Using the Submergent *Triglochin huegelii* for

Domestic Greywater Treatment

by

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Abstract

In recent years, there has been increased interest in alternative and innovative technologies which are used in the treatment of wastewaters, with the aim of developing efficient systems which are low-cost and low-maintenance. However, greywater reuse from domestic houses appears to have received very little attention and the role of indigenous wetland plants, especially submergents, in contributing to nutrient reduction in wastewater is largely unknown.

Species of *Triglochin*, commonly known as water ribbons, are fast growing submersant macrophytes. In Western Australia, *Triglochin huegelii* is mainly a submersant plant but as water recedes, the leaves become emergent. *Triglochin huegelii* can tolerate a range of water regimes and high nutrient concentrations, and this is useful in wastewater treatment applications. The aims of this present study were to examine the use of *Triglochin huegelii* for domestic greywater treatment, to compare the effectiveness of this plant with other better known, and more frequently used, emergent macrophytes, and to investigate why *Triglochin huegelii* is so successful in nutrient accumulation.

A series of investigations using *Triglochin huegelii* in greywater treatment experiments showed that *Triglochin* has consistently removed more nitrogen and phosphorus, in all parts of the plant - leaves, tubers and roots, than most other indigenous emergent macrophyte species, including those of *Schoenoplectus, Baumea* and *Juncus* which are commonly used for
wastewater nutrient-stripping. In some cases, such as in the leaves, twice as much nitrogen (N) and one and a half times more phosphorus (P) is assimilated in the *Triglochin* tissue. In all parts of the plant there has been an increase in Total N and Total P.

Investigations were conducted using different environmental conditions for the plants. A comparison was made between root zone (substrate-only) and complete pond conditions, with some changes to loading rate and retention times. *Triglochin huegelii* has many practical applications in wastewater management, especially if the level of influent/wastewater can be controlled, thus allowing sufficient time for *Triglochin huegelii* to respond with changed structure and morphology. Proline, a substance known to be produced by plants under stress (such as changing water levels), was detected in *Triglochin huegelii*.

In a pond, the leaves of *Triglochin* can be directly involved in nutrient absorption and assimilation. A study of leaf structure and other aspects of its biology showed that nutrients can easily pass into leaf tissue and then into other regions in the plant. In *Triglochin huegelii*, nitrogen was primarily stored or found in leaves then tubers then roots, while levels of phosphorus were higher in tubers then roots then leaves.

The above-ground:below-ground (AG:BG) ratio of *Triglochin huegelii* also depends on the water regime. For all samples, whether pond or substrate-only, the ratio was 0.84. However, when consideration is given to pond conditions the ratio increases to 1.11. It appears that in pond conditions, and
especially with long retention times, proportionally more above-ground
growth (leaves) occurs and in substrate-only conditions, proportionally more
biomass is found below-ground, with the number and size of leaves reduced
in these plants.

The highest nutrient levels recorded for *Triglochin huegelii* were 11.74 mgP/g
and 35.7 mgN/g dry weight. *Triglochin huegelii* has been found to have a
protein content of at least 1.7 g/100 g wet weight in the leaves, and less in roots
and tubers. *Triglochin huegelii* could have potential as a fodder source
because of its high protein content, similar to that of lucerne.

*Triglochin huegelii* seems to remove nitrogen and phosphorus at a greater
rate than many other types of aquatic macrophytes. Other parameters such
as BOD, Suspended Solids and fecal coliforms were also examined, with
reductions of up to 90%, 84% and 99% respectively. The implication is that
instead of only planting the perimeter of lagoons, artificial wetlands and
constructed basins we should be planting the bulk of the waterway with
submergent species such as *Triglochin spp* which are far more effective in
stripping nutrients than emergents currently used for that purpose. In
addition, systems need to be designed that mimic natural ecosystems, and yet
are economical and functional.

This current research can be used as a basis for further study to establish the
extent of nutrient removal by *Triglochin huegelii* and its interactions with
other macrophytes in polyculture systems.
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Glossary of Terminology and Abbreviations

**Aerobic** - processes using oxygen.

**Anaerobic** - no free oxygen or nitrate present (or used in reactions).

**Anoxic** - no free oxygen, but with nitrate present.

**Batch Feeding** - intermittent supply of nutrients/water to the system.

**Biological Oxygen Demand (BOD)** - a measure of organic material in suspension and solution. It is the total amount of oxygen taken up by bacteria as they decompose the organic material.

**Black water** - wastewater from household toilet systems.

**BOD$_5$** - BOD determined over 5 days.

**CBOD** - carbonaceous BOD.

**Chemical Oxygen Demand (COD)** - total amount of oxygen required for all types of chemical reactions. Determined by oxidation of matter, usually using potassium dichromate.

**Denitrification** - reduction of nitrate to nitrogen gas by (denitrifying) bacteria.

**DO** - dissolved oxygen.

**Downflow system** - water enters the top of the system and moves downwards.
**Ecosystem pond** - stable water environment where a variety of different organisms exist. A complex food web, with many interactions, is a common feature of these ponds.

**Enteric** - intestinal. Organisms which are found in a human’s digestive tract.

**Greywater** - wastewater from all internal household water fixtures, other than toilet wastes. Also called sullage.

**Hydraulic conductivity** - the permeability of water to move through a soil, which is dependent on the available pore space and degree of clogging or biomass within it.

**Kjeldahl nitrogen** - the combination of organic nitrogen and ammonium-nitrogen (generally nitrogen sources other than nitrate and nitrite).

**N** - nitrogen.

**NBOD** - nitrogenous BOD.

**Nitrification** - oxidation of ammonia to nitrate by bacteria (called nitrifying bacteria).

**P** - phosphorus.

**Plug Flow** - flow of water where it is assumed that each amount of inflow remains as one unit as it passes through the system.

**Rhizosphere** - the immediate area/environment surrounding the roots of plants.
Sessile - organisms which are permanently attached to an object, stationary.

SS - suspended solids.

TN - total nitrogen.

TP - total phosphorus.

TSS - total suspended solids.

Upflow system - water enters the bottom of the system and percolates upwards.

Water regime - the integration of continuously changing depth over time, and includes the depth, duration, frequency, rate, magnitude, timing and predictability of inundation and drying phases.
Chapter 1  Introduction

11 Household Wastewater

The increasing extent of residential and commercial development in the outer metropolitan areas of major cities has resulted in demands for high water quality before discharge into watercourses or for reuse applications. Additionally, the rising costs of building, maintaining or upgrading sewage and wastewater treatment plants is of increasing concern to the public and to health and water authorities. An effective greywater reuse system would reduce the need for increased capital expenditure on the building and use of municipal treatment systems.

Accordingly, in recent years, there has been increased interest in alternative and innovative technologies, with the aim of developing low cost, low maintenance and efficient treatment of wastewaters. Wang (1991) describes how China has placed special emphasis on the research and development of appropriate technologies with such merits as energy saving, resources recovery, easy operation, wide spectrum removal of various pollutants, as well as those features already mentioned. Odendaal (1991) adds reliability to the list of features required in the choice of technology.

Instead of discharging polluted or excess wastewater, it can be reclaimed, purified and reused. There is no hope for creating a partnership with
nature without sustainable systems. Technologies to limit the effects of pollution have been developed. Nutrient removal, both chemical and biological, now makes it possible to remove 90 to 99% of all organic and inorganic pollutants, and improvements on these figures are continually being made.

Water reuse is becoming more common throughout the world, although mostly at the municipal level, where it is used to water parks, golf courses and other landscapes. Greywater reuse from domestic houses has received very little attention.

It has been estimated that at least 500 000 hectares of cropland in some 15 countries are now being irrigated with municipal wastewater (Ramsdale, 1995). By using the water twice, once for domestic use and again for irrigation, potential pollutants such as nitrogen, phosphorus and potassium become valuable fertilisers, rivers and lakes are protected from contamination, the irrigated land boosts crop production, and the reclaimed water becomes a reliable, local supply.

Good treatment of greywater is especially important in areas which have soils of low infiltration rates, are close to natural waterways or have high ground water tables. In small communities where liquid waste disposal is difficult and/or expensive, recycling can be a cost effective option. For example, Karpiscak et al. (1990) discuss the experimental house, Casa del Agua, in Arizona where greywater reuse represents the largest source of
water savings, averaging 32% of its total water used. This is supported by Jepperson (1994) who contends that water savings in the order of 30 to 50% could be realised if all household greywater was reused.

Land capability investigations for subsurface effluent disposal within sensitive water catchment areas suggest that septic tank systems are unsuited in many areas. It is for this reason that Geary (1991) suggests that on-going management and maintenance of both conventional and alternative wastewater treatment systems should be continually reviewed.

The poor performance of septic tank systems is related to the unsuitability of many soils for effluent absorption, the hydraulic overloading of under-designed systems and the lack of land capability assessment in site selection (Geary, 1991). In particular, the widespread use of modern appliances, which utilise large volumes of water, contribute to the hydraulic overloading. Insufficient detention times in septic tanks further compound the problem.

As a consequence of these types of problems, recommendations have been made by the NSW State Government, for example, to increase the septic tank size from 1620 L to 3000 L and to significantly increase the soakage trench (leach drain) system from 18 m to 45 m for subsurface effluent disposal. Similarly, health authorities in Western Australia require two drains, up to 13 m long, which are alternated every six months or so.
Domestic wastewater can also be treated biologically by the use of wetland macrophytes. Aquatic plants offer a technically simple, low cost, energy-efficient method of treating greywater. Aquatic plant systems require little technical back-up and are easy to maintain. Certain rooted aquatic plants have bacteriocidal properties and the ability to breakdown chemical pollutants, while submerged aquatics are important as oxygenators. Effluent passes through various stages and the wastewater is gradually stripped of nutrients and pollutants. Treatment of wastewater depends on factors such as system design, the chemistry of the plant root-water-sediment environment, plant uptake, available carbon (for microbe activity), nutrient volatilisation (e.g. ammonia) and type of substrate (House et al., 1994).

12 Triglochin huegelii

Common wetland plants, mainly reeds, rushes and grasses, have been used in wastewater treatment, including that of greywater from domestic sources. To date, most of the trials of wetland macrophytes have involved emergent reeds and grasses, such as species of Juncus, Schoenoplectus and Phragmites (Fisher, 1990). The use of submergent macrophytes has received little attention, even though some have demonstrated good nutrient-stripping capability. Species of Triglochin, commonly known as water ribbons throughout coastal Australia, are fast growing submergent macrophytes which seem to be adapted to high nutrient concentrations.
Species of *Triglochin* have the potential for water treatment as their growth mainly depends on adequate water and nutrient levels, compared with the seasonal growth of some other grassy wetland plants. Furthermore, *Triglochin* shows no seasonal senescence. Growth occurs throughout the Australian winter period, unlike some macrophytes which “die back” during this time. There does not appear to be any climatic influence, other than water level, on the growth of the plant (Rea and Ganf, 1994D).

*Triglochin huegelii*, found in the south-west of Western Australia, is mainly a submergent plant but its leaves tend to float on the surface in shallow waterways and it has been found seasonally in some ephemeral swamps and lakes. As water recedes, the leaves become emergent. The characteristics of *Triglochin huegelii* are discussed in more detail in the Literature Review in Chapter 2 and in this work in Chapter 4.

Some research (Adcock and Ganf, 1994) has shown that a similar species, *T. procerum*, removes nitrogen and phosphorus about five times more effectively than species of *Baumea* and *Phragmites*. Their research, along with others from the University of Adelaide, centres on storm and run-off wastewaters rather than domestic wastewater.

This research has focussed on the use of *Triglochin huegelii*, which has not been previously studied, to remove excess nutrients from domestic greywater.
13 Aims of Research

Three main aims were identified which led to a series of investigations.

These aims were:

1. To examine the use of a mainly submergent Western Australian wetland plant for domestic greywater treatment. While wetlands and wetland plants have been used for wastewater treatment, little has been done to treat greywater only. Greywater generally contains less BOD, suspended solids and levels of nutrients than the complete domestic waste stream which also contains toilet wastes (blackwater).

2. To compare the effectiveness of this plant with other better known, and more frequently used, emergent macrophytes. Most of the plants used in wastewater treatment are emergents such as Phragmites and Typha in subsurface flow systems. Again, other than work by Rea, Adcock and Ganf, little has been researched about the use of submergents in ponds for wastewater treatment.

3. To examine the particular characteristics and strategies of Triglochin huegelii which enable it to survive in a range of water regimes and cope with water stress. Some plants are known to produce proline, for example, as a response to stress. No research about the morphology, nutrient levels, wet and dry weight ratios of various plant organs and changes to its structure under different water regimes has been
conducted on *Triglochin huegelii*. This research is the focus of Chapter Four.

From these aims, several hypotheses were developed and these directed individual investigations. The background to these hypotheses is outlined in Chapter 3. Each investigation set out to examine whether particular hypotheses were supported or disproved. Results from some investigations (and their interpretations) led to further investigations which were developed to satisfy the general aims of the research.

### 14 Scope and Layout of Thesis

This thesis presents the results of a series of investigations which examines the use of *Triglochin huegelii* as a nutrient-stripping mechanism for domestic greywater. The various methodologies are outlined in Chapter 3, while research about *Triglochin huegelii* is discussed in Chapter 4. All of the results (and their interpretations) of the various investigations are given in Chapter 5, and conclusions about the suitability of *Triglochin huegelii* for domestic greywater treatment are discussed in Chapter 6. Finally, some suggestions are made of ideas for further research.
Chapter 2  Literature Review

This chapter examines and discusses the current findings of researchers undertaking work in the fields of greywater and wastewater treatment, and especially using wetland macrophytes. Various sections that follow examine the types of plants used for such systems, the results of nutrient removal efficiency studies, concerns about health, design considerations for greywater treatment and literature about *Triglochin*.

2.1 Introduction

The growing recognition that the quality and availability of water as a commodity on the one hand, and as a natural resource on the other, are inter-related, and raises questions about the relative value of water. For a sustainable urban future, society must move towards the goal of efficient and appropriate water use, including the re-use of domestic greywater and, as Dixon *et al.* (1999) also advocate, the capturing and using of rainwater.

In the developed global community it is Japan, USA and Australia who maintain the highest profile in greywater re-use (*Mustow et al.*, 1997). Even so, wastewater recycling, in general, is in its infancy in Australia, and direct water recycling is only now been seriously considered in the United Kingdom, Europe and other countries (*Surendran and Wheatley 1998, Dixon et al. 1999*). Anderson (1994) believes, however, that considerable technological improvement would be needed on current
individual household systems to achieve acceptable public health and environmental outcomes in urban areas.

There also appears to be a general lack of understanding of the contaminants in greywater (and associated health risks) and as Jackson and Ord (2000) point out there is a need to change public attitudes, as the re-use of greywater has tremendous potential for safely conserving a precious resource. Public acceptance of wastewater recycling is currently seen as major barrier to the widespread application of reuse schemes (Jeffery, 2000).

Water savings have two important implications for society (Henze, 1997). One is the reduced amount of energy used for the water supply and wastewater treatment, and the second is the savings of freshwater resources. Harvested rainwater can even be used for toilet flushing and the washing of clothes (Dixon et al. 1999). Denny (1997) contends that a domestic wastewater treatment system should be able to prevent the transmission of water-borne diseases, ensure clean water resources and reduce pollution (especially eutrophication sources) of the environment. One of the simplest methods to sterilise water is to place water bottles on a frame heated by sunlight. Wegelin and Gremion (2000) were able to reduce *Escherichia coli* by 99.9% after only one hour at 50°C, and this technique is most suitable for improving poor water quality for drinking purposes in low-income countries.
Most of the research (Surendran and Wheatley 1998, El-Hoz and Apperley 1994, Imura et al. 1995, van der Graaf 1999, and Kalker et al. 1999) in wastewater and greywater systems has been too narrow in focus and, in many cases, environmentally-unfriendly, costly and difficult to set-up and maintain. Other natural systems, based on sustainable practices, should be the focus and direction for future work. For example, Todd (1988) designed a system of large, clear tanks which utilise the sun’s energy to drive the various aquatic ecosystems. Organisms, such as bacteria, algae, zooplankton, higher plants, fish and crustacea, have the capability to transform sewage waste into other products, some of which would be incorporated into their living tissue. Ammonia toxicity, for example, had been reduced to concentrations which allowed Todd to raise rainbow trout in the outfall effluent. Denny (1997) advocates the use of a similar integrated production system for all constructed wetlands. These kinds of systems will be the future of wastewater treatment.

The major advantage of using macrophytes in greywater treatment is their ability to produce an effluent with very stringent bacteriological standards (Badkoubi et al., 1998). Furthermore, they have great potential in being used in systems of wastewater treatment for small to medium-sized communities, particularly in rural areas where land is more readily available.

Greywater, after treatment, has a variety of uses. For example, recycled greywater would be most suitable for aquaculture activities, which
generally require periodic changes of large volumes of water. Water could also be piped to trenches or beds where plants could be grown. Alternatively, ponds, which contain a variety of plants and animals, could be set up. One of the more interesting studies, by Garland et al. (2000), is the re-use of greywater in hydroponic systems during space travel.

2.2 Greywater

The wastewater stream produced by a household can be divided into two fractions; the toilet wastes, commonly called black water, and the other household wastewater, commonly called greywater (see Figure 2.1).

![Figure 2.1. Segregation of household wastes (after Siegrist, 1977).](image)

Greywater contains soaps, detergents, hair, lint and bacteria and other materials which include a wide range of foodstuffs. Rose et al. (1991) estimates that the proportion of greywater from different house fixtures is
5% bathroom basin, 40 - 80% bath and shower, 10 - 15% kitchen and 5 - 20% from laundry facilities. Untreated greywater, especially kept in storage and heavily contaminated with food particles, cooking oils and grease, would quickly turn septic and emit unpleasant odours, and clog pumps and irrigation systems (Foster et al., 1988 and Jeppeson and Solley, 1994).

Separating the greywater from blackwater sources is an advantage, as greywater contains less nutrients and is mainly limited to COD (Zeeman and Lettinga, 1999) and easier to treat (Bahlo and Wach, 1990) using aerobic conditions and biofilms (Otterpohl et al., 1997). The quality of greywater effluent primarily depends on the duration of the biological and chemical treatment of the wastewater; that is, the detention time (usually in days) as the greywater is undergoing treatment.

The concentration of nitrogen in greywater is only 2 mg/L in a total of 11 mg/L of total Kjeldahl nitrogen in combined wastewater. Fifteen percent of nitrogen in greywater and ninety percent of nitrogen in black water is in the form of ammonia (Surendran and Wheatley, 1998). Table 2.1 lists typical nutrient concentrations in greywater samples, with effluent from washing machines and laundry facilities having the highest nutrient concentrations and wastewater from the wash basin generally having the lowest, and Table 2.2 lists typical values of greywater volume produced by households.

<table>
<thead>
<tr>
<th></th>
<th>Bath/shower</th>
<th>Wash basin</th>
<th>Washing machine</th>
<th>Kitchen sink</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium as N.mg/L</td>
<td>1.56</td>
<td>0.53</td>
<td>10.7</td>
<td>4.6</td>
</tr>
<tr>
<td>Nitrate as N.mg/L</td>
<td>0.9</td>
<td>0.34</td>
<td>1.6</td>
<td>0.45</td>
</tr>
<tr>
<td>Phosphate as P.mg/L</td>
<td>1.63</td>
<td>45.5</td>
<td>101</td>
<td>15.6</td>
</tr>
<tr>
<td>Fecal coliforms cfu/100 mL</td>
<td>600</td>
<td>32</td>
<td>728</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2.2. Typical values of greywater produced per person per day.

<table>
<thead>
<tr>
<th>Author/s</th>
<th>Volume greywater/person/day (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeppeson and Solley (1994)</td>
<td>100</td>
</tr>
<tr>
<td>Public Health Department of W. Australia</td>
<td>180</td>
</tr>
<tr>
<td>Terpstra (1999)</td>
<td>145</td>
</tr>
<tr>
<td>Fittschen and Niemczynowicz (1997)</td>
<td>200</td>
</tr>
</tbody>
</table>

2.3 Greywater Treatment Using Chemical and Physical Methods

Very few, if any, greywater treatment systems rely solely on chemical and physical processes. Mels et al. (1999) give examples of these types of processes which include micro-screening, filtration, magnetic separation, flotation, activated carbon adsorption and ion exchange. Reverse osmosis and ammonia stripping/volatilisation could also be added to this list (Machlum, 1995). Biological treatment, in some form or another, is generally part of the whole treatment system, and the use of wetland plants in wastewater treatment is discussed in the next section.
Besides macrophytes, biological treatment also includes using algae, bacteria, and animals such as earthworms. For example, Madan et al. (1991) and White (1996) discuss a variety of processes to treat waste material. These include anaerobic digestion to produce biogas, enzymatic hydrolysis to produce glucose (as a source of energy for micro-organisms), as fertiliser for mushrooms and other plants, and as food for earthworms to produce compost. Some of the more common methods are shown in Table 2.3, all having a positive effect on nutrient removal.

Table 2.3. Summary of physical-chemical methods for greywater treatment.

<table>
<thead>
<tr>
<th>Author/s</th>
<th>Treatment and Reductions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skjelhaugen (1999)</td>
<td>two step filter system - filter bags to mechanically remove large</td>
</tr>
<tr>
<td></td>
<td>particles followed by a sand filter BOD 95%, P 85% and N 45%.</td>
</tr>
<tr>
<td>Surendran and Wheatley (1998)</td>
<td>screening, anaerobic solids treatment, aerated bioreactor and</td>
</tr>
<tr>
<td></td>
<td>then an active reticulated foam bead filter. SS 97%, fecal</td>
</tr>
<tr>
<td></td>
<td>coliforms 99.9%, BOD 98.6% and ammonium-N 91.9%. However,</td>
</tr>
<tr>
<td></td>
<td>phosphate removal is only 4.5% and dissolved solids 12.3%.</td>
</tr>
<tr>
<td>van Buurn et al. (1999)</td>
<td>sand filter - BOD 95%, TN 30% and TP 40%.</td>
</tr>
<tr>
<td>Ratanamskul et al. (1995)</td>
<td>used a membrane separator bioreactor and zeolite-iron column,</td>
</tr>
<tr>
<td></td>
<td>which alone removed up to 70% phosphorus and, with oxygenation,</td>
</tr>
<tr>
<td></td>
<td>increased this to 92%.</td>
</tr>
<tr>
<td>Maurer and Boller (1999)</td>
<td>chemical precipitation, to form metal hydroxo complexes, by the</td>
</tr>
<tr>
<td></td>
<td>addition of iron (Fe³⁺) and aluminium (Al³⁺) salts. However,</td>
</tr>
<tr>
<td></td>
<td>Meinhold et al. (1999) point out that at pH 7 phosphate precipitation is minimal.</td>
</tr>
</tbody>
</table>

Physical and chemical methods often supplement biological treatment. For example, Wittgren and Tobiason (1995) discuss a wastewater treatment plant in the town of Oxelösund, Sweden, which has mechanical and chemical treatment for removal of BOD and phosphorus. However, little nitrogen is removed and biological treatment is therefore required.
As adding some types of biological treatment to the existing plant was expensive, the town opted for a constructed wetland which is far cheaper to install and maintain. Ratanamskul et al. (1995) also advocate wastewater treatment at the source in areas where a centralised large-scale treatment system is not cost-effective.

A study by Mels et al. (1999) indicates that physical-chemical pre-treatment leads to energy saving when biological post treatment is applied, although this results in a greater sludge build-up which ultimately needs disposal and/or further biological treatment. This may include the use of a upflow anaerobic sludge blanket reactor which relies on anaerobic treatment for solids digestion (El-Hoz and Nasr, 1999).

2.4 Greywater Treatment Using Wetland Plants

2.4.1 Constructed wetlands

A constructed wetland is a man-made, engineered, marsh-like area designed and constructed to treat wastewater (Yang et al., 1995). Constructed wetlands are a cost-effective alternative to conventional treatment systems, simple to both install and operate (Juwarker et al., 1995). Furthermore, Ayaz and Akea (2000) contend that constructed wetlands are low-cost technologies which are able to control environmental pollution.

Constructed wetland technology is currently evolving into an acceptable, economically competitive alternative for many wastewater treatment
applications, and reed beds effectively remove N and P and the quality of effluent is better than secondary treatment at a conventional wastewater treatment plant (Yin and Shen, 1995 and White, 1995).

Reed bed treatment systems are now accepted in the UK as an appropriate solution for village treatment (Surendran and Wheatley, 1998 and Dixon et al., 1999), and constructed wetlands are not only for treating domestic sewage, but for treating abattoir wastewater, landfill leachate, highway run-off, contaminated groundwater, and agricultural and animal wastes (Haberl et al., 1995).

Reed bed systems have many applications in low-income countries, because of the low operating and maintenance costs; maintenance which local people could be trained to do. In their study with Typha beds Mashawri et al. (2000) found that local people in Tanzania could also use Typha for biogas production, compost, raw material for basket weaving and for roofing essentials. However, Hazin (2000) believes that there is a general lack of knowledge of sanitation and water recycling techniques in middle and low-income countries, which often cannot meet their water supply effectively.

Bhamidimarri et al. (1991) contend that all three biodegradation processes, namely aerobic, anoxic and anaerobic, are expected in wetlands and thus are applicable in greywater treatment. Aquatic plants remove pollutants by directly assimilating them into their tissue and by providing a suitable
Most systems worldwide are based on rooted emergents. The design is either a surface flow system or a subsurface flow system with either soil or gravel as a substrate and with a horizontal or vertical flow regime.

Wastewater can be effectively treated through a free-water surface system, in which water flows over land in a thin stream, and where purification takes place through contact with plant stems and root systems. However, there are many different systems currently being trialed and used worldwide.

Surface flow constructed wetlands typically consist of trenches or basins with emergent macrophytes and free water on the surface (water depth 30 - 40 cm). Such a trench system initially acts as a filter - for both suspended solids and bacteria and other disease organisms (Fisher, 1991).

In shallow wetlands with gentle elevation gradients, small changes in water level will expose or flood large areas, altering the oxygen and nutrient status of the sediments and water. In addition, Rea and Ganf (1994A) believe that the flooding or exposure of shoots will influence the availability of light, inorganic carbon and oxygen. These factors are known to influence plant morphology. Changes from fluctuating to stable water levels have led to grasses, sedges and herbaceous species dying out.

Subsurface flow systems, ideally, have no water on the surface and can either have horizontal or vertical water movement. Horizontal flow systems are usually rectangular beds planted with emergent macrophytes
in a soil or gravel medium. Horizontal flow systems are very popular. Most systems in the UK and Europe are horizontal flow, which have proved adequate for BOD and suspended solids removal, but not for ammonia removal because they are oxygen-limited (Cooper and Green, 1995). A typical sub-surface flow system is shown in Figure 2.2.

![Diagram of a typical greywater treatment system](image)

**Figure 2.2.** A typical greywater treatment system. Source: Helix No 15. CSIRO.

However, vertical flow systems have shown the capability to oxidise ammonia as well as BOD. In vertical flow systems, the wastewater is led onto the surface of a planted bed from where it percolates through the medium (usually fine sand or small-sized aggregate) to a drainage system located in the bottom of the bed.

In combined systems, several horizontal and vertical systems operate in series, which intensifies the treatment process and therefore reduces the surface area requirements. Sapkota and Bavor (1994) have developed a formula showing an empirical relationship between the influent and effluent suspended solids, hydraulic application rate and filter length of an unplanted gravel-based trench system.

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Although vertical flow systems are more efficient than horizontal flow (Burka and Lawrence, 1990), horizontal flow systems are more effective at nutrient removal once the initial high organic load is removed, due to the longer contact time.

The most important factor for good nutrient removal in horizontal flow reed beds is a subsurface flow (Platzer and Netter, 1994), with beds typically long and narrow to avoid short circuiting. A ratio of 4:1 to 6:1 (length to width) is recommended, with 2:1 a minimum for large systems (Crites, 1994). For example, the beds used by Tanner (1994A) measured 9m x 2m x 0.4m. In comparison, upflow system models usually use tanks, such as polyethylene tanks with 1.5 m diameter and height.

Tanner (1994A) also found that the vertical flow system is not likely to increase treatment efficiency per unit of wetland volume. More important is the surface to depth ratios in determining the supply of atmospheric oxygen to wetland treatment systems and the relative importance of plant mediated nutrient uptake and oxygen release (discussed in section 2.6.1).

Even so, Bucksteeg (1990) recommends at least 30 cm depth for horizontal flow and 60 cm depth for vertical flow systems, with 5 m² of treatment area per person equivalent for settled sewage. Fisher (1990) adds that it may be more efficient to construct treatment areas to correspond to the known species depth of plant roots. If deep trenches or ponds were used, short circuiting and by-passing the root system would occur. These problems
were encountered by Liénard et al. (1990) and Hiley (1995) who found that horizontal beds which were continuously fed did not have a high efficiency and clogged rather rapidly. They advocate the use of planted alternating horizontal beds, where one bed is rested.

