and lead had been found in aquatic ecosystems. This work indicated a more than additive toxicity to the indigenous microbial population of the LL sediment. However, this experiment was carried out with pure PPT, and it should be noted that throughout these experiments it was found that the toxicant effect of the commercial formulation was far greater. Also some of the more sensitive species (microorganisms) could have been strongly damaged even at the $EC_{10}$ values concentrations calculated here. In conclusion, a review of the Maximum allowable concentration (MAC) imposed by the Environment Agency (Table 1.3) for PPT might be appropriate.

# Chapter 6. Conclusions.

Here conclusions have been drawn from the information collected about the chemistry of the sediment and water from the Conwy River and Estuary to enable a better understanding of the environmental behaviour and toxicology of PPT. The mobility and interactions of metals with NOM have also been considered alongside the ecotoxicological effects of a "cocktail" of metals and PPT.

Figure 6.1 is a schematic representation of the interactions between colloidal and particulate NOM, PPT and selected metals found in fresh water and sediment from the Conwy River. The diagram is therefore sub-divided first into fresh water and sediment with the principal physicochemical characteristics shown, (pH and salinity). The metal ions are shown in their most likely species along with their likely interactions with NOM as NOM-metal ion complexes. In fresh water, NOM is sub-divided into colloidal and particulate organic matter. The colloidal NOM is further divided into high (HMW) and low molecular weight (LMW). Within this, calcium is preferentially linked with HMW NOM, sodium, magnesium and potassium are correlated with both HMW and LMW NOM fractions, and zinc is preferentially related to LMW NOM. By comparison, the particulate NOM was found to contain high concentrations of aluminium, iron and zinc. In solution calcium, magnesium, aluminium, iron and zinc were all found and these could be present as free ions. When PPT is released into water bodies it is suspected to bind weakly to colloidal NOM mainly because of its low $K_{ow}$, as the Chapter 4 data suggested, but a large proportion will remain in solution due to the relatively slow partitioning process onto solids. In the solid phase (sediment)

