Mechanisms of phosphorus removal by constructed wetland systems

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PLEASE NOTE

The greatest amount of care has been taken while scanning this thesis,

and the best possible result has been obtained.
CERTIFICATION OF ORIGINAL CONTRIBUTION

I hereby certify that the material contained within this thesis is original work by the author and has not been submitted in part or in full to any other University or Institution. The work is entirely that of the author except where referenced or where noted for particular analyses and acknowledged in the text.

Greg Ryan
8/4/03
DEDICATION AND ACKNOWLEDGEMENTS

Dedication
I wish to dedicate this Thesis to my wife Janine, without whose patience and support it would not have been possible to complete this substantial undertaking.

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ABSTRACT

The objective of this thesis is to provide a detailed investigation of phosphorus transformations in constructed wetlands. Five replicate Wetland Units were constructed adjacent the wastewater treatment plant in Richmond, Australia. Each wetland was supplied with secondary or tertiary sewage effluent and planted identically with species of Schoenoplectus, Phragmites, and Triglochin. Detention times for each Unit were established at 5 or 15 days. Phosphorus concentrations were monitored routinely at the inlet and outlet of each Unit, with a number of specific studies conducted to investigate internal transformations. These studies, undertaken in 1994 and 1995, determined that plants were the dominant phosphorus store in the short term, during wetland establishment and that sediments were the dominant long-term phosphorus storage compartment.

The primary form of phosphorus entering the wetlands was filterable phosphorus (FP). This reacted directly with the sediment surface through passive diffusion or was actively pumped into the sediment by transpiration driven water movement. There was no association between phosphorus forms or concentrations and suspended solids material in any of the wetland Units. There was also limited association of phosphorus concentration changes with nitrogen and organic carbon in the water column. Investigations that compartmentalised the wetland based on plant species indicated that possibly greater phosphorus was removed to the lignified grass species Phragmites, with lowest phosphorus removal occurring in stands of the submergent plant Triglochin.

Evaluation of flow variation and movement through Unit 4, receiving alum-dosed effluent at a low flow rate, demonstrated phosphorus removal predominantly occurred through the central region of the Unit. It was likely that removal was related to settling or removal of floating plant material derived from the upstream stands of submergent Triglochin. Evaluating the role of material deposited in the wetland, either as suspended solids material or through litterfall indicated that this material accounted
for less than 0.1% of the phosphorus sequestering capacity of the wetland and was not a significant factor in overall phosphorus removal or release.

Laboratory investigations indicated that there was no significant role for bacteria or algae in the water column relating to phosphorus sequestering, although microorganisms appeared to have some role in the translocation of phosphorus to soil binding sites. After phosphorus contacted the soil surface, transpiration related entrainment of surface water and direct phosphorus uptake by plants were the dominant mechanisms for causing phosphorus to move deeper through the soil substrate. Removal of phosphorus from the interstitial water was by incorporation to biomass or direct sorption to soil binding sites.
CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

The purpose of this thesis was to determine the ability of constructed wetlands to remove phosphorus from an effluent water stream and store it within the wetland such that it was not released and detected at the outlet weir. Constructed wetlands were chosen over natural wetlands, as it was desired to determine the effectiveness of phosphorus removal processes in a controlled, defined and replicated environment.

1.1 PHOSPHORUS AND EUTROPHICATION

Phosphorus is a commonly occurring element, comprising approximately 0.1% of the total environment by weight (Holtan et al. 1988) and up to 2% of the dry weight of biota (Stevenson 1986). In the environment the natural valence state of phosphorus is 5 (Richardson et al. 1993) and its predominant form in water and wastewater is orthophosphate (Stevenson 1986). Orthophosphate is a highly soluble compound that is readily available to aquatic organisms.

In Australia, the largest contributors to phosphorus contamination of waterways are detergent and fertiliser, with approximately 80M tonnes of fertiliser and 40M tonnes of detergent consumed annually by the population (NSW blue-green algal task force 1992). It is estimated that up to 20% of this material could end up in waterways, where it presents a problem because of its ability to promote eutrophication and the growth of cyanobacteria (Ekhholm et al. 1992; GH&D 1992; Hynes et al. 1970; NSW blue-green algal task force 1992). Eutrophication is caused by the accumulation of excessive amounts of carbon, nitrogen, and phosphorus in the water inducing the proliferation of algal growth. Sequentially, microorganisms then exhaust available oxygen supplies in the water through the metabolism of organic carbon, causing the death of fish and other marine life.

Potentially more serious are “blooms” of cyanobacteria or blue-green algae in a lake or waterway. These algae are capable of producing hepato- and neuro-toxins that affect higher mammals including humans. During summer, a combination of high
temperature, still conditions and nutrient loads often results in the proliferation of blue-green algae (GH&D 1992; NSW blue-green algal task force 1992). Consequently, toxin levels in the water may increase to levels where they can sicken or kill domestic animals and livestock. The 1991 Murray Darling bloom of Anabaena circulans which spanned over 1000km of river, heightened awareness of the issues including the need to address point sources along Australian rivers, such as wastewater treatment plants (WWTP), abattoirs and stormwater discharges (GH&D 1992; Murray Darling Basin Ministerial Council 1994). It also led to the formation of the Algal Management Task Force in NSW and fostered the development of nutrient management plans throughout NSW and Victoria (GH&D 1992; NSW blue-green algal task force 1992).

**Wastewater Treatment Plants**

WWTP have traditionally represented significant phosphorus sources, primarily associated with the breakdown of faecal material. However, the quantity of this input into the riverine environment is currently declining due to the attachment of pollution reduction targets to discharge licences (25% reduction between 1992 and 1995 (EPA 1997)), and the move toward zero or minimal net change to water quality from point source inputs by 2010, at least in Victoria (EPA (Victoria) 2000). This is currently not possible without land discharge of wastewater, the use of environmental technologies such as wetlands or very expensive upgrades to conventional WWTP.

Wastewater treatments can be divided into three broad classes, based on the level of treatment provided:

- Primary treatment - separation and removal of gross solids.
- Secondary treatment - treatment of the primary wastewater by aeration and occasionally trickling filter, prior to ponding for up to 30 days. Natural UV is utilised for disinfection and ponding allows time for settling of turbid materials.
- Tertiary treatment - more complex and removes not only solids and bacteria, but also nutrients. Tertiary treatment requires solids removal, the precipitation of phosphorus by aluminium or iron compounds then pH correction followed
by nitrification/denitrification and final settling of sludge in a clarifier. This process results in effluent retention times in the order of hours to days (Masters 1997). Final filtration, chlorination and dechlorination are typically part of the overall tertiary WWTP process (Gakstatter et al. 1978).

Many non-metropolitan WWTP's provide secondary treatment to achieve outputs of total nitrogen (TN) of less than 10 mg.L\(^{-1}\) and total phosphorus (TP) between 10-15 mg.L\(^{-1}\) (Gakstatter et al. 1978; Rohlick et al. 1972). Reduction of phosphorus concentrations to less than 8 mg.L\(^{-1}\) requires the addition of coagulant and flocculant (Rohlick et al. 1972). The coagulant binds phosphorus and suspended material. The flocculant causes the coagulated material to coalesce into large aggregates that settle rapidly from the water column. The coagulating agents most commonly used in WWTP are derivatives of aluminium sulphate or iron oxide, whilst flocculants are typically organic compounds (Masters 1997). Both of these additions have the potential to affect downstream water quality and are an added cost to the treatment process. Reducing TN concentrations below 10 mg.L\(^{-1}\) can be problematic for conventional treatment plants as this process is currently not chemically driven, relying on microbial biotransformations.

For secondary polishing and tertiary treatment of wastewater, biological methods have the potential to offer an economic alternative. This thesis investigates the potential to use constructed wetlands for phosphorus removal by polishing tertiary and secondary treated wastewater.

1.2 FORMS OF PHOSPHORUS

In nature, phosphorus can be divided into the following five groups: elemental phosphate, orthophosphates, polyphosphates (chain phosphates), metaphosphates (ring phosphates) and ultraphosphates (branched ring structures) (Van Wazer 1958). The predominant and most bioavailable form of phosphorus in soil and water is orthophosphate (PO\(_4\)) (Schurmann 1964; Bostrom et al. 1988; Richardson et al. 1993; Stevenson 1986). Orthophosphate can exist in several valence states as determined by solution pH (Table 1-1). At pH = 7.0 orthophosphate exists as both hydrogen-
orthophosphate (HPO$_4^{2-}$) and dihydrogen-orthophosphate (H$_2$PO$_4$) with valence states of -2 and -1 respectively (Table 1-1) (Bolt et al. 1976; Stevenson 1986; Vymazal 1995). If the pH remains between 5 and 8, the concentrations of the other phosphate forms (orthophosphate and trihydrogen orthophosphate, Table 1-1) are negligible (Stevenson 1986).

Table 1-1: Chemical formation constants (log K$^o$) and pH ranges for particular phosphorus forms (Adapted from Lindsay 1979).

<table>
<thead>
<tr>
<th>Form of orthophosphate</th>
<th>pH range for occurrence in significant concentrations</th>
<th>Log K$^o$ (at 25°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO$_4^{3-}$</td>
<td>&gt; 10</td>
<td>-19.55</td>
</tr>
<tr>
<td>HPO$_4^{2-}$</td>
<td>5 - 14</td>
<td>-12.35</td>
</tr>
<tr>
<td>H$_2$PO$_4^-$</td>
<td>0 - 10</td>
<td>-7.2</td>
</tr>
<tr>
<td>H$_3$PO$_4$</td>
<td>&lt; 5</td>
<td>-2.15</td>
</tr>
</tbody>
</table>

Other bioavailable phosphorus forms are typically organic and can range from 0 to 90% of the TP pool (Young et al. 1985). Inorganic phosphorus compounds (other than orthophosphate) may also be bioavailable but tend to be formed only transiently in a wetland.

1.2.1 Organic phosphorus compounds

Organic phosphorous compounds fall into the following groups:

1. Inositol phosphorus (10-50%);
2. Phospholipids (1-5%);
3. Nucleic acids (0.2-2.5%);
4. Phosphoproteins (trace);
5. Phospho sugars (trace);
6. Metabolic phosphates (trace); and
7. Unidentified phosphorus compounds such as those found in humic material (Anderson 1964; Martin et al. 1966; Cowardin et al. 1979; Stevenson 1986; Harrison 1987).
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By far the greatest number of organic phosphorus compounds (35-40%) are found in peat (Moyer et al. 1970). Immobilisation into this material is mainly controlled by chemical sorption (Richardson et al. 1986). However, the percentage of peat and the type of organic phosphates differs between wetlands and includes phospholipids, nucleic acids, inositol phosphates, glucose-1-phosphate, glycerophosphate, phosphoproteins, polymeric organic phosphorus compounds of high molecular weight and phosphonates (Harrison 1987). Inositol phosphates are the most predominant, singly identifiable organic phosphate form found in the environment, being present as a polymer capable of forming insoluble complexes with proteins and lipids (Harrison 1987).

Inositol phosphorus is an ester of hexahydrro-hexahydroxy benzene, commonly found in plant phytin or generated by microorganisms (Stevenson 1986). Phospholipids are commonly found in the cell walls of microbial cells. Nucleic acids, phosphoproteins and metabolic phosphates are essential components of living cells. They are readily metabolisable and are rapidly degraded or adsorbed by microorganisms once outside the cell. Humin and humic materials are formed as a result of the degradation of complex organic matter such as lignin or cellulose (Schnitzer et al. 1972). These compounds are the least bioavailable forms of organic phosphorus, taking years to centuries to decompose if sufficient oxygen is not available. It has been speculated that the phosphorus content of this material may not be significant unless the compounds present are chelated or complexed with iron (Carignan 1985).

1.2.2 Inorganic compounds
The predominant inorganic phosphorus (Schnitzer et al. 1972) forms that occur in wetlands, other than orthophosphate or its derivatives, include phosphorous acid (H₂PO₄⁻), pyrophosphoric acid (H₂P₂O₇²⁻), phosphine (PH₃(g)), polyphosphate (H₉₋₃P₅O₁₇₋₁) and elemental phosphorus (Bolt et al. 1976). The relatively neutral pH and strong buffering capacity of water in a wetland causes rapid dissociation of pyrophosphoric and phosphorous acids to orthophosphate (Martin et al. 1966). Polyphosphates are unstable in soils and rapidly dissociate to orthophosphate due to solubility and equilibrium constraints around pH 7 (Anderson 1964).
Phosphine gas production has only been observed in lakes and wetlands at neutral pH where production is insignificant unless the redox potential (Eh) falls below -300 mV i.e. completely anaerobic. The amount of phosphine gas produced will be minute, in the order of $10^{-47.73}$ atm (PH$_{3(g)}$) at pH 7 in the presence of $10^{-4}$ M H$_2$PO$_4^-$ (Burford et al. 1975; Stevenson 1986), however at the sediment interface the pH needs to be less than 5 (Anderson 1964). The redox conditions necessary to allow the gas to escape the water column are such that it would be sorbed by the soil prior to entry into the water column, unless the water column had become completely anoxic (Johnson 1991). These conditions can be established transiently in a wetland but if sustained a permanent wetland would be unlikely to persist. Therefore, the formation of gaseous phosphorus is not a viable mechanism of removing phosphorus from a wetland.

Polyphosphates are biologically formed after exposure of orthophosphate to sequential cycles of aerobic and anaerobic conditions. They are unstable in aqueous solution, dissociating to H$_2$PO$_4^-$ within hours of formation (Davelaar 1993). They may also form pyrophosphate compounds with iron (Fe$_3$P$_2$O$_7$) or calcium (Ca$_3$P$_2$O$_7$) (Davelaar 1993). Although these compounds are bioavailable to algae and some bacteria, their transitory nature prevents them being significant phosphorus reservoirs within wetlands. However, they may facilitate the movement of phosphorus from the water column to the sediment (Davelaar 1993). Elemental phosphorus cannot be formed in-situ but originates from parent rock material. This material is weathered to soils and other particulates, which can enter the water column during rain events. However, for this phosphorus to become bioavailable it must first be solubilised to orthophosphate (Bolt et al. 1976).

1.3 PHOSPHORUS INTERACTIONS
Orthophosphate can interact with a number of metals: iron (Fe), Aluminium (Al), calcium (Ca), manganese (Mn), magnesium (Mg), strontium (Sr), ytterbium (Y), barium (Ba) and other rare earth metals (Bolt et al. 1976). Reactions involving cations more abundant than phosphorus can control phosphorus solubility, whereas those less abundant than phosphorus do not (Lindsay 1979). The elements Ca, Fe and Al are
CHAPTER 1

among the most abundant on earth and are highly significant in the phosphorus cycle (Lafferty et al. 1995). All three are capable of forming highly stable compounds with phosphorus that can persist for long periods (Bolt et al. 1976). Phosphorus becomes associated with these elements by direct chemical reaction, hydrogen bonding, or attraction due to Van Der Waals forces (Sample et al. 1980).

Phosphorus complexes with Mn are highly soluble and unlikely to have a significant effect on phosphorus removal from the water column (Gachter et al. 1993). Magnesium phosphate compounds have a transitory existence. The initial reaction is to form an Mg-phosphorus complex from which the phosphorus is rapidly released to other more stable compounds. One notable exception is the microbially formed compound, struvite (MgNH₄PO₄.H₂O), which requires a minimum of 0.01 M ammonium at pH 8.0 for its formation (Anderson 1964). Due to the specific requirements for formation, magnesium compounds can be discounted as permanent fixation products of phosphorus in sediments (Anderson 1964). Little is known about phosphorus interactions with Sr, Y, Ba and other rare earth metals but because of their scarcity, they are not considered to have a significant effect on phosphorus cycling in wetland environments.

1.3.1 Iron and aluminium
Al and Fe are soluble in water at pH less than 3, as Al³⁺ and Fe²⁺. Below pH 3, phosphorus may react directly with soluble Al and Fe to form insoluble compounds and precipitate out of solution (Berkheiser et al. 1980; Lindsay 1979). For aluminium these compounds include: variscite (AlPO₄.2H₂O); berlinit (AlPO₄); potassium-(H₆(NH₄)₃Al₅(PO₄)₈.18H₂O), and ammonium-taranakites (H₆K₃Al₅(PO₄)₈.18H₂O (Berkheiser et al. 1980; Lindsay 1979). With iron, they are stregnite (FePO₄.2H₂O) and vivanite (Fe₃(PO₄)₂.8H₂O (Lindsay 1979; Berkheiser et al. 1980)). Berlinit is 10000 times more soluble than variscite and is not expected to form in wetlands (Anderson 1964). Both potassium and ammonium taranakites may be present in high concentrations with ammonium or potassium, however they will break down to variscite, which is the primary form of aluminium in soils (Anderson 1964). Both
stregnite and vivanite are stable in water and may be present in high concentrations in the environment (Anderson 1964; Berkheiser et al. 1980; Davelaar 1993).

As pH increases, Fe and Al ions become less soluble, with a lower binding affinity for phosphorus exchanging it to form charged molecules of oxides (eg FeO\textsubscript{4})\textsuperscript{4-}, hydroxides (eg Fe(OH)\textsubscript{3})\textsuperscript{3-} and oxyhydroxides (eg Fe(OOH)\textsubscript{2})\textsuperscript{2+}, due to competition with hydroxyl ions (Lijklema. 1977). These compounds comprise a group known as sesquioxides, which have a high binding affinity for phosphorus (Bolt et al. 1976; Lijklema 1977; Lindsay 1979). The molecules are charged, with the net charge on the molecule dependent on the solution pH. As pH increases the charge tends toward neutrality then becomes negative (Holtan et al. 1988). Orthophosphate, possessing a net negative charge, adsorbs to these particles when they possess a net positive charge. When the charge on Fe or Al compounds becomes neutral or negative, phosphorus is released. The reaction is through Van Der Waals forces or displacement of water and hydroxide molecules by orthophosphate (Holtan et al. 1988).

In addition, Al and Fe can form octahedral crystal structures with water or hydroxide groups, with Al having the capacity to exchange for Fe in such groups (Bolt et al. 1976). Octahedral structures help form the layer lattice structure of clay, accounting for the abundance of these compounds in soil (Bolt et al. 1976). The layer lattice structure of clay allows for greater surface area within the clay structure, increasing the availability of binding sites and potential for phosphorus removal.

1.3.2 Calcium
Calcium forms a variety of compounds with orthophosphate, the most common being apatite (Ca\textsubscript{5}(PO\textsubscript{4})\textsubscript{3} (F, Cl, OH) - fluorapatite, chlorapatite, or hydroxyapatite). The presence of fluoride from a WWTP may result in greater formation of fluorapatite (Richardson 1985). Other less commonly formed compounds include brushite, monetite, and octacalcium phosphate (Lindsay 1979). These compounds are formed at neutral to high pH. Acidic pH ranges result in the formation of monetite (CaHPO\textsubscript{4}) or mono- and di-calcium phosphates (Ca\textsubscript{2}(HPO\textsubscript{4})\textsubscript{2} and Ca\textsubscript{3}(PO\textsubscript{4})\textsubscript{2} respectively). Low pH’s increase the solubility of Ca compounds with an associated release of phosphate.
CHAPTER 1

The most insoluble forms of calcium phosphates are fluorapatite and hydroxyapatite, which are only solubilised under extremely reducing conditions (Richardson 1985).

Phosphorus reacts with Ca oxides, hydroxides and oxyhydroxides in a similar manner to those for Fe, and Al, except that Ca molecules possess positive charges at high pH values that change to neutral or negative as pH decreases. As a result, Fe and Al control phosphorus deposition below pH 7 with Ca becoming dominant at higher pH ranges (Richardson 1985). Therefore, in the more acidic peat and bog type wetlands iron and aluminium related processes will tend to dominate. In wetlands containing water of a more neutral pH a combination of these and calcium related processes is possible.

1.3.3 pH
There has been some conflict regarding the significance of pH effects, with earlier studies (Ryding 1985) concluding that it was not significant. Although later investigations using more reproducible methodologies indicated a significant role for pH in controlling phosphorus concentrations (Boers et al. 1988; Drake et al. 1987). pH indirectly influences phosphorus removal from metal-phosphorus complexes through the solubilisation of iron- and aluminium-oxides and -oxyhydroxides at low pH, and Ca forms at high pH. The pH also influences the solubility and activity of various physical processes and can influence plant growth (Rayment 1992). In wetlands and lakes, a high pH may be induced by photosynthesis, which acts by withdrawing carbon dioxide from water, altering the carbonate balance. This can result in the pH increasing to 10 units or above (Boers 1991). Low pH may be caused by the formation of organic acids from the biodegradation of organic matter such as detritus (Ehrlich 1990). Whilst extremes and fluctuations in pH are possible in wetlands under certain conditions, the wetlands examined in this study had a relatively neutral pH range of between 6 to 8.5 pH units. Under these conditions, the effect of pH in affecting the release of phosphorus to the water was expected to be minimal.
1.3.4 Redox

Redox has the potential to affect all phosphorus forms. At redox potentials of less than 200 mV, oxygen and nitrate become less available, and bacteria may use oxidised forms of Fe to generate energy for metabolism (VanDemark et al. 1986a). In this process Fe$^{3+}$ (as Fe oxides, hydroxides or oxyhydroxides) is reduced to soluble Fe$^{2+}$, releasing it to the aqueous phase, along with any adsorbed or bound phosphorus (VanDemark et al. 1986a). Soluble or free Fe$^{2+}$ tends to readily adsorb to any remaining oxidised Fe, taking up space on the surface of these insoluble particles, reducing the area available for phosphorus binding and reducing the observed concentration of Fe$^{2+}$ in solution (Hsu 1976). Therefore, it may not be possible to correlate an increase of phosphate in the water column with changes in Fe$^{2+}$ concentration, which may account for a number of conflicting observations of the correlation between concentrations of released Fe and TP (Hsu 1976). In addition, lower redox potentials decrease the degradation rate of organic materials, causing organic phosphates to become more persistent.

1.3.5 Organic acids

The solubility and ease with which many organic compounds are degraded affects their impact on the phosphorus cycle in wetlands. The most significant group of organic compounds are the acids (Ehrlich 1990). Organic acids that directly affect the phosphorus cycle have either a carboxylic (-COOH), or a carboxylic and hydroxyl (-OH) side chain. Compounds possessing only a carboxylic side chain may be short chain carbon molecules (formic, acetic and oxalic acid), or long chain carbon molecules (humic acids). Both types of compounds alter solution pH, which directly affects phosphorus binding to Fe, Al and Ca compounds (Bolt et al. 1976). Organic compounds that have both carboxylic and hydroxyl side groups may also be short chained, for example citric, malic, aspartic, tannic and p-hydroxybenzoic acids, or long chained, for example fulvic acids (Ehrlich 1990). These compounds influence phosphorus cycling by altering pH and acting as chelating agents of metals (Jackson et al. 1975).
An increase in the concentration of organic acids causes a corresponding decrease in pH. The closer the carboxylic and hydroxyl side groups on the organic molecule, the greater is its binding affinity for metal ions (Ehrlich 1990). The order of binding efficiency for short chain organic compounds is of the order citric acid > malic acid > tannic acid > aspartic acid > p-hydroxybenzoic acid and corresponds to values for binding affinity ($\log K_i$ (Ehrlich 1990)).

The size of the organic acid affects the fate of the metal ions. Short chain molecules (molecular weight (MW) <150) are soluble in water. Thus, when they chelate free metal ions they tend to solubilise them. Organic molecules with a MW > 150 (fulvic acids) are generally not highly soluble in the water column (Ehrlich 1990). These compounds tend to settle from the water column, forming a detrital layer on the sediment, which has the capacity to bind metal ions. When a chelating agent encounters a clay it can interrupt the layer lattice structure formed by Al and Si, preventing hydrolysis (release of protons) as the soil ages, reducing soil acidity and increasing the available surface area of the clay (Ehrlich 1990). The increased surface area equates to an increase in phosphorus binding sites.

Organic acids also prevent the formation of new lattice structures within the soil by inhibiting the formation of Al hydroxides (Ehrlich 1990). This effectively destabilises and permits weathering of the clay. Destabilisation may solubilise bound phosphorus and possibly release adsorbed phosphorus. In preventing soil hydrolysis the organic acids can occupy binding sites that may have been available to phosphorus. In clays, organic acids may be either beneficial or detrimental. The deciding factor will be the concentration and form of the organic acid. The greater the concentration of organic acid the less likely it is that phosphorus will be adsorbed (Ehrlich 1990). The minimum concentration at which these acids have an effect is $10^{-6}$ M (malic acid) (Ehrlich 1990).

Humic material is the residue formed during the degradation of plant and other organic material and constitutes the major proportion of organic matter in soil and sediment. It is conventionally divided into three broad categories based on solubility:
fulvic acid; humic acid; and humin. Fulvic acid is soluble at all pH ranges. Humic acid is soluble at alkali but insoluble in neutral to acidic pH. Humin is an insoluble precipitate at all pH ranges. Fulvic and humic acids show similar carbon and hydrogen composition but different TN content and total acidity. The total acidity of fulvic acids is approximately twice that of humic acids due to the presence of both carboxyl and phenolic hydroxyl groups. Humic acids are thought to possess only phenolic hydroxyl side chains. The charge characteristics of humic material depend on the dissociation of carboxyl and phenolic hydroxyl groups, giving the molecule a net negative charge (Ehrlich 1990).

Compounds from these groups can form a protective layer over the colloidal sesquioxides of Fe, Al and Ca that inhibits binding with phosphate (Stevenson 1986). However, this is dependent on the size and type of humates. If they are of low molecular weight and readily degraded, then they may have a priming effect on the degradation of larger organic molecules, depending on oxygen availability (Stevenson 1986). This may reduce the protective layer formed through microbial degradation of the organic compounds involved, and increase the solubility of Ca and Mg phosphates through carbonic acid formation (Stevenson 1986).

Microorganisms have two primary roles in the cycling of organic material, the formation of humic material from biomass and the conversion of organic phosphorus compounds to orthophosphate (Ehrlich 1990). Humic material is primarily formed from lignified plant tissue and consists of combinations of oxidised aromatic, alicyclic and aliphatic hydrocarbons (Schnitzer et al. 1972). These combinations make microbial attack difficult, and even under aerobic conditions it proceeds slowly. Such compounds constitute the organic mixture known as peat, which can persist for thousands of years (Schnitzer et al. 1972). Conversion of organic compounds results in carbon dioxide, water and phosphate if sufficient oxygen or other electron acceptors are available otherwise the resultant degradation compounds may prove refractory. These processes are an integral component of wetland processes and phosphorus sequestering. Their impact on wetland functioning and phosphorus removal within wetlands is more fully discussed in Section 1.5.
1.4 WETLANDS

1.4.1 Overview
Wetlands offer a comparatively low cost, low technology alternative for polishing secondary or tertiary effluent to reduce nitrogen and possibly phosphorus concentrations from discharge water (Kadlec et al. 1996; Rohlick et al. 1972) whilst adding to the aesthetic qualities of an area. The relative cost of a WWTP is in the order of hundreds of millions of dollars, whereas a comparable constructed wetland may be less than one tenth of this, depending on land costs (Cooper 1993; Hammer 1992; Kadlec et al. 1996; Walbridge 1991). The major disadvantages of using wetlands include questions over their ability to remove phosphorus over long time intervals (Johnson 1991) and their large footprint. Wetlands currently require several hectares of land to reduce nutrient concentrations in sewage effluent from a small source (e.g., a town of 500 people) below the limits (Table 1) set by the State Environmental Protection Agency (EPA) (DLWC 1998; EPA 1997; Kadlec et al. 1996).

The wetland size is governed by the loading of each parameter. If the effluent has undergone tertiary treatment, then the limiting factor for sizing may be ammonium removal (DLWC 1998; Kadlec et al. 1996), however, if only secondary treatment has been undertaken then the size limiting factor may become phosphorus concentration because of the difficulty wetlands have in removing high concentrations of TP (Kadlec et al. 1996). In the short term, during wetland establishment, large percentage reductions in phosphorus concentrations have been consistently demonstrated. However, as the wetland reaches maturity the percentage removal of phosphorus often declines, and may actually cease. Reasons for this are unclear, although thought to be from saturation of available phosphorus binding sites within the soil and maturation of plants within the wetland (Carignan 1985; Craft et al 1995; Gumbricht 1993). If the wetland is not well maintained and operated at this stage phosphorus may actually be liberated from the sediment to the water column causing an increase in concentrations of phosphorus observed at the wetland outlet (Carignan 1985; Craft et al 1995; Gumbricht 1993). Wetlands are biologically systems, which are perceived as being
difficult to manipulate and control. There is a degree of uncertainty associated with their long-term ability to reproducibly and predictably reduce nutrient concentrations. Hence, there has been a reluctance to use wetlands for nutrient removal.

1.4.2 Wetland definition
A wetland is a region that is periodically or permanently flooded with water and contains aquatic plants. It can vary in size from a small puddle less than a meter in diameter up to a large swamp of several hundreds or thousands of hectares and can be in a broad range of terrain from floodplains to ponds and bogs (Holland et al. 1990). Wetlands that are supplied by a river are termed Riverine, those on a lake are Lacustrine systems, and those not confined by channels or marginal lakes are Palustrine systems (Davis et al. 1978; Sloey et al. 1987). This thesis is concerned with the study of constructed wetland systems. These are regions that have been manipulated to maintain a specific water depth and flow rate, established with emergent and submergent aquatic plants. There are a number of similarities between natural and constructed wetlands. Therefore, a brief summary of the salient features of natural wetlands is presented to aid in understanding the processes operating in constructed wetlands.

Natural Wetlands
Natural wetlands have been described as “lands transitional between terrestrial and aquatic systems where the water table is usually at or near the surface and the land is covered by shallow water” (Cowardin et al. 1979). All wetlands have a proportion of plant species, although the percentage of wetland surface covered by plant species can vary from as low as 30% (Cowardin et al. 1979; Lawrence 1986) to full coverage (Cooper 1993).

Stagnant or low flow wetlands have been subjected to a rigorous classification scheme in the United States. They are classified based on soil pH, plant type and origins of the surface water (Johnson 1991). Those with primarily inorganic soils (comprising not more than 12 to 18% organic carbon (Cowardin et al. 1979)) are termed a marsh if they contain mainly herbaceous vegetation, or a swamp if larger woody species are
present (Johnson 1991). Wetlands with soils high in organic material typically receive little or no surface water and are classified as peatlands (Cowardin et al. 1979). These fall into two categories: fen and bog. A fen is a minerotrophic peatland receiving nutrients from surface water, groundwater and precipitation, with a neutral or basic soil dominated by species of Gaminoids such as Carex (Cowardin et al. 1979; Verhoeven et al. 1990). A bog only obtains water by precipitation and has an acid soil dominated by peat mosses including Sphagnum (Cowardin et al. 1979; Verhoeven et al. 1990). The mineral content of Bogs is significantly higher than fens (Waughman 1980).

Functions of a natural wetland

In the natural environment, wetlands serve many functions, including:

1. Habitat for wildlife;
2. Retarding of floodwaters to diminish the stress on downstream waterways (Niering 1968); and
3. Acting as sinks to trap nutrients and other materials from point and non-point sources, including WWTP (Klopatek 1978).

The plants and the open body of water within the wetland form a significant habitat for waterfowl and other wildlife. Wetlands that retard floodwaters may be found within streams, where the plants within the wetland serve as a natural barrier to flow, reducing the velocity for downstream areas.

Nutrient removal occurs on differing time scales and through a variety of mechanisms. Fens and bogs tend to accumulate nutrients over time intervals ranging from tens of years to centuries (Craft et al. 1995), and are typically supplied from low nutrient sources. In riverine systems, littoral wetlands have a major role in reducing the concentration of various substances entering river systems from specific land sources, whilst 'in stream' wetlands treat the existing stream water quality to improve the quality downstream (Davis et al. 1978).

A number of studies have shown that natural wetlands are efficient in the removal of suspended solids (SS), organic material, biochemical oxygen demand (BOD), nitrogen (nitrate (NO$_3^-$), nitrite (NO$_2^-$)), and ammonium (NH$_4^+$) and heavy metals
(Cowardin et al. 1979; Davis et al. 1978; DeLaune et al. 1980; DLWC 1998; Klopatek 1978). Based on these studies wetlands are seen as a simple, low cost technology, competitive with nutrient polishing processes used in conventional WWTP systems, and an augmentation to gross pollutant traps and retention basins for treating stormwater (DLWC 1998). However, their ability to consistently remove phosphorus remains questionable (Davies et al. 1993; Richardson 1985), limiting their widespread adoption for nutrient removal, particularly if this is linked to ongoing licensing limits or regulations.

**Constructed Wetlands**

Constructed wetlands may be designed to simulate natural wetland conditions. As with natural wetlands, constructed wetlands are typically designed for habitat and nutrient removal, and more rarely flood mitigation (DLWC 1998; Hammer 1992). These wetlands may have an impervious liner (clay or plastic) to avoid groundwater communication (loss of wetland water to groundwater or entry of material from groundwater into the wetland) and water depths between 0 to 2.5 m (DLWC 1998; Kadlec et al. 1996). If a wetland is not restored from a pre-existing marsh, it may have no historical natural peat accumulation. Mature wetlands typically have saturated soil binding sites and a lower redox potential (Craft et al 1995). Although variants of wetland systems have been in use since before Roman times, the applied use of reeds for sewage treatment was initiated in Germany by Seidel and Kickuth in the 1960’s (Cooper 1993).

Constructed wetlands are of three basic types:

1. surface flow or free water wetlands (FW);
2. subsurface flow (SSF); and

FW wetlands are regions where water flows overland, above a surface substrate, typically of soil and sometimes clay. In SSF wetlands, the water flow is below the ground surface through substrates of clay, soil or gravel. There appears to be a predominance of SSF wetlands in Europe, designed to process all forms of sewage
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effluent (from primary to tertiary (Cooper 1993; Kowalik et al. 1995)). Whilst in America and Australia wetlands that treat secondary and tertiary effluent are predominantly flooded FW marshes (Bavor 1992; Bavor et al. 1987; Cooper 1993; Knight et al. 1993). In the main, these preferences are dictated by land availability and treatment load (Cooper 1993).

Vertical flow wetlands are planted beds of porous substrate, where the majority of flow is in the vertical direction, either upflow or downflow. These systems can be used in isolation for small-scale treatment, or more commonly (particularly in Europe), they are used as a pre-treatment step to enhance the performance of the more traditional SF and SSF wetlands (Brix 1995; Radoux et al 1995).

Most European beds are constructed with straight sides, between 0.3 to 0.6 m deep with a soil or gravel substrate (Cooper 1993). Gravel beds typically have gravel in the range 3-6 or 5-10 mm with a water flow velocity (Kf) of $10^{-3}$ m.$s^{-1}$ or higher or soil with $K_f = 10^{-2}$ m.$s^{-1}$ or less (Cooper 1993). Stone gabions (60-100 mm) are used to stop large amounts of organic material entering the wetland and plugging the soil pores. Weekly maintenance is required (Cooper 1993). The surface area of these wetlands is calculated by equations such as: $A = \frac{Q(\ln C_o - \ln C_i)}{K}$. Where $C_o$ = concentration of TP at the inlet ($C_o$) or outlet ($C_i$), $K$=decay constant, $Q$ = average daily flow and $A$ = area (Cooper 1993). A commonly applied rule of thumb is 5m$^2$ per person connected (i.e. equivalent person EP (Cooper 1993)).

American and Australian systems are more varied, from large flooded marshes or swamps up to several hundred hectares in size, to smaller scale wetlands that are lined and have steep batters and a variety of water depths. Several equations are available to predict efficiency and nutrient removal. They are similar to those for European systems, using the assumption of uniform plug flow and that phosphorus decay can be explained by a simple first order equation (Cooper 1993; Reed et al. 1995). However, caution is required in their use as the majority of equations do not take into account:

- water and nutrient losses through evaporation and groundwater recharge;
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- additions through communication with the aquifer, precipitation and surface runoff; or
- background sediment nutrient concentrations and the phosphorus adsorption potential of the sediment.

Sizing and landscaping of wetlands is dependent on the nutrients to be removed. Sizing can be determined by either rule of thumb or simple equation as noted above. The two models recommended for use the NSW DLWC (DLWC 1998) are those by Reed et al. (1995) and Kadlec and Knight (1996, the k-C* model). Both of these models assume uniform plug flow and use a first order decay model to predict parameter removal. Reeds’ method uses different equations for suspended solids and total phosphorus than for BOD and nitrogen species. Each method has its own set of limitations and assumptions. Landscaping can be either to attract wildlife or to address diffuse source inputs.

1.5 MECHANISMS OF PHOSPHORUS ACCRETION BY WETLANDS

The ability of wetlands to store and transform organic matter and nutrients has lead to their definition as “the kidneys of the landscape” (Mitsch et al. 1993). Phosphorus accumulation by wetlands primarily occurs in the initial years after establishment due to uptake into plant biomass and binding to soil (Cooper 1993; Craft et al. 1995). Wetlands are preferred to open ponds as the vegetation not only adsorbs phosphorus but has the effect of knitting the soil particles together, providing up to 3 times the binding strength of unvegetated soils (Knutson 1988), minimising resuspension and loss during high flows. In addition, the vegetation provides an impediment to flow, trapping incident sediment. During establishment this process is more pronounced due to the high rate of root growth and as a result an establishing marsh can collect 2-2.5 times the amount of sediment of an established marsh (up to 5 cm.m⁻².yr⁻¹ (Craft et al 1995; Knutson 1988)). On a mass basis this can be around 2-3 g.m⁻².yr⁻¹ (DeLaune et al. 1980).

As the system ages the soil redox decreases, which can cause a subsequent reduction in phosphorus storage capacity due to the accumulation and degradation of litter.
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(Craft et al 1995). The long-term removal of phosphorus by wetlands is dependent on uptake by the biomass, or immobilisation by sorption and precipitation processes (Gumbricht 1993). The efficiency of these processes is largely determined by the biological composition of the wetland and the chemical and physical structure of the substrate (Gumbricht 1993).

1.5.1 Phosphorus pathways

Phosphorus enters a wetland through stream flow, rainfall, runoff, atmospheric deposits and biota (Figure 1-1). Within the wetland it exists in one of four compartments: biological; dissolved; waterborne particulate material or SS; and sediment (Broberg et al. 1988). On entering the water column phosphorus is readily taken up by biofilms and free-floating microorganisms (Figure 1-1). The role of these biofilms is unclear but is suspected to facilitate the transport of phosphorus into plant tissue or through the sediment (Tate 1985).

Phosphorus may move unassisted through sediment interstitial water where it can bind to sediment particles or become cycled at the plant roots (Figure 1-1). Sediment particles can bind phosphorus for long periods (8 – 5,600+ years (Johnson 1991)). In addition, phosphorus also has the potential to move through the interstitial soil water to soil biofilms and into the roots of plants, where it can be translocated to the leaves and stems of the plant (Figure 1-1). The presence of plants serves two functions, to open the soil pores and to increase hydraulic conductivity (i.e. movement of water through the soil), as the plant acts as a water pump during evapotranspiration (Harbler et al. 1990). Once in the plant, phosphorus can be re-released into soil or water biofilms and interstitial water. Plant tissue may also senesce to form detritus that deposits on the sediment surface (Figure 1-1 (Richardson et al. 1986)). Fish and other detritivors consume both this tissue and microbiota, incorporating phosphorus into their biomass and recycling it into the water column through their excrement or as they die. This material, together with sloughed biofilm, free floating microorganisms and some sedimentary material is present in water that elutes from the wetland (Figure 1-1) and is typically washed out of the wetland during heavy rains (Devito et al.
1993). These components together constitute the net amount of phosphorus released from the wetland.

![Diagram of phosphorus cycle in a constructed wetland](image)

**Figure 1-1: A simplification of the phosphorus cycle in a constructed wetland**

### 1.5.2 Water column

The water column represents only a transient storage compartment. Its main function is in the transport of phosphorus to and from long-term storage compartments (such as the sediment or litter), or out of the wetland. Movement of phosphorus through the water column to the sediment surface occurs by several mechanisms:

1. Incorporation to algae and bacteria;
2. Biological incorporation into polyphosphate;
3. Settling with SS material by gravity;
4. Precipitation with Fe, Al and Ca salts; and
5. Direct interaction with sediment and detritus (Sloey et al. 1987).
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Algae and Bacteria

The characteristics of phosphorus uptake in bacteria and algae has been well studied (Bostrom et al. 1988; Broberg et al. 1988; Burkholder et al. 1990; 1992; Craft et al. 1991; Nicholls et al. 1979; Richardson et al. 1986; Rodhe 1969). Although, direct incorporation of phosphorus into algae and bacteria can occur with virtually all orthophosphate entering the wetland (Bostrom et al. 1988; Schurmann 1964), phosphorus incorporation into microorganisms is considered only a short term storage mechanism (Richardson et al 1995). Cyanobacteria and algae represent the largest potential for microbial phosphorus storage, being capable of storing 300-500 times more phosphorus than bacteria (Andersson et al. 1988). However, bacterial population numbers are often several orders of magnitude larger than for algal populations, which has been shown to offset the benefits of increased storage capacity by up to 5 to 10 fold (Broberg et al. 1988). The phosphorus concentration in algae can range from 0.04% to 0.96% by dry weight in freshwater lakes (Rodhe 1969) and has been shown to constitute up to 3% of the TP pool in a wetland (Richardson et al. 1986). Transport to the sediment may occur via migration through the water column using gas vacuoles or flagella, aggregation to each other or death (primarily of algal cells) (Rodhe 1969).

Algae

Algae are autotrophic microorganisms that photosynthesise using chlorophyll (a, b or c). They may exist in the water column or at the sediment surface, provided adequate light is available. A subgroup of organisms that are intermediate between algae and bacteria are the cyanobacteria or blue green algae (true freshwater algae are typically referred to as green algae). These organisms differ from the green algae in being able to control their buoyancy and produce toxins that inhibit the growth of competitor species (DLWC 1998). Algae utilise phosphorus in cellular processes and a number of equations linking light, nitrogen, phosphorus and carbon levels to algal growth have been derived using laboratory investigations, however their applicability to natural systems is questionable and requires much modification (Leihr et al. 1990; Sweazy et al. 1975).
Algae are able to utilise high and low MW phosphorus compounds through excreted extracellular enzymes (Broberg 1985). These enzymes split organic compounds to generate orthophosphate (Broberg 1985; Janjean et al. 1974). The ability of algae to utilise available phosphorus is affected by the species present, enzyme pool, starvation status, phosphate uptake rate, the nature of the phosphorus compounds and the environmental conditions (Broberg 1985; Janjean et al. 1974). The algae species present control the potential enzyme pool, which is in turn influenced by available nutrients and environmental conditions. Enzymes provide the mechanism by which organic phosphorus compounds may be mineralised and affect the rate of uptake, which is also species dependent (Broberg 1985; Janjean et al. 1974). The nature of the phosphorus compounds influences their ability to be degraded (Section 1.2.2).

Phosphorus starved algae can take up phosphorus in excess of their needs and store it as inorganic polyphosphate for later use (Rhee 1973). In addition, algae can form a film or layer on sediment, oxidising the upper few centimetres of sediment and preventing phosphorus release to the water column (Van Luijn et al. 1995). This provides a competitive advantage by capping the sediment and limiting the potential for algal growth in the water column. Such algae or diatoms can contribute 0.08 to 0.63 mg P.m$^{-2}$.d$^{-1}$ phosphorus to the sediment surface (Van Luijn et al. 1995).

Bacteria

Bacteria predominantly exist on surfaces, forming biofilms with algae and fungi. They have a high phosphorus demand and are more likely to sequester phosphorus than release it to the water column directly, with the rate of uptake being temperature dependent (Andersson et al. 1988). Within wetlands, bacteria have three predominant functions in phosphorus transformation:

1. Sequestering of phosphorus; and
2. Degradation and solubilisation of organic phosphorus forms
3. Alteration of solution pH to allow release of inorganically bound phosphorus compounds (Holtan et al. 1988).

The bacterial population, their protozoan predators and the supply of nutrients including dissolved organic carbon (DOC (Hamilton et al. 1987)) determine the
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dynamics of these functions. The bacterial population will determine how sequestering might occur and whether polyphosphate compounds are formed. Protozoan predators control population dynamics, limiting numbers of bacteria available for transformation or degradation. DOC can provide either an alternate carbon source or readily degradable organic phosphorus compounds (Hamilton et al. 1987). The degradation of organic compounds can acidify the surrounding water, allowing solubilisation of iron and aluminium bound phosphorus.

When bacteria are stressed they tend to utilise alternate metabolic pathways to conserve energy, one such pathway is the formation of polyphosphate and PHB (poly $\beta$ hydroxy butyrate), which are produced when bacteria are deprived of oxygen and nitrate (Nicholls et al. 1979). This metabolism allows these bacteria to consume organic matter under severe oxygen limitation (Nicholls et al. 1979). Instead of the organisms utilising the Krebs cycle to produce ATP they may use the Acetyl coenzyme A pathway (Acetyl coenzyme A $+\text{ADP} + \text{P} \rightarrow^{+2\text{H}} \rightarrow \text{ATP} + \text{Acetic acid} + \text{CoA}$H (Stanier 1975)). A by-product of this pathway is the formation of polyphosphate. However, for the reasons noted in Section 1.2.2, such products are only transient in the natural environment.

**Biofilms**

Biofilms are thin films of microorganisms that occur on soil particles and plant surfaces. They may comprise of algae, bacteria, fungi and small eukaryotic organisms. Although ubiquitous in occurrence, biofilms generally retain less than 1 % of the annual input of phosphorus from the water column (Burkholder et al. 1990). Cells at the surface of the biofilm accumulate almost 10 times the amount of nutrients as those deeper in the biofilm (Burkholder et al. 1990). The factors influencing the size and depth of a biofilm are the available surface area, nutrient availability and water flow (Burkholder et al. 1990). Given a constant flow rate a biofilm will reach a maximum size and density. Once this is achieved the organisms will continue to cycle phosphorus internally, with sloughing occurring mainly during high flow events (Bryers et al. 1989). Biofilm sloughing is the release of the outer biofilm layers to the water column with the possibility of nutrients leaving the wetland. This will be
followed by an increase in nutrient removal from the water column as the biofilm reforms.

In soil, biofilms are less affected by sloughing events, but will reach a maximum density dependant on soil porosity, particle size and a variety of environmental parameters (VanDemark et al. 1986b). The maximum population achieved will be primarily constrained by pore space and nutrient availability. The composition of the microbial populations and their respective metabolism's may be able to be manipulated by controlling the microenvironments and external nutrient conditions (Konings et al. 1992; Lewis et al. 1986). For example, presenting soil bacteria with nutrients (C, N, P) has been demonstrated to increase the phosphorus concentration in the biomass by at least 50% relative to soils receiving no treatment (Hynes et al. 1970; Thien et al. 1992).

**Suspended Solids**

SS are particles able to be filtered from the water column. They have settling times ranging from days to weeks, determined by charge, size, shape and density (Masters 1997). The majority of waterborne particles have a net negative charge (Masters 1997). This is a force of repulsion, which may act in opposition to gravity. The shape of a particle affects its ability to migrate through the water column (Masters 1997). The size of the particles affects the number per unit area and hence the charge density of those particles. The charge density on colloidal sized particles is very high and if the repulsion forces exceed gravitational attraction, they can remain in suspension for periods of months to years (Masters 1997). The primary reactions between organic matter and dissolved ions occur with these colloidal size particles (Koenings et al. 1976). At low charge densities, the force due to gravity prevails and in a non-turbulent system such as a wetland, the particles sink at a rate dependent on their density (Masters 1997). The greater the density the more rapid will be the rate of settling. By increasing either the mass of the particle or its density (through the attraction or reaction with other particles), it should be possible to increase the rate of deposition from the water column.
The smaller size fractions of colloidal material in most instances have a higher phosphorus content than other particle size fractions (Oliver et al. 1993). Fe and Al adhere to organic fractions of all sizes in the water column (Bostrom et al. 1982) and may therefore lead to additional adsorption of TP onto small particles due to the greater surface area to volume ratio.

It is in the aggregation of colloidal material that microorganisms may have a role. Microorganisms show a great affinity for solid/liquid interfaces, forming biofilms on the solid surface to extract nutrients from the surrounding water column (Tate 1985). Microorganisms can also concentrate phosphate from the water column by direct incorporation into their biomass and by formation of polyphosphate or organic phosphate compounds. The formation of microbial films or the deposition of phosphorus containing compounds on SS particles may be one way to increase their density sufficiently to precipitate them out of solution, enhancing phosphorus removal to soil and sediment substrates.

**Particle accretion**

Within a wetland significant variations in accretion rates can occur, with the accretion rate for alluvial or high sediment input wetlands typically observed at approximately 1,700 g.m\(^{-2}\).yr\(^{-1}\), although the highest rates approach 7,840 g.m\(^{-2}\).yr\(^{-1}\) (Johnson 1991). Clay particles and high molecular weight humic acids can aid precipitation through flocculation of suspended particles (Boto et al. 1979). Phosphorus deposition by sedimentation (1.5 mg P.m\(^{-2}\).yr\(^{-1}\)) can be an order of magnitude greater than by litter deposition (0.26 mg P.m\(^{-2}\).yr\(^{-1}\) (Johnson 1991)).

**Inorganic compounds**

Precipitation of Fe, Al and Ca compounds or complexes is due to their oxygenation in solution and the subsequent adherence of other compounds (Hsu 1976; Prenki et al. 1978). The formation of oxygenated compounds is assisted through the maintenance of neutral pH and aerobic conditions (i.e. positive redox (Prenki et al. 1978)). The minimum precipitation ratios for Fe and Al phosphorus complexes are 1:1 in simple systems (Hsu 1976). However, in the environment ratios from 1.5 (Rhine estuary (Van Eck 1982)) to 10:1 (Lakes (Williams et al. 1971)) have been observed. Similar
ratios are expected for Ca. The precipitation of Fe/Al/Ca-P complexes is optimised by high collision rates (induced by elevated salinity), high rate gradients (i.e. flow rates, nutrient concentrations), and high concentrations of turbid particles (providing flocculation nuclei (Stumm et al. 1979)).

The formation of low molecular weight organic acids by heterotrophic bacteria under anaerobic conditions will tend to solubilise Fe and Al bound phosphorus, due to the lower pH (unless it can bind to Ca compounds) and the potential for oxidised iron to be utilised in microbial metabolism (Maine et al. 1992). However, larger carbon molecules, such as fulvic acids formed from detritus, may trap and chelate phosphorus compounds through the formation of organo-metallic complexes and transport them to the sediment (Koenings et al. 1976).

1.5.3 Animals
Almost no studies of wetlands have taken into account the effects of macrobiota such as fish in nutrient budgets. Animals play a role in phosphorus cycling through consumption and digestion whereby organic phosphorus compounds are mineralised and released by excretion or egestion. Animal foraging particularly by benthic fish species actively contributes to translocation of phosphorus to or from epilimnetic water. The high mobility of fish species precluded them from this study. In addition, small zooplankton have been shown not have a significant effect on phosphorus availability in the water column or its uptake by algae (Hamilton et al. 1987).

1.5.4 Plants and litter

Plants
In a wetland, environment plants provide an active water pump to cycle nutrients from the water column through the sediment. Accumulation of phosphorus directly into plant tissue is related to plant growth. During establishment plants can remove 70% or more of the incoming phosphorus (Adcock et al. 1995). Phosphorus removal by mature plants is often insignificant (Craft et al 1995). Management of mature wetlands through the removal of vegetation only results in the removal of less than 5 g
P.m$^{-2}$.yr$^{-1}$ which usually accounts for less than 20% of the mass of phosphorus entering a wetland (Sloey et al. 1987).

In general, phosphorus removal by plants is influenced by temperature and growing season, with greater removal during summer as phosphorus is incorporated into plant biomass (Devito et al. 1993; Johnson 1991). Plants obtain nutrients from the water column and interstitial water via their roots. Roots and stems also stabilise sediments by reducing erosion velocities and entrapping sediment particles (Odum E.W. 1990; Thorne 1990). Microorganisms, particularly fungi, have a role in assisting phosphorus sequestering by plants (Stevenson 1986). This is primarily through the translocation of nutrients to the root surface (Burford et al. 1975).

The maximum uptake of nutrients by emergent plants is low and varies depending on species. Typical figures include 4.3 g P.m$^{-2}$ (summer) and 2.2-2.5 g P.m$^{-2}$ (winter) for *Typha Latifolia* (Prenki et al. 1978), 1.0 g P.m$^{-2}$ for *Carex* spp. (Richardson et al. 1986), and 3.7 g P.m$^{-2}$ annually for *Scirpus Fluvatilis* (Klopatek 1978). An average apportioning of phosphorus would be of the order 0.9 to 1.35 mg.g$^{-1}$ dry weight for stems, 1 to 1.7 mg.g$^{-1}$ for leaves and 0.9 to 1.63 mg.g$^{-1}$ for whole shoots (Hocking 1989). These studies indicate potential variations for individual plants. However, investigations by Richardson demonstrated that overall nutrient removal in wetlands could be independent of the plant species (Richardson 1985). Typical measurements indicate phosphorus can average 0.2% of the dry weight and up to 0.5% of free floating vegetation in a wetland (Davis et al. 1978).

The concentrations of phosphorus absorbed into the tissues of floating plants, such as *Eichehonia* spp. and *Lemna* spp., can vary between 3 to 80 g P.m$^{-2}$ (Korner et al. 1998; Zirschky et al. 1988) annually under ideal conditions, equivalent to or much higher than emergent plants. However, their location and movement are difficult to control. Phosphorus is absorbed into the leaf structures but is transported to the sediment surface when the plant cells die or the wetland dries out, as they have no emergent root structure. Detritus from these plants is highly susceptible to biodegradation due to the low lignin content (Bouchard et al. 1998; Greenway 1994;
Rice et al. 1981), and its role in long term phosphorus sequestering in low maintenance wetlands thought to be minimal, but not well understood or characterised. Nutrient contributions from floating plants were not directly investigated in this study.

**Sediment aeration**

In addition to nutrient accumulation, plants also may aerate the sediment substrate and affect soil chemistry to produce or retain oxygenated iron and aluminium compounds capable of adsorbing phosphorus, although the extent to which this mechanism has an impact on wetland processes is questionable (Banoub 1977; Brix et al. 1993). At Silver Lake in south western Washington, *Moenchites tripoliata* L. was shown to create an oxygenated rhizosphere by translocation of oxygen to the roots. This resulted in lower concentrations of total soluble iron and manganese near the roots and strong pH buffering, promoting phosphorus sequestering (Moore et al. 1994).

**Plant Senescence**

A net effect of rooted vegetation may be the movement of phosphorus into the water column via detritus as the plant senesces during late summer and autumn (Carignan 1985; Richardson et al 1995). On senescence between 35% to 80% (Richardson 1985; Richardson 1985; Richardson et al. 1986; Richardson et al 1995; Simpson et al. 1978) of phosphorus will be lost to the environment, with the remainder being sequestered to below ground biomass in autumn and winter (Johnson 1991; Richardson et al. 1978). Nutrient below ground stocks can range from 1:1 to 1:3 of the above ground stocks (Johnson 1991).

Detritus generated by plant senescence is either slowly degraded or forms a permanent storage such as peat. In addition, such material may also be washed out of the wetland during periods of high flow. Litter decay is calculated as the decay constant \(-k = \ln(X/X_0)/t\) and the half life as 0.693/k (Jenny et al. 1949). Where \(X =\) mass of detritus remaining, \(X_0\) is the mass at time zero, and \(t\) is time. Decay constants for herbaceous plants and their leaves ranges from 0.2 to 2.0 kg.ha\(^{-1}\).yr\(^{-1}\), with slowing decaying twigs and wood between 0.1 to 0.3 kg.ha\(^{-1}\).yr\(^{-1}\) (Johnson 1991) and up to 18.1 kg.ha\(^{-1}\).yr\(^{-1}\) for wetland sedges such as *Sagittaria latifolia* (Davis et al. 1978).
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The primary converters of organic detritus are microorganisms (Hedley et al. 1982). The rate for transformation of material into peat varies from 0.05 (Richardson 1985) to 5 kg.ha\(^{-1}\).yr\(^{-1}\) (or 0.5 g.m\(^2\).yr\(^{-1}\), with the upper end being for Narrow leaved sedges – eg Carex spp (Richardson et al. 1986)). The percentage of phosphorus stored in peat varies but has been observed at greater than 90% (Richardson et al. 1978). The thickness and mass of peat accumulation decrease with depth due to decomposition and compaction (Johnson 1991).

The rate of detritus accumulation is affected by:

1. **Plant structure/composition – degree of lignification**: lignin is a refractive carbon compound that is difficult to biodegrade and so will persist in the environment for long periods. Plants that are less lignified should degrade more rapidly, acting as a short to medium term phosphorus store (Bouchard et al. 1998; Greenway 1994; Rice et al. 1981).

2. **Climate**: biodegradation proceeds at greater rates in warmer climates.

3. **Fire and animal disturbance**: fire in wetlands results in significant export of nitrogen and phosphorus into the water immediately after the event from plant tissue and in forests the export rates can remain high for up to 6-9 years after the fire (Bayley et al. 1992).

4. **Flooding**: will reduce the diffusion of oxygen to detrital material, adversely affecting biodegradation rates. If an elevated water depth is maintained for a period, it may significantly reduce the rate of oxygen diffusion. In addition, flooding can also result in passage of detrital material out of the wetland because of elevated water flow.

5. **Two other factors - geologic factors and human intervention** (eg logging), influence accumulation rates but are associated with very large wetlands and have not been considered here (Polunin 1984).

1.5.5 **Sediment/water interface**

The sediment/water interface is the region of initial contact of phosphorus with the sediment. In unplanted regions, most soil phosphorus is concentrated near the surface.
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with slow movement through the soil by diffusion, accelerated by the presence of plant roots (Tate 1985).

Nutrient fluxes across the sediment/water interface are due to:
1. Precipitation and settling of solid particles;
2. Movement of dissolved material with water flow;
3. Hydrostatic pressure, which can be due to communication with an aquifer or evapotranspiration driven fluxes;
4. Molecular diffusion from the overlying water;
5. Microbial sequestration and metabolism; and

Losses of phosphorus to the water column in the interface region can be as high as 650\( \mu \text{g P.m}^{-2}.\text{day}^{-1} \) (240 mg P.m\(^{-2}.\text{yr}^{-1} \) (Neame 1977)) although the typical range is from 0 to 100 mg P.m\(^{-2}.\text{yr}^{-1} \) (Richardson et al 1995). Primary sources of phosphorus generation at the soil/water interface are microbial degradation of detrital material (380 \( \mu \text{g P.m}^{-2}.\text{day}^{-1} \) and insect emergence and fish feeding (averaging 33 and 23 \( \mu \text{g P.m}^{-2}.\text{day}^{-1} \) respectively (Neame 1977)). In certain circumstances, it has been demonstrated that if the oxygen concentration at the sediment water interface is maintained above 2 mg.L\(^{-1} \) then phosphorus will not be released from the sediment to the overlying water (Mortimer 1971).

1.5.6 Soil
Phosphorus concentrations in wetland soils range from 0.001 to 7.0 mg P.g\(^{-1} \) (Richardson et al 1995). Phosphorus removal and release from soils is a highly complex phenomenon. It includes interrelationships between the biota, sediment chemistry and physical processes (Boström et al. 1988). However, soil type (i.e. organic or inorganic) has little effect on the net amount of phosphorus removed (Johnson 1991). Nor does the concentration of organic or inorganic compounds present (Johnson 1991), which can range significantly, e.g inorganic phosphorus has been shown to range from 90.6% in oligotrophic lakes (Petterson 1986) to a minimum of 5.8% in inorganic lakes (Messner et al. 1984).
CHAPTER I

The majority of phosphorus (97%) is trapped in the upper 15 to 90 cm of sediment (Klotz 1991; Stevenson 1986). Movement of phosphorus through the sediment is predominantly through flow of interstitial water and chemical concentration gradients, microorganisms appear to have no significant role in the bulk transfer of nutrient through large regions of soil (Hynes et al. 1970). Binding of phosphorus compounds to sediment is a different process, affected by interstitial phosphorus concentrations, wind induced turbulence, bioturbation, temperature, pH, redox conditions, carbon concentration, microbial metabolism, and planktonic growth on the sediment (Boström et al. 1988; Mitsch et al. 1995).

Phosphorus uptake by sediment depends on substrate characteristics and oxygenation. Removal mechanisms (in contrast to net removal amounts) vary depending on whether the phosphorus compounds are organic or inorganic. When phosphorus contacts soil particles it may be adsorbed to the soil grains due to Van Der Waals forces; react with compounds in the soil; or be assimilated into the indigenous microbial biomass. Most phosphorus compounds are negatively charged. Therefore, Van Der Waals forces will attract them to positively charged ions within the soil. However, this form of binding is weak compared to formation of stable compounds with elements in the soil matrix (Petterson et al. 1988). Microorganisms are capable of accumulating phosphorus as part of their cellular matrix, however their main role appears to be in facilitating and regulating the movement of phosphorus to sediment surfaces (Maher et al. 1995). Microcosm experiments indicate that fungi and yeasts are predominantly responsible for initial phosphorus removal (within the 1st hour) (Richardson et al. 1986). There are three mechanisms by which phosphorus may become bound to a sediment surface, through:

1. Direct soil sorption;
2. Decomposition and subsequent sorption to soil particles; and
3. Isomorphic replacement.

Soil sorption is described on Page 38. Isomorphic replacement involves the direct substitution of silica with iron or aluminium primarily in Kaolinite clays (Patrick et al. 1968) and was of limited application in the wetlands investigated in this study.
CHAPTER 1

Long-term accumulation of phosphorus in wetlands occurs via sorption to sesquioxides of iron and aluminium in acid soils or calcium in basic soils (Brady 1990). This material may be further stabilised by complexation with humic compounds in the soil. Initial investigations into lake phosphorus storage mechanisms in the 1930’s and 1940’s (Einsele 1936; Einsele 1938; Mortimer 1941; Mortimer 1942) provided convincing evidence that association with iron compounds was the dominant removal mechanism for phosphorus. More recently the phosphorus removal ability of a wetland has been stated as being best predicted by the Fe and Al content of the soils (Richardson 1985). In addition, the amount of iron in soil has been shown to regulate the amount of phosphorus binding sites in the sediment of a number of European lakes (Jensen et al. 1992). By maintaining soil iron to phosphorus (Fe:P) ratios above 15 and aerobic conditions it has been possible to minimise phosphorus release from the sediments of these lakes (Jensen et al. 1992).

However, more sensitive instrumentation indicates that overall phosphorus release is not well predicted by iron chemistry alone (Boström et al. 1988). In lake sediments the predominant inorganic phosphorus forms are complexes of iron and aluminium, comprising up to 62% for inorganic lakes (Chang et al. 1957) and around 43% in peaty sediment (Boers et al. 1984). Within wetlands, iron and calcium phosphates arguably represent the greatest fractions of inorganic phosphorus (Richardson 1985), with precipitation of Ca occurring above pH 6.5 (Patrick et al. 1968). The predominance of each mechanism depends on pH, the availability of each ion, and with the rate of reaction proportional to temperature (Berkheiser et al. 1980). The presence of insoluble iron oxides increases the number of phosphorus ions able to be bound (Anderson 1964; Lake et al. 1977), although more weathered soils such as those in Australia bind aluminium in preference to iron (Anderson 1964).

Humic complexes with iron and aluminium may account for more than 50% of phosphorus in soil (Gerke 1992). The optimal pH for the formation of these complexes is 7.5 (Gerke 1992). Humic complexes of iron and aluminium appear to readily bind phosphate ions, although the evidence is only circumstantial (Jackson et al. 1975). In the US studies of phosphate adsorption index indicated a high
correlation between extractable amorphous aluminium and iron $r = 0.929$ and 0.621 respectively, rather than with pH, extractable calcium or organic matter in certain wetland systems (Richardson 1985).

**Microbial interactions**

The microbial pool of phosphorus in soil is typically small and rapidly becomes saturated (Richardson et al. 1996). However, microbial biomass can be a significant phosphorus storage compartment (13 to 21% of total soil phosphorus) when the surface water contains very low phosphorus concentrations ($<100 \mu g.L^{-1} P$) (Boström et al. 1988). The average phosphorus content of microbial tissue ranges from 0.5-2.5% for bacteria to 4.8% for fungi (Stevenson 1986). However, the dominant role for microorganisms in the phosphorus cycle of soil is in the immobilisation and sequestering of phosphorus rather than storage within living tissue (Richardson et al. 1996; Walbridge 1991). Microbes can immobilise up to 90% of added phosphorus, although this proportion diminishes as the concentration of phosphorus increases (Walbridge 1991).