All parts of a plant can be involved in the wastewater treatment process. Besides rocks and soils, roots provide the all-important surfaces on which bacteria collect and grow. Stems and leaves act as natural aerators to funnel oxygen to the roots, shelter the water from wind, and shade the aquatic environment, preventing the growth of algae. Symbiosis ensures: marsh plants absorb the metabolites produced by the bacterial degradation of the organic compounds while the microbes exploit the metabolites released from the plant roots. In essence, both use each other's wastes.

2.5 Types of Plants in Reedbed Systems

2.5.1 General studies

Suitable plants for greywater trials include members of the families Cyperaceae, Juncaceae and Typhaceae. The selected species should have a high production rate and show a high standing crop throughout the year. Other criteria include: high oxygen transport capability, tolerance to adverse concentrations of pollutants, tolerance to adverse climatic conditions, resistance to pests and disease and ease of management (Brix, 1994). Thomas et al. (1995) adds that aquatic plant species should be
Almost without exception, the UK beds have been planted with *Phragmites* (Cooper and Green, 1995) and species of *Phragmites*, *Iris* and *Typha* have been demonstrated to remove heavy metals, such as lead, copper, zinc, nickel and cadmium (GschlöBl and Stuible, 2000), but less effectively than that of soil/substrate (Mungur *et al.*, 1995). However, *Typha* and *Phragmites* should not be used in domestic wastewater treatment systems in Australia because of the massive seasonal release of wind-blown seeds (Mitchell *et al.*, 1990).

Relatively few native Australian species have been studied in detail and, therefore, many would be suitable for future research. Examples include the emergent macrophytes *Phragmites karka* and *Triglochin spp.*, and submergents such as *Vallisneria* (local species *V. spiralis*).

Fisher (1991) and Osborne and Totome (1994) describe a constructed marsh in Papua New Guinea where *Phragmites karka* was used. This reed is also a native of north-western Australia and can exist in areas which are inundated for most of the year. It is a fast-growing, clumping plant. It is a good possibility for research in greywater treatment. Furthermore, Juwarker *et al.* (1995) showed that *Phragmites karka* is more efficient in N removal than *Typha latifolia*. *Phragmites karka* establishes quickly and grows profusely, as this author has observed.
2.5.2 Species of *Triglochin*

*Triglochin* is a genus in the family *Juncaginaceae* which is characterised by the presence of basal leaves which are usually sheathed, small wind-pollinated flowers, fruit which separates into carpels, buoyancy in water, and are perennial herbs associated with freshwater or saline marshes. Species of *Triglochin* are perennial freshwater macrophytes which form singular clumps of primarily vegetative shoots of large fleshy flattened leaves (Aston, 1995).

*Triglochin huegelii* is a recent reclassification of a Western Australian variety of the very common (Australia-wide) *T. procerum* (Aston, 1995). Leaves are typically up to 900 mm long and 3 to 20 mm wide. It has tubers (for underground storage only - not reproductive), a flower spike (infrafruitescence) which rises up above the leaves during spring to early summer and flowers and fruits from August to January. Its carpels are all fertile, unlike some other species of *Triglochin* which have only half fertile and half infertile (aborted) carpels.

*Triglochin huegelii* is confined to the south-west of Western Australia and can be found in still to flowing water to one metre deep. Its known distribution is shown in Figure 2.3.

Western Australia has several species of this genus; few have been studied in detail and certainly none have been examined as possible macrophytes for water treatment. To date, only one species of *Triglochin* has been
studied in wastewater treatment trials. Research by Adcock and Ganf (1994) found that nitrogen and phosphorus removal in trench studies was five times higher for *Triglochin procerum* than for species of *Baumea* and *Phragmites*.

![Graph showing distribution of *Triglochin huegelii* in Western Australia.](image)

**Figure 2.3** Distribution of *Triglochin huegelii* in Western Australia  
Source: Aston, 1995

Experimental results also demonstrate that small differences in depth and water regime have a significant effect on the accumulation and allocation of nutrients and biomass (Rea and Ganf, 1994C). For example, *Baumea arthropylla* was largely unaffected by different water depths, whereas the other species in the study, *Triglochin procerum*, performed better in deep water than in shallow water (Rea and Ganf, 1994D).
Rea and Ganf (1994D) found that, in deep water conditions, nutrients were primarily held above ground in *B. arthrophylla* stands and below ground in *T. procerum* stands. Changes in the concentration of nutrients were directly related to changes in biomass, and were not due to nutrient reallocation within the plant. A decrease in tuber mass is offset by an increase in shoot mass. For *B. arthrophylla* mass was unrelated to depth, whereas *T. procerum* showed a positive linear correlation of mass with depth. In shallow water, then, *B. arthrophylla* had a greater biomass and nutrient content than *T. procerum*, but the reverse is true in deep water.

As water level changes so does the availability of above and below-ground resources such as inorganic carbon, oxygen, light and nutrients. *T. procerum* may use dissolved carbon and nutrients directly from the water whereas other macrophytes might rely upon gaseous exchange with the atmosphere and other nutrients from sediments (Rea and Ganf, 1994B).

Rea and Ganf (1994B) suggest that the ability of *T. procerum* to maintain net shoot mass without its population being adversely affected depends on several attributes: the presence of tubers, which are storage organs rather than reproductive structures; rapid leaf growth and recruitment, which bestows morphological plasticity as new leaves adjust their height and diameter according to depth (Rea and Ganf, 1994A); and the possibility that *T. procerum* needs less energy to mobilise resources because it can access resources from below and also from above the water, as indicated by the thin cuticle of its spongy leaves.
*Triglochin procerum* has also been studied by Warwick and Bailey (1997) who examined the effect of salinity on the plant. They noticed that the leaves of *T. procerum* die back from the tip to the base after becoming colourless and mucilaginous. There was a reduction in leaf area of newly formed leaves, with a noticeable reduction in leaf width. Leaf size was reduced in high salt solutions (6 gNaCl L\(^{-1}\)). *Triglochin procerum* had a high Na\(^+\)/K\(^+\) ratio, and that the plant may be capable of absorbing Na\(^+\) into leaf vacuoles which could be balanced by a high concentration of a compatible solute such as proline in leaf cell cytoplasm. The high levels of Na\(^+\) in old leaves and K\(^+\) in young leaves may explain the plant's tolerance to NaCl.

Warwick and Bailey (1998) found similar results in a later study, with a reduction in leaf dry weight in 6 gNaCl L\(^{-1}\) (about 0.1 M) solution but no difference in size with the control at 2 gNaCl L\(^{-1}\). *Triglochin procerum* had a much slower growth rate compared to other submergents such as *Potamogeton tricarinatus*. *Triglochin procerum*, even though non-halophytic, still exhibits halophytic responses to increased salinity.

Naidoo (1994) demonstrated that other species such as *Triglochin bulbosa* and *T. stricta* can also tolerate high levels of salinity (23.5 gNaCl L\(^{-1}\)), although reduced growth was evident at this level, but no adverse effects showed at 12 gNaCl L\(^{-1}\). This contradicts the findings of Warwick and Bailey (1998) who had detrimental effects at 6 gNaCl L\(^{-1}\).
Many macrophytes, which rely on nitrogenous organic solutes for osmotic adjustment, grow slowly in high salinities, probably due to nitrogen limitation. Naidoo (1994) recorded increasing levels of proline in leaf tissue with increasing salinity. In *T. maritima*, 13-20% of N is proline at low salinities (12 gNaCl L\(^{-1}\)) and 45% is proline in hypersaline conditions.

Proline is an amino acid which is oxidised in the mitochondria (Pahlich, 1992) and may contribute up to 20% of the energy produced in respiration. Proline levels are often observed to increase dramatically (up to 200 fold) under stress conditions, such as increased salinity, drought or high temperatures (Pahlich, 1992) and osmotic stresses (Shinozaki and Yamaguchi-Shinozaki, 1998). While it accumulates in plant tissue under stress, levels of proline rapidly reduce when the stress is removed.

In addition to acting as an osmoprotectant, proline also serves as a sink for energy to regulate redox potentials, as a hydroxy radical scavenger, as a solute that protects macromolecules against denaturation and as a means of reducing the acidity in cells (Hayashi and Murata, 1998).

*T. maritima* is capable of synthesising proline. *T. procerum* and other species of *Triglochin* may also be able to do this (Warwick and Bailey, 1997). High levels of proline accounted for high levels of total N in the leaves treated with saltwater. The production of nitrogenous osmotic solutes make demands on the nitrogen economy of the plant.
Other amino acids are also found in higher concentrations when plants are under stress. For example, glycine, which is oxidised in plant mitochondria, and betaine make up 16% and 17% respectively of the total N in the grass *Spartina townsendii* (Dennis *et al.*, 1997).

In a study of *Triglochin maritima*, Jefferies and Rudmik (1991) suggest that phenotypic plasticity in response to a changing environment is likely to be well developed in long-lived perennial halophytes. Leaf plasticity in *T. maritima* is expressed as changes in the size and number of leaves, and nutrient and water content of the leaves.

### 2.6 Nutrient Removal Mechanisms

#### 2.6.1 Role of plants

Wastewater treatment by wetland plants has little to do with the plants, but is primarily accomplished by aerobic and anaerobic micro-organisms attached to the surface of the substrate in which the plants are established (Brix, 1994).

The inundated roots of aquatic plants provide a mainly anaerobic environment for bacteria to convert nitrate into nitrogen gas and metabolise solids. Root growth in aquatic environments is prolific, providing an interface where nutrients are absorbed and bacteria flourish as they metabolise organic matter. A variety of algae then grow in the
water, on the roots and on the walls of tanks, ponds or aquatic systems, and this also contributes to nutrient removal and assimilation.

The root systems of plants growing in water-saturated substrates must obtain oxygen from their aerial organs via internal transport mechanisms. Oxygen leaks from the roots into the water. This, in turn, provides oxidised conditions in the otherwise anoxic substrate (Brix, 1994) and stimulates both aerobic decomposition of organic matter and growth of nitrifying bacteria. This general belief is not supported by Rogers et al. (1991), who contend that the growth of emergent aquatic plants does promote aeration of the below-ground environment, but it doesn’t improve treatment of nitrogenous wastes by improving microbial activity. They contend that plants are the major nutrient removers (see section 2.7.2 later in this chapter for more discussion).

Wood (1995) recognises that the amount of oxygen expected to be released by plants is nominal, and the limited aeration around the roots effectively ensures that anaerobic conditions will predominate unless the organic load to the wetland is low and/or aeration is possible by other means (for example, other types of plants, shallower water or mechanical devices).

Plant roots can generate a portion of this demand for oxygen, but direct oxygen transfer from the atmosphere or by artificial aeration may be required to achieve effective nitrification. Some oxygen can be expected to be supplied from the photosynthetic process of algae or submerged plant
species. If photosynthesis does occur it would remove some carbon dioxide and, as Findlater et al. (1990) suggests, the pH would be raised causing the volatilisation of ammonia and destruction of some bacteria.

Brix, Sorrell and Orr (1992) demonstrated internal pressurisation and convective through-flow of air are common mechanisms for internal gas transport for many wetland species including *Schoenoplectus validus*, *Baumea articulata* and species of *Typha*, *Eleocharis*, *Cyperus* and *Juncus*. *Schoenoplectus validus* has a relatively high resistance to air flow and thus is restricted to water less than one metre deep.

The role of plants in the treatment mechanism depends on two main parameters, as described by Findlater et al. (1990). These are the rate at which oxygen diffuses into the root zone and the permeability or hydraulic conductivity of the region containing the roots. This is why most systems use gravel rather than soil. Better filtration of solids occurs in gravel beds, although these types of beds may not significantly remove nutrients such as nitrogen and phosphorus.

The rate of oxygen release is highest near the tips of new roots and minimal in old roots and rhizomes. Brix (1994) estimated the oxygen release rate in *Phragmites* to be from 0.02 gm⁻²/day to 12 gm⁻²/day. He believes that wetland plants attempt to minimise their oxygen losses to the rhizosphere, which is contrary to the widely-held belief that the design of wetland systems relies on high oxygen leakage from roots.
Furthermore, species possessing an internal convective through-flow ventilation system have higher internal oxygen concentrations in the rhizomes and roots than species relying exclusively on the diffusive transfer of oxygen. Internal transportation of oxygen in wetland plants usually occurs by passive molecular diffusion and by convective flow (bulk flow) of air through the internal gas spaces of the plants.

Plants such as pennywort (*Hydrocotyle umbellata*) transport oxygen 25 times more rapidly than water hyacinth (*Eichhornia crassipes*), which, in turn, transports oxygen four times more rapidly than water lettuce (*Pistia stratiotes*) (Reddy and deBusk, 1987). Enough oxygen was transported in the pennywort and water hyacinth to cause 90% BOD reduction while the remaining 10% of BOD removal was due to oxygen from the air.

Wetlands have been shown to have nutrient conservation strategies involving internal cycling (Osborne and Totome, 1994). Nutrients absorbed during growth are translocated to the below-ground storage organs during senescence of above-ground parts. Later, these nutrients are mobilised upwards for use by the young shoots (stems and leaves) in the next growing period.

Reallocation of biomass between compartments is essential for surviving water level changes. Species that can maintain allocation to shoots without an adverse effect on total or below-ground mass are at a distinct advantage. It seems that wetland macrophytes do possess high storage
capabilities and can translocate stored nutrients from one part of a plant to another (Chu et al., 1998).

The uptake capacity of emergent macrophytes is roughly in the range 50 to 150 kgP.ha⁻¹.year⁻¹ and 1000 to 2500 kgN.ha⁻¹.year⁻¹ (Brix, 1994 and 1997). Figures by Reddy and deBusk (1987) are similar. They add that more than 50% of nutrients were found to be stored in below-ground portions of the plants, tissues which may be difficult to harvest to achieve effective nutrient removal.

The highly productive floating plants, such as water hyacinth, have generally higher uptake capacities whereas the capacity of submerged macrophytes is lower (Brix, 1997). Even so, the area needed for wastewater treatment for phosphorus removal solely by water hyacinths would still be 30 - 50 m² per person equivalent and that of emergents about 100 m² per person equivalent. These figures are for whole domestic wastewater - both black water and greywater. Less area would be required for greywater treatment alone.

Regular harvesting of submerged and emergent plants removes as little as 6% of nutrients (Simpson, 1993). Harvesting can upset the ecological cycles; hence, the nutrient removal process can be interfered with. If this is the case, then harvesting could be undertaken to maintain plant vigour rather than for nutrient reduction. Crites (1994) contends that harvesting is only required for mosquito control, promoting new growth, and
maintaining hydraulic capacity. Harvesting for nutrient removal in large systems is not practical, but for small domestic systems may be necessary.

While plants seem to only have a small role in wastewater treatment, they are an essential part of a system devised to maximise nutrient removal. For example, domestic waste treatment may involve a system where greywater is passed through a sedimentation tank, a root zone horizontal flow bed, a sand filter and an artificial pond as a final stage. In such a system, as devised by Fittschen and Niemczynowicz (1997), the treated water had very low BOD, total N and P and heavy metals, and was then able to be used for irrigation.

Plants can absorb nutrients directly and provide growing areas and conditions for micro-organisms. Unfortunately, conventional reedbed systems are little more than monocultures of *Phragmites*, *Baumea*, Water Hyacinth, *Typha* or *Schoenoplectus*. Pond systems employing a wider range of species are a means to recycle more nutrients, improve treatment potential and mirror natural ecosystems. A combination of floating plants, submergents and emergents would seem to be ideal as Greenway and Woolley (1999) found with their results of nutrient accumulation in a range of these types of plants.
2.6.2 Nitrogen removal

Nitrogen removal processes are very complex with nitrifying bacteria converting ammonia to nitrite and nitrate (nitrification) and denitrifying bacteria converting these compounds to nitrogen (denitrification) via a series of intermediates such as HNO₂, NO and N₂O (van Loosdrecht and Jetten, 1998).

Nitrification is generally performed by slowly growing bacteria (Behrendt, 1999), most of which are immobilised, whereas both immobilised bacteria and suspended bacteria contribute to denitrification (Aravinthan et al., 1998). Nitrification requires oxygen and alkaline conditions. The general equations are:

\[
\begin{align*}
\text{NH}_4^+ + 1.5 \text{O}_2 + \text{H}_2\text{O} & \rightarrow \text{NO}_2^- + 2\text{H}_3\text{O}^+ \\
\text{NO}_2^- + 0.5 \text{O}_2 & \rightarrow \text{NO}_3^-
\end{align*}
\]

Wetland systems are generally not successful at nitrification (primarily due to oxygen limitations). Nitrification does occur but it is a limiting step for nitrogen removal (Wittgren and Tobiason, 1995) and typically requires long hydraulic retention times (White, 1995). For example, planted trenches had 70% ammonia removal after four days.

Nitrification and denitrification, together with plant uptake was most likely responsible for all ammonium-N removal in a study by Wittgren and Tobiason (1995). Ammonia volatilisation was ruled out since the maximum pH was 7.6 in their reed bed, and this should not have been
sufficient for significant ammonia production (estimated at 2%, Kadlec and Knight 1996).

Furthermore, Wittgren and Tobiason (1995) found that nitrifying bacteria attach to the epiphyton of living plants and much higher numbers of both ammonia and nitrite oxidisers are found on the branching leaves of submerged plants than in the water column itself. However, the contribution by plants is generally accepted as being minimal. For example, Cooke (1994) found that 60 - 70% of nitrate in wastewater samples was denitrified, 25 - 35% was changed to ammonium ions (dissimilatory reduction) and only 5 - 10% was assimilated in plant tissue (6% in plants and 13% in the sediment - Van Oostram, 1995).

Lund et al. (2000) found that denitrification accounted for 89-95% of nitrogen removal, although denitrification may be masked by mineralisation and plant uptake, as both vegetated and unvegetated ponds can be effective in reducing nitrate concentration. While Bachand et al. (2000A) also found denitrification to be the major nitrogen removal process, Newman et al. (2000) found that denitrification accounted for less than 1%, while plant uptake was about 3%, with most of the nitrogen stored in sediments (10- 35%) or lost in system outflow.

Plants also contribute to nutrient removal by supplying organic carbon material which is necessary for denitrification (Bachand et al., 2000B, Van Oostram, 1995, House et al., 1994, Breen, 1990, van Buurn et al., 1999 and
Zhu and Sikora, 1995). In systems with low C, plant uptake accounts for 70-
75% N removal. Denitrification processes with available carbon results in
55-70% N removal, but without carbon still results in 15-25% N removal.
Denitrification was limited by a C:N ratio >5:1, resulting in 90% nitrate
removal efficiency (Baker, 1998). A typical equation for denitrification is:

$$5(CH_3COOH) + 8NO_3^- + 8H^+ \rightarrow 10CO_2 + 4N_2 + 13H_2O$$

Due to nutrient uptake in plant tissue and enhanced denitrification (root
exudates) a slightly higher TN and TP removal is expected than in a
mechanical-only treatment such as sand filters, and denitrifying bacteria,
such as species of *Pseudomonas*, *Micrococcus* and *Bacillus*, seem to
flourish in hypoxic conditions.

Plants may also contribute in other ways. For example, *Phragmites* was
found to make indirect contributions to nutrient removal by stimulating
nutrient absorption onto soil particles (Wathugala et al., 1987) and
Bachand et al. (2000B) recommend a mixture of labile (submerged, 
floating) and more recalcitrant (emergents and grasses) plants as a
reasonable approach to improving denitrification rates.

Nitrate removal and nitrification are also dependent on temperature, with
a reduction in these processes below 10°C (Crites, 1994) and faster rates
during warmer conditions and climate (Mandi et al., 1998). While lower
temperatures do slightly decrease the level of metabolism processes, the
growth rate of nitrifiers decreases markedly. As nitrifying bacteria, such
as species of *Nitrosomonas* and *Nitrobacter* (Platzer and Netter, 1994) and
*Nitrospira* (Burrell *et al.*, 1998) are long-living sessile organisms, nitrification can still be achieved even at low temperatures.

Other factors also influence nitrification and denitrification. Platzer and Netter (1994) found that, with detention times ranging from 20 to 40 days, up to 70% of the influent evaporates. This results in a rising residual concentration. Even though 88% of the ammonia is removed, higher than expected nitrate readings show that the nitrate is not completely denitrified. The elimination rate of nitrogen rose from 40 to 70% with rising evapotranspiration rates, as bacteria have more substratum per time equivalent and show a higher productivity which promotes a higher purification rate.

Thus, it can be suggested that alternating systems for greywater treatment would have much higher nitrification potential than beds which are continuously fed. The ammonia adsorbed can be nitrified during the drying period, and the nitrate so formed is washed out in the next loading cycle and enables new ammonia fixation. This is supported by Platzer and Netter (1994) and Findlater *et al.* (1990), who have found that the best results for nitrification were obtained in systems based on under-draining sandy soils with periodic resting.

Ammonia can also be stripped from wastewater by making the water basic, which converts ammonium ions to ammonia, and then aerating the waste stream to drive off the ammonia into the atmosphere (Ho, 1987).
nitrification of 20 mg/L ammonia requires 100 mg/L oxygen (Crites, 1994) and thus the transfer and availability of oxygen in the water is essential. It takes 4.3 g oxygen to change 1 g ammonia into nitrate compared with 1 g to oxidise 1 g BOD (Hiley, 1995).

In cases of high requirements of nitrogen removal, it seems promising to use a combination of vertical and horizontal flow reed systems. Due to their efficient soil aeration, the vertical flow beds require much less area for nitrification and removal of organic compounds than horizontal flow beds. As vertical flow beds show very poor denitrification rates, horizontal flow beds should be added as a second stage for denitrification. An aerobic environment followed by a mostly anaerobic one should enhance nitrification and subsequent denitrification to remove most of the nitrogen from wastewater.

2.6.3 Phosphorus removal

Phosphorus and nitrogen are key elements contributing to eutrophication of surface water, and thus they should be removed (Ratanatamskul et al., 1995 and El-Hoz and Apperley, 1994). Brandes (1978) estimates that the output of phosphorus in greywater per person is about 200 mg/day whereas black water is ten times this figure (over 2 g/day). Most (88%) of the phosphorus in greywater is in a soluble form as phosphate (House et al., 1994). Phosphorus in wastewater is found in three forms: organic phosphorus, polyphosphate and orthophosphate (Ho, 1987). As a result of
biodegradation, most organic and polyphosphate end up as orthophosphate which significantly contributes to eutrophication. It is soluble reactive P which causes algal blooms, not organically-bound P (Greenway and Simpson, 1996).

Table 2.4 lists some of the physico-chemical methods to remove phosphorus from wastewater. It should be noted that Ann et al. (2000) contend that high rates of chemical amendments are needed to reduce phosphorus levels. e.g. 12 gKg\(^{-1}\) (of soil) for alum. It seems that the removal of phosphorus could be sustainable, provided that the relative supply of reactants continues.

Table 2.4. Summary of common physico-chemical methods for phosphorus removal.

<table>
<thead>
<tr>
<th>Author/s</th>
<th>Treatment and Reductions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ho et al. (1992)</td>
<td>red mud (containing iron and aluminium oxides)</td>
</tr>
<tr>
<td></td>
<td>TP 96-100% (and TN 58-85%).</td>
</tr>
<tr>
<td>Cooke (1994)</td>
<td>formation of Fe-P-humic floc - precipitated up to 70%</td>
</tr>
<tr>
<td>Maurer and Boller (1999)</td>
<td>addition of iron (Fe(^{3+})) and aluminium (Al(^{3+})) salts - up to 80%.</td>
</tr>
<tr>
<td>Brandes (1978)</td>
<td>sand filtration - 48%</td>
</tr>
<tr>
<td>Nasimian (1994)</td>
<td>adding alum and lime, for flocculation and precipitation processes respectively.</td>
</tr>
</tbody>
</table>

However, wetlands are constructed with the mistaken assumption that the substratum soils must have a high P adsorptive capacity. This implies a finite life span of the soil as all adsorptive sites are used (and which would be expensive to replace) and, if this was the case, then the low porosity of this substratum after years of phosphorus build-up would reduce loading
capacity and treatment efficiency (Rogers et al., 1990). Vandaele et al. (2000) also found that initially phosphorus is quickly removed, but this rate diminishes with time; for example, from 100% to 71% after seven months.

Even though the water/soil interface can become saturated with phosphorus, it can be regenerated by the use of a cycle of wetting and drying the soil. A number of chemical changes occur in soil during flooding and drainage, including changes in pH, ionic concentration, and redox potential (Kaynalp, 1990). The wetting and drying cycle, together with changes in pH, may cause more minerals, such as iron and calcium, to become available in solution as possible adsorption sites. Furthermore, some precipitation and crystallisation of insoluble phosphate compounds can occur. Nguyen (2000) found that phosphorus in sediments is found as either organic-P, carbonate-bound P or attached to the weakly-bound aluminium and iron oxides.

Plant uptake of phosphorus can also be rapid (Crites, 1994) and following plant death, phosphorus may be quickly recycled to the water or deposited in sediments. The soil is the major sink for phosphorus in most wetlands, and may, in time, reach its adsorption capacity. For example, White et al. (2000) found that while about 60% of phosphorus was stored in sediments, after six years the wetland had reached 66% phosphorus saturation. Significant phosphorus removal requires long detention times (15 to 25 days) and generally low phosphorus loading rates - typically less than 0.3 kg/ha.d, which is much lower than the average domestic greywater level.
In the last decade, increasing research has been undertaken on phosphorus accumulating organisms (PAO). Phosphorus accumulating organisms are of two types: denitrifying, which use oxygen as nitrate, and non-denitrifying which only use oxygen. PAO are heterotrophic microorganisms which store phosphorus as polyphosphate granules (Meinhold et al., 1999), and they can remove both phosphorus (Van Veldhuizen et al., 1999) and nitrates (Stevens et al., 1999).

Biological phosphorus removal occurs by micro-organisms typically found in activated sludge (Maurer and Boller, 1999), but Bond et al. (1999) have shown that biological phosphorus removal is not by the bacteria *Acinetobacter*, as generally believed, but by beta protobacteria and various types of gram positive bacteria. *Acinetobacter* seems to have a minor role (Wang and Park, 1998). Other bacteria species, such as *Microlunatus phosphovorous* and *Arthrobacter spp.*, are also phosphorus accumulators (Ubukata and Takii, 1998 and Wang and Park, 1998).

Recently, Choung and Jeon (2000) used sulfate-reducing bacteria, acting on iron particles in anaerobic conditions, to reduce phosphorus levels without adverse effects on nitrification and denitrification.

### 2.7 Results of Nutrient Removal Studies

#### 2.7.1 Processes in the substrate and soil

Some of the nitrogen and most of the phosphorus can be removed from wastewater by the soil. For example, some wastewater treatment systems
have used unplanted sand and/or stone filters, with fine-grained sand generally showing better nitrogen removal than coarse-grained sand (Platzer and Netter, 1994). The higher elimination rate can be explained by the higher cation exchange capacity of fine-grained soil.

The principal removal mechanisms of phosphorus are plant uptake and subsequent harvesting, and chemical and biochemical fixation in the sediments (Bhamidimarri et al., 1991). Eugelink (1998) used tracer studies of phosphorus movement to show that while some phosphorus was assimilated in plant tissue, most phosphorus seemed to be adsorbed in the soil/substrate, and Ratanatamskul et al. (1995) found that aeration of soil containing iron, to produce iron oxides, was essential for phosphorus fixation and removal.

On the other hand, Fisher (1991) found that filtering and sedimentation of particulate matter was a significant nutrient removal mechanism. Results from several authors, including Fisher (1991), show that inert gravel or sand in trenches have only limited adsorption sites to chemically absorb phosphorus, and gravel and fine sand had relatively poor phosphate retention compared to clay (Mann and Bavor, 1993).

Plants also contribute to the removal of nutrients by the substrate. In plants that permit the translocation of oxygen to their roots, the total phosphorus and other concentrations of redox-sensitive species such as Fe$^{2+}$ were reduced in the nearby water (Moore et al., 1994). Total alkalinity
and pH also were lower and oxidation-reduction potentials were higher in sediments with plants than those in which the plants were removed.

### 2.7.2 Emergent macrophytes

The use of macrophytes in wastewater treatment systems is becoming more commonplace. Breen (1990) concluded that plant biomass was found to be the major nutrient storage compartment with plant nutrient uptake being the major removal mechanism of nutrients in wastewater. He maintained that the variations in results and conclusions made by different researchers was partly due to an inadequate understanding of the removal mechanisms responsible for wastewater treatment. If this is true, then as some of the effectiveness of wetlands to remove pollutants is due to their vegetation, full performance of the wetlands may not be achieved until the vegetation is well established (Dunkerley, 1995).

The amount of nutrient removal and storage in plants does vary. Table 2.5 lists the range of TN and TP values for a variety of different macrophytes.