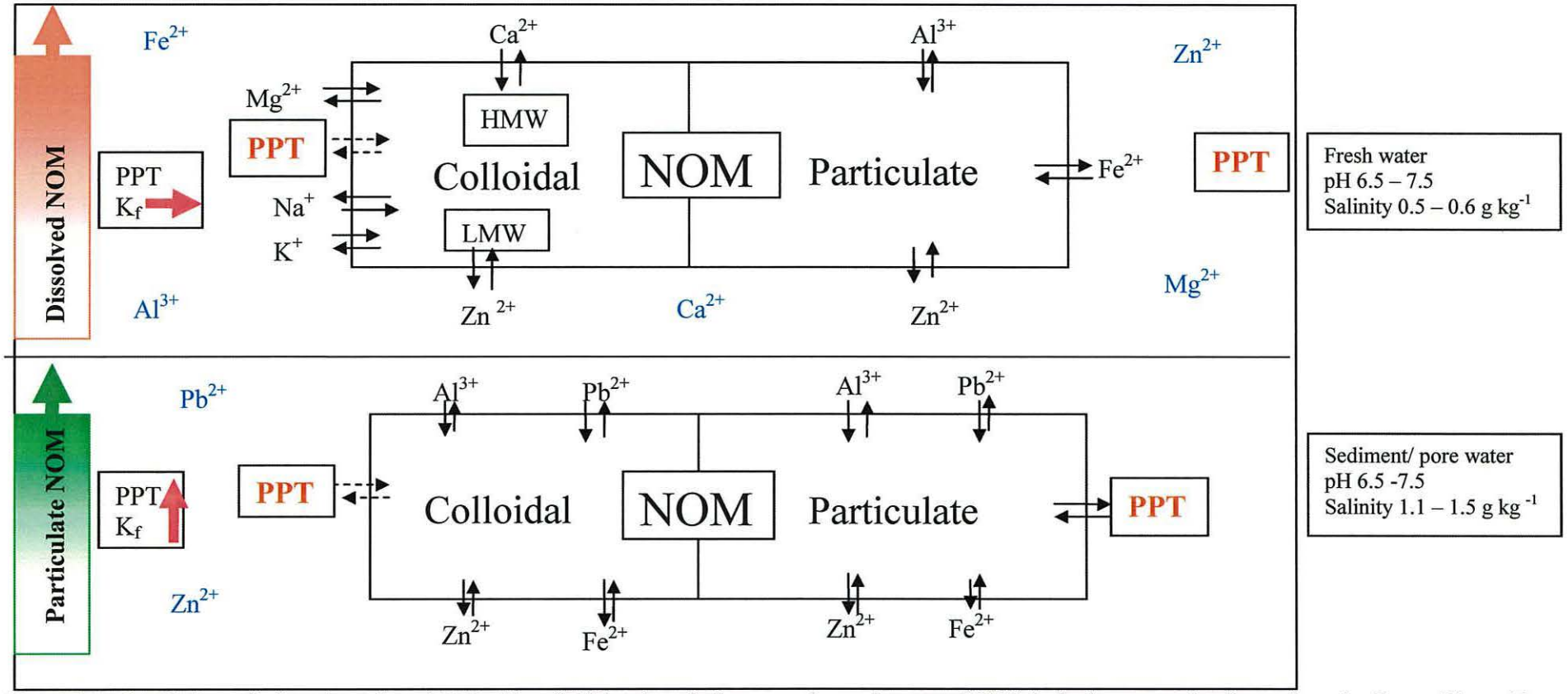



**Figure 6.1.** Correlations of selected metals and PPT with colloidal and particulate natural organic matter (NOM) in fresh water and sediment from the Conwy River. The PPT partition coefficient (red arrow), increases with particulate NOM in sediment (green arrow), but remains constant with increasing dissolved NOM in water (orange arrow). The metals ions in the aqueous phase sediment are also present in the sediment pore water (blue). Strong bonds are represented as solid arrows and the weaker bonds are dashed arrows. PPT forms strong bonds with particulate NOM in sediments but weak bonds with colloidal dissolved NOM in water and pore water in sediment.

NOM is also divided into colloidal, if it is found dissolved in pore water, and particulate fraction, if it is coating the mineral phase. Pore water was rich in aluminium, lead and zinc with these metals mainly bound to organic matter. PPT strongly binds to the solid phase which appears to be largely controlled by the content of organic matter. The presence of metals or NOM in solution does not affect PPT partitioning processes indicating that the binding sites or mechanism of PPT partitioning are different to those of the metal ions or NOM.

A similar scheme for the estuary system is presented in Figure 6.2. The major difference with the fresh water system is that salinity increases from left to right. In the estuarine water, iron and zinc were preferentially found in the low salinity zone, aluminium and manganese were found in the low and medium salinity waters, whilst the earth metals (Ca, Mg, K and Na) were distributed throughout the estuary. In the sediment, in addition to the alkaline metals, aluminium, iron and zinc were found throughout the estuary sediments at significant concentrations. This is an indication of metal transport from upstream and subsequent precipitation due to increasing pH and salinity. Aluminium, iron, lead and zinc concentrations were particularly well correlated with the organic fraction of the sediment. Further to what is shown in the fresh water system, the partitioning of PPT was dependent on the salinity of the water.

Bioavailability of sediment-associated contaminants has been defined by Landrum and Robbins (1990) as "the fraction of the total contaminant in the interstitial water and on the sediment particles that is available for bioaccumulation", whereas bioaccumulation is "the accumulation of contaminant via all routes