One explanation for such observations is that the microorganisms assist with sequestering phosphorus from the interstitial water – at least in the short term, allowing direct transfer to the soil surface (Richardson et al. 1986). The process occurs due to natural bacterial metabolism and biofilm interaction. The metabolism of phosphorus compounds results in a concentration gradient to the sediment surface. The close proximity to the sediment surface may allow more direct adsorption and a more active pumping mechanism in addition to direct chemical adsorption to soil particles. An additional mechanism may also be the formation of poly-phosphates. Whist this mechanism has been postulated, its significance and occurrence in wetlands are not known (Davelaar 1993).

Microorganisms indirectly affect the binding of phosphorus to inorganic material through changes to pH and redox potential (Gachter et al. 1993). Changes in pH are brought about by the formation of organic acids, primarily under anaerobic conditions (Gachter et al. 1993). Eh changes result from the microbial use of certain oxidised
compounds as terminal electrons acceptors during the metabolism of organic compounds. The order in which the compounds are utilised depends on the energy available from each, indicated by their redox potential (Table 1-2), with more highly oxidised compounds being reduced preferentially.

Table 1-2: Relative oxidation potentials for common microbial electron acceptors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Oxidation Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>+200 mV</td>
</tr>
<tr>
<td>Nitrate</td>
<td>+100 mV</td>
</tr>
<tr>
<td>Fe-oxides</td>
<td>-100 mV</td>
</tr>
<tr>
<td>Mn-oxides</td>
<td></td>
</tr>
<tr>
<td>Sulphate</td>
<td></td>
</tr>
<tr>
<td>Carbonate</td>
<td></td>
</tr>
<tr>
<td>Methane</td>
<td>-300 mV</td>
</tr>
</tbody>
</table>

Based on VanDemark et al. (1986)

The effect on phosphorus compounds is through the change in hydrogen ion concentration, and by direct conversion of Fe from Fe$^{2+}$ to Fe$^{2+}$ or Al$^{3+}$ to Al$^{3+}$. This reaction causes the solubilisation of Fe and Al bound phosphorus (Section 1.3.1).

Microorganisms have the potential to degrade organic phosphorus compounds, resulting the direct release of phosphorus to the water column (Hedley et al. 1982). Photosynthetic degradation of organic phosphorus compounds is highly attenuated by the water column and is confined only to the top few cm of the surface water (Francko et al. 1979). When aerobic conditions occur within the soil, microorganisms can rapidly degrade and often completely mineralise most organic compounds. Under anaerobic conditions, organic matter is slowly degraded and incompletely mineralised, resulting in the formation of large quantities of organic acids. The formation of organic acids lower pH and can induce the release of phosphorus inorganically bound to Fe or Al complexes (Fleischer et al. 1988).

At Lake Lugarno in Switzerland observations have clearly indicated that the degradation of organic carbon resulted in the solubilisation of iron and manganese into the water column, with subsequent increases in lake phosphorus concentrations, the entire process being driven by microorganisms (Lazzaretti et al. 1992). In other
European lakes, sediment dwelling bacteria not only release phosphorus but also contribute to refractory organic phosphorus compounds through incomplete mineralisation (Gachter et al. 1993). The production of such refractory compounds appears higher in oligotrophic than eutrophic lakes (Gachter et al. 1993).

**Organic interactions**

Organic phosphorus constitutes 20-80% of soil phosphorus, its turnover is regulated by microbial transformation but the compounds involved are less reactive than inorganic phosphorus complexes (Berkheiser et al. 1980). A flooded soil is termed organic if it comprises:

- 18% or more organic carbon and the mineral fraction is 60% or more clay;
- 12% or more organic carbon and the mineral fraction has no clay; or
- Between 12 and 18% organic carbon and a clay content of less than 60% (Cowardin et al. 1979).

Inositol phosphorus is the major portion of identifiable organic phosphorus in soils (McKercher et al. 1968). It has been isolated from organic matter with a MW of less than 50,000 (Hong et al. 1981; Moyer et al. 1970). If a high amount of biological activity is present in flooded wetland sediments i.e. under aerobic conditions, then microorganisms are able to degrade inositol phosphates such that they are not a significant component of the sediments (Islam et al. 1973; Suzumura et al. 1992). Degradation is greatest at neutral pH with high background carbon concentrations (compounds more readily degraded than inositol) (Islam et al. 1973).

Many other organic phosphorus forms occur in soil, but no more than one third of these compounds have been identified. The low identification rate is due to the limitations of current extraction methods and the complexity of some organic phosphorus compounds (eg components of humic material – humic and fulvic acids, and humin). The distribution of phosphorus compounds associated with humic and fulvic acid fractions have been observed in the range from 4% (in New Zealand soils (Martin et al. 1966)) to 85% in Bangladesh soils (Appiah et al. 1982; Stevenson 1986).
(24-58% in British soils (Anderson 1964)). The percentage of organic phosphate compounds in soil reflects their relative persistence.

**Interstitial water**

Interstitial water is the water that exists between soil particles, it is the medium by which soluble material is transported to surfaces and binding sites within the sediment (Brinkman *et al.* 1982; Enell *et al.* 1988). It is thought to have higher phosphorus concentrations than the overlying water but is extremely difficult to sample (Brinkman *et al.* 1982; Enell *et al.* 1988; Leman A. 1978). Studies indicate that phosphorus in interstitial water ranges between 0.01 mg.L\(^{-1}\) to 24 mg.L\(^{-1}\) (Carignan 1985; Craft *et al.* 1991). The pool of iron sesquioxides is highly significant for controlling concentrations of TP in the interstitial water (Lijklema. 1977).

**Soil sorption**

Sorption is the removal of inorganic phosphate from solution to a solid phase (Holtan *et al.* 1988). Phosphorus sorption has been shown to occur in two stages, an initial rapid uptake over a few hours thought to be from initial precipitation, which then slows due to a shift from direct sorption to chemotransformation (Berkheiser *et al.* 1980). It may occur due to:

- Precipitation - concentration from the liquid phase; and
- Chemisorption - concentration/bonding to the solid substrate, which includes ligand exchange and incorporation into the clay structure (Holtan *et al.* 1988).

Precipitation reactions are dominated by iron-based interactions (Patrick *et al.* 1974). Phosphorus release being associated with the solubilisation of these compounds under anaerobic conditions (Patrick *et al.* 1974). In flooded soil the redox potential can vary between 200 to -300 mV with the range between +150 to -100 mV critical for reduction and solubilisation of iron based compounds (Patrick *et al.* 1968). Lowering the water level in a wetland can result in rapid oxidation of iron compounds. The initial oxidation is often so rapid that amorphous oxides rather than crystalline oxides form (Anderson 1964). These amorphous forms are more readily able to adsorb phosphorus compounds (Anderson 1964).
CHAPTER 1

Phosphorus desorption

Phosphorus release can occur under both aerobic and anaerobic conditions. Release under anaerobic conditions generally occurs after a change from an aerobic state (Kleeberg 1993). Such removal tends to be more rapid and significant than under aerobic conditions, observed rates vary between 175.2 and 236.3 mg P.m\(^{-2}.d\(^{-1}\) (Kleeberg 1993)). The re-establishment of aerobic conditions will halt such release above 10% oxygen saturation (>1.2 mg.L\(^{-1}\) dissolved oxygen) (Frevert 1980). Hence, drawdown of the water column has the effect of releasing phosphorus to the surface water on refilling (Klopatek 1978). Wetland drying aerates sediments with the conversion of iron sulphide to iron sesquioxide, allowing greater phosphorus retention whilst facilitating the degradation of organic-phosphorus compounds and complexes (DeGroot et al. 1993a; DeGroot et al. 1993b).

The following factors influence phosphorus desorption:

1. Dissolved oxygen concentrations in the water. Oxygen promotes the formation of oxidised forms of iron and aluminium, which bind phosphorus compounds (Section 1.3.1).

2. Dissolved ion concentrations and redox. High redox and dissolved ion concentrations (phosphate, carbonate, sulphide and hydroxyl) promote the formation of sesquioxides, which bind phosphorus (Lijklema. 1977).

3. Phosphorus desorption is promoted by the presence of biodegradable organic matter (including organic acids), which has a significant role in the reduction and chelation of phospho-metallic compounds (Patrick et al. 1968).

4. pH changes. Lower pH’s solubilise iron and aluminium bound phosphorus, whist pH above 7 tends to solubilise calcium compounds (Anderson 1964; Lijklema. 1977).

5. Net immobilisation of phosphorus will occur when the carbon to organic phosphorus is 300 or more, net mineralisation will result when the ratio is 200 or less (Richardson et al. 1995; Stevenson 1986).

Environmental factors that induce the release mechanisms listed above include: diffusion; wind induced turbulence; bioturbation; rapid surface water movement (i.e. flooding); and gas convection (Richardson et al 1995).
The rate of release from the sediment is controlled by the ratios of Fe to P, the age of the complex and pH (Lijklema. 1977). In 1977, Fleischer determined bacteria had a role in release of phosphorus from sediments (Fleischer 1977). The predominant enzyme used by microorganisms in this process is phosphatase, which cleaves organic phosphorus compounds. Depending on the makeup of organic phosphorus compounds the ability of phosphatase to cleave organic phosphorus varies, with the amount of orthophosphate formed ranging from 9 to 100% (Klotz 1991). Phosphorus release from sediment by phosphatase is of the order 10.4 µmol phosphate-P.hr⁻¹.L⁻¹ (Francko et al. 1979). Phosphorus mineralisation is highest in the upper 0.9 cm of soil (Kadlec et al. 1996).

**Dosing of Iron to sediments**

The sorption properties of iron and phosphorus are well recognised, with many European countries trialling the direct application of iron based compounds to lake sediments with mixed results (James et al. 1992; Keizer et al. 1992; Quaak et al. 1993). Iron and iron oxides added to peat and sand can result in up to 95% removal of phosphorus from the overlying water column at a TP concentration of 0.65mg.L⁻¹ (James et al. 1992). The addition of nitrate can cap the release of iron bound phosphorus, primarily by acting as an alternative electron acceptor, being reduced in preference to iron sesquioxides (Anderson 1982; Boström et al. 1988). Such reactions depend on the concentration of nitrate being at or above 1.0 g N.m⁻³ or higher, with phosphorus release typically occurring when the nitrate concentration is less than 0.1 g N.m⁻³ (Anderson 1982).

The use of alum for removal of phosphorus within a wetland has been attempted, but mixing is a critical issue and needs to be undertaken external to the wetlands. Concentrations of Al up to 50 mg.L⁻¹ have been shown to be non-toxic to plants (Davies et al. 1993).
1.6 REMOVAL RATES

There is a large variation in the results observed for nutrient removal between different wetlands, which is in part due to the variety of wetland types in existence (Table 1-3). However, it is difficult to compare the results from different wetlands because not often all the required data is reported. This is highlighted in a review conducted by Kadlec and Knight (Knight et al. 1993). Figures for nutrient removal are frequently reported as percentage removal from inlet to outlet of the wetland. There may be no concentration values or if they are given, readings will primarily be based on average values without standard deviations (Table 1-3), Note the number of missing parameters, and that phosphorus removal varies from 0 to 97%, although the rate of phosphorus export from wetlands through the water column can exceed 0.4kg.ha$^{-1}$.yr$^{-1}$ (Richardson 1985)). It is uncommon for the concentration changes over time to be reported nor the range of inlet and outlet values. Thus it is difficult to determine the variability of results from different sites. Few wetland studies quantify fluxes between wetland vegetation, litter, surface water, groundwater, soil and atmospheric compartments, and even fewer studies present data for multiple years (Johnson 1991). Long-term studies are seldom undertaken, short-term studies indicate initial trends but not long-term retention, which may fluctuate or be reversed over time (Johnson 1991).

The minimum information necessary to compare between sites should be based on a water balance that accounts for additions and losses to the system, along with temporal variations and changes due to detention time. Loads of the nutrients of interest at the inlet and outlet to the wetland should be presented using either averaged or preferably total figures, rather than concentration values. Final information would include the size and shape of the wetland, retention time, evapotranspiration rates, temperature and plant species. To further complicate the issue quality assurance procedures are unable to be determined from published literature i.e. accreditation of testing laboratories, introducing further uncertainty.
1.6.1 Phosphorus removal

In 1993, Knight et al. produced a detailed analysis of some 500 wetlands (Knight et al. 1993). Typical performance of SF constructed wetlands is for removal in the order of: BOD 80-90%, TN 20-30%, TP 30-40% (Mitsch et al. 1995). Table 1-3 gives examples of typical removal rates and conditions.

Highest phosphorus removal efficiencies for surface flow wetlands in the USA have been shown to occur at phosphorus loading rates between 1-5 mg-P.m\(^{-2}\).yr\(^{-1}\) (60-90%) reducing to 30% at 15 g-P.m\(^{-2}\).yr\(^{-1}\) (Mitsch et al. 1989); (Knight et al. 1993). The mass of phosphorus able to be adsorbed by wetland sediments increases with phosphorus load in a logarithmic fashion i.e. proportionally less mass is absorbed as the loading rate increases (Richardson et al 1995). Maximum storage rates appear to be of the order 1 g.m\(^{-2}\).yr\(^{-1}\) (Florida everglades) (Knight et al. 1993; Richardson et al 1995). Richardson has used some of this data to postulate that phosphorus loadings into freshwater wetlands above 1 g.m\(^{-2}\).yr\(^{-1}\) will not significantly increase phosphorus storage once short-term uptake processes become saturated (Richardson et al 1995).

1.7 WETLAND DESIGN

Despite the problems with interpreting data generated from various wetlands it should be possible to achieve predictable long-term phosphorus removal from constructed wetlands provided the internal removal mechanisms are understood (Richardson et al 1995). Within a wetland, there are only a number of variables available to be manipulated. It is through the adjustment and maintenance of these variables that optimal phosphorus removal can be achieved. Factors that cannot be controlled or regulated may have a random and unpredictable effect on phosphorus removal. These factors must be negated or minimised to ensure reliable and reproducible phosphorus removal. Long-term phosphorus removal requires that long-term phosphorus storage compartments be maximised through appropriate selection of soil and plant types. In addition, uptake rates can be maximised by the use of designs that optimise retention time and sediment contact. Such optimisation should not only allow long-term phosphorus removal but also reduce the area required by constructed wetlands for the removal of phosphorus or increase the phosphorus-loading rate.
### Table 1-3: Summary details for a range of constructed wetlands and waste types for Phosphorus Removal (data independent of Knight et al. 1993)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Flow in (m³.d⁻¹)</th>
<th>P in (g.m⁻².yr⁻¹)</th>
<th>P out (g.m⁻².yr⁻¹)</th>
<th>Removal rate (g.m⁻².yr⁻¹)</th>
<th>% removal</th>
<th>Wetland size (m²)</th>
<th>Duration of investigation</th>
<th>Effluent type</th>
<th>Wetland type</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Hosari et al. 1994)</td>
<td>12.7</td>
<td>3.8</td>
<td>8.4</td>
<td>0.4</td>
<td>77</td>
<td>1224</td>
<td>4 yrs</td>
<td>Night soil</td>
<td>Rice Paddy</td>
</tr>
<tr>
<td>(Richardson et al. 1993)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Mitsch et al. 1989)</td>
<td>12 - 23</td>
<td>0.24 mg.L⁻¹ (min)</td>
<td>5-7</td>
<td>30-49</td>
<td></td>
<td>69,000,000</td>
<td>20 yrs</td>
<td>Sewage Effluent</td>
<td>Grass – fill/draw</td>
</tr>
<tr>
<td>(Richardson et al. 1986)</td>
<td>32.3</td>
<td>32.1</td>
<td>97</td>
<td></td>
<td></td>
<td>195,000</td>
<td>3 yrs</td>
<td>85% dissolved inorganic phosphorus</td>
<td>Fen</td>
</tr>
<tr>
<td>(Kowalik et al. 1995)</td>
<td>850</td>
<td>162.3</td>
<td></td>
<td>70-96</td>
<td></td>
<td>22,100</td>
<td>2-3 yrs</td>
<td>Phragmites planted, sand base.</td>
<td></td>
</tr>
<tr>
<td>(Haberl et al. 1993)</td>
<td>3 - 4.5</td>
<td></td>
<td></td>
<td>approx 70 (Range 50-90)</td>
<td>0.25 yrs</td>
<td>150m² (10x15m)</td>
<td>Primary sewage and biological sewage</td>
<td>Phragmites australis plots (lined)</td>
<td></td>
</tr>
<tr>
<td>(Brix et al. 1993)</td>
<td>≥ 0.2</td>
<td></td>
<td></td>
<td>20-40</td>
<td></td>
<td></td>
<td></td>
<td>Sewage</td>
<td>SSF clay</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.2</td>
<td></td>
<td></td>
<td>&gt;50 (significant)</td>
<td></td>
<td></td>
<td></td>
<td>Sewage</td>
<td>SSF clay</td>
</tr>
<tr>
<td>(Gehrels et al. 1989)</td>
<td>0.2</td>
<td>0.7</td>
<td>0.4</td>
<td>50</td>
<td></td>
<td>180,000</td>
<td></td>
<td>TP</td>
<td>FRP</td>
</tr>
<tr>
<td>(Bavor et al. 1987)</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td></td>
<td>500</td>
<td>3 yrs</td>
<td>Secondary effluent (P 8-16 mg/L⁻¹)</td>
<td>SSF - gravel</td>
</tr>
<tr>
<td>(Mitsch et al. 1995)</td>
<td>190 - 434</td>
<td>0.64 - 4.97</td>
<td>0.12 - 0.96</td>
<td>0.04 - 0.48</td>
<td></td>
<td>19,000 - 34,000</td>
<td>3 yrs</td>
<td>River water</td>
<td>Typha latifolia and T. augustifolia dominated basins</td>
</tr>
</tbody>
</table>

* Values calculated from literature data.

* Values are only given in non-standard units where insufficient data was available for calculation, based on details in the manuscript.
CHAPTER 1

In a constructed wetland, decisions must be made both pre- and post-construction. Before construction, factors such as topographic profile (location and shape of deep and shallow regions), site location, soil characteristics (type, bulk density, porosity, structure, mineral composition, structure and permeability), waste type/characteristics (BOD, SS, TP, TN etc) must be determined and optimised (DLWC 1997; Kadlec 1996; Mitsch 1995). On establishment, the following become dominant effectors:

- loading rate;
- retention and contact time (as determined by surface area, flow rate, flow path length);
- standing stock (plant density, surface area, shading and species);
- plant litter (amount of lignin, cellulose and hemicellulose; depth of litter);
- periods of flooding/drying; and

Based on the previous discussions, wetland design should optimise the processes that lead to long-term phosphorus storage including:

1. Movement of phosphorus through the water column to biofilms, plant surfaces, detritus and sediment surfaces (Frevert 1980; Johnson 1991);
2. Medium to long-term adsorption of phosphorus from the water column to the sediments and litter (Prenki et al. 1978; Richardson et al. 1986);
3. Incorporation of phosphorus into plant tissue (Brix et al. 1993; Gumbricht 1993); and
4. Formation of stable phosphorus compounds within soils and detritus (Richardson et al. 1993; Richardson et al. 1986; Sloey et al. 1987).

Mechanisms to control these processes include manipulation of the hydroperiod, loading rate, retention time, flushing, outflow regulation (to prevent nutrient flushing), harvesting and chemical treatment (Richardson et al. 1993; Sloey et al. 1987).

It is anticipated that mainly the more readily controllable factors will be altered to affect levels of phosphorus being removed from the system once it has become established. In the short to intermediate term these factors are the water depth,
loading, flow rate and external additions of flocculating material such as alum. In the longer term the soil substrate or plant biomass are likely to be the main storage locations. These may become saturated with phosphorus and it may be necessary to take the wetland offline from time to time to allow replenishment of these storage compartments (Mitsch et al. 1989).

1.7.1 Loading rate
The loading rate is the mass of material entering a wetland over a period of time. It is determined by multiplying the concentration of the parameter (eg phosphorus) by the flow entering the wetland for a given time interval. It can further be divided by the surface area of the wetland to determine the loading per unit area (Mitsch et al. 1989). Hydraulic Retention Time (HRT) is the time water resides within the wetland. It is related to the hydraulic efficiency (HE, refer to chapter 4 for a more detailed discussion). Longer HRT’s allow for greater interaction of physical and biological processes with nutrients such as phosphorus, in the water column. The theoretical HRT = (A x d)/Q where A = surface area, d = average water depth and Q = flow rate into the wetland. The actual detention time in FW wetlands will typically be at least 60-85% of the predicted or theoretical HRT due to short-circuiting (Fisher 1992). The velocity of water through the wetland can be determined by the Manning equation: \( V = 1.49/n x (R^{2/3}S^{1/2}) \) where V = velocity, R = hydraulic radius, S = stream gradient and n = roughness coefficient (Johnson 1991). The size of the standing stock refers to the plant biomass. During establishment, phosphorus removal is related to the standing stock (Johnson 1991) because a significant proportion is adsorbed into the growing biomass.

1.7.2 Nutrient removal equations
Removal of nutrients and particles from the wetland can be described using first order kinetics.
\[ C_t = C_0 \exp(-tK_{20}b^{(0-20)}) \]
\( C_t \) = pollutant concentrations at time t
\( C_0 \) = pollutant concentrations at time 0
T = detention time
$K_{20}$ = rate constant at 20°C = approx 0.1

$\theta$ = the temperature coefficient, approximately equal to 1

$T$ = temperature (Fisher 1992)

A simplified version of this formula would be:

$$C_t = C_0 \exp(-t^*K),$$

where $t$ = retention time, $K$ = constant – approx 0.11 (Haberl et al. 1993).

Essentially these equations are stating that phosphorus removal should occur in an exponential manner as a function of time, initial concentration and temperature. They are a simplification of reality and do not take into account inputs of phosphorus from precipitation, birds etc or losses due to aquifer intrusion or concentration effects due to evaporation. Hence, they can be used as rough rules of thumb, which need to be confirmed by actual observation.

1.8 SUMMARY

Constructed wetlands show a variable ability to remove phosphorus. The dominant mechanisms of phosphorus removal vary as a wetland ages. During establishment, the dominant mechanism of phosphorus removal is suggested to be adsorption to sediment substrate and incorporation into plant tissue. In the longer term, phosphorus should be retained in detritus and sediment. The purpose of this investigation was to confirm the predominant removal and storage mechanisms within a constructed wetland receiving secondary and tertiary treated effluent under Australian conditions and understand the nature of the phosphorus removal processes occurring. Several key questions were investigated.

1. Was phosphorus removed consistently from the wetlands under investigation?
2. If so, were there specific regions of removal?
3. Was removal specific to plant type i.e. lignified, semi-lignified, emergent or submergent?
4. What is the role of microorganisms in the phosphorus removal process?
CHAPTER 2: THE POTENTIAL FOR SMALL SCALE CONSTRUCTED WETLANDS TO REMOVE PHOSPHORUS

2.1 INTRODUCTION

This chapter describes the initial design of the constructed wetland systems at Richmond, NSW, Australia. Of interest were changes in phosphorus removal over time and its association with changes in other water quality parameters. It has been hypothesised that if phosphorus removal was biologically mediated, either by plants or microorganisms there would be a decrease in removal activity during winter months. A decline in activity would be from plant senescence over winter or the reduced metabolic activity of microorganisms at lower temperatures.

In addition to temporal variations in removal rates it was desired to ascertain whether release or removal of phosphorus was associated with changes in other water quality parameters including TN, SS, BOD, pH, turbidity and conductivity.

2.2 AIM

The aim of this chapter is to outline the study sites, gain an overall view of the phosphorus removal potential of selected wetland systems, and establish apparent trends in phosphorus removal from the wetlands investigated. Detailed studies proceeded as follows:

i) Monitor the inlet and outlet of six wetlands over a two-year period to assess the ability of each to remove TP.

ii) Determine quarterly trends within the data set for each wetland.

iii) Evaluate potential correlations between phosphorus removal and changes in the concentration of nitrogen compounds, SS, BOD, pH, turbidity and conductivity.

iv) Investigate the partitioning of phosphorus into inorganic and organic fractions within the soils of each wetland Unit.
2.3 OVERVIEW OF THE STUDY SITE

Five constructed wetlands were used for all investigations reported in this thesis. These wetlands are described below.

2.3.1 Location and design

The Richmond pilot plant wetlands were located in Richmond NSW (Lat. 33° 37' S, Long. 150° 45' E, elevation 20 m), approximately 60 km north west of the Sydney central business district. They were established in November 1993, adjacent the Richmond Sewage Treatment Works. The region averaged 799 mm of rainfall annually, falling over an average of 112 days with average maximum daily temperatures of 23.6°C (NSW Department of Meteorology 1995). The pilot plant consisted of five pilot scale wetlands supplied with secondary treated sewage effluent (primary effluent passed through a trickling filter, settling, aeration and oxidation ponds) from the adjacent treatment works. The wetlands were established to evaluate their ability to polish effluent containing low concentrations of nitrogen, phosphorus, carbon and SS.

The five wetlands at Richmond were constructed identically, each being 27 m long by 5 m wide, sunken below ground level to obtain vertical sides to a height of 1 m, designed to contain a water depth of 0.5 m above the added clay and topsoil substrates. The banks of each wetland were raised a further 75 cm above ground level using a 60° batter to prevent erosion, facilitate stabilisation and prevent inflow of possible flood waters from surrounding lands. They were lined with 3 mm "Dantuff" plastic, with the base overlaid with 2 to 3 cm of sand to prevent tearing during construction. Overlaying the sand was 30 cm of clay then 40 cm of mixed topsoil, obtained from adjacent agricultural land. Each wetland was divided into seven segments or bands from the inlet (Figure 2-1). The initial six bands were 5 m wide x 4 m long, with the seventh segment adjacent the outlet only 5 m wide x 3 m long. Each segment of the five wetlands was planted identically in terms of plant density per m² and plant species (Figure 2-1). The plant species (from inlet to outlet) were *Phragmites australis*, *Schoenoplectus validus*, *Triglochin procera*, *Phragmites*
*australis, Triglochin procera, Schoenoplectus validus and Phragmites australis* (Sainty *et al.* 1981) (Figure 2-1). Plants were transplanted as seedlings at a density of 16 plants per m² with two exceptions due to low plant numbers. Unit 5 and the final stand of *Phragmites* adjacent the outlet in each wetland were planted at a density of nine plants per m².

Figure 2-1: Schematic plan of the pilot plant wetland at Richmond, NSW.

The pilot plant facility had the capacity to dose incoming effluent with liquid alum, reducing inlet phosphorus concentrations from 7 - 12 mg.L⁻¹ to 0.5 - 3 mg.L⁻¹ (Section 2.5.4) The planned inlet TP concentration, hydraulic loading rate, water depth and retention time for each pilot scale wetland are outlined in Table 2-1.
Table 2-1: Major operational parameters for the five pilot scale wetland systems at Richmond.

<table>
<thead>
<tr>
<th>Wetland Number</th>
<th>Water depth (m)</th>
<th>Theoretical Hydraulic Loading Rate (L-day$^{-1}$)</th>
<th>Theoretical Retention Time (days)</th>
<th>TP (mg.L$^{-1}$)</th>
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<tbody>
<tr>
<td>1</td>
<td>0.50</td>
<td>13 500</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
<td>5 400</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
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<td>0.20</td>
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<tr>
<td>4</td>
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<td>1 800</td>
<td>15</td>
<td>1</td>
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<tr>
<td>5</td>
<td>0.20</td>
<td>1 800</td>
<td>15</td>
<td>8</td>
</tr>
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</table>

2.3.2 Operating structures and mechanisms
The pilot plant wetlands at Richmond were planted, maintained and monitored by students (including the author) and staff from the Water Research Laboratory at UWS-Richmond. Due to difficulties with the establishment and operation of water flow monitoring equipment it was not until three months after wetland commissioning that inflow and outflow rates were measured on a continuous basis. It was at this stage that collection of water samples commenced on a weekly basis, with sample collection occurring fortnightly after the first month. Alum dosing commenced on the 27/4/94. A detailed description of the mechanism of alum dosing at the pilot plant wetlands in Richmond, along with inlet, outlet and flow monitoring structures for each of the study sites is presented in Sections 2.3.4, 2.3.5, 2.3.6 and 2.3.7.

2.3.3 Effluent supply
Water for each wetland was obtained from the Richmond WWTP by using a centrifugal pump (Flyte Pump (Model WP3) capable of delivering a maximum of 20,000 L of water per hr) placed at the end of the final maturation pond, immediately prior to the chlorine dosing point. This arrangement permitted maximum treatment of the wastewater before processing by the pilot plant wetlands. A gate valve regulated water flow from the Flyte pump, and an overflow pipe was used to route excess flow back to the maturation pond. Flow into the pilot plant facilities was further regulated by a 100 mm butterfly valve, which permitted isolation of the pilot plant facilities for maintenance and other procedures.
2.3.4 Alum dosing

Plumbing for the pilot plant was designed so that secondary treated effluent was available at the inlet of each wetland unit and the alum-dosing unit. Individual gate valves controlled the flow of effluent into each wetland, and alum-dosing unit.

The alum dosing unit comprised of a 23,000 L rainwater tank (2.5 m diameter) which was supplied with 20,000 L of secondary treated effluent per day, dosed with a 10 % solution of liquid alum (20 kg of industrial grade aluminium sulphate in 200 L of tap water) at the rate of 1,000 L.day⁻¹. The flow rate of effluent to the alum-dosing unit was regulated by gate valve (100 mm), and the supply rate of alum by a dosing pump (Alldos 16 L.hr⁻¹ variable speed dosing pump model 205-5.0-DO4 by Water Technology and Engineering Pty. Ltd.). Alum was originally fed into the secondary effluent supply line 10 m before it entered the alum-dosing tank. However, a precipitate was continually formed at the dosing point inlet, blocking the dosing line because of its small diameter. Increasing the diameter of dosing tube was not an option because it would cause back flow and pressure problems at the dosing pump due to the flow rate of effluent from the main supply line. To overcome this problem alum was injected at the point where the secondary treated effluent entered the dosing tank.

As effluent entered the alum-dosing unit, it was passed through a 1 m long baffle box to promote mixing of alum with wastewater (Figure 2-2). The outlet from the primary tank was directly opposite the inlet, 10 cm below the top. Alum reacts with phosphorus to form aluminium phosphate which exists as a white precipitate with a high bulk density, commonly know as alum floc. It was not possible to pump directly from the larger tank because of the potential for disturbing the alum floc, resulting in alum being pumped into each wetland. Therefore, the outlet of the primary alum-dosing tank drained into a smaller 10,000 L holding tank before being pumped to each of the wetlands. Effluent was pumped by a "Grundfoss 350" centrifugal pump.
2.3.5 Inlet design for each wetland

Constant head (water pressure) was supplied to each wetland using a riser tube (Figure 2-3). This consisted of a pipe with a large internal diameter (100 mm) plumbed into the alum-dosed and secondary treated effluent lines, elevated to a height of 2 m above the inlet valves. The pipe functioned as an overflow for both secondary treated and alum-

dosed effluent and fed back to the main return sump. The 2 m of water in the tube and the large internal diameter of the tube (90 mm), compared to the smaller diameter of the inlet structures (25 mm), provided equal pressure to each of the inlet structures.
2.3.6 Flow monitoring
The flow of effluent into the wetland units was controlled by 25 mm gate valves. Using this system it was possible to supply a wetland with either dosed effluent, secondary treated effluent or a mixture of both, while regulating the flow rate. Monitoring of flow rates into each wetland was by tipping bucket (holding capacity 1000 mL, supplied by Manly Hydraulics Laboratory, Figure 2-4). The number of tips over a period of time was detected by an electronic reed switch and logged to a liquid crystal counting device. The amount of water that flowed into a wetland was determined by monitoring the number of tips that occurred in a known time interval.

Figure 2-4: Configuration of the tipping bucket apparatus used to measure inflow to the Richmond pilot plant wetlands (Front View).

Flow rates into each wetland were checked twice weekly and calibrated by using a stopwatch to determine the time for 10 tips. This reading was confirmed by observing the time taken to fill a 5 or 10 L calibrated container. The tipping bucket apparatus was calibrated every 3 months. Effluent entering the wetland was passed through the tipping bucket and into a distribution manifold. This was a 25 mm diameter pressure pipe with holes along its length placed across the entrance of the wetland (Figure 2-1) to allow dispersion of effluent across the full width of the inlet area and avoid short-
circuiting. Due to continuous fouling problems, this structure was removed in January 1994. Despite the absence of the distribution manifold, water entering the wetland became mixed with water already present within 8 m of the inlet (refer Chapter 4).

2.3.7 Outlet design
The rate at which water flowed out of the wetlands was determined by observing the height of water flowing over a V-notch weir. Weir plates of stainless steel, 4 mm thick, bevelled on the trailing edge, with an angle of 19°, fitted into a slotted channel located in the centre of the outflow wall. Water height was determined by shaft encoder (Unidata, Australia), which comprised of a float attached to a counterweight via 3 m of steel cable passed over a grooved wheel. The float (10 cm diameter) was placed at water level inside a PVC pipe (internal diameter of 150 mm, length 1 m) that was buried (50 cm depth) into the sediment to provide structural support. The pipe was perforated to facilitate water permeability while minimising the effect of wind on the float. The wire support was 2 mm thick with brass nodes (4 mm diameter) every 5 cm along its length. The grooved wheel over which the wire was passed was scored to accept the nodes, thus preventing slippage. A lead sinker of identical weight to the float was attached to the other end of the wire. Movement of the float caused the wheel to turn and movements of 1 mm or greater were registered by an electronic counting device with a liquid crystal display, attached to the shaft encoder.

Rates of flow over the v-notch weir were obtained concurrently with water height measurements. Flow was determined by the time taken to fill a 2 L container, as determined by stopwatch. By keeping the height of the weir constant, it was possible to derive a relationship between the flow rate of water over the weir and the height of water above the v notch. The water height was relatively easy to determine through the use of water level measurements obtained from the shaft encoder, allowing quantitation of the flow rate over the weir. After passing over the weir plate, the water entered a concrete sump, which drained by gravity or was pumped (Grundfoss 350 - centrifugal) to the main return sump. Wastewater from the main return sump was pumped (using a Grundfoss 550 - centrifugal pump) back to the Richmond WWTP, as EPA licensing requirements did not permit discharge to the environment.
2.4 METHODS

To accomplish the aims stated in Section 2.2 the protocol detailed in Figure 2-5 was implemented. Note: all investigations using the Pilot Plant Wetlands were conducted between 1994 and 1995.

Figure 2-5: Experimental design overview for a preliminary evaluation of phosphorus removal from the wetlands at Richmond.

Design of wetland shape, planting layout, inlet structures, outlet structures, supply lines and alum dosing unit.

Build wetlands and associated structures.

Undertake long term monitoring of wetland inlets and outlets for flow and chemical parameters.

Evaluate nutrient removal trends

Plan and perform detailed investigations of nutrient removal processes

2.4.1 Overview of the experimental design

The ability of each of the wetland system to reduce phosphorus concentrations from incident water was evaluated. Initial investigations quantitated phosphorus concentrations at the inlet and outlet of each wetland, along with nitrogen, SS, BOD,
pH, turbidity and conductivity. These parameters covered a range of water quality parameters, providing a preliminary assessment of changes occurring in water quality through each wetland. Monitoring of each wetland was conducted on a weekly basis, providing a long-term record of concentration changes. This record was used to derive the expected variance of each parameter. Flow was also measured simultaneously with nutrient concentration, providing an estimate of retention time and hence the time taken for each parameter to travel from the inlet to outlet of a given wetland. When removal of phosphorus occurred it was therefore possible to determine the significance of such removal. Evaluation was made of removal duration, timing, extent and association with other water quality parameters. Based on these initial studies more detailed investigations of regions of phosphorus removal were undertaken to better understand removal processes (Chapters 3, 4 and 5).

2.4.2 Flow
In addition to the detection and analysis of water quality parameters, it was also necessary to determine flows through each wetland. Computation of flows determined the mass loading of each water quality parameter into each wetland and allowed calculation of a nutrient mass balance of nutrients for a given wetland.

Flow from the pilot plant wetlands at Richmond was recorded three times a week. Monitoring of inflow and outflow points initially occurred as noted in the site description. However, difficulties were encountered with the electrical connections due to the length of cable between each shaft encoder and the data logger. Therefore, flow monitoring was modified: inflow readings were obtained using a cumulative digital coulter attached to the tipping bucket mechanism with calibration for volumes done twice weekly; and outflow data was obtained by measuring the time taken for a known volume of water (2L) to exit over the v-notch weir. Manual samples were taken twice weekly at discrete time intervals. The outflow readings were directly influenced by rainfall and evapotranspiration, unlike inflow readings. In addition, the observed outflow at a given time could be influenced by the time of day, rainfall or the presence of material obstructing the v-notch. Even though this material was removed before taking a reading, the measurement itself was then biased because it
was difficult to ascertain when the flow had returned to equilibrium, the time depending on the size of the obstruction and how long it had been in place. For these reasons outflow measurements were only used to provide a rough indication of actual flow out of each wetland over a period of time (see Table 2-3 for a comparison of actual inflow and outflow values). A more reliable indication of flow out of each wetland was gained through the use of inflow data corrected to account for rainfall and evapotranspiration. Calculated outflow figures were used when deriving the nutrient mass loading for each wetland.

2.4.3 Water loss and evapotranspiration

To evaluate the performance of each wetland unit from the pilot plant at Richmond it was necessary to derive a mass balance for nutrients and water entering and leaving the wetland. To obtain this balance it was necessary to know how material was leaving the system. Apart from losses to atmosphere by evapotranspiration, transfer of all nutrients through a wetland was via the water column. Therefore, it was important to know the proportion of water exiting over the outlet weir, infiltrating to groundwater and being lost to the atmosphere.

Water loss figures for the pilot plant wetlands at Richmond were determined on three separate occasions, November/December 1994, July 1995 and January 1996. On each occasion, flow into the wetlands was stopped for 13 to 18 days (the exact period depended on the number of rain free days). It took approximately 3 days after turning off the flow of water into each wetland unit for the water level to drop below that of the outlet weir. Measurements of water height were commenced once flow out of the wetlands had ceased. Water height was monitored twice daily at 9 am and 5 pm using the shaft encoder, with confirmation by ruler. Precipitation was measured by an on-site rain gauge and compared to the values from the University of Western Sydney – Hawkesbury, Meteorological Station (UWSH-MS), 1 km from the site. Comparison of precipitation readings from both sites was used to indicate microclimate variations between the two sites. Pan evaporation measurements were provided by the UWSH-MS.
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The relationship between precipitation and water loss was determined by mm rain, time of day it fell, duration, prevailing wind conditions, hours of sunlight, degree of cloud cover and daily temperature fluctuations. Since it was not possible to determine all of these parameters with the precision required to accurately evaluate water loss during rainfall periods, figures for water loss were only obtained from days that had no rainfall and compared to pan evaporation at the UWSH-MS.

2.4.4 Water quality parameters

Samples were taken over a two-year period from each wetland to evaluate trends, statistical variation and significant changes between wetland inlets and outlets. The following parameters were observed: phosphorus (TP and orthophosphate (PO₄)), nitrogen (TN, nitrate and nitrite (NOₓ), ammonium (NH₄) and total Kjeldahl nitrogen (TKN)), BOD SS, pH, turbidity and conductivity. Inlet samples at Richmond pilot plant wetlands were obtained immediately after the tipping bucket apparatus, before water entered each wetland. Outlet samples were taken of water flowing over the v-notch weir. Analysis methods are detailed in Section 2.4.8.

Water samples were initially collected from the inlet and outlet of each wetland. However, the level of variation between inlet samples of the same effluent was insignificant and inlet sampling was reduced to one sample from alum-dosed and secondary treated effluent at each time (i.e. inlets to wetland Units 1 and 3, on a fortnightly basis).

2.4.5 Derivation of mass loading values

Values obtained for flow were averaged on a weekly basis. Flow into each wetland did not match flow out due to evapotranspiration and rainfall. The mass of a given parameter entering each wetland was calculated from inlet concentration and flow data. The mass of material exiting the wetland each week was determined from outlet flow and concentration. The difference between the two parameters was deemed the net removal or input of nutrients into the wetland. Note that each wetland had a different retention time. Therefore it was necessary to adjust the timing for observed
inlet and outlet concentrations to allow for the time delay caused by retention time (calculated from inlet flows).

Due to the time of commissioning and the duration of the investigation the data did not lend itself to seasonal breakdown, therefore each year was broken into quarters; January to March, April to June, July to September, and October to December. To account for variations in flow during each period, concentrations of each parameter were converted to a mass loading value. The mass loading was calculated by multiplying average weekly flow by average weekly concentration over the quarter. This enabled calculation of the mass of each substance entering and leaving a wetland, the difference being the mass of that component retained by the wetland.

2.4.6 Statistical analysis of water quality parameters

Only a limited number of statistical tests were available to analyse the summary data for the pilot plant wetlands because the datum generated were non-parametric (Zar 1996). Changes in the concentration of various parameters in the water samples at the outlet were a function of the concentration of these parameters observed at the inlet. Therefore the data was paired. In addition, the concentrations of a substance observed at a wetland inlet at any given time were not totally random because the wetlands were supplied by the effluent from a single oxidation pond with a 30-day retention time. Over a short time span the concentration of a parameter entering a wetland was in similar proximity to the preceding reading eg if TP was 8.0 mg.L⁻¹ on the 27/9/94 then on the 28/9/94 it would only be within 0.3 to 0.5 mg.L⁻¹ of this value. Therefore, concentration variations of substances entering each wetland were neither totally random nor independent (Zar 1996). Thus it was not possible to compare inflow and outflow data by parametric statistical tests. To evaluate significant differences the non-parametric Wilcoxon paired sample test (Zar 1996) was used. Differences were observed at the $\alpha = 0.05$ or 0.01 levels of significance, as noted.

2.4.7 Soil analysis

Soil profiles of each wetland were obtained both before and after wastewater was applied to each system. Cores of soil were obtained from each of the Richmond pilot
plant wetlands prior to water entering the systems and after 24 months of operation. As noted in Section 2.3 each wetland was divided into 7 segments. Three cores of soil were obtained from each segment except Segment 7 (the smallest), from which only two soil cores were removed. Using a stainless steel coring device (15 cm diameter, 1.5 m length) sediment was cored to a depth of 35 cm. Coring to this depth ensured that the entire topsoil layer of the wetland was sampled. Sampling at this depth also resulted in a small sample of clay being collected in the base of the corer, which acted as a plug to help prevent loss of soil during extraction of the coring device.

The corer was externally marked with the appropriate depth to avoid rupturing the plastic liner of the wetlands. A rubber plunger was used to obtain an airtight seal over the coring tube, preventing the entry of water and maintaining a vacuum over the soil core to prevent it falling out of the corer. When the corer was placed in a wetland the plunger was placed at the bottom of the corer tube, flush with the base. This arrangement prevented water from entering the corer. Once the sediment was contacted, the plunger was retracted into the corer barrel. When the required depth had been reached, the plunger was secured in place to maintain a vacuum over the column of soil in the coring tube. A pin was placed through the top of the corer, perpendicular to the barrel with a steel cable attached. This cable was passed to a winch supported by a tripod arrangement to provide leverage for removing the corer.

After the corer was removed from the wetland, the plunger was used to extrude the soil sample. Extrusion was onto a wooden board that was washed and dried between samples. On the board the core of soil was measured by ruler and divided into the following segments from the top of the core 0-2, 2-5, 5-10, 10-15, 15-20, 20-25, 25-30, and >30 cm. Each segment was sliced at 90 degrees to the length of the core and placed in separate, labelled plastic bag for transport to the laboratory.

In the laboratory the soil was analysed for TP, IP and OP according to the method of Page et al. (1982). The purpose of such fractionation was to evaluate partitioning of phosphorus with inorganic and organic material. It should be emphasised that these investigations were not to determine uptake or release rates, but rather provide initial
information on possible phosphorus sequestering pathways and indicate directions for further investigative work.

Initially, each soil sample was air dried at 23°C. When the soil was dry, it was ground to a fine powder with a mortar in a pestle until it was able to pass through a 0.5 mm sieve. A subsample of 2g air dried soil was then taken for analysis. The sample was divided into two x 1g fractions, one portion which was weighed into a 200 mL polypropylene tube, the other 1g was weighed into a ceramic crucible. The sample in the crucible was heated to 550°C for 2 hours. The sample was then reweighed and the entire mass transferred to a 200mL polypropylene container. A 50mL volume of a solution of 0.5M H₂SO₄ was added to each sample container. The container was then capped and the sample placed on an orbital shaker and shaken for 48 hours at 200 rpm at room temperature. The extract was then filtered by gravity through a Whatman No. 11 filter paper to remove the soil particles and the eluent analysed to determine the concentration of phosphorus using the methodology described in Section 2.4.10.

The concentration of phosphorus observed in the sample combusted at 550°C represented the concentration of TP in the soil sample. The concentration of phosphorus on the sample that was not heated to 550°C represented the concentration of phosphorus associated with inorganic soil compounds (IP). The difference between these two fractions represented the concentration of OP. Analysis of these phosphorus fractions was conducted with assistance from Dr. K. Sakadevan of the Water Research Laboratory.

TP and TN were determined for air dried soil samples using a nitrogen, carbon, sulphur analyser based on standard analytical methods (Abbott 1987). Soil was air dried at 23°C and then homogenised. Soil subsamples (100 µg) were weighed into platinum crucibles using a 6 decimal place balance (Sartorius). They were then placed in the Autosampler tray of a Carlo Erber 1500 CNS analyser, and analysed as described in Section 2.4.10. Analysis for trace elements was undertaken on homogenised soils samples by the Department of Water Resources (DWR) laboratories at Arncliffe, NSW. An Inductively Coupled Plasma spectrophotometer
equipped with a mass spectrum detector (ICP/MS) was used to analyse the soil samples after hydrofluoric acid digestion based on USEPA standard methods.

2.4.8 General analytical methodologies
This section outlines the general methods used in field and laboratory analysis for the majority of tests undertaken during the project. Where a specific modification was made or where non-standard testing was used this is mentioned in the investigations for that Section.

2.4.9 Field samples
Water samples were collected in the containers types and volumes noted in Table 2-2. Any exceptions to this protocol are annotated. All glassware and plastics were first rinsed with tap water, soaked in Extran detergent for 24 hours, rinsed twice with distilled water then three times with MilliQ water before being dried in an incubator at 37°C. Containers for BOD and SS were rinsed with distilled water only.

<table>
<thead>
<tr>
<th>Table 2-2: Details of sample collection containers</th>
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<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
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</tr>
<tr>
<td>TP, TN, TKN, NH₄, NO₃,</td>
</tr>
<tr>
<td>SS, BOD</td>
</tr>
<tr>
<td>TP/BR</td>
</tr>
<tr>
<td>TC, TN, TS</td>
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</table>

Samples were placed on ice at the time of collection. All parameters were analysed at the University of Western Sydney – Water Research Unit laboratory or the Department of Water Resources (DWR) laboratories in Arncliffe. Where possible, samples for SS were filtered on-site through a 1.2 μm GFC filter within 30 minutes of collection. SS samples analysed by the DWR laboratories were not filtered on site but were delivered by courier to DWR within 6 hours. All samples kept at 4°C were analysed within 7 days. If nutrient analysis could not occur within 7 days then the sample was frozen at -20°C for a maximum of 30 days and thawed immediately prior to analysis.
Field measurements

_In situ_ field measurements were undertaken for pH, conductivity, dissolved oxygen (DO) and temperature. pH was measured using a standard meter (Beckman Φ 12), equipped with a research grade probe – accuracy 0.01 Units (Hanna Instruments 8521, HI 1332 probe); conductivity was measured using an hand held meter (Activon AS 302); DO and temperature were by a portable meter (YSI model 58 DO meter) equipped interchangeable probes (a 15795 probe for DO and a 5730 probe for BOD). In the laboratory, pH was measured using a bench meter (Orion 8103 meter equipped with a Ross - research grade combination pH probe). pH was calibrated with standard pH solutions at pH’s 4, 7 and 10. Conductivity was calibrated using a standard solution of 1400 μS cm⁻¹. DO was calibrated against water saturated air as stipulated by the manufacturer, corrected for altitude and temperature on the day of measurement.

2.4.10 Laboratory analysis

Phosphorus

TP was determined by a modification of persulphate method (Method 4500-P E (APHA 1992)). This method uses a combination of persulphate and sulphuric acid at elevated temperature and pressure to break down organic and inorganic phosphate compounds. In both reactions, orthophosphate is released from the parent molecule by substitution with sulphate ions. The persulphate reacts with organic phosphorus according to the equation \((\text{NH}_4)_2\text{SO}_4 + \text{C}_n\text{H}_m\text{PO}_4 \leftrightarrow \text{N}_2 + \text{H}_2\text{PO}_4^- + \text{C}_n\text{H}_m\text{SO}_4\) (APHA 1992). Sulphuric acid is responsible for dissociation of inorganic compounds according to \(\text{MPO}_4 + \text{H}_2\text{SO}_4 \leftrightarrow \text{H}_2\text{PO}_4^- + \text{M}^+ + \text{SO}_4^{2-}\) (APHA 1992) – where M is a monovalent metal ion). These reactions are endothermic and require either a catalyst or a combination of temperature and pressure. The required temperatures and pressures were achieved using an autoclave.

Once phosphorus compounds have been converted to orthophosphate the concentration of orthophosphate ions can be evaluated. The concentration of orthophosphate was determined by the molybdate blue method. In this method, the
combining of molybdenum and antimony with orthophosphate at a pH of less than 1 results in the formation of a complex with the formula Mo$_{11}$Sb(PO$_4$)$_{12}$. When this complex is exposed to ascorbic acid, a blue discoloration is caused in the original solution proportional to the concentration of phosphorus present, which has peak light absorbance at 660 and 882 nm. The concentration of orthophosphate is determined through the use of standard solutions and the application of Beer-Lamberts law.

**Digestion**

When digesting TP to orthophosphate 10 mL of sample was transferred to a 25 mL test tube with 2 mL of digest reagent. The digest reagent consisted of 10 mL of 7.8N H$_2$SO$_4$ and 5 g of ammonium persulphate (NH$_4$)$_2$SO$_4$, made to 100 mL with MilliQ water. The mixture of sample and digest reagent was then vortexed and the tubes covered with 3 layers of aluminium foil (to prevent loss of solution during autoclaving) before being placed in an autoclave at 121°C for 60 minutes. After autoclaving, the solution was allowed to cool before being analysed for orthophosphate. Where possible, samples were analysed on cooling. The maximum time interval that was allowed to elapse between digestion and analysis was 24 hours. Quantitation of phosphorus concentrations was performed both manually and by autoanalyser. Samples tested by each method were checked against each other and with a NATA accredited laboratory (DWR).

**Manual phosphorus detection**

The manual phosphate detection method required the addition of 2 mL antimony-molybdate reagent to the digested sample solution (Appendix 1, Table 1-A). This mixture was then vortexed for 30 seconds before adding 0.4 mL of ascorbic acid solution (Appendix 1, Table 1-A) and vortexing for a further 30 seconds. The final solution was then allowed to stand for a minimum of 10 minutes to allow colour development prior to analysis and analysed within 24 hours. Absorbance was measured by a spectrophotometer (Unicam UV/Vis model 8620, 5 nm bandwidth) with a sipper arrangement. The unit was equipped with a 5 cm flow cell to increase the light path and hence sensitivity. A volume of 2 mL was required to fill the flow cell. Between each sample, the flow cell was rinsed with two volumes of MilliQ
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water. Prior to taking a spectrophotometer reading, the cuvette was flushed with an initial 2 mL of sample. This removed any residual MilliQ water from the tubing and avoided artificial dilution of the sample. Absorbance values at 882 nm were recorded from the second and third 2 mL aliquot's taken by the sipper. Thus, two readings were taken per sample. If the two readings differed by more than 0.005 absorbance units, they were repeated. Standards were prepared in the same way as samples, blanks were of digested MilliQ water. A minimum of six standards was used to cover the expected concentration range in equidistant increments eg for 4 to 10 mg.L⁻¹ standards were at 4, 5, 6, 7, 8, 9, 10 mg.L⁻¹.

Automated phosphorus analysis

The automated method for phosphorus detection eliminated the vortex and mixing steps of the manual phosphorus method. It was performed using a segmented flow analyser (Technicon Mk II Autoanalyser (configurations detailed in Appendix 1, Figures 1-A and 1-B)). The chemistry of this method was similar to the manual method. However, the concentrations of antimony/molybdate and ascorbic acid solutions were slightly different (Appendix 1, Table 1-B).

Samples (3 mL) were placed in polyethylene vials, which were fitted into an automated sampling device. For sample analysis, a volume of between 0.1 to 1 mL was withdrawn from the vial into the analyser. The sample solution was combined with the antimony-molybdate and ascorbic acid solutions (Appendix 1, Figures 1-A and 1-B, Table 1-B) at a constant rate. Flow was achieved using a peristaltic pump moving at a constant rate and the flow rate of each solution determined by the internal diameter of the tube that passed over the peristaltic pump apparatus. Air was used to separate the sample into discrete components - segmented flow. A buffer solution (Appendix 1, Table 1-B) was used to further separate samples and provide diluent.

Coils of a set number of turns (2 cm in diameter), through which the solution passed, were used to achieve mixing. The air bubbles used to segregate the samples were removed just prior to the sample passing into the flow cell to prevent interference with absorbance readings. The solution in the tubes was heated at 37°C for 10 minutes to
catalyse the reaction before passing through a flow cell with a 5 cm light path. As the solution passed through the flow cell the absorbance at 660 nm was monitored continuously by an electronic sampling device.

The absorbance signal was transmitted to an analogue to digital converter (Waters™ Satin Bus), which digitised the signal for transmission to a computer. On receipt of the signal, it was stored in a database (Millennium 2010™ by Waters™) and the height of each absorbance peak determined. The peak heights of standard calibration solutions (prepared in the same way as for the manual analysis) were used to calibrate the system and determine the concentration of orthophosphate in sample solutions. Decreases in light intensity (light absorbance) were measured as peaks. Phosphorus concentration was quantitated by measuring the height of the peak and comparing the results to a regression curve generated from the standards. If the regression curve showed an $r^2$ of less than 0.99 it was rejected and the samples repeated (this allowed for a maximum error of 5-10 % depending on the range of standards).

At the commencement and conclusion of a sample run a set of six calibration standards were run. After every 20 samples two duplicate standards and one blank were analysed to detect shifts in baseline and determine any inconsistencies. The set of standards comprised of a minimum six standards arranged in duplicate from highest to lowest followed by two blanks. Standards were prepared from a stock solution of 1000 mg.L$^{-1}$ PO$_4$ as potassium orthophosphate dibasic (K$_2$HPO$_4$.7H$_2$O). The setup for the autoanalyser is shown in Appendix 1, Figures 1-A and 1-B.

**Nitrogen**

During this study, four forms of nitrogen were quantified: TN, TKN, NH$_4$ and NOx. Samples were analysed by DWR Laboratories for all four forms, and by the Water Research Laboratory (UWS) for TKN and TN. Analysis of TKN was by autoanalyser (Technicon AA Mk II) and TN by nitrogen, carbon sulphur (NCS, Carlo Erber 1500) analyser.

**Digestion**

TKN is a measure of all ammonium forms of nitrogen. For analysis of TKN in water
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samples, it was necessary to perform a digestion step prior to detection to convert organic ammonium to an inorganic form. The digestion step used concentrated sulphuric acid in the presence of selenium and potassium sulphate at 340°C to cleave ammonium from organic compounds in soil and water, producing free ammonium ions. The low pH generated by the acid prevented these ions from volatilising at this temperature. Ammonium ions in solution were detected through the formation of a complex with salicylic acid and nitroprusside buffered to a pH of 11. When this complex was reacted with hypochlorite, it produced a blue discoloration of the solution proportional to the concentration of ammonium ions, with an absorbance maximum at 660 nm (Method 4500-P E. (APHA 1992)).

TKN digestion was obtained by heating in the absence of pressure, using selenium to catalyse the reaction. When digesting a sample, 0.5 to 1 g of solid material or 2 to 10 mL of liquid was added to a 70 mL Kjeldahl digestion tube. A 4 mL aliquot of TKN digest reagent (Appendix 1, Table 1-C) was added to each tube. The tubes, including controls and blanks were then transferred to an aluminium block digester situated inside a fume hood. A temperature controller (Harco) was pre-programmed to heat the tubes according to the following specification:

- Heat from room temperature to 200°C at a rate of 200°C per hour,
- Hold at 200°C for 30 min
- Heat to 340°C at 300°C per hour,
- Hold at 340°C for 60 min
- Allow to cool to room temperature.

When samples returned to room temperature, 40 mL of MilliQ water was added to each Kjeldahl tube, which were then vortexed for 30 seconds before being allowed to stand for 24 hours. The supernatant was then decanted into a 3 mL autosampler vial for analysis. The configuration of the autoanalyser is shown in Appendix 1, Figure 1-C. At the commencement and conclusion of a sample run a set of six calibration standards were run. After every 20 samples two duplicate standards and one blank were analysed to detect shifts in baseline and determine any inconsistencies. The set of standards comprised of a minimum six standards arranged in duplicate from
highest to lowest followed by two blanks. Standards were prepared from a stock solution of 1000 mg.L\(^{-1}\) NH\(_4\) as ammonium chloride.

**Total Nitrogen, Carbon and Sulphur**

TN, TC and TS concentrations were determined using an NCS analyser (Carlo-Erba 1500 NA). TN was also calculated from TKN and NOx. Before analysis, samples of soil and plant tissue were air dried (80\(^\circ\)C) and ground to pass through a 0.5 mm sieve. Then 20 to 60 mg of dried matter was weighed into a 100 \(\mu\)L tin NCS autosampler capsule to an accuracy of 0.1 mg. Water samples were obtained by adding 500 \(\mu\)L of water to a 100 \(\mu\)L silver or tin capsule. Since the capsule could not contain this volume of solution at one time, a smaller volume (100 \(\mu\)L) was added and evaporated at 97 ± 2\(^\circ\)C on a heating block. When the initial 100 \(\mu\)L had evaporated, a further 100 \(\mu\)L was added and evaporated. This process was repeated until a total of 500 \(\mu\)L of sample material had been added to the container. The capsules were crimped to prevent loss of dried material. Each capsule was placed, in order, onto an autosampler tray. Sample names and masses were entered into a sample analysis database (Eager 2000 software), which then proceeded to drive the NCS analyser.

The NCS analyser comprised of an autosampler, furnace, gas chromatograph (GC) column and thermal conductivity detector (TCD). The autosampler was of a rotating turret type, driven by compressed air. The autosampler was designed to feed samples directly into a packed glass column that passed through the furnace. Samples dropped by gravity from the autosampler into a holding position. When analysis was required, the desired sample was guided from the holding position and dropped into the column by a piston mechanism. The interior of the column was maintained at 1020\(^\circ\)C and as the sample entered the column, a small amount of pure oxygen was injected along with the helium carrier gas to facilitate flash combustion of the sample. Flash combustion elevated the sample temperature to 1700\(^\circ\)C, effectively vaporising it.

Two catalysts - tungstic anhydride and reduced copper, were placed in the column to remove residual oxygen and prevent contamination of the GC column by larger molecules. The vaporised sample then passed through a water trap before entering the
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GC column, which provided separation of the compounds based on molecular weight. Quantitation of each gas was by TCD. The TCD generated an electronic signal that was continuously monitored by the Eager 2000 software. The percentage of N, C or S in a sample was proportional to peak area. Compound identification was based on retention time.

Before commencing analysis of a batch of samples, two blanks (tin capsules with no sample) were run to ensure the column was clean. If excessive background noise was observed during the injection of the blanks then it was necessary to replace the column before continuing. After the blanks were run, two standard calibration mixtures were injected to calibrate the instrument. The composition of the calibration mixtures was chosen to match the expected N and C concentrations in the samples. BBOT was used for plant samples, sulphanilamide for soil and water. The percentage composition of these materials were: Sulphanilamide C - 12.46, N - 4.69, S - 0.15; and BBOT C - 82, N - 8, S - 0.54. After every 10 samples, one standard and one blank were injected into the column to check for baseline drift, determine changes to the integration parameters, confirm the calibration and detect for column deterioration.

Suspended solids

SS is a measure of the concentration of particulate material present in the water column. It was determined by filtering a known volume of water through a filter paper of known pore size. Commercially available filter papers were pre-treated before use by rinsing with MilliQ water three times, drying at 103-105°C for one hour, then cooling in a desiccator and determining the weight to the nearest 0.1 mg. The drying and weighing process was then repeated until the mass between successive observations differed by less than 0.5 mg (Method 2540 Solids E. (APHA 1992)). This mass was then noted and the filter paper stored in a desiccator ready for use. Through experimentation it was shown that no significant changes in filter mass were observed after four hours drying. This interval was then used as a minimum interval for drying filter paper to save time and manipulation.
Analyses for SS were performed in duplicate. The concentration of SS in a water sample was determined by filtering 100 mL of solution through a pre-treated and weighed GFC filter (pore size 1.2 μm) within 30 minutes of sample collection. In the field, this was accomplished using a hand held vacuum pump (Nalgene model 6130-0020) equipped with a vacuum pressure gauge. A standard Millipore vacuum pump was used when a 240 V power supply was available. Prior to filtering the sample, the filter paper was rinsed with 10 mL of MilliQ water to pre-wet the filter paper. After the sample was filtered, the filtration apparatus was twice rinsed with MilliQ water and the filter paper was then removed into a numbered plastic Petri dish (50 mm diameter) using forceps. The Petri dish was large enough to contain the filter paper and was attached to a wooden board to prevent cross contamination and movement or loss of samples during transport to the laboratory. This board could also be covered with aluminium foil to minimise light entry when used to transport filter papers for chlorophyll determinations. It was not necessary to place the filter papers at 4°C during transport to the laboratory as they were to be heated to 105°C on arrival. When filter papers arrived at the laboratory, they were transferred to a numbered position on a sheet of folded aluminium foil. The foil was folded to prevent the filter paper adhering to it. The filter paper and foil were then placed in a oven at 105°C for four hours before being allowed to cool to room temperature (Method Solids D. (APHA 1992)). The paper was then weighed and this weight was then reported as the mass of SS and converted to units of mg.L⁻¹.

Algae

Algal biomass was determined by measurement of chlorophyll a using standard methods (Method 10200 Plankton - H. (APHA 1992)). Analysis was by filtration to concentrate the chlorophyll from the sample, then an extraction using acetone to remove any chlorophyll present to an organic phase for detection by spectrophotometer. Whilst it is noted that fluorometry can be used with greater accuracy at low concentrations of chlorophyll, it is non-specific for simultaneous detection of different chlorophylls (Coveney 1988). A total sample volume of 100 mL was filtered through a GFC filter (where possible, the amount of sample was adjusted to give a chlorophyll reading between 0.1 and 1.0 absorbance unit (Neveux 1988)).
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The filter and algal material were then ground in a mortar with a pestle in 2-3 mL of aqueous acetone (90%, balance with MilliQ water) until macerated (APHA 1992; Neveux 1988). The macerate was then transferred to a graduated 15 mL screw cap centrifuge tube. Any residual algal on the mortar was rinsed off into the centrifuge tube with 4 to 6 mL of 90% (w/v) acetone. The final volume of acetone and algae was adjusted to 10 mL. Centrifuge tubes were capped and samples kept for 4 ± 2 hrs at 4°C in the dark. After this period, the resultant solution was clarified by centrifuging at 500 g for 20 minutes and the supernatant transferred to a glass cuvette for determination of absorbance. When analysing soils, 0.1 g of sediment was transferred to a glass test tube and 8 mL of acetone added. The tube was capped and vortexed for 30 seconds, before being left overnight at 4°C in the dark. The solution was clarified by centrifuging at 5 000 rpm for 5 minutes (Abbott 1987).

The chlorophyll concentration was initially quantitated by two different methods, both from the standard methods for the analysis of water and wastewater 18th edition (Method number 10200 H. (APHA 1992)).

Method 1 - chlorophyll a

Transfer 3 mL of supernatant to a cuvette of 1 cm path length. Read the OD at 750 and 664 nm. Acidify with 0.1 mL of 0.1 N HCl, gently agitate and measure the OD at 750 and 665 nm. (It is essential that exact measures of extract and acid were used to ensure reproducibility). Note that the spectrophotometer (Cary MK III) was required to have a bandwidth of less than 2 nm for this measurement.