<table>
<thead>
<tr>
<th>Author</th>
<th>Plant/s</th>
<th>TN mg.g⁻¹</th>
<th>TP mg.g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanner (1996)</td>
<td>8 macrophytes</td>
<td>15 to 32</td>
<td>1.3 to 3.4</td>
</tr>
<tr>
<td>Adcock <em>et al.</em> (1995)</td>
<td>6 macrophytes</td>
<td>9.9 to 19.7</td>
<td>1.9 to 5.8</td>
</tr>
<tr>
<td>McJannet <em>et al.</em> (1995)</td>
<td>41 macrophytes</td>
<td>2.5 to 21.4</td>
<td>1.3 to 10.7</td>
</tr>
</tbody>
</table>

Total biomass has also been determined. Tanner and Sukias (1995) calculated *Schoenoplectus validus* biomass at 4 kg.m⁻² to 9.5 kg.m⁻² and Adcock *et al.* (1995) measured *Phragmites australis* in combination with
species of *Typha*, *Leersia* and *Urochloa* as 4.2 to 8.1 kg.m\(^2\). Table 2.6 lists the typical values of N and P in their planted wetland, where water held less than 1% of all nutrients.

Table 2.6. Nutrient concentrations in a wetland. All units g.m\(^2\).


<table>
<thead>
<tr>
<th></th>
<th>Plant</th>
<th>Sediment</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP</td>
<td>up to 35</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>TN</td>
<td>up to 130</td>
<td>30</td>
<td>5</td>
</tr>
</tbody>
</table>

A large number of emergent plants have been used in wastewater treatment systems. For example, Table 2.7 lists some of the results from various planted beds, and the nutrient removal rates by these plant systems, which do help to reduce a range of nutrients in wastewater.

Table 2.7. Amount of nutrient reduction by various plants.

<table>
<thead>
<tr>
<th>Author/s</th>
<th>Plants used</th>
<th>Results - reductions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urbanc-Bercic and Bulc (1995)</td>
<td><em>Phragmites australis</em></td>
<td>NH(_3)-N 97.5%, NO(_3)-N 74.5%, org-N 84.8%, TP 97.1% and COD 94.4%</td>
</tr>
<tr>
<td>Williams <em>et al.</em> (1995)</td>
<td><em>Phragmites australis</em></td>
<td>BOD 81-93%, NH(_3)-N 84-93% and org-N 53%</td>
</tr>
<tr>
<td>Juwarker <em>et al.</em> (1995)</td>
<td><em>Phragmites karka</em> and <em>Typha latifolia</em></td>
<td>BOD 78-90%, TP 28-41%, TN 65-73%</td>
</tr>
<tr>
<td>Fisher (1991)</td>
<td><em>Typha spp</em>, <em>Schoenoplectus spp</em> and <em>Myriophyllum spp</em></td>
<td>BOD 95%, SS 94% and TN 67%</td>
</tr>
<tr>
<td>Thomas <em>et al.</em> (1995)</td>
<td><em>Schoenoplectus validus</em> and <em>Juncus ingens</em></td>
<td>SS 85%, BOD 75% and up to 80% NO(_3)</td>
</tr>
<tr>
<td>Heritage <em>et al.</em> (1995)</td>
<td><em>Schoenoplectus validus</em></td>
<td>BOD 92-97%, up to 57% TP and up to 66% TKN</td>
</tr>
<tr>
<td>Bolton and Greenway, (1997, 1999)</td>
<td><em>Melaleuca spp</em></td>
<td>SS 98%, BOD 93%, fecal coliforms 100% and up to 84% nitrate</td>
</tr>
<tr>
<td>Liu <em>et al.</em> (2000)</td>
<td><em>Pennisetum purpureum</em></td>
<td>TP 83%, TN 76%, BOD 86%, NH(_4) 96%</td>
</tr>
</tbody>
</table>
Many researchers (Yang et al. 1995, Chick and Mitchell 1995, and Cooper and Green 1995), including some of those listed in Table 2.7, have noticed that phosphorus removal is generally low in reed bed treatment systems and minimal total phosphorus (TP) was removed except with long retention times (Tanner, 1994A, Karnchanawong and Sanjitt, 1995).

_Schoenoplectus validus_ has been used in many wastewater treatment plants, some of which are mentioned in Table 2.7. The annual removal rates of nitrogen and phosphorus by _Schoenoplectus validus_ in ammonia-rich effluent in dairy farm wastewaters was 0.15 to 1.4 gm²d⁻¹ N and 0.13 to 0.32 gm²d⁻¹ P (Tanner, Clayton and Upsell, 1995). The nutrient removal rate increases with loading rate, but increases at a slightly decreasing rate.

Tanner (1994B) found that plant uptake in _Schoenoplectus validus_ to be 15% N and 25% P of the inputs and rising if the initial nutrient levels were diluted. A later study by Tanner (1996) also found that _Schoenoplectus_ had the highest reduction of ammonium. He thought that the deeper root penetration may have resulted in greater root zone aeration and thus increased levels of nitrification. Similarly, Sikora et al. (1995) also noticed that NH₄-N removal was greatest in planted areas, due to some combination of sorption onto gravel, microbial assimilation and nitrification at the air-water interface, with some uptake mediated by seasonal macrophyte growth. However, sorption of NH₄-N onto gravel is rapidly reversible, and ammonium is mainly lost by nitrification.
Nitrogen removal was found to increase with longer detention times and higher system operating temperatures. For example, if the temperature is less than 7°C the following results were obtained by Yin and Shen (1995) in their reed bed treatment system: reductions of 44% SS, 30% TN, 44% TP, and 32% NH$_4^+$-N. Temperature also affects evapotranspiration, permitting more water to be lost from the system to the atmosphere than unplanted areas (Heritage et al., 1995).

There seems to be some disagreement about how much nutrients plants take up. Estimates range from more than 90% of the nitrogen input in microcosm tanks (Rogers et al., 1991) to 20 to 30% of the influent nitrogen and 10 to 30% of the phosphorus loading (Fisher, 1991) to 15% N and 25% P (Tanner, 1994B). These values would be dependent on the rate of harvesting of plant material, which some researchers feel is essential (Hosoi et al., 1998 and Breen, 1990), even though plant harvesting is not a particularly effective method of removing nutrients in a low maintenance aquatic macrophyte system.

Finally, Bolton and Greenway (1997, 1999) state that most constructed wetlands rely on soft-tissue macrophytes, and few studies have considered trees as few tree species can tolerate wetland conditions. Constructed *Melaleuca* wetlands, for example, have shown high nutrient reductions, as shown in Table 2.7, and have performed well in reducing nutrients in secondary effluent (Greenway and Simpson, 1996).
2.7.3 Submergent macrophytes and floating plants

Several submergents and floating macrophytes have been used in wastewater treatment systems, and these have demonstrated good nutrient removal. Table 2.8 lists examples of nutrient removals in either floating or submergent plants, or both, and these results compare well with emergents as shown in Table 2.7.

Greenway (1997) found that surface floating plants, such as duckweed (*Lemna spp*), had higher nutrient concentrations than submergents, followed by emergents. Concentrations of nitrogen and phosphorus ranged from 10 to 58 mgN.g⁻¹ and 2 to 18 mgP.g⁻¹ respectively.

Table 2.8. Levels of nutrient reduction by various floating and/or submergent plants.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Plants used</th>
<th>Results - reductions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhu and Zhu (1998)</td>
<td>floating plants - water hyacinth (<em>Eichhornia crassipes</em>) and Azolla (<em>A. filiculoides</em>) and submergents - <em>Potamogeton (P. crispus)</em>, parrot-feather (<em>Myriophyllum spicatum</em>) and eelgrass (<em>Vallisneria spiralis</em>).</td>
<td>TN 58% TP 38%</td>
</tr>
<tr>
<td>Van der Steen <em>et al.</em> (1998)</td>
<td>duckweed (<em>Lemna spp</em>)</td>
<td>About 18% of the nitrogen removed was assimilated as duckweed growth.</td>
</tr>
<tr>
<td>Körner and Vermaat (1998)</td>
<td>duckweed (<em>Lemna spp</em>)</td>
<td>TN 30-47% and up to 52% TP</td>
</tr>
<tr>
<td>Alaerts <em>et al.</em> (2000)</td>
<td>duckweed (<em>Lemna spp</em>)</td>
<td>BOD 95-99%, TKN 74-77%</td>
</tr>
<tr>
<td>Rose <em>et al.</em> (1987)</td>
<td>water hyacinth (<em>Eichhornia crassipes</em>)</td>
<td>Total coliform and fecal coliform up to 94%, turbidity 93.6% and BOD 97.3 %</td>
</tr>
<tr>
<td>Krolak (1991)</td>
<td>water hyacinth (<em>Eichhornia crassipes</em>)</td>
<td>cadmium 99% and zinc 97% benzene, toluene and other organic molecules 100%</td>
</tr>
<tr>
<td>Erikson and Weisner (1997)</td>
<td>submergent <em>Potamogeton pectinatus</em></td>
<td>8% nitrogen removal</td>
</tr>
</tbody>
</table>
Water hyacinth (*Eichhornia crassipes*) has been used in the northern hemisphere, in tropical and sub-tropical regions. Zhu and Zhu (1998) found that water hyacinth had the greatest nitrogen and phosphorus uptake, with its tissues estimated to be 0.185% N and 0.026% P of the plant wet weight. The water hyacinth was continually being used as green fodder for animals, waterfowl and herbivorous fish. Water hyacinth has other uses as Kim and Kim (2000) point out. It was used to reduce algal growth in their wetland, as the algae adsorbed onto the hyacinth’s roots. Water hyacinth, while one of the best bio-accumulators of all plants, is a declared noxious weed in WA and cannot be kept, grown or used in any capacity.

Water peanut (*Alternathera philoxeroides*) ponds were even more efficient than water hyacinth (Xu *et al.*, 1991) because of the larger numbers of useful bacteria which were involved in nitrogen and phosphorus removal processes. However, it too is a declared noxious weed in Australia.

One of the more difficult aspects of wastewater treatment is the high levels of ammonia, which is normally converted into nitrate by biological activity, and then nitrate into nitrogen gas in marshes (Spencer, 1992). Tanner (1994A) maintains that the high level of NH$_4$-N in some effluents is of particular concern because of both its direct toxicity to fish and invertebrates, and its oxygen demand (NBOD) which is commonly four-fold higher than the CBOD. Furthermore, many researchers assume that most of the nitrate loss is due to denitrification, but other mechanisms are
possible, such as assimilation by plants and microbiota and dissimilatory reduction to ammonium-nitrogen (Kadlec and Knight, 1996).

2.8 Health Concerns

A major concern with greywater reuse is the presence of pathogenic micro-organisms. Fecal contamination of greywater can occur, but the health risk associated with greywater reuse is limited and the extent of information about microbial quality of greywater is poor. Some details about the guidelines in Australia and risk assessment of wastewaters can be found in Gregory *et al.* (1994).

The presence of large amounts of organic matter in greywater discharge, from the kitchen, for example, may contribute to high levels of bacterial growth - sometimes higher than septic tank effluent. This is because kitchen scraps have undergone little breakdown, whereas toilet wastewater contains only material which has undergone considerable breakdown during its passage through the human digestive tract.

The level of coliform organisms in greywater is quite high, with an average about $2 \times 10^7$ cells/100 mL (Hypes, 1974 and Rose *et al*., 1987). The number of total coliforms and fecal coliforms are about ten times higher in shower or bath water than in laundry wash and rinse water. The coliform count is usually low in greywater from families without children, and considerably higher in families with small children (x1000s).
The physical and chemical properties of greywater may also contribute to the growth of micro-organisms stored in greywater (Rose et al., 1987). The phosphates, ammonia and turbidity in greywater indicate that nutrients are available for micro-organisms, and the recovery and survival of bacteria would also appear to be dependent on the physico-chemical nature of the sediment (Jefferies et al., 1990). For example, a pH of 9.2 for 24 hours will provide a 100% kill of E. coli and presumably most pathogenic organisms (Oswald, 1991), but bacteria have a high survival rate at a pH less than 9 (Strauss, 1991).

Although plant, soil and food debris can contribute to the coliform population, the presence of high levels of fecal coliforms \((10^6)\) and other bacteria would indicate the possible presence of enteric (intestinal) pathogens. Indicator bacteria include *Escherichia coli*, *Clostridium perfringens*, *Pseudomonas aeruginosa*, *Salmonella* and fecal streptococci.

In addition to enteric organisms, Siegrist (1977) believes that non-enteric organisms, such as those discharged in sputum and washed from the skin, deserve attention. However, he concedes that transmission of these types of organisms in greywater is not a major concern.

Some residual enteric pathogens can survive in the environment for weeks or months and some up to a year (helminth eggs were still viable after eighteen months at 18-20°C - Strauss, 1991), but their numbers are greatly reduced (Shuval, 1991). For this reason, the worm (helminth)
diseases are of concern while viruses would be the least effectively transferred through wastewaters. Bacterial and protozoan diseases rank between these two extremes.

In most wastewater treatment plants, coliform removal was probably achieved by a combination of sedimentation and natural die-off, supplemented by the effects of UV radiation in the open water sections. Rose et al. (1991) found that most enteric pathogens died within days but there was some persistence, thus greywater needs some treatment, such as storage, sedimentation, filtration, biological treatment or disinfection, prior to re-use. Coliform removals were shown to increase with longer detention times and higher systems operating temperatures. Some of the common methods of coliform removal using macrophytes and/or wastewater treatment processes are given in Table 2.9.

Fisher (1991) noted that the gravel-based aquatic macrophyte systems consistently achieved greater reductions in fecal coliform densities than tertiary maturation ponds operating at similar detention times. The greatest reductions were found in those trenches which had sections of Typha, open water and gravel. However, it seems that anaerobic conditions, typically found in subsurface flow systems, prolongs fecal coliform survival (Williams et al., 1995).

Bacterial contamination and the associated health issues are of primary importance to households. However, it has been shown that little reliance
can be placed on the individual householder to maintain and manage conventional or alternative on-site systems (Geary, 1991). Centralised management and control may be a necessary option if the total removal, or at least to acceptable levels, of pathogens cannot be demonstrated.

Table 2.9. Examples of systems used for coliform and pathogen removal.

<table>
<thead>
<tr>
<th>Author/s</th>
<th>System used</th>
<th>Results - reductions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shuval (1991)</td>
<td>stabilisation ponds</td>
<td>all helminths and 99.99% and more of coliform bacteria.</td>
</tr>
<tr>
<td>House and Broome (1990)</td>
<td>retention time of 7 to 10 days in ponds</td>
<td>most enteric bacteria and viruses.</td>
</tr>
<tr>
<td>Brandes (1978),</td>
<td>sand filters</td>
<td>99.5% of coliform bacteria and 82% of fecal coliforms.</td>
</tr>
<tr>
<td>Rivera et al. (1995)</td>
<td>gravel-filled subsurface flow reed beds</td>
<td>removal of pathogenic protozoa and helminths more effectively than soil-based systems.</td>
</tr>
<tr>
<td>Decamp and Warren (2000)</td>
<td>subsurface beds with Phragmites australis</td>
<td>98.9% reduction of E. coli</td>
</tr>
<tr>
<td>Badkoubi et al. (1998)</td>
<td>macrophyte Phragmites</td>
<td>fecal coliforms 99%.</td>
</tr>
<tr>
<td>Ottová et al. (1998)</td>
<td>macrophytes such as Glyceria maxima</td>
<td>fecal coliforms 99%.</td>
</tr>
<tr>
<td>Shi and Wang (1991)</td>
<td>range of macrophytes</td>
<td>coliform bacteria &gt;95%.</td>
</tr>
<tr>
<td>Hirata et al. (1991)</td>
<td>sedimentation</td>
<td>eliminated most fecal and coliform bacteria, while aeration effectively reduced all microorganisms by a factor of one hundred times or more.</td>
</tr>
</tbody>
</table>
2.9 Design Considerations

2.9.1 Structures and treatment processes

Alternative system options for wastewater treatment are generally of three types:

1. Those which modify the existing site and soil absorption characteristics.

2. Those which involve in-house modifications of wastewater quality and quantity.

3. Those which involve the installation of new treatment and disposal technology on-site.

The third option is the main one for consideration here, where plants will be used for effluent treatment. Breen and Chick (1989) have found that plant uptake is a major nutrient removal mechanism. Consequently, if plants are to be used in the system, then the wastewater-rootzone contact must be optimised. It seems that mixing, flow path and water-plant contact are critical factors in system design and performance.

Any system design has to be based on plant species and growth rate, soil type and permeability, inflow and outflow volume, and bed gradient (Krolak, 1991). Variables such as organic loading, daily water usage, average winter temperatures and average humidity need to be considered. The design should also be sized for extreme conditions such as maximum influent, minimum temperatures and minimal plant and microbial activity.

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A settling tank is essential as resistant solids such as human hair will settle out in the sludge at the bottom of the tank. Sludge accumulation has been estimated by Brandes (1978) as about 10 L/y/person. The settling tank, also called a surge tank, would accommodate a high influx of water at certain times, preventing the system from being too overloaded at any one time. A submersible pump would be activated at a particular level in the tank and then discharge the water to the treatment area.

As already discussed, various bacteria are essential for nutrient removal, even though the re-introduction of small amounts of bacteria-laced effluent from one tank into another (as a seed) was not beneficial (Spencer, 1992). This was because recycling made the mixtures in the tanks too homogenous and bacterial diversity, found to be important in each of the different tanks, could not be maintained. However, in any system there is a slow build-up phase where bacteria need to colonise, grow and develop in the soil matrix (DeBusk et al., 1990) and “seeding” a system with bacteria may be beneficial to get it up and running.

Initial aeration of effluent is seen as essential (Todd, 1988). Aeration effectively reduces odours from the waste stream and helps in the reduction of organic matter by bacteria. Organic matter is removed far more efficiently under aerobic conditions than under anaerobic or anoxic conditions. For example, Nasiman (1994), in a study of textile effluent, found that not all organic matter readily biodegrades, and aeration would be necessary for a better COD removal.
2.9.2 Algae and mosquitoes

One of the biggest concerns is that of overgrowth of algae in open aquatic systems. Many researchers keep the effluent level always below the soil surface as algal solids will move in open water and clog gravel or soil interfaces, thereby reducing the permeability to water. Effluent kept below the soil surface also prevents ponding which would act as a breeding ground for a variety of mosquito species (Fisher, 1990), many of which are disease carriers (Jeppeson and Solley, 1994), and this is a major health concern.

However, algae can be used in the system because they purify efficiently even during cloudy, winter conditions. The difficulty in most other systems is the control and harvesting of algae. Todd (1988) and Spencer (1992) used animals such as water snails to eat the algae and keep it from reducing light penetration. Algal growth is a function of nutrient levels (N and P) in a pond and if the availability of these nutrients can be limited, then the algal levels can be effectively controlled (Wang, 1987).

A range of strategies for mosquito control, including the use of fish, maintenance of aerobic conditions and moving water, use of biological controls (such as growth hormones) and the encouragement of predators have all been recommended (Karpisak, 1990 and Crites, 1994).

Algae and mosquitoes are only two problems which have to be dealt with. It is easy to see why many researchers prefer systems which use
subsurface flow. Subsurface disposal systems depend on the hydraulic
capacity of the soil, the purification ability of the soil and the wastewater's
infiltration rate. However, Jenssen (1991) states that a simple set of design
criteria which would include these factors does not exist.

2.9.3 Ponds or trenches?

Trenches, where the water level can be kept below the soil surface, are the
most common methods for biological wastewater treatment using
macrophytes. Furthermore, greywater should be disposed directly into
soil, as the disposal of greywater into open drains and/or waterways has
the potential to cause large-scale pollution in waterways (Jelliffe, 1995) and
produce unpleasant odours.

The main limitation of subsurface flow systems is oxygen availability,
particularly for nitrification (and most COD is eliminated by oxidation -
Jetten et al., 1997), and thus these systems are less effective at organic and
ammonium nitrogen removal, even though King and Mitchell (1995) found
that subsurface flow wetlands are particularly effective in BOD removal.
However, conditions are normally satisfactory for denitrification
processes.

The soil used as the growing medium for the macrophytes also needs
consideration. Tam and Wong (1994) have found that the application of
wastewater to soil alters the capacity of the soil to immobilise nutrients
and heavy metals. They found a significant negative correlation between
soil pH and the concentration of nutrients and heavy metals, and a positive correlation between soil organic matter and nutrients and heavy metals. Their studies with mangrove *Avicennia spp.* sediments showed that over 99% of phosphorus and heavy metals were removed from the wastewater.

Some consideration also needs to be made about the water regime and its possible effect on the efficiency of particular macrophytes in waste treatment processes. Changing water levels will also affect the amount of aeration and hence nitrification and denitrification which occurs in the system.

Ponds, rather than trenches, may be more beneficial in wastewater treatment, and even though Yang *et al.* (1995) found gravel beds to perform far better than ponds, ponds were recognised as important for the removal of nitrogen and phosphorus.

Ponds contain a variety of organisms and can be stocked with macrophytes and algae. Algae are important in that they produce a strong pH shift towards alkaline conditions which favours ammonia volatilisation and nitrification.

The greatest benefit of the pond system is its rapid response to shock loading events. As bacteria and algae have short generation times, they are able to adjust their population levels according to the loading received. Macrophytes, too, have a broader function than simply supplying a large surface area for micro-organisms.
In systems where water levels can be controlled, such as in ponds, there are widespread practical applications. As elevation decreases, the depth and duration of inundation increases, the duration of drying will decrease and the timing of flooding and drying will vary accordingly.

Ponds, whether oxidation or maturation, with or without plants, are common methods of wastewater treatment for smaller communities and industries (Sapkota and Bavor, 1994). They are relatively cheap to construct, maintain and operate, given that the land available is inexpensive.

Nutrients in ponds not only are assimilated, they are also produced by the continual recycling of matter. Ponds must be correctly designed as the average nutrient level in the outflow may progressively increase year after year. In particular, excess phosphorus/phosphate must be minimised as it may lead to toxic blooms of blue-green algae. Summers (1994) discusses the application of red mud from bauxite processing to retain phosphorus. However, the high alkaline condition of red mud (pH ≥ 11) would be unsuitable in a pond as these conditions reduce the availability of trace elements such as manganese, zinc and copper which plants need for their metabolic processes.

The size of ponds and infiltration areas must be calculated to reflect the amount of nutrient intake (number of people), the seasonal temperature variations and the characteristics of the plants themselves (some species
undergo winter senescence). Pond systems normally require 5 to 8 m² per person (Shields, 1995), but Burka and Lawrence (1990) believe that only 1 m² per person is possible when a more holistic approach is taken to wastewater treatment. They achieved a 90% reduction in BOD and SS in the first two stages of their reed bed system, using flowforms to aerate, alternating beds which were used and then rested for a week or more, and a combination of vertical and horizontal flow beds.

Ponds will also allow the natural water levels in the system to be varied to find the optimum treatment process. In this way, the life cycle of plants can be enhanced, plant diversity can be encouraged and a greater range of plants, with their special conditions for growth, can be maintained.

The number of ponds needs consideration. One pond may be sufficient to treat greywater but Oswald (1991) used four ponds, so that distinct environments could be set up and all conceivable manner of short circuiting of influent and effluent would be avoided.

All of these considerations for the design of the “best treatment” system lead to the idea of a holistic, integrated design - something Wang (1991) calls an ecosystem pond. These are different from oxidation ponds which rely on an algae/bacteria system that often results in high concentrations of algae in effluents, thus causing secondary pollution to receiving waters. This may not become a problem because Reddy and deBusk (1987) have
found that about an 80% coverage of the surface of a pond, with plants such as water hyacinth, is sufficient to shade out suspended algae.

Wang’s ecosystem ponds consist of various food chains formed from fish farming, duck and geese raising, and several macrophytes growing together. The biological, chemical and physical processes, which occur in the ecoponds, result in efficient removal of nutrients and disease organisms, while reclaiming them as recoverable resources in the production of aquatic plants, fish, duck and geese.

Ecosystem ponds tend to be stable. The components of the community and the number of individuals in each population remain fairly constant. Conversely, in most other treatment systems, such as activated sludge, population numbers fluctuate and, at times, the system becomes inoperable or highly inefficient.

Wastewater treatment using the submergent *Triglochin huegelii* in a pond set-up has not been undertaken by any other researchers and this species is to be the focus of this current research.
Chapter 3 Materials and Methods

3.1 Experimental Design Considerations

This section briefly describes the steps taken to determine the scientific method and apparatus used for the investigations, which are both described later in this chapter.

It was felt that, as a minimum for the first four investigations, four set-ups with three replications were needed. Ideally, a larger sample size with a greater number of replicates will increase the reliability of the experimental results and will account for the variation which exists in the plant samples used. As this was not possible, both physically and economically, some aspects of these trials were repeated seasonally so that a picture of climatic influence on the effectiveness of the nutrient stripping capability of the plants could be determined. The twelve tanks were randomly spaced at the test site, and used vertical flow water movement.

Vertical flow systems have shown the capability to oxidise ammonium ions as well as BOD much more efficiently than horizontal flow systems (Rogers, Breen and Chick, 1991). It can be expected that the up and down movement of water in vertical flow will enhance aeration, there will be some loss of ammonia directly to the air (especially in water with high pH) and greater conversion of ammonium to nitrate, which can then be acted upon by the plants and micro-organisms present in the system. High levels of oxygen will help kill pathogenic, enteric organisms and increase
the general levels of beneficial organisms in the wastewater treatment process.

The tanks were kept “outdoors” as rainfall can automatically be drained to a constant water level and evaporation can be compensated for by the addition of scheme (town) water.

In a normal domestic situation, protection from wind, rain and sun is not always possible and as long as all tanks are subject to the same climatic conditions the effects of rainfall and evapotranspiration would be partially accounted for by the control. Planted tanks would have proportionally more evapotranspiration and in Western Australia the rate of evapotranspiration exceeds the annual rainfall, so water loss can be expected from the system.

Pond tanks normally contained about 400 mm water as *Triglochin* is a submergent and needs to be covered by water for much of the year. However, water regime may influence the life cycle and effectiveness of the plant and subsequent lowering of the water level to the soil surface, for Investigation 4 at least, enabled the investigation of this possibility.

Furthermore, most wetland plants do not have root systems that extend much more than 400 mm and so deeper ponds will have water in the lower areas not in direct contact with the root interface. This will result in less interaction with the plants and the bacteria on the root and leaf surfaces, which are important in the nutrient removal process.
Concentrations of nutrients initially added were similar to those quoted in Jeppesen and Jolley (1994) for typical greywater analysis. For example, 20 mg/L nitrogen, 10 mg/L phosphorus and 200 mg/L BOD. Similar figures are given by Heritage et al. (1995) and Juwarkar et al. (1995) in their respective studies of domestic wastewater treatment using constructed wetlands.

Specially designed tanks were mainly used for this research. These are discussed initially in this chapter. Investigation 5 had a different set-up, and this is discussed in detail in section 3.2.2. The methods of analysis follow and all procedures are discussed. However, each investigation only used some of these analytical procedures.

3.2 Materials

3.2.1 Experimental tanks for Investigations 1 to 4

The experimental tanks, made of plastic, were designed and built to allow both upward and downward vertical flow and permit water addition or removal from either the top or bottom of each tank. The tanks could be set up as a pond or a subsurface only system. Water flow, either as inflow or outflow, was controlled by taps which directed water in particular directions.

In these tanks, the inlet was at the top but effluent entered at the bottom through the PVC pipe arrangement. The outlet was generally at the top, as an overflow pipe. As new effluent was added, the same volume of treated water passed out of the system.
All tanks were identical, with inlet and outlet fittings at the same position, and each contained the same volume of sand, stone and water. One series of tanks, as the control, did not contain any plants. The other tanks either contained a number of *Triglochin huegelii* or *Schoenoplectus validus* plants or a mixture of both species.

Twelve identical 200 L tanks (diameter = 53 cm, surface area of each = 0.22 m²), as shown in Figure 3.1 and Plates 3.1 to 3.3, were built and set up such that four different tank plantings were repeated three times.

For the first three investigations, each tank contained 300 mm of 20 mm stone, 100 mm of washed sand and up to 400 mm of water. For Investigation 4, the stone and sand medium was replaced by new screened river sand only, to a depth of 300 mm. The volume of water in the sand medium was 30 L, while a further 100 L of water was added for the pond set-up. Figures 3.3 and 3.4 and Plates 3.4 and 3.5 show the new arrangements for the tanks.

![Figure 3.1. Cross-section of a typical tank.](image)
Plate 3.1. Inside a tank showing a tap to direct water flow and the dispersion pipes on the bottom to spread water evenly over the tank floor.

Plate 3.2. Tanks were either planted with one or two species of macrophytes or remained unplanted (as controls).
Investigation 5 did not involve the tanks. Figures 3.2 and 3.5, and Plates 3.6 to 3.8, show the special subsurface tank system which was built for this investigation. In this investigation, *Triglochin* and seven other local macrophytes were examined in a subsurface flow system. The layout of the plantings and the tank arrangement is shown in Figure 3.2.

### 3.2.2 Plant descriptions in subsurface tank system

*(Investigation 5)*

Eight species were randomly planted in a small artificial wetland, which was constructed and described in section 3.3.5. A brief description of these plants is as follows.

- *Juncus pallidus* R. Br. (Pale Rush). Medium to large plant (stems up to 2 m) that prefers partial or temporary inundation. Stems are up to 7 mm diameter.
Figure 3.2. Layout of mini-wetland with planted species. Scale: 1 cm = 0.3 m (1:30).