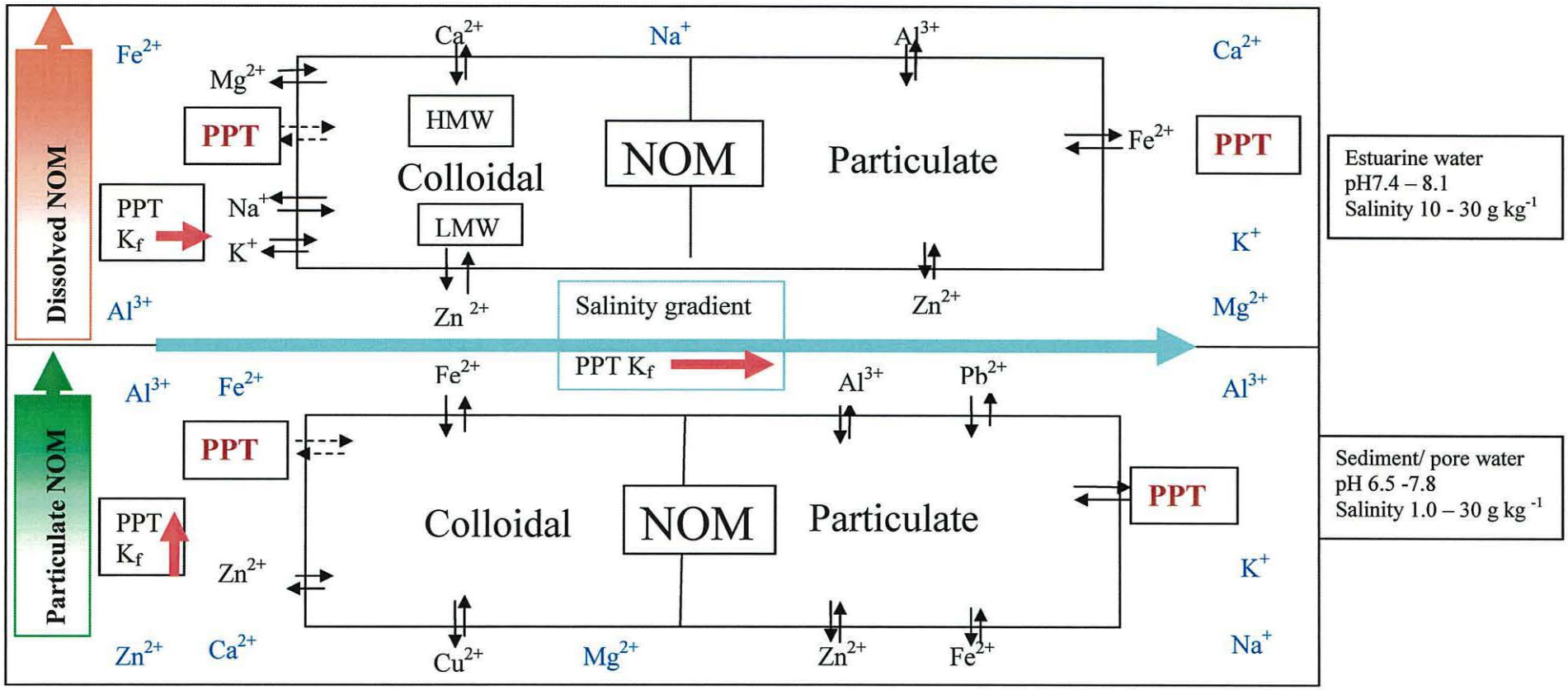



**Figure 6.2.** Correlations of selected metals and PPT with colloidal and particulate natural organic matter (NOM) in estuarine water and sediment from the Conwy Estuary. The PPT partition coefficient (red arrow), increases with particulate NOM in sediment (green arrow) and also with increasing salinity (turquoise), but remains constant with increasing dissolved NOM in estuarine water (orange arrow). The metals ions (blue) in the aqueous phase are also present in the sediment pore water. The strong bonds are represented as solid arrows and the weaker bonds are dashed arrows. PPT forms strong bonds with particulate NOM in sediments but weak bonds with colloidal dissolved NOM in water and pore water in sediment.

available to the organism". The major factors affecting the rate or extent of sediment contaminant bioaccumulation, particularly for organic contaminants are: the characteristics of the contaminants, the composition and characteristics of the sediment, and the behaviour and physiological characteristics of the organisms. This outline has been applied to benthos macro organisms. In this work the emphasis is on sediment microorganisms. However, the definition given previously works well with some adjustments. For instance, the composition and characteristics of the organic matter either dissolved or particulate needs to be included as this is of great importance along with physicochemical characteristics of the aqueous phase.

The environmental phenomena measured (sorption, desorption, degradation and toxicant effect) were investigated at different time scales. It is known that PPT does not undergo chemical degradation for up to 70 hours (*ca.* 3 days) (Tomlin 1994). This period covers the most critical stages of change in the environmental parameters measured here. Thus it can confidently be said that up to 70 hours any effects reported here were produced by PPT and not by its breakdown products. The kinetics of sorption, desorption and biodegradation have been combined in Figure 6.3, showing the critical window for microbial toxicity. This does not rule out the potential toxic effect to other aquatic organisms, since enough free PPT in solution is found after the one hour window. In the graph, the toxic zone corresponds to the area above the sorption curve.

In river sediments where the effect of salinity is minimal, metals are omnipresent sometimes at high (pollutant) levels in pore water and in fresh water. The major