- Chlorophyll a (mg.m⁻³) = (26.7 x (664b - 665a) x V1) / (V2 x L)
- Pheophytin (mg.m⁻³) = (26.7x(1.7 x (665a - 664b)) x V1) / (V2 x L)
- 665a = A664 - A750 nm
- 664b = A664 - A750 nm
- L = path length in cm
- V1 = volume of extract (in centrifuge tube)
- V2 = volume of sample (amount initially filtered)
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Method 2 - Chlorophyll a, b, and c
Measure absorbance at 664, 647, 630, 750 nm. Correct all values to OD by subtracting them from the absorbance obtained at 750 nm i.e. OD 664 = A_{664} - A_{750}

- Chlorophyll a = 11.85 x (OD 664) - 1.54 x (OD 647) - 0.8 x (OD 630)
- Chlorophyll b = 21.03 x (OD 647) - 5.43 x (OD 664) - 2.66 x (OD 630)
- Chlorophyll c = 24.52 x (OD 630) - 7.60 x (OD 647) - 1.67 x (OD 664)

Turbidity
Turbidity is a measure of light scattering, which provides a coarse indication of the amount of particulate matter in a volume of water. It provides different information to SS because the measurement is independent of particle size. Turbidity is determined by the amount of light scattered at 90 degrees an incident source. The result is expressed in nephelometric turbidity units (NTU). The turbidity meter (Hanna Instruments) was initially blanked using MilliQ water. It was calibrated using the stock reference solution recommended by the manufacturer (BaSO$_4$ 50 mM concentration). Each sample was shaken before being transferred to a cuvette (2 cm light path - optically aligned for the turbidity meter) and taking a reading.

Mass
The mass of samples and reagents were determined using either a 4 digit (AE 260 Delta Range balance - Mettler) or a 2 digit electronic balance (AND FA 2000). Autoclaving was done in a freestanding autoclave (Atherton 110A series) or a vertical autoclave (Labec 3084-P-85). Heated digestions without pressure were performed on an aluminium heating-block (Windrift instruments P5) regulated by a controller box (Harco Electronics).

BOD
All samples for BOD analysis were measured by DWR laboratories using Standard Methods for the Analysis of Water and Wastewater (Method 5210 BOD (APHA 1992)).
2.5 RESULTS

2.5.1 Flow
Measured values for flow into each wetland were slightly different from the theoretical flow rates set out in Table 2-3. The flow rate for Unit 1 was lower than the ideal because of limitations in the inlet pump: it was not possible to supply water at a faster rate. The flow rates for the other systems were slightly higher than the ideal. Between each flow setting and calibration event there was a gradual build up of biofilm or other material in each outlet pipe, which resulted in a slowing of the flow over time.

Since it was not physically possible to check and calibrate the flow every few hours throughout the investigation it was checked and reset every 2 to 3 days. To compensate for the decline in flow it was necessary to set the initial inflow rate slightly higher than the ideal. Since the rate at which the flow declined was variable, flows had to be set for the worst expected blockage rate, resulting in an average flow rate higher than the ideal. There was a decrease of approximately 50% between values for water flowing out of each wetland relative to inflows for Units 2 to 5. This change in flow was not observed in Unit 1.

Table 2-3: Theoretical and average actual daily flows measured at the inlet and outlet points for each of the pilot plant wetlands over the 2 years since commissioning.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Flow in (KL.day$^{-1}$)</th>
<th>Flow out (KL.day$^{-1}$)</th>
<th>Theoretical flow (KL.day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.6 ± 3.2</td>
<td>9.2 ± 4.6</td>
<td>13.5</td>
</tr>
<tr>
<td>2</td>
<td>6.3 ± 2.1</td>
<td>3.4 ± 1.9</td>
<td>5.4</td>
</tr>
<tr>
<td>3</td>
<td>7.0 ± 2.9</td>
<td>2.9 ± 1.7</td>
<td>5.4</td>
</tr>
<tr>
<td>4</td>
<td>2.8 ± 1.4</td>
<td>1.1 ± 0.9</td>
<td>1.8</td>
</tr>
<tr>
<td>5</td>
<td>2.1 ± 0.6</td>
<td>1.0 ± 0.7</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Rainfall into each system ranged from 0 to 18 KL.day$^{-1}$. Pan evaporation for the period ranged from 1 to 7 KL.day$^{-1}$.

2.5.2 Water loss and evapotranspiration
Average daily water loss was measured by stopping the flow into each wetland for a period of two weeks on three separate occasions. The amount of water lost represented a combination of evaporation and transpiration. Statistical analysis of
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water loss data from each wetland using ANOVA indicated no significant difference between each unit ($\alpha = 0.01$, $p=0.99$). Therefore, values for each unit were combined to obtain the averages presented in Table 2-4.

Table 2-4: Average daily water loss, rainfall and pan evaporation for five experimental wetlands from the Richmond Pilot Plant, measured over three different periods.

<table>
<thead>
<tr>
<th>Period</th>
<th>Pan Evaporation</th>
<th>Net Water loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average (mm.day$^{-1}$)*</td>
<td>Average (mm.day$^{-1}$)*</td>
</tr>
<tr>
<td>November 1994</td>
<td>6.2 ± 2.4</td>
<td>9.7 ± 2.1</td>
</tr>
<tr>
<td>July 1995</td>
<td>2.0 ± 1.5</td>
<td>1.5 ± 1.0</td>
</tr>
<tr>
<td>January 1996</td>
<td>4.7 ± 1.7</td>
<td>6.1 ± 1.5</td>
</tr>
</tbody>
</table>

* Excluding Rainfall events

Overall, pan evaporation represented between 54 to 76 % of total water loss from the wetland units. There was no discernible correlation between pan evaporation and evapotranspiration during the periods observed. Pan evaporation was always less than evapotranspiration except during rain events. There was no correlation between water loss and daily average, minimum or maximum temperatures.

2.5.3 Rainfall
Rainfall at both the pilot plant and UWSH-MS did not differ by more than 1 mm in 10 mm. Variations were higher than this only when readings were for less than 1 mm, most likely from site specific differences. Thus, rainfall at the UWSH-MS could be used to estimate continuous rainfall data for the site.

2.5.4 Water quality parameters
When water commenced flowing into the pilot plant wetlands at Richmond in December 1993, each unit was initially supplied with secondary treated sewage effluent. Alum-dosed effluent was supplied to wetland Units 3 and 4 from April 1994. To avoid confusion in the interpretation and presentation of average inlet and outlet values and due to the low number of available readings for the pre-alum dosing period in Units 3 and 4, only the post alum dosing data are presented here. The average inlet and outlet values for physical and chemical parameters entering wetland Units 1, 2
and 5 (continuously receiving secondary treated effluent) are presented in Table 2-5 and Units 3 and 4 (receiving alum-dosed effluent) in Table 2-6. Note that the similarity of inlet readings (<5% difference) meant that it was possible to consolidate them into the single value presented for each effluent type.

Wetlands receiving secondary treated effluent evidenced a reduction in the concentrations of TP, FP, TN, TKN and NH₄ at the outlet relative to the inlet, in average terms, over the 2-year period of the investigation (Table 2-5). This decrease was more pronounced at lower loading rates. The reduction in TP and FP from inlet to outlet was of a lesser percentage than the nitrogen species, with the proportion of FP increasing at lower loading rates. Despite the high standard deviations observed for NOx, SS and BOD it was possible to discern a significant difference between inlet and outlet readings for some Units using the Wilcoxon paired T-test. SS and BOD decreased at the outlet of Units 1 and 2 relative to the inlet, with NOx being slightly elevated at the outlet of Unit 5 (Table 2-5).

Table 2-5: Average values for physical and chemical parameters monitored at the inlet and outlet to each of the pilot plant wetland units receiving secondary treated effluent over the two years since commissioning. Units are presented in order of decreasing hydraulic loading, Unit 1 receiving the highest, Unit 5 the lowest.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inlet</th>
<th>Unit 1 outlet</th>
<th>Unit 2 outlet</th>
<th>Unit 5 outlet</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (mg.L⁻¹)</td>
<td>8.2 ± 0.6a</td>
<td>7.3 ± 1.8**</td>
<td>7.1 ± 1.7**</td>
<td>6.4 ± 1.8**</td>
</tr>
<tr>
<td>FP (mg.L⁻¹)</td>
<td>6.7 ± 1.1</td>
<td>6.1 ± 1.7*</td>
<td>6.0 ± 1.6*</td>
<td>5.6 ± 1.7*</td>
</tr>
<tr>
<td>TN (mg.L⁻¹)</td>
<td>36.3 ± 4.7</td>
<td>27.7 ± 9.2**</td>
<td>24.2 ± 8.6**</td>
<td>11.4 ± 7.7**</td>
</tr>
<tr>
<td>TKN (mg.L⁻¹)</td>
<td>35.5 ± 4.8</td>
<td>26.3 ± 9.5**</td>
<td>21.2 ± 8.1**</td>
<td>8.1 ± 5.0**</td>
</tr>
<tr>
<td>NH₄ (mg.L⁻¹)</td>
<td>32.6 ± 4.9</td>
<td>28.0 ± 6.5**</td>
<td>21.4 ± 8.0**</td>
<td>6.3 ± 5.7**</td>
</tr>
<tr>
<td>NOx (mg.L⁻¹)</td>
<td>1.0 ± 0.6</td>
<td>1.7 ± 2.1</td>
<td>3.3 ± 3.6*</td>
<td>3.6 ± 5.9</td>
</tr>
<tr>
<td>SS (mg.L⁻¹)</td>
<td>10.0 ± 7.5</td>
<td>6.4 ± 5.2*</td>
<td>5.3 ± 4.7*</td>
<td>10.7 ± 14.6</td>
</tr>
<tr>
<td>BOD (mg.L⁻¹)</td>
<td>27.1 ± 27.9</td>
<td>24.4 ± 24.4*</td>
<td>18.4 ± 17.7*</td>
<td>15.0 ± 20.1*</td>
</tr>
<tr>
<td>pH</td>
<td>7.5 ± 0.3a</td>
<td>7.1 ± 0.2</td>
<td>6.8 ± 0.2</td>
<td>7.0 ± 0.4</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>15 ± 9</td>
<td>16 ± 14</td>
<td>19 ± 21</td>
<td>20 ± 24</td>
</tr>
<tr>
<td>Conductivity (µScm⁻¹)</td>
<td>762 ± 217</td>
<td>756 ± 137</td>
<td>696 ± 204</td>
<td>667 ± 222</td>
</tr>
</tbody>
</table>

a Standard deviation.

* Significant difference by Wilcoxon paired T-test compared to inlet, α = 0.05

** Significant difference by Wilcoxon paired T-test compared to inlet, α = 0.01
In alum-dosed wetlands, both TP and FP decreased between the inlet and outlets. The reduction in TP was greater than for FP. However, the reduction in each parameter was independent of the loading rate (Table 2-6). Nitrogen species (with the exception of NOx) declined between the inlet and outlet of both units. The extent of decline correlated with loading and followed the similar trends (at the same loading rate), as observed for secondary treated effluent. Again, BOD, SS, and NOx results evidenced high standard deviations, with BOD and SS evidencing a decrease in concentration from inlet to outlet while NOx increased (Table 2-6). Changes in these parameters appeared to be independent of loading rates.

Table 2-6: Average values for physical and chemical parameters monitored at the inlet and outlet to each of the pilot plant wetland units receiving secondary treated effluent over the two years since commissioning. Units are presented in order of decreasing hydraulic loading, Unit 3 receiving the highest, Unit 4 the lowest.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inlet</th>
<th>Unit 3</th>
<th>Unit 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (mg.L⁻¹)</td>
<td>2.8 ± 2.2</td>
<td>1.7 ± 1.3**</td>
<td>1.7 ± 1.4*</td>
</tr>
<tr>
<td>OP (mg.L⁻¹)</td>
<td>1.8 ± 1.7</td>
<td>1.3 ± 1.0</td>
<td>1.5 ± 1.3</td>
</tr>
<tr>
<td>TN (mg.L⁻¹)</td>
<td>34.1 ± 5.3</td>
<td>24.9 ± 8.4*</td>
<td>11.2 ± 7.4*</td>
</tr>
<tr>
<td>TKN (mg.L⁻¹)</td>
<td>33.6 ± 5.3</td>
<td>22.1 ± 7.8*</td>
<td>8.0 ± 6.4*</td>
</tr>
<tr>
<td>NH₄ (mg.L⁻¹)</td>
<td>31.5 ± 4.5</td>
<td>21.4 ± 7.6**</td>
<td>8.4 ± 7.6**</td>
</tr>
<tr>
<td>NO₃ (mg.L⁻¹)</td>
<td>1.1 ± 0.6</td>
<td>2.9 ± 2.7*</td>
<td>2.6 ± 3.1*</td>
</tr>
<tr>
<td>SS (mg.L⁻¹)</td>
<td>9.9 ± 7.3</td>
<td>5.6 ± 4.7*</td>
<td>5.8 ± 5.1*</td>
</tr>
<tr>
<td>BOD (mg.L⁻¹)</td>
<td>21.3 ± 21.0</td>
<td>14.0 ± 13.2*</td>
<td>10.4 ± 13.6**</td>
</tr>
<tr>
<td>pH</td>
<td>7.1 ± 0.2</td>
<td>6.7 ± 0.1</td>
<td>6.5 ± 0.3</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>5 ± 4</td>
<td>8 ± 10</td>
<td>8 ± 10</td>
</tr>
<tr>
<td>Conductivity (µScm⁻¹)</td>
<td>834 ± 126</td>
<td>762 ± 127</td>
<td>709 ± 138</td>
</tr>
</tbody>
</table>

* Standard deviation.
** Significant difference by Wilcoxon paired T-test compared to inlet, α = 0.05

There was no significant difference between turbidity readings for each unit regardless of effluent source. However the decrease in pH and conductivity observed in water passed through each wetland was significant at α =0.01 using the Wilcoxon paired sample test (Tables 2-5 and 2-6). The decline in conductivity corresponded to reductions in hydraulic loading and was slightly higher in alum-
dosed systems. There was a slight decrease in pH between the inlet and outlet of each unit that was independent of hydraulic loading rates.

2.5.5 Trace elements

Due to the expense of analysis, trace elements were only examined on one occasion to provide an indication of background concentrations within each wetland (Table 2-7).

Table 2-7: Trace metals in water samples taken from the inlet and outlet of each of the pilot plant wetlands on 21/11/95

<table>
<thead>
<tr>
<th>Element</th>
<th>Secondary effluent</th>
<th>Alum-dosed effluent</th>
<th>Unit 1 outlet</th>
<th>Unit 2 outlet</th>
<th>Unit 3 outlet</th>
<th>Unit 4 outlet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>0.1</td>
<td>3.4</td>
<td>0.3</td>
<td>0.4</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Ca</td>
<td>12</td>
<td>12</td>
<td>12.5</td>
<td>13</td>
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Cd, Cu, Pb and Se were all below the detection limits for these elements (Table 2-7). Despite high Al concentrations at the inlet to Units 3 and 4, there were no apparent differences between the outlet concentrations of Al in each wetland. Ca, Fe, Mg, and Zn were not significantly different for each wetland unit (Table 2-7). Concentrations of Mn detected at each outlet were markedly higher than at the inlet, regardless of effluent source (Table 2-7).

2.5.6 Mass loadings of phosphorus, nitrogen and suspended solids

To provide more detail on wetland functioning, fortnightly data for TP, TN and SS were expressed as 3-monthly averages. Mass loading results are shown in Appendix 2. For TP these are in Figures 2-A to 2-E, TN in Figures 2-F to 2-J and SS in Figures 2-K to 2-O.
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The greatest mass of TP removed from the water column of each wetland occurred in the first two quarters of the year, immediately after establishment (Appendix 2, Figures 2-A to 2-E). On an annual basis, the lowest mass of TP sequestered within each wetland occurred in the December Quarter - equivalent to late spring - mid summer. Only wetlands receiving alum-dosed effluent experienced net phosphorus export. This occurred during the December 1994 quarter. The highest net masses of TP were accumulated in Units 1 and 3, the lowest in Units 4 and 5. However, the highest average percentage of phosphorus retained was in Units 3, 4 and 5 (38.1, 42.9 and 38.2% respectively), the lowest in Units 1 and 2 (12.4 and 13.5% respectively).

Total nitrogen removal

The TN removal profile was slightly different to that for TP. Removal of TN by each wetland did not follow any apparent annual cycle and was independent of the hydraulic loading rate (Appendix 2, Figures 2-F to 2-J). There was a trend in 3 of the 5 wetlands for decreasing TN removal from the water column over the 2 years after establishment. The wetland Units that did not follow this trend were 3 and 5. Unit 3 had a high initial TN retention in the first 6 months after commissioning (from January to June). TN retention then dropped to a steady state of between 4 to 8 kg per 3-month interval. Unit 5 commenced with a much lower TN loading and maintained a relatively constant retention of 3 to 6 kg of TN per 3-month interval, over the 2-year period. At the end of the 2-year period, it appeared that all wetlands were achieving approximately the same removal of TN from the water column of between 4 to 5 kg per 3-month interval.

SS removal

SS removal from the water column followed an apparently random pattern, although peaks were observed for all wetland units during the March and September quarters in the second year of operation (Appendix 2, Figures 2-K to 2-O). The two alum-dosed wetlands evidenced similar quarterly patterns of SS removal, with the greatest removal appearing in the September quarter of each year. All wetlands except Unit 5 experienced a sharp increase in SS accumulation during the March '95 quarter after depressed removal in the preceding December quarter. Unit 5 evidenced this trend in the December and September quarters respectively possibly indicating the event was
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not entirely seasonally driven. Units 1 and 2 did not export SS. There was no direct correlation between SS and TP removal.

2.5.7 Soil analysis
Average concentrations of TP, IP and OP at each depth in cores of soil removed from four of the Richmond pilot plant wetlands after 2 years are presented in Figure 2-6 to Figure 2-9. In each wetland, there was noticeably greater concentration of all phosphorus forms within the top 0-2 cm of soil than at other depths. The soil layer below 0-2 cm did not evidence any significant change in phosphorus concentration or type. The clay layer (<30 cm) had a lower TP content than the soil layer. Within the 0-2 cm band of soil TP accumulation was greatest at high flow rates and was associated with an increase in both IP and OP. At slower flow rates, the increase was less, with the primary change being due to the accumulation of IP. TP was as expected for Australian soil, with a significant proportion in the inorganic form within the topsoil. The maximum P adsorption figure indicated the potential for further phosphorus removal, particularly at a depth of greater than 2 cm.

Figure 2-6: A depth profile of the mass of organic, inorganic and total phosphorus forms within the soil of wetland Unit 2 after two years of receiving sewage effluent.
Figure 2-7: A depth profile of the mass of organic, inorganic and total phosphorus forms within the soil of wetland Unit 3 after two years of receiving sewage effluent.

Figure 2-8: A depth profile of the mass of organic, inorganic and total phosphorus forms within the soil of wetland Unit 4 after two years of receiving sewage effluent.
2.6 DISCUSSION

2.6.1 Flow
Actual flow into and out of each unit differed to that calculated from the theoretical loading rate. Variations in the inlet flow rate appeared to be related to the sloughing and accumulation of biofilm and other debris on the interior of the supply pipes, causing restriction of flow between reading events. To account for the expected drop in flow over this time the inlet flow was set slightly higher than the theoretical flow.

Flow out of each wetland was significantly lower than both the theoretical value and the inlet value. This was due to three factors, evapotranspiration, decreases and variations in inlet flow, and measuring methodology. Although evapotranspiration should have had a noticeable effect at lower flow rates, the observed amount of water lost was independent of flow. Variations in the inlet flow and the time interval between flow measurements were expected to introduce a further source of
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discrepancy between inflow and outflow rates although both were monitored at the same time.

The method of measurement was unable to take into account the time lag between a change in inlet flow and the time to detection at the outlet, as this fluctuated randomly. Further, low rates of flow caused difficulty in the accurate calibration and measurement of flow over the v-notch weir, with resultant values being underestimates of true flow. However, the observed losses could also have been to groundwater (in Units 2 through 5). In which case it would not be possible to derive an accurate mass balance for flows nor be certain that changes in phosphorus concentrations between the wetland inlet and outlets were due to internal processes, and not communication with groundwater. Confirmation that water loss was not occurring to groundwater was obtained through the investigation outlined in Section 2.4.3, which is more fully discussed in Section 2.6.2.

Inflow readings were more stable than outflow readings because of the manner in which the measurements were made. The more stable nature of inflow readings was obtained using tipping buckets equipped with a reed switch, to continuously monitor the volume of water entering the wetland. Outflow readings were taken manually every few days resulting in less consistency between results. The greater frequency of inlet result readings meant that they were more accurate over the longer term, and so were used in mass balance calculations in preference to outlet measurements.

Calculations using the theoretical flow into each wetland indicated that Unit 1 would receive effluent at 2.5 times that of Units 2 and 3, and 7.5 times that of Units 4 and 5. In actuality, this rate was 1.6 that of Units 2 and 3, and 4.5 times that of Units 4 and 5 or approximately 60% of the theoretical values relative to Unit 1 (Table 2-3). The change in these ratios was caused by the reduced maximum flow rate through Unit 1 and the higher average flow rates through Units 2 through 5. However, between Units 2/3 and Units 4/5 the actual ratio of flow rates was 2.7, which only differed slightly from the theoretical ratio of 3. The noticeable change was the ratio of flow between
UNIT 1 and all other units, with the relative flow through each of the other units being comparatively unaffected.

This meant that although the flow rates into Units 2 through 5 were higher than expected from theoretical calculations that it was still possible to use flow ratio of 3:1 to compare between high and low flow units. The flow rate through Unit 1 was lower than expected theoretically. Therefore, the ratio for nutrient loading had to be revised downward. Data analysis was adjusted in line with these changed ratios.

2.6.2 Water loss

The large difference between inflow and outflow measurements indicated the possibility of water loss to groundwater. Water loss to groundwater would be indicated by losses in excess of evapotranspiration (calculated relative to pan evaporation and Unit 1) or by a greater amount of water being lost by one wetland Unit relative to all others. Unit 1 was used as a benchmark due to the minimal change in flow rates between inlet and outlet, which implied minimal water loss. Significant differences were not observed in the amount of water lost by each wetland over the three selected time intervals, relative to each other and Unit 1 (Table 2-4), with the exception of Unit 2 in January 1996. Unit 2 appeared to be losing approximately 10% of flow to groundwater for some of the period between July 1995 and January 1997.

For all wetland units, water losses due to evapotranspiration were greater than pan evaporation in the warmer months of November (Spring) and January (Summer) but less than pan evaporation during July (Winter). The absence of a difference in water loss between wetlands and the occurrence of water loss at rates less than pan evaporation during winter provided strong evidence that water was not being lost to groundwater. The placement of an impervious plastic liner in each of the wetlands during their construction meant that water loss to groundwater could only occur through damage to this material. If such damage did occur then it would be unlikely to have occurred simultaneously in all systems to the same extent unless severe deterioration of the plastic had occurred. Physical observation of the plastic after 2 years indicated only minor surface weathering.
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Based on the three trials to measure evaporative loss there appeared to be no water loss from each of the pilot plant wetlands, with the exception of Unit 2 during the final 7 months of the two-year investigation. It was possible that there was some leakage of Unit 2 during this time, possibly through root penetration of the plastic liner and bottom clay layer, allowing water to seep out of the wetland (note that this loss represented less than 10% of the net flow into the wetland).

The variations observed in the rate of evapotranspiration with time of year were not unexpected. The energy for evapotranspiration is derived directly from the sun. During the warmer months of late spring, summer, and early autumn the solar radiation is at its highest, hence the energy available for evapotranspiration is greatest, and rates would be concurrently high. During winter the energy available for evapotranspiration is much lower, hence the observed lower water losses.

In addition, evapotranspiration consists of evaporation and transpiration. Rates of transpiration are controlled by a combination of environmental and plant associated factors, and varies for each plant in a stand and between plant species. The primary mechanism by which plants control water losses are stomata, which depend on plant species for their size, number and orientation (Salisbury et al. 1985). Stomata are openings in the surface of the plant leaf, which can be opened and closed to regulate the amount of water lost. A number of environmental parameters affect their opening, including wind strength, leaf temperature, air temperature, carbon dioxide concentration and water stress (Salisbury et al. 1985). When fully open, stomata facilitate the movement of water from the plant and the exchange of carbon dioxide into the plant (Salisbury et al. 1985). This is an active process and results in water losses above normal pan evaporation (Salisbury et al. 1985). During colder months, the abovementioned factors may combine to promote the closing of stomata, closing down this water pumping mechanism (Salisbury et al. 1985). The covering of the water body by plant biomass would also serve to reduce water temperature and further inhibit evaporation from the water surface, contributing to the lower water loss values observed during winter.
It was not possible to determine a predictive relationship between rainfall in millimetres and any increase in water depth through each wetland or pan evaporation and net water loss. This may have been from variations between wetlands (including the small catchment area), the distance between the meteorological station and the study site or other physiological and physical parameters. Slight variations in construction of each wetland meant each had a slightly different catchment area associated with the 60° batter used to prevent the wetland sides collapsing. Wind moving through the site would flow across the wetlands differently depending on its intensity and direction because of the proximity of the wetlands and surrounding structures. Although a direct correlation between evapotranspiration and pan evaporation could not be derived, pan evaporation figures could be used as a conservative estimate of water loss, with the precaution that pan evaporation may underestimate evapotranspiration (Table 2-4).

2.6.3 Water quality parameters

Averaging the concentrations of various water quality parameters provided a general indication of net removal from the water column and an overall pattern of the effects of loading rates. All water quality parameters monitored, except for NOx and turbidity evidenced a decline between inlet and outlet over the two years of the investigation (1994 and 1995).

Phosphorus

In wetland units receiving alum-dosed effluent, changes in TP and FP concentration from inlet to outlet were independent of hydraulic loading, differing from wetlands receiving secondary treated effluent. In units receiving secondary treated effluent the proportion of TP relative to FP removed from the water column increased at lower flow rates i.e. a greater proportion of larger particles were being removed from the water column relative to smaller particles. Note that although the amount of larger particles being removed increased, FP was always the predominant form that was being removed. In contrast, alum-dosed systems only removed TP, not FP from the water column. TP removal suggested that a proportion of phosphorus removed from
the water column was associated with particulate matter. The removal of TP but not FP in wetlands receiving alum-dosed effluent may have been from alum entering these systems causing precipitation with TP in the wetland. In wetlands receiving secondary treated effluent, particulate matter would most likely be algal and bacterial biomass. Removal of this material would be from settling and contact with sedimentary material. The mechanism for FP removal was hypothesised to be direct binding to sediment or detrital material, and incorporation into biomass.

It is not clear why FP removal was not observed to a significant extent in alum-dosed systems. One possible explanation was that the concentrations of FP had reached an equilibrium between the water and sediment, preventing additional FP from being removed. However, in laboratory investigations (Chapter 7) these sediments still had residual phosphorus adsorption capacity. It would be expected that saturation of such binding sites would occur first in wetlands receiving secondary treated effluent. The more likely source of removal was precipitation with alum particles. TP removal in alum-dosed wetlands may have been accompanied by degradation of these compounds and re-release to the water column, which may have masked FP adsorption. Further investigation of FP removal processes is described in Chapters 3 and 4.

Nitrogen

Alum dosing had little apparent effect on the concentrations of nitrogen compounds in secondary treated effluent. The removal rates of these nutrients were similar at each flow rate irrespective of the effluent source, reflecting the similar nitrogen loadings. The removal rates for TN, TKN and NH₄ were significantly increased at lower loading rates. Dropping the loading from 10.6 KL.day⁻¹ to 6.6 KL.day⁻¹ (40%) caused an increase of approximately 20% in TN removal, however lowering the loading to 2.5 KL.day⁻¹ (75% reduction in loading) produced removal of 60% incident TN. TKN and NH₄ directly followed the trends shown by TN. Therefore, lowering the effluent loading rate should result in enhanced removal of TN, TKN and NH₄, with gains in removal in excess of the mass loss due to the lower loading. Therefore, based on these figures, in order to remove a greater mass of these nitrogen forms from the water
column, a lower loading rate would be the more effective solution, resulting in higher percentage removal rates.

Commensurate with the decrease in ammonium forms of nitrogen was an observed increase in the concentrations of oxidised nitrogen. This indicated that ammonium and TKN were probably being converted to NOx by nitrification. However, the increase in oxidised nitrogen concentrations were less than proportional to that predicted if all of the ammonium and TKN were converted to NOx. Suggesting that denitrification was also a significant process within each wetland. There was no observable correlation between NOx production and hydraulic loading rate. This may be due to adsorption of residual ammonium and TKN by plants or microorganisms, complicating the removal pathways.

**Biochemical oxygen demand and suspended solids**

A lack of SS removal from Unit 5 may have been due to a poor establishment of *Phragmites* in the zone adjacent the outlet of this wetland. This permitted the penetration of light to the water column, facilitating the growth of *Lemna, Azolla* and algae adjacent the outlet weir. Despite the presence of protective screens around the weir structure, it is possible that some of this material exited over the weir and was collected during sampling, appearing as a net export or zero removal of SS from a wetland. The amount of BOD lost between the inlet and outlet declined at higher flow rates, with similar removal percentages for both alum-dosed and secondary treated effluent. Alum dosing resulted in a slightly lower BOD and SS in water entering wetlands 3 and 4. Most likely due to the adsorption of carbon to the alum floc and the retention time of effluent in the dosing Unit allowing precipitation of SS and oxidisable organic matter.

**Conductivity**

Conductivity decreased with hydraulic loading. The conductivity of alum-dosed effluent was higher than secondary treated most probably because of the salts produced in the dosing process. The more rapid decline in conductivity values with hydraulic loading in alum-dosed systems may have been from the precipitation of aluminium salts within the wetlands. This was partially confirmed by an observed
decrease in aluminium concentration from inlet to outlet (Table 2-7) for wetlands receiving alum-dosed effluent.

Turbidity
A decrease in turbidity between wetland inlet and outlets would be expected if there was a correlation with SS, however no such correlation existed within the pilot plant wetlands. In the absence of a decline in turbidity it must be assumed that turbid particles were conserved or recycled within each wetland and that SS particles i.e. material greater than 1.2 μm were only a minor component of turbidity. Laboratory studies (Chapter 7) were conducted to further elucidate the association between turbidity and SS.

pH
A slight but consistent decrease in pH was observed between the inlet and outlet of each wetland. This may have been from the production of organic acids through the degradation of leaves and other organic material within each wetland (Section 1.5.4).

Trace elements
Due to the low number of samples, it was not possible to draw detailed conclusions from the trace element data. However, the following points should be noted. At the time of measurement, aluminium was present in effluent entering the wetlands receiving alum-dosed effluent. This allows the possibility that aluminium was entering these wetlands on a regular basis. Concentrations of aluminium were reduced by several milligrams at the outlet of alum-dosed wetlands, with concentrations not significantly different from non-alum-dosed outlets. This would imply either a one off event or that the incident aluminium had precipitated between the inlet and outlet of Units 3 and 4.

Concentrations of manganese would indicate that this element was being released from all wetlands during the sample event. The reason for this is unclear, but may relate to inflow concentration variability or incorporation of manganese into detritus, algal or floating plant cells.
2.6.4 Mass balance

A mass balance was estimated for TP, TN and SS entering each wetland unit. These parameters were chosen to evaluate possible associations of phosphorus removal with nitrogen turnover and the production or removal of SS.

TP removal

Except for the December 1994 quarter for alum dosed Units, TP was removed from the water column of each pilot plant wetland over the 2 years after commissioning. The largest removal of TP mass from the water column of each wetland occurred in the six months after establishment. The high accumulation levels observed during this period were most likely from phosphorus uptake into growing plant seedlings. However, as the seedlings matured this rate would have declined into autumn and winter as senescence began, with most seedling growth occurring in spring and summer. The observed high TP removal rates continued from the initial late spring/summer planting into the June 1994 quarter - autumn/winter. The continued storage of TP during late autumn and early winter could be from secondary storage in the soil. This would occur either from the translocation of nutrients from the plant to below ground tissue or adsorption of phosphorus to sediment (Craft et al. 1995).

The newly flooded sediment was shown to still have available phosphorus binding sites 2 years after establishment, particularly at a depth of greater than 2 cm (Analysis based on compositied samples). This observation was consistent with analysis conducted by Sakadevan et al. (1998), who determined the adsorption maxima for these soils to be in the order of 1153 mg P kg⁻¹. Rapid filling of these available binding sites would account for continued TP accumulation rates during autumn and winter months. However, the accumulation of TP re-occurred during winter and autumn in the second year of operation, suggesting a viable medium to long-term storage mechanism not simply explained by adsorption to soil. This general trend occurred in all wetlands except for Unit 2 and was therefore not an isolated incidence.

Apart from the period immediately after establishment, the greatest mass of phosphorus deposition occurred in the June and September quarters of each year,
equating to late autumn, winter and early spring. This is the period when biological activity was expected to be at its lowest (Sections 1.5.2, 1.5.4, and 1.5.6). The period of greatest biological activity would be expected to be the December quarter due to the higher temperatures. This was the quarter showing the lowest phosphorus accumulation. These results were consistent with observations in Chapters 4 and 5.

A number of possible explanations were available for the summer release and winter accumulation of phosphorus. Lower accumulation of TP in each wetland during summer months could be from increased biological activity or rainfall. Increased algal or floating plant growth would create a higher demand for phosphorus in the water column. The plant or algal biomass could then either: settle, contacting the wetland sediments and depositing its phosphorus load during senescence; or be washed out of the wetland. If the cells were washed out then there should be a correlation between increased turbidity, SS and TP concentrations during summer months. This was not observed.

Bacterial metabolic rates during summer would be expected to be higher than in winter due to the increased water temperature. Increased bacterial metabolism of detrital and other organic material in the water column may induce anoxic conditions at the sediment water interface resulting in the release of inorganically bound phosphorus to the water column. Anoxic conditions may stimulate the growth of iron-reducing bacteria able to convert insoluble oxidised forms of Fe to soluble Fe$^{2+}$, allowing the resultant release of phosphorus bound to Fe particles. It is hypothesised that the subsequent decrease in solution Eh could also have allowed the release of Al bound phosphorus.

The degradation of the organic compounds could also be a mechanism for the direct release of organically bound phosphates to the water column. Microbial breakdown of detrital material may lead to the formation of organic acids, which was hypothesised as possible explanation for the pH reductions observed in each wetland (The lower inlet pH for alum-dosed effluent could likely have been caused by the addition of aluminium sulphate during alum dosing, which was not buffered at neutrality).
lower pH can cause the solubilisation of phosphorus bound to Fe and Al compounds within sediment. It is hypothesised that if this reaction was occurring it may have been secondary to the generation of anoxic conditions because the decrease in pH observed between inlet and outlet was less than 1 pH Unit i.e. the water in the wetland remained at essentially a neutral pH. A more detailed description of litterfall interactions is provided in Chapter 5.

Higher rainfall may cause turbation of sediment and detrital material, which could then become available for subsequent export from the wetland. Stratification of the water column has been observed in shallow wetlands during summer months (Waters 1996). Stratification would enhance the formation of an anoxic zone at the sediment-water interface. In winter and autumn detritus would be more likely to accumulate from senescing plants. In addition, algal numbers were typically lower during this time of year. Therefore export of TP bound into cells would be expected to be lower than in spring and summer, when stratification should be absent and bacterial metabolic rates likely to be depressed.

A greater mass of TP was retained at higher flow rates, although for the same flow a lower loading rate produced greater TP retention (Appendix 2, Figures 2-A to 2-E). This phenomenon was most easily explained by the hypothesis that chemical adsorption to sedimentary material was the dominant phosphorus removal mechanism. An alternative was that phosphorus might have been adsorbed directly into plant biomass. Phosphorus was also taken up by other organisms but not to the same extent. Since approximately the same amount of biomass was present in each wetland, TP accumulation in plant material should be similar, only influenced marginally by flow rates.

Adsorption should at least partially be driven by equilibrium considerations. As flow increased the contact time between the sediment and the water column decreased. Thus at high retention times the percentage of phosphorus removed from the water column increases as there is more opportunity for the TP in the water to reach an equilibrium with the soil. At high flows, the transfer and binding processes in both
water column and sediment become less efficient, but the mass of phosphorus passing over the sediment could have resulted in a greater net removal.

The more efficient removal of TP from wetlands receiving alum-dosed effluent relative to those receiving secondary treated effluent at the same flow rates was more complicated. If the same principles applied irrespectively of the effluent source then a higher net concentration of TP in the water column should increase absolute but not percentage TP retention. Higher concentrations would allow the soil to have a higher equilibrium concentration of TP (if adsorption occurs as a first order equation). Therefore, a greater mass of TP should have been removed in systems receiving secondary treated effluent. In both absolute and percentage terms, alum-dosed units removed more phosphorus.

The most compelling explanation is that there was a fundamental difference in the way phosphorus is removed from the two effluents. One likely hypothesis is that alum was a participant in the deposition of TP in wetlands receiving alum-dosed effluent. Evidence for this hypothesis was the higher incident Al concentration for Units 3 and 4 (Table 2-7). Figures 2-A to 2-E (Appendix 2) indicated that wetlands receiving alum-dosed effluent had a higher average percentage TP removal than units receiving secondary treated effluent. In addition, Units 3 and 5 had a more consistent temporal pattern of TP removal, whereas non-alum dosed units showed a declining trend in TP removal over time. This, coupled with the previous discussion suggests that in wetlands receiving alum-dosed effluent two phosphorus removal mechanisms were operating, sediment binding and alum induced precipitation. Hence, the greater removal of TP from these Units relative to non-alum dosed systems.

2.6.5 Total nitrogen removal
On a mass loading basis TN removal declined over time in three out of the five wetland systems. Only Unit 5 showed a uniform net removal for the sampling period, averaging only 5 kg of TN removal per quarter. While in Unit 3 TN removal was high at around 18 kg per quarter, dropping to a sustained average of 5 kg per quarter after the initial two quarters. However, Unit 4, along with Units 1 and 2 evidenced a slow
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decline in TN removal over time. This observed decline was unexpected because the majority of TN removal should be through microbial metabolism, which would be primarily affected by temperature, season and available nutrients. Available nutrients were not likely to have declined consistently over the study period and both temperature and season follow annual cycles.

One possible explanation would be that the plants within each wetland were reaching maturity during this period and it was possible that on maturity they may supply less oxygen to the root zone, resulting in lower nitrification rates. Another hypothesis was that a removal or deposition rate of around 5 kg per 3-months may be the naturally sustainable rate for nitrification processes in these wetlands. The decline in nitrification over time may have been due to the accumulation of biofilm in each wetland, which produced a consistent turnover of nitrogen once an equilibrium population was achieved.

2.6.6 Suspended solids removal

On a mass loading basis SS removal increased as the wetland matured. This was probably due to the increasing number of plant stems present in each wetland over the two years (1994 and 1995). This is consistent with the higher removal rates occurring in the September Quarter, a period of potentially rapid plant growth. During the December quarters, when TP accumulation was lowest, SS accumulation was also depressed and SS was being released from Units 3, 4 and 5 in greater concentrations than it was entering. The hypothesis being, that export of biomass may be at least partially responsible for phosphorus release or reduced accumulation rates observed during the December quarters for the majority of pilot plant wetlands.

2.6.7 Soil

TP values at the end of 24 months indicated that none of the pilot plant wetlands soils were saturated with phosphorus. The accumulation of phosphorus in the top 0-2 cm of each wetland was not unexpected because this was the region in direct contact with the water column. TP in the water column must first pass through the upper layers of sediment before penetrating deeper. Therefore, sediment in the upper regions will
have first contact with TP in the water column and it would be expected that any available soil phosphorus binding sites in this region would become saturated before those at a lower depth. This is what was observed.

It should be noted that there were still available binding sites in this upper region. However, the depth of soil that interacted with the water column in this way was not determined in the current study. If only the top 0.5 to 0.25 cm of soil interact with the water column then there may be saturation of this, the most interactive soil layer. If the upper layer of soil becomes saturated with phosphorus, it may prevent phosphorus penetrating further into the soil. The extent of migration may also be influenced by the flow rate of water. At high flow rates, there would be less contact time to allow penetration of sedimentary material, with lower flow rates permitting greater penetration.

During this study, the most significant factor influencing phosphorus forms in soils from each wetland was the source of effluent. Secondary treated effluent produced increases in soil FP and IP, while alum-dosed effluent was associated only with accumulation of IP. Accumulation of IP in these systems would probably be from deposition of alum-associated phosphorus entering these systems. Data for aluminium concentration in the top 0-2 cm of wetlands receiving alum-dosed effluent provide support for this theory. Aluminium concentrations at the end of 2 years were higher in alum-dosed systems, with greater accumulation at higher loading rates. The uniform distribution of Al throughout each wetland would imply either high background soil concentrations or movement of alum through the wetland. Such movement would be expected if the aluminium was loosely bound and could be exported from the wetlands during periods of high flow. The occurrence of such an event was not confirmed.

2.7 CONCLUSIONS

With the exception of Unit 2 during the last 6 months of the study, water was not lost to groundwater in any wetland Unit. From the available data, inlet flow rates adjusted for evaporative losses were used to calculate mass loadings. It was not possible to
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derive a predictive formula to determine evapotranspiration from evaporation readings. Therefore average evaporation figures were used due to the frequency of measurement, allowing that this value may under-represent evapotranspiration losses by up to 70%. Note however, that the energy available available for evapotranspiration varies with season and it may be possible to derive a more predictive relationship between evaporation and the rate of water loss if water loss was investigated on a quarterly basis. Further work would be required to determine whether such a relationship could be established. This was beyond the scope of the current thesis.

In average terms, removal of TP, FP, TN, NH₄, TKN, SS and BOD occurred for wetland Units 1 through 5. There was a net increase in the concentration of NOx between inlet and outlet of each wetland. Mass loading data indicated different patterns of removal for TP, TN, SS. TP removal was greatest in June and September quarters with lowest removal, and some export observed during the December quarters. The suspicion is that much of this material was being sequestered to sediment and incorporated into plant and detrital material during the June and September quarters and released due to increased microbial activity during the warmer moths of late spring and early summer. TP removal in alum-dosed systems appeared to be related to aluminium entering each system and precipitating TP. These investigations highlighted the need to elucidate the role of different plant species, detritus formation, the ability of phosphorus to adsorb to sediment and the interactions between sedimentary material and microorganisms in different plant stands in order to gain a more predictive understanding of phosphorus removal in these wetlands.

TN evidenced a gradual decline in removal since establishment, tending toward an apparent equilibrium condition, unrelated to trends in phosphorus removal. However, SS removal increased or was highest in the September quarters when TP removal was also high, and lowest (even being exported from various wetlands) during the December quarter, indicating that some of the observed phosphorus losses may have been associated with the export of SS material.
CHAPTER 3: DETAILED EXAMINATION OF SECTORS WITHIN THE RICHMOND PILOT PLANT WETLANDS

3.1 INTRODUCTION

Data presented in Chapter 2 indicated that on average, over a 2-year period, TP was being removed from the water column of all wetland units at the Richmond Pilot Plant. This chapter details analysis undertaken to establish the association of TP removal with various size fractions of phosphorus, concentrations of TN and SS, water quality parameters, plant species and distance through each wetland. A comparison was made of these parameters at two different retention times, in wetlands receiving secondary and alum-dosed effluent. Units 2 through 5 at Richmond represented four permutations of flow rate and effluent composition (two different retention times and two effluent compositions) at the same water depth. The non-replicated water depth and flow in Unit 1 precluded its inclusion in this and subsequent investigations (Chapters 4 and 5).

3.2 AIM

This chapter details investigations to locate regions of phosphorus removal and determine possible phosphorus removal mechanisms within four of the wetlands at Richmond. These four wetlands (Units 2, 3, 4 and 5) are described in Chapter 2. Each of these wetlands had been planted in seven distinct bands with the plants Phragmites, Triglochin and Schoenoplectus (Figure 2-1). Investigations were designed to address:
1. whether TP was removed between the inlet and outlet of each wetland during the period of investigation;
2. whether phosphorus was being removed proportionally with distance into each wetland;
3. if different plant stands stimulated or repressed the removal of TP;
4. how different flow rates affected the pattern of phosphorus removal;
5. the association between phosphorus removal or release and the parameters: TN; SS; DO; pH; temperature; conductivity; chlorophyll a; pheophytin; and total organic carbon (TOC); and
6. the proportion of different size phosphorus fractions removed with distance through each wetland.

3.3 MATERIALS AND METHODS

To accomplish the aims outlined in Section 3.2 the detailed sampling protocol outlined below was used:

Preliminary Investigations
Variability of phosphorus concentrations at the inlets to wetland Units 2, 3, 4, and 5 was evaluated to confirm the required sampling frequency.

Detailed Investigations
Detailed examination of each wetland (Units 2, 3, 4 and 5) was undertaken. This involved dividing each wetland into 7 segments based on plant species. Three quadrats of 25 cm² were randomly selected from each segment for sampling and analysis of physico-chemical water quality parameters (detailed in Section 3.2). Statistical analysis was performed on all results to determine associations of TP with distance, plant species and physico-chemical parameters.

3.3.1 Inlet variations
The inlets to Units 2 and 3 were used as representative samples of secondary treated and alum-dosed effluent respectively. Monitoring was undertaken over 5 days, on 3 separate occasions, with samples taken hourly by autosampler (Isco) to determine the variability of TP and SS concentrations over this period. Ice was placed in the autosampler on a daily basis to maintain the temperature below 4°C. Samples were removed from the autosampler and processed within 24 hours of collection. Samples were analysed as outlined in 2.4.8.

3.3.2 Water sampling
Since the water column in these units was 20 cm deep, samples were taken 10 cm above the sediment surface. Due to the shallow water depth in all units (20 cm), it was assumed that stratification would be negligible. Water samples were taken from three
randomly selected sites within each of the 7 zones of plants (only 2 sites were selected from Stand 7 due to its length, see Figure 2-1). Samples were obtained using a plastic tube (4m long, ID 0.5 cm) attached to a stainless steel metal rod (1.0 m x 0.5 cm) such that the open end was 10 cm from the base of the metal rod. The tube was secured along the length of the rod (Figure 3-1). The metal rod was secured to a wooden pole (3m x 2.5 cm) by a metal chain and the plastic tubes secured along the length of the pole.

Figure 3-1: The design of the hand sampling apparatus used for obtaining water samples.

The pole was designed so that it could be extended over the wetland and lowered into the water column from the bank, minimising disturbance to the system. The purpose of the metal rod was to allow penetration of wetland vegetation and provide a method of locating a suction tube 10 cm above the wetland sediment. The banks of each wetland were marked at 4m intervals from inlet to outlet with white marker poles. By
use of a tape measure and these poles as reference points, it was possible to locate the sampling tube in a chosen quadrat. Once the tube was in position, a suction apparatus was used to siphon two dead volumes of water (approximately 500 mL) through the tube to prevent cross contamination of samples.

The suction apparatus consisted of a hand operated vacuum pump fitted with a pressure gauge (Nalgene model 6130-0020). To prevent movement of solution into the pump mechanism it was attached to two vacuum containers connected in series. The first of these containers was used to collect the sample. This container was rinsed with MilliQ water between the collection of each sample. An initial 500 mL of solution from each site was used to flush the water collection tube (this material was collected in two portions, the container being rinsed between each). After siphoning through the two dead volumes of water, 500 mL was collected for analysis. A small metal clamp was used to prevent the solution in the tube from flowing back to the wetland by gravity whilst the collection container was being emptied or rinsed. The collection tube was emptied by gravity between samples. Water samples were analysed for SS, TP and FP using the persulphate method (Section 2.4.8).

3.3.3 Detailed examination of sectors within each wetland

The objective of this study was to determine and compare regions of phosphorus removal in four wetland units from the Richmond Pilot Plant. To achieve this objective it was necessary to obtain a profile of TP, FP and SS prior to within wetland testing to determine the expected range and variance in concentrations that should be observed during the investigation. Samples were taken from the inlet for a period equivalent to one retention time (15 days for wetland Units 4 and 5, 5 days for wetland Units 2 and 3) prior to sampling within that Unit. Sampling of sites within each wetland was then undertaken and sampling of water from the outlet commenced, continuing for one full retention time. Outlet samples were taken to provide an indication of overall phosphorus removal by the Unit relative to each plant stand in the wetland and provide data on the variability of readings.
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Water samples from both inlet and outlet were obtained by Isco autosampler (models 1680 and 6800), filled with ice to maintain a temperature of 4°C. Samples were collected hourly and combined into four-hour composites by each autosampler. As noted in the site description (Figure 2-1) each wetland was divided into 7 sectors corresponding to the location of different plant species. During this investigation each of these sectors was subdivided into a series of squares measuring 25 cm x 25 cm, each representing a separate quadrat. Three quadrats were randomly selected from each sector. Samples were obtained using the apparatus described in Section 3.3.2. Water samples were analysed for conductivity, pH, temperature, DO, chlorophyll a, SS, TP and various phosphorus fractions as outlined below and in Section 2.4.8.

Filter papers of four different pore sizes were used to determine the association of phosphorus with particles of various sizes. The pore sizes used to separate phosphorus particles were 1.2 μm (GFC filter - to determine FP), 0.45 μm, 0.2 μm and 0.1 μm (the last three filters being of the Nucleopore type). A separate volume was filtered for chlorophyll determinations. All filters were rinsed in MilliQ water prior to use.

- Initially, 100 mL of sample was filtered through the preweighed GFC filter (1.2 μm).
- The filtrate was then divided into two components; 40 mL was kept for analysis and 60 mL was filtered through a 0.45 μm filter.
- Of this material, 20 mL was kept and 40 mL filtered through a 0.2 μm filter.
- Again, 20 mL was kept and the residual filtered through a 0.1 μm filter.

The eluent solutions were then digested by the persulphate method and analysed for TP (Section 2.4.10). Results were reported as concentration of phosphorus passing through a filter of pore size x (where x = 1.2, 0.45, 0.2 or 0.1 μm).
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3.4 RESULTS

3.4.1 Initial study - inlet variations

Variations in inlet phosphorus concentrations were observed for alum-dosed and secondary treated effluent over a number of time intervals as set out in Table 3-1. There was no significant difference between readings in samples taken as far apart as 4 hours, at the 95% confidence level.

Table 3-1: comparison of readings taken of total phosphorus in alum-dosed and secondary treated sewage effluent

<table>
<thead>
<tr>
<th>Time interval (hr)</th>
<th>Number of readings (n)</th>
<th>Average (mg.L⁻¹)</th>
<th>Standard Deviation</th>
<th>Significant Difference¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alum-dosed effluent</td>
<td>0.5</td>
<td>10</td>
<td>1.48</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>63</td>
<td>1.39</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>31</td>
<td>1.61</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>40</td>
<td>1.87</td>
<td>0.28</td>
</tr>
<tr>
<td>Secondary treated effluent</td>
<td>0.5</td>
<td>10</td>
<td>8.47</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>40</td>
<td>7.92</td>
<td>0.09</td>
</tr>
</tbody>
</table>

¹ Two tailed test, α = 0.05, t-test, two sample, paired mean.

Note that the 0.5 and 1 hour intervals were sampled separately to the 2 and 4-hour intervals, hence the difference concentration readings. The significant difference column indicated whether a significant difference existed between n samples taken over a stated time interval.

During this study, SS concentrations evidenced random fluctuations with high standard deviations even at time intervals of 0.5 hr (4.4 ± 3.6 mg.L⁻¹) with a range of 0 to 14 mg.L⁻¹.

3.4.2 Detailed examination of sectors within each wetland

Unit 2 - High phosphorus concentrations

Phosphorus

Over the five day period of monitoring the concentration of TP at the inlet to Unit 2 was consistent at 7.92 ± 0.09 mg.L⁻¹. The concentration of TP at the outlet was slightly lower and more variable, averaging 7.50 ± 0.58 mg.L⁻¹. This difference was
significant (Wilcoxon’s paired sample test, $\alpha = 0.05$) and an average of 0.42 mg.L$^{-1}$ of TP was removed from the water column between the inlet and outlet of Unit 2.

Overall, TP concentrations declined with distance into the wetland. However, there were three regions (S1, S4 and S6 - Figure 3-2) that produced significant rises in TP against this general trend (Kruskal-Wallis, followed by a Tukey Test, $\alpha = 0.05$). These rises were also observed for the FP fraction. The other fractions evidenced slightly different but unrelated trends (Figure 3-2).

Figure 3-2: Phosphorus concentrations associated with different particle sizes entering pilot plant wetland Unit 2 (supplied with secondary treated effluent).

Note that the predicted TP concentrations were not included in the above graph for simplicity, as the concentrations at the inlet evidenced such low variation during the time of this investigation.

Other parameters

Physico-chemical parameters monitored within the wetland showed a range of responses. Several parameters did not vary significantly through the wetland. These included water temperature (averaging 17.6 ± 0.9°C), TN (9 ± 5 mg.L$^{-1}$), TC (18 ± 11 mg.L$^{-1}$) and DO (except for a peak in S3, Table 3.1). As expected, SS concentrations fluctuated over a range of values, with high standard deviations preventing detailed interpretation.

A linear response was observed for pH, which gradually declined from 7.4 to 6.9 through the wetland. However, a number of parameters evidenced variations that were
more distinct. These included conductivity, where readings were slightly elevated in S1, fluctuated randomly between S2 and S5, but dropped between S5 and S6 (Table 3-2). Both chlorophyll a and pheophytin decreased from the inlet to S4 and S3 respectively (Table 3-2), before steadying and fluctuating randomly through the remainder of the wetland; hence their high correlation ($r = 0.92$). These two parameters also correlated well with TP and FP phosphorus fractions ($0.76 < r < 0.81$).

<table>
<thead>
<tr>
<th>Plant Stand</th>
<th>DO (mg.L$^{-1}$)</th>
<th>Conductivity (µS)</th>
<th>Chlorophyll a (mg.m$^{-2}$)</th>
<th>Pheophytin (mg.m$^{-2}$)</th>
<th>SS (mg.L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>3.04 ± 1.44</td>
<td>780 ± 25</td>
<td>1590 ± 1940</td>
<td>2365 ± 1220</td>
<td>63 ± 68</td>
</tr>
<tr>
<td>S2</td>
<td>3.47 ± 1.46</td>
<td>740 ± 15</td>
<td>590 ± 300</td>
<td>1210 ± 525</td>
<td>44 ± 26</td>
</tr>
<tr>
<td>S3</td>
<td>5.87 ± 0.92</td>
<td>720 ± 30</td>
<td>260 ± 145</td>
<td>755 ± 290</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>S4</td>
<td>2.21 ± 0.19</td>
<td>750 ± 20</td>
<td>85 ± 90</td>
<td>895 ± 275</td>
<td>65 ± 86</td>
</tr>
<tr>
<td>S5</td>
<td>3.30 ± 0.84</td>
<td>720 ± 20</td>
<td>105 ± 35</td>
<td>865 ± 425</td>
<td>17 ± 8</td>
</tr>
<tr>
<td>S6</td>
<td>2.53 ± 0.85</td>
<td>690 ± 25</td>
<td>355 ± 320</td>
<td>1185 ± 275</td>
<td>32 ± 7</td>
</tr>
<tr>
<td>S7</td>
<td>2.64 ± 0.30</td>
<td>685 ± 5</td>
<td>120 ± 50</td>
<td>800 ± 95</td>
<td>75 ± 98</td>
</tr>
</tbody>
</table>

Unit 3 - Low phosphorus, 5 day retention time

Phosphorus

TP concentrations measured over the 5 day sampling interval averaged $1.21 ± 0.34$ mg.L$^{-1}$ at the inlet and $1.57 ± 0.2$ mg.L$^{-1}$ at the outlet. Using the Wilcoxon paired sample test the outlet was significantly higher than the inlet for 90% of measurements at the $\alpha = 0.001$ level of significance (two tailed test).

The values for inlet TP shown in Figure 3-3 were derived from extrapolating the concentrations of TP observed at the inlet to Unit 3 based on the theoretical hydraulic retention time. Phosphorus removal within Unit 3 was evaluated by comparison of this extrapolated value to the actual in-situ phosphorus reading.

Within Unit 3, the actual TP concentrations differed significantly from the predicted concentrations in sectors 1, 4, 5 and 6 (Figure 3-3). In all other sectors, there was no
significant difference. The greatest difference between actual and predicted TP occurred in S5, with the difference decreasing towards the outlet.

Despite the changes occurring to phosphorus concentrations at the wetland inlet, the concentrations of FP (relative to TP within the wetland) evidenced apparently random fluctuations through the wetland from S1 to S4, decreasing thereafter (between S5 and S7, Figure 3-3). There was a convergence of FP with all smaller phosphorus fractions between S5 and S7.

Through the first half of Unit 3, fluctuations in the 0.45μm phosphorus fraction closely followed those of the FP fraction (Figure 3-3). There was no significant difference between the concentration of phosphorus in the 0.45 and 0.2μm phosphorus fractions, with the possible exception of S1 and S2, where readings for the 0.45μm fraction were slightly higher.

Figure 3-3: Average phosphorus concentrations associated with different particle size fractions within wetland Unit 3.
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Phosphorus concentrations in the 0.1\μm fraction followed the trend of the other fractions from S1 to S4, with the 0.1\μm fraction comprising some 80 to 85% of the 0.2\μm fraction. However, in S5 through to S7, the 0.1\μm fraction comprised 100% of the 0.2\μm and 0.45\μm, and averaged 90% of FP (between S1 and S4 it only constituted 70% of FP).

The high standard deviations observed in S5 (Figure 3-3) indicated that phosphorus concentrations in this stand were undergoing a transition during the sampling period, with concentrations declining through the stand. The highest TP concentration was observed in the sample taken from the central stand of *Phragmites* (S4, TP = 2.7 mg.L\(^{-1}\)), the other two samples were of lower concentration (TP = 1.34, 1.34) and taken towards the outlet end of the stand.

Other parameters

Physico-chemical parameters within Unit 3 produced different trends to Unit 2. Water temperature and DO both varied through Unit 3. Temperatures were elevated in water samples taken from S1, S3 and S5, by 1.5 to 2°C compared to surrounding regions which averaged 21.8 ± 0.5°C. DO concentrations in the two plant stands adjacent the inlet and outlet structures were similar, averaging between 2.2 to 2.6 mg.L\(^{-1}\) (Table 3.3). However, the central three sectors (S3, S4 and S5) had elevated DO concentrations, which ranged between 5.0 and 6.0 mg.L\(^{-1}\).

TN and TC fluctuated widely through the wetland (Table 3-3). TC was 5 to 10 times higher than TN, but values for TC did not correlate with those for TN. TC concentrations experienced a minimum in S2. SS also fluctuated randomly throughout the wetland, with consistently high standard deviations preventing meaningful correlations.
Table 3-3: Concentrations of dissolved oxygen, chlorophyll a, pheophytin, suspended solids, total nitrogen and total carbon within Unit 3

<table>
<thead>
<tr>
<th>Plant Stand</th>
<th>DO (mg.L⁻¹)</th>
<th>Chlorophyll a (mg.m⁻²)</th>
<th>Pheophytin (mg.m⁻²)</th>
<th>SS (mg.L⁻¹)</th>
<th>TN (mg.L⁻¹)</th>
<th>TC (mg.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>2.2 ± 1.1</td>
<td>&lt;1</td>
<td>338 ± 55</td>
<td>195 ± 335</td>
<td>1.1 ± 1.0</td>
<td>17.0 ± 18.3</td>
</tr>
<tr>
<td>S2</td>
<td>2.4 ± 0.8</td>
<td>1 ± 1</td>
<td>322 ± 47</td>
<td>25 ± 15</td>
<td>1.8 ± 0.8</td>
<td>11.2 ± 3.1</td>
</tr>
<tr>
<td>S3</td>
<td>5.5 ± 1.4</td>
<td>159 ± 139</td>
<td>902 ± 151</td>
<td>50 ± 60</td>
<td>1.1 ± 1.9</td>
<td>26.1 ± 10.1</td>
</tr>
<tr>
<td>S4</td>
<td>5.6 ± 2.1</td>
<td>24 ± 15</td>
<td>169 ± 63</td>
<td>2.6 ± 3.5</td>
<td>42.7 ± 41.6</td>
<td></td>
</tr>
<tr>
<td>S5</td>
<td>5.8 ± 1.2</td>
<td>15 ± 11</td>
<td>273 ± 66</td>
<td>140 ± 115</td>
<td>1.2 ± 1.2</td>
<td>39.3 ± 18.3</td>
</tr>
<tr>
<td>S6</td>
<td>2.6 ± 1.1</td>
<td>44 ± 50</td>
<td>269 ± 122</td>
<td>25 ± 20</td>
<td>2.8 ± 3.1</td>
<td>28.8 ± 11.8</td>
</tr>
<tr>
<td>S7</td>
<td>2.3 ± 0.8</td>
<td>48 ± 56</td>
<td>237 ± 119</td>
<td>370 ± 500</td>
<td>2.1 ± 2.9</td>
<td>34.1 ± 12.4</td>
</tr>
</tbody>
</table>

Unlike Unit 2, the pH of the water column did not vary significantly through Unit 3, averaging 7.2 ± 0.1. Conductivity fluctuated randomly through the wetland with an average of 696 ± 31 µS.cm⁻¹. Chlorophyll a concentrations were below detection limits until the initial stand of *Triglochin* (S3). After S3, concentrations averaged between 15 to 48 mg.m⁻³ with no apparent correlation with distance or plant type. Pheophytin evidenced a coincident peak with chlorophyll in S3, however concentrations in other parts of the wetland showed no correlation, ranging from 169 to 338 mg.m⁻³. There was no correlation between low concentrations of pheophytin and distance or plant type. Neither chlorophyll a nor pheophytin evidenced any correlation with phosphorus concentration.

**Unit 4 - Low phosphorus, low flow**

Phosphorus

Using the Wilcoxon paired sample test to compare TP concentrations from the inlet and outlet of Unit 4, concentrations at the outlet (1.35 ± 0.26 mg.L⁻¹) were significantly higher than the inlet (1.19 ± 0.34 mg.L⁻¹) for 100% of observations (α = 0.001 level of significance for a two tailed test), when a theoretical retention time of 14 days was applied. If a retention time of 8 days (Chapter 4) was assumed then only 87% of paired observations show higher TP concentrations at the outlet, making the outlet concentrations significantly higher than the inlet at the α = 0.005 level of significance during the period of observation, regardless of the hydraulic retention time.
When the phosphorus association with different particle sizes within the wetland was examined a number of trends emerged. Predicted and actual TP concentrations did not differ significantly in the final three plant stands S5 to S7. However, significant differences were observed upstream of these locations, with values significantly higher than the inlet in S3, but lower in S4 (Figure 3-4).

Comparison of phosphorus fractions indicated that TP and FP followed similar trends, with rises in TP occurring in S3 and S5 (Triglochin), but decreasing slightly in the central stand of Phragmites (S4). There was no significant difference between the FP and 0.45µm phosphorus size fractions, and only slight differences between the FP and 0.20µm fractions (Figure 3-4). The 0.1µm fraction was slightly less than the 0.2µm fraction, and differed in trend from the other fractions in S1 and S7, where concentrations were lower compared to other size fractions.

Other parameters
Similar to Unit 3, water temperatures varied through Unit 4, with peaks in S2 and S3, which averaged 2.1°C higher than the other regions of the wetland (21.4 ± 0.6°C). DO concentrations evidenced random fluctuations through the wetland with a range of 1.3 to 3.2 mg.L⁻¹ (Table 3-4). The only exception occurred in S3 (Triglochin) which evidenced much higher DO concentrations in all replicates, giving an average DO concentration of 9.97 ± 3.8 mg.L⁻¹. TN and TC were noticeably higher than in the
other wetland units. There was no direct correlation between TN and TC, except that both evidenced peaks in segment 3 of Unit 4, with only negligible concentrations of each detected in the water column towards the latter part of the wetland (Table 3-4).

SS again fluctuated widely through the wetland but produced very low values in S4, 6 and S7 (Table 3-4). The pH of the water column was neutral throughout Unit 4 at 6.9 ± 0.2 units. Conductivity was relatively constant, averaging 660 ± 10 µS.cm⁻¹. Regions of high SS concentration were found at S3 and S5, although the high standard deviation of these readings brings into question the reproducibility of such results.

Table 3-4: Concentrations of dissolved oxygen, chlorophyll a, pheophytin, suspended solids, total nitrogen and total carbon within Unit 4.