- **Juncus microcephalus** Kunth. (Jointed Rush). Medium perennial plant to 0.7 m, stems mainly hollow (7 mm diameter) with articulations spread along its length. Prefers temporary inundation.

- **Juncus pauciflorus** R. Br. Small to medium plant, stems to 3 mm diameter.

- **Baumea articulata** R. Br. (Jointed Twig Rush). Large (to 2.5 m) stems up to 13 mm diameter, hollow, articulate. Edge plant in permanent waterlogged soil.

- **Baumea juncea** R. Br. (Bare Twig Rush). Small up to 1 m stems to 3 mm diameter. Prefers seasonally waterlogged soil.
• *Eleocharis acuta* R. Br. Small to 0.7 m. Prefers temporary inundation (wet depressions). Stems to 3 mm diameter.

• *Schoenoplectus validus* Vahl. (Lake Club Rush). Stems to 2m and 8 mm diameter with longitudinal grooves. Can tolerate permanent inundation.

• *Triglochin huegelii* Endl. (Water Ribbons). Strap-like leaves to 1 m. Singular plant which can tolerate conditions from wet soil to being submergent.

### 3.3 Investigation Procedures

#### 3.3.1 Investigation 1: Comparison of nitrate and phosphate removal (reduction) from greywater in mesocosms (tanks) containing *Triglochin huegelii* and *Schoenoplectus validus*.

This investigation was based on *Schoenoplectus validus*, which has been used in many wetland studies. Nutrient levels typically found in *Schoenoplectus validus* are well documented (Tanner, 1996; Greenway and Woolley, 1999). No work had ever been undertaken on the nutrient-stripping ability of *Triglochin huegelii*. The hypothesis tested in this investigation was:

“That *Triglochin huegelii* removes more nitrogen and phosphorus from domestic greywater than *Schoenoplectus validus*”.

This hypothesis was developed to consider the fate of two of the main nutrient substances in typical greywater samples; namely soluble forms of nitrogen and phosphorus. Furthermore, most researchers focus on the
• *Eleocharis acuta* R. Br. Small to 0.7 m. Prefers temporary inundation (wet depressions). Stems to 3 mm diameter.

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ability of wetland macrophytes to remove these nutrients from wastewater because of their known contribution to environmental problems such as eutrophication. Furthermore, high levels of phosphorus are toxic to many Australian native plants (Greenway and Simpson, 1996), and amounts in soils need to be limited.

Studies by Greenway (1997) and Tanner (1996) have confirmed that Schoenoplectus validus was able to store levels of N and P comparable to many other emergent and submersent macrophytes, and this current study set out to determine if Triglochin huegelii was also able to store high levels of N and P in its tissues.

The planted tanks (nine in total) contained either four Triglochin, two Schoenoplectus, or one Schoenoplectus and two Triglochin. Schoenoplectus is a larger clumping plant, and there was an attempt to make the initial biomass similar for each planted tank. The plants were left to grow and develop for several months before the experiment began. Three replicates of each tank system were set up. The twelve tanks therefore contained one of the following:

1. Triglochin huegelii only.

2. Schoenoplectus validus only.

3. Triglochin huegelii and Schoenoplectus validus combined.

4. No plants - as the control.

Ten litres of solution, with an initial nutrient concentration of 19.60 mg/L nitrate and 9.90 mg/L phosphate (as KNO₃ and NaH₂PO₄ respectively),
was added to each tank. These concentrations are typically found in
domestic greywater. Thus, the total initial nitrate in each tank = 196 mg
and total initial phosphate in each tank = 99 mg.

Each day, 10 L of scheme or town water was added to the 20 L drums as
shown in Plate 3.3. This permitted a constant, slow water flow into each
tank. Water flowed from the bottom upwards (vertical flow) and was
collected, sampled and tested after it passed through the overflow outlet.
Samples were tested for both nitrate and phosphate concentration using a
HACH DR 2000 Spectrophotometer and standard procedures from the

The duration of this investigation was thirty days. The tank volume (water
component) is approximately 130 L. By passing ten litres of water into each
tank each day, the retention time would be expected to be 13 days.
Extending this time to 30 days would allow enough time for most nutrient
to be either absorbed or flushed out of the system.

The experiment was only a preliminary investigation to gauge the
effectiveness of submergents for nutrient removal. The outcome
determined future investigations with this plant. Samples of plant tissue
were analysed for total Kjeldhal nitrogen and total phosphorus (standard
APHA tests, 1995) before any nutrient was added and at the completion of
the investigation. Overflow due to rainfall was collected and used in the
calculations.
3.3.2 Investigation 2: The effect of wetland plants on nutrient, BOD, Fecal coliform and suspended solids reduction in greywater.

The first hypothesis revealed that *Triglochin huegelii* did lower nitrogen and phosphorus concentrations in wastewater. This investigation was developed to expand this idea and examine the effect of *Triglochin huegelii* on a range of other parameters typically found in greywater. These include BOD, fecal coliforms and suspended solids (SS) which are often studied in wastewater treatment trials.

It is commonly believed that much of the suspended solids will simply be filtered out by the substrate. As the greywater slowly moves through the soil medium, material is dropped out of suspension. It is unknown if tanks containing *Triglochin* enhance or inhibit this process.

If *Triglochin* performs like most other wetland plants then the extensive root system will act as a filter and provide numerous sites for bacterial attachment. Bacterial action will greatly contribute to BOD reduction. Even the stone and soil in the substrate provide these bacterial sites. The aerobic nature of the surface waters will also reduce BOD.

The presence of fecal coliforms is used as an indicator of the potential presence of other disease organisms. Fecal coliforms are reduced by the action and presence of wetland plants, although no research has been
undertaken on the ability of species of *Triglochin* to reduce coliforms in wastewater.

Thus, the hypotheses tested in this investigation were:

"*Triglochin huegelii* lowers nutrients and other constituents, such as BOD, fecal coliforms and Suspended Solids, in greywater".

"That planted tanks reduce the concentration of nutrients, such as nitrates, phosphates and ammonium, in greywater more than unplanted tanks”.

Domestic greywater was collected and stored for five days in a large galvanised rain water tank. A submersible pump mixed the total volume of greywater before samples were taken.

Measurement of the daily greywater production by the four-person household was undertaken and found to be 540 L/day, on average. Daily output varied from 100 L to 800 L/day depending on the amount of use of the washing machine, number of showers by family members and kitchen water use.

The general method followed for this experiment was:

1. Using upward vertical flow in the existing tanks and wetland plant set-ups, a 50 L volume of homogeneous (mixed) sample of domestic greywater was slowly passed into each tank - in two 20 L and then one 10 L aliquot - over a five minute period.
2. An initial analysis of this wastewater was undertaken for BOD and SS (standard APHA tests). Fecal coliforms, pH, TDS, and nitrate, ammonium and phosphate concentrations were also measured.

3. Every five days, a further 50 L of greywater was added and samples taken of the overflow. At all times, up to 50 L of overflow was collected from each tank. A one litre homogeneous (thoroughly mixed) sample was taken from the collection tank and this was used for all tests and analysis. Levels of SS and BOD in this water, along with other parameters, were determined.

4. This procedure was repeated five times over the course of four weeks. Three samples of scheme water were added in the last part of the experiment to flush the residual greywater through the system.

Evaporation in each of the twelve tanks occurred during the test period. The same volume of additional scheme water was added (usually 5 to 8 L) to each tank before greywater was added.

3.3.3 Investigation 3: Nutrient reduction in the root zones of *Triglochin huegelii* and *Schoenoplectus validus*.

The first two investigations indicated that nutrient concentrations were reduced in pond conditions, with *Triglochin* and *Schoenoplectus* having an overall N gain of 16% and 29% respectively and a P gain of about 68% for both species. How *Triglochin huegelii* performed in a subsurface
environment and, in particular, the effect of the root zone on nutrient stripping was examined here. The two hypotheses tested in Investigation 1 and 2 were able to be examined again, but for Investigation 3 the pond system was replaced by a substrate-only (subsurface) study. The same plants and tanks were used, so a comparison between *Schoenoplectus* and *Triglochin* could be made again. The hypotheses were:

"*Triglochin huegelii* removes more total nitrogen and total phosphorus from domestic greywater than *Schoenoplectus validus*".

"*Triglochin huegelii* lowers nutrients and other constituents, such as BOD and Suspended Solids, in greywater".

"That the root zones in tanks planted with *Triglochin huegelii* will reduce concentrations of nutrients in greywater more than *Schoenoplectus validus*".

Domestic greywater was collected and stored for five days in a large galvanised rain water tank. A submersible pump mixed the total volume of greywater before samples were taken.

The general method followed for this experiment was:

1. A 20 to 40 L volume of homogeneous (mixed) sample of domestic greywater was slowly passed onto the surface of each tank - in combinations of 20 L and 10 L aliquots. This volume was that required to permit the water level to remain below the surface. Each tank
system was repeated three times, as shown in Figure 3.3, and the arrangement was:

(a) Control - no plants. Tanks 4, 8 and 12.

(b) *Triglochin huegelii*. Tanks 1, 5 and 9.

(c) *Schoenoplectus validus*. Tanks 2, 7 and 10.

(d) *Triglochin* and *Schoenoplectus* combined. Tanks 3, 6 and 11.

![Diagram of tank layout](image)

Figure 3.3. Plan view of tank layout for Investigation 3.

2. An initial analysis of this wastewater was undertaken for BOD and SS (standard APHA tests). A selection of other parameters, such as nitrate, ammonium and phosphate concentrations were also measured.
3. Every five days, the tanks were individually drained and pumped out into another holding container. The volume removed was equivalent to what was added. A one litre homogeneous sample was taken from this holding or collection tank and this was used for all tests and analysis.

4. This procedure was repeated every five days, six times over the course of four weeks.

Data were calculated by multiplying the concentration of each nutrient (in mgL⁻¹) by the volume (L) that was removed from each tank. The total data for each trial was collated and averaged, with standard deviations and the standard error of the differences between means calculated, as well as the level of significance from ANOVA and t tests, to give clear indication of the statistical significance of the data.

3.3.4 Investigation 4: Comparison of nutrient reduction in substrate-only and pond systems in mesocosm tanks planted with Triglochin huegelii.

Earlier investigations were either pond or substrate-only (subsurface). This investigation combined the two different treatments. New hypotheses were formulated to examine the effect of different retention times and to determine if Triglochin huegelii was able to absorb nutrients through its leaves, as well as its roots. The hypotheses tested in this investigation were:

“More nitrogen and phosphorus nutrients are removed by Triglochin huegelii in a pond system than in a subsurface-only system”.

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“Long retention time (low hydraulic loading) will increase nutrient-stripping efficiency”.

“That absorption of nutrients can occur through the leaves of *Triglochin huegelii*”.

Twelve 200 L tanks were set up, nine (at random) were planted with the submergent *Triglochin huegelii* and the other three were not planted. These served as control tanks. The tank layout is shown in Figure 3.5. Each planted tank had six plants. Although the plants were of varying sizes, samples of each plant contributed to an analysis of initial total N and total P in the tissues - leaf, roots and tubers.

Furthermore, details about plant height and number of leaves was recorded at the beginning and end of the experiment. This gave some indication of the growth of individual plants. At the completion of the experiment, all plants were removed, their wet and dry weights determined and individual nutrient analysis took place. Analysis of the soil before and after the experiment was also undertaken so that its effect on nutrient adsorption could be determined.

Three different combinations of conditions, for three tanks each, were set up. These were:

- 5 L of greywater every day to a pond system. Retention time = 20 days.
• 3 L of greywater every third day to a subsurface (substrate only) system. Retention time = 10 days.

• 10 L of greywater every day to a pond system. Retention time = 10 days.

This arrangement permitted the following comparisons:

• substrate versus pond systems with the same hydraulic loading rate (10 days).

• 20 d turnover pond versus 10 d turnover pond systems.

• plant versus no-plant 10 d pond systems.

The control tanks were subject to 10 L of greywater every day to a pond system (retention time = 10 days). It was important to have the same retention time even though the volume of water in the pond system (100 L) and the substrate only system (10 L) differed. The variables were hydraulic loading (volume and nutrient concentration of greywater) and retention time. All other conditions, such as temperature, were the same for each tank. Tanks contained 300 mm of washed sand and up to 400 mm water, depending on the experimental design, as shown in Figure 3.4.

Thoroughly-mixed homogeneous greywater was added to the top of each tank so that it moves in downward vertical flow. This was to simulate plug flow water and nutrient movement through the tank system. Samples are removed from the bottom tap, the volume corresponding to the amount of
greywater added to that tank. In the substrate only tanks, the water level was kept just below the soil surface. All water was drained from the bottom before refilling with new greywater. Only the activity of the root zone, including any uptake or involvement by tubers or roots, was examined. Due to the position of taps and outlets in each tank and the movement of the water through the soil, only downward vertical flow could be used for the below-ground investigation. This then set the criteria for the whole investigation. Plates 3.4 and 3.5 show the differences in plants and conditions between a typical substrate-only tank and a typical pond tank.

Plate 3.4. A typical substrate-only tank. *Triglochin huegelii* plants were generally smaller than those in ponds.
Plate 3.5. A typical pond tank. *Triglochin huegelii* plants were generally larger with leaves emerged above the water surface.

Figure 3.4. Cross-section of typical planted tank system for pond conditions.

The general procedure of greywater addition was repeated up to 30 times over the course of one month, with up to another forty daily
additions of scheme (town) water. This ensured that all greywater had moved through the system (as plug flow) and that each tank was subject to two to three complete flushes of scheme water, depending on the retention time.

Domestic greywater was pumped into the system as part of normal household usage. Plants were established for one month before greywater was added. The study was conducted over four months.

![Diagram of tank layout](image)

**Figure 3.5.** Plan view of tank layout and applied conditions.

Sample analysis took place for nitrate, phosphate and ammonium concentrations in both the greywater added and the water samples taken. Digestion of plant samples was undertaken to determine total N and total P of the plant parts. In this way, a N and P mass balance was able to be
performed on each tank. Total nitrogen and total phosphorus in greywater were not determined as greywater can be assumed to contain little organic matter, and any nutrients in this form would probably be degraded into inorganic forms which are easier to measure.

Table 3.1 lists values of nitrate-N, ammonium-N and phosphate concentrations, measured colorimetrically using the HACH spectrophotometer, in four typical greywater samples. These were compared to values obtained from a commercial laboratory, after digestion and analysis. Rather than digesting a large number of samples during the course of the investigations, only the nitrate, ammonium and phosphate concentrations in solution was measured, with the assumption that these values would give a good indication about the nutrient changes which occurred in the tanks.

Table 3.1. Comparison of nutrient concentrations in typical greywater samples.

* Samples analysed by spectrophotometric methods, after digestion, by Marine and Freshwater Laboratory, Murdoch University. TKN = Total Kjeldahl-N. Total Nitrogen = TKN + Nitrate-N (Ammonium-N is also determined in TKN).

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<tr>
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<th>Sample 1 mgL$^{-1}$</th>
<th>Sample 2 mgL$^{-1}$</th>
<th>Sample 3 mgL$^{-1}$</th>
<th>Sample 4 mgL$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKN*</td>
<td>23.5</td>
<td>25.5</td>
<td>35.1</td>
<td>30.1</td>
</tr>
<tr>
<td>Nitrate-N</td>
<td>1.8</td>
<td>3.5</td>
<td>7.3</td>
<td>6.2</td>
</tr>
<tr>
<td>Ammonium-N</td>
<td>2.8</td>
<td>1.5</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td>25.3</td>
<td>29.0</td>
<td>42.4</td>
<td>36.3</td>
</tr>
<tr>
<td>Total P*</td>
<td>5.9</td>
<td>9.3</td>
<td>15.4</td>
<td>15.5</td>
</tr>
<tr>
<td>Phosphate</td>
<td>4.8</td>
<td>6.9</td>
<td>9.1</td>
<td>9.8</td>
</tr>
</tbody>
</table>
The data in Table 3.1 shows that the soluble orthophosphate fraction represents about 70% of the total P present in greywater (range 58 to 79%, average 68%). That most of the phosphorus in greywater is in the orthophosphate form is commonly accepted and described by House et al. (1994) and Ho (1987).

The concentrations of nitrate and ammonium, as measured by the HACH, are much lower than the TKN values obtained by Marine and Freshwater Laboratory, indicating that the majority of nitrogen in these greywater samples is found as organic-nitrogen. HACH values range from 19.6 to 25.6% (average 22%) of digested sample estimates. Thus, only a small fraction of greywater contains nitrate ions, with most nitrogen found as organic nitrogen and ammonium ions. Similar concentrations of nutrients in greywater were measured by Surendran and Wheatley (1998) and these are found in Table 2.1, and Hypes (1974) lists average nitrate, ammonium and total Kjeldahl nitrogen concentrations as 0.3, 5.3 and 11.6 mg/L respectively.

3.3.5 Investigation 5: Comparison of nutrient uptake between eight wetland macrophytes.

Two different hypotheses were tested in this investigation as the study of only one or two macrophyte species in previous investigations was replaced by a study of eight species. Earlier studies had established that concentrations of nitrogen and phosphorus in Triglochin huegelii plant
tissue were high, and usually higher than that found in *Schoenoplectus validus*. How *Triglochin huegelii* compared to other emergents was the focus of this investigation. The hypotheses tested were:

“*Triglochin huegelii* removes more total nitrogen and total phosphorus from domestic greywater than other emergent macrophytes”.

“That *Triglochin huegelii* has the highest storage of nitrogen and phosphorus in its tissues, compared to other wetland macrophytes”.

The tank used in this investigation was an impervious fibreglass pond, about 300 mm deep. It was set up by placing 20 mm stone, subsurface coil pipe and sand in an arrangement as shown in Figure 3.6. Plate 3.6 shows the subsurface coil and inlet pipe in the tank.

The perforated subsurface coil pipe, which was attached to the inlet pipe, was to allow the movement of wastewater throughout the substrate area. Vent pipes from the coil to the surface further increases the amount of oxygen made available to the subsurface area. Oxygen was able to diffuse from the atmosphere and make contact with the wastewater. Early planting and growth can be seen in Plate 3.7, while Plate 3.8 shows the planted system three months later.

Domestic greywater was pumped into the system as part of normal household usage. Plants were established for one month before greywater was added. The study was conducted over four months. Plant samples were taken before the start of the investigation and at the completion to
determine total nitrogen and total phosphorus in plant parts such as leaves, tubers roots or rhizomes.

Figure 3.6. Cross-section of pond system for Investigation 5. The vent pipe is connected to the subsurface coil so that oxygen can diffuse into the water.

Plate 3.6. The subsurface coil allowed greywater to circulate throughout the bottom part of the tank.
Plate 3.7. The fibreglass tank held up to 1000 L. Usually, three to four samples of eight different macrophytes were randomly planted.

Plate 3.8. The planted tank three months later.
Plate 3.7. The fibreglass tank held up to 1000 L. Usually, three to four samples of eight different macrophytes were randomly planted.

Plate 3.8. The planted tank three months later.
3.4 Analytical Methods

3.4.1 Suspended solids

Suspended solids were measured by filtering a known volume of water through dry, pre-weighed filter paper and then redrying and reweighing.

A 200 mL water sample was filtered through a 47 mm Whatman glass microfibre filter using a vacuum filter system. Usually, two samples of only 100 mL of greywater were filtered. The filter papers were dried in a desiccator for at least one day and weighed before water was passed through.

After filtration, the filters were oven-dried for twenty four hours at 104°C and then re-weighed after a brief cooling period in a desiccator. The filter papers were individually placed in aluminium dishes for the oven drying.

3.4.2 Biochemical Oxygen Demand (BOD)

BOD was determined using a HACH 2137 manometric apparatus and 500 mL BOD bottles. A 157 mL water sample was added to each standard BOD bottle. Two potassium hydroxide pellets were placed in the rubber cup and the bottles were left to mix, without barometric tubes connected, for half an hour. Each manometer mercury level was adjusted to zero after re-connecting the tubes. Bottles were kept at a constant temperature of 20°C.

Checks were made over the course of five days to make sure that the equipment was functioning properly and thorough mixing was taking
place. The final barometric readings were taken after the five day period and are expressed as mg/L BOD.

3.4.3 Fecal coliforms

Fecal coliforms were examined by a membrane filtration method. Agar broth, containing mFC agar, rosolic acid and mFC broth, was prepared according to the standard procedure in APHA (1995). The sterilised agar was poured into 55 mm petri dishes, allowed to set and then stored in the refrigerator (at 4°C) until used. Usually, one millilitre of greywater or water sample was added to each plate, and incubated for 24 hours at 44.5°C. Colonies of fecal coliform showed up as blue dots on the plates (APHA, 1995).

3.4.4 Plant digestion and solution preparation

Roots, rhizomes and tubers were thoroughly washed to remove any soil particles. Plants were divided into their components before drying. All plant samples were dried for 3 days at 104°C and then ground in a stainless steel mill grinder to a fine powder. Samples were allowed to cool in a desiccator before grinding and weighing. Samples were kept in a sealed screw-top vial and placed in a dark cupboard until required. A sample weighing 0.2 g was placed in a pyrex digestion tube. Replicates were made. The procedure for the digestion was adapted from APHA (1995) and the Technicon Autoanalyser II Industrial Method booklet (1977). Essentially, one gram of sodium sulphate and mercury (II) oxide
catalyst (10 g HgO to 500 g NaSO₄) was added to each tube. Four millilitres of conc. sulphuric acid was pipetted in.

The block digester apparatus was programmed for different temperatures and times for a total of four and a half hours to a maximum of 360°C. The solution became clear at the completion of the digestion.

After partial cooling, each solution was diluted to 20 mL. This stock solution was used for both total N and total P determinations. Before analysis, one millilitre of stock solution was neutralised with the addition of up to one and a half millilitres of 4N NaOH (red litmus just changes to blue). It was found that the high acidic nature of the stock solution did affect the nitrate and phosphate readings with the HACH Spectrophotometer. For all nitrate and ammonium determinations, one millilitre of stock solution was diluted to 25 mL in the HACH bottles. For phosphate, 0.25 mL of stock solution was used.

3.4.5 Nitrate concentration

Nitrate concentration was determined by the cadmium reduction method, using powder pillows with a HACH 2000 spectrophotometer. The method is outlined in the HACH User's Manual (1989). The results were expressed as nitrate-nitrogen in mg/L. If initial testing yielded over-range data, and thus particular nutrient levels were too high, samples were diluted x10 before further analysis.
3.4.6 Ammonium concentration

Ammonium was determined by using Nessler's Reagent and the HACH 2000 spectrophotometer. A simple colorimetric method was used to ascertain ammonium ion levels. The method is described in the HACH Manual (1989). Ammonium-N is the total Kjeldahl N in the plant sample.

3.4.7 Total Nitrogen determination

The digestion process usually converts organic nitrogen into ammonium nitrogen. However, some nitrate ions are also produced and/or are present in the plant samples. It was necessary to test for both ammonium-N and nitrate-N in all samples. To calculate the total Nitrogen, the nitrate-N and ammonium-N (total Kjeldahl N) results were added.

3.4.8 Phosphate concentration

Phosphate, as orthophosphate, was determined by the ascorbic acid method using powder pillows with the HACH spectrophotometer. This method was adapted from the Standard Methods for the Examination of Water and Wastewaters (1995), and was suitable for reactive phosphorus with a concentration of zero to 2.5 mg/L phosphate ions. A description of the method is found in the HACH Manual (1989).

3.4.9 Total phosphorus determination

Phosphate, as orthophosphate, was determined by the ascorbic acid method using powder pillows with the HACH spectrophotometer as
discussed above. The total phosphorus in a sample, after digestion, is determined as an orthophosphate concentration.

3.4.10 pH

Levels of pH were measured with a hand-held microprocessor-based tester - pH Scan 1 (Eutech Cybernetics) - with an accuracy of ± 0.2 units.

3.4.11 Total Dissolved Solids (TDS)

A hand-held digital conductivity meter, a TDS Scan 4, measured samples in milliSiemens/centimetre (mS/cm). The accuracy of the meter was ± 0.05 mS. The meter was lowered into each water sample and the electrical conductivity was measured. Samples were not filtered.

3.4.12 Proline determination - acid ninhydrin method

Three replicated samples of young and old leaves in emergent and submergent conditions, making a total of twenty-four samples, were taken. A number of plants were kept in these particular conditions for over one month, which should be sufficient for differences in proline or other substances to be evident. These plant samples were independent of any used in the investigations with greywater, and proline determination was only carried out after further reading and research following the main investigations.

Leaf samples (0.5 g) were ground in a mortar and pestle. Five millilitres of 3% aqueous sulfoalicylic acid (assa) was added and grinding continued until all material was homogenised. The contents were rinsed into a
centrifuge tube. Another 5 mL of assa was added to the mortar to wash the remainder of plant material into the same tube.

Samples were centrifuged for twenty minutes at 4°C. The rest of this procedure was carried out in a fume hood. Two millilitres of the supernatant liquid were pipetted into a digestion tube. Two millilitres of acid-ninhydrin and 2 mL of glacial acetic acid were added and the mixture was then heated for one hour at 110°C. The tubes were removed and placed directly into an ice bath to terminate the reaction. Three replicates of proline standards of concentrations of 5 µg, 10 µg, 20 µg and 40 µg/mL were also prepared and digested.

After cooling, 4 mL of toluene was added and the tube vortexed for 10 seconds. Two layers formed: the top toluene layer which is used for analysis and a bottom aqueous layer.

Each toluene sample was analysed in a UV/Vis spectrophotometer (Shimadzu UV-1601) at 520 nm using toluene as a blank. Proline concentration was determined from a standard curve and calculated on a fresh weight basis by the formula given in Bates et al. (1973).

\[
\text{Formula is: } (\text{µg proline mL}^{-1} \times \text{mL toluene})/115.5 \text{ µg. µmole}^{-1}/(\text{g sample/5}) = \text{µmoles proline/gfw}\text{eight. (Note: gfweight = gram freshweight.)}
\]

Acid-ninhydrin was prepared by combining 5 g ninhydrin, 120 mL glacial acetic acid and 80 mL 6M Phosphoric acid. This mixture was heated to 70°C until all the ninhydrin dissolved (15 minutes). This reagent is stable for up to 24 hours at 4°C.
Table 3.2 and Figure 3.7 show the standards' measurements and curve, which was used to interpolate the proline content of leaves.

Table 3.2. Average absorbance values of proline standards.

<table>
<thead>
<tr>
<th>Concentration µg/mL</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.224</td>
</tr>
<tr>
<td>10</td>
<td>0.477</td>
</tr>
<tr>
<td>20</td>
<td>1.011</td>
</tr>
<tr>
<td>40</td>
<td>2.063</td>
</tr>
</tbody>
</table>

Figure 3.7. Standard absorbance curve for proline standards. Line equation is \( y = 0.0582x - 0.0687 \). \( R^2 = 0.989 \).
Chapter 4 *Triglochin huegelii*

This chapter discusses data collected about the main species under study, *Triglochin huegelii*. These data include observations on morphology, wet and dry weights, nutrient concentrations in its plant parts, proline concentration in leaves, above-ground to below-ground ratios, seed production and germination, and microscopic examination of leaf structure.

4.1 Introduction

Species of *Triglochin* are classified in the family *Juncaginaceae*, as described in Chapter 2.5.2. They are common aquatic plants, found throughout coastal Australia in wetlands, streams and seasonal waterways.

Species of *Triglochin* are also known to provide food in the form of leaves, seeds and tubers, as well as shelter (both above and below water) for a diverse variety of animals, such as frogs and aquatic macro-invertebrates. Thus species of *Triglochin* play an important role in aquatic ecosystems.

*Triglochin huegelii*, one of several species of *Triglochin* in Western Australia, is confined to the south-west of the state. It is mainly a submergent plant but its leaves become emergent in still water and tend to float in running water.
4.2 Morphology and Characteristics

_Triglochin huegelii_ has fleshy, straplike leaves which are sheathed over the lower sixth-to-third of their length. Leaf structure changes from base to tip, as further discussed in section 4.3.

It has a short underground rhizome and an extensive root system up to 300 mm long, with some root projections terminating in tubers. These root projections are normally 2-3 times longer than the tuber. Aston (1995) describes the tubers as 8-25 mm long and 4.5-8 mm diameter (length usually 2-3 times diameter). This study found that samples were generally larger than this, with tubers up to 43 mm long and 11 mm diameter (and length 2.7-4 times the diameter). The average length of tubers was 25 mm, with smaller tubers in plants from wet soil only and larger tubers from plants in ponds. Aston does not indicate the general number of tubers found in _Triglochin huegelii_ but a range of 8 to 24 with an average of 14 per plant was found during this investigation.

Aston (1995) also lists the leaves as being up to 89 cm long, but in the present work I found them to 110 cm long (and many samples had leaves 100 cm or more). Each leaf has a thickened mid region and edges which were slightly curled (Figure 4.1). The longer leaves may be due to the influence of higher-than-normal levels of nitrogen and phosphorus nutrients from the surrounding greywater.
By altering the water level in a system, leaf length, flowering, seed production (and thus seed dispersal) were often affected. *Triglochin* appears to respond to a changing water regime during its life cycle, by adjusting its height and diameter according to depth, and with the development and lengthening of its leaves (see section 4.5).