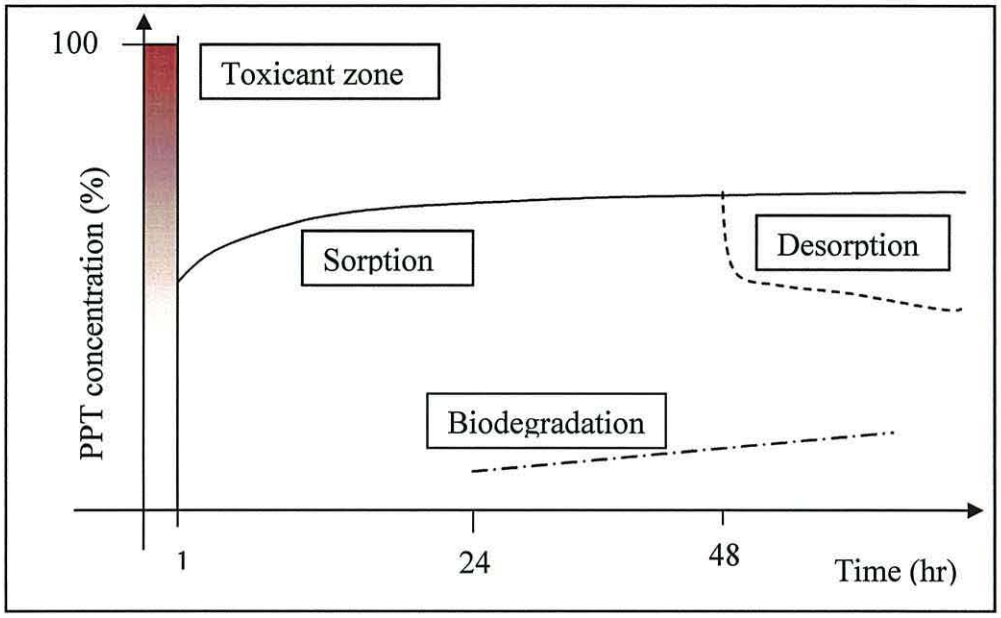



**Figure 6.3** Kinetics of sorption (solid line), desorption (dashed line), microbial biodegradation (dashed and dotted line) and measured toxicant window of PPT (red box) in a water and sediment system for microorganisms.

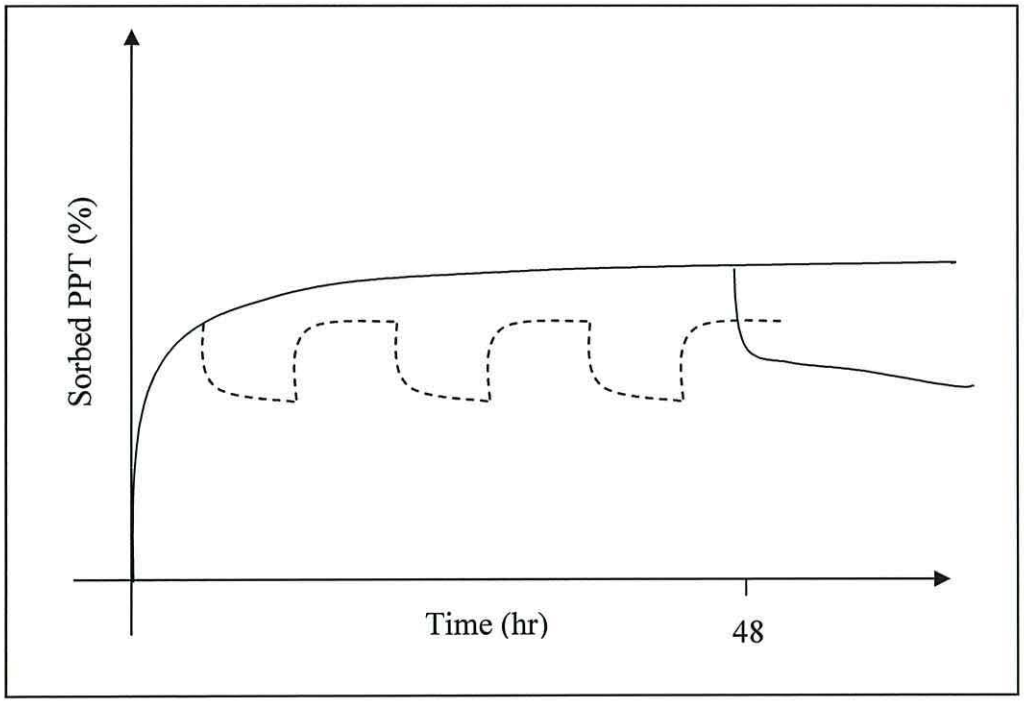



**Figure 6.4**. Experimental sorption and desorption in a sediment water laboratory system (solid line) and a hypothetical sorption and desorption in a dynamic river system, (dashed line).