<table>
<thead>
<tr>
<th>Plant Stand</th>
<th>DO (mg.L⁻¹)</th>
<th>Chlorophyll a (mg.m⁻³)</th>
<th>Pheophytin (mg.m⁻³)</th>
<th>SS (mg.L⁻¹)</th>
<th>TN (mg.L⁻¹)</th>
<th>TC (mg.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>2.44 ± 0.93</td>
<td>&lt; 1</td>
<td>261 ± 19</td>
<td>62 ± 60</td>
<td>&lt; 1</td>
<td>140 ± 60</td>
</tr>
<tr>
<td>S2</td>
<td>2.45 ± 0.73</td>
<td>13 ± 14</td>
<td>416 ± 265</td>
<td>78 ± 23</td>
<td>17 ± 16</td>
<td>461 ± 156</td>
</tr>
<tr>
<td>S3</td>
<td>9.97 ± 3.8</td>
<td>449 ± 371</td>
<td>527 ± 253</td>
<td>582 ± 581</td>
<td>77 ± 24</td>
<td>764 ± 178</td>
</tr>
<tr>
<td>S4</td>
<td>3.11 ± 1.27</td>
<td>15 ± 17</td>
<td>281 ± 34</td>
<td>4 ± 5</td>
<td>&lt; 1</td>
<td>61 ± 105</td>
</tr>
<tr>
<td>S5</td>
<td>2.23 ± 0.22</td>
<td>33 ± 28</td>
<td>234 ± 151</td>
<td>157 ± 256</td>
<td>2 ± 2</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>S6</td>
<td>1.77 ± 0.83</td>
<td>11 ± 19</td>
<td>426 ± 292</td>
<td>10 ± 5</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>S7</td>
<td>1.53 ± 0.35</td>
<td>3 ± 5</td>
<td>251 ± 77</td>
<td>10 ± 7</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

Concentrations of chlorophyll a peaked in stands 3 and 5 (*Triglochin*) at 449 and 33 mg.L⁻¹ respectively. Through the remainder of the wetland chlorophyll a concentrations ranged from 0 to 15 mg.m⁻³ giving an average of 8 ± 6 mg.m⁻³. Although pheophytin concentrations peaked in S3, along with chlorophyll a, the difference was not significantly higher than values for the remainder of the wetland. Chlorophyll a correlated well with temperature, TN and TC (r = 0.68, 0.97, 0.82), as did pheophytin (r = 0.83, 0.79, 0.79). There was moderate correlation between TN and TC with chlorophyll a and SS (0.83 < r < 0.66). Correlation was also observed between the fraction of phosphorus larger than 1.2 µm (TP) and chlorophyll a, SS and TN (r=0.92, 0.97, 0.93).
Unit 5 - High phosphorus, low flow

Phosphorus

Samples taken from the inlet and outlet of Unit 5 indicated TP removal occurred in 97% of paired samples, assuming a 15 day retention time; average inlet TP concentration $6.83 \pm 1.62$, outlet TP concentration $6.74 \pm 0.4$. Differences observed were statistically significant at the $\alpha = 0.001$ confidence level (two tailed test). Therefore, Unit 5 was consistently removing TP from the water column during the period of observation.

With the exception of S2 and S5 there was a gradual decrease in TP with distance (Figure 3-5). TP concentrations changed independently of other phosphorus fractions (Figure 3-5). This is highlighted by the low correlation between TP and these fractions ($r = 0.17$ to $-0.34$) with the correlation decreasing with particle size. There was high correlation between FP, 0.45 and 0.2 $\mu$m phosphorus fractions ($r \geq 0.97$), and a lower correlation between these fractions and phosphorus particles smaller than 0.1 $\mu$m ($0.82 \leq r \leq 0.93$).

Figure 3-5: Concentrations of different phosphorus fractions within pilot plant wetland Unit 5.
Other parameters

Temperature through the wetland averaged 21.5 ± 0.6°C, with no significant variations throughout Unit 5. DO in the wetland averaged less than 3.4 mg.L⁻¹, with the only exception a high of 6.7 ± 3.6 mg.L⁻¹ through S3 (Triglochin). There was a marked decrease in DO from this section into S4 (Phragmites) to 1.9 mg.L⁻¹. TN and TC concentrations correlated (r = 0.91), with TN declining from S2 to S5 and TC from S2 to S6 (Table 3-5). TN and TC concentrations were the highest of any wetland observed thus far.

SS concentrations again fluctuated widely through the wetland with high averages observed in the plant stand adjacent the outlet, which was mostly open water due to the failure of Phragmites to establish in this zone. The highest concentrations of SS occurred in the sections of the wetland with exposed water surface.

Similar to observations of Unit 2, pH declined steadily from 7.22 ± 0.12 at the inlet to 6.84 ± 0.07 adjacent the outlet. Conductivity followed pH over the range 740 ± 15 to 600 ± 5 μS.cm⁻¹ resulting in a high correlation with pH (r= 0.89) and negative correlations with distance (r = -0.82 (conductivity), -0.96 (pH)).

Table 3-5: Concentrations of dissolved oxygen, chlorophyll a, pheophytin, suspended solids, total nitrogen and total carbon within Unit 5.

<table>
<thead>
<tr>
<th>Plant Stand</th>
<th>DO (mg.L⁻¹)</th>
<th>Chlorophyll a (mg.m⁻³)</th>
<th>Pheophytin (mg.m⁻³)</th>
<th>SS (mg.L⁻¹)</th>
<th>TN (mg.L⁻¹)</th>
<th>TC (mg.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>1.79 ± 0.45a</td>
<td>74 ± 87</td>
<td>480 ± 773</td>
<td>220 ± 370</td>
<td>0.6 ± 1.0</td>
<td>200 ± 93</td>
</tr>
<tr>
<td>S2</td>
<td>3.43 ± 1.44</td>
<td>217 ± 172</td>
<td>250 ± 217</td>
<td>1180 ± 830</td>
<td>83.6 ± 67.0</td>
<td>862 ± 475</td>
</tr>
<tr>
<td>S3</td>
<td>6.65 ± 3.65</td>
<td>199 ± 142</td>
<td>518 ± 698</td>
<td>5950 ± 6900</td>
<td>58.3 ± 73.0</td>
<td>711 ± 612</td>
</tr>
<tr>
<td>S4</td>
<td>1.92 ± 0.33</td>
<td>28 ± 8</td>
<td>78 ± 11</td>
<td>70 ± 60</td>
<td>25.6 ± 20.6</td>
<td>302 ± 44</td>
</tr>
<tr>
<td>S5</td>
<td>2.88 ± 0.80</td>
<td>88 ± 90</td>
<td>230 ± 287</td>
<td>5600 ± 9700</td>
<td>13.8 ± 24.9</td>
<td>149 ± 148</td>
</tr>
<tr>
<td>S6</td>
<td>2.63 ± 1.17</td>
<td>28 ± 27</td>
<td>73 ± 32</td>
<td>190 ± 320</td>
<td>&lt; 0.5</td>
<td>322 ± 150</td>
</tr>
<tr>
<td>S7</td>
<td>1.84 ± 0.09</td>
<td>235b</td>
<td>56 ± 26</td>
<td>4230 ± 5370</td>
<td>37.6 ± 15.6</td>
<td>759 ± 229</td>
</tr>
</tbody>
</table>

¹ Values are SD, n=3.
² Only 1 replicate.

Chlorophyll a concentrations peaked in S2, S3 and S7. The lowest concentrations of chlorophyll a were in S4 and S6 (Table 3-5). Pheophytin values did not follow the trend evidenced by chlorophyll a, large fluctuations in concentration were observed.
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with peaks in S1, S3 and S5. Minimum values were observed at S4, S6 and S7. There was good correlation of TC with chlorophyll a and the fraction of TP greater than 1.2 \( \mu \text{m} \) (\( r = 0.88, 0.83 \)). However there was very slight correlation of TP with chlorophyll a or TC (\( r = 0.44, 0.39 \)) and none with SS (\( r = 0.10 \)).

3.4.3 Comparison of wetland Units 2 through 4

Table 3-6 summarises the key findings across each segment of Units 2 through 5. Overall rates and regions for TP removal or release were not consistent between wetlands, except that wetlands receiving higher inlet nutrient concentrations (Units 2 and 5) reduced TP concentrations between the inlet and outlet, whereas wetlands receiving low inlet nutrient concentrations appeared to have an increased concentration of TP at the outlet, during this investigation. There were no other associations between flow rates or inlet nutrient concentrations.

In general, TP concentrations in S1 were higher than inlet concentrations and there was a drop in TP from S1 to S2 for all except Unit 5, with a lower planting density of *Phragmites* in S1 (9 plants per m\(^2\) instead of 16 plants per m\(^2\)). However, the relative change in TP concentration from inlet to S2 was similar for all wetlands. In S2, changes in phosphorus fractions were mixed where chlorophyll or pheophytin decreased (Units 2 and 3) but the majority of phosphorus fractions decreased in concentration if chlorophyll or pheophytin concentrations increased (in Unit 5 TP also increased). Chlorophyll, pheophytin and DO increased in S3 for all wetland Units except Unit 2. In wetland Units receiving high flows TP was removed, but released in low flow units (Unit 3 and Unit 4).

In S4, pheophytin and chlorophyll decreased in all Units. The concentration of all P fractions increased in Units 2, 3 and 5 (except for TP in Unit 5). All TP fractions decreased in Unit 4 between S4 and S3. In S5, if pheophytin increased then TP concentrations (and in Unit 2 concentrations of all other fractions) increased. If pheophytin remained constant in S5, then concentrations of phosphorus decreased in all size fractions within S5.
Table 3-6: Summary of consistent and key observations across each wetland sector for pilot plant wetland Units 2, 3, 4 and 5.

<table>
<thead>
<tr>
<th>Sector</th>
<th>Unit 2</th>
<th>Unit 3</th>
<th>Unit 4</th>
<th>Unit 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall wetland performance (relative to inlet)</td>
<td>Phosphorus removed from all stands but S1.</td>
<td>Phosphorus release in all stands but S7 on occasion.</td>
<td>Phosphorus released from all stands except S7 on occasion.</td>
<td>Consistent phosphorus removal from all stands except S2 and S3.</td>
</tr>
<tr>
<td>1</td>
<td>TP &gt; inlet, TP release from detritus suspected.</td>
<td>TP &gt; inlet, TP release from detritus suspected.</td>
<td>TP &gt; inlet, TP release from detritus suspected.</td>
<td>TP &lt; inlet, S1 has lower planting density than other Units.</td>
</tr>
<tr>
<td>2</td>
<td>Slight drop in TP from S1, various responses from all other phosphorus fractions. Decrease in chlorophyll a and pheophytin, increase in DO.</td>
<td>Slight drop in TP from S1, various responses from all other phosphorus fractions. Decrease in chlorophyll a and pheophytin.</td>
<td>Slight drop in all phosphorus fractions from S1. Increase in chlorophyll a and pheophytin.</td>
<td>Slight increase in TP but a drop in all other phosphorus fractions from S1. Increase in chlorophyll a, pheophytin and DO.</td>
</tr>
<tr>
<td>3</td>
<td>Slight drop in all phosphorus fractions except 0.2 μm. Decrease in chlorophyll a and pheophytin from S1 to S3 (although levels were high relative to other wetlands). Elevated DO.</td>
<td>Increase in all phosphorus fractions, associated with increased chlorophyll a and pheophytin. Elevated DO.</td>
<td>Increase in TP, drop in other phosphorus fractions, associated with increased chlorophyll a and pheophytin. Elevated DO.</td>
<td>Decrease in all phosphorus fractions relative to S2 and inlet. Increase observed in chlorophyll a and pheophytin.</td>
</tr>
<tr>
<td>4</td>
<td>Slight increase in all phosphorus fractions, decreases in chlorophyll a, and increase in pheophytin.</td>
<td>Increase in all phosphorus fractions, decrease in chlorophyll a and pheophytin. Consistent DO.</td>
<td>Decrease in all phosphorus fractions, decrease in chlorophyll a and pheophytin.</td>
<td>Decrease in TP but increase in all other phosphorus fractions, decrease in chlorophyll a and pheophytin. Elevated DO.</td>
</tr>
<tr>
<td>5</td>
<td>Decrease in all phosphorus fractions. Consistent chlorophyll a and pheophytin.</td>
<td>Increase in all phosphorus fractions, TP greatest. Low chlorophyll a, elevated pheophytin. Consistent DO.</td>
<td>Decrease in all fractions. Low chlorophyll a and consistent pheophytin. Slight decrease in DO.</td>
<td>Increase in TP, decrease in all other fractions. Large increase in chlorophyll a, pheophytin and DO. Lowest TC.</td>
</tr>
<tr>
<td>6</td>
<td>Increase TP, decrease OP but no change other fractions. Increase in chlorophyll a and pheophytin</td>
<td>Increase in all phosphorus fractions overall. Increase in chlorophyll a, consistent pheophytin. Decrease in DO.</td>
<td>Decrease in all fractions. Low chlorophyll a and increase in pheophytin. Slight decrease in DO.</td>
<td>Decrease in TP, increase of all other phosphorus fractions. Drop in chlorophyll a and pheophytin.</td>
</tr>
<tr>
<td>7</td>
<td>Decrease in all phosphorus fractions except OP. Decrease in chlorophyll a and pheophytin.</td>
<td>Increase in all phosphorus fractions with the exception of OP. Consistent chlorophyll a, and pheophytin. Low DO</td>
<td>Decrease in all phosphorus fractions. Low chlorophyll a and decrease in pheophytin. Slight decrease in DO.</td>
<td>Increase in TP, decrease in all other phosphorus fractions. Consistent chlorophyll a, large increase in pheophytin.</td>
</tr>
</tbody>
</table>
In S6, a mixed set of results was observed with no clear trend apparent. In S7, phosphorus concentrations decreased if there was a decline in chlorophyll or pheophytin concentrations.

3.5 DISCUSSION

3.5.1 Inlet variations
Observation of the variability of inlet TP concentrations indicated no significant difference in readings taken at four hourly intervals, relative to shorter time intervals for the pilot plant wetlands at Richmond. Sampling at time intervals as low as 30 minutes did not significantly increase the precision of phosphorus measurements and there was no significant difference between the samples obtained for a given run. However, there was a significant difference when comparison was made between runs, indicating that phosphorus concentrations underwent significant change in the longer term. Refer to the two-year study of pilot plant nutrient concentrations (Appendix 2, Figures 2-A to 2-E. ‘TP in’ on the graphs) for an indication of the extent of this change. These figures highlighted the need for four hourly composite sampling. Thus samples were taken every hour from water entering each wetland and composited into four hourly batches to smooth out any fluctuations. Composite samples were taken to simulate mixing, which should occur as the effluent entered a wetland.

Monitoring of SS concentrations indicated that they fluctuated widely and independently of phosphorus concentrations in both secondary treated and alum-dosed effluent. The high variance of SS concentrations over short time intervals meant it would be difficult to discern whether changes in SS concentrations observed within a wetland were due to inlet fluctuations or internal wetland processes. Therefore, caution was needed in the interpretation of SS concentrations measured within each wetland.

Possible sources of the inlet variations in SS could have been algal biomass from the secondary pond, sloughing of biofilm from the interior of the pipes into the wetland,
or abiological material from the treatment plant. Alum dosing did not decrease the average concentration of incident SS and so was not a successful strategy for removing SS at the levels present in secondary treated effluent.

3.5.2 Detailed examination of sectors within each wetland

Unit 2 - High phosphorus, high flow

Unit 2 was the only wetland unit which evidenced consistent inlet TP concentrations throughout the entire period of investigation, removing an average of 5% TP from the water column between inlet and outlet. Note however that TP concentrations at the outlet were less consistent than the inlet, fluctuating randomly. Generally, TP was removed with distance travelled through the wetland.

The principle size fractions of phosphorus that were removed most consistently from the water column between inlet and outlet were TP (i.e. particles greater than 1.2μm) and FP. Smaller size fractions of phosphorus did not evidence consistent trends for concentration changes. Changes in phosphorus size fractions appeared unrelated to plant type, unless associated with the sequence of plant species through the wetland (See below).

Phosphorus release to the water column

Chlorophyll a and pheophytin evidenced a high correlation with FP and TP, whilst other physical chemical parameters did not correspond well with any phosphorus size fractions. This correlation would lead to a tentative hypothesis that phosphorus removal and release were associated with algal and floating plant biomass, or plant detrital material. Removal of this material (either live or dead) from the water column, and the likely adsorption of its phosphorus to biofilm or sediment may have caused the observed drop in TP concentrations through Unit 2. In all sections of the Unit 2, pheophytin concentrations were higher than chlorophyll a, suggesting that the larger portion of biomass was dead.

The elevation in TP concentrations relative to other phosphorus fractions in S1 was accompanied by slightly higher pH (0.21 units higher than S2), conductivity,
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chlorophyll a and pheophytin compared to the remainder of the wetland. SS concentrations were elevated but not significantly so. The combination of these factors indicated a slightly alkaline environment with elevated concentrations of inorganic ions and the presence of material containing chlorophyll a. If the increase in chlorophyll a was influenced by algal or floating plant biomass there would need to be growth of algae or floating plants within S1. The most likely source of any algal material was the inlet pipe because of the inability of light to penetrate the water surface in S1. Support for the absence of light can be derived from pheophytin readings, which indicated that a large proportion of the plant material was dead. The alternative would be that the chlorophyll and pheophytin readings were from dead and newly fallen Phragmites litter. Some of this material would be fragile enough to slough off during sampling. This material would also contain a proportion of phosphorus, accounting for the elevated phosphorus concentrations in this region and its apparent correlation with chlorophyll and pheophytin. Further discussion of these factors is provided in Chapters 4, 5, 6 and 8.

Support for a detrital based pheophytin and phosphorus source was given by the lower DO concentrations in S1 relative to the S2 and S3. The biodegradation of plant carbon is an energy expensive process and the most efficient electron donor for the reaction is oxygen. Two secondary reactions that typically occur in conjunction with oxygen removal are a decrease in redox potential and changes in pH. Redox declines directly as a result of oxygen consumption, which lowers the oxidation potential of the environment (Refer Section 1.3.4). Changes in pH occur because of the production of organic acids from oxidised organic material or the formation of ammonia and other inorganic compounds (Section 1.3.3). Redox within the wetland was not determined but pH values at S1 were elevated compared to downstream regions. Observations of average pH values entering Unit 2 (Section 3.5.1) indicated that the pH in S1 was slightly elevated, relative to the mean. This suggested that some form of chemical reaction was occurring within S1.

The increase in TP within S4 and S6 occurred slightly differently to S1. Concentrations of chlorophyll a, pheophytin and conductivity did not differ
significantly from adjacent plant stands, and concentrations of SS material did not correlate with the observed phosphorus increases. DO concentrations at both sites however, did follow the trend of S1 and were lower than the surrounding regions. Note that the dominant phosphorus fractions that altered between stands S3/S4 and S5/S6 were TP and 0.1μm. Phosphorus removal occurred slightly differently between S4 and S6, with TP, FP and 0.1μm fractions increasing in S4 (relative to S3), but in S6, TP increased, FP remained stable (relative to S5), and the 0.1μm fraction decreased. Note that fluctuations in the 0.1μm fraction were not significantly carried through into higher fractions (Figure 3.1) and hence can be discounted in influencing TP concentrations. Variations in FP concentration were higher in S6 relative to S5 and S4. The increase in TP in S4 and S6 was difficult to explain, the only consistent factor was that both stands were preceded by a stand of Triglochin.

Phosphorus removal from the water column

Removal of TP from the water column was greatest immediately proceeding a region of high TP release to the water column (S2, S5, S7). TP removal at these points ranged from 0.3 to 0.4 mg.L⁻¹ (relative to the previous plant stand). As with TP release, the removal of TP occurred slightly differently in each stand, most noticeably between S2 compared to S5 and S7. In S2, removal appeared driven by a reduction in TP concentration, whilst the concentrations of other phosphorus size fractions increased. The most obvious reason for TP removal was the setting and subsequent degradation of biotic SS material. This is supported by a reduction in average SS concentrations at S2 compared to S1. The settled particles may have been dead algal and floating plant biomass or detritus because of the high pheophytin concentrations observed in the water column. The increase in other phosphorus size fractions could have been from the degradation of settled material. A proportion of this material would also have been adsorbed to detritus, plants or sediment, resulting in a net decrease in TP.

Whilst a similar mechanism may have operated from the water column within S5 and S7, it occurred under different physico-chemical conditions coincident with removal of other phosphorus size fractions. The decline in TP was accompanied by reductions
in phosphorus concentrations in the 1.2 to 0.2 μm fractions, although the proportion in each fraction varied slightly. The decline in phosphorus concentrations within S5 correlated with SS and may have been from particle settling. The reduction in the phosphorus concentration of other particle size fractions could be due to adsorption to plant, sediment or detritus. The absence of any significant decrease in the 0.1 μm phosphorus fraction would imply that colloidal phosphorus was not simply being adsorbed but that larger particles were settling or adsorbing to surfaces. The reduction in the 0.45 μm fraction at S7 indicated this material might have been algal or bacterial. The reduction in chlorophyll a and pheophytin support this hypothesis. The correlation of TP reduction and DO concentrations in S5 further highlight that degradation and transfer of phosphorus between various size fractions may have been occurring. In S7, the absence of such a correlation would imply that settling and binding were dominant mechanism i.e. in S5 there was more likely degradation of detrital material, with commensurate phosphorus release, while at S7 the main removal mechanism may have been direct settling and binding. A number of wetlands need to be tested to confirm whether these mechanisms were operating, which mechanisms were more effective and the factors that induced them.

In this study, phosphorus removal was associated with either removal of TP, FP or changes in several different phosphorus size fractions. Removal rates were highest when conversion or involvement of multiple phosphorus fractions was observed. Such changes imply that phosphorus is being converted or transferred between fractions by metabolism or chemical reaction, ultimately resulting in the adsorption or sequestering of the smallest phosphorus fraction. It is this process, rather than what appeared to be a simple settling process, that was more effective in phosphorus removal within Unit 2.

Unit 3 - Low phosphorus, high flow

Inlet/outlet TP

Unit 3 received alum-dosed sewage effluent and had a theoretical retention time of 5 days. Comparison of inlet and outlet TP concentrations for Unit 3 indicated that there was a net release to the water column (30%) within the wetland. Inlet TP
concentrations were more variable than Unit 2 or Unit 3 outlet. TP concentrations appeared to rise from S1 to S4, decreasing from there to the outlet. Other phosphorus size fractions tended to decrease in concentration through the wetland except within S4, where there was a significant increase in the concentration of phosphorus in all size fractions.

Actual TP concentrations within Unit 3 were lower than the theoretical for all plant stands except S7. One explanation for the large difference between the expected and actual TP concentrations was the theoretical retention time. Since the theoretical retention time of the wetland was most likely longer than 5 days (Chapter 2) then it is possible that not all of the inlet phosphorus variations were observed during the period of inlet monitoring which only occurred for 5 days prior to sampling. Alternatively, there could have been a high TP spike entering the wetland prior to analysis or between the hourly sampling intervals (considered unlikely). Since monitoring only occurred for a single theoretical retention time, it was not possible to discern the true cause for these occurrences.

Even allowing for these factors, TP was released to the water column within Unit 3 during the period of investigation. However, actual zones of release or removal were difficult to determine. There was a reduction in phosphorus concentrations within Unit 3, from S4 to S7, which may have been due to internal removal processes, variations at the inlet or an artefact of mixing processes within the wetland. Further investigations were conducted using tracers to determine flow paths and retention times, in an attempt to separate these factors (Chapter 4).

Phosphorus fractions

Despite the complications described, it was possible to determine some associations that were occurring in Unit 3 regarding various phosphorus size fractions. In S4, there was a convergence of the 0.45 and 0.2 μm phosphorus fractions, which was joined by the 0.1 μm fraction at S5 and continued to the outlet. As mentioned, the five observed phosphorus fractions formed essentially two distinct groups: Particles greater than or equal to 1.2 μm, and particles less than 1.2 μm.
Particles that were less than 1.2μm in size converged between S4 and S5, coupled with a continual decline in concentrations from S5 to S7. Convergence suggested that phosphorus removal in this region of the wetland may have been associated with colloidal or ortho-phosphorus fractions. Between S1 and S3 it appeared removal of colloidal phosphorus was occurring but this was offset by changes in TP, which increased between S2 and S4. Note that at S4 the concentration of all phosphorus fractions increased, in reverse to the trend seen in previous sectors. This was accompanied by high concentrations of chlorophyll a and pheophytin in S3 followed by very low concentrations of these parameters in S4. Suggesting that phosphorus concentrations in this region of the wetland may be associated with plant or algal material, which settled on entering the densely planted area of Phragmites in S4.

Other parameters
There was a general lack of correlation between the measured physico-chemical parameters and phosphorus concentrations. Changes observed in pH, temperature, pheophytin, chlorophyll a and DO did not correlate with phosphorus concentrations through the wetland and it was unlikely that they were directly related to phosphorus cycling at the time of observation. The lower DO concentrations in plant stands adjacent the inlet and outlet would indicate a higher level of oxygen consumption in these regions, possibly from biological degradation of organic material. The increased concentrations of SS in these regions could be from the degradation of detrital material, the release of material from the sediment or biomass growth. Conductivity evidenced random fluctuations, as did TN and TC, negating links with phosphorus removal.

Unit 4 - Low phosphorus, low flow

Inlet/outlet TP
Unit 4 was the second wetland receiving alum-dosed effluent, with a theoretical retention time of 15 days. There was a net release of phosphorus to the water column passing through Unit 4, with the average phosphorus concentration released being 0.16 mg.L⁻¹ (approximately 12%). Phosphorus release did not occur uniformly
through the wetland. On entering the wetland TP concentrations became elevated relative to the inlet, remaining high until the second Phragmites stand (S4) where TP was consistently removed from the water column for all samples collected. The TP concentration then increased slightly through stands S5 to S7, consistent with inlet variations. As in Unit 2, TP concentrations at the outlet were more variable than the inlet.

phosphorus fractions

An interesting and unusual phenomenon was observed with all phosphorus fractions within Unit 4. Unlike all other Units, there was a decrease in TP concentrations from inlet to S4 (which had the lowest TP concentrations in this Unit), increasing again towards the outlet. Similar to Unit 3, the correlation of various phosphorus size fractions formed two distinct distributions: particles 1.2 μm or larger (TP) and particles smaller than 1.2 μm.

As in Units 2 and 3, TP in Unit 4 increased between the inlet and S1. In Unit 4, this was coupled with a high standard deviation relative to other phosphorus fractions, implying that TP concentrations were changing across the stand (most obviously caused by a decrease in the inlet TP concentration). As mentioned for Unit 2, this may have been from incident allochthonous material, litter decomposition, sedimentary release of orthophosphate or sloughing of biofilm. The decrease in phosphorus concentrations in S2 was primarily from a reduction in colloidal and orthophosphorus (the phosphorus fraction smaller than 0.2 μm), rather than TP. This would imply definite removal of TP via adsorption and chemical interaction. S3 evidenced a higher TP concentration that S2, coupled with a decrease in the concentrations of smaller size phosphorus fractions, suggesting metabolism or assimilation of phosphorus.

S4 experienced the lowest TP concentrations. Within S4, the majority of TP was removed from the water column, accompanied by a slight decrease in other size phosphorus fractions (less so than within S2). The low standard deviation present within S4 indicated that phosphorus removal most likely occurred at the upstream
boundary between *Triglochin* (S3) and *Phragmites* (S4). The reduction in phosphorus concentration at this point was primarily from the removal of particles larger than 1.2 μm from the water column and averaged 1.2 mg.L\(^{-1}\).

Phosphorus associated with other particle sizes evidenced only a slight decline from S3 to S4 (≤ 0.3 mg.L\(^{-1}\)). After this point, the concentration of TP gradually increased towards the outlet. The increase was initially driven by a rise in the size fraction greater than 1.2 μm before slowing and being moved up by increases in the 0.2 μm phosphorus fraction. The higher standard deviations observed in S5 and S6 suggested that these stands were in transition and that changes in phosphorus concentration were occurring across each stand.

Other parameters

There was variation of physico-chemical parameters within Unit 4. Whilst there was little correlation of parameters with phosphorus fractions, they assist in explaining variations at specific sites. At S3, TP concentrations were elevated relative to other phosphorus fractions, correlating with elevated temperature, DO, chlorophyll a, pheophytin, SS, TN and TC in this region. The occurrence of increased concentrations of these parameters would indicate that algae or floating plants might have been involved in increasing the concentration of TP in the water column through this plant stand. Although chlorophyll a and pheophytin were also elevated in S5, concentrations were an order of magnitude lower and so did not have the same impact on TP. Note however, that TP concentrations were elevated within S5, relative to other phosphorus fractions. Chlorophyll a, pheophytin, TN and TC concentrations implied that the increase in TP may have been due to a biotic source of phosphorus. DO concentrations in the water column were almost at saturation point through S3, implying abundant algal or floating plant growth. The lower DO concentrations in S5 were consistent with lower chlorophyll readings and algal/floating plant growth in this plant stand.

The drop in chlorophyll a and pheophytin and DO within S4, coupled with their low standard deviations would indicate that only a limited number of algae or floating
plants were able to penetrate this stand of plants. The low standard deviations for these results implied that removal probably occurred near the boundary of the two stands and not across S4. This would also explain the rapid removal of phosphorus entering S4. As the larger particulate matter of algal/plant biomass and other material settled in S4 it may have also bound or attracted smaller particles, inducing the decreases observed in other particle size fractions. Plant density as well as species could have contributed to the removal of algal or floating plant material.

The low DO concentrations within S4 would indicate that degradation of organic material was probably occurring within this stand. However, there was not a discernable increase in orthophosphate or other phosphorus fractions. The low TP in S4 may have been an isolated occurrence, indicating the binding of all material to the sediment or other surfaces. Alternatively, it may have been attributable to the height of the sampler tube or short-circuiting through specific channels. The theory being that if material was saltating along the sediment surface then the height of the sample tube may have prevented this material from being sampled (although this would be the only wetland sector in which this occurred). The relatively high pheophytin readings and low chlorophyll readings would imply any algal or plant material present was dead but not lysed. Chapter 4 looks at short-circuiting and phosphorus levels throughout Unit 4 in more detail.

The increase in TP through S5 correlated with an increase in algal or floating plant biomass. At this point, an increase in SS, chlorophyll a, pheophytin, TC, DO and the phosphorus fraction greater than 1.2 μm were indicative of increased floating plant or algal biomass. An increase in bacterial biomass would have involved a reduction of DO without changes in chlorophyll a and pheophytin. In the final portion of the wetland, the increase in TP may have been associated with changes in inlet concentration and mixing processes with the wetland.

In S7, increases in TP concentration were related to increases in the concentration of intermediate phosphorus size fractions. Through this sector, the average concentration of orthophosphate (the size fraction < 0.1μm) decreased slightly, while the
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concentration of all other phosphorus fractions increased. The reason intermediate size fractions of phosphorus were released to the water column within this stand was unclear, although concentrations of pheophytin were elevated but decreasing relative to S6, possibly implying breakdown of algal or plant detritus.

The high standard deviation through S6 indicated that phosphorus might have been released to the water column in S6 as well as in S7. In which case, the increase in some of the intermediate size fractions of phosphorus through S7 may have been from the degradation of detrital material present in S6. Some support for these hypotheses is seen from the lower DO readings obtained in S6 and S7.

Unit 5 - High phosphorus, low flow

Inlet/outlet TP
There was net removal of phosphorus from the water column of Unit 5 (approximately 1.3%) throughout the period of investigation. The variability of TP concentrations at the outlet was greater than the inlet over the time of analysis. The observed inlet and outlet TP variability observed for this wetland was random. Variations were not consistent with either phosphorus concentration or hydraulic retention time.

Phosphorus fractions
Unlike the other wetlands, the concentration of TP through S1 was not higher than the inlet concentration. This may have been from the lower plant density in this region relative to other wetland units (9 plants per m² compared to 16 plants per m²). The lower plant density would have resulted in a lower amount of litter deposition throughout the stand (Section 5.2). If litter deposition were the main difference then litter decomposition may be causing higher phosphorus concentrations in the water column through the release of particles to the water column. Alternatively, the stems of the emergent *Phragmites* may have had a retarding effect on any allochthanoous material entering each wetland, facilitating settling to the sediment surface. The lower DO concentrations in S1 relative to S2 may indicate degradation of organic material.
Further investigations were conducted in Chapter 5 to more conclusively determine the role of detrital and settled material in phosphorus cycling.

Similar to Units 3 and 4, phosphorus size fractions in Unit 5 could be divided into two correlation groups: TP, and all smaller fractions. Phosphorus size fractions smaller than 1.2 μm were well correlated, and showed a different removal pattern to TP. Relative to the inlet, TP was removed from the water column in all plant stands, except for S2 and S5. Allowing for increases through these stands and fluctuations associated with the inlet supply, the removal TP from the water column was gradual and almost linear through Unit 5. Phosphorus particles smaller than 1.2 μm showed a similar trend, but with increases in concentration through S4 and S6, immediately after open water stands planted with Triglochin.

In S4 TP decreased (relative to S3), with a concurrent rise in phosphorus concentration within each of the fractions smaller than 1.2 μm, as observed in all other wetland Units.

In the last four plant stands of Unit 5, TP concentrations were inversely correlated with all other phosphorus fractions. This may have been associated with the incorporation of TP into floating biomass in regions of lower shading from emergent plants (S5 and S7) and the settling and/or degradation of this material in more densely planted regions of the wetland.

Other parameters
TP increases (relative to adjacent plant stands) observed in S5 and S7 were accompanied by increases in chlorophyll a and SS, while decreases in TP through S4 and S6 were accompanied by declines in chlorophyll a and SS. Providing supporting evidence that changes in the concentration of TP through the wetland may have been associated with incorporation of phosphorus into algal or floating plant biomass.

Similar correlations were observed through S2 where a rise in TP (relative to S1) was associated with an increase in chlorophyll a and SS concentrations. It is possible that the slightly lower plant density in this wetland (9 plants per m² compared to 16 plants...
per m$^2$) may have allowed penetration of sunlight to the water surface in this stand of *Schoenoplectus*. However, in S3 the reduction in TP was only accompanied by a decrease in chlorophyll a but not SS. Implying that the association of SS may have been an isolated occurrence with the increase in SS observed in S2 possible attributable to other particles in the water column, disturbance of sediment by waterfowl or some other cause.

Despite internal cycling of phosphorus through Unit 5, there was a gradual decline in TP concentrations with distance. Where phosphorus removal was associated with a decrease in the 0.1μm fraction (S2 and S3) this was probably through adsorption of colloidal phosphorus to soil, plant or detrital material. However, through the remainder of the wetland it would appear that the decline in TP was from the removal of particles larger than 1.2 μm from the water column, possibly bound in algae, floating plants or other SS material.

Reductions in TP from S3 to S7 occurred in plant stands that had a dense canopy with only a low probability of light reaching the water surface (S4 and S6). The low standard deviation of TP concentrations in each of these stands implied that removal probably occurred at the edge of the stand because concentrations within the stand were relatively uniform. The lack of light penetration, plant density and the slow flow rates within these stands may have acted as barrier to the growth of algal or floating plant biomass. Reduced light penetration minimises the energy available for algal and floating plant growth; plant density and slow flow rate act to maximise settling and allow crude filtration of algae, floating plants and other SS material. Thus material entering S4 and S6 would appear to rapidly settle on the edge of the stand, allowing adsorption of phosphorus to sediment, plant and detrital material which were abundant within.

3.5.3 Comparison of wetland Units 2 through 5

Phosphorus release only occurred wetland Units receiving high concentrations of TP. TP release from wetland Units receiving low concentrations of TP may have been associated with inlet variations and short-circuiting within each wetland Unit. A more
detailed investigation of flow paths, concurrent with changes in inlet TP concentrations was undertaken to confirm this (Chapter 4).

For the majority of wetland Units, water in S1 gained phosphorus relative to the inlet. In Unit 5, where this did not occur the planting density in this stand was lower than in other Units. It was suspected that phosphorus was being released to the water column through the degradation of allochth nous algae, floating plants and possibly autochthonous detrital material in this region. The change in phosphorus fractions detected in this and the proceeding sector could indicate transfer between different fractions and some adsorption to sediment and possibly incorporation into emergent plant material.

Changes occurring from S1 to S2 appeared to be related to chlorophyll and pheophytin concentrations. When the concentrations of these two parameters decreased, then changes in phosphorus concentrations between the different size fractions were not consistent. This could be explained by a mixture of degradation – forming smaller fractions and other transformation processes eg incorporation into biomass. When chlorophyll and pheophytin increased the smaller size fractions of phosphorus were observed to decrease, implying possible uptake of orthophosphorus or more bioavailable phosphorus forms into biomass. In Unit 4, this was associated with a decrease in TP, suggesting possible settling or absorption of phosphorus-laden material. In Unit 5, the concentration of TP increased coupled with a much greater increase in chlorophyll and pheophytin than Unit 4, suggesting algal or floating plant growth may have been associated with this change, using mechanisms noted earlier.

In Sector 3 of each wetland, the submergent Triglochin appeared to allow significant growth of floating plant and/or algal biomass. In Units with a low phosphorus input, this resulted in elevated phosphorus concentrations in the water column. However, wetlands with a high phosphorus input did not have such a noticeable increase. A possible explanation was that floating plants and algae were only able to absorb a certain amount of phosphorus into their biomass. The net concentration of which was relatively consistent between wetlands. At a higher background phosphorus
concentration this amount might not have been significant compared to that found in the surrounding water. However, at lower phosphorus concentrations it appeared to be significantly greater than that detected in the surrounding water column, as seen in Units 3 and 4.

Note that in wetlands receiving higher nutrient concentrations the smaller size fractions of phosphorus continued to be removed from the water column through S3, consistent with the above observations. Hence, even though algae or plants were probably obtaining their nutrients directly from the water column they did not appear have a significant affect on absorption and sequestering processes in wetlands receiving high inlet concentrations of TP. However, in Units 3 and 4 algae and floating plants appeared to directly affect TP concentrations in the water column, particularly in S3 and to a lesser degree S5 and S7 (see below).

As water moved into the next sector (S4) concentrations of each size fraction of phosphorus generally increased. It is suspected that such an increase may have been due to the death of algal or floating plant material and the presence of decaying detritus within S4. The net decrease observed in phosphorus concentrations in Unit 4 contrasted sharply with the results for other wetland Units and may have been due to short-circuiting. This was investigated further in Chapter 4. The reason for the decrease in TP in S4 for Unit 5 is unknown. It may have been that degradation of the algal species was more rapid in this Unit, although this could not be confirmed.

Between S5 and S7, the processes relating to phosphorus release and uptake appeared to be influenced by the concentration of chlorophyll and pheophytin. As the concentration of these increased so did the overall phosphorus concentration in the water column. Whether this material was from decaying plants or algae was not determined in this investigation, but the relative contribution of detrital material to phosphorus concentrations was evaluated further in Chapter 5, and the contribution from algae in Chapter 6.
CHAPTER 3

3.6 CONCLUSIONS

3.6.1 Inlet variations
In the pilot plant wetlands, TP concentrations did not vary significantly over a 4-hour interval, and 4 x hourly composite samples provided representative sampling of inlet and outlet water quality. However, significant variation did occur in SS readings over the 4-hour period, preventing association of SS readings with values obtained either at the wetland outlet or within the wetland. Alum dosing did not significantly reduce SS concentrations or their variability.

3.6.2 Detailed examination of sectors within each wetland
Wetlands receiving secondary treated effluent reduced the concentration of TP between the inlet and outlet during the period of these investigations. Wetlands receiving alum-dosed effluent had a higher concentration of TP at the outlet relative to the inlet. Due to possible short-circuiting, mixing and fluctuations in inlet TP concentrations these results may not be an accurate reflection of actual wetland processes or the phosphorus removal and release characteristics of each wetland Unit. To address these issues, a more detailed investigation was required to characterise wetland flows and accurately correlate them with inlet phosphorus concentrations. This investigation is described in Chapter 4.

However, in the course of the current investigations it was possible to observe some of the internal functioning of each wetland. Through these observations there appeared to be some commonality in processes occurring within various pilot plant wetland Units. Overall, the initial or front four sectors of each Unit appeared to show a greater variety of interactions and patterns than the final 3 sectors. These variations were only indirectly related to plant species or inflow rates.

Processes within the initial four sectors of the wetland appeared to be a combination of settling, surface and sediment absorption, partial and full degradation of phosphorus compounds, and incorporation into biomass, all acting almost independently or antagonistically. This is believed to have resulted in a number of
marked differences between each wetland and making separation of individual mechanisms difficult. In the latter part of each Wetland Unit, these mechanisms occurred but seemed to be acting in synergy rather than antagonistically, with greater similarities observable between wetland Units.

In increase in TP between a number of wetland Sectors may have been related to the amount of litter deposited in each region and the plant density. Assessment of the effect of litter on phosphorus concentrations was therefore undertaken, the results of which are described in Chapter 5. In S1, elevated TP concentrations may have been related to the breakdown or fragmentation of detrital material (plant or algal) through this plant stand (Section 3.7.4). If plant litter was a significant phosphorus source or sink then phosphorus removal in this region could be expected to be seasonal. This hypothesis was further evaluated in Chapters 4 and 5.

Phosphorus removal and release in S2, S4 and the latter half of each wetland appeared to be associated with changes in the amounts of chlorophyll and pheophytin. However, no direct correlation could be observed along the length of each wetland between these parameters and TP. It was suspected that a change in the concentration of these compounds was also associated with changes to a number of other phosphorus size fractions, further preventing a direct correlation. The source of this pheophytin containing material was unclear, whether from rooted plants, algae or floating plants. In addition, although pheophytin and chlorophyll were measured it was unclear to what extent detrital material was involved in this process, as opposed to live plant or algal tissue. It is clear that algae or floating plants were likely to be involved in some of this process, particularly in S3 and S4. Characterisation of the contribution from algae was assessed in Chapter 6, while the contribution from detritus was assessed in Chapter 5. A summary of these results and a comparison with possible inputs from other sources is given in Chapter 8.

3.6.3 Physico-chemical parameters
Phosphorus concentrations did not directly correlate with any particular combination of physico-chemical parameters through each wetland unit. However, it was possible
to note that in certain open water sections (primarily S3, planted with *Triglochin*), there was a correlation of chlorophyll a, pheophytin, DO and occasionally SS with TP. The occurrence of high concentrations of these parameters often correlated with rising TP concentrations and lower concentrations of phosphorus fractions less than 1.2 μm, implying adsorption of phosphorus into algal or floating plant biomass. However, decreases in pheophytin, chlorophyll a and DO did not always correspond with a decrease in TP but rather, within S4 especially, changes in these parameters were accompanied by a shift from TP to other phosphorus forms, which were not adsorbed or removed in S4 but rather S5, suggesting degradation or transformation and then absorption.

3.6.4 Removal mechanisms

Two dominant mechanisms for removal of phosphorus from the water column appeared to be present in the pilot plant wetlands. Phosphorus was removed through from particles larger than 1.2 μm or smaller than 0.1 μm in each wetland Unit. The proposed mechanisms would be that particles larger than 1.2 μm settle from the water column when they encounter a region of slower flow i.e. dense stands of plants. Particles smaller than 0.1 μm were considered either dissolved orthophosphate or colloidal phosphorus. These phosphorus forms may interact directly with sediment and biofilm particles, binding through adsorption or able to be directly metabolised. Both mechanisms are effective but for long-term storage, particles need to penetrate deeper into the soil matrix, being bound to either soil particles or in plant root material.

Settled SS had the disadvantage that they could easily be resuspended into the water column, as could surface biofilms. Transfer of phosphorus from the soil to plant material was also less desirable if it the transfer was to the above ground plant components, where it might eventually senescence and be re-released to the water column as degraded detrital tissue. Investigations into the ability of phosphorus to penetrate deeper into the soil substrate are detailed in Chapter 7.
3.6.5 Flow

Retention time had a significant impact on the ability to interpret phosphorus removal data. The data generated and the predicted time of travel for inlet TP concentrations had a significant impact on whether phosphorus removal or release was deemed to have occurred within plant stands and at the wetland outlet. The variable nature of inlet TP concentrations meant that an accurate retention time was essential to allow meaningful interpretation of regions of phosphorus removal or release to the water column.

In addition to complications arising from the need to accurately measure the hydraulic retention time, there was the issue of short-circuiting. Throughout a number of the plant stands examined in this study, phosphorus concentrations in the water column showed a high standard deviation. These variations may have been from processes occurring within the plant stand, water short-circuiting through the wetland Segment or inlet variations. Since inlet variations and short-circuiting could not be prevented, it was proposed that a tracer be injected into the wetlands to determine the flow path through each unit and correlate this with phosphorus removal. This was the subject of Chapter 4.

From the results obtained, it appeared that the degradation of detrital material and formation of algal biomass had a significant impact on phosphorus concentrations within each wetland unit. The contribution of detrital material to phosphorus removal or release was not measured in this investigation. Changes in phosphorus concentrations, which may have been caused by algal or floating plant biomass, were at most 0.5 mg.L⁻¹ over the surface area (15-20 m²) of a plant stand. Further investigations were conducted to evaluate the relative contribution of detrital material to water column phosphorus concentrations. These are covered in Chapter 5. Investigations were also undertaken to better quantify the role of algae in phosphorus removal/release from sediments and the possibility of stimulating either process, these are detailed in Chapter 6. An overall summary, combining these findings is provided in Chapter 8.
CHAPTER 4: THE ROLE OF FLOW IN THE INTERPRETATION OF PHOSPHORUS REMOVAL DATA

4.1 INTRODUCTION

Chapters 2 and 3 highlighted significant issues with the interpretation of phosphorus removal results. Difficulties arose in determining whether changes in phosphorus concentration through each wetland or even stands of plants within each wetland Unit were due to changes in flow patterns, inlet concentrations or actual removal and release. To separate these factors a detailed investigation was undertaken of a single wetland Unit – Unit 4.

Unit 4 was selected for detailed analysis as it was receiving low concentrations of phosphorus at a low loading rate. It was suspected that if removal processes were occurring then they would be easiest to observe in this Unit. In addition, the results for Unit 4 had been equivocal prior to this investigation and it appeared that a number of different processes were occurring simultaneously throughout this wetland. Thus, it was thought that investigation of this wetland would provide the greatest chance for observing and being able to differentiate between the various contributing factors for changes in phosphorus concentration in the pilot plant wetland Units.

The investigations described in this Chapter were designed to discriminate the role of flow paths, variable inlet phosphorus concentrations and hydraulic retention time in influencing phosphorus-sequestering processes. In undertaking this work an inert tracer – Bromide was used to evaluate flow paths, time of travel and observe changes in phosphorus concentrations due to dilution, dispersion, mixing and variations in inlet concentration. Concurrent determinations of tracer movement with phosphorus concentration were planned to allow accurate assessment of these processes.

By evaluating the flow path, time of travel and mixing for bromide, it should then be possible to apply this information to calculating the average time of travel for phosphorus through the wetland. Understanding and measuring these parameters should enable accurate determination of regions where phosphorus was being either...
removed from or released to, the water column. If such regions could be consistently
determined, it should be possible to identify causative factors and evaluate their
potential for performance prediction or control. Selected parameters could then be
altered to optimise phosphorus removal or release, and maximise wetland
performance.

4.2 AIM

The aim of these investigations were to:

- Evaluate the effectiveness of using a bromide tracer to characterise flow; and
- Through this analysis, assess phosphorus removal through Unit 4 of the pilot plant
  wetland units at Richmond.

Definitions used in this Chapter for flow and dispersion are set out below.

4.3 DEFINITIONS

4.3.1 Flow

The volume of water passing through a known cross sectional area in a given time
interval is defined as the flow (Rutherford 1994) and is expressed in m$^3$.s$^{-1}$. Flow can
be measured by flow meter, neutrally buoyant markers or a tracer. Flow meters are
commonly used for evaluating flows greater than 0.8 m$^3$.s$^{-1}$. For slower flows,
eutrally buoyant markers, tracers or sonic techniques are more effective (Standards
Australia 1991b). Neutrally buoyant markers are able to float with a current,
estimating the mean stream velocity (the time taken for a marker to travel a known
distance). By multiplying the velocity by the cross sectional area (at 90 degrees to the
direction of flow) it is possible to determine the flow between points. Markers are of
limited use in a wetland, as they require an unobstructed flow path, free of vegetation
or rocks in which they tend to become entangled.

The injection of a tracer into a moving body of water is referred to as dilution gauging
(Standards Australia 1991a), and was the method used for flow measurement in Unit
4. A soluble tracer was used to overcome the limitations of physical markers.
Solubility also allowed the tracer to be used in the measurement of dispersion. It is the
method of choice for slow shallow flow with many obstructions (Herschy 1995). Velocity is calculated from time interval between initial tracer injection and detection of the median tracer concentration (see Section 4.3.3 below).

4.3.2 Hydraulic residence time and hydraulic efficiency
The theoretical hydraulic residence time (HRT) is the time required to completely replace the entire volume of water in a wetland at the current inlet flow rate. Actual HRT is obtained by physically measuring the average flow within the wetland using a tracer or other method and dividing this by the volume of the wetland. An ideal flow occurs when the water entering a wetland moves as a uniform plug to the outlet, causing the theoretical HRT to equal the actual HRT.

The ratio of the actual HRT to the theoretical HRT is termed the Hydraulic efficiency (HE). For an ideal flow the HE equals 1 i.e. the theoretical HRT equals the actual HRT, and the flow moves as a uniform front through the wetland, from inlet to outlet. In a wetland, the actual HRT is typically less than the theoretical HRT due to short-circuiting (Kadlec 1997). Short-circuiting occurs because water follows the path of least resistance. As obstructions arise within a wetland, water flow becomes channelled, causing it to bypass certain regions of the wetland in favour of the path of least resistance. This results in a low contact time of water in some parts of the wetland and allows the development of dead zones with little or no flow.

Regions with low contact times (dead zones) do not allow effective interaction of the bulk flow with sediment, plants, detritus or biofilms. Dead zones do not permit effective delivery of nutrients, allowing stagnation. Low HE ratios indicate the presence of both short-circuiting and dead zones. The most efficient flow of water for maximizing overall contact time is achieved as the HE approaches unity (Kadlec 1996, DLWC 1998).

4.3.3 Tracer injection
There are two methods by which a tracer can be introduced into a flow, continuous and sudden injection. Continuous injection is the long-term introduction of tracer into
the flow at low concentration. Sudden injection involves the introduction tracer to inlet flow at a high concentration over a period of a few minutes (Standards Australia 1991a). The sudden injection method was preferred over continuous injection because of the shorter sampling duration, the lack of complicated injection equipment (injection was by steady emptying of a flask of tracer solution) and the added ability to detect dispersion. The brief injection period also reduced the time for total elution, shortening the sampling duration.

When undertaking the sudden injection method the volume and mass of tracer must be known accurately (Standards Australia 1991a; Standards Australia 1991b). Tracer selection must be done with care to avoid problems of environmental toxicity at the concentrations used. Injection must be slow enough to prevent the tracer from partitioning from the inlet water flow due to differences in density. Such partitioning may delay tracer detection, resulting in an underestimation of flow.

**Tracer selection**

Several types of tracer were available for dilution gauging: salts, fluorescent dyes and radioisotopes. Each type of tracer had its own unique limitations. Fluorescent dyes suffer significantly from photodecay, β emitting radioisotopes such as phosphorus are unable to be introduced into the environment, and salts may need to be introduced at high concentrations to allow downstream detection (Standards Australia 1991a).

Standards Australia (1991a) lists the following as commonly used tracers: sodium chloride, chromium, iodide, and lithium. The Standard also notes that “Bromide and fluoride are satisfactory but are not in common use”. Sodium chloride, fluoride and chromium were not considered because of the potential adverse impact on the wetland biota. Iodine has the potential for considerable tracer losses by absorption when significant amounts of organic material are present e.g. plant litter. This left lithium and bromide as suitable tracer candidates.

Tracer selection in wetlands is complicated by significant adsorption affects, background colour (due to such factors as humic material and iron based compounds),
and the chemical matrix of the water within the wetland. Tracer conservation and stability are essential, if the tracer interacts with material within the wetland then its passage may be unduly slowed, underestimating the flow of water or it may take a longer time to elute, making detection of low concentrations difficult, affecting mass balance calculations. Tracer losses occur when the tracer is photochemically affected by sunlight, adsorbed to surfaces or chemically interacts with material within the wetland. The chemical salt Bromide was chosen for use in the pilot plant wetlands because of its chemical stability and extremely low sorptive properties (Behrens 1983), along with its favourable conservation relative to other salts such as Lithium (Netter 1994). Using comparative studies of soil filters, Netter et al. (1990) indicated that recovery of bromide could be almost twice that of lithium.

4.3.4 Tracer movement
Injection of tracer using the sudden injection method typically occurs from a point source. At the pilot plant wetlands in Richmond, it was possible to introduce the tracer directly into the inlet flow. Water entering the wetland then passed through an inlet structure (Figure 2-1). From this point source, the tracer should begin to disperse in all directions (Figure 4-1, dispersion and the factors which cause uneven dispersion are more fully described in Section 4.3.4). Since the tracer could be injected with the inlet flow, mixing was expected to be instantaneous. However, this needed to be confirmed, because typically when the sudden injection method is used, dispersion does not occur instantaneously but at a certain distance of travel downstream. It is only once the tracer has become fully dispersed that it is possible to effectively measure the velocity of flow.
Since it is doubtful that a wetland will have conditions of ideal flow, the tracer (and also TP) is likely to spread out as noted in Figure 4-1: non-ideal flow, producing a tracer 'cloud' rather than a 'plug' (Netter et al. 1990). Note that this dispersion pattern has been idealised, as in reality the dispersion cloud may only flow down one side or even through only the central region of the wetland depending on the flow path through the wetland. Hence, several observation points across the flow are needed to determine the pattern and rate of water movement through each given cross section.

Plotting the concentration of bromide flowing past a single observation point over time should produce a curve similar to that of Figure 4-2. The time to initial tracer detection was the time for the fastest moving particles to reach a point and was defined as the minimum time of travel (Figure 4-2). Conversely, the maximum time of travel was the time taken for the last residue of tracer to pass a given sampling point (Figure 4-2). The apex of the curve determined the time of travel for the maximum concentration of tracer (Figure 4-2).

The median time of travel was the point equal to half the total concentration of bromide (as determined by *in situ* measurement). In theory, the median time of travel divided by the distance travelled should equal the mean velocity of the water column, irrespective of dispersion (Chadwick *et al.* 1993; Netter 1994). Wetland Unit 4 at Richmond had a depth of 0.2 m and a width of 5 m, giving a constant cross sectional area, simplifying the calculation of flow.
The geometric mean (Figure 4-2) represented the movement of 50% of the mass of injected bromide past a given point. The time for full bromide elution, when coupled with the time to initial and peak bromide concentration (Figure 4-2) was used to evaluate dispersion and was used to calculate the dispersion coefficient for the wetland (Section 4.3.5).

Figure 4-2: Expected distribution of bromide tracer, compared to ideal flow conditions, highlighting parameters important for data interpretation (adopted from Netter et al. 1990).

![Diagram showing plug flow and normal flow](image)

<table>
<thead>
<tr>
<th>Key:</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>Exact time tracer was injected into the wetland.</td>
</tr>
<tr>
<td>Tmin</td>
<td>Time from injection to when the tracer was initially detected.</td>
</tr>
<tr>
<td>P</td>
<td>Time from injection to peak concentration.</td>
</tr>
<tr>
<td>Tav</td>
<td>Time from injection to the mean bromide concentration.</td>
</tr>
<tr>
<td>Tm</td>
<td>Time from injection to the median bromide concentration.</td>
</tr>
<tr>
<td>Tmax</td>
<td>Time from injection to when all of the tracer has exited the wetland.</td>
</tr>
</tbody>
</table>

### 4.3.5 Dispersion

As a tracer enters the wetland, a number of forces act upon it. These forces include the downstream current (bulk flow), turbulence and eddy currents. Bulk flow carries the tracer downstream. In the pilot plant wetlands, the rate of flow was directly
proportional to the average stream velocity. Turbulence and eddy currents were caused by shear stresses from the bulk flow interacting with the bottom, sides and other obstacles within the channel. Turbulence acted to increase mixing at the boundaries of the flow. Eddy currents are small secondary currents arising as the main flow moved past the sides of the wetland or internal obstructions such as plant stems and detritus.

Dispersion is the movement of tracer away from a point due to the above factors, random Brownian motion or the effects of a concentration gradient. Movement along a concentration gradient is described by Fick's Law, which states that rate of movement of a solute per unit area in a given time is proportional to the concentration gradient for that solute (Chadwick et al. 1993). Thus to establish an equilibrium, bromide will move from a region of high concentration to a region of low concentration. The greater the concentration difference the faster is the rate of movement.

**Dispersion and mixing on injection**

Dispersion can occur in the vertical, lateral or longitudinal direction. The shallow water depth of Unit 4 meant that mixing due to dispersion should have commenced in all three directions as tracer entered the wetland (Standards Australia 1991b). As detailed above the ability to add tracer directly to the inlet flow should have eliminated or significantly reduced the mixing zone. The shallow water depth in the wetlands meant that dispersion in the vertical direction should have occurred rapidly. Dispersion in the lateral direction was assessed by comparing three points perpendicular to the flow at the end of each wetland segment (Figure 4-3). It was expected that lateral dispersion should occur by the end of the first segment. Dispersion in the longitudinal direction should be the major factor influencing the distribution of the tracer cloud. The following equations by Yotsukura (Rutherford 1994) and Rutherford (1994) were used to determine the theoretical mixing distances.

Yotsukura (Rutherford 1994) gives the distance to complete mixing for a mid-stream discharge as
$L_m = 1.3U(H^2/B)$ \textit{Equation 4-1}

(This equation approaches it limits at a channel width of 5m).

$L_m$ = distance from the source to the zone where the discharge has been well mixed laterally in m.

$U$ = average stream velocity in ms$^{-1}$.

$B$ = average stream width in m.

$H$ = average stream depth in m.

The values of these parameters in Unit 4 were: average width 5 m; average depth 0.2 m; and average stream velocity 113 L.hr$^{-1}$, equivalent to $3.1 \times 10^{-2}$ m.s$^{-1}$. Therefore, the distance to complete lateral mixing $L_m$ should have been $3.2 \times 10^{-4}$ m from the inlet structure.

By the simple method of Rutherford (1994) the equation

$L_m = 0.057 HU$ \textit{Equation 4-2}

is used giving a mixing length of $4.2 \times 10^{-5}$ m. The more complex formula of Rutherford (1994) (derived from Prandtl’s mixing length formula for gases) is

$L_m \approx \beta U(H^2/Ey)$ \textit{Equation 4-3}

Where $\beta$ is a constant derived by the location of the source and degree of mixing (e.g, for a point source at mid-depth $\beta = 0.134$), $Ey$ = the depth averaged diffusivity (for this width and depth of channel $Ey$ would range between 0.2 and 0.4). Taking $\beta = 0.134$, $Ey$ as 0.2 (worst case) then $L_m \approx 8 \times 10^{-4}$ m (Equation 4-3).

**Lateral mixing**

Within the wetland, lateral mixing was evaluated by comparing the total concentration of bromide passing through the three sampling points comprising a given cross section (Figure 4-3, points a, b and c). Uniform lateral mixing was deemed to have occurred if the net concentration of bromide passing each point in a given transect was equivalent, after accounting for background noise (Rutherford 1994).
Longitudinal dispersion

Once the tracer has become mixed laterally and vertically, the two primary factors affecting its movement are longitudinal dispersion and bulk flow. Bulk flow affects the relative time of travel of the tracer - forcing the 'cloud' down the channel. Longitudinal dispersion is measured by the shape of the tracer cloud, as shown in Figure 4-1 and 4-2. It is the amount by which the tracer becomes spread with distance. Under ideal flow, the time for detection of such a tracer cloud should equal the injection period. Under conditions of non-ideal flow, the time for detection of the tracer cloud is much longer than the injection period due to wind induced mixing, edge effects and eddy currents. Interaction with plants, sediments and other surfaces within the wetland can further result in tracer absorption, reducing maximum tracer concentration and tracer recovery. However, the time to maximum peak concentration and the relative dispersion around each point should remain equal (Chadwick et al. 1993).

The dispersion of tracer past any given sample point in the wetland is given by the dispersion coefficient, which is calculated by the equation

\[ D = \frac{\sigma^2}{2P} \]  
(Rutherford 1994) 

\text{Equation 4.4}

Where \(D\) is the dispersion coefficient, \(\sigma^2\) is the variance and \(P\) is the time to detection of maximum tracer concentration.

4.3.6 Bromide recovery

The total mass of bromide recovered from water passing each detection point was measured at discrete intervals. The cross sectional area, flow rate and time interval between each sample were known. Multiplication of bromide concentration by the flow past each sample point for a known time interval enabled calculation of the mass of bromide passing that point. This could be done most effectively by plotting the mass of bromide passing a point against time and calculating the integral of the curve. To express this mathematically
Chapter 4

\[ m = CV = \int_0^{t_\infty} (C_0 - C_1) \, dt \]  \hspace{1cm} \text{Equation 4.5}

(Standards Australia 1991a; Standards Australia 1991b).

\( m \) = mass of bromide
\( C \) = bromide concentration
\( V \) = volume of solution injected
\( Q \) = flow rate in m\(^3\).s\(^{-1}\)
\( C_0 \) = concentration of bromide detected at each time interval
\( C_1 \) = background concentration of bromide
\( t \) = time since injection

Discrete samples were taken to conduct this analysis, with the time interval between samples recorded. When taking discrete samples it was essential that greater than 20 samples per sample point were taken to comply with Australian standards AT3778.5.1 and 5.2 (1991) (Standards Australia 1991a; Standards Australia 1991b).

4.4 Methods

Unit 4 was selected for detailed analysis as it was receiving low concentrations of phosphorus at a low loading rate. Investigations of Units 2 and 5 indicated that phosphorus removal was definitely occurring between inlet and outlet, but without the internal variations seen in Unit 4. For Unit 3, the high loading rate meant that differences in TP removal were more difficult to discern against background noise. Investigations undertaken in Chapter 3, were unable to definitively show whether total phosphorus was removed between the inlet and outlet of Units 3 or 4, although investigations within a individual plant stands indicated patterns of removal may be occurring with distance into these wetlands. It was suspected that if removal processes were occurring then they would be easiest to observe at the lower loading rate present in Unit 4.

In particular, Unit 4 was unique in the pattern of phosphorus removal within plant Segment 4 – Phragmites, in the centre of the wetland. The high and definite removal in this sector of the wetland needed to be confirmed, or shown to be due to other factors such as inlet variations or wind mixing. If removal could be reproducibly
demonstrated, it might represent a manipulable process. The lower phosphorus loading also meant a greater potential (as outlined in Chapter 3) to confirm whether phosphorus removal in Wetland Segment 3 was related to algal or floating plant biomass, and if so whether there was an equilibrium in phosphorus concentrations incorporated into biomass. There was an opportunity to evaluate whether alum dosing and settling were occurring in Wetlands Segments 1 and 2, along with the potential to evaluate the contribution of detrital settling towards the end of Unit 4 (in conjunction with investigations described in Chapter 5). Thus, it was thought that investigation of this Unit would provide the greatest chance for observing and being able to differentiate between the various causes for changes in phosphorus concentration within the pilot plant wetland Units.

The lower loading rate also meant that the water column had a longer period in contact with sediment and plant surfaces within this wetland; suspected major phosphorus sinks (Sakadevan et al 1995). The longer contact time should allow sharper distinction between regions of TP removal than could be observed at higher flow rates. The low flow rates would allow proportionally more contact with these surfaces. The greater the contact time the more chance for adsorption. In addition, the facilitation of phosphorus sequestering by bacteria predominantly occurs at surfaces, therefore it would be expected that longer contact times with sediment surface would increase the potential for phosphorus absorption through this mechanism.

Sampling points were established to evaluate changes in bromide concentration and total phosphorus through each stand of wetland plants (Figure 4-3). Three tracer injections were performed on separate occasions to determine flow and how it related to phosphorus removal within Unit 4.

4.4.1 Overall experimental design
The method for injection of the tracer was the sudden injection or integrated flow method and followed the principles outlined in AS 3778.5.2 – 1991 (Standards Australia 1991b). Analyses of results from this method become complicated if flows through the region under investigation were not constant. Flows at the inlet were
initially controlled through a centrifugal pump and gate valve (injection 1, Table 4-1). Flows obtained using this type of control were subject to significant variation over the period of investigation (15-30 days). To minimise any fluctuations the inlet pump was changed to a variable speed positive displacement Mono Pump for injections 2 and 3 (Table 4-1). The tracer was combined with the flow after the tipping bucket and prior to entry into the wetland. Allowing the tracer to mix with the inlet flow and disperse with it into the wetland. Samples analysed from the wetland prior to injection of the bromide indicated a consistent average background concentration of bromide entering and within the wetland was $0.55 \pm 0.1$ mg.L$^{-1}$. Since this concentration did not change between wetland inlet and outlet, or over time it was suspected to be a background “artefact” of the effluent.

Table 4-1: The relative mass and concentration of bromide for each of the three injections into Unit 4 with the date of each injection.

<table>
<thead>
<tr>
<th>Injection No.</th>
<th>Date</th>
<th>Mass of Bromide (g)</th>
<th>Concentration of bromide injected (mg.L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2/6/95</td>
<td>388</td>
<td>194</td>
</tr>
<tr>
<td>2</td>
<td>23/10/95</td>
<td>510</td>
<td>255</td>
</tr>
<tr>
<td>3</td>
<td>13/11/95</td>
<td>515</td>
<td>257.5</td>
</tr>
</tbody>
</table>

The total volume of each solution was 2 L. Each injection occurred at 10 am on the dates specified, over a 3-minute interval to minimise concentration spikes and problems with density. For all injections, the time to full elution was derived using the equation set out in the Australian Standard (Standards Australia 1991a) for calculating the final tail end of tracer elution.