The number of tubers is not stable. There was, on average, about one extra tuber in subsurface (substrate-only or non-pond) conditions. The ratio between tuber weight and that of other plant parts is affected under these conditions, and this is discussed in section 4.5.

![Leaf sections and tubers of *Triglochin huegeli*](image)

**Figure 4.1.** Cross-section of leaves and tubers in typical samples of *Triglochin huegelli*.

Table 4.1 shows the changes to leaf number and size during Investigation 4. There were more leaves and larger leaves in a pond situation. Large plants initially grown in a pond and then transferred to a substrate-only treatment reduced their overall leaf size. Larger leaves were replaced by leaves which were more rigid, thinner and less spongy. At the same time, nutrients from the leaves of these plants were transferred to tubers, hence
the greater proportion of tuber mass and corresponding reduction in leaf proportion in the whole plant weight measurements as shown in Table 4.3.

Table 4.1. Effect of different environmental conditions on leaf number and size.
* no net change in number of leaves.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Number of leaves</th>
<th>Size of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 L substrate</td>
<td>_*</td>
<td>↓</td>
</tr>
<tr>
<td>5 L pond</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>10 L pond</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

Plate 4.1. A typical specimen of *Triglochin huegelii*, showing leaves and flower stalk.

4.3 Microscopic Examination

Wetland macrophytes are successfully used in wastewater treatment because their spongy leaf and stem tissue permits oxygen transfer to roots
and below-ground parts, such as tubers and rhizomes. Microscopic examination of *Triglochin* helped to determine if this species also possessed structural mechanisms to allow oxygen movement throughout the plant.

Thin sections of leaf tissue were microtomed and stained with either Safranin and Fast Green or Toluidine Blue (O'Brien and Cully, 1981). Material from three common wetland macrophytes (*Triglochin, Schoenoplectus* and *Juncus spp*) were sectioned and internal leaf structure documented in Plates 4.2 to 4.7.

The large intercellular spaces (aerenchyma) in the leaves of *Triglochin huegeli* (Plate 4.2), permit oxygen diffusion throughout the plant. Plate 4.3 is a section taken from the lower part of the leaf, where photosynthetic cells were not present.

Plate 4.2. Gross section of *Triglochin huegeli* leaf.
Magnification x40. Scale 1:25 (1 cm = 0.04 mm).
Plate 4.3. Transverse section of lower part of *Triglochin huegelii* leaf. 
Magnification x160.

Plate 4.4 is a section higher up along the leaf. It shows photosynthetic cells 
as stained longitudinal cells around the perimeter and especially its upper 
surface.

Plate 4.4. Transverse section of upper part of *Triglochin huegelii* leaf. Magnification x160.
Plate 4.5 is a razor blade (gross) section of *Schoenoplectus validus*, a plant which was used in many investigations. Again, large air spaces can easily be seen to permit air transport to the root zone.

Plate 4.5. Gross section of leaf of *Schoenoplectus validus*. Magnification x40. *Schoenoplectus validus* (Plate 4.6) has spongy leaves with a large amount of aerenchyma present, indicating that oxygen is able to move throughout the plant. This is one of the reasons why *Schoenoplectus validus* is used in wastewater treatment systems, as bacteria and other microscopic organisms are able to access this oxygen in the rhizosphere, as discussed in Chapter 2.6.1. Photosynthetic cells are seen completely around the perimeter (shown as the dark blue region).

The leaf structure of *Juncus pallidus* is more pithy, with less air spaces. Plate 4.7 shows distinct vascular tissue as a ring near the outer surface.
The leaf is more rigid and photosynthetic cells are clustered around the outer perimeter as shown by the dark blue ring of stained palisade cells.

Plate 4.6. Transverse section of leaf of *Schoenoplectus validus*. Magnification x160.

What is evident from the drawings and photographs of the leaf structure of *Triglochin huegelii* is that the leaf structure changes along its length. The lower section (the sheathed part which covers about a quarter of the length) has no photosynthetic cells, and the part of the leaf which does contain photosynthetic cells has only a thin layer of them around the leaf perimeter, and these are concentrated on the top surface. This outer layer is thin as shown by the microscope cross-section (Plate 4.3).

As water levels change in natural wetland environments, so does the availability of above and below-ground resources such as inorganic carbon, oxygen, light and nutrients. Species of *Triglochin* may use
dissolved carbon and nutrients directly from the water, as experimental results suggest in the next chapter, whereas most emergent macrophytes usually rely upon gaseous exchange with the atmosphere and a nutrient supply from the sediments (Brix et al., 1992).

![Plate 4.7. Transverse section of leaf of Juncus pallidus. Magnification x100.](image)

Furthermore, species of *Triglochin* may need less energy to mobilise resources which can be accessed from below as well as above the water, as indicated by the thin cuticle of their spongy leaves.

### 4.4 Seed Germination Experiments

Species of *Triglochin* propagate by seed, whereas many wetland macrophytes can propagate by both seed and rhizome sections. If *Triglochin* had potential for wastewater treatment it was important to ascertain its ease of germination and the rate of seedling survival.
However, while data was not collected, regular observations enabled discussion about the results obtained.

Specimens of *Triglochin huegelii* were obtained from Lake Goollelal and Lake Cooloongup, two lakes on the coastal plain north and south of Perth respectively, were raised to maturity and their seed collected. Plants used for investigations were germinated from this seed to reduce genetic variability and enhance comparability and uniformity of performance of the plants.

*Triglochin huegelii* is easily propagated by carpels/seeds sown on waterlogged soil, with the soil being moist but not flooded. There is no need to bury the carpels/seeds. Light seems essential for good germination, so seeds were not buried.

A germination rate was estimated, through observation, as 60-70% for seeds less than three months old. Plate 4.8 shows a thick mat of young *Triglochin huegelii* plants in seedling trays on the right-hand side and individual plants pricked out in pots on the left-hand side. Figure 4.2 details the structure of the carpels and location of seed.

The leaf raceme of *Triglochin huegelii*, about 25 cm long, produces large numbers of carpels/seeds (typically 300). This large seed production is common in plants that do not reproduce vegetatively and rely on seed dispersal mechanisms. In *Triglochin huegelii*, carpels (each containing
one seed) float and are dispersed by water flow or wind to banks or shallower areas, where they are able to germinate.

Plate 4.8. Young germinated seedlings of *Triglochin huegelii*.

Rea and Ganf (1994C) found that the seeds of the related species *Triglochin procerum* were short-lived - less than twelve months. In contrast, I have found that the seeds of *Triglochin huegelii* are viable for at least 12 months, as seed was readily propagated after this time. This is because the seeds remain protected inside the carpel.

Figure 4.2. Carpels and seed structure in *Triglochin huegelii*. 

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4.5 Wet and Dry Weights

Data were collated as part of Investigation 4 which examined nutrient uptake under different hydraulic loading, retention time and other experimental conditions.

At the completion of Investigation 4, all plants were carefully washed and then the wet and dry weight measurements were determined as described in Chapter 3. The percentage of dry weight to the original wet weight was found to be 6.3\% \pm 1.6, with a range of 4.6 - 10.1\%. These figures are low and suggest that much of the bulk of the plants was a small amount of water in a spongy, air-filled mass.

Individual dry weights for leaves, roots and tubers were measured and their percentage proportion of the whole plant determined. When the raw data was examined there was a large range of values which seemed random. A summary of all of the data is found in Table 4.2. It appears that the percentage of leaf and root dry weights is the same, with a much lower amount of tuber biomass. However, re-grouping the data according to environmental conditions to which the plants were subject, gives the results shown in Table 4.3.

Table 4.2. Mean percentage dry weight for plant parts. Standard deviations are shown. n = 18.

<table>
<thead>
<tr>
<th></th>
<th>Leaf</th>
<th>Root</th>
<th>Tuber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean %</td>
<td>42 ± 15</td>
<td>43 ± 9</td>
<td>15 ± 12</td>
</tr>
<tr>
<td>Range %</td>
<td>8 - 60</td>
<td>36 - 63</td>
<td>2 - 50</td>
</tr>
</tbody>
</table>
In substrate-only (non-pond) tanks there was a greater proportion of below-ground mass. This was concluded from visual inspection and measurements during the investigation; there was a reduced leaf size in these tanks.

Table 4.3. Proportion (as %) of plant parts under different conditions. Standard deviations are shown. n = 18.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Leaf</th>
<th>Root</th>
<th>Tuber</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 L substrate</td>
<td>24±8</td>
<td>51±9</td>
<td>25±15</td>
</tr>
<tr>
<td>5 L pond</td>
<td>56±5</td>
<td>35±5</td>
<td>9±2</td>
</tr>
<tr>
<td>10 L pond</td>
<td>47±7</td>
<td>43±6</td>
<td>8±3</td>
</tr>
</tbody>
</table>

Pond tanks with a longer retention time of nutrients yield proportionally more leaf. This provides further evidence that leaves take in nutrients directly from water and respond accordingly.

Another way of examining the data was to group the plants into one of three categories: large, medium and small, irrespective of which tanks they came from. This grouping was based on wet weights and their corresponding dry weights. The data from this re-organisation of the plant dry weights is shown in Table 4.4. A selection of plants, typical for the range of wet weights given, were selected.

Table 4.4. Proportion (as %) of dry weight plant parts for different plant sizes. Standard deviations are shown. n = 19.

<table>
<thead>
<tr>
<th>Number of plants</th>
<th>Large</th>
<th>Medium</th>
<th>Small</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet weight range</td>
<td>280 - 970 g</td>
<td>125 - 190 g</td>
<td>60 - 80 g</td>
</tr>
<tr>
<td>% leaf - dry weight</td>
<td>63±9</td>
<td>41±13</td>
<td>23±11</td>
</tr>
<tr>
<td>% root - dry weight</td>
<td>40±10</td>
<td>47±10</td>
<td>45±5</td>
</tr>
<tr>
<td>% tuber - dry weight</td>
<td>7±2</td>
<td>12±4</td>
<td>32±14</td>
</tr>
</tbody>
</table>
There appears to be a general shift to less leaf mass and more tubers from large plants to small. This may be an adaptation for the plant in its natural environment to store nutrients until flooding occurs. As discussed below, large plants (from pond conditions) have a higher percentage of above-ground parts, and small plants (mainly substrate-only conditions) have a much greater below-ground biomass. In this analysis, medium plants were a mixture of pond and substrate-only plants.

Adcock and Ganf (1994) found that *Triglochin procerum* (as a submergent in a pond) had an above-ground:below-ground ratio (AG:BG) of 6. This seems very high compared to their study of *Baumea* (1) and *Phragmites* (0.4). This present study has found that *Triglochin huegelii* has a much lower AG:BG ratio, depending on water regime. Table 4.5 is a summary of AG:BG ratio under different environmental conditions. For all samples the ratio is $0.84 \pm 0.45$. However, when consideration is given to pond tanks/conditions the ratio increases to $1.11 \pm 0.32$. It appears that in pond conditions proportionally more above-ground growth (leaves) occurs.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Mean AG:BG ratio</th>
<th>Range</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 L substrate</td>
<td>0.31 ± 0.20</td>
<td>0.12 - 0.51</td>
<td>low load, substrate only</td>
</tr>
<tr>
<td>5 L pond</td>
<td>1.30 ± 0.24</td>
<td>0.88 - 1.62</td>
<td>longest retention time</td>
</tr>
<tr>
<td>10 L pond</td>
<td>0.92 ± 0.27</td>
<td>0.67 - 1.40</td>
<td>higher load, but shorter retention time</td>
</tr>
<tr>
<td>All samples</td>
<td>0.84 ± 0.45</td>
<td>0.09 - 1.62</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.5. Above-ground to below-ground ratio under different conditions. Standard deviations are shown. Standard error of means = 0.12. $p = 0.00001$
The standard error of the differences between means as shown in Table 4.5 is 0.12. This suggests that the difference between the means of the AG:BG ratios for pond compared to substrate-only conditions is statistically significant. ANOVA and t tests confirm that $p = 0.00001$ (ANOVA) and no more than $p = 0.02$ when comparing the means of the two pond systems (5 L and 10 L). *Triglochin huegelii* does change its biomass distribution according to the environmental conditions.

Information from Tables 4.2 to 4.4 inclusive were used to calculate the ratio of leaf:tuber:root which were used in Investigation 1 to estimate total nutrient amounts in particular plant tissues. For example, using the dry weights listed in Table 4.2 the ratio would be approximately 3:1:3, but this would not be a true reflection of the actual ratio as the AG:BG ratio changes markedly under different water regimes (Table 4.5). Instead, the dry weight ratio in the 5 L pond in Table 4.3, with the highest AG:BG ratio was used, and it was found that the leaf:tuber:root ratio approximates 6:1:4.

### 4.6 Nutrient Content

The amounts of nutrients assimilated and stored by *Triglochin huegelii* tissue are listed in the next chapter, which discusses the results of many investigations into the nutrient-stripping ability of this plant.
Rea and Ganf (1994D) found that nutrients were primarily held below-ground in the roots of *Triglochin procerum* and that changes in the concentration of nutrients was directly related to changes in biomass and were not due to nutrient reallocation within the plant. This seems unusual, as the study by Adcock and Ganf (1994) found a AG:BG ratio of 6, suggesting that more nutrients were to be found in the above-ground leaf matter.

These studies (for example, Investigation 1 and 5) with *Triglochin huegelii* show that nitrogen is primarily stored or found in leaves then tubers then roots, while phosphorus is higher in tubers then roots then leaves.

Adcock and Ganf (1994) found nutrient concentrations in the related species *Triglochin procerum* to be 5.19 mgP/g and 22.6 mgN/g dry weight. This concentration of N would equate to 16 - 18% protein. The highest concentrations recorded for *Triglochin huegelii* were higher - 11.7 mgP/g in roots (average = 9.1) and 35.7 mgN/g in leaves (average 25.8) dry weight.

Table 4.6 lists the TN and TP averages and ratio for several plant samples taken during the course of Investigations 1, 4 and 5 (see next chapter). The TN:TP ratio is given for three species - *Triglochin huegelii* and *Schoenoplectus validus* from this present work and *Triglochin procerum* from work by Adcock and Ganf (1994).

The low TN:TP ratio of the whole plant in *Triglochin huegelii* was due to the high concentrations of phosphorus in roots and tubers and the low
nitrogen content in these organs. Conversely, when the leaves only are
considered the ratio rises from 2.6 (whole plant) to 8.1. This latter value is
consistent with, but higher than, the other two species and falls within the
range of 5:1 to 10:1 reported for wetland macrophytes (Kadlec and Knight,
1996).

Table 4.6. A comparison of total nitrogen and total phosphorus in plant samples.
* T. procerum from Adcock and Ganf (1994).

<table>
<thead>
<tr>
<th>Plant</th>
<th>Whole plant</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TN mgN/g</td>
<td>TP mgP/g</td>
</tr>
<tr>
<td>T. huegeli</td>
<td>20.3</td>
<td>7.7</td>
</tr>
<tr>
<td>T. procerum</td>
<td>22.6</td>
<td>5.2</td>
</tr>
<tr>
<td>S. validus</td>
<td>8.1</td>
<td>2.2</td>
</tr>
</tbody>
</table>

The TN:TP ratio in *Schoenoplectus* rises from 3.7 (whole plant) to 5.2
because of the greater TN content in leaves compared to below-ground
tissue. In contrast, *Triglochin procerum* had a slightly higher whole plant
ratio. This is possibly due to the high nutrient environment these plants
were subject to (from secondary treated effluent, TN = 35 mg/L and TP =
20 mg/L) and hence greater absorption of N and P.

*Triglochin huegeli* has been found in this present work to have a protein
content of at least 1.7 g/100 g wet weight in the leaves, and less in roots and
tubers. This figure is calculated by multiplying the total N concentration
by the dry weight of leaves and expressing a value/100 gram wet weight.
This is multiplied by a constant of 6.25 to determine the amount of protein,
as protein is assumed to have a nitrogen content of 16% (method based on Brand-Miller, personal communication).

Brand-Miller et al. (1993) found that *Triglochin procerum* has an average protein content of 1.4 g/100g wet plant. This is similar to the well-known edible native vegetable *Tetragonia tetragonoides* (New Zealand Spinach) which has a protein content of 1.7 g/100 wet weight (Brand-Miller et al., 1993).

Nutrients absorbed during growth are translocated to below-ground tubers and then mobilised for use by the young shoots in the next growing period. On-going observations during the course of the investigations with *Triglochin huegelli* revealed that there is rapid leaf growth and replacement - new leaves are often produced within 2 to 3 weeks. Loss of leaves is common in wetland plants. In his study of submerged *Phragmites australis* shoots, Gessner (2000) found that about half of the shoots were lost within six months. Furthermore, *Triglochin* does not show winter senescence, unlike many other macrophytes such as *Phragmites* and *Typha*.

This reallocation of biomass between plant organs is critical for survival if water levels change. Measurements on leaf length and water level changes supports that of Rea (1992) with her study on *Triglochin procerum* in South Australia. This author believes that the most recent water depth for *Triglochin huegelli* determines its morphology, with the length,
structure and diameter (and thus the above-ground biomass) of new leaves changing according to depth.

Wetland plants display several strategies for dealing with seasonal water level changes. *Triglochin huegelii* can survive under a wide range of conditions from permanent inundation to damp soil, whereas many other wetland macrophytes could not survive rapidly changing water levels from flooding to exposure and desiccation.

4.7 Proline Content in Leaves

Proline is an amino acid, commonly found in plants which undergo stress-related conditions such as waterlogging, drought and changing water regimes. Many wetland plants contain proline, and this investigation was undertaken to examine the possibility of *Triglochin huegelii* containing proline as an adaptation for survival in changing water regimes.

Quantitative amounts in individual leaves were determined by measuring their absorbance in a spectrophotometer and comparing readings with those of a range of proline standards (see Table 3.2 and Figure 3.7). A total of twenty-four leaf samples of *T. huegelii* were analysed for proline content. Raw data is available in the appendix. Leaf samples were grouped as old or young in either emergent or submergent conditions. Old leaves
are generally found on the outside parts of the plant and young leaves, which are also smaller, arise from the inner part of the plant.

Table 4.7 is a summary of the average fresh weight of proline per gram of leaf and, while initial results indicate that levels of proline are low, there appears to be more proline in old leaves and more proline in submersent samples.

Table 4.7. Comparison of proline concentrations between old and young leaves of *Triglochin huegelii*. n = 6 for each pond condition. Standard deviations are shown. p = 0.21

<table>
<thead>
<tr>
<th>Plant Conditions</th>
<th>Leaf sample</th>
<th>Average Fresh weight proline (μmoles/g leaf)</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergent</td>
<td>Old</td>
<td>1.14 ± 0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Young</td>
<td>0.79 ± 0.15</td>
<td>31</td>
</tr>
<tr>
<td>Submergent</td>
<td>Old</td>
<td>1.39 ± 0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Young</td>
<td>1.05 ± 0.37</td>
<td>24</td>
</tr>
</tbody>
</table>

When samples were compared to either their emergent or submergent condition, there is an increase in proline in both old and young leaves when the plant leaves are submerged. However, ANOVA and t tests reveal that there is generally no significant difference between emergent and submergent concentrations (p = 0.21), except between old and young emergent proline concentrations (p = 0.001). Here, there was a reduction of 31% of proline concentration from the old to the newer leaves. The submergent samples also had a 24% reduction in proline concentration, but this was not statistically significant (p = 0.19).
Table 4.8 shows that younger leaves tend to have greater increases in proline content (even though the concentrations of proline are lower than in older leaves), and Table 4.9 lists the results for samples from different regions of the leaf. While most samples were leaf tips, with half-a-gram samples ranging from 3 to 13 cm in length, some samples were taken from the next section from the tip. In most cases, there is a reduction in proline from the tip to the middle section of the leaf, especially in older leaves. No middle sections in young leaves of submergent plants were analysed, as three individual leaf tips were able to be tested.

Table 4.8. Comparison of proline concentrations between emergent and submergent T. huegelii plants. Standard deviations are shown.

<table>
<thead>
<tr>
<th>Plant Conditions</th>
<th>Leaf sample</th>
<th>Average Fresh weight proline (μmoles/g leaf)</th>
<th>% Increase of proline in submergent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergent</td>
<td>Old</td>
<td>1.14 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>Submergent</td>
<td>Old</td>
<td>1.39 ± 0.86</td>
<td>22</td>
</tr>
<tr>
<td>Emergent</td>
<td>Young</td>
<td>0.79 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>Submergent</td>
<td>Young</td>
<td>1.05 ± 0.37</td>
<td>33</td>
</tr>
</tbody>
</table>

Table 4.9. Reduction of proline concentrations between tip and middle sections of leaves. * Two samples showed slight increases in proline concentration from tip to middle section.

<table>
<thead>
<tr>
<th>Plant Conditions</th>
<th>Leaf sample</th>
<th>% Reduction of proline from tip to middle section of leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergent</td>
<td>Old - 1</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Old - 2</td>
<td>+12*</td>
</tr>
<tr>
<td></td>
<td>Young - 1</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Young - 2</td>
<td>18</td>
</tr>
<tr>
<td>Submergent</td>
<td>Old - 1</td>
<td>+7*</td>
</tr>
<tr>
<td></td>
<td>Old - 1</td>
<td>9</td>
</tr>
</tbody>
</table>
One further observation, only measured in young submergent plants, was that very small, young leaves had higher concentrations of proline than the longer "young" leaves. Table 4.10 lists a few results indicating the range of proline concentrations in these leaves. These results are significant (p = 0.018) and indicate that the cells of very young leaves contain high levels of proline as they begin to form and grow.

Table 4.10. Proline concentrations in young submergent leaves.
Standard deviations are shown.  p = 0.018

<table>
<thead>
<tr>
<th>Leaf description</th>
<th>Range of proline concentration (µmoles/g leaf)</th>
<th>Average Fresh weight proline (µmoles/g leaf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long young</td>
<td>0.59 - 0.97</td>
<td>0.77 ± 0.19</td>
</tr>
<tr>
<td>Short, very young</td>
<td>1.09 - 1.59</td>
<td>1.34 ± 0.25</td>
</tr>
</tbody>
</table>

Concentrations of proline in this species seem low compared to other plants which are subject to similar stress conditions. For example, Woodward (personal communication, 1999) has found levels typically 3 to 5 times greater (and sometimes more than ten times the proline level) in *Eucalyptus camaldulensis* than those found in this study. In his work, absorbance figures are usually in the range 0.5 to 1.0, but up to 2.2, which corresponds to proline concentrations from 3.5 to 7.0 (and up to 13.5) µg/gfw. The highest proline level in any sample of *Triglochin huegelii* was 2.6 µg/gfw. (Note: gfw = gram fresh weight.)

When the proline content was compared to the total N of the leaf samples, there are some positive correlations. For example, while there was only
marginally more total N in younger emergent leaves, young submergent leaves have far greater total N (about 70% more) than older submergent leaves. By way of comparison, older emergent leaves have marginally more total P than young emergent leaves, but in submergent leaves the average levels of total P in young and old leaves are the same.

Table 4.11 summarises the total N and total P levels in young and old leaves. ANOVA and t tests confirm that there is significant difference in the total N concentrations \( p = 0.001 \) but not in total P concentrations \( p = 0.37 \). In particular, the difference in total N between the means of submergent old and young leaves is significant, with \( p = 0.009 \). However, comparisons between emergent leaves are not significantly different \( p = 0.09 \). This may be due to the small number of samples analysed.

Table 4.11. Comparison of TN and TP concentrations between emergent and submergent leaves of *Triglochin huegeli*. Standard deviations and standard error (SE) of the differences between means are shown. \( n = 3 \) for each condition. \( p \) (TN) = 0.001. \( p \) (TP) = 0.37.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Leaf</th>
<th>Average total N (mg.N/g)</th>
<th>SE (between means)</th>
<th>Average TP (mg.P/g)</th>
<th>SE (between means)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergent</td>
<td>Young</td>
<td>31.9 ± 0.7</td>
<td></td>
<td>3.9 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>29.6 ± 1.2</td>
<td>0.8</td>
<td>5.5 ± 1.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Submergent</td>
<td>Young</td>
<td>29.7 ± 3.6</td>
<td></td>
<td>4.6 ± 1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>17.2 ± 2.8</td>
<td>2.6</td>
<td>4.6 ± 2.2</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Chapter 5  Results and Discussion

5.1 Investigation 1: Comparison of nitrate and phosphate removal (reduction) from greywater in mesocosms (tanks) containing *Triglochin huegellii* and *Schoenoplectus validus*.

The research hypothesis for the first investigation was that “*Triglochin huegellii* removes more nitrogen and phosphorus from domestic greywater than *Schoenoplectus validus*”.

Analysis of the main plant parts, as shown in Table 5.1, indicates that *Triglochin* has higher nitrogen and phosphorus concentrations than corresponding parts in *Schoenoplectus*. In some cases, such as in the leaves, twice as much nitrogen and one and a half times more phosphorus is accumulated in the *Triglochin* tissue. Raw data and all statistical analysis findings are found in Appendix 1.

<table>
<thead>
<tr>
<th>Species and part</th>
<th>Total Nitrogen mg/g dry mass</th>
<th>Total Phosphorus mg/g dry mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Average</td>
<td>Final Average</td>
</tr>
<tr>
<td><em>Triglochin</em> leaf</td>
<td>20.8 ± 2.1</td>
<td>25.2 ± 0.8</td>
</tr>
<tr>
<td>root</td>
<td>10.9 ± 0.8</td>
<td>11.7 ± 2.1</td>
</tr>
<tr>
<td>tuber</td>
<td>7.7 ± 0.1</td>
<td>6.6 ± 0.1</td>
</tr>
<tr>
<td><em>Schoen. leaf</em></td>
<td>9.65 ± 0.78</td>
<td>13.5 ± 0.8</td>
</tr>
<tr>
<td>root</td>
<td>5.85 ± 0.21</td>
<td>6.35 ± 0.07</td>
</tr>
<tr>
<td>rhizome</td>
<td>5.1 ± 0.1</td>
<td>4.55 ± 0.49</td>
</tr>
</tbody>
</table>
At the end of the first study, some tissues in both *Triglochin* and *Schoenoplectus* generally increased levels of total nitrogen and phosphorus in their tissues. In both plants, the relative concentration of nitrogen in the tubers (*Triglochin*) and rhizomes (*Schoenoplectus*) decreased, most probably as it was translocated to growing leaf regions.

In half of the twelve cases, the increase in levels is statistically significant \( (p < 0.05) \). T Tests were conducted on all pairs of means (as the sample size <30) and \( p \) values determined. Those values where \( p < 0.05 \) are marked in the table.

Just using nutrient concentrations to ascertain whether plants assimilate nutrients is not reliable - higher N and P concentrations does not necessarily mean greater removal rates. Relative proportions of each plant part and the total biomass of the plants must also be considered. Total nitrogen and total phosphorus in plant parts were calculated and these are shown in Table 5.2. Here, the nutrient levels in each tissue were added after considerations of the relative proportions of each plant part. The calculations were weighted by applying the following ratios, which were based on crude wet weight measurements (listed and discussed in Chapter 4.5): *Triglochin* leaf:tuber:root - 6:1:4 and *Schoenoplectus* leaf:rhizome:root - 7:2:3. The determination of the former ratio was discussed in Section 4.5, and the latter ratio was based on Tanner (1996) who calculated a AG:BG ratio for *Schoenoplectus validus* of 1.85 and crude wet weight observations by this author.
The nutrient level was calculated by multiplying the N or P concentration in a particular plant part by the relative proportion of that plant part (as compared to the whole plant). The difference in initial nutrient content and final content of the whole plant was determined and the percentage increase of each nutrient was then calculated. Clearly *Schoenoplectus* has gained proportionally more nitrogen than *Triglochin*, with phosphorus increases about the same.

Table 5.2. Summary of nutrient changes in both species.

<table>
<thead>
<tr>
<th>Species</th>
<th>% N increase</th>
<th>% P increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglochin</td>
<td>16</td>
<td>63</td>
</tr>
<tr>
<td>Schoenoplectus</td>
<td>29</td>
<td>63</td>
</tr>
</tbody>
</table>

Table 5.3 compares the increase, or decrease, of total nitrogen and total phosphorus in each plant part. *Schoenoplectus* seems to have the greatest increase in nutrient concentration, except for phosphorus in the rhizomes compared to the tubers of *Triglochin*. *Schoenoplectus* may have greater ability to store more nutrient, although the amount stored per gram of dry mass is much less than *Triglochin* (Table 5.1). While *Triglochin* had large increases in P levels in roots and tubers these are not significant results due to the variation in P levels in the samples and the large standard deviations and standard errors calculated for the means.

All nutrient level changes, except four as shown in Table 5.3, were significantly different. The standard error of the differences between means (before and after experiment) are shown in Table 5.1 and these
values were used in the calculations for Table 5.3. ANOVA and t tests were also undertaken, comparing means of the initial and final amounts of N and P, and p values are listed in the table.