variables influencing the behaviour and interactions of metals and organic pollutants were thought to be NOM and pH. The sorption kinetics of PPT showed a rapid sorption phase during the first few minutes of contact followed by a slower rate over the next few hours. Generally, less than 50 % of the PPT was sorbed onto the sediment. The Freundlich sorption coefficient of PPT correlated well with the organic matter content in the sediment. However, dissolved OM concentration or type had little impact on sorption during the first 24 hours of equilibration period. Whilst the effect of increasing the dissolved NOM concentration was not statistically significant, it was noticed that NOM tended to suppress PPT sorption at the beginning of the process. On this basis, it might be possible that, in a non-static system NOM would reduce PPT sorption at low initial concentrations. By comparison, the presence of aluminium, iron, lead, zinc and an equimolar mixture of the former did not affect the PPT sorption. Desorption after 48 hours of equilibration presented hysteresis and seemed to be sediment dependent, probably in correlation with organic matter content. The desorption kinetics were slow and in a river system it would be expected that desorption and sorption would occur simultaneously, regardless of whether the water column was free of pesticide or not. The quality and source of the NOM had a mixed effect at the sorption equilibration time. As it has been said previously no effect was seen during the first 24 hours, but after 48 hours of equilibration the source of NOM did affect PPT sorption. High and low MW HA, Aldrich and NOM-SR decreased PPT sorption whilst low MW FA (Norway) increased sorption. By comparison, after 24 hours (i.e. 72 hours after initial contact), NOM-SR retarded PPT desorption. The chemical composition of these humic substances might be directly related to their behaviour observed, rather

than their MW, unfortunately due to their complexity, the chemical characterisation of the humic substances groups was not attempted. In any case these effects were only observed after a considerable time period has elapsed in static systems (sediment-water). In a dynamic system (e.g. a river), it is unlikely that the partitioning processes of PPT would reach this point before passing through several sorption and desorption cycles/phases (Figure 6.4). From the point of pollution, (e.g. sheep dip discharges into a small tributary, accidental spillage or sewage treatment), it would be expected that the proportion of PPT that did not become sorbed onto the sediment would travel down stream to the river and progressively become sorbed onto "clean" sediment. The soluble PPT would therefore decrease from the point source of pollution, along with the toxicant effect and microbial biodegradation. Desorption would then occur later depending on the concentration of PPT in solution; thus the toxic effect would last longer.

The rate of biodegradation of PPT in river sediments increased each day up to 6 days and then slowed. The half life of PPT in river sediment was calculated to be 377 hours (*ca.* 16 days) in aerobic conditions. The proportion of PPT being biodegraded by the microbial population was very low (less than 10%) after 24 hours and high dissolved NOM concentrations had little effect on this. In contrast, the presence of high levels of zinc or lead reduced biodegradation by the fifth day. The addition of zinc and or lead along with NOM also reduced PPT biodegradation but not beyond the reductions observed for single metal. Considering that the levels of dissolved metals in the pore water from BC and LL in the Conwy River were high it would be expected that the biodegradation

kinetics would be slowed due to the constant leaching of metal from the nearby mines.

The toxic effect of PPT was also measured measuring the respiration of glucose after one hour of contact with PPT. The addition of NOM to the aqueous solution tended to reduce the toxic effect measured by half (i.e. $EC_{50}$ doubled). Though, the 95% confidence limits showed that statistically NOM did not influence the toxicant effect of PPT. During this small window of time, the most important process that could affect the bioavailabity of PPT is sorption. It is known that more than 50% of PPT remains in solution and that only a small proportion of this is biodegraded, but probably after a lag time that covers the 1 hour window. Theoretically, PPT should be expected to bind to DOM because of its hydrophobic nature, and this process might also be expected to occur rapidly. This assumption is based on a thermodynamic gradient favouring the partitioning of a water-insoluble solute from an aqueous environment into the non polar domain of a second physicochemical phase, the humic substance (McCarthy, 1989). Under this scenario, the toxicant effect of PPT would be expected to reduce. The small effect may be because PPT is a mildly hydrophobic compound ($K_{ow}$ = 6600), and possibly because the interactions with NOM are weak. The binary combination of PPT with metals were found to be additive when added in equitoxic ratios implying that PPT and zinc or lead combine to produce more or less the same effect as on their own. This implies that these species do not interact strongly during their action. In the equitoxic ternary combination, the joint action was also additive. Here again the addition of NOM to the aqueous solution had a tendency to decrease toxicity but not enough to be statistically

significant. This could imply that the interaction that these metals have with NOM did not affect their bioavailability. During the fractionation of NOM-SR with zinc solutions it was found that approximately 66% was equally divided between the low molecular weight (54 to 1,000 MW) and dissolved fractions. Thus a considerable proportion of zinc was hypothetically bioavailable to the microbes and a significant reduction on toxicity would not be expected. Unfortunately the data to identify the lead rich fractions were inconclusive (data not shown), thus it is not possible to reach any conclusion.