The recommended configuration to obtain flow through a cross section was one sample point near each bank and one in the centre of the channel (Figure 4-3) (Standards Australia 1991a). A black plastic tube (Irrigation drip feed pipe, internal diameter 4 mm) was used to obtain samples. Black tubing was selected to minimise light penetration and the growth of algal biofilms. Tubing was placed in the water column 10 cm above the sediment surface. Tubes were permanently located at each sampling site and extended to the bank of the wetland as indicated in Figure 4-3. Tubes were colour coded for ease of identification. This design was to minimise
disturbance of the wetland and provide a rapid and efficient sampling system that could be operated by one person.

Samples were taken twice daily from each sample point within the wetland, at 9 am and 5 pm. Samples were obtained by 50 mL syringe fitted with a small three-way stopcock (Luer fitting - cat no. 63603.000, Indoplas. Pty. Ltd., Sydney). Approximately three times the void volume was pulled through each tube and discarded to remove debris and loose biofilm from the tubing before obtaining a sample. The tubing was not backflushed to minimise disturbance to the water column and avoid dilution or the introduction of foreign water into the wetland. Samples were collected in prewashed 70 mL polyethylene containers. Samples from the inlet and outlet of the wetland were taken every hour (100 mL) and combined to make a composite 4 hourly sample. The wetland inlet and outlet were sampled by autosamplers (Inlet - ISCO model 1680, outlet - ISCO model 6800). The inner compartment of each autosampler (between the sample bottles) was filled with ice to maintain a temperature of 4°C during sampling runs. All samples were analysed to determine the concentrations of phosphorus and bromide in solution, as per Sections 2.4.10 and 4.4.3.

Figure 4-3: A schematic indicating the location of sampling sites within wetland Unit 4 for investigations detailed in Chapter 4.

Results from the injection of bromide tracer were used to determine flow past each sample point within Unit 4. The mass of bromide recovered was derived for each
sample location using the protocol outlined in Section 4.3.6. Times to initial and final bromide detection were used to estimate dispersion.

The curve of bromide concentration over time for each sample location was used to derive the theoretical concentration of TP that should have occurred at that location based on inlet TP concentrations, assuming dispersion was the only process occurring (Figure 4-4). The difference between predicted and actual TP concentrations was then used to evaluate phosphorus removal or release within the wetland profile. Derivation of the theoretical TP concentration at each location (assuming no net release or adsorption within the wetland) was done by assuming TP followed the same pattern of movement as the bromide tracer (Figure 4-4). The mass of TP entering the wetland at each 4-hour time interval (as measured by autosampler) was then apportioned as per the bromide tracer curve (Figure 4-4).

Figure 4-4: Technique for apportioning the concentration of phosphorus passing the inlet, using the observed distribution of the concentration of bromide tracer at a given site within Unit 4 to predicted the theoretical concentration of phosphorus passing that site.

\[ P_1 \text{ to } P_6 \text{ are the concentrations of TP at the inlet that have been proportioned to the bromide tracer curve passing a given point in the wetland then summed to give the predicted TP concentration at that point in the wetland.} \]
By summing the concentrations derived from these distribution patterns it should be possible to determine the theoretical or predicted TP concentration at a particular location if none was absorbed, removed or lost from the wetland in any way (Figure 4-4). The advantage of such a technique was that it should account for processes such as dispersion. Through constant monitoring of inlet TP concentrations and correlating them with flow it should be possible to take into account such variations in determining the theoretical TP concentration past a given point, as predicted from observations of bromide tracer. Since the apportionment of phosphorus concentrations using the bromide tracer curves was based on proportionality, not the absolute mass of bromide, the effect of any non-conservative bromide losses should be minimal.

4.4.2 Bromide injections

Injection 1

Unit 4 was sampled from over a one-month period during winter. The inlet supply pump was a Flyte 350 Centrifugal pump. The flow from this pump was regulated by gate valve. Samples were collected as noted. The inlet flow from this pump showed a degree of variability, which was thought to have a negative effect on the reproducibility of flow times (see below). The centrifugal pump mechanism slows the rate of flow when it encounters a restriction to flow eg sloughed biofilm or algal biomass, protecting the supply pipe from undue pressure and minimising the potential for pipe breakage, but at the cost of a reduction in flow rate. Therefore, it was decided to replace the centrifugal pump with a positive displacement pump, which provided a constant flow rate regardless of obstructions or constrictions in the line for injections 2 and 3. This necessitated a higher level of maintenance, as there was a greater chance for pressure build up in the pipes and potential for pipe breakage or leaking.

Injections 2 and 3

Thus, for injections 2 and 3 the original centrifugal pump supplying the effluent was replaced with a positive displacement piston pump (Mono Pumps Model CP 3, three phase feed). Note that approximately the same flow rates were maintained throughout each investigation. The CP3 pump was a positive displacement piston pump capable
of accurately delivering a constant volume of water with each pumping stroke irrespective of any obstructions within the plumbing.

The pump was plumbed into the inlet supply for Unit 4 for a period of one week to test the accuracy of flow. The flow consistency was measured by observing the change in the time for a set volume of water (10 L) to flow through a tipping bucket flow analyser at 24-hour intervals over a period of 5 days. Over a 24-hour period the mono pump showed a variation of only 3 to 4 seconds in the time taken for a set volume of water to pass through the analyser, compared to a variation of between 20 to 40 seconds for the centrifugal pump. Through the use of a more constant inlet flow, it was hoped to more accurately estimate TP removal and correlate this with distance or plant type.

4.4.3 Bromide detection

Bromide analysis was based on a modification of the Standard Methods for the Analysis of Water and Wastewater (APHA 1992), Method number 4500B (phenol red colorimetric method). The same reagents were used but concentrations were adjusted to automate the reaction process using a Technicon Mk II Autoanalyser System. The schematic for detection of sodium bromide is shown in Figure 4-5. The wastewater entering wetland 4 contained high amounts of ammonium, which interfered with detection of bromide. To overcome this problem, it was necessary to ensure that the lines supplying the analysis reagents contained the same background concentration of ammonium to compensate. Therefore, the buffer solution used in analysis conducted using the Technicon Autoanalyser contained ammonium at the average concentration present in Unit 4 (20 mg.L⁻¹).

At the commencement and conclusion of each set of samples analysed by the Technicon instrument a set of 6 calibration standards were run. After every 20 samples two duplicate standards and one blank were analysed to detect shifts in baseline and determine any inconsistencies. The set of standards comprised of a minimum six bromide concentrations analysed in duplicate, covering the expected bromide concentration range from highest to lowest followed by two reagent blanks.
Standards were prepared from a stock standard of 1000 mg.L\textsuperscript{-1} Bromide, prepared as per Table 4-2. Note that the standard also contained 20 mg.L\textsuperscript{-1} ammonium as background, consistent with concentrations within the wetland. Background levels of bromide were determined by taking samples prior to injection of bromide or in effluent entering (at the inlet to) Unit 4 during the sample run. The concentration of bromide was determined by comparison to the standard calibration curve for the run in question. The average concentration of bromide in background samples was between 0.46 and 0.6 mg.L\textsuperscript{-1}. The bromide concentration needed to exceed this concentration before detection of tracer was considered to have occurred.

Figure 4-5: Schematic of tubing requirements for Technicon Mk II Autoanalyser to detect Br\textsuperscript{-} (0.5 to 5 mg.L\textsuperscript{-1}), adapted from method 4500-Br B. (APHA 1992).

Key:
All coils were of glass, 2 cm outside diameter, internal pipe diameter 1 mm.
Abbreviations are indicative of the colour coding for each pipe diameter, colours were:
Gry – Grey
Blk – Black
Table 4-2: Reagents for Bromide analysis

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>22.5 g</td>
</tr>
<tr>
<td>NaC₂H₃O₂</td>
<td>10.25 g</td>
</tr>
<tr>
<td>Glacial Acetic Acid</td>
<td>7.5 mL</td>
</tr>
<tr>
<td>MilliQ Water qs.</td>
<td>1 L</td>
</tr>
</tbody>
</table>

Note that addition of ammonium to the buffer solution was through the Brij 35 solution.

<table>
<thead>
<tr>
<th>Phenol Red</th>
<th>52 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol Red</td>
<td>1 L</td>
</tr>
<tr>
<td>MilliQ Water qs.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chloramine-T</th>
<th>500 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramine-T</td>
<td>1 L</td>
</tr>
<tr>
<td>MilliQ Water qs.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sodium Thiosulphate</th>
<th>49.6 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Thiosulphate (Na₂S₂O₃·5H₂O)</td>
<td>1 L</td>
</tr>
<tr>
<td>MilliQ Water qs.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Brij 35</th>
<th>1 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brij 35 stock solution</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stock Ammonium Solution (2000 mg.L⁻¹ NH₄)</th>
<th>1 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock Ammonium Solution (2000 mg.L⁻¹ NH₄)</td>
<td></td>
</tr>
<tr>
<td>MilliQ Water qs.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stock Bromide Solution</th>
<th>744.6 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Bromide (NaBr)</td>
<td>1 L</td>
</tr>
<tr>
<td>MilliQ Water qs.</td>
<td></td>
</tr>
</tbody>
</table>

* Store in an amber bottle for up to 1 month
* Make up on day of analysis

### 4.4.4 Phosphorus removal calculations

TP concentrations were measured in the same samples collected for bromide analysis, as detailed in Section 4.4.2, using the methods outlined in Chapter 2. TP removal was estimated by two different approaches. The initial approach involved a simplistic calculation based on the measured average HRT of the wetland, the second approach was a more complicated calculation using the median time of travel results for each sample location within Unit 4, based on the results of the bromide tracer investigation. The simpler approach (Approach 1) was based on using the measured average HRT to
CHAPTER 4

represent the median time of travel for TP to move through the wetland. This time was used to provide a theoretical match between the inlet TP concentration and the outlet TP concentration, which would coincide with that incident TP concentration, displaced by 1 HRT. For example, if the measured HRT was 7 days, then water detected at the inlet today would have entered the wetland 7 days previously. By comparing the TP concentrations at the outlet today with TP concentrations obtained from the inlet 7 days prior, a more accurate estimate of the net change in TP concentration in water passing through the wetland should be obtained.

However, Approach 1 did not consider dispersion, mixing or other processes affecting flow within the wetland. To account for these processes a comparison was made with the inlet TP concentration and TP concentrations at each point within the wetland, with the time of median time of travel for TP being calculated from the bromide tracer results using the methods described in Section 4.4.1 (Figure 4-4). This was done to simulate actual water movement through the wetland. Values obtained by the simple retention time adjustment for inlet and outlet samples from Unit 4 are presented in Table 4-10 (Section 4.6.1). The values extrapolated from bromide tracer results are presented in Section 4.6.2.

4.5 RESULTS PART 1 – FLOW

4.5.1 Time to initial detection of tracer

The time to initial tracer detection was used to estimate the shortest time that the tracer would take to move through a section of the wetland. Given the similar physical properties of bromide and orthophosphate then the movement of tracer should provide and estimate of phosphorus movement in the absence of absorption or other losses. When coupled with peak and median detention times it provided an indication of the degree of short-circuiting occurring in a given region. The time to initial detection at each transect varied by up to 24 hours, for the same inlet flow rate for each separate bromide injection (Table 4-3, individual tracer curves for run 1, to show the typical variation in results are presented in Appendix 3, Figures 3-A to 3-F). However, the
time for initial bromide detection at the outlet to the wetland varied by only 10 hours between the three injections (Table 4-3).

The distribution pattern of initial detection sites varied extensively between each bromide injection. During injection 1 (Table 4-3), the initial movement of bromide into the wetland was rapid and uniform with bromide being detected almost simultaneously at T1 and T2 within 24 hours of injection, then at T3 through to T6 within 48 hours. A further 30 hours was required before bromide from the initial injection was detected at the outlet to Unit 4.

**Table 4-3: Time (in hours) to initial bromide detection for each detection point through Unit 4 during each of the three separate bromide injections.**

<table>
<thead>
<tr>
<th>Transect</th>
<th>Adjacent left bank</th>
<th>Sample location</th>
<th>Centre of flow</th>
<th>Adjacent right bank</th>
<th>Average time to detection (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Injection 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>23 ± 8⁺</td>
</tr>
<tr>
<td>T2</td>
<td>23</td>
<td>23</td>
<td>31</td>
<td>26 ± 8</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>31</td>
<td>47</td>
<td>31</td>
<td>36 ± 9</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>47</td>
<td>47</td>
<td>47</td>
<td>47 ± 8</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>47</td>
<td>47</td>
<td>47</td>
<td>47 ± 8</td>
<td></td>
</tr>
<tr>
<td>T6</td>
<td>47</td>
<td>55</td>
<td>55</td>
<td>52 ± 8</td>
<td></td>
</tr>
<tr>
<td>Outlet</td>
<td>80</td>
<td></td>
<td></td>
<td>80 ± 4</td>
<td></td>
</tr>
<tr>
<td><strong>Injection 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T11</td>
<td>7</td>
<td>7</td>
<td>23</td>
<td>12 ± 9</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>7</td>
<td>23</td>
<td>23</td>
<td>18 ± 9</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>31</td>
<td>23</td>
<td>31</td>
<td>28 ± 8</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>31</td>
<td>31</td>
<td>31</td>
<td>31 ± 8</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>47</td>
<td>31</td>
<td>47</td>
<td>42 ± 9</td>
<td></td>
</tr>
<tr>
<td>T6</td>
<td>95</td>
<td>79</td>
<td>95</td>
<td>90 ± 9</td>
<td></td>
</tr>
<tr>
<td>Outlet</td>
<td>86</td>
<td></td>
<td></td>
<td>86 ± 4</td>
<td></td>
</tr>
<tr>
<td><strong>Injection 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7 ± 4</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7 ± 4</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>23 ± 8</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>71</td>
<td>31</td>
<td>71</td>
<td>58 ± 23</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>47</td>
<td>79</td>
<td>95</td>
<td>74 ± 24</td>
<td></td>
</tr>
<tr>
<td>T6</td>
<td>95</td>
<td>71</td>
<td>95</td>
<td>87 ± 14</td>
<td></td>
</tr>
<tr>
<td>Outlet</td>
<td>90</td>
<td></td>
<td></td>
<td>90 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

Key: T1, T4 = Phragmites; T2, T6 = Schoenoplectus; T3, T5 = Triglochin.

*Standard deviation.
CHAPTER 4

For injection 2 (Table 4-3), the penetration of bromide into the wetland occurred similarly to injection 1, with rapid movement of tracer to T1, T2 and the central region of T3 within 24 hours. Tracer was next detected near the banks at T3, all transects of T4 and the central region of T5, indicating more rapid movement through the centre of the wetland. This was further supported by the early detection of the tracer in the central region of T6. The time for initial detection at T6 was approximately double that at T5 for all sample points along the transect.

During injection 3 (Table 4-3), bromide was rapidly detected at T1, T2 (7 hours) and T3 (23 hours) after injection, similar to injection 2 (same inlet flow rate and both supplied by Mono Pump). Again a rapid penetration of dye to the central region of the wetland was observed, culminating in detection at the outlet earlier than at the banks of T6, although a slight deviation towards the left bank was observed at T5. Movement of the tracer front occurred in two stages, penetrating over half the wetland in 24 to 48 hours before appearing through the remainder of the wetland over the next 48 hours.

4.5.2 Time to median bromide concentration

The time to the median bromide concentration provided an indication of the average time taken for the bromide tracer and hence water to reach each of the sampling points. Allowing the mean water velocity to be calculated from the formula:

\[
velocity = \frac{distance}{time}
\]

Equation 4-6

The time for detection of the median bromide concentration was significantly longer for all sectors including the outlet during injection 1 compared to injections 2 and 3 (Table 4-4), despite the same nominal inlet flow rate. A review of weather data from the University of Wester Sydney – Hawkesbury Meteorological Station indicated strong prevailing north and northwest winds over the days immediately following injection 1, but not injections 2 or 3 (UWS-Hawkesbury 1995).

For injection 1 (Table 4-4), the time for detection of the median concentration did not arrive at T1 until much later than injections 2 and 3. The median passed T2 approximately 40 hrs later (in average terms) and then appeared to be uniformly
distributed between T3 and T6 some 20 hrs later still. However, it was not until approximately 448 hrs into the run (100 hrs after the median appeared at T3 to T6) that the median passed through the wetland outlet.

Table 4-4: Time (in hours) to median peak height for triplicate injections as noted for the three points across the border of each plant stand and the outlet of Unit 4.

<table>
<thead>
<tr>
<th>Transect</th>
<th>Sample location</th>
<th>Adjacent left bank</th>
<th>Sample location</th>
<th>Adjacent right bank</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (hr)</td>
<td>Time (hr)</td>
<td>Time (hr)</td>
<td>Time (hr)</td>
<td></td>
</tr>
<tr>
<td>Injection 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>261 ± 24</td>
<td>269 ± 36</td>
<td>245 ± 48</td>
<td>258 ± 109</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>281 ± 16</td>
<td>281 ± 16</td>
<td>341 ± 36</td>
<td>301 ± 38</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>329 ± 4</td>
<td>325 ± 8</td>
<td>329 ± 4</td>
<td>328 ± 9</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>349 ± 8</td>
<td>301 ± 24</td>
<td>317 ± 16</td>
<td>322 ± 28</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>337 ± 8</td>
<td>333 ± 16</td>
<td>277 ± 16</td>
<td>316 ± 24</td>
<td></td>
</tr>
<tr>
<td>T6</td>
<td>341 ± 16</td>
<td>349 ± 16</td>
<td>341 ± 16</td>
<td>344 ± 22</td>
<td></td>
</tr>
<tr>
<td>Outlet</td>
<td>448 ± 8</td>
<td></td>
<td></td>
<td></td>
<td>448 ± 8</td>
</tr>
</tbody>
</table>

Injection 2

| T1       | 174 ± 16        | 150 ± 8            | 166 ± 8        | 163 ± 10            |         |
| T2       | 174 ± 16        | 174 ± 16           | 190 ± 8        | 179 ± 8             |         |
| T3       | 198 ± 16        | 190 ± 8            | 190 ± 8        | 193 ± 4             |         |
| T4       | 262 ± 16        | 222 ± 8            | 222 ± 8        | 235 ± 19            |         |
| T5       | 270 ± 8         | 286 ± 16           | 358 ± 16       | 305 ± 38            |         |
| T6       | 334 ± 8         | 310 ± 8            | 286 ± 16       | 310 ± 20            |         |
| Outlet   | 318 ± 8         |                    |                |                     | 318 ± 8 |

Injection 3

| T1       | 158 ± 8         | 62 ± 8             | 110 ± 8        | 110 ± 30            |         |
| T2       | 118 ± 16        | 142 ± 8            | 166 ± 8        | 142 ± 20            |         |
| T3       | 210 ± 8         | 198 ± 8            | 198 ± 8        | 202 ± 6             |         |
| T4       | 334 ± 16        | 262 ± 8            | 238 ± 16       | 278 ± 41            |         |
| T5       | 294 ± 16        | 270 ± 8            | 294 ± 8        | 286 ± 11            |         |
| T6       | 294 ± 8         | 222 ± 16           | 294 ± 16       | 270 ± 34            |         |
| Outlet   | 334 ± 4         |                    |                |                     | 334 ± 4 |

Key: T1, T4 = Phragmites; T2, T6 = Schoenoplectus; T3, T5 = Triglochin

During injection 2, the general movement of the tracer plume median (Table 4-4) was spread across the channel. Appearing at almost at the time through all three observation points in a transect, with some regions of short-circuiting at T2 – left bank, T6 – right bank, and slower flow through T5 – right bank. The largest time interval for the median to pass between two transects was between T4 and T5 (Triglochin), a region of open water. The interval between detection at T6 and the
outlet was the shortest of all tracer runs, occurring some 8 hours after detection at the central point of T6.

During the third injection (Table 4-4), times of travel for the median bromide concentration to T1 and T2 were quicker than observed during injections 1 and 2. A regular increase in time of travel was observed with distance into the wetland until T4. Here the tracer cloud was more widely spread, with regions of short-circuiting occurring at T6 centre, and a dead zone at T4 – left. The time for detection of the median tracer mass at the outlet was intermediate between injections 1 and 2, occurring some 50 hours later than at T6 (using average figures).

The time of travel for the median concentration of bromide varied between each injection. In injections 1 and 3, progression through the wetland occurred in two stages – the initial region where times of travel increased into the wetland from (T1 to T3 or T4) then a temporary stagnation through T3 or T4 to T6 before exiting the wetland (Table 4-4). Injection 2 differed from injections 1 and 3, as there did not appear to be any widespread areas of stagnation.

### 4.5.3 Flow

The time of travel for the median concentration of bromide through the wetland allowed observation of flow patterns through the wetland during each injection of tracer. This travel time was used to calculate the relative velocity of water past a given sample point in the wetland during each of the three bromide injection runs. The velocity was calculated as the product of the distance from the inlet by the time of travel for the median bromide concentrations (Equation 4-6).

The average velocity for each transect was divided by the cross sectional area (5m x 0.2 m = 1 m²) for the entire transect to determine the flow past that transect (Section 4.3.1). The values for average flow at the outlet of each wetland were similar for all three injections if the time delay for initial and median bromide detection during injection 1 was taken into account as being attributable to wind associated effects.
(Table 4-5 and Section 4.7.2). Variations in flow rates were observed between different transects for each injection.

Velocities of water past each sample point during injection 1 (Table 4-5) were relatively consistent between T1 to T3 and the wetland outlet. Slightly higher velocities were observed through T4 to T6. However, this difference was not significant at the 10% level (Kruskal-Wallis test).

During injections 2 and 3 (Table 4-5), the observed differences in velocity between sample transects were not significant at the 5% level using the Kruskal-Wallis test. The variance through some transects of the wetland was higher than others, and not consistent for each sector during the three injections.

Table 4-5: Average flow rates past each transect within Unit 4 during each of the three bromide injections calculated from time of travel for the median bromide concentration.

<table>
<thead>
<tr>
<th>Transect</th>
<th>Injection 1 (m³.day⁻¹)</th>
<th>Injection 2 (m³.day⁻¹)</th>
<th>Injection 3 (m³.day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>3.1 ± 0.2²</td>
<td>3.5 ± 0.5</td>
<td>2.8 ± 0.7</td>
</tr>
<tr>
<td>T2</td>
<td>3.3 ± 1.6</td>
<td>4.6 ± 1.3</td>
<td>2.9 ± 0.7</td>
</tr>
<tr>
<td>T3</td>
<td>2.9 ± 0.4</td>
<td>5.1 ± 0.9</td>
<td>5.8 ± 3.0</td>
</tr>
<tr>
<td>T4</td>
<td>4.4 ± 1.6</td>
<td>3.9 ± 0.8</td>
<td>3.4 ± 1.8</td>
</tr>
<tr>
<td>T5</td>
<td>5.7 ± 1.5</td>
<td>3.0 ± 0.8</td>
<td>3.4 ± 0.6</td>
</tr>
<tr>
<td>T6</td>
<td>5.0 ± 0.4</td>
<td>3.4 ± 0.6</td>
<td>5.0 ± 2.0</td>
</tr>
<tr>
<td>Outlet</td>
<td>3.1 ± 1.1</td>
<td>3.3 ± 0.8</td>
<td>3.4 ± 1.1</td>
</tr>
</tbody>
</table>

² all units are in m³.day⁻¹

4.5.4 Time to maximum bromide concentration

The similarities in physical characteristics between bromide and orthophosphate meant that the time to maximum bromide concentration was considered to indicate the time of travel for a sudden spike or peak in phosphorus concentration to reach a particular point for each injection run. Differences between the times of travel for the peak concentration between each run should indicate how reproducible the time of travel estimates were, and how useful this measurement was in predicting the likely movement of peak or sudden phosphorus fluctuations. The time for maximum concentration of bromide to reach various sections of the wetland fluctuated widely.
between each injection and did not appear to be associated with the times for detection of the median bromide concentration. In all cases, the times for detection of maximum bromide concentration were shorter than for detection of the median bromide concentration.

During each injection, the peak bromide concentrations were detected at longer average time intervals as the distance into the wetland increased (Table 4-6). The average time to detection of the peak at T1 was within 12 hours for all injections. There were only two locations, during injection 1, where short-circuiting was observed (injection 1: T1 left, T2 right). Peak detection during injection 1 was as a series of fronts, with areas of short-circuiting as mentioned. During injection 2 the peak bromide concentrations moved through the wetland as an almost uniform front, being detected at similar times at each site in a given transect, with the time for detection increasing with distance into the wetland. During injection 3, the detection of peak bromide concentration occurred almost simultaneously at sites within T1 and T2, then there was a delay of some 70 hours before peak bromide concentrations were observed at T3 through to T6, and only some 7 hrs later, at the wetland outlet.
Table 4-6: Time (in hours) to detection of maximum bromide concentration for bromide injection 1 as noted for the three points across the border of each plant stand and the outlet of Unit 4.

<table>
<thead>
<tr>
<th>Transect</th>
<th>Sample location</th>
<th>Adjacent left bank</th>
<th>Centre of flow</th>
<th>Adjacent right bank</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection 1</td>
<td></td>
<td>Time (hr)</td>
<td>Time (hr)</td>
<td>Time (hr)</td>
<td>Time (hr)</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>27</td>
<td>101</td>
<td>93</td>
<td>72 ± 43</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>117</td>
<td>141</td>
<td>29</td>
<td>92 ± 61</td>
</tr>
<tr>
<td>T3</td>
<td></td>
<td>93</td>
<td>93</td>
<td>117</td>
<td>96 ± 18</td>
</tr>
<tr>
<td>T4</td>
<td></td>
<td>93</td>
<td>117</td>
<td>101</td>
<td>100 ± 16</td>
</tr>
<tr>
<td>T5</td>
<td></td>
<td>93</td>
<td>141</td>
<td>149</td>
<td>124 ± 36</td>
</tr>
<tr>
<td>T6</td>
<td></td>
<td>149</td>
<td>149</td>
<td>165</td>
<td>152 ± 11</td>
</tr>
<tr>
<td>Outlet</td>
<td></td>
<td>188 ± 8</td>
<td></td>
<td></td>
<td>188 ± 8</td>
</tr>
</tbody>
</table>

| Injection 2 | | Time (hr) | Time (hr) | Time (hr) | Average |
| T1         | | 79        | 79        | 79        | 79 ± 4  |
| T2         | | 79        | 95        | 103       | 92 ± 12 |
| T3         | | 163       | 159       | 163       | 162 ± 4 |
| T4         | | 191       | 191       | 191       | 191 ± 4 |
| T5         | | 215       | 215       | 215       | 215 ± 4 |
| T6         | | 231       | 231       | 231       | 231 ± 4 |
| Outlet     | | 278 ± 8   |           |           | 278 ± 8 |

| Injection 3 | | Time (hr) | Time (hr) | Time (hr) | Average |
| T1         | | 79        | 95        | 79        | 84 ± 9  |
| T2         | | 71        | 95        | 79        | 82 ± 12 |
| T3         | | 147       | 151       | 151       | 150 ± 4 |
| T4         | | 151       | 151       | 151       | 151 ± 4 |
| T5         | | 151       | 151       | 167       | 156 ± 9 |
| T6         | | 151       | 151       | 151       | 151 ± 4 |
| Outlet     | | 158 ± 8   |           |           | 158 ± 8 |

Key: T1, T4 = Phragmites; T2, T6 = Schoenoplectus; T3, T5 = Triglochin

4.5.5 Maximum residence time

The maximum residence time indicated the time taken for bromide to elute from each sector of the wetland (Table 4-7) i.e. for bromide concentrations to approach background levels. It was used to calculate the amount of dispersion occurring within the wetland and determine the time that the tracer or other similar soluble materials such as orthophosphate remained in the wetland.
Table 4-7: Maximum time (in hours) between initial injection and the time for bromide concentrations returned to background levels at each transect and transect during the three bromide injections into pilot plant wetland Unit 4.

<table>
<thead>
<tr>
<th>Transect</th>
<th>Adjacent left bank</th>
<th>Sample location</th>
<th>Adjacent right bank</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection 1</td>
<td></td>
<td>Centre of flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>576</td>
<td>612</td>
<td>612</td>
<td>600 ± 25</td>
</tr>
<tr>
<td>T2</td>
<td>576</td>
<td>576</td>
<td>624</td>
<td>592 ± 32</td>
</tr>
<tr>
<td>T3</td>
<td>564</td>
<td>564</td>
<td>684</td>
<td>604 ± 73</td>
</tr>
<tr>
<td>T4</td>
<td>672</td>
<td>672</td>
<td>672</td>
<td>672 ± 4</td>
</tr>
<tr>
<td>T5</td>
<td>672</td>
<td>672</td>
<td>672</td>
<td>672 ± 4</td>
</tr>
<tr>
<td>T6</td>
<td>672</td>
<td>660</td>
<td>684</td>
<td>672 ± 16</td>
</tr>
<tr>
<td>Outlet</td>
<td></td>
<td></td>
<td>697</td>
<td></td>
</tr>
<tr>
<td>Injection 2</td>
<td>718</td>
<td>694</td>
<td>766</td>
<td>726 ± 30</td>
</tr>
<tr>
<td>T1</td>
<td>702</td>
<td>622</td>
<td>678</td>
<td>667 ± 34</td>
</tr>
<tr>
<td>T2</td>
<td>622</td>
<td>598</td>
<td>598</td>
<td>606 ± 11</td>
</tr>
<tr>
<td>T3</td>
<td>694</td>
<td>646</td>
<td>622</td>
<td>654 ± 30</td>
</tr>
<tr>
<td>T4</td>
<td>798</td>
<td>798</td>
<td>862</td>
<td>819 ± 30</td>
</tr>
<tr>
<td>T5</td>
<td>814</td>
<td>910</td>
<td>790</td>
<td>838 ± 52</td>
</tr>
<tr>
<td>T6</td>
<td>822</td>
<td>822</td>
<td>822</td>
<td></td>
</tr>
<tr>
<td>Outlet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection 3</td>
<td>478</td>
<td>486</td>
<td>478</td>
<td>481 ± 4</td>
</tr>
<tr>
<td>T1</td>
<td>462</td>
<td>574</td>
<td>702</td>
<td>579 ± 98</td>
</tr>
<tr>
<td>T2</td>
<td>750</td>
<td>766</td>
<td>750</td>
<td>755 ± 8</td>
</tr>
<tr>
<td>T3</td>
<td>870</td>
<td>822</td>
<td>798</td>
<td>830 ± 30</td>
</tr>
<tr>
<td>T4</td>
<td>798</td>
<td>814</td>
<td>814</td>
<td>809 ± 8</td>
</tr>
<tr>
<td>T5</td>
<td>814</td>
<td>726</td>
<td>822</td>
<td>787 ± 43</td>
</tr>
<tr>
<td>T6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outlet</td>
<td>944</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: T1, T4 = Phragmites; T2, T6 = Schoenoplectus; T3, T5 = Triglochin

Times for full elution were lower during injection 1 relative to injections 2 and 3. Using average values, the maximum residence times for injection 1 divided Unit 4 into a front (T1, T2, T3) and a back (T4, T5, T6) compartment with significantly higher maximum elution times occurring in the second half of the wetland (Table 4-7), similar to the minimum times for bromide detection. For injection 2, bromide appeared to reside for a long period in T1 and T2. Apparently being eluted more rapidly from T3 and T4 than these regions near the inlet, before eluting almost as a front through T5 and T6 to the outlet. Residence times near T5 and T6 (for injection 2) were similar to injection run 3, although time to final elution from the outlet was
shorter than for injection 3. Injection 3 produced a more rapid elution of bromide from T1 and T2 than observed during the previous injections. The rate of elution slowed markedly in T3 relative to the earlier transects with bromide tending to approach the limits of detectability after a similar time interval for all transects during this injection run.

4.5.6 Bromide recovery
The percentage of bromide recovered from the wetland determined how well bromide acted as an “ideal” tracer, providing an indication of the type and likely size of errors in calculations of the dispersion coefficient that were based on tracer observation. By integrating the tracer breakthrough curve and multiplying each integrated area by the flow for that sample time, the mass of bromide recovered was estimated. The percentage of bromide recovered was determined by dividing the mass of bromide recovered by the mass of bromide injected, after allowing for the inherent or background concentration of bromide as found at the inlet to the wetland. The concentration of bromide in inlet samples was between 0.46 and 0.6 mg.L⁻¹. The bromide concentration needed to exceed 0.6 mg.L⁻¹ before detection of tracer was considered to have occurred.

Percentage recovery of bromide was less than 100% at all sites (Table 4-8). The majority of bromide losses occurred between the inlet and T1 for all injections (Table 4-8). During injection 1, a significant difference in the percentage of bromide recovered from various sectors of the wetland was observed at the 5% level of significance (Kruskal-Wallis test) but this difference was random and not associated with distance. During injections 2 and 3, no significant difference was observed between the percentage recovery of bromide from each sector of the wetland (Kruskal-Wallis test, α = 0.05). Recovery varied between injections and was generally lower for injections 2 and 3 compared to injection 1. There were no regions of consistently high or low recovery through the wetland (Table 4-8).
Table 4-8: Summary of the percentage of bromide recovered from each sample point within Unit 4 during each of the three injection runs.

<table>
<thead>
<tr>
<th>Transect</th>
<th>Percentage of bromide recovered from each sampling point</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Adjacent left bank</td>
<td>Centre of flow</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>57</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>55</td>
</tr>
<tr>
<td>5</td>
<td>66</td>
<td>63</td>
</tr>
<tr>
<td>6</td>
<td>64</td>
<td>58</td>
</tr>
<tr>
<td>Outlet</td>
<td>59</td>
<td>60</td>
</tr>
<tr>
<td>Injection 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Adjacent left bank</td>
<td>Centre of flow</td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>41</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>44</td>
<td>45</td>
</tr>
<tr>
<td>Outlet</td>
<td>43</td>
<td>45</td>
</tr>
<tr>
<td>Injection 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Adjacent left bank</td>
<td>Centre of flow</td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>39</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Outlet</td>
<td>28</td>
<td>29</td>
</tr>
</tbody>
</table>

4.5.7 Hydraulic retention time and hydraulic efficiency

The HRT was calculated based on the average flow throughout Unit 4 during for each of the three bromide injections. HE is also presented because of its close links with HRT. Using average values, the HRT predicted during each bromide injection was similar (Table 4-9). This was also reflected in the respective values for HE for each of the three injections (Table 4-9). The average HRT for the wetland was 7.0 days, giving a HE of 0.7. The theoretical HRT, based on the inlet flow (2.2 kL/day) and system dimension was 12 days.
Table 4-9: HRT and HE for Unit 4 as measured by bromide tracer over three separate injections, presented with the average for all three injections.

<table>
<thead>
<tr>
<th></th>
<th>Injection 1</th>
<th>Injection 2</th>
<th>Injection 3</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT (days)</td>
<td>6.9 ± 2.1</td>
<td>7.1 ± 1.5</td>
<td>7.1 ± 2.3</td>
<td>7.0 ± 2.0</td>
</tr>
<tr>
<td>HE</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.2</td>
</tr>
</tbody>
</table>

4.6 RESULTS PART 2 - PHOSPHORUS REMOVAL

4.6.1 Phosphorus removal calculated using hydraulic retention time

Observations of TP concentrations at the inlet and outlet of Unit 4 during the 3 injection runs indicated inlet TP concentrations appeared to fluctuate randomly during each injection run (Table 4-10). Variations in TP concentration at the wetland outlet also appeared random, but with a lower variance than the inlet (Table 4-10). A simple comparison of inlet vs outlet results, with no correction for HRT (Column 4 of Table 4-10, ‘TP removed comparing average values’) showed that TP concentrations:

- reduced between the inlet and outlet during injection 1;
- showed partial or no reduction between inlet and outlet during injection 2 (depending on whether mean or median values were used); and
- increased slightly between inlet and outlet during injection 3 (note however the high standard deviations for the results from this injection meant the difference between inlet and outlet concentrations were not significant).

Paired inlet and outlet values based on HRT, as outlined in Approach 1 (Section 4.4.4, TP corrected for HRT) are presented in Table 4-10, Column 5 (TP removed using paired values). Using this approach, the concentration of TP consistently decreased between the inlet and outlet of Unit 4 during each bromide injection run. The concentration change was less variable and more consistent between injections than when values were not paired (Table 4-10, Column 4). The concentration change was greatest when mean values were used as opposed to median values, and overall more TP was retained by the wetland during each run than was released.
CHAPTER 4

Using Approach 1, the number of samples that evidenced TP removal was 43 % for injection 1, 60 % for injection 2, and 52 % for injection 3. Due to fluctuations of inlet TP concentration it is arguable that the median value for concentration rather than the mean should be presented because median values would typically be more representative of this type of time series data (Zar 1996). Therefore, both the median and mean phosphorus concentrations for the inlet and outlet of Unit 4 have been presented in Table 4-10.

<table>
<thead>
<tr>
<th>Injection</th>
<th>Inlet TP (mg.L⁻¹)</th>
<th>Outlet TP (mg.L⁻¹)</th>
<th>TP removed comparing average values (mg.L⁻¹)</th>
<th>TP removed using paired values (mg.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Injection 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>3.53 ± 2.55</td>
<td>2.31 ± 1.18</td>
<td>1.21 ± 1.87</td>
<td>0.62 ± 1.88</td>
</tr>
<tr>
<td>Median</td>
<td>3.57 ± 2.54</td>
<td>2.26 ± 1.18</td>
<td>1.30 ± 1.87</td>
<td>0.43 ± 1.88</td>
</tr>
<tr>
<td><strong>Injection 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>2.01 ± 1.64</td>
<td>1.33 ± 0.49</td>
<td>0.68 ± 1.15</td>
<td>0.69 ± 1.31</td>
</tr>
<tr>
<td>Median</td>
<td>1.29 ± 1.64</td>
<td>1.60 ± 0.49</td>
<td>-0.31 ± 1.15</td>
<td>0.24 ± 1.31</td>
</tr>
<tr>
<td><strong>Injection 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.97 ± 1.14</td>
<td>1.04 ± 0.24</td>
<td>-0.08 ± 0.90</td>
<td>0.23 ± 0.87</td>
</tr>
<tr>
<td>Median</td>
<td>0.67 ± 1.14</td>
<td>1.00 ± 0.24</td>
<td>-0.32 ± 0.90</td>
<td>0.07 ± 0.87</td>
</tr>
</tbody>
</table>

4.6.2 Phosphorus removal calculated from bromide tracer observations

The estimation of TP removal using values calculated from injections of bromide tracer was used to provide a more accurate indication of removal within different wetland segments and account for dispersion and other internal wetland processes (Approach 2, Table 4-11, refer to Appendix 4, Figures 4-A to 4-G for a graphical representation). Using a Kruskal-Wallis test on this data, TP was consistently removed between the inlet and outlet of Unit 4 during each bromide injection.

During injection 1, the concentration of phosphorus removed increased with distance into the wetland (Table 4-11), although the net removal declined in the last plant stand by 50%. Removal rates were highest through the central portion of the wetland between T2 and T5. The stands of Phragmites adjacent the inlet and outlet were the only stand which released TP to the water column.

Greg Ryan  
PhD Thesis – UWSH
Table 4-11: A summary of total phosphorus removal from the water column at each transect in Unit 4 during each of the three bromide injections, based on results predicted from observation of the respective bromide tracer curves. Values represent phosphorus removal relative to concentrations at the wetland inlet, adjusted for bromide time of travel.

<table>
<thead>
<tr>
<th>Sector</th>
<th>Adjacent left bank</th>
<th>Centre</th>
<th>Adjacent right bank</th>
<th>Average (mg.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP concentration (mg.L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Injection 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-0.02 ± 0.13 (-0.13)</td>
<td>-0.19 ± 0.24 (-0.76)</td>
<td>-0.18 ± 0.13 (-0.18)</td>
<td>-0.13 ± 0.17 (-0.36)</td>
</tr>
<tr>
<td>2</td>
<td>-0.02 ± 0.17 (-0.20)</td>
<td>-0.11 ± 0.20 (-0.35)</td>
<td>-0.09 ± 0.12 (-0.08)</td>
<td>-0.07 ± 0.16 (-0.21)</td>
</tr>
<tr>
<td>3</td>
<td>0.51 ± 0.12 (0.48)</td>
<td>0.30 ± 0.14 (0.20)</td>
<td>0.42 ± 0.14 (0.36)</td>
<td>0.41 ± 0.13 (0.35)</td>
</tr>
<tr>
<td>4</td>
<td>0.85 ± 0.11 (0.99)</td>
<td>0.48 ± 0.12 (0.30)</td>
<td>0.63 ± 0.12 (0.38)</td>
<td>0.65 ± 0.12 (0.56)</td>
</tr>
<tr>
<td>5</td>
<td>1.04 ± 0.10 (0.87)</td>
<td>0.71 ± 0.13 (0.73)</td>
<td>0.93 ± 0.11 (0.75)</td>
<td>0.89 ± 0.11 (0.78)</td>
</tr>
<tr>
<td>6</td>
<td>1.10 ± 0.14 (0.90)</td>
<td>0.80 ± 0.10 (0.75)</td>
<td>0.84 ± 0.11 (0.79)</td>
<td>0.91 ± 0.12 (0.81)</td>
</tr>
<tr>
<td>Outlet</td>
<td>0.46 ± 0.12 (0.24)</td>
<td>0.46 ± 0.12 (0.24)</td>
<td>0.46 ± 0.12 (0.24)</td>
<td>0.46 ± 0.12 (0.24)</td>
</tr>
<tr>
<td><strong>Injection 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.45 ± 0.11 (0.56)</td>
<td>0.74 ± 0.13 (0.82)</td>
<td>0.49 ± 0.11 (0.68)</td>
<td>0.56 ± 0.12 (0.69)</td>
</tr>
<tr>
<td>2</td>
<td>0.40 ± 0.06 (0.41)</td>
<td>*</td>
<td>0.58 ± 0.05 (0.61)</td>
<td>0.49 ± 0.05 (0.51)</td>
</tr>
<tr>
<td>3</td>
<td>0.61 ± 0.06 (0.69)</td>
<td>0.57 ± 0.05 (0.52)</td>
<td>0.38 ± 0.03 (0.42)</td>
<td>0.52 ± 0.05 (0.54)</td>
</tr>
<tr>
<td>4</td>
<td>0.38 ± 0.09 (0.59)</td>
<td>0.43 ± 0.06 (0.55)</td>
<td>0.39 ± 0.06 (0.50)</td>
<td>0.40 ± 0.07 (0.55)</td>
</tr>
<tr>
<td>5</td>
<td>0.47 ± 0.06 (0.45)</td>
<td>0.47 ± 0.05 (0.43)</td>
<td>0.74 ± 0.05 (0.83)</td>
<td>0.56 ± 0.05 (0.57)</td>
</tr>
<tr>
<td>6</td>
<td>0.54 ± 0.06 (0.47)</td>
<td>0.50 ± 0.05 (0.48)</td>
<td>0.47 ± 0.06 (0.36)</td>
<td>0.50 ± 0.06 (0.44)</td>
</tr>
<tr>
<td>Outlet</td>
<td>0.56 ± 0.05 (0.55)</td>
<td>0.56 ± 0.05 (0.55)</td>
<td>0.56 ± 0.05 (0.55)</td>
<td>0.56 ± 0.05 (0.55)</td>
</tr>
<tr>
<td><strong>Injection 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.43 ± 0.10 (0.6)</td>
<td>0.28 ± 0.13 (0.34)</td>
<td>-0.35 ± 0.18 (-0.19)</td>
<td>0.12 ± 0.14 (0.25)</td>
</tr>
<tr>
<td>2</td>
<td>0.04 ± 0.13 (0.11)</td>
<td>*</td>
<td>0.16 ± 0.03 (0.16)</td>
<td>0.10 ± 0.13 (0.08)</td>
</tr>
<tr>
<td>3</td>
<td>0.11 ± 0.05 (0.2)</td>
<td>0.02 ± 0.05 (0.03)</td>
<td>-0.10 ± 0.05 (-0.07)</td>
<td>0.01 ± 0.05 (0.05)</td>
</tr>
<tr>
<td>4</td>
<td>-0.11 ± 0.05 (-0.1)</td>
<td>-0.12 ± 0.03 (-0.15)</td>
<td>-0.46 ± 0.05 (-0.41)</td>
<td>-0.23 ± 0.04 (-0.22)</td>
</tr>
<tr>
<td>5</td>
<td>-0.08 ± 0.10 (0.03)</td>
<td>0.04 ± 0.03 (0.07)</td>
<td>0.33 ± 0.10 (0.26)</td>
<td>0.10 ± 0.08 (0.12)</td>
</tr>
<tr>
<td>6</td>
<td>0.11 ± 0.05 (0.09)</td>
<td>0.25 ± 0.05 (0.26)</td>
<td>-0.07 ± 0.03 (-0.05)</td>
<td>0.10 ± 0.04 (0.10)</td>
</tr>
<tr>
<td>Outlet</td>
<td>0.23 ± 0.03 (0.22)</td>
<td>0.23 ± 0.03 (0.23)</td>
<td>0.23 ± 0.03 (0.23)</td>
<td>0.23 ± 0.03 (0.23)</td>
</tr>
</tbody>
</table>

Key: negative values indicate TP release to the water column, positive values indicate TP removal. The initial value is the mean, the variation is the standard error (due to the non-normal distribution of data, caused by the interdependent nature of the results), and the number in brackets is the median.

* During injections 2 and 3, the sample point at T2 middle, had consistently high phosphorus concentrations associated with sedimentary material being drawn into the sample cup. Repositioning and cleaning of the sample line were unsuccessful at removing the problem. Therefore these results were regarded as outliers and not included in this investigation.

Net TP removal between the inlet and outlet of Unit 4 during injection 2 (19%) was similar to injection 1 (11% - due to variations in inlet TP concentration from the use of the centrifugal pump). Within the wetland during injection 2, phosphorus was predominantly removed by the initial stand of *Phragmites* (using average values).
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However, observations of changes in phosphorus concentrations during this run were complicated by problems with alum dosing during the initial few days of the investigation. This resulted in unusually high (4-6 mg.L\(^{-1}\)) phosphorus concentrations at the inlet for a brief period. For injection 2, TP appeared to be removed from the water column between the inlet and T1 with concentrations fluctuating randomly thereafter, but little or no removal throughout the remainder of the wetland (Table 4-11).

Throughout injection 3, TP removal was not consistent between the 3 sites comprising a given transect. On most occasions, TP was removed from the water column between the wetland inlet and T2. Phosphorus release occurred to some extent at T3 and all sites along T4 (stands of Phragmites and Triglochin), with removal occurring at a number of sites between T4 and the outlet. Overall there was a net removal of 38% TP between the inlet and outlet of the wetland.

4.6.3 Correlation of total phosphorus concentration with flow.

One of the major factors that could affect phosphorus absorption to sediment surfaces or plant material was the flow rate. A correlation analysis was undertaken to determine whether there were transects within Unit 4 that demonstrated a consistent association between flow and TP removal (Table 4-12).

Table 4-12: Correlation coefficients of concentrations of total phosphorus removed from the water column and flow in each sector from Unit 4 during the three bromide injections.

<table>
<thead>
<tr>
<th>Segment</th>
<th>Injection 1</th>
<th>Injection 2</th>
<th>Injection 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation coefficient (r)</td>
<td>Correlation coefficient (r)</td>
<td>Correlation coefficient (r)</td>
</tr>
<tr>
<td>1</td>
<td>0.87</td>
<td>0.15</td>
<td>0.35</td>
</tr>
<tr>
<td>2</td>
<td>-0.19</td>
<td>-0.70</td>
<td>-0.23</td>
</tr>
<tr>
<td>3</td>
<td>-0.99</td>
<td>-0.76</td>
<td>-1.00</td>
</tr>
<tr>
<td>4</td>
<td>-0.72</td>
<td>0.08</td>
<td>-0.93</td>
</tr>
<tr>
<td>5</td>
<td>0.62</td>
<td>-0.84</td>
<td>0.73</td>
</tr>
<tr>
<td>6</td>
<td>-0.99</td>
<td>-0.85</td>
<td>0.62</td>
</tr>
<tr>
<td>7</td>
<td>0.50</td>
<td>0.05</td>
<td>-0.01</td>
</tr>
</tbody>
</table>

The only transect with a consistently significant correlation between flow and TP removal for all three injections was T3, where the correlation was high and negative (Table 4-12) i.e. when flow was high, TP removal was low. Elsewhere in the wetland,
both positive and negative correlations occurred between flow and TP removal. Significant correlations were observed for 11 of the 21 observations ($r > 0.7$). The correlation coefficient was negative for 9 of these observations.

4.7 DISCUSSION PART 1 - FLOW

4.7.1 Time to initial tracer detection

The time to initial bromide detection indicated that the tracer moved through the first half of the wetland as a front following different paths for each tracer run and on occasions (injection 3) short-circuiting through the second half of the wetland. The time to initial tracer detection at T1 was slower during injection 1 relative to injections 2 and 3, most likely due to the strong prevailing wind, as inlet flow rates through the wetland were kept relatively constant ($\sim 2.2$ kL.day$^{-1}$). Variations further into the wetland may have been from channelling or short-circuiting, wind effects or other perturbation of the wetland. Tracer movement during injection 1 was fairly rapid (with the exception of the first 24 hrs) and uniform (simultaneously detected at all 3 sample points in a transect). Injections 2 and 3 evidenced regions of short-circuiting and obvious dead zones. Indicating channelling, wind effects and perturbation were probably occurring, although the specific locations of these effects differed between injections. The short time (only 3 weeks), between injections 2 and 3, meant that regions of short-circuiting, flow reversal or wind effects would appear to be localised and relatively transient.

Note that despite such variations the minimum times for bromide to reach the outlet of the wetland between each dye run only varied by a maximum of 10 hours. This would further support the argument that short-circuiting and other factors were only transient and localised in their effect. Essentially, water entering the wetland followed the minimum path length to the outlet and took virtually the same minimum amount of time to move between the inlet and outlet in each injection run. This suggests that the shortest path through the wetland may alter slightly with each run but overall a similar path length was travelled. Obstructions and external effects such as wind appeared to affect localised regions but overall they did not delay the minimum time of travel for...
water from inlet to outlet i.e. transient effects did not unduly detain water in the wetland, consistent with Kadlec’s (1997) theories of stochastic effects on wetland performance.

4.7.2 Time to median bromide concentration
The time to median bromide concentration was used as a measure of the average velocity of water past a given sample point. The times for elution of the median bromide concentration differed between injection 1 and injections 2 or 3. This was associated with the time to detection of the median tracer concentration at sites comprising T1, adjacent the wetland inlet during injection 1. Once the tracer was detected at T1, the relative time of travel through the remainder of the wetland was similar for each injection. Some of the reason for the delay in time to detection of the initial and median tracer concentrations at T1 appeared to be related to the strong northerly wind prevailing at the time of injection 1, which may have had a substantial effect on tracer movement through the initial Phragmites stand during this run. Stratification could have been an influencing factor, had the sample run not taken place in mid-winter. Stratification, may have caused some form of density related separation of the tracer with the water column, however, this was unlikely given the depth of the water column (20 cm), and the height of the sample tube (10 cm) above the sediment surface.

Injection 1 had a number of unique properties. This injection showed the longest time delay to initial detection, consistent with the time to median detection. This was most likely associated with wind direction affecting water and tracer movement at the time of injection. However, some factor also delayed the exit of bromide from the wetland during injection 1. It may have been from wind or another factor allowing mixing within the wetland (hence the uniform tracer cloud distribution) but not the majority of tracer cloud to exit the wetland. The similarity in time to initial tracer detection between injections at the outlet implied that some material was able to exit the wetland during this time. The times to median tracer concentration observed during injection 1 were not reproduced in the other two-tracer injection runs.
4.7.3 Mixing and dispersion

Despite bromide being injected with the main flow into the wetland, it was hypothesised that a distance was required before this point source was fully mixed with the water already present in the wetland (Figure 4-6). Results from Section 4.5.2, Table 4-4 suggest that full mixing did not occur until after T2 for all three bromide injections, some 8 m into the wetland. A suggested dispersal pattern is shown in Figure 4-6.

Figure 4-8: A proposed dispersal pattern for the inlet to Unit 4.

Regions where the bromide concentration uniformly appeared to stagnate or mix could be from wind-induced movement of the water column. Wind has been shown to have a significant effect in the mixing and dispersion processes occurring in wetlands (Waters 1998). Wind movement against the bulk flow would be expected to delay the movement of tracer through the wetland. If the initial delay present during injection 1 was removed then the total elution times become very similar for injections 1, 2 and 3. The opposite effect could occur when the wind movement was in the direction of bulk flow, resulting in an apparently shorter time of travel.
Comparing results from Sections 4.5.1 and 4.5.2, indicated that between 3 to 5 of the 20 sites comprising each bromide run showed significant variation in travel times of the initial tracer front, relative to the time for detection of the median bromide concentration. Possible factors that could have affected dispersion include short-term flow barriers to flow posed by litter fall, bird and animal desiccation, or wind driven water movement.

The observed pattern of dispersion for the bromide tracer cloud during this investigation meant that it did not follow an idealised theoretical flow pattern, where the median tracer concentration elutes from the wetland shortly after the tracer front. Instead, bromide remained in some regions of the wetland longer than others, however, the locations of these dead spots were not constant and therefore unlikely to be readily manipulated or directly controlled. Note that these dead spots or regions of short-circuiting did not significantly affect the overall dispersion through the wetland during any injection. However, the wind effects observed during injection 1 did have significant effect, increasing dispersion. Since bromide was used to model TP in this investigation, it was assumed that TP distributions would follow similar patterns.

4.7.4 Flow rates
Flow rates were calculated from the median time of travel for bromide, and corrected to allow time for the unexpected delay in the time to detection of median bromide concentration at T1 during each injection as outlined in Section 4.5.3. Significant variations in average flow rates occurred not only between bromide injection runs but also between sites for each transect. This was most likely attributable to wind effects because of the low flow rates and variations in the flow paths taken by water including short-circuiting during and between each injection. One way to minimise such paths impacting on flow through next plant stand would be to use a weir structure to ensure uniform flow. Such a structure could be used to channel the net flow through either a single point or preferably across the entire stand to ensure a more uniform distribution and avoid a mixing zone.
CHAPTER 4

The high variation in flow rates within and between each wetland transect made it difficult to accurately determine the flow rate passing through that transect during a given bromide injection. Hence, although numerical differences were observed these were not statistically significant. The similarity in time of detection for the median bromide concentration at the wetland outlets was consistent with previous observations (Sections 4.7.1 and 4.7.2). Flow was not uniformly passing down the left, right or centre of the wetland but appeared to be short-circuiting past specific regions based on litter fall, plant stem and root growth, and other factors.

4.7.5 **Effect of different pumps types on net flow**

The standard deviation for inlet flow results obtained during injection 1 was much higher than for injections 2 and 3 (Section 4.7.2). The higher variance observed during injection 1 was most likely because the inlet supply was fitted with a centrifugal pump rather than a Mono pump for this injection run. During injections 2 and 3 the centrifugal pump was replaced with a Mono pump. As can be seen by the greater consistency in flow rates between injections 2 and 3 the Mono pump produced a more stable flow than the centrifugal pump, as reflected by their respective pumping mechanisms.

When water being pumped through a sealed pipe encounters a resistance such as a blockage or a restriction in pipe diameter (due to engineering, biofilm growth or other factors) then an increase in pressure is experienced in the pipe and at the pump. A centrifugal pump uses a rotary turbine to provide a pumping force. When this turbine experiences a back pressure it slows down, reducing the flow in the pipe, keeping the pressure constant and preventing damage to the downstream pipe, fittings and valves. A Mono pump uses a piston mechanism to displace an equal volume of water with each stroke. Whilst this mechanism was more accurate, there was also more potential for damage to the downstream pipe, fittings and valves if a resistance is encountered, unless a pressure release valve is fitted. Fitting such a release valve would tend to negate the constant volume supplied by the mono pump and was not installed. Pressure changes that may have occurred during the operation of a Mono Pump on the
inlet to Unit 4 did not damage any portion of the inlet line or fittings during this investigation.

4.7.6 **Time to maximum bromide concentration**

The time to maximum bromide concentration was used to indicate the time interval from the detection of a spike or peak at the inlet to a given transect within the wetland. In all cases, the maximum bromide concentration was observed as occurring after the initial tracer front and before the median concentration. The time for detection of the maximum bromide concentration appeared independent of median times of travel and times to initial detection. Each injection produced a different pattern for the distribution pattern of time to peak bromide concentration. Injection 1 indicated significant short-circuiting along the sides of the wetland, injection 2 showed almost plug flow like results, and injection 3 showed a two-stage movement through the wetland. These results highlighted that times of travel for peak bromide concentrations were highly variable and inconsistent. It was likely that the pattern of results observed in Unit 4 during these investigations were caused by wind and possibly short term obstructions, given the great difference in distribution patterns and timing between injections, and the lack of any association with other tracer measurements such as minimum and median times of travel. These large variations and inconsistencies meant that time of travel for the maximum bromide concentration was not a usable predictive tool for bromide and hence phosphorus movement.

4.7.7 **Maximum residence time**

The maximum residence time was used to indicate the maximum time that dissolved material would remain in each sector of the wetland. The pattern for the maximum residence time differed between injection runs. Bromide generally remained near the inlet for the shortest time interval and lingered more towards the outlet. However, for injection 2, bromide was unable to be detected in the central regions of the wetland despite some material still being detectable near the inlet. This may have been associated due to wind effects. For injection runs 1 and 3, bromide concentrations decreased to nondetectable levels most rapidly between T1 and T2, with greater variability further into the wetland. Due to the similarities observed for the time to
initial detection of bromide, the longer estimated total residence times between T3 and T6 translated to greater dispersion through these transects, increasing with distance into the wetland. This observation would be expected as part of the normal mixing and elution process (Rutherford 1994).

4.7.8 Bromide recovery
Percentage figures for the mass of bromide recovered at various sampling points within the wetland indicated that between 30 to 60% was recovered. The only significant change in bromide concentration occurred between the wetland inlet and T1, for each injection. This change was most likely due to the interaction of bromide with the bulk flow. There was no net decrease in the percentage recovery of bromide with distance within Unit 4 and the recovery of bromide at the outlet to the wetland was not significantly different to that observed at sites within the wetland. Hence, bromide was not removed from the water column between T1 and the wetland outlet, acting as a conservative tracer during each tracer run. The change in bromide concentration between the inlet and T1 was most likely due to interactions with material in the bulk flow, which was suspected to have complexed with, or otherwise reacted, to mask bromide concentrations. Note that all bromide concentrations were relative to concentrations observed in water entering the wetland (background concentration).

The question arises, that given the low recovery of bromide was it the correct tracer for these investigations. In Section 4.3.3 a number of tracer options were evaluated based on published literature, indicating the most comparable tracer to bromide was lithium. Tracer losses for lithium were also significant (up to 55-85%) when long contact times with sediment material (9 – 24 days) occur (Netter et al. 1990). Hence it could be concluded that for investigations of flow in constructed wetlands bromide should be at least equivalent to lithium as a conservative tracer, and based on data at the time bromide was the tracer of choice for constructed wetlands receiving alum dosed sewerage effluent.
4.7.9 Hydraulic retention time and hydraulic efficiency

Using average outlet flows derived from tracer observations, a similar HRT (at the wetland outlet) was observed for each bromide injection. Therefore, the net average flow through the wetland did not change significantly during each injection run. The HE of less than unity indicated that short-circuiting was occurring within Unit 4. The variations in flows at individual sites within Unit 4 during each bromide injection, coupled with homogeneous flow rates at the outlet to the wetland would imply localised regions of variable flow. This would mean that trying to define exact regions of short-circuiting in this wetland would be difficult, with the data gained of questionable use due to its transitory nature.

4.8 DISCUSSION PART 2 - PHOSPHORUS REMOVAL

4.8.1 Total phosphorus removal calculated from retention time

General observations

Inlet TP concentrations appeared to fluctuate randomly during each injection run. Fluctuations in TP at the wetland outlet had a lower variance than the inlet, highlighting the capacity of mixing processes within the wetland to dampen fluctuations in TP concentrations. A direct comparison of median TP concentrations at the inlet and outlet (with no time correction) indicated questionable TP removal or release due to the high standard deviation of the data. However, a more indicative method was the use of paired, time displaced values (Approach 1, Section 4.4.4). This method generated a similar standard deviation to the previous but allowed direct comparison of removal using paired inlet/outlet median or average values. It indicated a net removal of TP from the water column during each of the 3 periods investigated.

If median values were used for comparison then TP removal occurred during each bromide injection, varying between 0.70 to 0.43 mg.L$^{-1}$. Median removal was between 10 to 19% of inlet TP. The concentration decrease for TP between wetland inlet and outlet was less at lower concentrations of TP. However, the greatest percentage removal was observed at the middle inlet TP concentration (median 1.29
mg.L⁻¹, injection 2). The percentage removal did not effectively differ between the other two injection runs. Therefore, TP removal was independent of inlet concentration during this investigation.

4.8.2 Total phosphorus removal calculated from bromide tracer results and correlation with flow

Analysis of TP removal by mapping inlet results to those for the bromide tracer curve (Section 4.6.2) confirmed the net removal of TP during each injection. Values lay within those obtained by using paired or average values but were more consistent. Percentage removal rates for TP using this method confirmed that removal was not proportional to or correlated with the inlet TP concentration. The similar net TP removal rates observed at the outlet for injections 1 and 2, taken some 4 to 5 months apart, with different inlet concentrations supported the hypothesis that the inlet concentrations (within the ranges observed in this investigation) did not have a significant impact on outlet TP concentrations. These findings were consistent with those of Richardson et al. (Richardson 1985; Richardson et al. 1993; Richardson et al. 1995) who indicated that phosphorus removal processes were related more to concentrations of iron and to a lesser extent aluminium in the wetland sediments, along with flow or contact time with those wetland sediments.

TP removal patterns differed during each injection run (consistent with the observations of (Kadlec 1997)). Removal rates for injections 2 and 3 (performed during spring) were similar. There was an initial removal of phosphorus from the inlet to T1 and then no significant removal through any other transect except between T6 and the wetland outlet (using average values – Table 4-11). TP also appeared to be released at T4 (through the central stand of *Phragmites*) during both injections. Implying TP removal occurred between T4 and T5.

The TP removal pattern during injection 1 (in autumn/winter) was markedly different to that observed for injections 2 and 3. TP was released in the initial stand of *Phragmites* (prior to T1) and removed from the water column in increasing amounts from T2 to T6, before further and significant release occurred between T6 and the
outlet. The differences in removal patterns between winter and spring sample runs indicated that a number of separate mechanisms for phosphorus removal might have been operating. Interestingly, despite these differences the overall net removal during injection 1 was similar to that observed during injection 2.

The results for injection 2 needed to be interpreted with caution. At the time of analysis, a failure in the alum dosing plant resulted in abnormally high TP concentrations entering the wetland during the first few days of this injection. The high concentrations mixed with and were diluted, by lower concentrations already present in the wetland and entering the wetland after this incident. Although it was possible to assess the movement and changes in bromide concentration, it was only injected as a point source, on separate occasions and so could not fully account for all phosphorus mixing processes. In this instance mixing was likely to have had a significant effect on TP concentrations, as there would be considerable dilution of the elevated inlet phosphorus concentrations by water already present in the wetland.

This event provided the opportunity to evaluate how well the use of the ‘sudden injection’ method for a tracer would model such mixing processes and predict phosphorus removal or release. A similarity in TP removal results (Table 4-11) was expected between injections 2 and 3 due to the relatively short time interval between injection runs. The similarity of results would appear to support the sensitivity and validity of using tracer results (under the ‘sudden injection method’) to calculate TP removal and release, as done in this investigation.

Regions of phosphorus removal within Unit 4 appeared to be predominantly associated with regions of low flow (Sections 4.7.4 and 4.6.3). Therefore, contact time was considered as one of the primary issues affecting TP removal. Contact time can be maximised by providing regions of low flow or by increasing the wetland surface area. This would suggest that to remove a greater mass of phosphorus a larger wetland footprint might be necessary. If 0.2 to 0.5 mg.L\(^{-1}\) of TP can be removed over a distance of 27 m, it should be possible to increase the concentration of phosphorus removed by extending the wetland. However, for this to be done a greater
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understanding of internal wetland processes is needed to design the wetland for consistent maximum removal.

A comparison of phosphorus removal by the methods proposed by the NSW Department of Land and Water Conservation (DLWC 1998), equations by Kadlec and Knight, and Reed indicated that wetlands of this size should remove TP to 0.5 mg.L\(^{-1}\) over a distance of 27m. The much higher TP concentration and low removal rate observed for Unit 4, and the pilot plant wetlands in general would indicate that the equations were not applicable to these wetlands. Further, they would indicate that such equations should be interpreted with caution as they are based on North American soils. The majority of Australian soils differ from typical North American soils because of their more weathered nature, and higher aluminium and iron content.