Table 5.3. Relative nutrient increase or decrease during experiment duration.
* = statistically significant results, p = < 0.05.

<table>
<thead>
<tr>
<th>Plant species and part</th>
<th>% N increase or decrease</th>
<th>p</th>
<th>% P increase or decrease</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Triglochin</em> leaf</td>
<td>21*</td>
<td>0.05</td>
<td>45*</td>
<td>0.006</td>
</tr>
<tr>
<td>root</td>
<td>7</td>
<td>0.33</td>
<td>76</td>
<td>0.13</td>
</tr>
<tr>
<td>tuber</td>
<td>- 14*</td>
<td>0.008</td>
<td>68</td>
<td>0.14</td>
</tr>
<tr>
<td><em>Schoenoplectus</em> leaf</td>
<td>40*</td>
<td>0.02</td>
<td>70*</td>
<td>0.001</td>
</tr>
<tr>
<td>root</td>
<td>9*</td>
<td>0.04</td>
<td>119*</td>
<td>0.003</td>
</tr>
<tr>
<td>rhizome</td>
<td>- 11</td>
<td>0.13</td>
<td>40*</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Another interesting observation, as shown in Table 5.4, is that both plants store different nutrients to different degrees in different parts. For example, nitrogen is primarily stored in the leaves of *Triglochin* and *Schoenoplectus*, while relatively more phosphorus is stored in the below-ground root and tuber regions of *Triglochin* but in the leaves in *Schoenoplectus*. Again, these results were calculated by considering the relative proportions of each plant part and the nutrient concentration in that part. Statistical tests could not be performed on the data in Table 5.4 as the numbers listed here are proportions of a whole (100%), rather than means of a large sample.

Preliminary investigation suggests that both *Triglochin* and *Schoenoplectus* shifted nitrogen upwards to above-ground parts. *Triglochin* shifted phosphorus downwards to below-ground parts, while
phosphorus levels in *Schoenoplectus* remained fairly constant in all plant parts.

Table 5.4. The relative amounts of nitrogen and phosphorus in above and below-ground plant tissues based on concentration changes (whole plant basis).

<table>
<thead>
<tr>
<th>Species</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial % Nitrogen</td>
<td>Final % Nitrogen</td>
</tr>
<tr>
<td><em>Triglochin</em> leaf</td>
<td>71</td>
<td>74</td>
</tr>
<tr>
<td>root and tuber</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td><em>Schoenoplectus</em> leaf</td>
<td>71</td>
<td>77</td>
</tr>
<tr>
<td>root and rhizome</td>
<td>29</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 5.5 shows the results of the thirty day experiment. As predicted, both *Triglochin* and *Schoenoplectus* have absorbed, utilised or stored some nitrate and phosphate. Individual plantings of *Triglochin*, for example, have taken in proportionally more nitrogen than *Schoenoplectus*; about 5% of the input is believed to be assimilated by *Triglochin* and only 1% by *Schoenoplectus*. These figures are similar, but lower to others (Tanner, 1996 - 10% N and 13% P), but Liu *et al.* (2000) found that the amount of plant assimilation for Teosinte (*Olzea mexicana schrad*) was much higher - about 42% N and 30% P.

Both types of plants in this investigation have absorbed a similar amount of phosphorus, which is four times the amount of nitrogen. *Schoenoplectus* had slightly better absorption of phosphorus than *Triglochin*, although, on the small sample size and due to the large variation of results for individual tanks, the results are not statistically
different. ANOVA and t tests confirm that $p > 0.05$ for all comparisons of means.

Table 5.5. Amount of nitrate and phosphate absorbed by plants. All units mg nitrogen as nitrate and phosphorus as orthophosphate. Standard deviations are shown. $p (P) = 0.24, p (N) = 0.69. n = 3$

Key: Th = *Triglochin huegelii* only, Sv = *Schoenoplectus validus* only and T/S = combined tanks with both *Triglochin* and *Schoenoplectus*.

<table>
<thead>
<tr>
<th></th>
<th>Th N</th>
<th>Th P</th>
<th>Sv N</th>
<th>Sv P</th>
<th>T/S N</th>
<th>T/S P</th>
<th>Control N</th>
<th>Control P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaving system</td>
<td>138 ± 20</td>
<td>25 ± 4</td>
<td>147 ± 7</td>
<td>24 ± 4</td>
<td>153 ± 11</td>
<td>54 ± 18</td>
<td>148 ± 5</td>
<td>49 ± 28</td>
</tr>
<tr>
<td>Removed</td>
<td>58</td>
<td>74</td>
<td>49</td>
<td>75</td>
<td>43</td>
<td>45</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>Absorbed by plant</td>
<td>10</td>
<td>24</td>
<td>1</td>
<td>25</td>
<td>-5</td>
<td>-5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: The values, denoted as 'leaving system' are those total amounts of N and P which passed through the tank system. The original input was 196 mg N and 99 mg P. The values denoted ‘removed’ are calculated by subtracting the ‘collected’ values from the initial input values. The values for absorption by plant is calculated by subtracting the amount estimated to be retained in the tank (by plants, sand and stone) minus the amount retained by the control set-ups (only sand and stone). In this way the nutrient budget is balanced, so that total input = total output.

The results in Table 5.5 show that the nutrient changes in both plant species are not significantly different to unplanted tanks even though there was a 16% increase in N in the *Triglochin* tissues, as shown by the digestion of plant material. Later investigations revealed that *Triglochin* could easily reach a wet weight of 900 g in mature plants and a nitrogen content of 0.27 gN/100 g wet weight, which suggests an overall N content of about 2.5 g/plant. Given a density of only 4 plants per square metre, and a gain of 16% in N content, the average N removal could be in the order of 123
1.5 g/m² over the course of ten days, which is comparable to other macrophytes. For example, Tanner, Clayton and Upsell (1995) calculated removal rates of nitrogen by *Schoenoplectus validus* in ammonia-rich effluent in dairy farm wastewaters from 0.15 to 1.4 gm⁻²d⁻¹ N.

What is surprising, and which will need further investigation, is that there has been less absorption of both nitrogen and phosphorus when both plants are grown together - about the same as the control set-ups. This suggests that there could be some inhibiting interaction between the plants, or some unknown factor may be responsible for these anomalous results. Nakai *et al.* (1999) have demonstrated that macrophytes do secrete inhibiting allelopathic compounds which influence other plants.

Brix (1997) discusses the work of Dr Seidel in the mid 60s who showed that a range of bacteria (including coliforms, salmonella and enterococci) were killed by the antibiotical secretions of a *Schoenoplectus* species. Furthermore, Brix (1997) states that some submerged macrophytes release compounds which inhibit the growth of other species as well as a range of organic compounds which may provide a carbon source for denitrifiers and thus increase nitrogen removal.

The control tanks, with no plants, still retained a high level of nitrogen and phosphorus, suggesting that these nutrients could have been adsorbed onto the substrate surface or been metabolised by various types of bacteria in the substrate layer. Some portion of the nitrogen load that was
designated ‘retained’ can be assumed to have been lost as gaseous nitrogen - through denitrification by microbial metabolism in the anaerobic section at the bottom of the tank.

Cooke (1994) has found that only 5 - 10% of nitrate in wastewater samples was assimilated. This low amount of nitrogen assimilation is supported by this experiment where about 5% of nitrate-nitrogen was believed to be taken up by *Triglochin*, but only 1% by *Schoenoplectus*.

While nitrate can be removed by denitrification or by plant uptake, phosphorus is more difficult to remove. It doesn’t form a gaseous phase, unlike nitrogen, so the phosphorus must be stored in plant tissue or elsewhere in the system.

Most phosphorus is believed to be stored in the substrate layer rather than in plant tissue. This is shown in the initial studies of *Triglochin* where more than 50% of phosphate in the control tanks had been removed after 30 days, presumably by adsorption, whereas only 25% of nitrogen was adsorbed or not collected in the effluent. White *et al.* (2000) confirmed that about 60% of phosphorus applied to a marsh wetland system had been stored in sediments.

More phosphorus (than the nitrogen) seems to be taken up by the plants. Table 5.5 shows that *Triglochin* may have absorbed about the same amount of phosphorus as *Schoenoplectus*. This is supported by the analysis of plant tissue in Table 5.2, where proportionally more phosphorus than nitrogen was found in plant tissue (a greater percentage increase in
phosphorus). While N increased by 16 to 29% in Triglochin and Schoenoplectus respectively, phosphorus increased by over 60% in both species.

Tanks with plants also grew filamentous algae. Analysis of the algae showed that some nitrogen and phosphorus was assimilated into the tissue. Concentrations of up to 17.8 mg/g N and up to 6.7 mg/g P were found in random samples of filamentous algae in the tanks. Excessive algae was found by Nairn and Mitsch (2000) to influence phosphorus retention though biological uptake, and chemical sorption and precipitation, and Sartoris et al. (2000) found that the algal biomass assimilated NH$_4$-N and thus was an important mechanism for nitrogen removal. Algae can be used as part of an ecosystem pond because they purify efficiently even during cloudy, winter conditions. The difficulty in most other systems is how the algae can be controlled and harvested.

It can be seen that changes in the amount of nitrogen and phosphorus in plant parts are directly related to changes in biomass and to nutrient reallocation within the plant. For example, a decrease in Triglochin tuber mass is offset by an increase in shoot mass. However, this re-allocation alone cannot account for the total increase in nitrogen and phosphorus in other plant parts.

Furthermore, it hasn't been established whether nutrient storage primarily occurs below-ground in shallow water and above-ground in deep water, or vice versa. It is suspected that the Triglochin tubers are
organs which gives this species added tolerance to changes (mainly decrease) in the depth of water. Additional studies in Investigation 4 have helped to clarify this, where differences in above-ground and below-ground biomass were evident when comparing plants in either subsurface or pond conditions.

Thus while both plants had increases in total N and total P, the hypothesis is not supported. Conversely, it was shown that tanks containing Schoenoplectus validus remove more nitrogen than tanks with Triglochin huegelii.

5.2 Investigation 2: The Effect of Wetland Plants on Nutrient, BOD, Fecal Coliform and Suspended Solids Reduction in Greywater.

The hypotheses tested in this investigation were that “Triglochin huegelii lowers nutrients and other constituents, such as BOD, fecal coliforms and Suspended Solids, in greywater” and “That planted tanks reduce the concentration of nutrients, such as nitrates, phosphates and ammonium, in greywater more than unplanted tanks”.

The collated results and discussion of the trends and outcomes for the parameters measured in each one of the eight trials, using either greywater or scheme water, follows.
5.2.1 Suspended solids

The total Suspended Solids output, as listed in Table 5.6, was calculated by multiplying the SS concentration by the volume collected; that is, 50 L. The individual tank values were then added and grouped according to the treatment. The percentage reduction was calculated by comparing the total tank output to the initial total input of the greywater.

Table 5.6. Percentage reduction of suspended solids in greywater. Standard deviations are shown. SE = Standard error of differences between the means of planted and unplanted tanks (control). p = 0.88

<table>
<thead>
<tr>
<th>Plant types</th>
<th>Average suspended solids collected (mg)</th>
<th>SE</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10700 ± 3200</td>
<td>-</td>
<td>44 ± 20</td>
</tr>
<tr>
<td><em>Triglochin huegelii</em></td>
<td>9000 ± 2500</td>
<td>2300</td>
<td>54 ± 27</td>
</tr>
<tr>
<td><em>Schoenoplectus validus</em></td>
<td>11000 ± 1400</td>
<td>2000</td>
<td>42 ± 15</td>
</tr>
<tr>
<td><em>Triglochin &amp; Schoenoplectus</em></td>
<td>10700 ± 1000</td>
<td>1900</td>
<td>44 ± 11</td>
</tr>
</tbody>
</table>

Total suspended solids input from greywater = 19200 mg.

Tanks with *Triglochin huegelii* appear to have the greatest reduction of suspended solids, but these results are not significant (t test p = 0.33). Thomas et al. (1995) and Fisher's (1991) study, both using *Schoenoplectus*, had, respectively, 85% and 94% reduction in suspended solids, which is about twice of the result in this study. While *Schoenoplectus validus* and *Triglochin* and *Schoenoplectus* combined had a similar effect to the control tanks with no plants, the *Triglochin* tanks, on average, seemed to have a further ten percent reduction in suspended solids. Even so, the
standard error of differences between the means ranged from 2011 to 2334, which suggests that there is no statistical significance between the different tanks. Further investigation will need to be carried out to examine the effect of plants on SS reduction. ANOVA and t tests between the means of planted tanks and the control confirm that these results are not significant, with $p = 0.33$ for a comparison between Triglochin and control tanks and $p = 0.88$ for all tanks.

5.2.2 Biochemical Oxygen Demand (BOD)

The results in Table 5.7 show that BOD is generally greatly reduced in all tanks, with the range of reduction for the various combinations of tanks being 86 to 90%. Some of the planted tanks had less reduction in BOD, on average, than the unplanted control tanks, but, again, there is no statistical significance between the different tanks (ANOVA, $p = 0.63$).

These types of results are consistent with other researchers such as Williams et al. (1995) and Bolton and Greenway (1999) who found 81-93% and 93% BOD reduction in their respective wetland macrophyte studies. Heritage et al. (1995), who used Schoenoplectus validus as well, found 92-97% BOD reduction.

In this investigation, the greywater was produced in summer months and due to higher temperatures during this time, more biochemical activity occurred. High algal content would also help increase BOD, and this might explain why the unplanted tanks had high BOD reduction.
Table 5.7. Percentage reduction of BOD in greywater. Standard deviations are shown. SE = Standard error of differences between the means of planted and unplanted tanks.

\[
p = 0.63, \ n = 3
\]

Total BOD load from greywater = 42600 mg.

<table>
<thead>
<tr>
<th>Plant types</th>
<th>Average BOD output (mg)</th>
<th>SE</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4800 ± 1500</td>
<td>-</td>
<td>89 ± 3</td>
</tr>
<tr>
<td><em>Triglochin huegelii</em></td>
<td>6000 ± 2900</td>
<td>1000</td>
<td>86 ± 3</td>
</tr>
<tr>
<td><em>Schoenoplectus validus</em></td>
<td>5100 ± 600</td>
<td>200</td>
<td>88 ± 1</td>
</tr>
<tr>
<td><em>Triglochin &amp; Schoenoplectus</em></td>
<td>4100 ± 1000</td>
<td>400</td>
<td>90 ± 2</td>
</tr>
</tbody>
</table>

5.2.3 Fecal coliforms

Fecal coliforms are identified as blue dots on the agar plates. Many other organisms were present as brown or colourless dots on the plates, suggesting that greywater contains a wide range of microscopic organisms.

Reduction in fecal coliforms in all tanks ranged from 87 to 99%, with control tanks, on average, having slightly lower reduction of fecal coliforms. However, the standard error of differences between the means ranged from 900 to 1100 (Table 5.8), which suggests that there is no statistical significance between the different tanks, and \( p \) was found to be \( p = 0.92 \) (ANOVA). These results are also consistent with the findings of others. For example, Badkoubi *et al.* (1998) and Ottová *et al.* (1998) both had 99% fecal coliform reduction using wetland plants.

Bacteria die-off is directly correlated with pH as shown by Fallowfield *et al.* (1996) and Rångeby *et al.* (1996). Generally, the die-off rate is high when the
pH is high, especially after pH = 9, which was achieved in some tanks (Table 5.12).

Table 5.8. Percentage reduction of fecal coliforms in greywater. Standard deviations are shown. SE = Standard error of differences between the means of planted and unplanted tanks. \( p = 0.92 \) (ANOVA)
Total fecal count was the sum of all coliform dots for each plate for the eight trials. Total fecal coliform count in greywater = 1625 in 5 mL. Average = 32500/100 mL.

<table>
<thead>
<tr>
<th>Plant types</th>
<th>Average total fecal count/100 mL</th>
<th>SE</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2200 ± 1420</td>
<td>-</td>
<td>93 ± 5</td>
</tr>
<tr>
<td><em>Triglochin huegelii</em></td>
<td>2040 ± 1300</td>
<td>1100</td>
<td>94 ± 5</td>
</tr>
<tr>
<td><em>Schoenoplectus validus</em></td>
<td>1400 ± 620</td>
<td>900</td>
<td>96 ± 2</td>
</tr>
<tr>
<td><em>Triglochin &amp; Schoenoplectus</em></td>
<td>1980 ± 1300</td>
<td>1100</td>
<td>94 ± 5</td>
</tr>
</tbody>
</table>

Bacteria are killed by UV light as well. With higher levels of algal growth the effect of light penetration in the water, and thus the degree of bacteria kill, will be reduced. Conversely, the higher algal content, and thus greater photosynthesis rate, will increase both the pH and diurnal oxygen content. Both of these variables will increase bacteria die-off. In this investigation, the tanks slowly became alkaline and this increased pH may have contributed to bacteria die-off. Because fecal coliforms were greatly reduced in all tanks, with control tanks yielding 93% reduction, the combination of long retention times, aerobic conditions and high pH would naturally kill coliforms.

5.2.4 Nitrate concentration

There is a wide range of results for the percentage nitrate removal even from the same types of plants. For example, *Triglochin* tanks had a range
of 61 to 90%, while *Schoenoplectus* tanks had 58 to 76% (the whole range for all tanks was from 49 to 91%).

In a pond situation, *Triglochin huegelii* seemed to have greater uptake of nitrate (or increase in nitrogen content) than *Schoenoplectus validus*, or at least provide the necessary conditions for nitrate reduction in the greywater. However, the standard error of differences between the means ranged from 37 to 67, which suggests that there is no statistical significance between the different tanks. ANOVA produces $p = 0.89$ which confirms that there is no statistical difference between the control and the planted tanks, and $t$ tests between *Triglochin* and *Schoenoplectus*, and *Schoenoplectus* and control, tanks both produce $p = 0.22$, so these results are not significant either. Further investigations are required to examine whether planted tanks do reduce nitrate by plant assimilation, or were other processes responsible for the high nitrate retention/loss in the tanks.

Table 5.9. Percentage assimilation or retention of nitrate in greywater. Standard deviations are shown. SE = Standard error of differences between the means of planted and unplanted tanks. $p = 0.89 \quad n = 3$

<table>
<thead>
<tr>
<th>Plant types</th>
<th>Total nitrate collected for 3 tanks (mg)</th>
<th>Average nitrate collected per tank (mg)</th>
<th>SE</th>
<th>% Assimilation or retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>575</td>
<td>192 ± 45</td>
<td>-</td>
<td>70 ± 7</td>
</tr>
<tr>
<td><em>Triglochin huegelii</em></td>
<td>515</td>
<td>172 ± 99</td>
<td>36</td>
<td>73 ± 15</td>
</tr>
<tr>
<td><em>Schoenoplectus validus</em></td>
<td>685</td>
<td>228 ± 64</td>
<td>26</td>
<td>64 ± 10</td>
</tr>
<tr>
<td><em>Triglochin &amp; Schoenoplectus</em></td>
<td>585</td>
<td>188 ± 135</td>
<td>47</td>
<td>71 ± 21</td>
</tr>
</tbody>
</table>

Total nitrate input from greywater = 637.5 mg.

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5.2.5 Ammonium concentration

The loss of ammonium ions from the system can be high. As the pH was steadily increasing (see Table 5.12) some of the ammonium ions would be changed to ammonia gas, which can be lost to the atmosphere by ammonia volatilisation. For example, a pH of 9 at 20°C will result in 50% of ammonium changed to ammonia, and this is possibly lost via volatilisation (Kadlec and Knight, 1996). Newman et al. (2000) also contend that ammonia volatilisation is only appreciable at pH>8.

Some ammonium ions will be oxidised to nitrates by nitrifying bacteria, and thus nitrification may be a mechanism for ammonia removal. Ammonium uptake by plants does occur (McBride and Tanner, 2000) and may be favoured over nitrate uptake. In any case, both of these mechanisms contribute to ammonium reduction, and thus ammonium removal can be high.

Furthermore, nitrate can be denitrified to nitrogen gas under anaerobic or anoxic conditions, which is often lost to the atmosphere. For these reasons, it is not surprising to suggest that much of the ammonium is lost from the aquatic system rather than being absorbed and then utilised by plant tissue. Results listed for the unplanted (control) tanks in Table 5.10 suggest that 76% of the ammonium input was lost or retained in the system and if 50% is volatilised due to the high pH (pH values were the highest (>9) in control tanks - see Table 5.12), then about 26% is estimated to be nitrified.
The values for the reduction in ammonium levels in each tank ranged from 67 to 92%, with a average of 82 ± 8%. This implies that another 6% or more of the ammonium is taken up by plants. When the tanks are grouped according to their particular treatment conditions, as shown in Table 5.10, we find that there is little difference between *Triglochin* and the control tanks. However, *Schoenoplectus* and the combined plant tanks have a significantly greater utilisation or effect on ammonium concentration. It could be that these plants provide suitable conditions for bacterial action around the root zone.

Other researchers have also noticed that ammonium reduction is usually greater than nitrate reduction. For example, Urbanc-Bercic and Bulc (1995) found 97.5% NH$_4$-N and 74.5% NO$_3$-N with *Phragmites australis*, and Williams *et al.* (1995) had a range of ammonium reduction 84-93%, similar to what this study has found.

The standard error of differences between the means ranged from 1 to 12, which suggests that there is statistical significance between the control tanks and tanks planted with *Schoenoplectus*, and the control tanks and tanks planted with both *Schoenoplectus* and *Triglochin*. ANOVA and $t$ tests confirm that for all tank systems $p = 0.008$, and between *Schoenoplectus* and control tanks $p = 0.01$, and between *Schoenoplectus* and *Triglochin* combined and the control tanks $p = 0.009$. 

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Table 5.10. Percentage assimilation or retention of ammonium in greywater. Standard deviations are shown. SE = Standard error of differences between the means of planted and unplanted tanks. \( p = 0.008 \)

Total ammonium input from greywater = 392.5 mg.

<table>
<thead>
<tr>
<th>Plant types</th>
<th>Total NH(_4)^+ collected for 3 tanks (mg)</th>
<th>Average NH(_4)^+ collected per tank (mg)</th>
<th>SE</th>
<th>% Assimilation or retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>287.5</td>
<td>96 ± 19</td>
<td>-</td>
<td>76 ± 6</td>
</tr>
<tr>
<td><em>Triglochin huegelii</em></td>
<td>291.0</td>
<td>97 ± 23</td>
<td>12</td>
<td>75 ± 3</td>
</tr>
<tr>
<td><em>Schoenoplectus validus</em></td>
<td>122.5</td>
<td>41 ± 8</td>
<td>3</td>
<td>90 ± 1</td>
</tr>
<tr>
<td><em>Triglochin &amp; Schoenoplectus</em></td>
<td>126.5</td>
<td>42 ± 4</td>
<td>1</td>
<td>89 ± 2</td>
</tr>
</tbody>
</table>

5.2.6 Phosphate concentration

The range of phosphate assimilation and/or retention in all tanks was 47 to 91%. Collectively, the control tanks had high phosphate retention. This could be partly due to assimilation into algal tissue. However, large amounts of phosphorus are stored in the substrate, bound to soil particles and organic matter. This is possibly the reason for high phosphate retention in the control tanks which did not contain plants.

Tanks containing wetland macrophyte plants had no greater phosphate retention than the control tanks, as shown in Table 5.11. The standard error of differences between the means, as shown in Table 5.11, ranged from 15 to 198, and \( p = 0.24 \) (ANOVA), which suggests that the differences between the means are not statistically significant.
Table 5.11. Percentage assimilation or retention of phosphate in greywater. Standard deviations are shown. SE = Standard error of differences between the means of planted and unplanted tanks. \( p = 0.24 \)  \( n = 3 \)

Total phosphate input from greywater = 1970 mg.

<table>
<thead>
<tr>
<th>Plant types</th>
<th>Total phosphate collected for 3 tanks (mg)</th>
<th>Average phosphate collected per tank (mg)</th>
<th>SE</th>
<th>% Assimilation or retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>728</td>
<td>240 ± 60</td>
<td>-</td>
<td>88 ± 4</td>
</tr>
<tr>
<td><em>Triglochin huegelii</em></td>
<td>1708</td>
<td>570 ± 340</td>
<td>198</td>
<td>71 ± 21</td>
</tr>
<tr>
<td><em>Schoenoplectus validus</em></td>
<td>843</td>
<td>280 ± 20</td>
<td>15</td>
<td>86 ± 1</td>
</tr>
<tr>
<td><em>Triglochin &amp; Schoenoplectus</em></td>
<td>631</td>
<td>210 ± 75</td>
<td>45</td>
<td>90 ± 5</td>
</tr>
</tbody>
</table>

5.2.7 pH

The level of acidity (pH) in the control tanks progressively increased during the experiment duration. This is probably due to increasing levels of algal growth. It appears that some plants have a buffering effect on acidity. This is particularly true of *Schoenoplectus validus* and *Schoenoplectus validus* combined with *Triglochin huegelii*. In these tanks the pH only marginally increased while the pH in the control and *Triglochin* tanks continually rose.

The growth of algae observed in most tanks would help explain the slow increase in pH over the study period. Algal photosynthesis causes an increase in pH due to the uptake of carbon dioxide. Hydrogen ions are then removed from the system as shown in the equation below. As carbon dioxide is removed from the system, the equilibrium shifts towards the
right, thus reducing hydrogen ions in the water. Furthermore, the assimilation of nitrate and its dissimilatory reduction to ammonium ions also increases the pH level.

\[ \text{H}^+ + \text{HCO}_3^- \rightleftharpoons \text{H}_2\text{O} + \text{CO}_2 \]

Not all wastewaters have this buffering capacity. For example, Fallowfield et al. (1996) contend that domestic sewage is poorly buffered by the carbonate-bicarbonate equilibrium, whereas piggery waste is well buffered by its phosphate content. Table 5.12 illustrates the changes in acidity during the duration of the experiment. The final column lists the overall change in pH for the duration of the investigation.

Table 5.12. Changes in average pH in tanks.
Note: Each 'Trial' represents the addition of greywater during the investigation.
Key: Th = Triglochin huetii only, Sv = Schoenoplectus validus only and T/S = combined tanks with both Triglochin and Schoenoplectus.

<table>
<thead>
<tr>
<th>Tank system</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
<th>Trial 6</th>
<th>Trial 7</th>
<th>Trial 8</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.0</td>
<td>7.4</td>
<td>7.9</td>
<td>8.4</td>
<td>8.4</td>
<td>8.6</td>
<td>9.1</td>
<td>9.4</td>
<td>+2.4</td>
</tr>
<tr>
<td>Th</td>
<td>6.9</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.8</td>
<td>8.4</td>
<td>8.6</td>
<td>+1.7</td>
</tr>
<tr>
<td>Sv</td>
<td>6.9</td>
<td>7.4</td>
<td>7.5</td>
<td>7.5</td>
<td>7.4</td>
<td>7.6</td>
<td>7.6</td>
<td>7.6</td>
<td>+0.7</td>
</tr>
<tr>
<td>T/S</td>
<td>6.9</td>
<td>7.4</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.8</td>
<td>8.0</td>
<td>7.9</td>
<td>+1.0</td>
</tr>
</tbody>
</table>

5.2.8 Total Dissolved Solids (TDS)

Conductivity remains high in the tanks - generally twice as much as the greywater itself. While evaporation can explain some increases in TDS measurements, there must be other mechanisms which contribute to the
total dissolved solids in each tank. It could be that chemical processes, by the plants and/or bacteria, change the nature of particular substances, thus increasing the amount of dissolved ions in solution.

The conductivity of the water system is affected by evaporation. Some output readings were higher than the input greywater level, possibly due to increasing concentration of ions as water is lost from the tanks (up to one-fifth of the input volume is lost by evaporation).

There could also be substances released by the plants, or unknown chemical reactions in the aquatic environment, which change the chemical nature of the solutes. Further research may need to be undertaken to investigate this phenomenon.

While the TDS of added greywater rose from 0.8 to 1.0 mS/cm, the TDS of the experimental tanks fell - usually from 1.2 or 1.3 to 0.9 mS/cm. Even so, there are no significant differences between the four experimental tank systems, with all planted tanks having the same types of readings as the control tanks.

The greatest change did occur in the combined plant tanks, suggesting that this combination may reduce total dissolved solids, but further investigation would need to be conducted to verify this, as only one average reading was taken for each tank.

The slow decrease, as shown in Table 5.13, could imply that particular soluble substances are utilised in the system, possibly chemically changed
by the plants, bacteria or some other organisms to substances which are less soluble.

Table 5.13. Changes in the average Total Dissolved Solids (conductivity in mS/cm) in the tanks.
Note: Each ‘Trial’ represents the addition of greywater during the investigation.
Key: Th = Triglochin huegelii only, Sv = Schoenoplectus validus only and T/S = combined tanks with both Triglochin and Schoenoplectus.