As stated earlier, in the estuary the major factor that could affect the behaviour and fate of propetamphos was salinity, apart from NOM and pH. It was observed that both the kinetics and the Freundlich sorption coefficients were slower and smaller for sediments from the estuary (TC and BS) than from LL (River). The environmental implication of increasing PPT sorption as salinity increases is that PPT would not be expected to reach the open sea before it becomes sorbed onto sediments or biodegraded by the indigenous microbial population. Desorption is also affected at higher salinities with results showing that PPT desorption also increases. This could imply that, after a tidal cycle, PPT sorbed into estuarine sediments might desorb more easily with the incoming tide and then be transported back to the upper estuary. After a whole tidal cycle the microbial population might then be able to partially degrade the organophosphorus pesticide. However, PPT would still be able to cause some ecological damage to estuarine organisms.

The biodegradation of PPT in sediments from the estuary (TC and BS) presented very different rates of biodegradation during the first few hours. This could indicate the state of "cleanliness" of each of the sites. The fastest rate was in the BS sediment, possibly because this site might have been exposed to pollution from the nearby sewage discharges. The slowest rate was in TC sediment, presumably a place with less chronic pollution. This difference could also be interpreted as being due to differences in microbial community structure between the sites. The impact than an increase in salinity had on the degradation of PPT was similar to that for glucose, which could be explained as an environmental (osmotic shock) effect on the microbes, since an increase in salinity would affect their membrane transport processes. The combined effect of increased NOM and salinity had a significant effect in reducing PPT degradation. It is well known that dissolved humic colloids coil in solution with higher ionic strength and this would have affected their hydrophobicity. PPT, being a mildly hydrophobic organic compound, would tend to bind better to the humic colloid, thus bioavailability would decrease and so would biodegradation. The joint effect of metals, salinity and NOM on PPT biodegradation was a significant decrease at the fifth day and a very low rate over the whole period of experimentation. This may indicate that the biodegradation of PPT in the upper inter-tidal zone in the estuary is low due to the environmental effects imposed upon the microbial population. This would agree with the earlier findings discussed here, since the slowest rate was registered for TC which is located in the upper estuary.

Considering the sorption/desorption processes, the tidal cycle in the estuary and the low biodegradation rate of PPT, it is hypothesized that PPT represents a

hazard to aquatic organisms in the estuary. Unfortunately salinity was not considered in the experimental design to measure the toxicant effect of PPT, thus it is not possible to test this.

The commercial formulation of PPT presented differences in behaviour compared with the pure PPT. These differences have to be considered to assess the "real" implications of PPT for the environment. For instance the Freundlich sorption coefficients ($K_f$) of PPT-Ind were considerably higher than for Pestanal PPT, particularly in the BS sediment. The desorption rate of PPT-Ind is slightly higher but not significantly different than the Pestanal PPT desorption rate. Overall, the biodegradation of PPT-Ind was similar to the Pestanal pesticide, and no significant differences were observed. The effect of metal, NOM, and salinity and in a "cocktail" had the same effects as for pure PPT.

It was also found that PPT-Ind was approximately 11 times more toxic than the Pestanal PPT considering the $EC_{50}$ values and 162 times more toxic considering the $EC_{10}$ values. This has important considerations because environmental policy is usually based upon data from Pestanal grade, even though the commercial formulations appear to be more hazardous to the environment. The addition of NOM had the same tendency to decrease toxicity as occurred with the Pestanal, however, this effect was not statistically significant. The combination of PPT:zinc:lead (ratio 1:100:10) to mimic pore water values, showed an $EC_{10}$ for PPT in this mixture below the Maximum allowable concentration (MAC) specified by the Environmental Agency in the UK. The metal pollution is a chronic situation in the Conwy River and the indigenous microbial population

from these sediments have developed a resistance to their presence, and even probably use them for their own benefit. In this work it has been found that even small quantities of PPT can affect the microbial physiological activity of the whole population, which means that more vulnerable species could be eliminated at low concentrations.

In summary this work reports the major metals found in the Conwy River and Estuary. Their behaviour was described along with in which phases were found. PPT environmental behaviour was described in river and estuarine conditions. It was found that the toxic effect of metals and PPT was additive in equitoxic ratios while in more realistic ratios the toxic effect was more than additive. The PPT-Ind was more relevant than pure PPT, and the PPT $EC_{10}$ was considerably lower than the current MAC.

The conclusions of this work are presented in relation to the four hypotheses proposed in the introduction to this study.

1. *The inorganic and organic characteristics of water and sediment samples of the Conwy River-estuary play an important part in the sorption and transport of chemicals and hence their bioavailability.*

    - Metals such as zinc and iron present conservative behaviour in the River and Estuary in relation to salinity gradient.