The reasons for variable phosphorus removal and release patterns within Unit 4 in areas subjected to a similar flow were unclear. If removal was only associated with soil binding (Richardson 1985; Richardson et al. 1993; Richardson et al 1995), then a removal pattern similar to that observed during injection 1 should have been prevalent i.e. the amount of phosphorus removed becomes greater with distance into the wetland. However, during injections 2 and 3, this pattern was not observed (nor was it observed between T6 and the outlet for injection 1).

If soil binding were the primary phosphorus removal mechanism occurring within Unit 4 then it would be expected that removal would occur in an incremental manner with distance through the wetland. That this was observed in winter but not spring or summer indicates that it is more probable that a range of mechanisms were working and with seasonality, flow and other variables interacting to change the relative magnitude of the contribution from each mechanism. The lack of consistency in removal rates between different stands of plants within Unit 4, as divided by each transect, would imply that all plants and/or their microenvironments within a stand and between stands were not behaving identically. The reasons for this were unclear but may have been related to local variations in dissolved oxygen content of the soil.
or water column, biofilm growth, plant health, mass and type of detrital material or other factors.

The localised nature of these effects and the large variations observed between stands of the same plant types would indicate difficulties in the ability to control or manipulate the wetlands ability to retain phosphorus. Investigations by Kadlec (1997) support these observations. Understanding the nature of the interactions involved will provide valuable information on whether finer control of phosphorus removal is possible. To this end the following Chapters are devoted to understanding the relative contribution of detritus, deposited particulate material, microorganisms and soil to phosphorus removal processes within the Richmond pilot plant wetlands. Chapter 8 then provides a summary and collation of the conclusions from Chapters 2 through 7, to create an overall picture of phosphorus removal processes and future research directions.

4.9 CONCLUSIONS

4.9.1 Bromide
Bromide measurements at the wetland outlet indicated consistency in wetland flow and HRT between the 3 dye injections. Internal variations were apparent between and within transects throughout the wetland for median and minimum times of travel for the tracer. These appeared to be localised, suggestive of small "microsites" whose location varied between sample runs. Although the occurrence of these regions appeared randomly throughout each tracer injection run, they did not have a significant affect on the overall flow, nor phosphorus removal through each wetland. There were no obviously consistent regions of short-circuiting or dead zones during each run, although the HE and tracer results at individual locations indicated these did exist within the wetland. Wind and other factors appeared to influence water movement, to a similar degree as bulk flow within the wetland, possibly because of the low internal flow and shallow water depth. Overall, there appeared to be a significant amount of variability or "noise" within the wetland during each injection
run, but the outlet results reflected a dampening of this effect, with similar flow rates for each injection run.

The large variations and inconsistencies in the time for detection of the maximum tracer concentration meant that this measure was not a useful predictive tool of bromide and hence phosphorus movement. The time to final tracer elution varied significantly between injection runs and was only useful in confirming that dispersion of bromide increased with distance through the wetland, as predicted by tracer injection theory (Standards Australia 1991b). Bromide recovery results confirmed that bromide recovery did not change significantly between each transect of the wetland. However, there appeared to be a loss of tracer between the inlet and T1, most likely caused by some interaction of the tracer with components of the bulk flow. This did not affect recovery through the remainder of the wetland. Providing confidence in the ability of bromide to act as a conservative tracer to model TP movement. In addition, from recovery rates observed it could be concluded that for investigations of flow in constructed wetlands bromide was likely to be at least equivalent to lithium as a conservative tracer, and possibly greatly superior, supporting the conclusions of Netter et al. 1990.

The use of a positive displacement pump provided a marked improvement in reducing flow variations at the wetland inlet. However, this improved flow consistency was not reflected at individual sample sites within the wetland where the flow variance past each location was not significantly improved due to the number of localised, transient variations in flow that appeared unrelated to inlet flow variations. The exact quantitation of these regions was not seen as viable or necessary due to their transitory nature, their localised effect and limited influence on outlet conditions.

4.9.2 Phosphorus

From the data, it would appear that Unit 4 could achieve TP removal of between 0.2 to 0.5 mg.L⁻¹. Over a 2-year period (Chapter 2), such removal was sustainable, at least in the short term. From the investigations on Unit 4 it did not appear as though any single stand of plants or location within the wetland was consistently removing or
releasing phosphorus. Localised variations in TP removal, similar to variations seen in flow past individual locations within the wetland were observed. These varied with each injection run and produced a degree of internal “noise” – stochastic behaviour, which in the context of the entire wetland tended to be significantly moderated, such that net TP removal at the outlet appeared consistent between injection runs. Therefore looking internally one finds variations in removal, release, and mixing but at the outlet a “merged” result is obtained.

The calculation of TP concentrations based on bromide tracer results provided a consistent indication of wetland performance by minimising mixing and dispersion effects. TP removal using both this method and time displace inlet results indicated that TP removal was independent of inlet TP concentration. However, there was some correlation between regions of TP removal and regions of slower flow within Unit 4. Suggesting that increased contact time with material in the wetland was a dominant mechanism for the control of TP sequestering. The two means by which contact time can be increased are a reduction in flow rate or an increase in available surface area. There is a need for improved understanding of internal TP processes to improve and sustain TP removal processes. However, the extent to which this can be done requires more detailed investigation, as stochastic effects appeared to have only a slight impact on overall wetland performance.

However, the non-uniform flows through Unit 4 were not helpful in attempting to fully understand the fate and transport of phosphorus in this wetland. The continuously altering flow regimes meant that accurate prediction of total phosphorus removal was extremely difficult. These variations suggested that wind had a significant influence in determining flow patterns. Since wind effects would appear to be ubiquitous, the assumption of plug flow should be questioned as not being valid for for the majority of wetlands, because even a wetland with a surrounding wind break (e.g. the embankment surrounding Unit 4), a narrow aspect (length to width of greater than 5:1), and uniformly planted, demonstrated significant short circuiting. For these reasons, researchers in this area should not assume plug flow as being an accurate model of flows typically encountered in a small wetland system.
A noticeable variation in TP removal patterns was observed between autumn/winter and spring. This indicated two important points, that the processes occurring within the wetland at each time of year were significantly different and that adsorption to sediment was not the dominant removal mechanism at all times, as proposed by Richardson (Richardson 1985; Richardson et al. 1993; Richardson et al. 1995). However, the overall net TP removal rates for each time of year were not significantly different. This again highlights that stochastic processes occurring within the wetland need not have a significant effect on the overall TP removal. TP adsorption to sediment appeared to be a likely dominant factor in the autumn/winter run, however during spring other factors appeared to interfere with TP removal. These may have been litter fall, biodegradation, microbial activity at the sediment surface or a combination of these and possibly other factors. The possible role of litter and detritus in this process is investigated in Chapter 5.

Chapters 2 and 3 indicated a possible role for the soil, plants and algae in phosphorus sequestering and storage. The role of other microorganisms was uncertain, as was the role of suspended solids deposition and litter fall. Chapter 5 investigated the role deposited solids and plant litter in phosphorus sequestering, Chapter 6 investigated the likely role of algae and settleable material in the water column in transporting phosphorus to the sediment, and Chapter 7 focused on phosphorus sequestering in the soil. Through understanding the nature of the interactions involved, it should be possible to determine the extent to which finer control of phosphorus removal might be achieved. A summary of the interactions between all of these mechanisms and their likely significance is provided in Chapter 8.
CHAPTER 5: THE ROLE OF SETTLED MATERIAL FROM DETRITUS AND THE WATER COLUMN IN THE ACCUMULATION OF TP IN CONSTRUCTED WETLANDS

5.1 BACKGROUND

Previous investigations (Chapters 2, 3 and 4) indicated SS might have been a significant source of TP entering the water column of various plant stands within the pilot plant wetlands at Richmond. Short-term measurements of SS indicated random variations were occurring within each wetland, which did not correlate with plant stands, distance or other environmental parameters (Section 2.6.3 and 2.6.6). It may be possible that although significant short-term variations were occurring that over the longer term there were consistent and observable differences in the deposition or settling of material through the wetland. In addition, the role of detrital material in phosphorus accumulation was unclear, as raised in Chapter 4. It was planned that the dynamics of sedimemented material in four of the pilot plant wetlands (Units 2 through 5, refer Chapter 3 for rationale) would be observed over intervals of several months to determine the relative deposition/mobilisation of material in different sections of each wetland system. The material would be collected in plastic containers and the concentrations of carbon, nitrogen and phosphorus associated with deposited material used to estimate the relative contribution of biological and abiological components to phosphorus deposition at each site.

Investigation of the mass of settled plant detritus and other material was undertaken in several ways. Evaluation of recently fallen litter has been typically undertaken using mesh bags (or in one particular case through use of a Perspex box with a mesh base (Conner et al. 1992) of 1-3 mm pore size that can vary from 20 cm to 1 m square (Greenway 1994; Hill et al. 1982; Neckles et al. 1994). Alternatively, above ground biomass has been harvested in quadrats that have varied from 25 cm to 1 m square (Adcock et al. 1995; Bouchard et al. 1998; Ford et al. 1998). Study of settling on sediment surfaces has been done by using a disc to measure accretion in wetlands or regions that are periodically flooded (Llyod et al. 2000), however this technique is limited by plant density. An alternative technique has used an artificial soil-marker
horizon such as feldspar clay as the base level over which accumulation can be measured. These regions are relatively fragile and subject to animal destruction (Ford et al. 1998). Below ground biomass is typically evaluated by coring (Ford et al. 1998). Decomposition rates have been looked at, primarily using litter bags as noted or through bundling together of litter material using string or similar material (Ryder et al. 1995). Alternatively some studies have looked at using cylindrical containers to observe litter decay (Rice et al. 1981; Ryder et al. 1995).

In the current investigation, it was desired to understand the total contribution of detrital material, particularly as senescence set in. The role of algae and smaller floating plants such as Azolla spp. and Lemna spp. were to be included if possible. Anecdotally, degrading detrital material within the pilot plant wetlands was extremely fragile, breaking apart with only slight disturbance. To capture all of these factors, a bag or quadrat type of sampling was deemed not suitable, as much of the material under investigation would leach through the pores, or not be captured by harvesting.

The placement of a solid disk on the sediment surface was possible, but this could only be of small dimension because of the planting density. However, removal of such a disk for analysis was seen to result in the resuspension of settled material into the surrounding water column during removal, despite careful handling. This was undesirable. Therefore, a walled container was selected for collection of deposited material. It was noted that such a container could act to accumulate biofilm, and hence bias the results. To counter this problem a control container, suspended in an inverted position was used to measure the extent of biofilm growth (Section 5.3.1).

Since no references were available for such a technique, a preliminary study, described below, was conducted in Units 2 and 3 to evaluate an appropriate time span for evaluation of accumulated materials. Once an appropriate timeframe had been determined, a full-scale investigation of four wetland Units (Units 2 through 5) was commenced. Samples for the full-scale study were taken over two seasons – spring and autumn: spring was selected as the period of greatest growth and hence
degradative potential; and autumn was selected as it was the most likely period for litter fall and commencement of plant senescence (Section 1.5.4).

5.2 AIM

Investigations in this chapter were designed to elucidate the mass and composition of sedimented material within four of the pilot plant wetlands at Richmond. Through examination of this material it should be possible to evaluate its relative contribution and hence significance to phosphorus cycling within these wetlands. Compositional analysis of the material would allow evaluation of the relative contribution of biological components in this process.

5.3 METHODOLOGY

5.3.1 Preliminary trials

An initial pilot study was proposed to evaluate the mass of material deposited in Units 2 and 3 of the Richmond pilot plant wetlands over a one-month period and determine associated concentrations of phosphorus, carbon and nitrogen. Duplicate samples were obtained from each stand of plants in Units 2 and 3 (14 samples per Unit). The accumulation of material from the water column and detrital material was determined by the placement of 70 mL sample containers (internal diameter 5 cm, height 7 cm, of polyethylene plastic - Figure 5-1). Larger containers were not feasible because of the shallow water depth (20 cm) and plant density.

Figure 5-1: sampling apparatus for in-situ detection of detrital material (not to scale).
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All sample containers were weighed prior to placement in the wetland. Containers were grouped into pairs, one control and one for sample collection, attached to wooden rods (0.5 cm diameter, 0.5 m length) marked with white tags to facilitate subsequent detection. The containers were attached to each rod by a length of steel wire (1 mm diameter). The two containers were positioned opposite each other: the sample collection container having the opening directed towards the water surface; and the control with the opening facing the sediment surface (Figure 5-1). The sample collection cup was oriented to allow collection of SS and detrital material settling from the water column. The control container was oriented to towards the sediment surface to avoid collection of SS and enable monitoring of the accumulated biofilm on the container surface. The wooden poles were situated so that lowest part of both containers was placed in the water column, approximately 2-3 cm above the sediment surface. This arrangement was to minimise the potential for collection of material from the sediment surface whilst maximising the retention of material from the water column.

At the end of the time period containers were carefully removed from the water column. This was most effectively achieved by removing the wooden pole and attached containers to the bank of the wetland where the sample cups were carefully detached from the wire clips. It was desirable not to decant or siphon the contents of the sample container as this may have resulted in disturbance or loss of finer detrital or other settled material. The entire content of each cup was retained and the cups placed in a drying oven at 50°C for 3 days (sample cups were of plastic and unable to withstand exposure to higher temperature). These conditions were a compromise. Rapid removal of the water was desirable but difficult to achieve without significant disturbance of the settled solids and biofilm material. Whilst it was appreciated that drying at this temperature may have resulted in some loss of volatile material, slow drying at a lower temperature could result in greater loss of semi-volatile material because of the longer time interval involved, whilst high temperatures would melt the sample cup. Note that in the published literature drying temperatures for plant and detrital material ranged from 22 to 105°C (Bouchard et al. 1998; Ford et al. 1998; Ganf et al. 1994; Greenway 1994; Hill et al. 1982; Neckles et al. 1994).
After drying, sample cups were cooled and reweighed to determine the dry weight of material collected. The material in the containers was ground to a fine powder in a mortar using a pestle. This powder was then analysed for TP by the molybdate blue method, TN and TC by CNS (as detailed in Section 2.4.8).

It was acknowledged that this process would result in collection of nutrients and SS from material settled in the sample container, biofilm on the sample container and material present in the water phase within the container. There was a significant problem with separating the water column fraction from the settled fraction. The primary methods to achieve such separation were on the basis of size i.e. dissolved vs settled, or removal of the liquid phase. However, it was likely that SS material from the water column would separate with the settled material in any size-based separation, biasing results. Any TP in the water phase would appear in the larger size fraction of such a separation, being counted as settled material. Simple removal of the water phase was not possible due to the extremely fragile nature of the detrital material, which appeared to fracture and dissolve with even the mildest of agitation.

Therefore, it was not possible to separate the solid from the liquid phase and the two were analysed as a composite. Hence results obtained represented an overestimate of the mass of material accumulated i.e. the mass of phosphorus present in detrital material was only a fraction of the total phosphorus mass obtained in a sample container. Therefore if the mass of phosphorus observed for the total amount of material collected was not significant in phosphorus sequestering then the mass bound in detrital material would be even less so. If the mass of deposited material proved to be a significant component then further investigations would be required to quantify the contribution of each contributing compartment.

Obtaining samples in this manner also meant that the final results needed to be interpreted with caution. An extrapolation of water column concentrations was possible by taking an average of inlet and outlet wetland concentrations to establish a baseline against which to compare sources of observed material. Average values for
each period were taken. Since there was likely to be a difference between inlet and outlet values the lower of the two was taken, for a given wetland and measurement time interval. This was to allow for the most favourable ratio of material being present in the solid (detrital and biofilm) phase. The data from this analysis is presented at the end of the results section, after the analysis for each wetland Unit.

5.3.2 Seasonal investigations of deposition
For the full scale investigation the procedure for the preliminary study (Section 5.3.1) was repeated with the following modifications:

- Four instead of two Units were investigated (Units 2 to 5).
- Sample containers were kept in the wetlands for two months instead of one, to increase the mass of sample collected and reduce the sample variability.
- Three containers were placed per stand of plants, with the exception of the final Phragmites stand, which only received two sample containers due to its smaller size (a total of 20 containers per wetland).
- Assessment of deposition was undertaken in spring and autumn, the periods of highest solids and litter turnover. Autumn samples were taken during April and May, spring in October and November.

5.4 RESULTS

5.4.1 Preliminary investigations of deposition (Units 2 and 3 only)

Unit 2
The preliminary investigation of accumulated solids material in Unit 2 produced only tentative results, as insufficient material was deposited to generate data for all plant stands. There was an apparent association between the net mass of solids accumulated (termed net mass or NM) and plant type, but not distance ($r = 0.79$ and $-0.06$ respectively, Figure 5-2). A high degree of correlation was also observed for TC, TN and TP with plant type ($0.94 > r > 0.75$) but not of these parameters against distance ($r < 0.20$, Figure 5-2). Accumulation of NM, TN and TC was greatest in the two stands of Triglochin (S3 and S5), lower in stands of Phragmites (S1 and S4), with lowest
accretion in the stands of *Schoenoplectus* (S2 and S6) and the *Phragmites* section (S7). TP was only observed in stands of *Triglochin*. The association of NM with TC, TN and TP was supported by high correlation coefficients ($r = 0.87, 0.94$ and $0.96$ respectively). There was also good correlation of TC with TN and TP ($r = 0.98$ and $0.77$) and TN with TP ($r = 0.89$).

Figure 5-2: Composition of material deposited within Unit 2 over a one-month period during preliminary investigations.

![Graph showing the composition of material deposited within Unit 2 over a one-month period during preliminary investigations.](image)

*NM refers to the net mass of settled material deposited in each sample container.*

The percentage composition of TN and TC deposited in Unit 2 differed between emergent and submergent plant species. The percentages of TN and TC were highest in stands with emergent vegetation S1, S4 and S6 (*Phragmites* and *Schoenoplectus*) and lower in the stands of *Triglochin* (S3 and S5, Table 5-1).

Table 5-1: Percentage total carbon, total nitrogen and total phosphorus in material deposited within Unit 2 over a one-month period during the preliminary investigation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
</tr>
</thead>
<tbody>
<tr>
<td>% TC</td>
<td>17.6 ± 11.1</td>
<td>-</td>
<td>8.68 ± 0.01</td>
<td>29.5 ± 5.14</td>
<td>15.9 ± 1.40</td>
<td>44.0 ± 16.9</td>
<td>-</td>
</tr>
<tr>
<td>% TN</td>
<td>2.36 ± 1.00</td>
<td>-</td>
<td>1.15 ± 0.17</td>
<td>2.74 ± 0.12</td>
<td>1.85 ± 0.17</td>
<td>4.80 ± 1.85</td>
<td>-</td>
</tr>
<tr>
<td>% TP</td>
<td>0.88 ± 1.90</td>
<td>-</td>
<td>0.26 ± 1.64</td>
<td>0.33 ± 0.63</td>
<td>0.31 ± 0.07</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* - insufficient sample to generate a result, when sample concentrations were corrected for the water column component.
CHAPTER 5

There was a strong positive correlation between percentage TN and TC in each sample ($r = 0.98$). There was a strong negative correlation between the percentage of TN and TC in each sample and the concentration of TP ($r = -0.76$ and -0.87). In samples where TP was detected, the percentage of phosphorus varied between 0.3 to 0.6% of the NM.

**Unit 3**

In the preliminary investigation of Unit 3, there was no correlation between the NM and plant species ($r = 0.34$). However, from the inlet through to the end of S5 there was an increase in the NM at each distance (Figure 5-3). Accumulation of TC and TN was greatest in S3, S4 and S5 (the central plant stands of Unit 3), achieving a maximum within S4 (*Phragmites*, Figure 5-3). Deposition of TP was greatest in the two stands of *Triglochin* (S3 and S5, Figure 5-3). There was a slight but significant correlation of NM with TC and TN ($r = 0.66$, 0.78 respectively) but not TP ($r = 0.20$). There was a very high correlation between TC and TN ($r = 0.95$).

**Figure 5-3:** Composition of material deposited within Unit 3 over a one-month period during preliminary investigations

![Composition of material deposited within Unit 3](image)

NM refers to the net mass of settled material deposited in each sample container.
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However, there was no correlation between TP and TC or TN ($r = -0.19$ and 0.01 respectively), contrasting sharply with the results for Unit 2.

In terms of percentage deposition there was a strong positive correlation between TC and TN ($r = 0.89$). Highest percentage composition of TC and TN in deposited material occurred in the central and end stands of *Phragmites* (S4 and S7), with the greatest variability in the two stands of *Schoenoplectus* (S2 and S6, Table 5-2). Consistently low percentages of TN and TC were observed in the two stands of *Triglochin* (Table 5-2). Note that stands that did not produce sufficient NM to detect TN and TC were excluded from this analysis. There was no correlation between the percentage of phosphorus in a sample and the percentage TC or TN ($r < 0.20$). In stands where phosphorus was detected, it ranged from 0.1 to 0.4% of the sample mass (Table 5-2). The maximum percentage TP deposition occurred in S3 (*Triglochin*) and S7 (*Phragmites*). Both the percentage and actual mass of TP deposited varied independently of NM, TC, TN, plant type and distance. However, TP was only detected once the NM reached 116 mg or greater, similar to Unit 2 where TP was detected when greater than 156 mg of deposited material was present.

Table 5-2: Percentage total carbon, total nitrogen and total phosphorus in material deposited within Unit 3 over a one-month period during the preliminary investigation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
</tr>
</thead>
<tbody>
<tr>
<td>% TC</td>
<td></td>
<td>9.5 ± 6.2</td>
<td>10.7 ± 2.7</td>
<td>23.7 ± 0.5</td>
<td>7.6 ± 0.75</td>
<td>42.1 ± 27.1</td>
<td>17.7 ± 11.7</td>
</tr>
<tr>
<td>% TN</td>
<td></td>
<td>1.17 ± 1.99</td>
<td>1.52 ± 0.71</td>
<td>1.86 ± 0.12</td>
<td>0.81 ± 0.10</td>
<td>2.47 ± 1.59</td>
<td>1.65 ± 1.28</td>
</tr>
<tr>
<td>% TP</td>
<td></td>
<td>-</td>
<td>0.37 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>0.11 ± 0.05</td>
<td>-</td>
<td>0.28 ± 0.17</td>
</tr>
</tbody>
</table>

- Insufficient sample to generate a result, when sample concentrations were corrected for the water column component.

Summary of results

The deposition pattern in the ‘preliminary investigation’ indicated that the average mass of NM, TC, TN and TP found in containers placed within Unit 3 was more than twice that observed in Unit 2 (Figure 5-4). This difference was statistically significant (t-test, $\alpha = 5\%$).

Differences were also observed between Units in the distribution of NM, which was correlated with plant type in Unit 2, but evidenced a correlation with distance through
the first five plant stands adjacent the inlet in Unit 3. In Unit 2, the maximum deposition of TC and TN occurred in the two stands of *Triglochin* (S3 and S5), while in Unit 3 the maximum deposition of TC and TN occurred in S4 (*Phragmites*), a region of low deposition within Unit 2. The distribution of TP deposition in both Units was similar, with high amounts of phosphorus observed in the two stands of *Triglochin* (Figure 5-2 and Figure 5-3).

**Figure 5-4:** Summary of average net mass of material deposited, total carbon, total nitrogen, total phosphorus (with standard deviations) accumulated over one month in wetland Units 2 and 3 during the preliminary investigation (plotted using a logarithmic scale).

![Graph showing deposition](image)

*NM refers to the net mass of settled material deposited in each sample container.*

### 5.4.2 Seasonal investigations of deposition (Units 2 through 5, spring and autumn)

**Unit 2**

**Autumn 1995**

The seasonal investigation of deposited material commenced in autumn 1995. In Unit 2, during this season, there was slight correlation between the average NM and plant species but not distance (r = 0.62 and 0.11 respectively, Figure 5-5). However, due to the high sample variance the differences between each stand of plants were not
significant at the $\alpha = 0.05$ or 0.1 confidence levels, using the Kruskal-Wallis (K-W) test (the K-W test was selected over a one way ANOVA because of the heterogeneity of the sample variances (Zar 1996), Section 9). There was no association of TC, TN or TP with distance (-0.20 < $r$ < -0.13), and varying degrees of association with plant type ($r = 0.66, 0.93, 0.91$ respectively). Due to the large variation between samples there were no significant differences in the mass of TC, TN or TP deposited within different plant stands through Unit 2 during autumn ($\alpha = 0.01$, K-W test, Figure 5-5). The average mass of TP accumulated was highest in the stands of *Triglochin* (S3 and S4) and lowest in the stands of *Phragmites* (S1, S4 and S7). The NM correlated well with TP ($r = 0.87$) but only slightly with TN ($r = 0.70$) and not with TC ($r = 0.49$). The correlation between TN and TC was again evident although the value was lower than observed in preliminary investigations ($r = 0.86$). TN also correlated highly with TP ($r = 0.94$), although a much lower correlation was observed between TP and TC ($r = 0.68$).

Figure 5-5: Composition of material deposited within each plant stand of Unit 2 over a two-month period during autumn 1995

In percentage terms there was no correlation of TC, TN or TP from settled material with distance, however an association with plant type was observed that was slightly different to that for total deposition (Table 5-3). Percentages of TC and TN deposited were lowest within stands of *Triglochin* (S3 and S5) and the final *Phragmites* section
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(S7) adjacent the outlet (Table 5-3). There was a high correlation between the percentage of TN and TC (r = 0.92). The percentage of TP was consistent through the wetland, except at S7 where the percentage composition decreased (Table 5-3), otherwise the percentage distribution of TP was not significantly affected by distance or plant type.

Table 5-3: Percentage total carbon, total nitrogen and total phosphorus in settled material within Unit 2 over a two-month period during autumn 1995.

<table>
<thead>
<tr>
<th>Compound</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
</tr>
</thead>
<tbody>
<tr>
<td>% TC</td>
<td>32.2 ± 4.2</td>
<td>36.9 ± 1.9</td>
<td>15.0 ± 6.7</td>
<td>35.0 ± 6.3</td>
<td>15.4 ± 3.1</td>
<td>37.8 ± 3.9</td>
<td>6.7 ± 2.5</td>
</tr>
<tr>
<td>% TN</td>
<td>2.4 ± 0.6</td>
<td>2.6 ± 0.1</td>
<td>1.6 ± 0.6</td>
<td>2.5 ± 0.7</td>
<td>1.9 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>0.32 ± 0.13</td>
</tr>
<tr>
<td>% TP</td>
<td>0.17 ± 0.06</td>
<td>0.13 ± 0.05</td>
<td>0.13 ± 0.06</td>
<td>0.14 ± 0.08</td>
<td>0.14 ± 0.05</td>
<td>0.11 ± 0.05</td>
<td>0.05 ± 0.01</td>
</tr>
</tbody>
</table>

Spring 1995

In the sampling from Unit 2 during spring, there was no correlation of NM, TC, TN and TP with distance (0.01 < r < -0.24). There was a significant correlation of NM, TC, TN (r = 0.56, 0.56 and 0.69, respectively) but not TP (r = 0.29) with plant type. There was a significant difference between the NM deposited in each plant stand (α = 0.05, K-W test). The NM was lowest in stands of Phragmites (S1, S4 and S7. Figure 5-6). Deposition in other regions was higher but the NM did not differ significantly between these regions (Dunn's Q Test, α = 0.1).

Figure 5-6: Composition of material deposited within Unit 2 over a two-month period during spring 1995

Despite the correlation of TC and TN deposition with plant type, the differences observed were not significant (α = 0.1, K-W test). There were no significant
differences in the mass of TP deposited in each plant stand within Unit 2 during spring 1995 (\(\alpha = 0.1\), K-W test). TN and TC had a high correlation (\(r = 0.96\)), while TP evidenced no correlation with NM, TN or TC (\(r > -0.17\)).

Table 5-4: Percentage total carbon, total nitrogen and total phosphorus in material deposited within Unit 2 over a two-month period during spring 1995.

<table>
<thead>
<tr>
<th>Compound</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
</tr>
</thead>
<tbody>
<tr>
<td>% TC</td>
<td>30.8 ± 4.7</td>
<td>25.7 ± 17.4</td>
<td>8.04 ± 1.83</td>
<td>57.5 ± 38.7</td>
<td>15.3 ± 1.9</td>
<td>44.2 ± 16.8</td>
<td>32.8 ± 15.7</td>
</tr>
<tr>
<td>% TN</td>
<td>2.76 ± 0.43</td>
<td>1.89 ± 1.00</td>
<td>0.89 ± 0.14</td>
<td>3.95 ± 2.82</td>
<td>1.65 ± 0.25</td>
<td>3.52 ± 1.88</td>
<td>2.84 ± 1.09</td>
</tr>
<tr>
<td>% TP</td>
<td>0.33 ± 0.21</td>
<td>1.32 ± 2.16</td>
<td>0.22 ± 0.35</td>
<td>0.56 ± 0.62</td>
<td>0.07 ± 0.02</td>
<td>0.12 ± 0.09</td>
<td>0.29 ± 0.07</td>
</tr>
</tbody>
</table>

The percentage of distribution of TC and TN evidenced a high correlation with each other (\(r = 0.98\)), but negative association with plant type (\(r = -0.74, -0.77\)) and no correlation with distance (\(r = 0.3, 0.23\)). The lowest proportions of each were located in the two stands of Triglochlin (S3 and S5, Table 5-4). There was no significant difference in the percentage distribution at other sites (\(\alpha = 0.1\), Dunn's Q test). Relative proportions of TP associated with NM did not correlate with plant type, distance or sample mass (-0.06> \(r > -0.47\)). Sites of high TP deposition, S2 (Schoenoplectus) and S4 (Phragmites), also evidenced large variations in NM (Table 5-4).

A comparison of deposition in Unit 2 during autumn and spring indicated that the NM correlated with plant species but not distance for each period. This correlation was only significant during spring, with Phragmites representing the regions of lowest deposition. This result was also observed in the preliminary investigation. There was a correlation of TC, TN and TP with plant type in autumn and TN, TC with plant type in spring, although the high variance of the data precluded the observation of significant differences between stands at this sampling frequency. TC, TN and TP were lowest in stands of Phragmites during autumn, corresponding to data from the preliminary study. Percentages of TN and TC were associated with plant type at both sampling times, with highest percentages in stands of Phragmites and Schoenoplectus, lowest in stands of Triglochlin. There was no association between the percentage of TP deposited and plant type, distance, NM, TC or TN during spring.
Unit 3

Autumn 1995

Analysis of material collected from Unit 3 in autumn 1995 indicated that there was no correlation of the NM, TC or TN with distance or plant type ($0.13 > r > -0.39$). There were also no significant differences in the mass of these substances between plant stands (K-W test, $\alpha = 0.10$, Figure 5-7). Deposition of TP was not correlated with plant type ($r = -0.22$) or distance ($r = -0.27$). The overall trendline for TP indicated a net decrease in the mass deposited with distance. The NM showed a very slight correlation with TN or TC ($r = 0.54$, 0.46) and evidenced a moderate correlation with TP ($r = 0.67$). Consistent with previous results, the correlation between TC and TN was high at $r = 0.96$. TP evidenced a moderate correlation with TC or TN ($r = 0.51$, 0.66).

Figure 5-7: Composition of material deposited within Unit 3 over a two-month period during autumn 1995

From the percentage of TC, TN and TP in deposited material within Unit 3 a number of trends emerge (Table 5-5). The percentage of TC and TN correlated quite highly ($r = 0.97$) and were related to plant type ($r = 0.65$, 0.64, respectively). The lowest percentages of TN and TC were in S3, S5 (Triglochin) and S7 (open water/Phragmites), highest were in S2 and S6 (Schoenoplectus), with percentages for the two other Phragmites stands (S1 and S4) only slightly lower (Table 5-5). This result was similar to that for Unit 2 in autumn. Except for the initial stand of plants (S1 - Phragmites) with elevated phosphorus concentrations, the remainder of the
wetland evidenced a relatively consistent association of TP with NM (Table 5-5), with TP comprising between 0.06 to 0.13 % of the total dry mass. There was no correlation between the percentage of TP detected and the percentages of TC or TN in any sample (r < 0.23).

Table 5-5: Percentage total carbon, total nitrogen and total phosphorus in material deposited within Unit 3 over a two-moth period during autumn 1995.

<table>
<thead>
<tr>
<th>Compound</th>
<th>SI</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
</tr>
</thead>
<tbody>
<tr>
<td>% TC</td>
<td>22.3±1.80</td>
<td>32.7±3.0</td>
<td>14.3±5.0</td>
<td>25.3±11.6</td>
<td>18.6±9.1</td>
<td>33.1±7.1</td>
<td>11.8±12.5</td>
</tr>
<tr>
<td>% TN</td>
<td>1.9±0.5</td>
<td>2.4±0.1</td>
<td>1.5±0.4</td>
<td>1.8±0.7</td>
<td>1.8±0.7</td>
<td>2.3±0.1</td>
<td>1.2±1.3</td>
</tr>
<tr>
<td>% TP</td>
<td>0.36±0.16</td>
<td>0.06±0.003</td>
<td>0.09±0.003</td>
<td>0.13±0.10</td>
<td>0.08±0.03</td>
<td>0.13±0.17</td>
<td>0.11±0.09</td>
</tr>
</tbody>
</table>

**Spring 1995**

During the spring sampling of Unit 3, there was no correlation of the NM, TC and TN with distance (-0.27 < r < -0.32, Figure 5-8). Deposition of TP did not follow this trend, evidencing a slight negative correlation with distance (r = -0.49). TP decreased with distance into the wetland until S5, fluctuating randomly thereafter (Figure 5-8). No correlation was observed between NM, TC, TN or TP and plant type (-0.20 < r < 0.04). The K-W test indicated that differences observed in the NM, TC, TN and TP deposited in each plant stand were not significant (α = 0.10). The NM correlated well with TC and TN (r = 0.91, 0.97) but slightly less so with TP (r = 0.57). TN and TC correlated closely with each other (r = 0.95).

In Unit 3, there was no measured association of distance or plant type with TC, TN or TP (Table 5-6, -0.29 < r < -0.15). However, there was a good correlation between percentage deposition of TC and TN (r = 0.84) but not between these nutrients and TP (-0.33 < r < -0.22). Percentage deposition of TP though Unit 3 was highest in S1, with the percentage composition fluctuating randomly thereafter (Table 5-6), in contrast to samples collected during autumn.
In Unit 3 during autumn, there was a slight correlation of NM, TC and TN with plant type. This was replaced by a negative correlation with distance during spring. However, it was not possible to conclude the significance of this variation due to the high sample variance. The NM correlated with TP in autumn, and TC and TN during summer. TP appeared independent of TC and TN at both times. There was no correlation of TP with plant type in autumn but an overall trend towards decreasing deposition with distance. There was no correlation between the NM, TC and TN with distance or plant type within Unit 3 during spring or autumn. However, for TP there was a trend towards decreasing deposition with distance into the wetland in both autumn and spring.

Table 5-6: Percentage total carbon, total nitrogen and total phosphorus in material deposited within Unit 3 over a two month period during spring 1995.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plant stand</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1</td>
</tr>
<tr>
<td>% TC</td>
<td>23.6±8.3</td>
</tr>
<tr>
<td>% TN</td>
<td>1.06±0.47</td>
</tr>
<tr>
<td>% TP</td>
<td>1.58±1.44</td>
</tr>
</tbody>
</table>
As with other units, the percentage of TC and TN correlated well at both sampling times. The percentages of TC and TN were related to plant type in autumn but not spring. The percentage of TP deposited was consistently highest in S1 (Phragmites) with no consistency in the percentage deposited thereafter between each season.

Unit 4

Autumn 1995

Deposition of NM and TC did not readily correlate with distance ($r = -0.17, 0.11$) or plant type ($r = 0.34, 0.24$, Figure 5-9). TP deposition showed no correlation with plant type ($r = -0.22$) and a very slight negative correlation with distance ($r = -0.37$) corresponding to an overall trend for decreasing deposition through the wetland. Deposition of TN did not directly correlate with plant type or distance ($r = -0.02, -0.31$) and was highest in S2 (Schoenoplectus), but lowest in S5 (Schoenoplectus) and S7 (Phragmites).

Figure 5-9: Composition of material deposited within Unit 4 over a two-month period during autumn 1995

Despite these variations, NM correlated moderately with TP and TC ($r = 0.66$) but not TN ($r = 34$). The correlation between TC and TN was much less than in Units 2 and 3 during this sampling period ($r = 0.68$). Correlations of TP with TC and TN were maintained at a low value ($0.37 < r < 0.42$).
The percentage composition of deposited material within the wetland did not evidence any trends with distance or plant species (Table 5-7). TC and TN evidenced random fluctuations but unlike other units, there was no correlation between the percentage values ($r = 0.27$). For TP the highest percentage deposition occurred in the two plant stands adjacent the inlet (S1 and S2) before reaching a uniform percentage through the remainder of the wetland (Table 5-7).

Table 5-7: Percentage total carbon, total nitrogen and total phosphorus in material deposited within Unit 4 over a two month period during autumn 1995.

<table>
<thead>
<tr>
<th>Compound</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
</tr>
</thead>
<tbody>
<tr>
<td>% TC</td>
<td>20.4±17.8</td>
<td>22.3±6.8</td>
<td>10.4±9.9</td>
<td>35.4±5.8</td>
<td>24.4±15.7</td>
<td>37.0±0.4</td>
<td>28.2±7.7</td>
</tr>
<tr>
<td>% TN</td>
<td>3.3±3.7</td>
<td>5.4±3.1</td>
<td>1.4±1.2</td>
<td>2.5±2.2</td>
<td>&lt;0.5</td>
<td>2.0±2.2</td>
<td>0.8±1.1</td>
</tr>
<tr>
<td>% TP</td>
<td>0.28±0.26</td>
<td>0.14±0.10</td>
<td>0.09±0.11</td>
<td>0.07±0.04</td>
<td>0.07±0.02</td>
<td>0.04±0.02</td>
<td>0.06±0.02</td>
</tr>
</tbody>
</table>

Spring 1995

Observations of material deposited in Unit 4 during spring 1995 indicated there was no significant correlation of the NM, TN, TC or TP with distance into the wetland (-0.23 < $r$ < -0.16) or plant species (0.12 < $r$ < 0.34). Using the K-W test there was no significant difference between the NM, TC, TN or TP within different regions of the wetland (Figure 5-10). A very high correlation was observed for TC, TN and TP with NM ($r = 0.94, 0.97, 0.96$ respectively). TN and TC were also highly correlated ($r = 0.98$). TP correlated well with both TC and TN ($r = 0.86$ and 0.93). The overall trend for TP was for decreasing deposition with distance into the wetland (Figure 5-10).
Figure 5-10: Composition of material deposited in Unit 4 over a two-month period during spring 1995

NM refers to the net mass of settled material deposited in each sample container.

Percentage distribution of TC, TN and TP for Unit 4 in spring (Table 5-8) indicated a high correlation of TC with TN \((r = 0.92)\), in contrast to the autumn results. Variations in the percentage composition of TC and TN were not associated with either distance or plant type. Percentage of TP reached a maximum in S2 from a high at S1, remaining lower but constant through the remainder of the wetland (Table 5-8), as was observed during autumn.

Table 5-8: Percentage total carbon, total nitrogen and total phosphorus in material deposited within Unit 4 over a two-month period during spring 1995.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plant stand</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1</td>
<td>S2</td>
<td>S3</td>
<td>S4</td>
<td>S5</td>
<td>S6</td>
<td>S7</td>
</tr>
<tr>
<td>% TC</td>
<td>39.9 ± 1.8</td>
<td>19.0 ± 3.7</td>
<td>35.5 ± 11.3</td>
<td>19.2 ± 27.2</td>
<td>19.2 ± 6.9</td>
<td>42.4 ± 6.9</td>
<td>12.6 ± 17.8</td>
</tr>
<tr>
<td>% TN</td>
<td>2.67 ± 0.62</td>
<td>1.84 ± 0.44</td>
<td>3.21 ± 1.36</td>
<td>1.24 ± 1.75</td>
<td>1.57 ± 0.59</td>
<td>2.80 ± 0.76</td>
<td>0.83 ± 1.17</td>
</tr>
<tr>
<td>% TP</td>
<td>0.34 ± 0.14</td>
<td>0.44 ± 0.09</td>
<td>0.19 ± 0.13</td>
<td>0.10 ± 0.13</td>
<td>0.20 ± 0.02</td>
<td>0.15 ± 0.14</td>
<td>0.16 ± 0.07</td>
</tr>
</tbody>
</table>

Correlation was evidenced between NM and plant type during the spring sampling event. In autumn there was a low correlation between TC and TN, this altered in spring to a high correlation. During autumn only NM correlated with TP but during spring this correlation was extended to TC and TN. TP evidenced an overall trend towards decreasing deposition from inlet to outlet in both seasons. The percentage composition of TP in NM was highest in the initial two plant stands S1 and S2 but relatively constant thereafter for both sample periods, similar to autumn results for Unit 3.
Unit 5

Autumn 1995

Throughout Unit 5 during autumn 1995, there was no correlation between NM, TC or TN and either plant type or distance (-0.08 > r > -0.17, and 0.25 < r < 0.34 respectively). However, TP evidenced a slight correlation with plant type (r = 0.48) but not distance (r = -0.03). The detected masses of TC, TN and TP correlated well with NM in each section of the wetland (r = 0.96, 0.80, 0.90) but there was only a significant difference (K-W, α = 0.01) between sites in the distribution of TN and TP not NM or TC. The highest masses of TN and TP were deposited in the open water section (S7), both stands of Triglochin (S3 and S4) and one stand of Schoenoplectus (S2, Figure 5-11). The lowest amounts of TN and TP were deposited in the two stands of Phragmites (S1 and S4), and the second stand of Schoenoplectus. Despite the absence of significant differences in the deposited mass of TC, it evidenced a high correlation with TN and TP (r = 0.87, 0.89).

Figure 5-11: Composition of material deposited within Unit 5 over a two-month period during autumn 1995

There was a correlation between the percentage of TN, TC and TP in material deposited at each site within Unit 5 (0.79 > r > 0.68), but not of these parameters with distance or plant type (Table 5-9). The greatest deposition of TP occurred in the two
stands of *Triglochin*, with little difference between other sites in the wetland (Table 5-9). There was no significant difference between the percentage distribution of TC and TN through Unit 5 during autumn 1995 because of the high sample variance ($\alpha = 0.1$, K-W).

Table 5-9: Percentage total carbon, total nitrogen and total phosphorus in material deposited within Unit 5 over a two-month period during autumn 1995.

<table>
<thead>
<tr>
<th>Compound</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
</tr>
</thead>
<tbody>
<tr>
<td>% TC</td>
<td>27.2 ± 3.2</td>
<td>26.6 ± 5.0</td>
<td>52.9 ± 35.3</td>
<td>13.8 ± 18.4</td>
<td>22.3 ± 1.4</td>
<td>35.6 ± 3.8</td>
<td>28.8 ± 1.0</td>
</tr>
<tr>
<td>% TN</td>
<td>4.8 ± 4.7</td>
<td>2.1 ± 0.74</td>
<td>5.2 ± 3.9</td>
<td>0.58 ± 1.00</td>
<td>4.2 ± 2.9</td>
<td>3.2 ± 0.3</td>
<td>3.20 ± 0.3</td>
</tr>
<tr>
<td>% TP</td>
<td>0.18 ± 0.05</td>
<td>0.12 ± 0.04</td>
<td>0.34 ± 0.21</td>
<td>0.12 ± 0.12</td>
<td>0.24 ± 0.21</td>
<td>0.17 ± 0.02</td>
<td>0.15 ± 0.02</td>
</tr>
</tbody>
</table>

**Spring 1995**

NM, TC, TN and TP through Unit 5 in spring did not correlate with distance, evidencing only slight correlation with plant species ($r = 0.47$, 0.30, 0.40, 0.35 respectively). The NM correlated highly with TC, TN and TP ($r = 0.92$, 0.93, 0.78, Figure 5-12) but because of sample variance TN was the only parameter of the four to evidence significant difference between sites ($\alpha = 0.1$, K-W test).

**Figure 5-12:** Composition of material deposited within Unit 5 over a two-month period during spring 1995

<table>
<thead>
<tr>
<th>NM</th>
<th>TC</th>
<th>TN</th>
<th>TP</th>
</tr>
</thead>
</table>
| ![Bar chart](chart.png)

NM refers to the net mass of settled material deposited in each sample container.

The mass of TN deposited increased through S1 to S3 with random fluctuations thereafter. Lowest masses of TN were accumulated in the second stands of
Phragmites and Schoenoplectus (S4 and S6, Figure 5-12). TP also correlated well with TN and TC (r = 0.82, 0.75), as did TC with mass of TN (r = 0.97).

There was no correlation between plant type or distance and percentage composition of material in Unit 5 during spring. Percentage composition of material indicated correlation between TC and TN (r = 0.96) with peaks occurring in S4, S6 and S7 (Table 5-10). The percentage of TP was highest in S1 and S6 although the large standard deviation of the results prevented this difference from being significant. The lowest percentage of TP was deposited in S3 (Triglochin, Table 5.10).

Table 5-10: Percentage total carbon, total nitrogen and total phosphorus in material deposited within Unit 5 over a two-month period during spring 1995.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plant Stand</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1</td>
</tr>
<tr>
<td>% TC</td>
<td>18.5 ± 5.3</td>
</tr>
<tr>
<td>% TN</td>
<td>1.76 ± 0.73</td>
</tr>
<tr>
<td>% TP</td>
<td>0.28 ± 0.22</td>
</tr>
</tbody>
</table>

Throughout Unit 5 during autumn, there was no correlation of NM, TC and TN with plant species with only a mild association during spring. TP deposition was mildly correlated with plant type during autumn. The pattern of TN deposition was similar in both autumn and spring and corresponded to that for NM, TC and TP, with greatest deposition in S2 and S3, lowest in S4 (Phragmites). The correlations between TC and TN were high at both sample times.

5.4.3 Summary of combined results
A summary of all results, with masses of deposited material (NM, TP, TC and TN) corrected for the amount likely to have been present in the water column (detailed in Section 5.4.3) are presented in Figure 5-13 and NM refers to the net mass of settled material deposited in each sample container.

Figure 5-14. These figures indicate that during autumn detectable TP deposition only occurred in wetlands receiving alum-dosed effluent.
Figure 5-13: Summary of average mass of material accumulated per month within pilot plant wetlands during the studies in autumn of 1995.

NM refers to the net mass of settled material deposited in each sample container.

Figure 5-14: Summary of average mass of material accumulated per month within pilot plant wetlands during the studies in spring of 1995.

NM refers to the net mass of settled material deposited in each sample container.

NM deposition appeared to be related to inflow rate irrespective of water treatment method. TN accumulation was related to effluent type, with higher deposition rates
observed in wetlands receiving secondary treated effluent. The lowest carbon deposition occurred in Unit 4, highest in Unit 2.

The overall NM was higher than autumn, by an additional 0.5 times for alum dosed systems and 2.5 to 4 times for Units receiving secondary treated effluent. Levels of TC deposited were almost twice as high as autumn for all Units. TN deposition patterns were also more than twice as high in spring than in autumn, with the majority of deposition in Units 2 and 5. Phosphorus deposition was relatively consistent between all Units except Unit 3, with a slightly higher deposition rate in spring. This appeared to be related to an unusually high TP concentration in S1, adjacent the wetland inlet. The reading appears anomalous. If it were removed then deposition would be consistent between Units.

The net amount of material deposited, along with the incident phosphorus loading and percentage removal were predicted using an average of spring and autumn figures, corrected for concentrations of SS, TN and TP likely to be present in the water column (Table 5-11). The deposition of NM and TC appeared to be related to wetland inlet loading rates. TN deposition appeared related to inlet concentration and to some extent loading, being greater at higher inlet concentrations.

**Table 5-11: Derived net annual deposition of the net mass of deposited material and associated total carbon, total nitrogen and total phosphorus for each Unit of the Richmond pilot plant wetlands, corrected to account for material present in the water column of containers. Calculations of the total mass of total phosphorus entering each wetland, removed by each wetland and the percent attributable to deposited material are presented.**

<table>
<thead>
<tr>
<th>Unit</th>
<th>NM (kg·Ha⁻¹·yr⁻¹)</th>
<th>TC in sedimented material (kg·Ha⁻¹·yr⁻¹)</th>
<th>TN in sedimented material (kg·Ha⁻¹·yr⁻¹)</th>
<th>TP in sedimented material (kg·Ha⁻¹·yr⁻¹)</th>
<th>TP entering each Unit* (kg·Ha⁻¹·yr⁻¹)</th>
<th>TP removal by each Unit* (kg·Ha⁻¹·yr⁻¹)</th>
<th>% TP removal associated with settled material (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1490</td>
<td>210</td>
<td>15.9</td>
<td>0.2</td>
<td>1400</td>
<td>100</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>830</td>
<td>150</td>
<td>9.4</td>
<td>2.1</td>
<td>530</td>
<td>210</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>480</td>
<td>100</td>
<td>6.3</td>
<td>0.7</td>
<td>210</td>
<td>80</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>880</td>
<td>160</td>
<td>14.4</td>
<td>0.3</td>
<td>470</td>
<td>100</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Figures summarised from Chapter 2.
For the same inlet concentration, there was a slightly higher deposition at a higher loading. This was primarily significant for Units 3 and 4. The greatest deposition of TP was in Unit 3, although this was primarily related to an anomalous result in S1, as noted above. In each wetland, the percentage of TP associated with deposited material was less than 1% of the total mass of phosphorus removed from the water column of any wetland.

5.4.4 **Nutrients present in the aqueous phase**

The concentrations of nutrients and the NM of settled material observed in the investigations previously were a composite of both water and solid phases i.e. detritus, settled solids and water. The concentrations of NM and nutrients assumed to be present in the water column were evaluated by taking the minimum average values from either the inlet or outlet of each wetland, for the period of each investigation (as described in Section 5.3 and summarised in Table 5-12).

Table 5-12: Minimum mass values for water column constituents in each sample container (as opposed to settled detrital, algal or other material) during the autumn and spring settling investigations, based on minimum inlet and outlet concentrations.

<table>
<thead>
<tr>
<th>Wetland</th>
<th>Autumn</th>
<th></th>
<th></th>
<th>Spring</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SS (mg)</td>
<td>TN (mg)</td>
<td>TP (mg)</td>
<td>SS (mg)</td>
<td>TN (mg)</td>
<td>TP (mg)</td>
</tr>
<tr>
<td>Unit 2</td>
<td>0.43</td>
<td>3.39</td>
<td>1.04</td>
<td>0.75</td>
<td>3.53</td>
<td>1.09</td>
</tr>
<tr>
<td>Unit 3</td>
<td>0.43</td>
<td>3.05</td>
<td>0.31</td>
<td>1.23</td>
<td>2.89</td>
<td>0.30</td>
</tr>
<tr>
<td>Unit 4</td>
<td>0.43</td>
<td>1.63</td>
<td>0.09</td>
<td>1.03</td>
<td>1.97</td>
<td>0.28</td>
</tr>
<tr>
<td>Unit 5</td>
<td>0.77</td>
<td>1.48</td>
<td>0.75</td>
<td>0.89</td>
<td>0.71</td>
<td>1.02</td>
</tr>
</tbody>
</table>

The average concentration of SS material during any sample run was less than 1% for most sites and not a significant component of NM. TN values at the inlet and outlet during each time interval appeared to be greater at higher flow rates through each wetland, accounting for almost all of the nitrogen observed in collection containers during autumn. The same was true of TP. This was not the case in spring, when TN in the water column only accounted for an average 10% of deposited TN and TP, and between 10 to 50% of deposited TP (the higher percentages being observed in wetlands receiving secondary treated effluent).
5.5 DISCUSSION

5.5.1 Preliminary trials

Unit 2

In the preliminary investigation, only a limited number of samples had sufficient quantity of material for determination of TC, TN, and TP, indicating that the time interval for analysis was insufficient. The minimum net sample mass for detection of all parameters in any given stand was between 116 and 156 mg (Section 5.4.1). The shortest practicable time to achieve this for the majority of stands appeared to be 2 months i.e. double the period used in the ‘preliminary investigation’. However, this may have missed one or two locations, at the observed rates of deposition. Three months would allow greater deposition but increase the risk of disturbance by macrobiota or remobilisation to the water column in the absence of sediment. Therefore, a time interval of 2 months was selected for the seasonal investigation.

Where significant deposition was observed, stands of *Triglochin* consistently reported amongst the highest net amounts. *Triglochin* is a plant with mostly spongy, basal leaves containing a low percentage of lignin (Sainty et al. 1981). The plant is a perennial with litter production occurring throughout the year (Sainty et al. 1981). By comparison, *Schoenoplectus* is a more lignified emergent plant with the leaves tightly bound around the growing stem, with slower growth and senescence in winter (Sainty et al. 1981). *Phragmites* is slightly more lignified than *Schoenoplectus* with an almost woody stem and leaves up to 70 cm long, and 1-3 cm wide (Sainty et al. 1981). *Phragmites* has an annual period of senescence during winter (Sainty et al. 1981).

The higher NM observed in the two stands of *Triglochin* may have been from the continuous deposition of litter within these stands during the sampling period. The deposition of material in *Phragmites* stands (S1 and S4) occurred to a greater extent than in the stands of *Schoenoplectus* and the final *Phragmites* section (S2, S6 and S7). This may have been from leaf senescence or decomposition of litter causing the
release of organic material to the water column. *Schoenoplectus* did not have leaves distinctly separate from the stem whereas *Phragmites* does, possibly allowing for a greater deposition the biomass in stands of *Phragmites* as leaves, in addition to stems, could be deposited.

In the preliminary investigation of Unit 2, a strong correlation was observed between NM, TC, TN and TP and plant type, implying that material was not simply settling with distance as it entered the wetland. The strong association of all four parameters with plant type suggested a differing depositional relationship for each plant species. Deposition was consistent between replicate stands of each plant type, suggesting upstream plant characteristics may not significantly affect the deposited material as upstream species differed through the wetland. Association with plant type could be due to direct input of litter and detritus, biofilm association or other factors such as changes in physical or chemical parameters through each stand, or the association of differing consortia of microorganisms through each plant stand (Vymazal 1995).

The correlation between NM, TC, TN and TP would suggest the deposited material was largely organic. The highest observed percentage deposition of TC and TN (where detectable) occurred in stands of *Phragmites* and *Schoenoplectus*: sites with more lignified plant tissue. The lignin in this plant material could cause it to degrade much slower than the fleshy leaved *Triglochin*. Although litter from *Triglochin* should fall more frequently than from the other plant species it should be more biodegradable because of its lower lignin content, hence the lower proportions of TC and TN per gram of NM (Section 1.5.4). As plant material was degraded, carbon and nitrogen compounds may be converted to either soluble organic compounds or gaseous nitrogen and carbon dioxide (Kadlec 1997; Vymazal 1995), and thus removed from the sample container. If this process was occurring then the higher percentage of TP relative to TC and TN would imply that TP was unable to be released to the water column because it may have been less labile or available to microorganisms. The more readily bioavailable TC and TN being removed but TP remaining, causing the negative correlations observed for percentage TP.
CHAPTER 5

An alternative hypothesis is that the plants in a stand induced a chemical change within that stand related to pH or redox (Vymazal 1995), inducing precipitation or deposition of inorganic iron, aluminium or calcium compounds from the water column within that stand (refer to Chapter 1 for a more detailed description of these mechanisms). The parameters measured through each wetland during investigations detailed in Chapter 3 did not indicate that significant chemical related differences existed between plant stands. Thus it is unlikely plant stands were inducing secondary precipitation through each wetland.

A third alternative was that each plant species allowed the growth of differing populations of microorganisms (algae, bacteria and fungi) either in the water column or as biofilms. Although this may have occurred to some extent, observations of others (Kadlec 1997; Richardson et al 1995; Vymazal 1995) indicated that that the concentration of phosphorus taken into microorganisms tended to be transitory, primarily associated with the movement of phosphorus to more permanent binding sites in plants or the soil.

In Chapters 3 and 4, consistently high phosphorus concentrations were observed in stands of Triglochin. However, other observations including those of Chapter 6, tended to indicate that the majority of this phosphorus deposition was likely to be associated with plant tissue, either attached or floating. Thus, it was unlikely that microorganisms in the water column contributed directly to the mass of phosphorus containing material deposited from the water column. Note that they would most likely have had a role in the subsequent degradation of that material. Alternately it could be that attached biofilms were dominant in the accumulation of deposited material. To cause the results observed such biofilms would need to be growing on the surface of specific plants, rather than the sample container (biofilms growing on the container surface were accounted for by the control). However, the relative size (based on number of organisms and physical characteristics) of attached biofilms in the pilot plant wetlands was Negligible (Flood 1999).
Thus, the most likely source of deposited material in Unit 2 was from the deposition or decomposition autochthonous of plant material rather than deposition of allochthonous material from the water column, as discussed in previous chapters. Note that this was only a preliminary hypothesis and required confirmation by results from the seasonal investigation (refer Section 5.5.2).

**Unit 3**

Unit 3 evidenced a different pattern of deposition to Unit 2, possibly related to the effluent source. Unit 3 was planted identically to Unit 2 and received a similar flow rate. The primary difference was that Unit 3 was supplied with effluent that had passed through an alum-dosing unit, while Unit 2 was provided with effluent from a settling pond, without alum dosing. Tables 2.4 and 2.5 (Chapter 2) indicated that the mass of SS material entering each wetland was not significantly different. However, the composition of this material may not have been the same in terms of inorganic content. It was likely, from observations in previous chapters (Chapters 3 and 4) that the incident SS material for Units 3 and 4 had a noticeable aluminium component.

The correlation of the NM with distance in Unit 3, was in contrast to observations in Unit 2, and supported an alum related settling regime. In Unit 3, the NM increased in an almost linear manner from the inlet of the wetland to S5. This may have been from the aggregation of aluminium phosphates and other complexes. As possible alum related material progressed through the wetland these aggregates could become larger until they achieved sufficient mass to cause them to precipitate from the water column. Note that the percentage of TC also increased between the inlet and S5 implying a biological deposition mechanism, but a greater sample mass would be required for more detailed interpretation.

The deposition pattern of TP appeared to be consistent between Units 2 and 3 for the central regions of the wetland (as has been noted previously – Chapter 3). The reasons for variations in the mass of TP within different plant stands were unclear from the preliminary investigation. There was an absence of any correlation between TP and the other parameters of NM, TC and TN, requiring a more representative sampling of
CHAPTER 5

NM through the wetland over a longer time interval for evaluation. However, there may be two primary mechanisms of deposition of settled material in Unit 3: (1) senescence and deposition of plant material; and (2) precipitation and settling of aluminium based complexes.

Combined results
Deposition NM was higher in Unit 3 relative to Unit 2. On a cursory examination, it would be easy to conclude this was due to the presence of alum in effluent supplied to Unit 3. However, the trend was for greater NM in all sectors of Unit 3 relative to Unit 2 except S1, adjacent the inlet, which could be expected to be higher in Unit 3 if alum related settling was a primary deposition mechanism. A longer sampling time and observations over at least two time intervals were required to confirm these preliminary results.

5.5.2 Seasonal study of deposition
This study observed settled material over a two-month time interval during autumn and spring, clarifying trends observed in the ‘preliminary investigation’.

Unit 2
In Unit 2, the association of NM with plant type but not distance was consistent with results from the preliminary investigation and indicated that deposition was not due to simple precipitation of SS as it moved through the wetland. The figures for autumn would imply that during this season, deposition of material was similar for all plant types. During spring, senescence from both Phragmites and Schoenoplectus were expected to be less than in autumn, with consequently lower NM due to the annual growth pattern of these plants (Sainty et al. 1981). However, deposition of material in both plant stands was greater in spring than autumn.

It is important to note that this measurement was based on the detection of material within a sample container, and it did not take into account standing but not yet fallen biomass. It was likely that the material observed in the sample cup was derived from the fall of standing detritus. A greater rate of microbial (bacterial, algal and fungal)
activity should occur during the warmer spring months, resulting in the degradation and weakening of standing litter, which may then have been deposited in the sample containers.

Regardless of season, there was a consistent trend for a greater mass of material to be deposited with particular plant species. The net deposition of settled material in Unit 2 was greatest in stands of *Triglochin*, and lowest in stands of *Phragmites*. This observation differed slightly from the ‘preliminary investigation’, which showed one stand of *Phragmites* with a moderately high amount of deposited material. The observation in the ‘preliminary study’ appears anomalous and may have been associated with the duration of sample collection and the number of samples taken per stand (2 rather than 3 containers per stand of plants).

The percentage of carbon and nitrogen varied inversely with the NM, with the lowest percentages in containers located within stands of *Triglochin* but similar, high percentages for *Schoenoplectus* and *Phragmites*. This observation was consistent with the degree of lignification and potential for biodegradation of the three plant types. It suggested that material from the stands of *Triglochin* was continually being senesced, producing a high NM, but that this material was rapidly degraded resulting in less organic carbon and nitrogen in the collected material than observed in other plant stands. The more lignified stems of *Schoenoplectus* and *Phragmites* would be slower to degrade, hence they tend to remain structurally intact for longer, preserving their carbon and nitrogen within the intact tissues (Bouchard et al. 1998; Greenway 1994; Rice et al. 1981).

The pattern and mass of TP deposited in each plant stand varied with season. In winter, TP was associated with TN and TC. However, the percentage composition of TP remained relatively constant for all plant stands, implying an association with deposited plant material. Complicating this picture was that the observed TP could be entirely attributed to the water column (based on figures from Section 5.4.4).
During spring, a different trend was observed. Deposited phosphorus concentrations were higher than those in the water column alone, most likely forming part of the deposited biomass. TP increased through S1 to S3, possibly confirming the presence of an allochthonous input, not associated with plant stands, which deposited with distance into the wetland. Deposition in the latter part of the wetland was very similar for all remaining plant stands except the final stand of *Phragmites* (S7) where deposition was noticeably lower, approaching levels attributable to the water column alone.

In addition, during spring NM was greatest in the first three stands of Unit 2, supporting the settling of incident SS and an association of TP with this material. The amount of material deposited in the latter part of the wetland appeared to be at a slightly lower level for similar plant stands, relative to stands near the inlet. This association was not observed for TN and TC. One theory would be that the higher NM could be associated with floating plant material – possibly from the upstream treatment lagoon, which settled with distance into the wetland. The less lignified nature of this material would mean that it should decay relatively rapidly, which could account for only a moderate correlation between TP with TC and TN during spring. If such a mechanism was present in the pilot plant wetlands then it should be observed in other wetland Units, particularly Unit 5, receiving water with the same TP levels.

**Unit 3**

NM, TC and TN material in Unit 3 were independent of both plant type and distance during both autumn and spring. The consistently high correlation between TC and TN in the approximate ratio of 10:1 would indicate the deposited material was likely to have been from biota. The consistent deposition through the wetland would further indicate this material may have been of plant, bacterial or fungal origin. The lack of variation in deposition rates through shaded stands (*Phragmites* and *Schoenoplectus*) would tend to preclude algal growth. Biofilm accumulation should be accounted for by the sample control, but was not seen as a significant mechanism (refer ‘pilot investigation’ Discussion, Section 5.5.1).
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TP deposition in Unit 3 was consistent with that seen in Unit 2. Deposition was greater in spring than in autumn, with deposition greatest adjacent the wetland inlet (S1 – Phragmites) and lowest in the central stand of Phragmites (S4). In both seasons, the amount of TP deposited was greater than that due to the water column alone. TP deposition did not appear to correlate with distance or plant type. However, this may have been due to more than one deposition mechanism operating. The high deposition of TP adjacent the inlet and the even higher percentage of TP in S2, would indicate a deposition mechanism consistent with settling of allochthonous material entering the wetland, as was observed in Unit 2. Whether this was due to aluminium or biological components was unable to be confirmed. The high correlation between TN and TC (although not TP) further complicated this picture.

In the central regions of the wetland, there was a pattern of TP accumulation that was consistent between seasons and also with all other Units. The amount of TP, NM, TC and TN deposited were high in the two stands of Triglochin (S3 and S5) and very low – almost always the lowest, in the central stand of Phragmites (S4). In addition, the average mass of these substances deposited in S6 was invariably lower than S5. These observations were consistent with observations of TP in the water column in Chapter 4, suggesting that some mechanism was occurring to migrate phosphorus into the water column in S3 and S5 (stands of Triglochin). This material was settleable, as observed in these investigations and it appeared to be plant species related.

However, when the material settled, it did not do so within the proceeding stand of plants. It appeared that the material was being removed to the sediment within the stand it was being formed or more likely, at the boundary of that plant stand, particularly when observations from Chapter 4 were included in the analysis. This would imply movement or sequestering of phosphorus to the sediment was occurring through the downstream plant stand (Phragmites – S4, and Schoenoplectus – S6). Such a mechanism would only be possible if significant degradation was occurring to the material deposited in S3 and S5. This appeared to be the case, based on observations of TN and TC in each wetland Unit.
Unit 4

Despite the absence of any significant trends for deposited material with distance or plant species during either season a few observations were possible. The highest percentage deposition of TP was in the initial 1 or 2 plant stands, consistent with observations of Units 2 and 3, confirming the likely presence of an allochthanoous deposition mechanism. During each season, the trend noted under Unit 3, for minimal deposition in the central stand of Phragmites (S4) and high deposition in the two stands of Triglochin (S3 and S5), along with lower deposition in S6, was also observed.