<table>
<thead>
<tr>
<th>Tanks</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
<th>Trial 6</th>
<th>Trial 7</th>
<th>Trial 8</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.20</td>
<td>1.10</td>
<td>1.10</td>
<td>1.05</td>
<td>1.05</td>
<td>1.05</td>
<td>0.90</td>
<td>0.90</td>
<td>- 0.30</td>
</tr>
<tr>
<td>Th</td>
<td>1.20</td>
<td>1.20</td>
<td>1.15</td>
<td>1.20</td>
<td>1.20</td>
<td>1.10</td>
<td>1.00</td>
<td>0.90</td>
<td>- 0.30</td>
</tr>
<tr>
<td>Sv</td>
<td>1.25</td>
<td>1.15</td>
<td>1.10</td>
<td>1.10</td>
<td>1.15</td>
<td>1.10</td>
<td>1.05</td>
<td>0.90</td>
<td>- 0.30</td>
</tr>
<tr>
<td>T/S</td>
<td>1.35</td>
<td>1.20</td>
<td>1.15</td>
<td>1.15</td>
<td>1.15</td>
<td>1.10</td>
<td>1.00</td>
<td>0.90</td>
<td>- 0.45</td>
</tr>
</tbody>
</table>

In conclusion, there is support for the hypothesis that “planted tanks reduce the concentration of nutrients, such as nitrates, phosphates and ammonium, in greywater more than unplanted tanks”, but not all of the results are statistically significant. Support for the hypothesis that “Triglochin huegelii lowers nutrients and other constituents, such as BOD, fecal coliforms and Suspended Solids, in greywater” is lacking. The current work discussed in Chapter 4 showed that the structure and morphology of Triglochin changed in different water regimes. There was a need to examine the nutrient-stripping ability of Triglochin when water levels were reduced to below-surface. This led to Investigation 3.
5.3 Investigation 3: Nutrient Reduction in the Root Zones of *Triglochin huegelii* and *Schoenoplectus validus*

Similar hypotheses to those already examined were tested in this investigation. However, the pond system was replaced by a study involving the root zones of the plants. The water level was kept below the surface. An additional hypothesis tested here was “that the root zones in tanks planted with *Triglochin huegelii* will reduce levels of nutrients in greywater more than *Schoenoplectus validus*”.

The collated “pooled” results of the six trials, using greywater as the only source of nutrients, follow. Raw data for individual tanks is presented in Appendix 1.

5.3.1 Suspended solids

Data was not analysed as the pumping action to drain the tanks seems to have dislodged particles in the substrate causing the SS to be higher at times than what was added through greywater. Furthermore, any released/dislodged organic matter will affect the BOD data, and this may explain the discrepancies encountered in the next section. If SS are to be determined, a slower, passive method may need to be employed to drain the water into the collecting tank.
5.3.2 Biochemical Oxygen Demand (BOD)

BOD is generally greatly reduced in all tanks, with the range of reduction being 83 to 93%. Although not statistically significant (p = 0.60, ANOVA), all planted tanks have greater reduction in BOD than the control tanks, with *Schoenoplectus* slightly higher than *Triglochin*.

The difference between the means of control tanks and the *Schoenoplectus* tanks is only twice the standard error, which is not great enough for statistical significance (p = 0.13, t test). These results are similar to those of Investigation 2 where 86 - 90% BOD reduction was obtained (Table 5.7).

Figure 5.1 shows the general relationship between the BOD loading rate and the amount of BOD removal. It seems that the amount of BOD removed is proportional to the loading rate, except at high loading rates where it decreases. At higher rates, a longer retention time may be needed by these types of systems to reduce BOD.

Table 5.14. Percentage reduction in BOD by individual tanks. Each ‘Trial’ represents the addition of greywater to the tanks. SE = Standard error of differences between the means of planted and unplanted tanks. p = 0.60

<table>
<thead>
<tr>
<th>Trial</th>
<th>Control tanks</th>
<th>Triglochin tanks</th>
<th>Schoenoplectus tanks</th>
<th>Triglochin and Schoenoplectus tanks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59.7</td>
<td>85.6</td>
<td>65.2</td>
<td>69.8</td>
</tr>
<tr>
<td>2</td>
<td>89.9</td>
<td>96.1</td>
<td>100.0</td>
<td>94.4</td>
</tr>
<tr>
<td>3</td>
<td>84.1</td>
<td>100.0</td>
<td>98.5</td>
<td>100.0</td>
</tr>
<tr>
<td>4</td>
<td>98.6</td>
<td>97.2</td>
<td>99.4</td>
<td>100.0</td>
</tr>
<tr>
<td>5</td>
<td>70.6</td>
<td>61.3</td>
<td>97.1</td>
<td>93.8</td>
</tr>
<tr>
<td>6</td>
<td>98.0</td>
<td>87.3</td>
<td>100.0</td>
<td>95.1</td>
</tr>
<tr>
<td>Mean</td>
<td>83.5</td>
<td>87.9</td>
<td>93.4</td>
<td>92.2</td>
</tr>
<tr>
<td>Std Dev</td>
<td>14.2</td>
<td>13.0</td>
<td>12.7</td>
<td>10.3</td>
</tr>
<tr>
<td>SE</td>
<td>-</td>
<td>4.5</td>
<td>4.5</td>
<td>4.1</td>
</tr>
</tbody>
</table>
A typical analysis of BOD removal is shown in Figure 5.2. Here, the amount of BOD input (loading) and then removal for each set-up is shown,
with planted tanks consistently removing more BOD than unplanted tanks. However, for this particular trial there doesn’t seem to be statistical significance between the control tanks and those of *Schoenoplectus* as the standard deviations overlap, but there is significance between the control tanks and tanks containing *Triglochin*.

### 5.3.3 Nitrate concentration

There is a wide range in the percentage reduction of nitrates even from the same types of plants. For example, *Triglochin* tanks had a range of 71 to 79%, while *Schoenoplectus* tanks had 73 to 91% (the whole range for all tanks was from 63 to 91%). These results compare favourably with those of Bolton and Greenway (1999) and Thomas *et al.* (1995) who found up to 84% and 80% (respectively) nitrate reduction in macrophyte wetlands.

Table 5.15 shows that tanks with *Schoenoplectus validus* seemed to have greater uptake or removal of nitrate than tanks with *Triglochin huegelii*. This supports Investigation 1 where similar results were obtained.

Again, as some previous experiments have indicated (e.g. Investigation 1), the combination of *Triglochin* and *Schoenoplectus* seem to cause lower assimilation of some nutrient parameters. There may be some antagonism between the plants.

Most of these results are not statistically significant. While all planted tanks have, on average, more nitrate removal and reduction than the unplanted control tanks, only the *Schoenoplectus* planted tanks are
significantly different from the control tanks \( (p = 0.02) \). T tests between the means of other planted tank systems and the control produce \( p \) values of 0.08 and 0.30.

Table 5.15. Percentage reduction of nitrate over all trials. SE = Standard error of differences between the means of planted and unplanted tanks. \( p = 0.11 \) \( n = 3 \)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Control tanks</th>
<th>Triglochin tanks</th>
<th>Schoenoplectus tanks</th>
<th>Triglochin and Schoenoplectus tanks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62.9</td>
<td>75.0</td>
<td>74.3</td>
<td>63.0</td>
</tr>
<tr>
<td>2</td>
<td>72.3</td>
<td>76.5</td>
<td>73.2</td>
<td>65.6</td>
</tr>
<tr>
<td>3</td>
<td>77.8</td>
<td>79.0</td>
<td>90.6</td>
<td>77.6</td>
</tr>
<tr>
<td>4</td>
<td>68.8</td>
<td>70.9</td>
<td>81.3</td>
<td>72.7</td>
</tr>
<tr>
<td>5</td>
<td>76.4</td>
<td>73.2</td>
<td>75.0</td>
<td>81.4</td>
</tr>
<tr>
<td>6</td>
<td>68.6</td>
<td>75.1</td>
<td>83.5</td>
<td>78.6</td>
</tr>
<tr>
<td>Mean</td>
<td>71.1</td>
<td>74.9</td>
<td>79.6</td>
<td>73.2</td>
</tr>
<tr>
<td>Std Dev</td>
<td>5.1</td>
<td>2.5</td>
<td>6.2</td>
<td>6.8</td>
</tr>
</tbody>
</table>

As the data which was analysed were percentages, and ANOVA assumes a normal distribution of data for analysis, the nitrate data was transformed in a process outlined by Zar (1984). Individual values for each tank were changed via arcsine transformation, and then statistical tests, such as ANOVA and \( t \) tests, applied. Transformed data for both nitrate and ammonium can be found in the appendix.

The arcsine transformation of the nitrate data did not make much difference to \( p \) values. Untransformed data had a \( p = 0.0853 \) and transformed data \( p = 0.0838 \), and this author believes that data may not need to be transformed for valid results to be obtained.

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The effect of macrophytes, and the unplanted control, in nitrate removal can also be seen in Figures 5.3 and 5.4. Figure 5.3 shows that relationship between nitrogen loading and the amount of nitrate-nitrogen reduction by the tanks. There seems to be a direct, almost linear, relationship between the two, at these low loading rates. Regression analysis was undertaken and the results are found in Table 5.16.

![Graph showing nitrate-nitrogen load mg vs % reduction in nitrate removal](image)

Figure 5.3. The effect of nitrate-nitrogen loading on nitrate removal.

Table 5.16. Regression analysis for the effect of nitrogen load on nitrate removal. The 95% confidence intervals for the slope are shown.

<table>
<thead>
<tr>
<th>Tank system</th>
<th>Slope 'b'</th>
<th>y intercept (a)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Triglochin</em></td>
<td>$1.28 \pm 0.14$</td>
<td>2.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Schoenoplectus</em></td>
<td>$1.35 \pm 0.49$</td>
<td>-4.79</td>
<td>0.001</td>
</tr>
<tr>
<td>Tri. &amp; SV</td>
<td>$1.77 \pm 0.57$</td>
<td>-20.49</td>
<td>0.001</td>
</tr>
<tr>
<td>Control</td>
<td>$1.50 \pm 0.54$</td>
<td>-4.30</td>
<td>0.001</td>
</tr>
</tbody>
</table>
The low p values confirm that each line is not horizontal, and the slopes are similar (not significantly different as each falls within the 95% confidence interval of the other gradients). The equation for *Triglochin*, for example, would be:

\[
\% \text{ Nitrate reduction} = 2.63 + 1.28 \text{ nitrate-nitrogen load. (} y = b + ax \)
\]

To examine the general trend of planted and unplanted tanks in their ability to reduce nitrates in the greywater, the results of one typical trial are shown in Figure 5.4. Again, the differences between the various plants can be easily seen, with planted tanks having greater nitrate removal than the unplanted control tanks (but not statistically different as shown in Table 5.15), and tanks with *Schoenoplectus* producing higher removal rates than tanks with *Triglochin*. Even so, only a small amount (3 to 5%) of the nitrate seems to be taken up by plants.

![Figure 5.4. Nitrate removal for Trial 6. Total nitrate-nitrogen loading = 50 mg. Standard deviations are shown.](image)
5.3.4 Ammonium concentration

As with Investigation 2, there is a significant difference between the planted tanks and the control tanks (p = 0.00003). *Schoenoplectus* and the combined plant tanks have again greater utilisation or effect on ammonium concentration, significantly different from the control tanks (p = 0.0003, and 0.0001 respectively). Results for *Triglochin* could arguably be said to be significant as the difference between these tanks and the control tanks is between two to three times the standard error, but p = 0.053. While there appears to be some antagonism between plants for nitrate reduction (see 5.3.3), there doesn’t seem to be antagonism between *Triglochin* and *Schoenoplectus* when ammonium reduction is concerned.

Table 5.17 lists the average ammonium reduction (as %) in all systems. It appears that about 64% of ammonium is lost from control tanks, mainly by nitrification as the pH was not high enough for appreciable amounts of volatilisation to occur. The planted tanks do contribute to ammonium reduction, either by direct absorption and assimilation or by providing further sites for nitrifying bacteria to convert ammonium to nitrate.

Again, as with nitrate, percentage data were transformed (arcsine) before ANOVA and t tests were applied. The *Triglochin* tank was re-analysed and the results for p did not change very much. Untransformed data had a p = 0.053 and transformed data p = 0.051. This re-affirms this author’s contention that there is no need to transform all data before statistical tests are applied.
Table 5.17. Percentage reduction of ammonium over all trials. SE = Standard error of differences between the means of planted and unplanted tanks. p = 0.00003 (ANOVA)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Control tanks</th>
<th>Triglochin tanks</th>
<th>Schoenoplectus tanks</th>
<th>Triglochin and Schoenoplectus tanks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>82.0</td>
<td>89.8</td>
<td>96.9</td>
<td>93.0</td>
</tr>
<tr>
<td>2</td>
<td>79.4</td>
<td>88.0</td>
<td>98.1</td>
<td>94.0</td>
</tr>
<tr>
<td>3</td>
<td>75.8</td>
<td>85.5</td>
<td>99.6</td>
<td>94.6</td>
</tr>
<tr>
<td>4</td>
<td>43.7</td>
<td>68.8</td>
<td>100.0</td>
<td>90.7</td>
</tr>
<tr>
<td>5</td>
<td>62.7</td>
<td>76.4</td>
<td>100.0</td>
<td>96.0</td>
</tr>
<tr>
<td>6</td>
<td>42.4</td>
<td>67.5</td>
<td>100.0</td>
<td>98.3</td>
</tr>
<tr>
<td>Mean</td>
<td>64.3</td>
<td>79.0</td>
<td>99.1</td>
<td>94.4</td>
</tr>
<tr>
<td>Std Dev</td>
<td>16.2</td>
<td>8.7</td>
<td>1.2</td>
<td>2.3</td>
</tr>
<tr>
<td>SE</td>
<td>-</td>
<td>4.2</td>
<td>3.8</td>
<td>3.8</td>
</tr>
</tbody>
</table>

As discussed in Investigation 2, the wide range of results from each of the trials could be partly due to the daily temperature variation that occurred throughout the experiment. Fluctuations in water temperature and changes in pH may affect the volatilisation of ammonia (as discussed in the last investigation) and activity of microbes. Even so, ammonium removal rates were generally very high and this is seen in Figure 5.5 which shows the results of a typical trial. Liu et al. (2000), Tanner (1996) and Sikora (1995) also found that ammonium reductions in their planted wetlands were very high (>90%).

At low loading rates (<100 mg/tank), there is a direct correlation with ammonium removal. Regression analysis was performed and the results are shown in Table 5.18. Figure 5.6 clearly shows the ability of Schoenoplectus and Triglochin to remove ammonium from the wastewater.
Figure 5.5. Ammonium-nitrogen removal for Trial 2. Total loading = 96 mg. Standard deviations are shown.

Figure 5.6. The effect of ammonium-nitrogen loading on ammonium removal.
Figure 5.5. Ammonium-nitrogen removal for Trial 2. Total loading = 96 mg. Standard deviations are shown.

Figure 5.6. The effect of ammonium-nitrogen loading on ammonium removal.
Table 5.18. Regression analysis for the effect of ammonium-nitrogen load on ammonium removal. The 95% confidence intervals for the slope are shown.

<table>
<thead>
<tr>
<th>Tank system</th>
<th>Slope ('b')</th>
<th>y intercept (a)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglochin</td>
<td>1.02 ± 0.23</td>
<td>8.73</td>
<td>0.0008</td>
</tr>
<tr>
<td>Schoenoplectus</td>
<td>1.02 ± 0.94</td>
<td>-0.92</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tri. &amp; SV</td>
<td>1.05 ± 0.10</td>
<td>0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Control</td>
<td>1.04 ± 0.36</td>
<td>14.73</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Table 5.18 and Figure 5.6 show that the gradient of curves for each tank system are similar, and that the equation follows the typical \( y = b + ax \).

The equation for *Triglochin huegelii*, for example, would be:

\[
\% \text{ Ammonium removal} = 8.73 + 1.02 \text{ ammonium-nitrogen load}
\]

We need to measure total N and total P in plant tissue over the course of the investigation to determine the amount of nutrient removal by plants themselves. Furthermore, there was a need to further examine the effect of substrate-only conditions compared to pond conditions. Both of these ideas were considered in Investigation 4.

### 5.3.5 Phosphate concentration

Table 5.19 shows that all tanks had exceptionally high phosphate retention. In comparison, the pond system in previous experiments had much less phosphate assimilated or retained (e.g. 47 - 91% in Table 5.11) - possibly due to some orthophosphate in solution passing out of the tanks. Although the tank systems had very similar percentages for phosphate retention/reduction these results are significantly different from each

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other, as seen by the \( p \) values listed in the table which compare planted tanks with the control (variance between trials for any one tank system is low). This is the first investigation where phosphate changes in planted tanks were statistically different from the unplanted tanks.

Table 5.19. Percentage reduction of phosphate over all trials. SE = Standard error of differences between the means of planted and unplanted tanks. \( p \) (all tanks) = 0.009

<table>
<thead>
<tr>
<th>Trial</th>
<th>Control tanks</th>
<th>Triglochin tanks</th>
<th>Schoenoplectus tanks</th>
<th>Triglochin and Schoenoplectus tanks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99.3</td>
<td>99.6</td>
<td>99.8</td>
<td>99.5</td>
</tr>
<tr>
<td>2</td>
<td>98.2</td>
<td>99.4</td>
<td>99.4</td>
<td>98.7</td>
</tr>
<tr>
<td>3</td>
<td>99.7</td>
<td>99.8</td>
<td>99.9</td>
<td>99.9</td>
</tr>
<tr>
<td>4</td>
<td>98.9</td>
<td>99.7</td>
<td>99.8</td>
<td>99.8</td>
</tr>
<tr>
<td>5</td>
<td>98.9</td>
<td>99.6</td>
<td>99.6</td>
<td>99.8</td>
</tr>
<tr>
<td>6</td>
<td>99.2</td>
<td>99.6</td>
<td>99.8</td>
<td>99.8</td>
</tr>
<tr>
<td>Mean</td>
<td>99.0</td>
<td>99.6</td>
<td>99.7</td>
<td>99.6</td>
</tr>
<tr>
<td>Std Dev</td>
<td>0.5</td>
<td>0.1</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>SE</td>
<td>-</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>( p )</td>
<td>0.0007</td>
<td>0.0007</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

5.3.6 Correlation of data

To examine the overall effect of tank systems on nutrient removal, and to see if there were any discrepancies or differences in the reduction of particular nutrients, data was pooled and the correlation of one nutrient with another was determined. The correlation co-efficients are shown in Table 5.20, and were calculated with the statistical package in Microsoft Excel.
In Table 5.20, correlation co-efficients are shown for combinations of nitrate, ammonium and phosphate percentage removal. The table indicates that there is strong correlation between nitrate and both ammonium and phosphate removal/reduction and an even higher correlation between ammonium and phosphate reduction (0.081).

Table 5.20. Correlation co-efficients for nutrient removal. Data for percentage nitrate, ammonium and phosphate reductions in tanks were used.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Nitrate</th>
<th>Ammonium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium</td>
<td>0.724</td>
<td>-</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.760</td>
<td>0.801</td>
</tr>
</tbody>
</table>

These correlation co-efficients suggest that whenever nitrate, for example, is able to be reduced in the tank systems, other mechanisms are generally present to reduce ammonium and phosphate as well. These mechanisms, as already discussed, may involve some combination of physical, chemical and biological processes, including plant uptake and the range of bacteria and other microbes acting on these nutrients.
5.4 A Comparison Between Pond and Substrate-only Conditions (Investigations 2 and 3)

Investigations 2 and 3 were conducted under similar conditions, using the same plants, tank combinations and hydraulic residence time (5 days), and similar environmental factors such as temperature and pH. The volume of greywater added to each tank in each investigation, however, was different. Fifty litres of greywater were added in Investigation 2 but only 20 L were added in Investigation 3 (enough to maintain the water level just below the surface). This still permitted a comparison between the substrate-only investigation and the pond system of Investigation 2.

5.4.1 Biochemical Oxygen Demand (BOD)

Table 5.21 compares the substrate to the pond system and lists the percentage of BOD reduced by each group of tanks.

Table 5.21. Percentage reduction in BOD by each system. Standard deviations are shown. SE = Standard error of differences between the means of planted and unplanted tanks. p (substrate) = 0.60, p (pond) = 0.63 (ANOVA). n = 3

<table>
<thead>
<tr>
<th>System</th>
<th>Control tanks</th>
<th>Triglochin tanks</th>
<th>SE</th>
<th>Schoeno. tanks</th>
<th>SE</th>
<th>Triglochin &amp; Sch. tanks</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>83 ± 14</td>
<td>88 ± 13</td>
<td>4.5</td>
<td>93 ± 12</td>
<td>4.5</td>
<td>92 ± 10</td>
<td>4.1</td>
</tr>
<tr>
<td>Pond</td>
<td>89 ± 3</td>
<td>86 ± 3</td>
<td>2.4</td>
<td>88 ± 1</td>
<td>1.8</td>
<td>90 ± 2</td>
<td>2.0</td>
</tr>
</tbody>
</table>

BOD is generally greatly reduced in all tanks, with the range of reduction being 83 to 93% over both investigations. In the sub-surface flow system all planted tanks have, on average, greater reduction in BOD than the control tanks, with Schoenoplectus higher than Triglochin.
In the pond system, *Schoenoplectus* has slightly greater reduction in BOD than *Triglochin*, with combined planted tanks higher still, even though none of these comparisons are statistically significant, as shown by p values listed in Table 5.21.

### 5.4.2 Nitrate concentration

From Table 5.22, all planted tanks, except *Schoenoplectus* in the pond, have, on average, more nitrate removal and reduction than the unplanted control tanks. In a pond, *Triglochin* had greater nitrate removal (by 3%) than the control tanks, which were themselves better than *Schoenoplectus* tanks. When only considering root zones, *Schoenoplectus* was 8% and *Triglochin* 4% greater than the control tanks. However, these results are not statistically significant because of the small number of tanks and trials. One-way analysis of variance testing produced p values as listed in the table below.

Table 5.22. Percentage reduction of nitrate by each system. Standard deviations are shown. SE = Standard error of differences between the means of planted and unplanted tanks. \( p \) (substrate) = 0.11, \( p \) (pond) = 0.89. \( n = 3 \)

<table>
<thead>
<tr>
<th>System</th>
<th>Control tanks</th>
<th>Triglochin tanks</th>
<th>SE</th>
<th>Schoeno. tanks</th>
<th>SE</th>
<th>Triglochin &amp; Sch. tanks</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>71 ± 5</td>
<td>75 ± 2.5</td>
<td>3.0</td>
<td>79 ± 6</td>
<td>4.5</td>
<td>73 ± 6.5</td>
<td>4.7</td>
</tr>
<tr>
<td>Pond</td>
<td>70 ± 7</td>
<td>73 ± 15</td>
<td>4.0</td>
<td>64 ± 10</td>
<td>6.7</td>
<td>71 ± 21</td>
<td>5.3</td>
</tr>
</tbody>
</table>
5.4.3 Ammonium concentration

In both studies, there is significant difference between some of the planted tanks and the control tanks. *Schoenoplectus* and the combined plant tanks have a significantly greater utilisation or effect on ammonium concentration than *Triglochin* tanks. It could be that these plants provide suitable conditions for bacterial action around the root zone.

Table 5.23. Percentage reduction of ammonium by each system. Standard deviations are shown. SE = Standard error of differences between the means of planted and unplanted tanks. p (substrate) = 0.0004, p (pond) = 0.008 (ANOVA). n = 3

<table>
<thead>
<tr>
<th>System</th>
<th>Control tanks</th>
<th>Triglochin tanks</th>
<th>SE</th>
<th>Schoen. tanks</th>
<th>SE</th>
<th>Triglochin &amp; Sch. tanks</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>64 ± 16</td>
<td>77 ± 10</td>
<td>4.5</td>
<td>99 ± 1</td>
<td>3.8</td>
<td>94 ± 2</td>
<td>3.8</td>
</tr>
<tr>
<td>Pond</td>
<td>76 ± 6</td>
<td>75 ± 3</td>
<td>3.9</td>
<td>90 ± 1</td>
<td>3.5</td>
<td>89 ± 2</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Results listed in Table 5.23 indicate that in the sub-surface flow study, ammonium reduction in *Schoenoplectus* was 54% greater than that of the control tanks. Even *Triglochin* was 20% better than the control tanks, whereas in the pond system the results were similar.

5.4.4 Phosphate concentration

All tanks in the substrate-only experiment had exceptionally high phosphate retention, with planted tanks being statistically different from the unplanted tanks (p = 0.009, ANOVA). In comparison, the pond system study had much less phosphate assimilated - possibly due to some orthophosphate in solution passing out of the tanks - and the results are not significant (p = 0.24).
Table 5.24. Percentage reduction of phosphate by each system. Standard deviations are shown. SE = Standard error of differences between the means of planted and unplanted tanks.  p (substrate) = 0.009, p (pond) = 0.24 (ANOVA).  n = 3

<table>
<thead>
<tr>
<th>System</th>
<th>Control tanks</th>
<th>Triglochin tanks</th>
<th>SE</th>
<th>Schoeno. tanks</th>
<th>SE</th>
<th>Triglochin &amp; Sch. tanks</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>99.0 ± 0.5</td>
<td>99.6 ± 0.1</td>
<td>0.1</td>
<td>99.7 ± 0.2</td>
<td>0.1</td>
<td>99.6 ± 0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Pond</td>
<td>87.3 ± 3</td>
<td>71.3 ± 17</td>
<td>10.0</td>
<td>86.0 ± 1</td>
<td>2.0</td>
<td>89.3 ± 4</td>
<td>3.0</td>
</tr>
</tbody>
</table>

It appears that plants do not assimilate or absorb much phosphate - rather it is absorbed onto substrate particles. Investigation 5, discussed later, shows that the concentrations of phosphate in many wetland species fluctuate and varies from plant to plant and within plant organs.

Until now, little consideration was given to the total biomass of the plant. This led to the next experiment which was conducted solely with *Triglochin huegelii*.

5.5 Investigation 4: Comparison of Substrate-only and Pond Systems in Tanks Planted with *Triglochin huegelii*

This investigation built upon the previous ones by focusing only on *Triglochin huegelii* in all tanks and examining the effect of retention time on nutrient removal ability.

The additional hypotheses tested in this investigation were: “Long retention time and/or low hydraulic loading will increase nutrient-stripping efficiency” and “That absorption of nutrients can occur through the leaves of *Triglochin huegelii*”.

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5.5.1 Total nitrogen in plant samples

The range of total N measured in plant parts was from 0.33 to 9.13 mg/g. The highest concentrations were generally found in leaves with tubers having the lowest concentration (but with a greater range of values than roots). Leaves ranged from 0.75 to 9.13 mg/g with an average of 3.68, roots 0.35 to 5.23 (av. 2.17 mg/g) and tubers 0.33 to 8.4 (av. 2.3 mg/g). Similar ranges of nitrogen concentration were obtained by Greenway and Woolley (1999) in their study of a large number of macrophytes, including submergents, used to treat municipal wastewater. Tubers are storage organs so changes in nutrient levels is expected as the plant translocates or stores nutrients as required to and from growing (e.g. leaf) regions.

Tables 5.25 and 5.26 list the overall change in total N levels after consideration of the dry weights of plant samples. The total N value was calculated by multiplying the N content in a particular plant part by its initial or final dry weight and then cumulatively adding these. Thus, the mean (average) of the amount of nitrogen in each of the plant parts was not calculated. Rather, the sum total of N in all leaves, roots or tubers from the tanks was calculated. Standard deviations and t tests were conducted in all data in the following tables. However, it must be noted that at the start of the investigation, plants were not uniform in weight and size, and only approximate wet weights were taken. The initial dry weights were calculated from the estimated ratio of wet weight to dry weight as discussed in Section 4.5. Thus standard deviations were high. For
example, from Table 5.26, initial and final N levels in leaves were 36.3 ± 26.2 and 72.9 ± 102.4 respectively, with p = 0.07.

Individual weights of all plants were taken and an initial and final analysis of N and P concentrations was determined. Generally, there were increases in plant tissue (size) and N and P levels in plants as indicated by the results tables. The last column in Table 5.26 shows by how much the N level has changed in each plant part, with greatest nitrogen increases in leaves and the least increases, on average, in roots.

Table 5.25. Average increase in total nitrogen tissue content during the investigation. n = 18

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Initial N level (mg)</th>
<th>Final N level (mg)</th>
<th>Difference (increase) (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>654</td>
<td>1312</td>
<td>658</td>
</tr>
<tr>
<td>Root</td>
<td>445</td>
<td>739</td>
<td>294</td>
</tr>
<tr>
<td>Tuber</td>
<td>527</td>
<td>926</td>
<td>399</td>
</tr>
</tbody>
</table>

Table 5.26. Average changes in nitrogen content per plant. n = 18

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Average N level - initial/plant (mg)</th>
<th>Average final N level/plant (mg)</th>
<th>Factor increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>36.3</td>
<td>72.9</td>
<td>2.01</td>
</tr>
<tr>
<td>Root</td>
<td>24.7</td>
<td>41.1</td>
<td>1.66</td>
</tr>
<tr>
<td>Tuber</td>
<td>29.3</td>
<td>51.4</td>
<td>1.76</td>
</tr>
</tbody>
</table>

Table 5.27 examines the three different experimental trials. The total nitrogen gain was calculated by the multiplying the N concentration changes by the dry weight and then cumulatively adding each plant in each tank system. The 10 L tanks, those having the largest hydraulic loading, had by far the greatest total nitrogen gain, with generally the
highest concentration changes in plant parts. The 3 L and 5 L tanks had both the lowest total N gain and the lowest N concentration increases. Overall, all planted experimental tanks registered increases in total nitrogen.