    - The physical phases (solid and liquid) present in the water column and

sediment throughout the River and Estuary are very important in controlling the transport and bioavailability of zinc, iron and aluminium in the following order; suspended particular matter > sediment > pore water > water.

- The average molecular weight of dissolved NOM in the Conwy River falls into the range of low MW molecules (≈ 1100 MW). In the autumn of 1999, two distinct groups of HS were found based on MW distribution.

- The molecular weight of the dissolved NOM present in water and pore water from the Conwy River correlates via a positive linear model with total phenol; the ratio of humification ($E_4/E_6$) and the dissolved organic carbon (DOC) but via a negative correlation with the molar absorptivity (ε). By comparison ε and DOC correlates well following an exponential decay model.

- Metals tended to be associated with specific molecular weight fractions of NOM. For instance, calcium and sodium prefer high MW NOM ($2 \times 10^6$ to $10 \times 10^6$ MW and 1000 to $2 \times 10^6$ MW respectively), while potassium and magnesium prefer either the 1000 to $2 \times 10^6$ MW or 54 to 1000 MW NOM fractions, and zinc favours the low MW fractions (54 to 1000 MW NOM and MW < 54 or dissolved).

2. *The sorption and desorption behaviour of propetamphos in river and estuarine sediment is affected by the salinity, but not by natural organic matter or metal concentrations.*

- PPT is sorbed onto sediment from the Conwy River and Estuary in correlation with the organic matter present in the sediment. The sorption kinetics follows two distinct phases, firstly a very fast rate of sorption occurs almost immediately after contact and this is followed by a slower rate after which an apparent quasi-equilibrium is reached after 8 hours. In low PPT concentrations, no more than 50% PPT is sorbed at this point.

- PPT is desorbed following a fast initial rate followed by an extremely slow rate of desorption. Partial desorption (47%) occurs after 48 hours.

- The sorption of PPT increases during the first hours of equilibration in aqueous solutions with high salinities ($P < 0.05$ ANOVA). In addition, more PPT desorbs at higher salinities ($P < 0.05$ ANOVA).

- Changing concentrations of NOM in solution does not affect either the initial sorption or desorption behaviour of PPT ($P > 0.1$ ANOVA). By comparison, changing the source or type of humic substances does increase sorption after 48 hours. It also decreases desorption after 24 hours ($P < 0.05$ ANOVA). This may have reduced ecological

relevance because of the long times taken to register an effect.

- The addition of metal to the aqueous phase does not affect the sorption or desorption behaviour of PPT ($P > 0.05$ ANOVA), regardless of the sorption behaviour of the metals.

- The sorption coefficient (Freundlich model, $K_f$) and the desorption coefficient (Linear model) of the "Ectomort Centenary" commercial formulation of PPT (Vericore Ltd.), is considerable higher than that for the Pestanal formulation (Sigma).

3. *The biodegradation of propetamphos by sediment microorganisms (measured by the half-life) is affected by changing salinity and the presence of dissolved metals, but not by natural organic matter.*

- PPT is degraded by the indigenous microbial population of the Conwy sediment in a water/sediment system under aerobic ($DT_{50}$ = 359 hours) conditions but more slowly in an anaerobic ($DT_{50}$ = 446 hours) atmosphere.

- The biodegradation of PPT is considerably decreased in aqueous solution containing higher salinity ($P < 0.05$ ANOVA)

- Increasing concentrations of metal and an increasing complexity of the

"cocktail" in the aqueous matrix significantly reduces PPT biodegradation ($P < 0.05$ ANOVA).

- Increasing quantities of NOM in solution do not affect PPT biodegradation ($P > 0.5$ ANOVA).

- The biodegradation rates and behaviour of the commercial pesticide are not significantly different to the Pestanal grade pesticide ($P > 0.01$ GLM)

4. *The toxicological effects of propetamphos measured as the respiration rate of sediment microorganisms, is increased by the presence of dissolved metals, but not by the presence of natural organic matter.*

- PPT has a toxic effect on the indigenous microbial population of the Conwy sediment by reducing the production of $^{14}CO_2$ from the intake of $^{14}C$ labelled glucose as a carbon substrate ($EC_{10} = 54\ \mu g\ g^{-1}$).

- An equitoxic binary combination of PPT and either metal zinc or lead has an additive toxicant effect ($AI \approx 0.3$).

- An equitoxic ternary combination of PPT with zinc and lead has an additive toxicant effect ($AI \approx 0.3$).

- The addition of dissolved NOM tends to decrease the toxicant effect of the pesticide on its own and in combination, though the effects are not statistically significant.