A number of seasonal differences were present. The mass of TP collected was greater in spring, and there appeared to be a close association with biological material during this period that was not present in the autumn sampling period. The reasons for these observations have been discussed previously and it should be noted that the mass of TP observed was significantly higher than that attributable to the water column alone.

The reason for the low correlation of TC and TN during autumn was unclear and may have been a sampling aberration because it was not observed in other wetlands during the investigation, nor was it observed during the spring sampling event.

Unit 5

The high correlation of NM with TC, TN and TP during autumn suggested an association between NM and organic matter. The lack of association of this material with plant type during autumn was similar to that observed in all other Units. Correlations between other parameters did not indicate any further trends except to confirm that a large proportion of deposited material was organic. Consistent with other Units was the observation that TP was elevated in the two stands of Triglochin (S3 and S5. Actually the highest concentrations were observed here), with the lowest mass being observed in the central stand of Phragmites using average values.

In spring, there was no definite association with distance and only slight association with plant type, but the high correlation between NM with TC, TN and TP implied a
significant biological component in the observed material. The average monthly data indicated the deposition rate in spring was more than twice that of autumn for all sectors. The most likely mechanism for deposition was degradation of plant litter and detrital material. The similar and constant deposition over two seasons through all plant stands would again tend to preclude algal material from this process. It was expected that algal processes would predominate in spring but not autumn.

5.5.3 Summary of seasonal data
In the seasonal investigation of Units 2 to 5, deposition of NM, TC, TN and TP were greatest in spring. During autumn, deposition rates were so low that in wetlands receiving secondary treated effluent it was not possible to separate deposition of TP due to detritus and settled material from concentrations of TP present in the water column. During spring, TP deposition was similar for all wetland Units except Unit 3, where it was slightly higher. The elevated mass of TP in Unit 3 appeared to be caused by a very high mass deposited in S1 of this wetland stand at this time, disproportionate to all other stands in this and other wetlands and not proportional to NM, TC, TN at this location. It is suggested that this result was anomalous, possibly due to a slug of alum entering Unit 3 at this time.

The percentage composition of TP in deposited material was consistent with litterfall from herbaceous plant litter of between 0.01 to 0.24% (Johnston 1991). However, the percentage nitrogen was slightly higher than observed in dry standing litter (between 0.3 to 1.3%, Johnston 1991). The difference was hypothesised as being due to preferential absorption and formation of nitrogen compounds by attached biofilm, as has been observed in other studies of litter decomposition (Rice et al. 1981, Neckels et al. 1994).

The elevated TP deposition in spring appeared to be associated with plant detrital material, based on: the percentages of TC and TN; the consistency of observations through each wetland; and observations from Chapters 2, 3, 4 and 6, which also appeared to preclude microbial growth as a significant contributor. The reason deposition was higher in spring than autumn for each plant stand within each wetland
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Initially appeared incongruous with expected plant growth and senescence cycles. Consideration needed to be given to what was being measured. During autumn, growth cycles for the *Triglochin* were most likely to be subdued hence deposition would be reduced. The same would also apply to other floating plants within the *Triglochin* stands. In the stands of more lignified emergent plants, senescence would be occurring to produce standing litter. However, it was suspected that this material remained relatively intact until the warmer spring months. During the warmer periods, microorganisms would be expected to be highly active, degrading standing litter. In addition, macrobiota such as birds and snakes become more prevalent, with the possibility of flattening and destruction of standing litter.

Variations in the ratios of TC and TN found in NM appeared to be related to the biodegradability of various plants, as predicted by their lignin content. A higher lignin content was associated with lower biodegradability. Hence, in stands with lignified plants the percentage mass of TC and TN tended to be higher reflective of more intact plant material being present.

It appeared that nutrient loading had a significant impact on plant growth and hence deposition NM and TC. The highest deposition was in Unit 2, the lowest in Unit 4 (Table 5-11). Deposition of TN appeared more related to nutrient source, and was higher in wetlands supplied with secondary treated effluent. The cause was unclear and not explained well by preferential degradation or accumulation of TN relative to TC, as has been observed by some authors (Bouchard *et al*. 1998; Neckles *et al*. 1994; Rice *et al*. 1981). It may have been associated with biofilm formation on detrital material within these wetlands, but its exact source was not determined.

Despite the absence of high correlations of TP with plant type, there was a consistent pattern of deposition observable in each wetland. This was for consistent deposition in the initial 1 or 2 sections of each wetland, most likely from allochthananous sources. These may have been algae or floating plants within the upstream treatment plant, due to the high correlation between NM, TC and TN in these regions. In Units 3 and 4, it was probable that alum contributed to the higher net deposition of TP observed.
Deposition rates for NM, TN and TC appeared related to loading rate or inlet concentration. However, TP deposition appeared to be higher in Units receiving alum dosed effluent (Units 3 and 4), with higher deposition at a higher inlet flow rate. There was no significant difference in deposition for Units 2 and 5.

Within each wetland, the two stands of *Triglochin* were consistent regions of high deposition. The two stands of plants immediately after each these regions showed low deposition rates. This would indicate that the material in the stands of *Triglochin* was settled out of the water column before it entered the proceeding stands. The material may have bound to the sediment or released to the water column. Investigations in Chapter 4 indicated that it did not release to the water column, perhaps binding to the sediment surface. This material was not observed as being released in the central stand of *Phragmites*, but appeared to be released occasionally to the water column in the second stand of *Schoenoplectus* (S6, Chapter 4).

The amount of phosphorus deposited in S4 and S5 was consistently lower than observed in the upstream region of *Triglochin*. However, the central stand of *Phragmites* almost invariably had the lowest mass of TP deposited of all plant stands. These observations would suggest that the sequence of plant types may influence phosphorus sequestering and deposition processes. It appeared that placing a stand of dense emergent plants after a stand of submergent plants resulted in a removal of significant concentrations of TP from the water column. This was most significant when the emergent plant was *Phragmites*.

The mechanism for such removal and deposition was unclear, as was the source of the biomass, which might be expected to have included *Triglochin* and floating plants such as *Lemma* spp. or *Azolla* spp. Determination of this mechanism was beyond the scope of this thesis. However, further investigations into the significance of this mechanism are recommended as a possible method to increase the efficiency of constructed wetlands in removing TP. These observations suggest that biological processes were accounting for a significant proportion of TP deposition, but that in Units 3 and 4, alum dosing most likely augmented the amount of phosphorus retained.
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TP sequestered into settled detrital and other material was calculated as forming less than 1% of the net mass of phosphorus removed from the water column, consistent with predictions by Craft and Richardson (Craft et al 1995). The highest deposition was observed in Units supplied with alum-dosed effluent. However, this result must be interpreted with caution. The low result observed in wetlands receiving secondary treated effluent was associated with their low deposition rate during autumn, where it is suspected that TP deposition did occur, but was masked by the high residual TP concentration in the water phase.

5.6 CONCLUSIONS

The deposition of settled material within Units 2 to 5 was determined to be primarily associated with the senescence and degradation of plant material and the settling of inorganic particulates. The NM and the associated TC, TN and TP was more than twice as high in spring, compared to autumn. The most likely source of this increase was the partially degraded plant material released from the breakdown of intact leaves and stems due to increased temperatures and biological activity in spring.

Nutrient loading had a significant effect on deposition of material from the water column, most probably because of its effect on increasing plant growth. The apparently consistent pattern of deposition within each wetland should be investigated further, but was beyond the scope of this work. It appeared that submergent stands of Triglochin represented significant and consistent regions for settling of material from the water column. This material was most likely biological but whether from Triglochin or floating plants such as Lemna spp. or Azolla spp. was not determined. Despite the presence of settled detritus in these stands, previous Chapters indicated that a significant concentration of TP also existed in the water column within these stands. The effect of downstream stands of Phragmites or Schoenoplectus proved beneficial in removing TP from the water column and reducing the amount of material settled from the water column. Investigations should be undertaken to confirm whether this could be developed into a viable mechanism for enhancing the phosphorus removal capability of constructed wetlands.
CHAPTER 5

Determination of the relative contribution from plants, microorganisms and inorganic sources would require detailed analysis of deposited material for lignin, chlorophyll degradation products and inorganic compounds such as complexes with aluminium. However, given the results observed from the studies in this Chapter and in view of the low contribution of deposited material to the net mass of TP removed from water column throughout each wetland (less than 1%) it was determined that deposited material (particularly detritus formation) would not be investigated further as a mechanism for TP removal by constructed wetlands.
CHAPTER 6: LABORATORY STUDIES ON PHOSPHORUS MIGRATION THROUGH THE WATER COLUMN

6.1 INTRODUCTION

Previous investigations indicated that phosphorus was removed from the water column of the pilot plant wetlands at Richmond. The dominant mechanisms for phosphorus removal within the wetland were hypothesised as settling, adsorption to plant or soil surfaces, and incorporation into biomass. The paths by which each of these processes can occur were schematically presented in Figure 1-1. As phosphorus entered the wetland it may have settled through association with particulate material, be adsorbed into biomass or onto sediments. Biomass may include plants, algae and bacteria and may be within the water column or on the sediment surface. Biomass in the water column may travel out of the wetland or attach to the sediment or plant surfaces. The significance of each of these processes and the extent to which microorganisms were involved them at the pilot plant wetlands are complex issues.

In order to optimise the removal of phosphorus from the water column in the pilot plant wetlands it was first necessary to identify the dominant removal mechanisms. The most significant phosphorus removal pathways could then be evaluated to determine the capacity for process optimisation or improvement. An understanding of the relevant removal pathways would allow further investigation of possible optimisation methods.

Larger scale investigations of the pilot plant wetlands did not allow effective distinction between different removal pathways (Figure 1-1, Chapters 2, 3, 4 and 5). To overcome this limitation the components of each pathway were teased apart and subjected to separate laboratory investigation.

The laboratory investigations described here were designed to attempt to evaluate individual components of potential phosphorus removal pathways observed in the pilot wetlands. Investigations were divided into processes occurring in the water column and those primarily associated with the sediment.
Processes occurring in the water column were:

- Settling - through association of phosphorus particles with SS material;
- Adsorption to algal biomass;
- Incorporation into non-algal biomass such as bacteria and microorganisms;
- Adsorption to biofilms on plant surfaces; and
- Dissolution and efflux from plant tissues.

Processes primarily associated with sediment were:

- Direct adsorption to sediment particles;
- Desorption from sediment particles;
- Dissolution of phosphorus;
- Translocation through the soil substrate (which may be plant facilitated, with the plant acting as a water/phosphorus pump); and
- Microbially facilitated transport and storage on sediment particles.

Processes in the water column are presented in this chapter, and processes associated with the soil are presented in Chapter 7.

6.1.1 Settling of particulate material from the water column

Observations of the Richmond pilot plant wetlands suggested that the majority of phosphorus compounds (> 90%) in the water column were smaller than 0.45 μm, most likely as ortho-phosphate (Chapter 2 and Chapter 3). These observations and the high variability of SS concentrations within each wetland (Chapter 2 and Chapter 3) suggested that larger phosphorus particles may not have a significant role in translocating phosphorus to the sediment surface. Bulk litter could be considered as a separate subset of these particles, but as determined in Chapter 5 this represented less than 1% of phosphorus storage. Investigations of the association of phosphorus with SS material were preformed in the laboratory to examine this hypothesis and quantify the amount of phosphorus likely to be removed from the water column through settling and association with particulate material.
6.1.2 Biological uptake of phosphorus from the water column

Investigations conducted in Chapter 3 (Sections 3.4.2 and 3.5.2) indicated that microorganisms within the pilot plant wetlands at Richmond might influence phosphorus removal from the water column to both the sediment and into biofilms. The presence of algae was inferred indirectly through the detection of chlorophyll a and pheophytin within a number of wetland units and at their outlets (Sections 3.4.2 and 3.5.2). The presence of fungi and bacteria was assumed but not specifically quantitated (Richardson et al. 1986).

Investigations described in Chapter 3 indicated that in some circumstances, high concentrations of TP in the water column were associated with elevated concentrations of chlorophyll a and pheophytin. The presence of these substances indicated the likely presence of algae in high numbers. Algae have the potential to incorporate phosphorus from the water column into their biomass as part of their normal metabolism (Section 1.5.2). Such accumulation may have acted to reduce the concentration of phosphorus in the water column if the algae were able to control their buoyancy, as can most cyanobacteria. Blue-green algae are able to control their buoyancy using gas vacuoles. These free floating algae could serve as a mechanism for transport of phosphorus through the water column by collecting phosphorus from the water near the water surface during their growth stages, and then settling to the sediment at other times of the day. The dominant species of algae in the wetland at the time of observation were cyanobacteria from the genus Microcystis (D. Roser pers com.). Initial investigations were to determine the ability of algae to remove phosphorus from the water column, along with the impact that such removal had on sediment phosphorus concentrations.

6.2 Aim

Investigations were conducted to determine factors influencing the migration and removal of phosphorus from the water column of the pilot plant wetlands. These investigations were to:

- Determine the contribution from SS material in transporting phosphorus from the water column to the sediment surface;
• Examine whether microorganisms such as algae could increase the concentration of phosphorus in the water column;
• Evaluate the role of microorganisms in sequestering phosphorus to sediment; and
• Determine whether these organisms were predominantly associated with the sediment or effluent.

6.3 MATERIALS AND METHODS

6.3.1 Settling of particulate material from the water column
Investigations were designed to evaluate the:
1) Association of phosphorus with turbid particles; and
2) Role of turbid particles in settling and movement of phosphorus to the sediment surface.

Samples of water were collected from the inlet and outlet of each of the Richmond pilot plant wetlands. The inlet and outlet were selected as representing two possible extremes of phosphorus and SS concentration through each wetland. Samples from each location were placed in duplicate cylindrical plastic containers (Volume 1,250 mL, height 25 cm), which were filled on site.

Containers were then removed to a laboratory, where investigation of particle settling was undertaken. Each container was kept in the dark using an aluminium foil cover to minimise algal growth. Containers were kept at 21°C for the duration of the investigation and capped with aluminium foil to allow oxygen diffusion.

Two methods were trialled to remove material from each tube: it was possible to obtain samples by either using 1) tubes that were permanently secured in place or 2) by inserting a tube to the required depth at time a sample was required. The first method resulted in less disturbance to the water column but unduly biased results due to biofilm growth in the tube. It was also not possible to flush such a tube, as this would significantly disrupt the water column. Therefore, it was decided to use a very small diameter tube and reinsert the tube during each sample event.
Samples were obtained by slow withdrawal of solution from the container using a 5 mL syringe with an attached capillary tube (Tygon, 1 mm ID) placed to a depth of either 5 or 15 cm from the bottom of the container (as marked on the tube). Each tube was placed carefully, to minimise disturbance to the water column. A separate syringe and tube were used for each sample and both syringe and tube were rinsed with deionised water three times before and after each use, and flushed once with sample water prior to sample collection. The same syringe and tube combination were used for each sample point over the period of investigation. Samples were collected at 24-hour intervals over five days and analysed for turbidity and TP using the manual methods outlined in Section 2.4.8.

The two depths selected for analysis were to determine whether particles that settled from the upper portion of the water column settled completely from solution. Water from the inlet and outlet of each wetland was selected to evaluate changes that were occurring as water moved through the wetland. Effluent from near the wetland inlet should contain more readily settleable particles that were removed as water entered the wetland. The outlet stream may contain either no settleable particles, as they would have been removed from solution, or a different mixture of turbid material from biofilms and plant detritus within the wetland. The depths of 5 and 15 cm were set to prevent collection of bottom or surface material. The significance of any association between TP and turbidity was determined by correlation and ANOVA.

6.3.2 Biological uptake of phosphorus from the water column
This investigation was to determine the role of algae and other microorganisms in influencing the concentration of TP in the water column overlying wetland sediments.
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To achieve this objective it was necessary to obtain sediment and water samples for inoculation into laboratory cultures. In addition, because algal numbers within the pilot plant wetland were highly variable it was necessary to undertake initial subculturings of dominant species in the laboratory to obtain consistent and detectable numbers. Further, because of the inherent variability of secondary treated sewage effluent, it was desirable to use an artificial sewage media. This media was derived from previous studies done using effluent modelled on the Richmond WWTP by Bavor et al. (1986).

Samples of algal material were collected from pilot plant wetlands Units 4 and 5 during spring and summer. The algal material was taken to the laboratory were it was cultured in artificial effluent media as detailed below (Bavor et al. 1987). Artificial effluent media was used to provide TP and TN concentrations similar to those in alum-dosed effluent.

Sediment samples

Soil obtained from Pilot Plant Unit 5 was utilised as the sediment substrate for this investigation as it had a relatively low phosphorus loading (Section 2.5.6). Soil from Unit 4, although preferable, was not used due to potential for residual aluminium in the sample and interference with other investigations on this wetland (Sections 2.5.5, 2.6.3 and 2.7). The soil was homogenised by air-drying for 5 days at room temperature and ground with a mortar in a pestle to pass through a 0.5 mm sieve.

Algal cultivation

Six mixed species algal cultures were established using water (10 mL) from wetland Units 4 and 5 (3 cultures per Unit) inoculated into artificial effluent medium (90 mL in a 600 mL glass container, Table 6-1). The cultures were incubated in a constant temperature room at 23°C, under Growlux™ grow lights. These were designed to produce light between wavelengths 350 to 750 nm to stimulate algal growth. The ideal illumination was provided by placing the cultures at sufficient distance (20 to 30 cm from the top of the container) to provide a light intensity of between 4304 to 2152 lux (measured by a standard photographic light meter) to simulate daylight conditions (Sweazy et al. 1975). After one week of incubation, 10 mL of culture material was
transferred into a new batch (90 mL) of artificial effluent medium and subcultured to maintain growth.

Table 6-1: Composition of Artificial Effluent Media for algal cultivation (adapted from Bavor et al. 1986)

**Primary solution**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentration (mg.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NH₄)₂SO₄</td>
<td>100</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>8</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>100</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>2000</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>5000</td>
</tr>
<tr>
<td>Trace Metals</td>
<td>1 mL</td>
</tr>
<tr>
<td>MilliQ water</td>
<td>1 L</td>
</tr>
</tbody>
</table>

**Trace Metals Solution**

<table>
<thead>
<tr>
<th>Trace Metal</th>
<th>Concentration (mg.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCl₃·6H₂O</td>
<td>540</td>
</tr>
<tr>
<td>EDTA</td>
<td>500</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>2655</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>30</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>2</td>
</tr>
<tr>
<td>CuSO₄</td>
<td>1</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>4</td>
</tr>
<tr>
<td>NaMoO₄·2H₂O</td>
<td>2</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 L</td>
</tr>
</tbody>
</table>

The artificial effluent media was prepared in a 1L volumetric flask. Aliquots (90 mL) were transferred to 600 mL glass bottle. The bottles were then capped and the media autoclaved.

Algae from Unit 5 did not grow well in the artificial effluent media, so only material from Unit 4 was able to be utilised. Predominant algal populations were identified as *Chlorella, Scenedesmus, Anabaena* and *Microcystis* (Dr David Roser pers com. 1995).

Prior to inoculation (see investigative components below), the cultured algal cells were concentrated by centrifugation at 5,000 rpm then washed with sterile ¼ strength Ringers solution to remove residual nutrients and possible contaminants from the culture mixture. The centrifuged cells were resuspended in ¼ strength Ringers solution (50 mL), recentrifuged at 5,000 rpm and rewash before being suspended in 20 mL of sterile ¼ strength Ringers Solution. The concentration of algal cells in the inoculum prior to injection to each treatment was between 10⁵ to 10⁷ cells per mL as determined by Sedgwick Rafter counting chamber (Method no. 10200 F. (APHA 1992)). One millilitre of this solution was transferred to each culture treatment as the inoculum.
Investigative components

Twenty grams of the dried soil (prepared as described above) were transferred to each of treatments specified in Table 6-2. This was then covered with 200 mL of artificial effluent media (Table 6-1). The nutrient amendments detailed in Table 6-2 were then added. Nutrient amendments were prepared at concentrations that allowed them to be added aseptically in aliquots of 1 mL to minimise volume changes on addition. The nutrient solutions were sterilised before use by autoclaving at 121°C for 15 minutes. Transfer to the culture solution was by sterile 1 mL pipette.

Table 6-2: Details of the components for each algal treatment

<table>
<thead>
<tr>
<th>Treatment Number</th>
<th>Light conditions</th>
<th>Oxygen Status</th>
<th>Nutrient Amendment</th>
<th>Inoculated</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Light</td>
<td>aerobic</td>
<td>Nil</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>Dark</td>
<td>aerobic</td>
<td>Nil</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>3</td>
<td>Light</td>
<td>oxygen limited</td>
<td>Nil</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>4</td>
<td>Light</td>
<td>aerobic</td>
<td>Acetate (0.1 g L⁻¹)</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>Light</td>
<td>aerobic</td>
<td>Lactate (0.1 g L⁻¹)</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>6</td>
<td>Light</td>
<td>aerobic</td>
<td>Glucose (0.1 g L⁻¹)</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>7</td>
<td>Light</td>
<td>aerobic</td>
<td>Acetate (0.1 g L⁻¹)</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>8</td>
<td>Light</td>
<td>aerobic</td>
<td>Lactate (0.1 g L⁻¹)</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>9</td>
<td>Light</td>
<td>aerobic</td>
<td>Glucose (0.1 g L⁻¹)</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>10</td>
<td>Light</td>
<td>aerobic</td>
<td>Ascorbic Acid (0.1 g L⁻¹)</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>11</td>
<td>Light</td>
<td>aerobic</td>
<td>Nil</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>12</td>
<td>Light</td>
<td>aerobic</td>
<td>Nil</td>
<td>Y</td>
<td>N</td>
</tr>
</tbody>
</table>

Acetate, lactate, glucose and ascorbic acid were each used as alternate carbon sources for simulation of bacterial growth. Sterile ringer’s solution was used to make up the volume for solutions where no nutrient amendment was added.

Treatments were prepared in triplicate and placed in a randomised pattern under the same conditions and distance from the grow lights detailed previously. The Eh of treatment 3 was evaluated with an Orion (model 8103) meter using an Orion research grade Eh electrode to confirm the degree of oxygen limitation achieved.

TP concentrations in the water column were evaluated over a 14-day period by removing culture solution (5 mL) at intervals of 2 and 3 days. Samples were obtained at 11 am on the day of sampling, approximately 5 hours into the photoperiod. At the conclusion of the investigation, the liquid in the culture flasks was carefully poured off and separated from the soil. The upper layer of water was transferred to a centrifuge tube (250 mL). The lower sedimentary layer was transferred to a separate
centrifuge tube (250 mL) using ¼ strength Ringers Solution (50 mL) to facilitate the transfer of sedimentary material. Since the soil matrix would be disturbed in the transfer to the centrifuge tube, the culture flask was shaken with Ringers Solution for 30 seconds prior to removal of the sediment. This was designed to promote a more homogeneous dispersion and collection of soil particles.

The soil and overlying water were centrifuged (3,000 rpm for 10 minutes) to separate algal cells into the supernatant and sediment material into the pellet (Osborne et al. 1986). The supernatant was then analysed for chlorophyll a and TP. The sediment was analysed to determine TC, TN, pheophytin and chlorophyll a using the methodology outlined in Section 2.4.10. TP was not analysed in the soil given the high background variability in readings and the small potential to confirm that changes were due to a particular treatment rather than normal sample variability (refer to Sections 2.5.7 and 7.5.3).

6.4 RESULTS

6.4.1 Settling of particulate material from the water column

The association between turbidity and phosphorus settling from the water column was observed for effluent from the inlet and outlet of several of the Richmond pilot plant wetlands. The results of these investigations are presented in Figure 6-2 and Figure 6-3.
Differences in turbidity and phosphorus concentrations were observed between the two sample depths for each sample day. Over the two day observation period turbidity decreased for all treatments but TP concentrations fluctuated. For a given depth, the percentage change in turbidity was constant for a treatment. Percentage change in TP
was not consistent between depths for each sample or for samples of the same effluent.

However, the change in turbidity and phosphorus concentrations were correlated at each depth. There was only slight correlation between changes in turbidity and phosphorus concentrations in the upper 5 cm of the water column \((r^2 = 0.38)\), but there was a highly significant correlation in the lower region of the water column \((r^2 = 0.88)\) as indicated in Figure 6-4. This correlation was only observed if the second observation for Unit 1 outlet was removed as an outlier due to problems with the abnormally high turbidity readings observed on day 0.

Figure 6-4: Correlation between changes in turbidity and total phosphorus concentration over a 2-day period.

6.4.2 Biological uptake of phosphorus from the water column
This investigation was to determine the importance of microorganisms in the water column in sequestering nutrients from the sediment and the potential of such nutrients to remain in the water column. Over the initial 3 days of the investigation between 80 to 90% of the applied TP was removed from the water column of all cultures (Figure 6-5 and Figure 6-6).
The decrease in TP concentration from the water column from the solution containing no soil was because of adsorption to the calcium present in the media. The Eh of the oxygen limited cultures was 229 ± 11 mV. Therefore, microaerophilic but not anaerobic conditions were established, consistent with the occurrence of some algae within this treatment.

Figure 6-5: Concentration of total phosphorus in the water column of algal treatments 1 to 6, using non-sterile soil.

Carbon sources in each treatment were: 1 - No carbon, 4 - acetate, 5 - lactate, 6 - glucose.
All treatments had soil present and algal culture added.

Figure 6-6: Concentration of total phosphorus in the water column of algal treatments 7 to 12, using non-sterile soil.

Carbon sources in each treatment were: 7 - acetate, 8 - lactate, 9 - glucose, 10 - ascorbic acid, 11 and 12 - nil.
All treatments but 12 had soil. Only treatments 10 and 12 had algal culture added.
CHAPTER 6

Using a two way ANOVA there was no significant difference between treatments in this study at the $\alpha = 0.05$ level. For most treatments, removal of TP was maximised between 7 to 10 days. There was no significant change in TP concentrations in the water column thereafter. Total chlorophyll a and pheophytin concentrations in the water for each treatment and are presented in Table 6-3.

Table 6-3: Concentrations of chlorophyll a and pheophytin in soil, from each of the 12 nutrient treatments (Unless indicated all cultures were grown in the presence of light, oxygen and soil, with algal inoculum. The carbon sources added were as noted in Section 6.3.2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chlorophyll a average (ug.g$^{-1}$ soil)</th>
<th>Pheophytin average (ug.g$^{-1}$ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No amendment</td>
<td>0.29 ± 0.10a</td>
<td>0.81 ± 0.21</td>
</tr>
<tr>
<td>Oxygen limited</td>
<td>0.22 ± 0.06</td>
<td>0.70 ± 0.13</td>
</tr>
<tr>
<td>Dark</td>
<td>0.00 ± 0.00</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>Acetate (0.1 g.L$^{-1}$)</td>
<td>0.18 ± 0.16</td>
<td>0.58 ± 0.33</td>
</tr>
<tr>
<td>Lactate (0.1 g.L$^{-1}$)</td>
<td>0.30 ± 0.21</td>
<td>0.62 ± 0.23</td>
</tr>
<tr>
<td>Glucose (0.1 g.L$^{-1}$)</td>
<td>0.40 ± 0.22</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>Acetate (0.1 g.L$^{-1}$) NA</td>
<td>0.09 ± 0.03</td>
<td>0.52 ± 0.05</td>
</tr>
<tr>
<td>Lactate (0.1 g.L$^{-1}$) NA</td>
<td>0.13 ± 0.09</td>
<td>0.42 ± 0.09</td>
</tr>
<tr>
<td>Glucose (0.1 g.L$^{-1}$) NA</td>
<td>0.04 ± 0.05</td>
<td>0.52 ± 0.07</td>
</tr>
<tr>
<td>Ascorbic Acid (0.1 g.L$^{-1}$)</td>
<td>0.02 ± 0.01</td>
<td>0.21 ± 0.12</td>
</tr>
<tr>
<td>No algae inocula</td>
<td>0.10 ± 0.05</td>
<td>0.46 ± 0.06</td>
</tr>
<tr>
<td>No soil</td>
<td>4.10 ± 1.12</td>
<td>0.68 ± 0.12</td>
</tr>
</tbody>
</table>

*± values are for the standard deviation

Concentrations of TN and TC in soil did not vary significantly between treatments and did not correlate with chlorophyll a or pheophytin in the sediment, nor TP
concentrations in the water column. The average amounts of TC and TN and TP found on soil, for all treatments were TC $14 \pm 0.5 \text{ mg.g}^{-1}$, TN $0.16 \pm 0.10 \text{ mg.g}^{-1}$.

Average TP concentrations in the water column were highly correlated between all treatments ($r > 0.98$) except for glucose inoculate and lactose control. In both cases, the deviation from the values of other treatments was due to anomalous readings. Physical observations indicated that these variations would most likely have occurred when the sampling apparatus disturbed sediments during sample collection in these two samples, because of the shallow water depths. If these 2 readings were omitted then there was no significant increase in TP concentration in the water column of each treatment after day 3. There was no significant correlation between percentage carbon and chlorophyll a. There was a correlation ($r = 0.80, 0.81$) between TP concentrations on day 15 and final values for chlorophyll a per gram of soil and percentage OM (as determined by ash weight). TP present in the water column at the end of the period averaged 10% of the initial concentration.

As expected, the samples without light evidenced zero algal growth, as verified by the absence of chlorophyll a (Table 6-3). Samples that were not inoculated with algae had smaller amount of chlorophyll a, presumably from cells present in the sediment, which was not sterilised during this experiment to allow the growth of sediment microorganisms (see Chapter 7 for investigations and comparisons involving non-sterile soil). There was also no noticeable effect of various nutrient additions on chlorophyll a or pheophytin concentrations.

6.5 DISCUSSION

The laboratory investigations outlined in this Chapter were carried out to assist in clarifying mechanisms by which phosphorus migrated through the water column to the sediment surface in the Richmond pilot plant wetland system. Two components were examined: SS and microorganisms. If neither of these mechanisms dominated then phosphorus migration to the sediment then it was hypothesised that phosphorus movement through the water column may have been through dispersion, mixing or
Brownian motion, which can be difficult to influence by external manipulations of the water column or possibly associated with uptake into floating plant biomass.

6.5.1 **Settling of particulate material from the water column**

Turbidity readings were used as a surrogate measure of SS concentrations in the water column to establish whether particles settled over time (Figure 6-4).

The decrease in turbidity over the period of observation for all treatments indicated that particles were settling out of solution. There was no direct association between turbidity readings or SS concentrations and the concentration of TP, as observed from *in-situ* investigations (Chapter 2). In laboratory investigations, changes in phosphorus concentration were observed in the upper portion of the water column that did not correlate with changes in the amount of turbid material present. However, in the lower portion of the water column an association was observed between changes in turbidity and concentrations of TP.

In samples with low turbidity at the start of the investigation, the concentration of TP in the lower portion of the water column increased, possibly through material settling from the upper portion of the water column. As turbidity increased above 1.5 NTU the amount of phosphorus removed from solution increased in a proportional manner, suggesting that there may be a minimum threshold below which no association exists. The linearity of this change suggested a simple binding and settling process. Up to 31% of phosphorus could be removed from the water column over a two-day period by such a process.

The composition of these particles - whether organic or inorganic was not determined in this investigation. However, other investigations (Chapter 2) have indicated that this material should be primarily organic. This is supported by the location of sites from which high turbidity material was obtained, Unit 5 and Unit 1 outlets. These wetlands receive no alum of inorganic inputs and therefore the material in these samples was most likely organic, from either biofilms or other organic material in the water column.
A key question, therefore, was how to increase the amount of material settled out of solution given that the majority was of varying size and probably physical characteristics, and once settled, how long would the phosphorus stay settled?

6.5.2 Biological uptake of phosphorus from the water column

This investigation focused on the role of microorganisms in affecting the concentration of phosphorus in the water column. Overall, the investigation indicated that more than 90% of TP was absorbed to the sediment of the microcosms within 3 days and that there was no significant change in the location of phosphorus after this time. The amount and rate of phosphorus adsorbed was independent of the treatment imposed, including addition of carbon sources, algal inocula, absence of light, reduced oxygenation, or the absence of microorganisms. Phosphorus was not released to the water column in significant concentrations, irrespective of treatment over the 15 days of the investigation.

Treatment conditions affected chlorophyll a concentrations. Treatments with no light had no chlorophyll a. Various nutrient additions had no effect on chlorophyll production but the addition of algal inocula produced a significant increase in chlorophyll concentrations. There was also, a small residual concentration of chlorophyll a (algal biomass) associated with soil material, observed in treatments that were exposed to light but had no algal inocula. Therefore, although light affected chlorophyll a levels there was not a significant change in TP concentrations at the levels used in this investigation.

These observations would imply that increases in TP observed within unshaded regions of each wetland were unlikely to have been due to incorporation of TP into algal biomass, given the conditions and species of algae observed in the pilot plant wetlands.
6.6 CONCLUSIONS

Based on laboratory investigations of water from the pilot plant wetlands there did not appear to be a significant role for microorganisms in migrating phosphorus from the water column. Phosphorus was associated with the removal of turbid material from the water column, and the concentrations removed indicated that up to 30% of TP could be removed from the water column in this manner, given quiescent conditions. However, the predominant mechanism for movement of phosphorus to the sediment in the investigations conducted in Chapter 6 appeared to be diffusion and direct absorption. This, together with settling processes accounted for approximately 90% of TP removal from the water column.

Algae isolated from the pilot plant wetlands were not able to significantly affect TP concentrations in the water column. Hence, it is unlikely that they were responsible for the increases in TP observed through sections of the wetland that were unshaded (stands of Triglochin). Note that these investigations did not encompass observations of nutrient dynamics and phosphorus transformations involving floating plants such as *Lemna* spp. or *Azolla* spp. This was beyond the scope of the current work. However, these may represent a significant translocation mechanism of TP from the water column.
CHAPTER 7: THE ROLE OF SEDIMENT AND ITS CAPACITY TO ADSORB DISSOLVED PHOSPHORUS COMPOUNDS

7.1 INTRODUCTION

Investigations of the Richmond pilot plant wetlands indicated that the wetland sediment was a major phosphorus sink (Sections 3.5, 3.6, 4.5, 4.7 and 6.5.2). The extent and rate at which phosphorus was sequestered to the sediment surface was dependent on the:

- Presence of microorganisms;
- Degree of oxygenation of the soil substrate;
- Presence of selected compounds which enhance phosphorus binding or release;
- Form in which phosphorus is present; and
- Ability of phosphorus to penetrate below the surface of the sediment.

An overall summary of these effectors is provided in Chapter 1.

Investigations into the degree of oxygenation of the substrate and the presence of selected compounds that might enhance phosphorus binding or release were beyond the scope of this thesis and not examined here. The forms in which phosphorus were present in sediment from the pilot plant wetlands has been partially covered in Chapter 2, by investigations undertaken by Sakadevan (1998). However, there was still some question over the degree of association of phosphorus with the various organic and inorganic soil components. Investigations were undertaken to clarify some these associations. In addition, the purpose of this chapter was to study the effect that microorganisms had on sequestering of phosphorus to the sediment in the short (1-10 hours) to medium term (28 days) under controlled conditions, and observe the extent and rate of phosphorus movement through the sediment in planted and unplanted regions of sediment.
7.2 BACKGROUND

7.2.1 The role of microorganisms in sequestering phosphorus to sediment
The sediment represents a significant phosphorus sink and it was desired to ascertain whether microorganisms on sedimentary particles were involved in phosphorus sequestering or whether the process was predominantly abiological. Evaluation of the process involved a comparison of sterile soil against non-sterile soil. If microorganisms did not have a significant role then there should be no difference between the two treatments.

A secondary issue was whether the microorganisms involved resided on the soil particles or whether they were associated with the effluent entering the wetland. Microorganisms would also have been present in biofilms within the wetland, but the movement of these organisms from a surface such as a plant leaf or stem, through the water column to the sediment was theorised to be sporadic and uncontrollable. A more constant source of microorganisms was assumed to be present in the incident effluent stream.

The mechanisms by which phosphorus could be bound to the sediment were abiological, biological or a combination of both. If the processes were entirely abiological then phosphorus sequestering would primarily be through a form of anion exchange, driven at a set reaction or adsorption rate (Section 1.3). However, if biological processes were involved then it was hypothesised that it may be possible to influence the extent and rate of sequestering by optimising the conditions under which the biological process occurred. These conditions could then be simulated in the wetland to optimise in-situ removal, possibly resulting in either a smaller wetland area or the ability to increase the loading rate for a particular wetland.

7.2.2 Phosphorus infiltration below the sediment surface
Phosphorus adsorption to wetland sediments predominantly occurs as a form of anion exchange, where the phosphorus becomes tightly associated with iron, aluminium and calcium compounds comprising the sediment matrix. The capacity for adsorption is
therefore limited by the available binding sites. The second factor influencing phosphorus adsorption was the ability of phosphorus to contact the available binding sites. If phosphorus is only able to penetrate the uppermost few millimetres of soil then the removal capacity will be much less than if phosphorus can penetrate deeper, simply because of the number binding sites which it has been exposed to.

The rate of phosphorus adsorption is affected by not only the number of available binding sites but also their configuration and the rate at which they were contacted and able to adsorb particles (Boström et al. 1988). Although water may be able to penetrate rapidly through the subsoil, the phosphorus it contains needs to meet with adsorption sites. A certain period of contact or speed of movement is then necessary for stable particle adsorption (Boström et al. 1988). Faster rates of migration allow contact with a greater number of soil binding sites, enhancing the probability for adsorption. However, slower water movement rates allow for more stable binding to the sediment and less likelihood of phosphorus re-entering the water column (Hynes et al. 1970; Stevenson 1986; Bostrom et al. 1988; Klotz 1991).

Phosphorus penetration of the soil surface is highly dependant on the physical and chemical properties of the soil substrate and the overlying solution. Soil properties that influence the movement of a solution include the geometry of soil pores, their texture, and the net charge of the soil and solute particles (Boström et al. 1988). Monitoring the movement of a particle through the soil matrix is difficult because the majority of methods for analysing soil involve harsh disruption of the soil matrix, preventing repeated in situ measurement (Lehrman 1978; Hynes et al. 1970; Moore et al. 1994).

To overcome this problem, the migration of phosphorus particles through the soil was analysed by autoradiography, using microcosms. The principle of this technique is that radioactive phosphorus particles are allowed to interact with soil particles and the rate and extent of movement of these particles is then mapped using x-ray film (Baker 1989; Rogers 1973). Radioactive particles interact with silver nitrate particles on photographic film causing them to darken when the film is developed, allowing
accurate tracking of particle movement (Baker 1989; International Biotechnologies 1991; Rogers 1973). Undertaking such investigations over a period of time allowed the rate and extent of phosphorus movement to be traced.

The isotope of phosphorus used for autoradiography was phosphorus-32 ($^{32}$P (Rogers 1973)). $^{32}$P is a strong $\beta$ emitter, giving off electrons with a maximum energy of 1.7 MeV (Rogers 1973). The attenuation of radiation intensity is proportional to the density of the material it is penetrating and the distance squared (Shapiro 1990). The radiation levels from $^{32}$P at an intensity of 0.1 mCi are almost 100 percent attenuated by 3 mm of Perspex, 8 mm of water or approximately 4 mm of soil (Rogers 1973; Shapiro 1990). To optimise the image obtained using autoradiography it was therefore desirable to minimise the amount of material between the microcosm and the recording film (Baker 1989; Rogers 1973).

A secondary problem of using $^{32}$P is the formation of Bremsstrahlung radiation (Rogers 1973; Shapiro 1990). This radiation is formed when a high-energy $\beta$ emitter encounters a high molecular weight material such as lead. The resultant reaction converts the $\beta$ radiation into highly penetrating x-ray radiation (Bremsstrahlung radiation). The heterogeneous composition of soil could facilitate the production of Bremsstrahlung radiation within the microcosms and cause fogging of the autoradiographic image (Rogers 1973; Shapiro 1990). This was minimised by optimising the exposure time and the concentration of radioisotope. To protect the operator, Bremsstrahlung radiation is shielded by an element with a heavy atomic mass such as lead (Rogers 1973; Shapiro 1990). The expense and hazard of radiation work necessitated only limited optimisation of isotope concentration, with the focus on optimising exposure times.

The exposure time for the film was affected by a number factors. Firstly, a sensitive x-ray film was required, for this purpose the Kodak X-omat AR film was selected (International Biotechnologies 1991). The exposure time for this film varied according to:

- the radiation levels present;
the energy of the radioactive particles;
the amount of shielding around the source;
the type and density of material the radiation must penetrate before reaching the film;
the half life of the isotope; and
fixing and developing times for the film (International Biotechnologies 1991).

Any shielding or materials of different density around the source ($^{32}$P) such as plastic, water or soil would attenuate the number of disintegration's on the film, complicating image interpretation by increasing exposure time and possible scattering (Baker 1989; Rogers 1973). To minimise the density of material that the radiation had to penetrate each microcosm was fitted with a thin plastic film window within the thicker plastic wall of the container.

The short half life of 14.2 days for $^{32}$P further complicated exposure times (Rogers 1973; Shapiro 1990). As the experiment progressed it was necessary to extend the exposure times of the film to compensate for decreases in the amount of radiation caused by decay of the $^{32}$P. It was also necessary to optimise the times for fixing the emulsion to allow optimal image development.

Radiation levels are commonly measured using the Becquerel (Bq) unit, one Bq equalling one radioactive disintegration per minute (Rogers 1973; Shapiro 1990). Up to a single disintegration could be recorded on a photographic film: depending on its sensitivity. At higher radiation levels, more particles are incident on the film, reducing the time required to create an image (Rogers 1973).

The energy of the radioactive particles affects the path they will take through the film. Low energy particles will hit the film and tend to follow a meandering path through it (Rogers 1973). This creates a wider region of darkening – commonly know as fogging, than a high-energy particle. The high-energy particle will often pass directly through the film following a straight path, much like a bullet, providing a smaller region of fogging. It is for this reason that high energy particles may require a longer
exposure time than lower energy particles to generate an image (Baker 1989; Rogers 1973). Thus, exposure is a compromise, with the overall aim to optimise the exposure of particles from each energy type, always under the constraint that the maximum exposure time is established by the more rapid reaction of the low energy particles and their tendency to cause fogging (Baker 1989; Rogers 1973).

7.2.3 Phosphorus storage compounds in sediment
Phosphorus has the ability to associate with, or be bound into, organic and inorganic compounds. The association with inorganic compounds is primarily through covalent bonding with, and the formation of, metal oxides and oxyhydroxides (Section 1.3). These can readily be dissociated by changes in pH and solubilisation of the associated metal (typically iron, aluminium or calcium). Phosphorus bound into organic compounds is less subject to pH changes and usually must be removed from the organic matrix or compound enzymatically (Sections 1.2.1, 1.3 and 1.5). This makes organically bound phosphorus compounds more stable.

Investigations of the pilot plant wetlands at Richmond indicated that organic phosphorus was a significant component of soil phosphorus (Sections 2.5.7 and 2.6.7). Longer chained organic phosphorus compounds have been shown to be more stable than shorter chained organics (Sections 1.2.1 and 1.5.6). If it were possible to influence phosphorus binding to these larger molecules, this could represent a mechanism for increasing the long-term phosphorus storage in the sediment.

However, determining the amount of phosphorus bound to each carbon fraction was problematic as there was no simple method for separating individual organic and inorganic phosphorus fractions e.g. humic acids vs soluble organic material, other than by a series of sequential extractions (Hedley et al. 1982; Jensen et al. 1993; Petterson et al. 1988; Ruttenburg 1992). These investigations are typically performed on dried soil or sediment, which was subjected to a series of acidification and alkalisation steps to selectively remove metal ions from the soil matrix. Once the metal components had been removed, it was then possible to evaluate the organic component of the soil. These methods were predominantly performed to determine
soil metal concentrations (Jensen et al. 1993; Petterson et al. 1988), with the notable exceptions of Headley et al. (1982) – freshwater analysis, and Ruttenburg (1992) - marine waters, and were still only experimental at the time of the investigation. They needed to be further evaluated to determine their ability to:

1. Selectively remove the target metal ion;
2. Remove an extract that could be analysed to detect phosphorus; and
3. Quantitatively the concentration of phosphorus in the extracted fraction (Hedley et al. 1982; Jensen et al. 1993; Ruttenburg 1992).

The primary basis for the extraction procedure involved using pH and mild oxidation conditions to solubilise the fraction of interest, which is then removed from the sediment by centrifugation and filtration for analysis. However, the initial extraction sequence proposed by Hedley et al. (1982) did not include a method to separate the components of humic material, leaving them in the insoluble residue from the analysis after iron, aluminium, calcium and bioavailable fractions have been extracted.

Fulvic acids are soluble under highly acidic conditions whereas solubilisation of humic acid occurs under alkaline conditions (Section 1.3.5). Hedley et al. (1982) used alkaline conditions to extract the iron and aluminium associated phosphorus fractions from soil. However, it is likely that this extraction would also include a proportion of humic acid associated phosphorus. To overcome this limitation, a modification was evaluated using oxalate and citrate dithionate as alternative extractants for the iron and aluminium associated phosphorus fractions, as outlined by Rayment (1992) and Ruttenburg (1992).

7.3 AIM

The following investigations were undertaken to evaluate:

- The role of microorganisms in sequestering phosphorus to sediment;
- The role of organic compounds in sediment based phosphorus sequestering;
- The ability of phosphorus to penetrate the sediment surface; and
- Phosphorus storage compounds in sediment.
7.4 MATERIALS AND METHODS

7.4.1 The role of microorganisms in sequestering phosphorus to sediment

The amount of TP sequestered to sedimentary material over short and medium time frames was observed. Short time periods were defined as less than 10 hours, medium as a period between 10 hours and 28 days. The organisms in the sample originated from either the soil or effluent. Sterile controls were used to indicate the significance of abiological processes.

Short term – 10-hours

This investigation was to evaluate the relative importance of microorganisms in the short-term removal of TP from the water column. The soil used in this investigation was obtained from pilot plant wetland Unit 5, which had been receiving high concentrations of TP (7-12 mg.L⁻¹) for 6 months prior to sampling. Therefore, this soil should better approximate a mature wetland than other Units receiving lower phosphorus loadings (Chapter 2, and Sections 1.5.6 and 1.6). Secondary treated effluent (as described in Section 2.3.3) was obtained from the Richmond WWTP (Figure 2-1, secondary effluent line).

Samples of sediment from four random locations within Segment 7 of Unit 5 were collected by steel tube (5 cm diameter, 40 cm length) to a depth of 10 cm and mixed while wet in a plastic container (2 L). On sampling, the overlying water was drained off. A final volume of 1 L sediment was collected. This was divided into two equivalent portions (500 mL) - one for gamma sterilisation, the other for use in non-sterile treatments. Soil for gamma irradiation was further dispensed into 70 mL plastic containers (approximately 50 mL of sediment per container).

Effluent (2 L) from the inlet to Unit 5 was collected in polypropylene containers (120 mL) for gamma irradiation. Additional, fresh effluent was collected from the inlet of Unit 5 on the day of the investigation to minimise the effects of effluent age. Both soil and effluent samples for gamma irradiation were sterilised by Steritec Pty Ltd, at 60 kgy for 2 hours (recommended by Steritec as being capable of sterilising this volume...
of soil and water). The concentration of TP in each of the two effluent samples collected was determined by the automated method outlined in Section 2.4.10.

Four separate treatments were established (Table 7-1) to confirm the effects of sterile vs non-sterile soil and effluent on phosphorus sequestering to the soil. Soil samples were obtained wet, to preserve the microbial biomass. To determine the appropriate volume of soil to be added to each sample container it was necessary to determine the equivalent wet mass that gave a dry mass of 10 g. To do this, approximately 50 mL of non-sterile soil was massed and the percentage moisture determined by accurately weighing three aliquots (5 g) of soil, which were then dried at 105°C for 24 hours to obtain the dry weight (Page et al. 1982). Comparison of the dry with the wet weight of the sample determined the moisture content. The values obtained from this measurement were used to determine the average mass of wet soil that gave the equivalent to 10 g dry weight of soil (12.3 ± 2 g wet soil).

Table 7-1: Soil and effluent treatments, designed to evaluate the role of microorganisms in the short-term removal of phosphorus from the water column.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sterile Effluent</th>
<th>Non Sterile Effluent</th>
<th>Sterile Soil</th>
<th>Non-Sterile Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
</tr>
</tbody>
</table>

Key: ✓ = added, - = not added.

Treatments (Table 7-1) were prepared in autoclaved cylindrical glass bottles (600 mL) to which was added 12.3 g of wet sediment, overlaid with effluent (200 mL). Transfer of soil material to the glass container was achieved using a sterile 5 mL plastic syringe (Termulo) with the end removed by a heated scalpel blade, to maintain sterility. Transfer of effluent was by sterile measuring cylinder (200 mL), in a laminar flow hood. Treatments were prepared in triplicate. Each glass bottle was stoppered with cotton wool and incubated at 21°C on a temperature controlled orbital shaker. Flasks for each treatment were placed on the orbital shaker at slow speed (50 rpm) in an effort to maximise contact of the soil with the water column.
Samples of the overlying water were taken every hour over a ten-hour period, including time zero. Sampling involved briefly stopping the shaker, removing the flask and aseptically removing a 10 mL aliquot from each flask using a disposable pipette. Each aliquot (10 mL) was filtered through filter paper by gravity (Whatman Number 41, 11.5 cm) according to the method of Page et al. (1982), and the filtrate analysed by the manual methods for TP (Section 2.4.10).

**Medium term - 28 days**

A second set of flasks was established to observe phosphorus adsorption to the sediment surface over the medium term using the same treatments as the investigation outlined above.

In this investigation samples were not shaken, as it was desired to represent conditions occurring in the wetland over the longer time interval more realistically rather than derive saturation rates of transfer. Flasks were stored at 21°C and exposed to indirect sunlight to stimulate the growth of algae, along with non-photosynthetic microorganisms. Samples were prepared in triplicate for each time interval - 0, 14 and 28 days (total of 9 samples per treatment) and sacrificially sampled at each time, to minimise the possibility for sample contamination. Water from these samples was analysed to determine concentrations of TP (manual methods for TP, Section 2.4.10). The presence of viable microorganisms was checked at 14 and 28 days using the viability stains DAPI and acridine orange as per the methods described by Gerhardt et al. (1994). This was not done for the 10-hour investigation as the time interval involved was presumed to be too short for significant growth of microorganisms.

**Methods for viability stains**

*Acridine orange*: a solution of acridine orange was prepared at a concentration of 0.01% in deionised water. This was placed onto the specimen for a period of 2 minutes, which had previously been air dried onto a standard microscope slide and removed by rinsing twice in tap water. Viewing was by use of a Leitz fluorescent microscope equipped with an appropriate filter block to observe acridine orange (Gerhardt et al.1994).
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**DAPI**: Prepare a stock solution by dissolving 3 μg per mL in acetone (100%). Store in the dark for up to 1 month. Prepare on the day of use and dilute by adding 100 μL to 50 mL of deionised water then adding to an air-dried slide containing the specimen, for a period of 2 minutes and rinse twice with tap water. Viewing was by use of a Leitz fluorescent microscope equipped with a filter block containing an excitation filter at 350 nm and an emission filter for 450 nm (Gerhardt et al. 1994).

7.4.2 **Phosphorus infiltration below the sediment surface**

In order to evaluate the movement and penetration of phosphorus through the soil using the technique of autoradiography, five trial microcosms were established in white polypropylene containers (250 mL volume, 18 cm high x 5 cm diameter, wall thickness 2 mm). The neck of each container was removed by scalpel and a window measuring 6 cm x 6 cm, created 0.5 cm above the base of the container (Figure 7-1). This window was covered by Clingwrap® that was taped in place and sealed with a commercial silicone sealant (Selly’s).

Sixty grams of soil was transferred to each microcosm. The soil was obtained from the central region of Unit 5 of the Richmond pilot plant wetlands using the soil corer described in Section 2.4.7. The soil was air-dried at 23°C for 5 days and ground with a mortar in a pestle to pass a 0.5 mm sieve. A two-month old seedling of *Phragmites australis* was planted into the soil of two microcosms immediately after the addition of soil to the microcosms. The artificial effluent described in Table 6-1 (40 mL, with calcium omitted as a buffer due to the presence of the soil and the large amount of calcium precipitate (as observed in Chapter 6, Section 6.4.2 and 6.5.2)) was slowly added to the soil of each microcosm to minimise disruption of the soil surface, creating an overlying depth of water (approximately 2 cm) above the soil.
Figure 7-1: Design of microcosms used in autoradiography (not to scale), indicating the location of the viewing window constructed of a thin wall of Clingwrap® to facilitate image collection by the x-ray film.

Microcosms were placed at 23°C under grow lights with a photoperiod of 12 hours (timer controlled) for 14 days to allow growth of biofilms and stabilisation of the system. The grow lights were two grow lux fluorescent tubes at a distance of 20-30 cm above each treatment (see Chapter 6 for the rationale of distancing). A control was established that replicated the test conditions in the absence of added $^{32}$P (Amersham), to determine whether other particles within the sample were capable of interacting with the film and background radiation.

Each microcosm was placed inside a larger (20 cm height, 10 cm internal diameter polypropylene container, 2 mm wall thickness) which was positioned inside a plastic tub (650 mm x 450 mm x 300 mm, wall thickness - 3 mm). The placement of these containers permitted a mechanism of double containment for any potential radiation spill. Radiation shielding comprised of clear lead/Perspex barriers (2 cm thick) for whole body shielding and Perspex pipette guards (3 mm thickness) for hand and arm protection. All procedures with samples were conducted using remote handling devices. Three layers of rubber gloves were worn at all times and procedures were
thoroughly rehearsed before handling the radiation source to minimise exposure times. A Bicron 50 Geiger Muller tube able to detect disintegration’s per minute (DPM) and dose rate per hour in milli-Roentgens was used to detect radiation levels emitted from the microcosms to check for leaks and confirm overall safe operating levels were maintained.

Prior to exposure of the film to the radioactive source, it was removed from the packaging and secured to a curved film holder by tape. This procedure was performed in a laboratory dark room and the light source was a 25 W household globe, filtered through a red filter at a distance of greater than 2 m (International Biotechnologies 1991). Exposure of film to the radiation source took place in light proof, radiation laboratory. The film was transferred from the darkroom in a light proof box (600 mm x 400 mm x 150 mm). A hand held timer equipped with an alarm was used to determine exposure times. The light source for working in this room was a red photographic safelight globe (15W).

The film used for autoradiography was Kodak X-AR 5 (13 mm x 18 mm) as recommended by Eastman Kodak. The film was developed using Kodak x-ray film developer and replenisher and fixed with Kodak x-ray film fixer and replenisher according to the manufacturers’ instructions (International Biotechnologies 1991). The procedure was: developer for 5 minutes, wash for 30 seconds, fix for 6 minutes, wash for 6 minutes. The film was then hung in a well-ventilated area to dry.

An initial study was conducted to determine exposure times for the x-ray film. A volume of $^{32}$P solution (equivalent to 1 mCi) was added to one of the microcosms containing soil and water only. Such a high dose was used to overcome expected dampening by water and soil. The control for this experiment contained no $^{32}$P. The film was attached to the film holder shown in Figure 7-2. This was manipulated into place, adjacent the window region of the microcosm. A variety of exposure times, from 24 hours to 20 seconds, were investigated to determine optimal film exposure times. This investigation was repeated in duplicate with 0.5 mCi of $^{32}$P, in
microcosms with and without plants, exposing film at days 1, 2, 4 and 7 for the optimal times determined by the initial studies above (Baker 1989).

Figure 7.2: Apparatus used to position and expose film to radiation from microcosms. Note the curvature of the film holder was used to minimise the distance between the film and the microcosm.

7.4.3 Phosphorus storage compounds in sediment

A laboratory investigation was conducted to evaluate the effectiveness of various extraction techniques in determining the association of TP with the iron, aluminium, calcium and organic fractions of soil. The procedure was conducted in two stages. The first was a coarse analysis to evaluate the association of TP with organic matter. The second was a sequential extraction to determine the concentration of TP bound in different organic and inorganic soil fractions. Soil used in the analyses below was a homogenised composite of that obtained from the upper 10 cm of soil adjacent the outlet from all wetland Units (Section 2.4.7). Dr K Sakadevan of the Water Research Laboratory undertook compositing and homogenisation of soil.

Coarse fractionation analysis

Stage 1, coarse evaluation, TP, OP and IP were analysed on triplicate samples by the ignition method (Page et al. 1982) outlined in Chapter 2 (Section 2.4.7). The purpose of this analysis was to confirm the background concentrations of TP, OP and IP on sediments prior to more detailed fraction based on the method of Hedley et al. (1982) outlined below.

Sequential extraction procedure

The second extraction procedure was more complex and was used to determine the association of phosphorus with individual organic and inorganic fractions within the
soil. The actual method used is shown in Figure 7-3. It represents a modification of a number of methods, primarily that of Hedley et al. (1982) incorporating humic acid/fulvic acid/humin separation extraction steps based on Snitzer et al. (1972) and Francko (1979), and an alternative iron/aluminium extraction step according to Rayment (1992) and Ruttenburg (1992).

Duplicate samples were created for each analysis path, making a total of 6 samples (Figure 7-3). These were prepared by adding 0.5 g of soil to separate 50 mL centrifuge tubes. Two samples were used to determine the concentration of phosphorus within microorganisms, along with labile and bioavailable phosphorus, using the right hand branch of the flow diagram (Figure 7-3). These two samples were treated to lyse any microbial cells on the sediment by adding 1mL of CHCl₃ to each centrifuge tube and shaking for 1 hour. At the conclusion of this period, each centrifuge tube was placed in a fume hood overnight. Labile and bioavailable phosphorus (including that from the lysed bacterial cells) was then extracted by adding 20 mL of 0.5 M NaHCO₃ to each centrifuge tube, which were then shaken for 16 hours. On completion of shaking, each tube was centrifuged and the supernatant removed for filtration and analysis (See below for details of filtration and centrifuging methods). The pellet was discarded.

The remaining four samples were analysed to determine the amount of phosphorus associated with the other soil fractions, detailed in the left hand side of the flow diagram (Figure 7-3). Labile and bioavailable phosphorus (excluding that from the lysed bacterial cells) was first extracted by adding 20 mL of 0.5 M NaHCO₃ to each centrifuge tube, which was then shaken for 16 hours. On completion of shaking, each tube was centrifuged and the supernatant removed for filtration and analysis (See page 253 for details). The difference between this fraction and that containing lysed microbial cells could be used to evaluate the amount of phosphorus contained in microorganisms.

The iron and aluminium associated phosphorus fraction was then evaluated by resuspending the pellet in a solution of either dithionate-citrate or oxalate media.
(Figure 7-3 - See below for solution details). On completion of shaking, each tube was centrifuged and the supernatant removed for filtration and analysis. The pellet was resuspended in 30 mL of 1 M HCl solution to extract the calcium associated phosphorus fraction (Figure 7-3). On completion of shaking, each tube was centrifuged and the supernatant removed for filtration and analysis (See below for details).

A stronger acid wash (using H₂SO₄, 5mL), as noted by Hedley et al. (1982) was used to dissolve and collect the fulvic acid fraction, done after a milder extraction with HCl to remove the calcium associated phosphorus fraction (Figure 7-3). The humic acid fraction was extracted by resuspending the pellet in 30 mL of 1 N NaOH to create alkaline conditions. The pellet obtained after centrifugation and filtration of this fraction should contain the residual humin material (Figure 7-3).

All shaking was performed on an orbital shaker (shaking speed 200 rpm) and all investigations were undertaken at room temperature. Centrifugation was by a Sorval ultracentrifuge (3000 g for 10 minutes), with the supernatant being filtered by gravity through a Whatman No. 11 filter paper prior to collection and analysis by the manual method for TP (Section 2.4.10). Concentrations of Fe, Al and Ca in the supernatant were determined by atomic absorption spectroscopy using the methods described in APHA (1992) (method numbers 3-66, 3-43, and 3-57) to evaluate the effectiveness of that extraction step.
Figure 7-3: A flow diagram of the methodology used for determining the association of phosphorus with various fractions of soil, based on the methods of Hedley et al. (1992) and Ruttenburg (1992).

Dry and homogenise 10 g of soil

Create samples by adding 0.5 g of soil to 50 mL centrifuge tubes (6 per run)

4 replicates
Add 30 mL of 0.5 M NaHCO₃
Shake for 16 hrs
Centrifuge
Supernatant = bioavailable P

2 replicates
2 replicates
Add 1 mL of CHCl₃ (1mL)
Shake for 1 hr then evaporate CHCl₃ overnight
Add 30 mL of 0.5 M NaHCO₃ and shake for 16 hrs
Centrifuge
Supernatant = microbial P

Resuspend pellet in Dithionate-citrate solution or oxalic acid solution
Shake for 16 hours
Centrifuge
Supernatant = Al/Fe associated P

Resuspend pellet in 30 mL of 1M HCl
Shake for 16 hrs
Centrifuge
Supernatant = Cu associated P

Acidify to pH < 1 with 5 ml of H₂SO₄
Shake for 2 hrs
Centrifuge
Supernatant = Fulvic acid associated P

Alkylate with 1 N NaOH
Shake for 2 hrs
Centrifuge
Supernatant = Humic acid associated P

Residue = Humin

Inorganic fractions

Organic fractions
CHAPTER 7

Reagents

These solutions were prepared using the methods described in Rayment (1992).

Dithionate-citrate solution

Dissolve 220 g of sodium citrate (Na$_3$C$_6$H$_5$O$_7$·2H$_2$O) in deionised water and dilute to 1.0 L on the day of analysis. Add 30 mL of this solution to each centrifuge tube as required (Figure 7-3), along with 0.6 g of sodium dithionite (Na$_2$S$_2$O$_4$·H$_2$O).

Oxalic acid solution

Dissolve 16.2 g of ammonium oxalate (COONH$_4$)$_2$·H$_2$O in approximately 300 mL of deionised water. In a separate container dissolve 10.8 g of oxalic acid (COOH)$_2$·2H$_2$O in approximately 300 mL of deionised water. Combine the two solutions and make up to 1 L with deionised water. Add 30 mL of this solution to centrifuge tubes as noted in Figure 7-3.

7.5 RESULTS

7.5.1 The role of microorganisms in sequestering phosphorus to sediment

Short term – 10 hours

This investigation was to determine the role of bacteria and algae in short term phosphorus mobilisation processes. TP concentrations differed between sterile and non-sterile effluent at time zero due to the several days required to sterilise the effluent. Sterile effluent contained 5.81 mg.L$^{-1}$ TP and non-sterile effluent contained 7.50 mg.L$^{-1}$ TP at time zero.

Results for sterile and non-sterile effluent are presented separately to allow clearer visualisation of results. At time zero there was an initial rapid adsorption of effluent to the sediment on combining the two in the treatment flask (Figures 7-4 and 7-5). For treatments involving sterile effluent, non-sterile soil had lower concentrations of TP in the water column between 1 hr and 7 hours after commencement (with one exception
Figure 7-4: Total phosphorus concentrations in the water column of containers with sterile effluent, and sterile and non-sterile soil from the pilot plant wetland over a 10-hour period.

Key: Treatment 1 – Sterile soil and sterile effluent
Treatment 3 – Non-sterile soil and sterile effluent
Initial concentration of TP = 5.81 mg.L⁻¹

Figure 7-5: Total phosphorus concentrations in the water column of containers with non-sterile effluent, and sterile and non-sterile soil from the pilot plant wetland over a 10-hour period.

Key: Treatment 2 – Sterile soil and non-sterile effluent
Treatment 4 – Non-sterile soil and non-sterile effluent.
Initial concentration of TP = 7.50 mg.L⁻¹
at 3 hrs, Figure 7-4). After 7 hours, there was no effective difference between treatments (Figure 7-4).

In treatments containing non-sterile effluent, the non-sterile soil treatment showed almost linear removal of TP over the 10-hour period. Removal from the sterile soil treatment containing non-sterile effluent followed a similar trend but with a more sporadic removal pattern. The concentrations of TP in both treatments that contained non-sterile effluent were not significantly different after 10 hours (Figure 7-5), although the average concentrations appeared to differ occasionally, on a random basis (4 and 9 hours, Figure 7-5). Flasks containing sterile effluent and non-sterile soil appeared to be the only treatment to reach an equilibrium concentration of TP in the water column, after some 5 hours, with only slight removal thereafter.

The net amount of phosphorus absorbed by each treatment per hour, averaged over the 10-hour period of this investigation, was similar for each treatment (Table 7-2). A regression equation was used to derive the slopes of the curve and hence the removal rate from each treatment.

**Table 7-2: Removal rates averaged over a 10-hour period to total phosphorus removed by various treatments of sterile and non-sterile effluent and sediment.**

<table>
<thead>
<tr>
<th>Treatment Number</th>
<th>Average TP removal rate $\text{mg.L}^{-1}.\text{hr}^{-1}$</th>
<th>Regression coefficient $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.27</td>
<td>0.91</td>
</tr>
<tr>
<td>2</td>
<td>0.28</td>
<td>0.82</td>
</tr>
<tr>
<td>3</td>
<td>0.26</td>
<td>0.84</td>
</tr>
<tr>
<td>4</td>
<td>0.27</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Note: for the initial 5-hour period in treatment 3, where a more rapid removal of total phosphorus was observed from the water column the average removal rate was 0.36 $\text{mg.L}^{-1}.\text{hr}^{-1}$ becoming negligible thereafter.

Key: Treatment 1 – Sterile soil and sterile effluent
Treatment 2 – Sterile soil and non-sterile effluent
Treatment 3 – Non-sterile soil and sterile effluent
Treatment 4 – Non-sterile soil and non-sterile effluent.

**Medium term – 28 days**

A medium investigation over 28 days was carried out to assess whether the TP removal rates observed over 10 hours continued into the medium term and reached a
stable equilibrium, or whether TP was released back to the water column. Cultures in the medium term investigation were established at the same time as those in the short-term investigation therefore concentrations of TP in the sterile and non-sterile effluent were as noted above. Water column results are summarised in Figure 7-6 (Time zero samples for TP were taken within 10 minutes of combining the soil and effluent).

Figure 7-6: Concentrations of total phosphorus in the water column of various sterile and non-sterile treatments over a period of 28 days.

In all treatments, the most rapid removal of TP from the water column occurred between days 0 to 14 (Figure 7-6). Between days 14 and 28, TP in the water column above sterile soil treatments evidenced a further slight decrease in TP concentrations, whilst TP in the water column above non-sterile soil did not change significantly (Figure 7-6).

The primary differences between treatments appeared to be related to soil sterility. The presence or absence of microorganisms in the effluent had little effect on medium term TP concentrations. Bacterial analysis of the sterile soils indicated no viable cells using DAPI or acridine orange after 14 or 28 days.

7.5.2 Phosphorus infiltration below the sediment surface
Images from the autoradiography investigation are presented in Appendices 5 and 6, Figures 5-A to 5-E and 6-A to 6-D. Exposure times ranging from 10 minutes to 24 hours resulted in excessive darkening of the film. Times of 20 and 30 seconds
provided optimal image resolution. Images taken in microcosms with soil only, indicated that only the surface layer of sediment had been penetrated by the radiolabelled phosphorus directly after addition to the microcosm. It was not until after 5 days that radiation was observed below the surface of the soil. This result was also observed on day 7, although there was no significant change in depth at which radioactive phosphorus was detected between these two days.

In Figures 5-A to 5-E (Appendix 5), darkened regions were observed at the soil surface, indicating an accumulation of isotope at the sediment surface over time. The lighter grey regions between these lines are suggested to be due to attenuation of radiation by the water column (refer Section 7.2.3). Note that there was no obvious region of phosphorus occurrence, despite some fogging, below the top 2-3 mm of soil in the first few days of the investigation. However, after day 5, a dark region was observed (Appendix 5, Figures 5-A to 5-E) some 5 to 10 millimetres below the sediment surface, indicating the presence of $^{32}$P in one of the two replicates, this was not observed in the second replicate.

Addition of a plant to the microcosm resulted in a rapid uptake of phosphorus into the plant within 2 days (Appendix 6, Figures 6-A to 6-D). Phosphorus was clearly visible within the plant structure. On day 2, phosphorus appeared to be visible below the soil surface (Appendix 6, Figure 6-B) was hypothesised to be due to translocation by the growing plant. In the planted culture, the amount of radiation detected above the sediment gradually increased over time. In the last few autoradiographs, the outline of the growing seedling was found to become obvious. The distinguishing dark lines at the surface of the water and soil columns were fainter, but still present. Radiolabelled phosphorus was not detected below the first few millimetres of soil in any tube.

7.5.3 Phosphorus storage compounds in sediment
Initial investigations were aimed at determining the concentrations of total, organic and inorganic phosphorus in composited soil samples from the pilot plant wetland (Table 7-3) to provide a background reading for benchmarking the results from the
CHAPTER 7

more detailed analysis. Approximately two thirds of the soil phosphorus was in the inorganic fraction (Table 7-3), consistent with results from Chapter 2, Section 2.5.7.

Table 7-3: Concentrations of total phosphorus, inorganic phosphorus and organic phosphorus at the Richmond pilot plant wetlands (average of composite samples taken adjacent the outlet of each wetland).

<table>
<thead>
<tr>
<th>Phosphorus fraction</th>
<th>Richmond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (mg P/kg soil)</td>
<td>354 ± 87</td>
</tr>
<tr>
<td>Inorganic (mg P/kg soil)</td>
<td>245 ± 87</td>
</tr>
<tr>
<td>Organic (mg P/kg soil)</td>
<td>109 ± 7</td>
</tr>
</tbody>
</table>

The modified method of Hedley et al. (1982), described in Section 7.4.3 was used to determine the association of phosphorus with the minerals iron, aluminium, calcium, miro-organisms and other organic material in soils from the Richmond pilot plant wetlands (Table 7-4).

Table 7-4: Phosphorus, iron (Fe), aluminium (Al) and calcium (Ca) concentrations in triplicate soil samples from the Richmond Pilot Plant wetlands which were fractionated according to modified method of Hedley (1982), as detailed in Section 7.4.3, to determine bioavailable phosphorus and phosphorus concentrations associated with microorganisms, Fe/Al, Ca, fulvic and humic acids.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Extractant</th>
<th>TP (mg.g⁻¹)</th>
<th>Fe (mg.g⁻¹)</th>
<th>Al (mg.g⁻¹)</th>
<th>Ca (mg.g⁻¹)</th>
<th>% TP²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbial + Labile</td>
<td>Carbonate/ Chloroform</td>
<td>59.3 ± 4.85b</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>32.1</td>
</tr>
<tr>
<td>Labile</td>
<td>Carbonate</td>
<td>47.9 ± 8.01</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>25.9</td>
</tr>
<tr>
<td>Fe/Al</td>
<td>Oxalic³</td>
<td>3.2 ± 0.07</td>
<td>3.7 ±0.15</td>
<td>1.1 ±0.57</td>
<td>0.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Fe/Al</td>
<td>Dithionite*</td>
<td>2.0 ± 0.12</td>
<td>17.7±1.07</td>
<td>1.8 ±0.20</td>
<td>0.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Ca</td>
<td>0.1 N Acid</td>
<td>79.6 ± 20.07</td>
<td>2.1 ±0.94</td>
<td>0.9 ±0.13</td>
<td>0.0</td>
<td>43.1</td>
</tr>
<tr>
<td>Fulvic acid</td>
<td>1 N Acid</td>
<td>26.9 ± 1.20</td>
<td>0.2 ± 0.01</td>
<td>0.3 ± 0.08</td>
<td>0.0</td>
<td>14.6</td>
</tr>
<tr>
<td>Humic acid</td>
<td>0.1 N Alkali</td>
<td>16.9 ± 0.28</td>
<td>0.1 ± 0.02</td>
<td>0.6 ± 0.15</td>
<td>0.0</td>
<td>9.1</td>
</tr>
<tr>
<td>Total¹</td>
<td></td>
<td>184.7 ± 27.42</td>
<td>20.1 ± 2.05</td>
<td>3.5 ± 0.56</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

¹ Percentage TP was calculated based on the percentage of the total amount of phosphorus extracted using this extraction method.

² Variations are expressed as standard deviations.

³ The total was derived from the sum of labile phosphorus, dithionite extracted Fe/Al, Ca bound phosphorus, fulvic and humic fractions.

The total was more than the sum of the Fe/Al and Ca fractions.

Oxalic and dithionite extractions produced a yellow discolouration, which was not removable using activated carbon, suspected to be the cause of the low percentage recovery in the iron and aluminium fractions.

Greg Ryan
PhD Thesis – UWSH
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An alternative TP detection method (the Vanado-molybdate method, APHA 1992, method number 4500-P C) was used in an effort to determine the phosphorus concentration in iron and aluminium fractions. Despite using this method, improved quantitation was not possible.

Values for iron and aluminium as determined in extracted fractions examined by Atomic Absorption Spectrophotometer were in agreement with those obtained by Dr Sakadevan from the Water Research Laboratory (Sakadevan 1998). TP concentrations were very low in the dithionite and oxalic fractions where it was expected to be high, due to interference caused by the yellow discoloration of the solution. Dithionite was more efficient than oxalate for extracting iron from the soils examined.

Extraction of the ‘calcium’ associated phosphorus fraction indicated a very high percentage recovery of phosphorus (Table 7-4), however, this fraction contained no extracted calcium, but rather had high concentrations of residual aluminium and iron. Recovery of the fulvic and humic acid associated material indicated very low recoveries of iron and aluminium. In addition, a lower amount of extractable phosphorus was associated with these fractions.

Microorganisms appeared to account for less than 0.1% of the total phosphorus in the sediment obtained from the pilot plant wetlands, using this extraction method.

7.6 DISCUSSION

7.6.1 The role of microorganisms in sequestering phosphorus to sediment

Short term

This investigation was designed to examine the role of microorganisms in the rapid, short term sequestering of phosphorus from the water column to sedimentary material over a period of 10 hours. Phosphorus adsorption was not significantly different between treatments. An exception was the initial more rapid removal of TP from the water column in sterile soil treatments containing non-sterile effluent. This may have been related to the ability of microorganisms from the water column to rapidly fill
binding sites on the soil, due to the absence of any competitors and the availability of sites on the soil free of microorganisms. Otherwise, the removal of TP from the water column appeared to be unrelated to the presence of microorganisms on the sewage or the soil. Rates of absorption averaged between 0.26 to 0.28 mg kg\(^{-1}\) hr\(^{-1}\).

The observation that microorganisms were may not have been significantly involved in short term phosphorus sequestering was consistent with the low amount of phosphorus observed in the microbial fraction of soil from the pilot plant wetlands (Section 7.6.3). However, observations by others (Richardson et al. 1986; Stevenson 1986; Vymazal 1995) would indicate that this observation may not be typical of all wetlands. In some North American wetland systems, it has been concluded that a significant portion of TP removal was due to the microorganisms, primarily fungi and yeasts (Richardson et al. 1986).

Medium term
The medium-term investigation of TP removal indicated that phosphorus removal could be sustained, possibly at a lower adsorption rate, with significant concentrations of TP being removed from the water column after 14 days. However, removal appeared to plateau between 14 and 28 days, consistent with observations by Richardson et al. (1986).

There were two apparent trends in TP removal observed from the medium term investigation. The first was that phosphorus was reduced by at least 90% in all treatments. The amounts of TP removed were significantly greater in non-sterile soil than sterile soil treatments, only approaching the removal rates of non-sterile soil if a microbial inoculum was provided in the overlying water column. The second factor was that the time to achieve this amount of phosphorus removal was significantly quicker in treatments containing non-sterile soil. By 14 days, phosphorus removal had effectively reached equilibrium in non-sterile soil treatments, whereas the rate in sterile soil had slowed but not yet reached equilibrium. After 28 days, the relative concentrations of TP in the water column were roughly equivalent. Richardson et al. (1986) observed that phosphorus concentrations in the water column did not differ between various soil treatments with different microbial loads, after 30 days.
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Given the known concentration of phosphorus in the overlying water column and a selected sediment substrate, an equilibrium phosphorus concentration appeared to be established in the overlying water column. Therefore, if a body of water was left over sediment from the Richmond pilot plant wetlands, without significant movement, for an indefinite time period, it is likely that the concentration of TP in the water column would not be significantly different from that observed after 14 days. It remains a point of conjecture as to what factors were controlling this equilibrium condition. They may be phosphorus concentration, phosphorus species and soil type.

This investigation would also appear to indicate that microorganisms may have a role in controlling the final concentration of phosphorus adsorbed to the sediment surface. The presence of microorganisms appeared to facilitate the binding of TP over the medium term, consistent with the observations of Richardson (1986), Stevenson (1986) and Vymazal (1995). However, during the first few hours the adsorption process, microorganisms appeared to have almost no significant role in affecting the rate at which adsorption occurred.

The concentration of phosphorus absorbed by the sediment under laboratory conditions was almost twice as high as under field conditions. There are two possible explanations for this observation: 1) availability of binding sites; and 2) degree of soil saturation. In undertaking these investigations a significant amount of soil disturbance occurred. Hence the soil was not as compacted as it was under field conditions. It was not possible to match the overall soil depth or level of compaction present in the field due to the scale of the laboratory investigations. Therefore it is likely that the added phosphorus had more available phosphorus binding sites than were present under field conditions. In addition, the soil for these investigations was obtained early in the establishment of the Pilot Plant Wetlands as so may have been less phosphorus saturated, which may have been an additional contributing factor to the high phosphorus absorption rates observed under laboratory conditions.
7.6.2 Phosphorus infiltration below the sediment surface

The autoradiography investigation involved the use of the radiolabelled isotope $^{32}\text{P}$ in microcosms to determine the time taken for phosphorus to penetrate the soil surface. It was also to determine whether phosphorus was retained at the sediment surface or able to penetrate below the first few millimetres of the sediment surface in a non-flowing system with a sealed base. The reason phosphorus might be retained only at the sediment surface may be due to saturation of binding sites and establishment of an equilibrium with the water column (Richardson et al. 1986). If this situation existed then it would be necessary to destabilise this equilibrium to drive phosphorus deeper into the sediment. Planted systems may provide such a mechanism, uptake of phosphorus or water from the soil by the plant would act to change the soil/water equilibrium, taking phosphorus or water into the plant, depleting the concentration on the soil, causing a concentration gradient to move phosphorus deeper into the soil (Brix et al. 1993). The non-flowing system was used because of the necessity to work with radioactive $^{32}\text{P}$. The flows within the Richmond pilot plant wetlands were extremely slow and the non-flowing system should act as a conservative estimate for phosphorus movement through the soil.

The results of the radiolabelled investigation indicated that within 7 days phosphorus addition to the overlying water column, radiolabelled phosphorus had penetrated the top few millimetres of soil. The distribution of phosphorus in these top few millimetres was relatively homogenous. After 7 days, a single area of movement deeper than a few mm was observed in one treatment. This was quite localised and while it indicated the potential for phosphorus to move deeper in the sediment, the effect was localised and not consistent between treatments. The most likely reason for this movement would be a more open soil pore structure in this region of the soil (Salisbury et al. 1978), possibly during establishment of the microcosm.

The important implication of the first stage of the investigation was that on initial contact phosphorus was able to contact and move through the top few mm of the sediment in a non-flowing system. Contact times with the water column and particular region of soil within the pilot plant wetlands at Richmond was in the order of minutes
to hours, not days due to flow rates (Chapters 2, 3 and 4). In this time frame, it was unlikely that overlying water would significantly penetrate the sediment pores and deposit phosphorus. The most obvious saturation point was the sediment surface. Points deeper within the sediment would contact phosphorus, but the rate of movement and adsorption may not result in a significant overall removal from the water column in the absence of some form of pumping mechanism.

The presence of plant material in the wetland appeared to act as a pump (Chapter 2, Sections 2.5.2 and 2.6.2, Appendix 6, Figures 6-A to 6-D), moving water through the sediment and most likely increasing the rate and depth to which phosphorus penetrated the soil surface through entrainment. However, this was not able to be confirmed by field measurements due to the high background TP concentrations present in wetland soil (Chapter 2, Sections 2.5.7 and 2.6.7). Phosphorus was sequestered in plant tissue through the adsorptive action of the roots or stem, as was observed in the autoradiograph. Note that this was a young establishing plant and as such, it would take up significantly more phosphorus than a mature plant. It was not possible to use mature plants in this investigation due to the problems of potential radiation containment on scaling up. Whether this translocation would have occurred in a mature plant is questionable and was not able to be determined by autoradiography.

At the time of planting, all plant roots were below the sediment surface. Thus, it could be hypothesised that the plant would rapidly utilise labile phosphorus in the regions surrounding the roots and was able to draw newly added radioactive phosphorus through the soil. The fainter band of radioactivity at the sediment surface indicated lower phosphorus concentrations in the soil, away from the plant roots (relative to the non-planted microcosms) and was consistent with the accumulation of phosphorus in the soil around the plant and in plant tissues. Since this was a young, establishing plant this result is not unexpected, however, as the plant matured the rate of uptake might be expected to decrease. To determine the extent and rate of uptake this investigation could be repeated with a mature plant. This would establish the role of the roots in increasing soil porosity, facilitating phosphorus removal and allow
evaluation of phosphorus incorporation into growing plants as an active phosphorus pumping mechanism.

To minimise disturbance to the soils and enable collection of data representative of a field situation these studies could be conducted in a mature wetland system, however, because of problems associated with radiation containment this was not attempted. Performing such a study on a smaller scale would be difficult because the size and thickness of the container used to grow the plant would preclude the development of meaningful images by autoradiography. Data could be obtained by scintillation counting but a number of difficulties would need to be overcome. A procedure for harvesting the sedimentary material in increments of 0.5 cm or less, while maintaining the integrity of the surrounding sediment, contaminated with radioisotope would need to be developed. Samples need to be obtained in discrete amounts to be comparable. The transfer to the scintillation vial without spillage or contamination, and problems of soil quenching the signal from the scintillation vial would also need to be overcome. One possible method would be to freeze the sample under liquid nitrogen, then cut out the required segments. Considerations of cost prevented this study from occurring.

However, despite the lower phosphorus uptake by mature wetland plants they can still act as efficient water pumps (refer Chapter 2 - Evapotranspiration). Thus, as water is pumped through the plant during transpiration it is suggested that this water also brings with it soluble orthophosphate, which can be adsorbed to the sediment, sequestered by microorganisms in the rhizosphere or taken into the plant (supported by arguments put forth by Brix et al. 1993). This may be one reason mature wetlands provide more efficient phosphorus removal than unplanted ponds.

7.6.3 Phosphorus storage compounds in sediment
The investigation of different phosphorus fractions was to evaluate the role of biotic and abiotic material in phosphorus sequestering in soils and the relative importance of calcium, iron and aluminium ions in this process. The simple extraction procedure provided results that were consistent with the simpler acid extraction for inorganic
and organic phosphorus used by Sakadevan et al. (1996). This confirmed that 71% of phosphorus in this sample was associated with inorganic soil components.

The more detailed extraction method based on the method of Headley et al. (1986) did not provide results consistent with the simple organic/inorganic fractionation scheme discussed. Using the detailed fractionation scheme it was not possible to directly quantitate iron and aluminium associated phosphorus (Section 7.5.3). In addition, evaluation of the extraction technique for calcium by atomic absorption spectrophotometer indicated residual iron and aluminium were present in the sample. This meant that the method did not totally separate aluminium and iron either of the extraction steps designed to capture this fraction and the ability of the proposed method to extract phosphorus associated with these fractions must be questioned. At the time of investigation no suitable alternative extraction method existed for all phosphorus fractions and therefore results were based on the current imperfect system.

Using the two proposed extraction methods it was not possible to colorimetrically determine the concentration of phosphorus associated with aluminium and iron extracts, due to interferences from oxalate and dithionite used to extract these metals. A preferable method of detection may have been the use of high-pressure liquid chromatography (HPLC), which could help remove this pigmentation, although this was not undertaken.

The concentrations of TP obtained by each soil extraction method were not in agreement. The concentration of TP obtained using the method of Hedley (Hedley et al. 1982) was approximately 50% of that obtained using the simple fractionation technique (Sakadevan et al. 1995), primarily due to the inability to quantitate phosphorus concentrations in the oxalic and dithionate extraction steps. If these fractions were assumed to contain the residual of TP then there is good agreement between the percentage organic and inorganic composition for each extraction method (in the method of Hedley et. al. (1982) the Fe, Al and Ca extracted fractions were assumed to be inorganic).
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Using this assumption the majority of inorganic phosphorus was bound in the aluminium and iron fractions. The majority of organic phosphorus was associated with the labile fractions, with almost none observed in the microorganism related fraction with slightly less organic phosphorus bound into humic material. Thus, the microbiota appeared not to be a significant store of phosphorus.

The extraction procedure did not remove calcium from the sediment matrix and should have concluded with ashing the sample at 550°C. This would have determined the true concentration of TP residual in the sediment (Abbot 1987). Investigations on the concentrations of inorganic and organic phosphorus in soils indicated that the majority was in the inorganic form regardless of soil type.

7.7 CONCLUSIONS

7.7.1 The role of microorganisms in sequestering phosphorus to sediment
In the short-term microorganisms did not appear to be responsible for the direct sequestering of phosphorus to the sediment surface. Over a 28-day period, phosphorus adsorption was more extensive and appeared to be positively influenced by the presence of microorganisms on the sediment surface. The absence of microorganisms had a noticeable slowing effect on phosphorus removal from the water column.

7.7.2 Phosphorus infiltration below the sediment surface
Phosphorus infiltration primarily occurred in the top few mm of soil with only localised regions where deeper infiltration was observed. Even here, movement was restricted to less than 0.5 cm over a 10-day period (slightly less than reported for a full scale wetland of 5 cm – Craft et al. 1995). The presence of a live seedling allowed more rapid movement of phosphorus through the sediment substrate. This movement was associated with the active uptake of phosphorus and movement of water through the sediment to replace water lost from the interstitial spaces through transpiration (consistent with mechanisms hypothesised by Brix et al. 1993). Therefore, plants were a significant factor in the translocation of phosphorus through the sediment.
7.7.3 Phosphorus storage compounds in sediment
Within the sediments of the pilot plant wetlands, inorganic phosphorus compounds, predominantly through association with iron and aluminium were the dominant forms stored. The organic phosphorus fraction made up less than 33% of the TP fraction and almost none of this appeared to be associated with microbial biomass.

The phosphorus sequestering investigations highlighted that abiological processes predominantly governed phosphorus removal, although microorganisms may have a role in assisting phosphorus sequestering to the soil. The movement of phosphorus through the sediment indicated that phosphorus would initially only contact the top few mm of sediment without the presence of plants, providing a very limited surface for adsorption. Plants significantly increase the surface area available for phosphorus adsorption by causing the more rapid migration of water and phosphorus through the sediment due to growth and transpiration. The contribution of plant roots to further phosphorus movement after plant maturation due to entrainment associated with transpiration should not be underestimated.
CHAPTER 8: THESIS SUMMARY

8.1 BACKGROUND

The purpose of the investigations detailed in this thesis has been to elucidate the predominant phosphorus removal and storage mechanisms within wetlands receiving secondary and tertiary treated effluent under Australian conditions. Several key questions were investigated:

1. Was phosphorus removed consistently from the wetlands under investigation?
2. If so, were there specific regions of removal?
3. Was removal specific to plant type e.g. lignified, semi-lignified, emergent or submerged?
4. What was the role of microorganisms in the phosphorus removal process?

Studies were undertaken on four pilot plant wetlands constructed at Richmond, NSW. Initial investigations examined the interaction of different effluent types and phosphorus loadings on removal of phosphorus when comparing inlet and outlet concentrations. In addition, a number of other parameters including suspended solids, BOD, turbidity, conductivity, pH and nitrogen species were evaluated to determine whether any correlations were present between them and total phosphorus removal for each wetland. The outcomes of these preliminary investigations were summarised in Chapter 2.

In addition, phosphorus removal processes within the plant stands of four wetland Units were evaluated by taking grab samples and comparing inlet and outlet phosphorus concentrations over time (Chapter 3). These investigations highlighted internal variations between each wetland and indicated that accurate quantitation of phosphorus removal processes would require a detailed correlation and assessment incorporating flow data.

The conservative tracer – bromide was used to model flow movement through one of the wetlands, providing an accurate indication of flow paths and patterns. Unit 4 was selected as the unit most likely to allow distinction of hypothesised phosphorus
removal processes in the Pilot Plant wetlands. Possible mechanisms for the observed phosphorus removal processes occurring within these wetlands included:

- incorporation into emergent plant species;
- incorporation into floating biomass and settling from the water column;
- litter fall and detritus formation; and
- absorption to sediment surfaces.

Chapter 5 evaluated the contribution of litter formation to phosphorus pools, along with the association of detritus formation with plant species, on a seasonal basis. Methods of settling from the water column, and the relative importance of algae in this process were examined in Chapter 6. Once settled, phosphorus could migrate to “longer term” binding sites in the sediment. Mechanisms and likely storage compartments for phosphorus within the sediment and sediment biomass were examined in Chapter 7.

These previous chapters have formed a suite of observations with the common thread that they report on phosphorus removal and mechanisms for that removal. The purpose of this chapter is to review these observations, formulate a series of hypothesis for phosphorus removal processes within the pilot plant wetlands, and establish recommendations and directions for future investigative work. Note that each of the previous sections has provided reference to the published literature, and therefore only minimal referencing will be provided in this final summary chapter.

8.2 OVERALL WETLAND PERFORMANCE

Overall wetland performance was evaluated through investigations in Chapters 2 and 3. These investigations were undertaken between 1994 and 1995. Observation of evapotranspiration through each wetland indicated that for the two-year period of this investigation there was no communication between the water table and the wetland water flow, with the exception of Unit 2 during the final 6 months. Therefore, almost all phosphorus removal and release rates discussed were due to processes occurring within each wetland.
8.2.1 General wetland parameters
In average terms, removal of TP, FP, TN, NH$_4$, TKN, SS and BOD occurred for all wetland Units. However, the patterns of removal for the primary components of interest: TP, TN, and SS varied between wetlands.

8.2.2 Nitrogen
The removal rates for TN, TKN and NH$_4$ were significantly enhanced at lower loading rates. Removal of other nitrogen forms was more complex and less predictable. Suggesting that decreasing loading rates or increasing the wetland size may be the most effective methods for promoting ammonia and organic nitrogen removal.

8.2.3 Suspended Solids
There was no consistent association of SS with TP concentrations or turbidity in any investigations involving the pilot plant wetlands (Chapters 2, 3, and 6). One exception can be seen from the overall wetland analysis (Chapter 2) where SS removal was high in the September and December quarters, a period when TP removal was most variable. It is postulated that some of the observed phosphorus losses were associated with the export of SS material, possibly as algae or small floating plants such as Azolla or Lemna at this time, although this was not conclusively demonstrated. Otherwise, SS did not appear to be directly associated with TP removal from these wetlands, based on field observations.

8.2.4 Turbidity
There was no significant change in turbidity through each wetland. Material in the water column of a size greater than 1.2 μm (SS) formed only a minor component of turbidity. Typically, turbid material was caused by smaller sized particles (Chapter 3). A correlation was observed between phosphorus concentrations and declining turbidity over time (Chapter 6). However, this was only observed in laboratory studies, during instances where the initial turbidity reading was greater than 1.5 NTU (Nephelometric Turbidity Units). This suggested that there might be a minimum
threshold before a noticeable interaction between phosphorus and particulate matter might be observed. The linear nature of this association suggested a simple first order interaction. Up to 31% of phosphorus could be removed from the water column over a two-day period by the settling of turbid material from the water column (Chapter 6).

The composition of the suspended solids, whether organic or inorganic, was not determined. However, investigations in Chapter 2 indicated that this material would most likely be organic, and derived from processes within the wetland. Given that suspended material settled from the water column over time, possibly the most effective method for its removal would be to increase the retention time within the wetland. However, the absence of a significant decline in turbidity through any wetland Unit would imply that these particles either remained continually in suspension or underwent a continuous cycle of settling and resuspension, possibly through incorporation into floating algal or plant biomass, where they might become entrained in the biological cycle of growth, settling, lysis and regrowth.

8.3 PHOSPHORUS REMOVAL

8.3.1 Removal rates
All wetlands removed TP during the 2 years of investigation, when comparing averaged data (Chapter 2). Units receiving secondary treated effluent removed from 0.9 to 1.8 mg.L\(^{-1}\) (11-22%, on the basis of concentration changes) TP, whereas Units receiving alum dosed effluent removed approximately 1.1 mg.L\(^{-1}\) (39%, based on concentration changes between inlet and outlet).

A retention time adjusted matching of inlet and outlet TP concentrations (Chapter 3) produced different results, Unit 2 the only wetland unit that had consistent TP removal showed an average of 5% TP removal (0.4 mg.L\(^{-1}\)), whereas Unit 3 showed a 30% increase in TP concentrations, indicating TP release, during the month over which the investigation was undertaken. Based on these results it was determined that a detailed investigation of flows within wetland was required to more accurately
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characterise net removal or release of phosphorus. This was undertaken in Unit 4 using the conservative tracer - bromide.

Detailed investigation of Unit 4 using flow modelling indicated 0.2 to 0.5 mg.L\(^{-1}\) of TP was removed between the wetland inlet and outlet, compared to 0.16 mg.L\(^{-1}\) for matched inlet outlet values (Chapter 3). Significantly less than the 1.1 mg.L\(^{-1}\) observed in Chapter 2 (although these figures represented an average for the 2 year period and included the wetland establishment phase). The use of tracer appeared to provide a more consistent method of matching phosphorus concentrations within the wetland with the respective concentration from the inlet (using a time displacement method, based on time of travel for the tracer).

The net removal rates using the tracer were significantly lower than the 1.5 mg.L\(^{-1}\) predicted using equations based on overseas system (as recommended in DLWC, 1998). Before applying these equations, it is necessary to understand the background behind their creation. They assume a certain soil type, based on North American conditions and that the soil used has a significant proportion of available binding sites. Investigations conducted shortly after the detailed flow modelling investigation (Sakadevan 1998) confirmed that soil from the pilot plant wetlands was essentially phosphorus saturated, consistent with the above removal observations.

8.3.2 Loading and flow rates

Different phosphorus removal mechanisms were observed in the pilot plant wetlands depending on loading rate (Chapter 2). At higher loadings, it appeared that a proportion of the TP and FP fractions were removed from the water column. Lower loading rates i.e. alum dosed effluent, appeared to remove the larger size fraction of TP, with very little change in the concentration of FP throughout the wetland. A comparison of inlet and outlet concentrations (Chapter 2) suggested that the overall TP removal mechanism in wetlands receiving alum-dosed effluent was settling of larger material, most likely through precipitation of aluminium-phosphorus compounds. Removal of both TP and FP in wetlands receiving secondary treated effluent suggested a combination of settling and binding/sorption processes within
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these wetlands. These processes may have been occurring in alum-dosed wetlands, however their effect could have been masked by the dominance of the TP removal process, such as alum flocculation.

A higher flow rate also appeared to be associated with greater TP retention. However, of the two wetlands receiving the same flow, the wetland receiving alum dosed effluent showed a greater percentage removal of TP between the inlet and outlet (Chapter 2).

The use of a positive displacement pump provided a marked improvement in reducing flow variations at the wetland inlet. However, this was not reflected at individual sample sites within the wetland, where the flow variance past each location was not significantly improved. This was most likely explained by localised obstructions, regions of short-circuiting or slower flowing more stagnant water (Chapter 4): the stochastic behaviour noted by Kadlec (1996). The exact quantitation of these regions was not seen as viable or necessary due to their transitory nature, their localised effect and limited influence on outlet flows, phosphorus and tracer concentrations (Kadlec 1996).

The variable nature of inlet TP concentrations (Chapters 2 and 3) meant that reliable estimates of water movement (through the use of a conservative tracer) were essential to identify regions of phosphorus removal or release to the water column. The calculation of TP concentrations within a wetland or at the outlet, based on bromide tracer results, provided a more consistent indication of wetland performance by allowing the attenuation of mixing and dispersion effects. This method of analysis indicated that TP removal was independent of inlet TP concentration, and related to regions of slower flow. Thus increasing the contact time within the wetland (in this case Unit 4 – Chapter 4), should be a significant control mechanism for increasing TP removal. Contact time could be increased by reducing the phosphorus loading rate or increasing the available surface area.
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One factor affecting contact time was the hydraulic efficiency (HE). Within Unit 4, and typically within constructed wetlands this figure is well below ideal flow, indicating significant short-circuiting. Internal variations in tracer movement were apparent between and within transects throughout the wetland for median, minimum and maximum times of travel, supportive of regions of short-circuiting and differing travel paths. The greatest factor affecting tracer and perhaps phosphorus movement in the wetland was wind. However, the consistency in hydraulic retention time between different analysis runs indicated that such variations were localised, suggestive of small “microsites” whose location varied between sample runs but which did not significantly affect overall flow or phosphorus removal.

8.3.3 Seasonality

Net TP removal appeared related to time of year and wetland age. The largest amount of TP removal was observed in the first six months of wetland establishment (late spring to early autumn), most likely due to seedling growth and establishment (Chapter 2). If this period is put aside, then removal was consistently high in June and September quarters, with lowest removal and some export observed during the December quarters. These observations appear counter intuitive, since it would be expected that the majority of plant growth, and hence phosphorus removal through incorporation to biomass should occur during spring and summer. The most likely explanation is that incorporation of phosphorus into plant biomass was occurring during spring and summer, but that release through degradation of detrital material was occurring in excess of plant uptake. During the winter months, a small amount of phosphorus may be sequestered to plant biomass, but the dominant removal mechanism would be expected to be adsorption to sediment.

Supporting this hypothesis was the net increase in the amount of detritus and organic material that was found to be deposited in the wetlands during spring. The deposition and degradation of this material could explain the elevated phosphorus concentrations in the water column at this time of year.
In the detailed analysis of Unit 4 (Chapter 4), a noticeable variation in TP removal patterns was observed between the autumn/winter and spring sample runs. During autumn and winter TP was actively removed from all but two segments of Unit 4, both of which contained Phragmites (a highly lignified plant which typically senesces in winter). During spring, TP removal occurred in the initial stand of plants (Phragmites), with no further net removal thereafter. This removal may have been associated with deposition of alum in the initial stand of plants, or possibly, plant growth. The pattern of removal observed in Unit 4 during autumn/winter was suggestive of direct phosphorus adsorption to the sediment or possibly biomass. TP adsorption to sediment appeared to be a likely dominant factor in the autumn/winter run, however during spring other factors appeared to interfere with TP removal. These may have been litter fall, biodegradation, microbial activity at the sediment surface or a combination of these and possibly other factors. Curiously, the net phosphorus removal rate for each time of year was not significantly different. This observation, although difficult to conceptualise, was consistent with the hypothesis for stochastic behaviour put forth by Kadlec (1996), where he recognised the internal variability or ‘noise’ within a wetland but noted that overall this had only a minor effect on overall wetland performance in most cases.

8.3.4 Locations of TP removal within the wetland

Through the investigations conducted in Chapter 3, it was possible to observe some of the internal functioning of each wetland. In the course of these observations there appeared to be some commonality of internal processing occurring in various wetland Units. Overall, the initial or front 4 sectors of each Unit appeared to show a greater variety of interactions and patterns than the final 3 sectors.

Processes within the initial four sectors of each wetland were markedly different, becoming more consistent from S5 onwards. Possible removal mechanisms in the initial sectors appeared to include a combination of settling, surface and sediment absorption, partial and full degradation of phosphorus compounds, and incorporation into biomass, all acting almost independently or antagonistically.
Phosphorus removal and release in S2, S4 and the latter half of each wetland appeared to be associated with changes in the amounts of chlorophyll and pheophytin (Chapter 3). This association was either not observed or inconsistent throughout other sectors of each wetland. The source of pheophytin and chlorophyll containing material was unclear, whether from rooted plants, algae or floating plants. In addition, although pheophytin and chlorophyll were measured it was unclear to what extent detrital material was involved in this process, as opposed to live plant or algal tissue.

Investigations on Unit 4 indicated localised variations in TP removal, similar to variations seen in flow past individual locations within the wetland were observed. These varied with each injection run and produced a degree of internal “noise” – stochastic behaviour, which in the context of the entire wetland tended to be significantly moderated, such that net TP removal at the outlet appeared consistent between injection runs. Therefore looking internally one finds variations in removal, release, and mixing but at the outlet, a “merged” result is obtained. This is considered to be a key element in evaluating performance of constructed wetland systems.

Phosphorus removal patterns in Unit 4, as seen in Chapter 3, were not always as marked as in the other wetlands. However, removal was observed in the winter periods, and limited regions of removal observed during spring months, consistent with observations from Chapter 2. Chapter 3 showed consistent TP release from T1 during autumn, in agreement with observations in Chapter 4.

In considering the investigations of Chapter 4, the overall trend in the data was evaluated, in addition to trends in TP removal which were observable between plant stands. The greatest amount of change occurred in the central three plant stands (consistent with patterns observed in Chapter 3), with minimal change towards the outlet. There was a large contrast between spring and winter in S3, at the end of the Triglochin stand (S3). In the winter run, TP removal was quite high, but in spring, TP was actually released within the water column, consistent with the hypothesised uptake into floating plants or algal biomass in this sector. Over winter, both stands of Triglochin (S3 and S5) showed high rates of TP removal. However, it would appear
that in spring this may be dominated by uptake of phosphorus into floating plant or algal biomass. Significant TP release to the water column or no change in removal was consistently observed through the central stand of Phragmites (S4/T4), consistent with lysis of algal cells. However, during winter this was not observed. The second stand of Triglochin (S5, T5) did not evidence this phenomena, as was also seen in other investigations (Chapter 3).

It should be noted that investigations in Chapters 3 and 4 were conducted differently, with significantly different objectives. In Chapter 3, investigations were undertaken to correlate a range of factors with observations of changes in TP concentration through a given stand of plants, with readings being taken from randomly selected sites within each plant stand. In Chapter 4, readings were taken at the boundary of each plant stand measuring only TP and flow, changes in phosphorus size fractions and association with other parameters such as pheophytin and chlorophyll were not determined.

8.3.5 Settling from the water column
Two dominant mechanisms for removal of phosphorus from the water column appeared to be present in the pilot plant wetlands. Phosphorus was removed in particles larger than 1.2 μm or dissolved in the fraction smaller than 0.1 μm. The proposed mechanisms would be that particles larger than 1.2 μm settle from the water column when they encountered a region of slower flow such as a more dense stands of plants. Particles smaller than 0.1 μm were considered to be either ortho- or colloidal phosphorus. These could easily interact with sediment and biofilm particles, possibly binding through adsorption or metabolism. Both mechanisms would be effective but for long-term storage, phosphorus would need to penetrate deeper into the soil matrix, being either bound to soil particles or incorporated into plant root material.

Material settled from the water column tended to be easily able to resuspend into the water column, as could surface biofilms. Therefore, some form of movement or transformation would be necessary for long-term storage. Transfer of phosphorus from the soil to above ground plant material would also be undesirable because this
material would eventually senesce with the potential for re-release into the water column as degraded detrital tissue. Preferably (for optimum immobilisation), phosphorus would be located below ground in root tissue (approximately 1/3 of plant tissue is in the roots) or sediment. When the root tissue senesced it would gradually release phosphorus back to the water column due to degradation processes, with a high potential for binding to the surrounding soil.

Laboratory investigations indicated that algal growth in the water column (using species from the pilot plant wetlands) did not result in the detection of significant concentrations of TP in the water column. The results of these investigations suggest that the higher TP concentrations observed in the water column may have been associated with species of floating plants such as *Lemna* or *Azolla*. The association of TP with these water borne plants is well documented. This would imply that microorganisms had only a minor role in the migration of TP through the water column in the wetlands observed.

8.3.6 *Detritus*

It appeared that the degradation of detrital material and formation of floating plant biomass and possibly algae had a significant impact on phosphorus concentrations within each wetland unit. Changes in phosphorus concentrations, which may have been caused by floating plant or algal biomass, appeared to account for as much as 0.5 mg.L$^{-1}$ over the width (5 m) of a plant stand (Chapter 3).

The possible deposition of alum within wetlands receiving alum-dosed effluent was highlighted by the lower proportion of TC and TN as determined from litter deposition investigations (Chapter 5). This would imply an increased deposition of inorganic material, which could possibly be alum from the treatment process. The greatest percentage of TP removal occurred in alum-dosed systems (Units 3 and 4), which may imply that maximal TP removal through deposition requires the addition of an inorganic precipitant such as alum.
During autumn, there was a very high correlation of TC and TN with TP in the detritus from each wetland Unit. The similar planting design for each wetland and results for each Unit would imply the material obtained was from emergent plants rather than algae or floating plants. During spring, the association between TC and TN with TP was not observed, consistent with the degradation of detrital material. The net mass of deposited material and the associated TC, TN and TP was more than twice as high in spring, compared to autumn. The most likely source of this increase was the partially degraded plant material released from the breakdown of intact leaves and stems due to increased temperatures and biological activity in spring.

The averaged annual deposition of detrital material through each wetland unit (Table 5-13) indicated the total mass of TP deposited was only a small fraction of the total amount of TP removed from the inlet water. However, this may have been because of a net release of TP during spring and summer. Implying that although the observed percentage of TP bound to detritus in the long term may be less than 0.1%, the amount of TP released from this material may have a significant effect on TP concentrations in the water column.

8.3.7 Sediment
At the end of the 2 years investigation of the pilot plant wetlands (Chapter 2), phosphorus was predominantly bound to the uppermost 2 cm of the soil substrata. None of the soil samples investigated were saturated with phosphorus.

Within the sediments of the pilot plant wetlands, inorganic phosphorus compounds with iron and aluminium predominated. Approximately 33% of the TP fraction was comprised of organic material, with only a small fraction of this in the microbial biomass. Given this composition, increasing potential phosphorus binding sites would be difficult for all but the non-microbial organic fractions, which showed only a slight phosphorus accretion rate (Chapter 5). Alum dosing into a wetland could also increase available binding sites but would only be acceptable under specific circumstances.
CHAPTER 8

Alum is typically supplied in the form aluminium sulphate, which is highly acidic and would need to be buffered to neutrality to prevent the acidity adversely affecting the wetland plants. In addition, aluminium is toxic to plants above certain concentrations, depending on species. Its long-term addition to a wetland is therefore not sustainable as it could eventually kill the wetland vegetation. Provision for aluminium removal on a regular basis would also be required.

The role of micro-organisms

Investigations of sediment microcosms (Chapter 6) indicated that more than 90% of TP was absorbed to sediment within 3 days and that there was no significant change in the location of phosphorus after this time. The amount and rate of phosphorus adsorbed was independent of overlying conditions in the water column such as anoxia or nutrient status.

In the short term (within 12 hours), microorganisms did not appear to have a significant impact on phosphorus adsorption to the sediment. In the medium term microorganisms appeared to stimulate phosphorus adsorption to the sediment. However, the potential to control or influence this sequestering mechanism requires further investigation.

Phosphorus infiltration below the sediment surface

In laboratory investigations, phosphorus infiltration primarily occurred in the top few millimetres of soil, with only localised regions of deeper infiltration, even here movement was restricted to less than 0.5 cm over a 7 day period (based on laboratory studies, Chapter 7). The presence of a live seedling allowed more rapid movement of phosphorus through the sediment substrate, most likely associated with bulk water and phosphorus transfer to the plant roots for evapotranspiration and growth. Indicating a direct role for plants in the migration of phosphorus through the sediment substrate.
8.4 FINDINGS

The Richmond pilot plant wetlands were able to consistently remove TP from influent water over the two years of this investigation. There appeared to be two primary long-term mechanisms for phosphorus removal:

- binding to sediment, which appeared to occur year round, but became a dominant mechanism in autumn and winter; and
- incorporation into the tissue of emergent and floating plants.

Binding of phosphorus with sediment predominantly occurred in the top few centimetres of wetland sediment, however, plant roots should allow water to penetrate deeper through the sediment. This may not have been observed at the pilot plant wetlands due to the high background TP concentrations in the soil, the high proportion of available binding sites in the sediment surface and the relatively short duration of the investigation.

Investigations of phosphorus sequestering processes in wetland soil indicated it was predominantly an abiological process. Microorganisms appeared to facilitate the movement of phosphorus to soil binding sites in the medium term (14 to 28 days), with sediment material eventually binding up to 90% of incident phosphorus in the absence of plants within 3 days of contact. However, radiation tracer investigations on the movement of phosphorus through the sediment indicated the potential to only penetrate the uppermost few millimetres of the sediment surface in the absence of plants, providing a very limited surface for adsorption. Plants may act to significantly increase the surface area available for phosphorus adsorption by causing the migration of water and phosphorus through the sediment due to growth and transpiration. The contribution of plant roots to continued phosphorus movement after plant maturation should not be underestimated.

To effectively improve phosphorus binding over the long term, using the soils from the pilot plant wetlands, it would be necessary to increase the contact time with the sediment through a lower flow rate or improve the wetland design to reduce short circuiting. Alternatively, the size of the wetland could be increased.
Equations to compare wetland sizing and phosphorus removal based on American systems, as recommended by the New South Wales Department of Land and Water Conservation (DLWC 1998) indicated the observed rates of removal were well below theoretical expectations. This was most likely because of the lack of available binding sites in the sediment at Richmond (Sakadevan 1998). These observations confirm the predictions of Richardson (1985) who indicated that the most significant phosphorus store was most likely the sediment.

Phosphorus incorporation into plant biomass was not measured directly and could only be inferred from field and laboratory investigations. Detailed examination of plant stands within each wetland was unable to indicate significant differences associated with plant type. However, investigations indicated that the phosphorus binding in open water sections was most likely related to floating plants rather than algal biomass. The apparent settling of this material in the subsequent stands of emergent plants may have resulted in significant re-release of the bound phosphorus, which was consistently removed from the water column in the latter parts of these wetlands.

From these observations it was concluded that the final sections of a constructed wetland should be planted with emergent plants to shade the water surface and prevent or minimise the growth of algae and floating plants. The potential would be that the floating plants absorb phosphorus into their tissues and then carry this material out of the wetland into the downstream waterways. Studies undertaken in the Pilot Plant Wetlands would indicate that once incorporated into the floating biota the phosphorus may readily be released to the water column in downstream regions. By establishing emergent plants in the areas leading to and adjacent the wetland outlet the potential for phosphorus bound into floating plant material to exit the wetland is minimised, keeping the phosphorus in the wetland and reducing possible adverse downstream impacts.
Deposition or settling of material from the water column, including litter fall was not a significant long-term phosphorus sequestering mechanism in the pilot plant wetlands. The degradation of this material, particularly in the spring and summer months appeared to provide a significant increase in phosphorus concentrations within the wetlands.

In laboratory investigations, settling of material from the water column appeared to account for up to 33% of TP movement through the water column to the sediment. However, field results indicated this mechanism to be variable and unpredictable. Further work should be done to evaluate whether it is possible to enhance or control the settling of this material, and confirm and possibly develop means to alter its potential for resuspension.

Within the water column of the wetlands, a number of processes affected movement of water and phosphorus containing particles. Wind induced mixing and flow had a significant effect on water movement, effectively preventing plug flow from occurring in the Pilot Plant Wetlands despite the presence of a surrounding wind break (e.g. the embankment surrounding each wetland unit), a narrow aspect (length to width of greater than 5:1), and a uniform planting density. Since wind effects would appear to be ubiquitous, researchers in this area should not assume plug flow as being an accurate and descriptive model of flows typically encountered in a small wetland system.

Curiously, it appeared that the variations shown in the paths taken by water through the wetland and the mean travel time did not appear to significantly affect overall phosphorus removal. The use of a conservative tracer – bromide was effective at elucidating water movement patterns and provided a more consistent and possibly accurate indication of regions of phosphorus removal than simple assumptions of plug flow.

It should be noted that the recovery of bromide observed in this investigation was consistent with the results from other dye tracer investigations eg Netter et al. 1990.
These other investigations also indicated superior recovery of bromide tracer relative to lithium (the other primary tracer that was indicated by the literature survey as being suited to the investigation of flow within constructed wetlands, given the typical interfering factors of: photodestruction due to sunlight, sediment, organic matter and the presence of living biota). As a result, it may be concluded that bromide should be the tracer of choice for investigations of flow in constructed wetlands.

8.5 RECOMMENDATIONS

- Future investigations into water flow within either pilot or full-scale wetlands must take into account the role of wind induced mixing. This would include measurement of wind intensity and velocity effects along with paths of water movement and actual times of travel through sections of the wetland.

- To obtain greater consistency in evaluating reported phosphorus removal processes and rates within the wetland environment, a conservative tracer such as bromide should be used to confirm the hydraulic retention time and paths of water movement. Simple estimations of phosphorus removal that assume plug flow, and that are based on the theoretical hydraulic retention time are likely to be highly inaccurate.

- Investigations should be conducted to refine sustainable processes for harnessing the nutrient removal ability of floating plants for removal of Phosphorus. This could include harvesting or the use of other structural devices to trap this material.

- A more detailed evaluation of substrate and plant types is needed to confirm the effect of root depth and distribution mechanisms to improve the movement and absorption of phosphorus through the soil substrate.

- Surface flow wetlands should be established with appropriate species to minimise litter fall, or designed to allow for downstream adsorption of phosphorus released during litter fall and subsequent degradation.
- Investigations should be undertaken to gain a greater understanding of settling processes within constructed wetlands, primarily with a view to developing improved mechanisms to promote the sustained removal and binding of this material.

- Phosphorus removal and release appears to have a seasonal component for internal wetland processes. This should further investigated, as this effect may need to be taken into account in wetland designs to minimise the release of material during key periods of the year.

- Equations should be developed for appropriately sizing constructed wetlands that take into consideration the phosphorus saturation and likely future retention capacity of wetland sediments. If these are not developed, very careful consideration needs to be given to the application of currently available models, as these may significantly underestimate wetland sizing.

- The data from this thesis should be used as the basis for a PhD into the mathematical modelling of phosphorus removal and transport. Such a model could provide the basis for optimising the design of constructed be used to develop a more effective method for the design of constructed wetlands.
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PLEASE NOTE

The greatest amount of care has been taken while scanning the following pages. The best possible results have been obtained.
APPENDIX 1: AUTOANALYSER CONFIGURATIONS FOR DETECTION OF NITROGEN, PHOSPHORUS, AND BROMIDE

Table 1-A: Reagents used for phosphorus analysis – Manual Method (APHA (1992) Method 4500-P E.)

**Sulphuric acid (SN)**
Dilute 70 mL of conc. H₂SO₄ to 500 mL in distilled water.

**Potassium antimonyl tartrate solution**
Dissolve 1.37 g of K(SbO)₂C₆H₃O₆.H₂O in 400 mL of distilled water in a 500 mL volumetric flask and dilute to volume with distilled water. Store in a glass stoppered bottle.

**Ammonium Molybdate Solution**
Dissolve 20 g of (NH₄)₃Mo₇O₂₄.4H₂O in 500 mL of distilled water. Store in a glass stoppered bottle.

**Ascorbic acid 0.1 M**
Dissolve 1.76 g of ascorbic acid in 100 mL of distilled water. The solution is stable for about a week at 4 °C.

**Combined reagent**
Bring all solutions to room temperature before commencing. Make the final solution to a final volume of 100 mL with distilled water using the reagents as follows: 50 mL of SN H₂SO₄, 5 mL of potassium antimonyl tartrate solution, 15 mL of ammonium molybdate solution and 30 mL of ascorbic acid solution. Mix solution well after addition of each reagent.

Table 1-B: Reagents used for phosphorus analysis – Automated Method

<table>
<thead>
<tr>
<th>Molybdate Working Solution</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Molybdate (NH₄)₃Mo₇O₂₄.4H₂O</td>
<td>2.36 g</td>
</tr>
<tr>
<td>Concentrated Sulphuric Acid (36 N)</td>
<td>17.8 g</td>
</tr>
<tr>
<td>Potassium Antimony Tartrate solution</td>
<td>1 mL</td>
</tr>
<tr>
<td>5 % sodium dodecyl sulphate (SDS, high purity)</td>
<td>50 mL</td>
</tr>
<tr>
<td>MilliQ water qs.</td>
<td>1 L</td>
</tr>
<tr>
<td><strong>Potassium Antimony Tartrate solution</strong></td>
<td>1.1 g</td>
</tr>
<tr>
<td>Potassium Antimony Tartrate (K₂SbOC₆H₃₂O₆·0.5H₂O)</td>
<td>100 mL</td>
</tr>
<tr>
<td>MilliQ water qs.</td>
<td>100 mL</td>
</tr>
<tr>
<td>10 % SDS</td>
<td>10 g</td>
</tr>
<tr>
<td>SDS (high purity, C₁₂H₂₂OSO₃·Na)</td>
<td>100 mL</td>
</tr>
<tr>
<td>MilliQ water qs.</td>
<td>100 mL</td>
</tr>
<tr>
<td><strong>Ascorbic Acid</strong></td>
<td>6.7 g</td>
</tr>
<tr>
<td>Ascorbic acid (C₆H₈O₆)</td>
<td>100 mL</td>
</tr>
<tr>
<td>MilliQ water qs.</td>
<td>100 mL</td>
</tr>
<tr>
<td><strong>Working Buffer</strong></td>
<td>50 mL</td>
</tr>
<tr>
<td>10 % SDS</td>
<td>1 L</td>
</tr>
<tr>
<td>MilliQ water qs.</td>
<td>1 L</td>
</tr>
</tbody>
</table>

*Store in a dark bottle and refrigerate. Stable for one month*
Figure 1-A: Setup of Technicon Autoanalyser Mk II for low phosphorus concentrations (5 $\mu$gL$^{-1}$ to 3,000 $\mu$gL$^{-1}$).

Key: Blk = black, Gry = grey, Orn = orange

(*Note: to cover this range it was necessary to prepare three sets of standards because the standard curve showed three distinct sections of linearity 5 to 200 $\mu$gL$^{-1}$, 200 to 1000 $\mu$gL$^{-1}$, and 1000 to 3000 $\mu$gL$^{-1}$).
Figure 1-B: Setup of Technicon Autoanalyser Mk II for high phosphorus concentrations (2 mg.L\(^{-1}\) to 50 mg.L\(^{-1}\)).

Key: Gry = grey, Om = Orange, Blk = Black

(*Note: to cover this range it was necessary to prepare two sets of standards because the standard curve showed two distinct sections of linearity 2 to 15 mg.L\(^{-1}\), 15 to 50 mg.L\(^{-1}\)*)
Figure 1-C: configuration of the Technicon Mk II autoanalyser for the detection of TKN.

Key: Orn = orange, Wht = white, Red = Red, Blk = Black, Yel = Yellow, Gry = grey

The concentration ranges for this configuration were 0.05 to 10 mg.L⁻¹.
### Table 1-C: Reagents used for TKN analysis

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium powder</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Potassium sulphate (K2SO4)</td>
<td>250 g</td>
</tr>
<tr>
<td>Sulphuric acid (conc.)</td>
<td>2.5 L</td>
</tr>
<tr>
<td><strong>Ammonium working buffer</strong></td>
<td></td>
</tr>
<tr>
<td>Sodium potassium tartrate</td>
<td>62.5 g</td>
</tr>
<tr>
<td>Sodium phosphate - dibasic (KH2PO4)</td>
<td>12 g</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>95 g</td>
</tr>
<tr>
<td>Brij 35 solution (TACS T21 0110 040)</td>
<td>1 mL</td>
</tr>
<tr>
<td>MilliQ water qv.</td>
<td>1 L</td>
</tr>
<tr>
<td><strong>Sodium nitroprusside/salicylate working solution</strong></td>
<td></td>
</tr>
<tr>
<td>Sodium salicylate</td>
<td>1.50 g</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>0.3 g</td>
</tr>
<tr>
<td>Brij 35 solution</td>
<td>1 mL</td>
</tr>
<tr>
<td>MilliQ water qv.</td>
<td>1 L</td>
</tr>
<tr>
<td><strong>Hypochlorite working solution</strong></td>
<td></td>
</tr>
<tr>
<td>Hypochlorite 12 %</td>
<td>3.5 mL</td>
</tr>
<tr>
<td>Brij 35 solution</td>
<td>1 mL</td>
</tr>
<tr>
<td>MilliQ water qv</td>
<td>100 mL</td>
</tr>
<tr>
<td><strong>Wash solution</strong></td>
<td></td>
</tr>
<tr>
<td>H2SO4</td>
<td>10 mL</td>
</tr>
<tr>
<td>MilliQ Water qv</td>
<td>100 mL</td>
</tr>
<tr>
<td>Brij 35 Solution d</td>
<td>1 mL</td>
</tr>
<tr>
<td>MilliQ water qv</td>
<td>1 L</td>
</tr>
</tbody>
</table>

* Add the selenium and potassium sulphate to sulphuric acid, boil the acid until they dissolve and the solution becomes clear. The solution will progress from black to straw to clear. Allow the solution to cool before transferring to an amber coloured bottle. Solution is stable for 6 months.

* Store in a dark bottle - stable for 1 month

* Reagents stable for 1 day

* Reagents stable for 1 month
APPENDIX 2: WETLAND LOADING RATE GRAPHICS FROM CHAPTER 2

Figure 2-A: Net and percentage mass of total phosphorus retained each quarter by Richmond pilot plant wetland Unit 1, relative to average inlet concentrations.

kg.qtr\(^{-1}\) refers to the mass of TP (in kg) retained within the wetland over a 3 month period.

Figure 2-B: Net and percentage mass of total phosphorus retained each quarter by Richmond pilot plant wetland Unit 2, relative to average inlet concentrations.

kg.qtr\(^{-1}\) refers to the mass of TP (in kg) retained within the wetland over a 3 month period.
APPENDIX 2

Figure 2-C: Net and percentage mass of total phosphorus retained each quarter by Richmond pilot plant wetland Unit 3, relative to average inlet concentrations.

Figure 2-D: Net and percentage mass of total phosphorus retained each quarter by Richmond pilot plant wetland Unit 4, relative to average inlet concentrations.

kg.qtr⁻¹ refers to the mass of TP (in kg) retained within the wetland over a 3 month period.
APPENDIX 2

Figure 2-E: Net and percentage mass of total phosphorus retained each quarter by Richmond pilot plant wetland Unit 5, relative to average inlet concentrations.

Figure 2-F: Net and percentage mass of total nitrogen retained each quarter by Richmond pilot plant wetland Unit 1, relative to average inlet concentrations.
Figure 2-G: Net and percentage mass of total nitrogen retained each quarter by Richmond pilot plant wetland Unit 2, relative to average inlet concentrations.

\[ \text{Mass of nitrogen (kg.qtr}^{-1}\] 

- **TN in**
- **Mass Retained**
- **% Retained**

\[ \text{Percentage nitrogen retained} \]

*kg.qtr}^{-1} refers to the mass of TN (in kg) retained within the wetland over a 3 month period.

Figure 2-H: Net and percentage mass of total nitrogen retained each quarter by Richmond pilot plant wetland Unit 3, relative to average inlet concentrations.

\[ \text{Mass of nitrogen (kg.qtr}^{-1}\] 

- **TN in**
- **Mass Retained**
- **% Retained**

\[ \text{Percentage nitrogen retained} \]

*kg.qtr}^{-1} refers to the mass of TN (in kg) retained within the wetland over a 3 month period.*
Figure 2-4: Net and percentage mass of total nitrogen retained each quarter by Richmond pilot plant wetland Unit 4, relative to average inlet concentrations.

kg.qtr$^{-1}$ refers to the mass of TN (in kg) retained within the wetland over a 3 month period.

Figure 2-J: Net and percentage mass of total nitrogen retained each quarter by Richmond pilot plant wetland Unit 5, relative to average inlet concentrations.

kg.qtr$^{-1}$ refers to the mass of TN (in kg) retained within the wetland over a 3 month period.
Figure 2-K: Net and percentage mass of suspended solids retained each quarter by Richmond pilot plant wetland Unit 1, relative to average inlet concentrations.

Figure 2-L: Net and percentage mass of suspended solids retained each quarter by Richmond pilot plant wetland Unit 2, relative to average inlet concentrations.
APPENDIX 2

Figure 2-M: Net and percentage mass of suspended solids retained each quarter by Richmond pilot plant wetland Unit 3, relative to average inlet concentrations.

\[ \text{Mass of SS (kg.qtr}^{-1} \]

% Retention

Quarter ending

kg.qtr\(^{-1}\) refers to the mass of SS (in kg) retained within the wetland over a 3 month period

Figure 2-N: Net and percentage mass of suspended solids retained each quarter by Richmond pilot plant wetland Unit 4, relative to average inlet concentrations.

\[ \text{Mass of SS (kg.qtr}^{-1} \]

% Retention

Quarter ending

kg.qtr\(^{-1}\) refers to the mass of SS (in kg) retained within the wetland over a 3 month period
Figure 2-O: Net and percentage mass of suspended solids retained each quarter by Richmond pilot plant wetland Unit 5, relative to average inlet concentrations.

?-g qir refers to the mass of SS (in kg) retained within the wetland over a 3 month period.
APPENDIX 3: BROMIDE TRACER GRAPHS

Figure 3-A: Bromide tracer graph from Segment 1 of Unit 4, taken during the Bromide Run 1, illustrating the scatter of datapoints and tracer elution time.

Key: 1a is 0.5 m from the left bank of the wetland, 1b is in the centre of the wetland and 1c is 0.5 m from the right bank of the wetland. All sample points were at the downstream side of the stand.

Figure 3-B: Bromide tracer graph from Segment 2 of Unit 4, taken during the Bromide Run 1, illustrating the scatter of datapoints and tracer elution time.

Key: 2a is 0.5 m from the left bank of the wetland, 2b is in the centre of the wetland and 2c is 0.5 m from the right bank of the wetland. All sample points were at the downstream side of the stand.
Figure 3-C: Bromide tracer graph from Segment 3 of Unit 4, taken during the Bromide Run 1, illustrating the scatter of datapoints and tracer elution time.

Key: 3a is 0.5 m from the left bank of the wetland, 3b is in the centre of the wetland and 3c is 0.5 m from the right bank of the wetland. All sample points were at the downstream side of the stand.

Figure 3-D: Bromide tracer graph from Segment 4 of Unit 4, taken during the Bromide Run 1, illustrating the scatter of datapoints and tracer elution time.

Key: 4a is 0.5 m from the left bank of the wetland, 4b is in the centre of the wetland and 4c is 0.5 m from the right bank of the wetland. All sample points were at the downstream side of the stand.
Figure 3-E: Bromide tracer graph from Segment 5 of Unit 4, taken during the Bromide Run 1, illustrating the scatter of data points and tracer elution time.

Key: 5a is 0.5 m from the left bank of the wetland, 5b is in the centre of the wetland and 5c is 0.5 m from the right bank of the wetland. All sample points were at the downstream side of the stand.

Figure 3-F: Bromide tracer graph from Segment 6 of Unit 4, taken during the Bromide Run 1, illustrating the scatter of data points and tracer elution time.

Key: 6a is 0.5 m from the left bank of the wetland, 6b is in the centre of the wetland and 6c is 0.5 m from the right bank of the wetland. All sample points were at the downstream side of the stand.
APPENDIX 4: GRAPHS OF TOTAL PHOSPHORUS REMOVAL AS PREDICTED FROM BROMIDE TRACER INJECTIONS

Figure 4-A: A comparison of actual and predicted total phosphorus concentrations at Segment 1a in Unit 4 based on observations from bromide tracer during bromide injection 1.

Figure 4-B: A comparison of actual and predicted total phosphorus concentrations through Segment 2a in Unit 4 based on observations from bromide tracer during bromide injection 1.
Figure 4-C: A comparison of actual and predicted total phosphorus concentrations through Segment 3a in Unit 4 based on observations from bromide tracer during bromide injection 1.

Figure 4-D: A comparison of actual and predicted total phosphorus concentrations through Segment 4a in Unit 4 based on observations from bromide tracer during bromide injection 1.
Figure 4-E: A comparison of actual and predicted total phosphorus concentrations through Segment 5a in Unit 4 based on observations from bromide tracer during bromide injection 1.

Figure 4-F: A comparison of actual and predicted total phosphorus concentrations through Segment 6a in Unit 4 based on observations from bromide tracer during bromide injection 1.
Figure 4-G: A comparison of actual and predicted total phosphorus concentrations past the outlet to Unit 4 based on observations from bromide tracer during bromide injection 1.
APPENDIX 5: AUTORADIOGRAPHIC PLATES OF SOIL

Figure 5-A: Autoradiograph of microcosm containing soil only on Day 1 – replicate 1.

Figure 5-B: Autoradiograph of microcosm containing soil only on Day 2 – replicate 1.
Figure 5-C: Autoradiograph of microcosm containing soil only on Day 4 – replicate 1.

Figure 5-D: Autoradiograph of microcosm containing soil only on Day 7 – replicate 1.
Figure 5-E: Autoradiograph of microcosm containing soil only on Day 7 – replicate 2.
APPENDIX 6: AUTORADIOGRAPHIC PLATES OF PLANTS AND SOIL

Figure 6-A: Autoradiograph of microcosm containing soil and plant seedling on Day 1.

Figure 6-B: Autoradiograph of microcosm containing soil and plant seedling on Day 2.
Figure 6-C: Autoradiograph of microcosm containing soil and plant seedling on Day 4.
Figure 6-D: Autoradiograph of microcosm containing soil and plant seedling on Day 7.