- The toxicant effect of the commercial formulation is approximately 11 times more toxic than the Pestanal grade based on $EC_{50}$ (14 $\mu g\ g^{-1}$) values and 162 times more toxic based on $EC_{10}$ values (0.33 $\mu g\ g^{-1}$).

- A ternary combination of PPT, zinc and lead in a toxicant ratio 1:100:10 indicates that small quantities of PPT (0.06 $\mu g\ g^{-1}$) when combined with lead and zinc present in concentration lower than those found in sediment pore water (51 and 3.5 $\mu g\ g^{-1}$ respectively) can affect the microbial population based on the $EC_{10}$ values.

**Appendix Chapter 4**

Freundlich sorption equations, linearized model

$$Log_{10} C_s = Log_{10}K_f + 1/n\ Log_{10}C_{aq}$$

| Sediment-pesticide | Equilibration time | Matrix | $Log_{10}K_f$ | 1/n | $r^2$ |
|---|---|---|---|---|---|
| LL-PPT | 5 min | DDW | 5.137 | 0.761 | 0.999 |
| LL-PPT | 2 hr | DDW | 9.097 | 0.799 | 0.999 |
| LL-PPT | 4 hr | DDW | 10.049 | 0.825 | 0.999 |
| LL-PPT | 8 hr | DDW | 10.724 | 0.834 | 0.999 |
| LL-PPT | 24 hr | DDW | 11.474 | 0.833 | 0.999 |
| BS-PPT | 5 min | DDW | 1.673 | 0.766 | 0.999 |
| BS-PPT | 2 hr | DDW | 2.236 | 0.703 | 0.999 |
| BS-PPT | 4 hr | DDW | 1.965 | 0.591 | 0.999 |
| BS-PPT | 8 hr | DDW | 2.510 | 0.726 | 0.999 |
| BS-PPT | 24 hr | DDW | 2.549 | 0.738 | 0.999 |
| TC-PPT | 5 min | DDW | 1.398 | 0.641 | 0.999 |
| TC-PPT | 2 hr | DDW | 1.960 | 0.844 | 0.999 |
| TC-PPT | 4 hr | DDW | 2.280 | 0.772 | 0.999 |
| TC-PPT | 8 hr | DDW | 4.408 | 0.821 | 0.999 |
| TC-PPT | 24 hr | DDW | 4.496 | 0.809 | 0.999 |
| TC-PPT | 24 hr | ASW | 4.408 | 0.822 | 0.999 |
| BS-PPT | 24 hr | ASW | 4.497 | 0.809 | 0.999 |
| TC-PPT | 24 hr | DOC | 2.537 | 0.914 | 0.999 |
| BS-PPT | 24 hr | DOC | 2.478 | 0.822 | 0.999 |
| BS-PPT-Ind | 5 min | DDW | 0.837 | 1.244 | 0.999 |
| BS-PPT-Ind | 2 hr | DDW | 2.549 | 0.972 | 0.999 |
| BS-PPT-Ind | 4 hr | DDW | 2.510 | 1.115 | 0.999 |
| BS-PPT-Ind | 8 hr | DDW | 3.822 | 1.003 | 0.999 |
| BS-PPT-Ind | 24 hr | DDW | 2.564 | 1.246 | 0.998 |
| LL-PPT-Ind | 24 hr | DDW | 11.197 | 0.960 | 0.999 |

**Appendix Chapter 4 (continuation)**

Linear desorption equation unless stated. (A) linear coefficient, (s) linear standard error.

$$C_{sorb} = A\ (C_{aq})$$

| Sediment- pesticide | Equilibration time | Matrix | A | s |
|---|---|---|---|---|
| LL-PPT | 48 hr (sorption) | DDW | 7.35 | 3.25 |
| LL-PPT | 1 hr | DDW | 21.3 | 14.06 |
| LL-PPT | 24 hr | DDW | 15.2 | 10.57 |
| LL-PPT | 48 hr | DDW | 14.9 | 3.97 |
| BS-PPT | 48 hr (sorption) | DDW | 1.55 | 1.59 |
| BS-PPT | 1 hr | DDW | 5.92 | 2.61 |
| BS-PPT | 24 hr | DDW | 4.88 | 3.11 |
| BS-PPT | 48 hr | DDW | 4.85 | 2.54 |
| TC-PPT | 1 hr | DDW | 2.30 | 2.47 |
| LL-PPT-Ind | 48 hr (sorption) | DDW | 8.38 | 31.11 |
| LL-PPT-Ind | 1 hr | DDW | 21.1 | 4.69 |
| LL-PPT-Ind | 24 hr | DDW | 19.3 | 3.06 |
| LL-PPT-Ind | 48 hr | DDW | 20.4 | 10.33 |

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