Table 5.27. Total nitrogen gain per tank system (mg).

<table>
<thead>
<tr>
<th>Experimental set-up and tank numbers</th>
<th>Total nitrogen gain in plants (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 L - 1, 7, 10</td>
<td>218</td>
</tr>
<tr>
<td>5 L - 2, 6, 9</td>
<td>230</td>
</tr>
<tr>
<td>10 L - 3, 5, 12</td>
<td>894</td>
</tr>
</tbody>
</table>

5.5.2 Total phosphorus in plant samples

Most plant parts increased their phosphorus content, especially the leaves and roots. Phosphorus seems to be stored in below-ground parts as the highest levels of total P (mg/g dry matter) were found in tubers, then roots, with leaves the lowest. The range of total P measured in plant parts was from 8.6 to 44 mg/g.

Table 5.28. Average changes in P concentration in plant tissues. Standard deviations are shown.

<table>
<thead>
<tr>
<th>Set-up</th>
<th>Leaf</th>
<th>Root</th>
<th>Tuber</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial conc. mg/g</td>
<td>Final conc. mg/g</td>
<td>Initial conc. mg/g</td>
</tr>
<tr>
<td>3 L</td>
<td>16.4 ± 2.2</td>
<td>16.3 ± 3.2 ↓</td>
<td>17.6 ± 4.2</td>
</tr>
<tr>
<td>5 L</td>
<td>15.3 ± 2.9</td>
<td>12.6 ± 1.3 ↓</td>
<td>27.0 ± 4.9</td>
</tr>
<tr>
<td>10 L</td>
<td>16.1 ± 4.1</td>
<td>14.3 ± 3.2 ↓</td>
<td>19.1 ± 2.4</td>
</tr>
</tbody>
</table>
Table 5.28 shows the changes in average P concentration levels in each of the three experimental set-ups. It appears that there is a decrease in total P in most tanks, as shown by the position of arrows indicating either an increase or decrease in levels. While there was a general decrease in concentration there is a definite increase in the total amount of phosphorus in the biomass. This was due to the increase in size and mass of the plants during the investigation. Again, due to the range of plant sizes and concentrations of total P in plant tissue, standard deviations are high, with $p > 0.05$ for all t tests comparing means for each plant part and tank system.

Table 5.29 shows the overall total P changes for each plant part and for each experimental tank system, with almost universal increases in total P levels. The final total P level for tubers in the 10 L set-up has decreased, but most of this was probably translocated to both the leaves and roots which increased their amounts. Again, standard deviations and statistical tests were not performed on this data, as the amount of total phosphorus was calculated by the multiplying the P concentration changes by the dry weight and then cumulatively adding each plant in each tank system.

Tanner (1996) found that only up to 10% of the N and 13% of the P removal was attributed to his plantings of *Schoenoplectus validus*. These investigations with *Triglochin huegeli* support these types of figures, although earlier studies (Investigations 1 and 2) suggest only about 5%
assimilation for nitrogen and 10% for phosphorus. This implies that plants, while mooted by Breen (1990) and Rogers et al. (1991) as potential sinks of high levels of nutrients which often pass into waterways, do not have a major role in the total reduction of nutrients.

Table 5.29. Average changes in total P storage in plant biomass.

<table>
<thead>
<tr>
<th>Set-up</th>
<th>Leaf Initial total P mg</th>
<th>Leaf Final total P mg</th>
<th>Root Initial total P mg</th>
<th>Root Final total P mg</th>
<th>Tuber Initial total P mg</th>
<th>Tuber Final total P mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 L</td>
<td>136</td>
<td>158 ↑</td>
<td>149</td>
<td>232 ↑</td>
<td>226</td>
<td>256 ↑</td>
</tr>
<tr>
<td>5 L</td>
<td>183</td>
<td>205 ↑</td>
<td>321</td>
<td>377 ↑</td>
<td>342</td>
<td>473 ↑</td>
</tr>
<tr>
<td>10 L</td>
<td>238</td>
<td>303 ↑</td>
<td>394</td>
<td>476 ↑</td>
<td>675</td>
<td>612 ↓</td>
</tr>
</tbody>
</table>

Some plants may increase growth to maximise production while others tended towards a more conservative strategy of nutrient accumulation. In this investigation leaves were occasionally replaced by new ones, and in many tanks both the number of leaves and leaf size increased. Table 5.36 lists some of the observations on plant size, and issues about biomass changes are discussed in Section 5.5.6.

Further investigations will need to be undertaken to see if Triglochin huegelii had apparent increased efficiency of nutrient use or it simply was able to store additional nutrients when required.

5.5.3 Nitrate concentration changes in water samples

Data on nitrate-nitrogen differences from the greywater addition and the subsequent comparison between total input and output also confirmed
nitrogen gain in the plants. The results summary, shown in Table 5.30, had a p value of 0.014 (ANOVA), suggesting significant differences in each experimental tank set-up.

All of the planted tanks had a much greater N difference than the unplanted control tanks. It would be expected that tanks with the longer retention time (5 L = 20 days) yield the greatest differences, but both sets of tanks with only 10 days retention time appear to have the higher values. However, it is necessary to calculate the proportion of nitrate that is retained in the tanks compared to the loading rate. When this is done the figures shown in the last column are obtained. Here, proportionally more nitrate is retained in the 3 L tanks (substrate-only), followed by the 5 L then 10 L tanks. Again, all planted tanks retain much more nitrate than the unplanted, control tanks.

Two observations can be made about the average nitrate-nitrogen differences in the control tanks. Firstly, the large standard deviation is due to a wide range of values for these tanks. Secondly, the low average nitrate-nitrogen difference is possibly due to the different substrate used for this investigation. The first three investigations used a mainly stone substrate, while coarse sand was used here. It is possible that bacteria levels did not build up sufficiently to have any effect on nutrient removal, either as the sand was deep mined and may have been reasonably sterile before use in the tanks or appropriate bacteria were in low numbers in the greywater samples, or both.
Table 5.30. Comparison of total nitrate-nitrogen gain for each experimental set-up. Standard deviations are also shown. Standard error of means = 48. Standard error of proportions = 0.04.  p = 0.014

<table>
<thead>
<tr>
<th>Experimental set-up</th>
<th>Average nitrate-nitrogen difference between input and output (mg)</th>
<th>Proportion of nitrate-nitrogen retained in tanks</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 L</td>
<td>250 ± 32</td>
<td>0.64 ± 0.08</td>
</tr>
<tr>
<td>5 L</td>
<td>173 ± 23</td>
<td>0.26 ± 0.06</td>
</tr>
<tr>
<td>10 L</td>
<td>273 ± 152</td>
<td>0.21 ± 0.12</td>
</tr>
<tr>
<td>Control</td>
<td>1 ± 49</td>
<td>0.0008 ± 0.0026</td>
</tr>
</tbody>
</table>

While it is true that some ammonium is oxidised to nitrate, some nitrate may be also reduced to ammonium (Cooke, 1994), so we would expect a balance between what is formed and what is reduced.

5.5.4 Ammonium concentration changes in water samples

Data shown in Table 5.31 has similar trends to that of nitrate-nitrogen. However, it appears that both the 3 L and 5 L tanks have, on average, greater differences than the 10 L tanks. The results are significant, with a p value < 0.001 (ANOVA) as the ammonium-nitrogen retained in all planted tanks is much greater than the control and often between each other. It could be that these submergent plants provide suitable conditions for bacterial action around the root zone.

The control tanks have a negative average difference, indicating a net average loss of ammonium-nitrogen from the system. However, this value is not significantly different from 0 and is therefore due to experimental error or sampling variation. It was found that, after analysis of the sand
medium, there were net losses of nitrogen from some of the tanks, including all of the control tanks. Initial and final nitrogen levels in the sand were small, but these still had a minor effect on the overall results. When the proportion of ammonium-nitrogen retained in the tanks is considered, the same trend as before is found; that is, the 3L tanks retain much more than the 5 L and then the 10 L tanks with the control the lowest of all set-ups.

Table 5.31. Comparison of total ammonium-nitrogen gain for each experimental set-up. Standard deviations are also shown. Standard error of means = 13. Standard error of proportions = 0.06. $p = 0.00036$

<table>
<thead>
<tr>
<th>Experimental set-up</th>
<th>Average ammonium-nitrogen difference between input and output (mg)</th>
<th>Proportion of ammonium-nitrogen retained in tanks</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 L</td>
<td>$135 \pm 2$</td>
<td>$0.94 \pm 0.01$</td>
</tr>
<tr>
<td>5 L</td>
<td>$105 \pm 28$</td>
<td>$0.44 \pm 0.12$</td>
</tr>
<tr>
<td>10 L</td>
<td>$88 \pm 32$</td>
<td>$0.18 \pm 0.07$</td>
</tr>
<tr>
<td>Control</td>
<td>$-7 \pm 16$</td>
<td>$-0.014 \pm 0.006$</td>
</tr>
</tbody>
</table>

The higher level of ammonium removal in the substrate-only tanks (3 L) may be due to the lower water level which would affect the amount of aeration and hence nitrification and denitrification which occurs in the system. It would be expected that with higher levels of oxygen there would be an increase in nitrification of ammonium to nitrate or other forms of oxidised nitrogen.

When the total input and output data for both nitrate and ammonium are combined and compared, an approximate mass balance can be determined. The totals for each tank and treatment system are simply
added together, and no distinction between the amount of nitrate compared to the amount of ammonium utilised by plants is made. No data about the proportion of nitrate to ammonium that plants take up was collected for this investigation. The assumption used for the calculations in Table 5.32 is that equal amounts of nitrate and ammonium are absorbed by plants. The total Input is derived from levels of nitrate and ammonium added in greywater and the small amounts that were measured in the tap water, which was added for the last 40 days. Typically, the levels in tapwater were up to 1 mg/L nitrate, 0.02 mg/L ammonium and up to 0.1 mg/L phosphate.

Table 5.32. Nitrogen balance in each tank system. Standard deviations for the measured output are also shown. Each tank for a particular set-up had the same input.

<table>
<thead>
<tr>
<th>Set-up</th>
<th>Input (mgN)</th>
<th>Output (mgN)</th>
<th>N retained in tanks (mg)</th>
<th>% retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 L</td>
<td>1166</td>
<td>261 ± 17</td>
<td>905</td>
<td>78</td>
</tr>
<tr>
<td>5 L</td>
<td>2417</td>
<td>1825 ± 238</td>
<td>592</td>
<td>24</td>
</tr>
<tr>
<td>10 L</td>
<td>4920</td>
<td>4187 ± 181</td>
<td>733</td>
<td>15</td>
</tr>
<tr>
<td>Control</td>
<td>4925</td>
<td>4761 ± 141</td>
<td>164</td>
<td>3</td>
</tr>
</tbody>
</table>

If the amount retained in the control tanks is deducted from the planted tanks then the percentage retained reduces to values of 64%, 18% and 11% respectively. Assuming that the value for the control tanks represents denitrification and other processes that reduce nitrogen in the system, then it seems that plants, and/or microbiota associated with plants, are responsible for a reasonable amount of nitrogen removal. The data in Table 5.32 again confirms that substrate-only tanks remove proportionally
more nitrogen than pond tanks, with tanks having the longest retention times being able to remove more nitrogen.

Finally, when the nitrogen gain by plants (Table 5.27) is compared to nitrogen gain by each tank system (Table 5.32), it appears that plants do have a significant impact on nitrogen removal, as shown in Table 5.33. Again, statistical analysis cannot be performed as these values are not means but are cumulative totals of measurements from each tank system. However, more nitrogen appears to be gained by plants in the 10 L set-up than what is available. This discrepancy could be due to several factors, such as N changes in the substrate (there was a net loss of nitrogen as determined by soil analysis) or a different N pathway involving nitrate, ammonium and the plants (the assumption for these calculations was based on equal absorption on nitrate and ammonium by plants).

Table 5.33. Proportion on nitrogen taken up by plants.

<table>
<thead>
<tr>
<th>Tank system</th>
<th>N gain by plants (mg)</th>
<th>N retained in tanks (mg)</th>
<th>Proportion of N gained by plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 L</td>
<td>218</td>
<td>905</td>
<td>0.24</td>
</tr>
<tr>
<td>5 L</td>
<td>239</td>
<td>582</td>
<td>0.40</td>
</tr>
<tr>
<td>10 L</td>
<td>894</td>
<td>733</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Whatever the reason, plants in the 10 L tanks had the greatest growth and nutrient absorption, and the discrepancy in the above data could simply be within normal experimental error or due to other factors, such as
differences in the rates of nitrate and ammonium assimilation. Further investigation is needed to clarify this.

5.5.5 Phosphate concentration changes in water samples

All tanks in the substrate-only experiment (3 L tanks) had exceptionally high phosphate retention. In comparison, the pond system study had much less phosphate assimilated - which was demonstrated by greater phosphate amounts being recorded in solution passing out of the tanks. Similar results were obtained in earlier investigations (2 and 3).

The results shown in Table 5.34 of phosphorus changes in the wastewater are inconclusive. While the results are significant, with a p value of 0.0098 (ANOVA), the control set-up seems to retain more phosphorus than the 10 L tanks. As with Investigation 1, microalgal mats may have influenced phosphate retention. Both the 3 L and 5 L tanks retain more phosphorus than the other set-ups, with the 10 L tanks having the lowest retention.

Table 5.34. Comparison of total phosphorus gain for each experimental set-up. Standard deviations are also shown. Standard error of means = 90. Standard error of proportions = 0.02. p = 0.0098

<table>
<thead>
<tr>
<th>Experimental set-up</th>
<th>Average total phosphorus difference between input and output (mg)</th>
<th>Proportion of total P retained in tanks</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 L</td>
<td>1400 ± 42</td>
<td>0.96 ± 0.03</td>
</tr>
<tr>
<td>5 L</td>
<td>2245 ± 79</td>
<td>0.92 ± 0.04</td>
</tr>
<tr>
<td>10 L</td>
<td>4034 ± 55</td>
<td>0.83 ± 0.01</td>
</tr>
<tr>
<td>Control</td>
<td>4267 ± 208</td>
<td>0.87 ± 0.04</td>
</tr>
</tbody>
</table>

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It appears that plants do not assimilate or absorb much phosphate - rather it is absorbed onto substrate particles, as shown by the high level of phosphate retained in the control tanks. When a comparison between the phosphorus levels retained in the 3L and 5L tanks (Table 5.34) was made to that taken into plant tissue (Table 5.29) it appears that about 10 to 11% of the phosphorus is incorporated in plant tissue. Lantzke et al. (1998) suggest that orthophosphate removal occurs through three processes, listed in decreasing order - sorption to the gravel substratum, conversion to complex phosphorus compounds and uptake by plant roots. Furthermore, phosphorus removal also depends on the acidity and oxidation/reduction state of the solution (Kadlec and Knight, 1996). Other studies (Kadlec and Knight, 1996, Greenway, 1997, and Greenway and Woolley, 1999), including Investigation 5 which is discussed next, have shown that the levels of phosphate in many wetland species fluctuate and varies from plant to plant and within plant organs.

5.5.6 Biomass studies

Summarising the results for planted and unplanted tanks with the same hydraulic loading and retention time (i.e. control and 10 L tanks) leads to Table 5.35. This table lists the total N or total P input from the greywater during the course of the investigation, the amount retained/removed in the tanks and the amount assimilated into new plant tissue. The amount in plant tissue was determined from dry mass measurements of sample plants and the nutrient concentrations in the leaves, tubers and roots.
Initial dry mass was estimated from crude wet weights of the plants before the investigation began. Only approximate wet weights were measured due to the potential of damage to plant tissue as they were planted in the tanks. The final wet weights and final dry weights are more accurate as plants were sacrificed at the end of the experiment.

It is clear from the table that much of the total nitrogen retained in the tanks was incorporated into plant tissue. Proportionally much less phosphorus is assimilated into plant tissue. The data suggests that while phosphorus is retained in some way, little is taken up and incorporated into new plant growth.

Table 5.35. Comparison of total nitrogen and total phosphorus input and biomass gain in plants. Standard deviations are also shown.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Greywater input (mg/tank)</th>
<th>Amount retained in tanks (mg/tank)</th>
<th>Amount in plant tissue (mg/tank)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Planted 10L</td>
</tr>
<tr>
<td>Total N</td>
<td>1733</td>
<td>-6 ± 50</td>
<td>362 ± 150</td>
</tr>
<tr>
<td>Total P</td>
<td>4882</td>
<td>4267 ± 208</td>
<td>4034 ± 55</td>
</tr>
</tbody>
</table>

Further observations were made on the growth and biomass changes which occurred in the tanks. The largest two plants were chosen in each tank. The number of leaves and the maximum length was recorded. A cumulative total was calculated to provide an indication of overall change. For example, the number of leaves in 3L substrate before experiment is listed as 24, but each plant had twelve leaves, and after experiment as 27 - the two plants had either fifteen or twelve leaves. These totals are shown in Table 5.36.
Table 5.36. Changes observed in plant size.

<table>
<thead>
<tr>
<th>Tank system</th>
<th>Tank no.</th>
<th>Before exp.</th>
<th>After exp.</th>
<th>Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. leaves</td>
<td>Max length</td>
<td>No. leaves</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(cm)</td>
<td>(cm)</td>
<td></td>
</tr>
<tr>
<td>3 L substrate</td>
<td>1</td>
<td>24</td>
<td>150</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>10</td>
<td>145</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>13</td>
<td>170</td>
<td>12</td>
</tr>
<tr>
<td>5 L pond</td>
<td>2</td>
<td>15</td>
<td>160</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>11</td>
<td>165</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>14</td>
<td>195</td>
<td>22</td>
</tr>
<tr>
<td>10 L pond</td>
<td>3</td>
<td>15</td>
<td>160</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>28</td>
<td>160</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>12</td>
<td>180</td>
<td>26</td>
</tr>
</tbody>
</table>

Table 5.36 shows that, in most cases, the number of leaves per plant increases, up to two times, while the leaf size decreases in substrate-only conditions and varies in pond conditions. Most of the plants in pond conditions increased leaf length, but where there were decreases in length this was off-set by large increases in leaf number.

Productivity was also calculated for *Triglochin huegelii* from changes in dry weight biomass and estimating plant density from the tank studies. Each tank was 0.22 m² and four plants were grown in each tank. This means that at this density, about 18 - 20 plants could be grown per square metre. The maximum dry weight biomass increase for plants in the substrate and pond systems is shown in Table 5.37. The productivity increase (biomass gain) was calculated by finding the average dry weight increase per plant and multiplying this by 18 (estimated number of plants per square metre) to obtain biomass/m² (grams dry weight/m²).
Table 5.37. Productivity and biomass changes in *Triglochin huegelii*.

<table>
<thead>
<tr>
<th>Tank system</th>
<th>Final biomass gdwm(^2)</th>
<th>Gain in biomass gdwm(^2)</th>
<th>TN gain (mg)</th>
<th>TP gain (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 L substrate</td>
<td>162</td>
<td>24</td>
<td>218</td>
<td>135</td>
</tr>
<tr>
<td>5 L pond</td>
<td>238</td>
<td>76</td>
<td>299</td>
<td>299</td>
</tr>
<tr>
<td>10 L pond</td>
<td>446</td>
<td>52</td>
<td>894</td>
<td>432</td>
</tr>
</tbody>
</table>

Table 5.37 also lists the TN and TP gains by these tanks systems, as already listed in Table 5.27 and derived from Table 5.28 respectively. By comparing one tank system with another, it again shows that *Triglochin* performs better in pond systems, having the greatest biomass gains and TN and TP increases.

To calculate the potential of productivity for *Triglochin huegelii*, the largest dry weight increase for any plant during the four month study was 6.46 g (about 150 g wet weight). This corresponds to about 130 gdwm\(^2\) growth or about 1 gdwm\(^2\)d\(^{-1}\). By way of comparison, Liu (2000) quotes a range of 180 to 2000 gdwm\(^2\) for several wetland macrophytes, and Tanner (1996) a range of 300 to 7400 gdwm\(^2\) for wetland plants he used in his study. The results for *Triglochin* are low, but this is only an estimate of growth per day. The largest plant collected at the end of this investigation was about 1 kg (about 60 gdw). Using this figure in the calculations, the biomass per square metre would be about 1000 - 1300 gdwm\(^2\), well within the range of other macrophytes. Further work will need to be undertaken to examine the potential productivity for *Triglochin huegelii*. 

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A scatter graph of the relationship between the productivity (as a final biomass measurement) and the TN or TP gain in each tank system (Table 5.37) is found in Figure 5.7.

![Graph showing the relationship between TN or TP gain and final biomass](image)

Figure 5.7. The relationship between TN or TP gain and productivity.

There may be a linear relationship between TN or TP and biomass increases as indicated by the points plotted in Figure 5.7. Regression analysis was undertaken on both sets of data, and p values determined. For biomass and TN, p was 0.3, and for biomass and TP, p = 0.16. In both cases, p values are too high to suggest such a relationship. However, only three points were plotted and more data would be required before we should dismiss the likelihood of a linear relationship between TN or TP and biomass changes.
5.6 Investigation 5: Comparison of Nutrient Tissue Content Between Eight Wetland Macrophytes.

This investigation was a departure from previous ones as a purpose-built mini wetland was constructed. The main hypotheses tested in this investigation were: "Triglochin huegelli removes more total nitrogen and total phosphorus from domestic greywater than other emergent macrophytes" and "That Triglochin huegelli has the highest storage of nitrogen and phosphorus in its tissues, compared to other wetland macrophytes".

5.6.1 Raw data and comment

Table 5.38 shows the initial and final total N and total P determinations in each plant species and the associated calculations to convert the nutrient concentration readings (usually in mg/L) to mg/g dry weight. The calculations also considered the dilutions of the stock solution. This table is the summary of differences, expressed as a percentage change, of the nitrogen and phosphorus concentrations in each plant part. The raw data is available in Appendix 1.

Table 5.39 is a summary of the total N and total P found in the whole plant. The totals were calculated by simply adding the mg/g dry weight values of each plant part, without consideration of the relative proportions of each plant part compared to each other and the whole plant. It was assumed, for simplicity at the time, that the proportion of above-ground and below-ground biomass would be similar for these common wetland plants.
Values of AG:BG biomass of 1.85 for *Schoenoplectus validus* and 2.86 for *Baumea articulata* (Tanner, 1996), two species used in this investigation, and this present work with *Triglochin huegelii* (AG:BG ratio range from 0.09 to 1.62, depending on water regime), suggest that this is not so. Tanner (1994) and Rea and Ganf (1994) have also shown that AG:BG ratios vary with water regime and nutrient concentration in the wastewaters and, ideally, the relative amounts of leaf, root and rhizome biomass should have been noted. However, so that some comparisons could be made, concentrations in leaf, root and rhizome biomass were added to obtain a cumulative total.

5.6.2 Total nitrogen

Of the averages of twenty four different plant samples, eleven increased their total nitrogen concentration, while the other thirteen had reduced concentrations of TN in their tissues. However, it is reasonable to suggest that all plants had nitrogen gains, as all plants increased their biomass. It was observed that plants increased their growth and size by at least 150% or more, as shown in Plates 3.7 and 3.8. However, initial and final biomass measurements were not taken and so total nitrogen gain in the plants could not be determined. This investigation was conducted before Investigation 4, where biomass changes were first considered. Until now, the investigations only focussed on nutrient concentrations in plant tissue. This investigation examined both the nutrient content changes in the eight wetland plants and nutrient re-allocation within the plant as they
were absorbing nutrients from greywater and growing. This being the case, the four highest TN concentrations were recorded in leaves and stems (above-ground parts). After this, both above-ground and below-ground parts in various plants have similar nitrogen concentrations.

The range of total N measured in plant parts was from 2.03 to 8.48 mg/g. The highest rhizome measurement was *Baumea juncea* at 4.73 mg/g. The highest root measurement was 5.0 mg/g in *Triglochin huegelii*, which was often up to twice the level of other plants. It should be pointed out that the values of TN are consistently about one-third to one-half of the values previously found in *Triglochin* plant tissue (see other values in Tables 4.6, 4.11 and 5.1) and are in the lower end of values quoted in Table 2.5 (Section 2.7.2). This author believes that the relative amounts found in the different species are still appropriate and comparisons are made with that assumption.

The highest leaf Nitrogen concentration was *Triglochin huegelii* at 8.48 mg/g, which was often three times higher than other wetland plants.

As discussed in Chapter 4, *Triglochin* is able to manufacture amino acids, such as proline, and possibly other nitrogen containing substances. The macrophytes used in this investigation were grown in substrate-only conditions. In other words, water was kept below the surface at all times, and from Investigation 4 and the work discussed in Chapter 4, the form of *Triglochin* would yield a higher below-ground to above-ground ratio. This water regime would, in effect, change the size and nutrient content of the
leaves. It would be expected to find relatively small leaves and low nitrogen concentrations.

Even so, Figure 5.8 shows that *Triglochin* has the highest nitrogen content in its leaves compared to all other plants. This amount of nitrogen equates to about 50% protein. Roots have similar results to that of leaves and stems, as shown in Figure 5.9. In some species there is proportionally more nitrogen in roots than in leaves and stems. *Triglochin* is still high. When you consider the whole plant, *Triglochin* has the highest level of total nitrogen (Figure 5.10).

![Graph showing nitrogen content of different plant species]

**Figure 5.8.** Total nitrogen content of leaf/stem plant parts.

**NB:** In all such Figures the following key and conventions are used:

- BA = *Baumea articulata*
- BJ = *Baumea juncea*
- JP = *Juncus pallidus*
- P = *Juncus pauciflorus*
- JM = *Juncus microcephalus*
- EA = *Elsocharis acuta*
- SV = *Schoenoplectus validus*
- TH = *Triglochin huegelii*

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Figure 5.9. Total nitrogen content of root plant parts.

Figure 5.10. Total nitrogen content of whole plant.  
NB: Values were calculated as a cumulative total of TN of leaves, roots and rhizomes or tubers.
Table 5.38. Summary of all data of the total N and P concentrations for all plants.

Key: TN = total nitrogen initial, TNF = total nitrogen final. TP = total phosphorus initial, TPF = total phosphorus final. Standard deviations are also shown.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Part</th>
<th>TN mg/g</th>
<th>TNF mg/g</th>
<th>N change %</th>
<th>TP mg/g</th>
<th>TPF mg/g</th>
<th>P change %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baumea articulata</td>
<td>stem/leaf</td>
<td>3.13 ± 0.09</td>
<td>2.78 ± 0.00</td>
<td>-11 ± 0.48</td>
<td>4.38 ± 0.03</td>
<td>3.00 ± 0.30</td>
<td>-32 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>rhizome</td>
<td>2.43 ± 0.15</td>
<td>2.03 ± 0.00</td>
<td>-16 ± 0.55</td>
<td>5.50 ± 0.00</td>
<td>7.50 ± 0.00</td>
<td>35 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>root</td>
<td>3.83 ± 0.02</td>
<td>3.95 ± 0.00</td>
<td>3 ± 0.05</td>
<td>3.23 ± 0.00</td>
<td>5.93 ± 0.00</td>
<td>83 ± 3.5</td>
</tr>
<tr>
<td>Baumea juncea</td>
<td>stem/leaf</td>
<td>3.43 ± 0.09</td>
<td>2.45 ± 0.19</td>
<td>-28 ± 1.00</td>
<td>3.75 ± 0.09</td>
<td>4.45 ± 0.00</td>
<td>19 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>rhizome</td>
<td>4.73 ± 0.00</td>
<td>2.80 ± 0.05</td>
<td>-41 ± 0.00</td>
<td>8.70 ± 0.01</td>
<td>8.88 ± 0.00</td>
<td>2 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>root</td>
<td>2.53 ± 0.00</td>
<td>3.08 ± 0.00</td>
<td>22 ± 0.00</td>
<td>7.65 ± 0.00</td>
<td>0.70 ± 0.00</td>
<td>-91 ± 3.5</td>
</tr>
<tr>
<td>Eleocharis acuta</td>
<td>stem/leaf</td>
<td>4.23 ± 0.03</td>
<td>4.55 ± 0.03</td>
<td>8 ± 0.04</td>
<td>10.15 ± 0.18</td>
<td>6.93 ± 0.00</td>
<td>-32 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>rhizome</td>
<td>3.33 ± 0.08</td>
<td>3.20 ± 0.00</td>
<td>-4 ± 0.25</td>
<td>9.80 ± 0.00</td>
<td>8.55 ± 0.00</td>
<td>-13 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>root</td>
<td>3.55 ± 0.08</td>
<td>3.25 ± 0.00</td>
<td>-8 ± 0.54</td>
<td>4.00 ± 0.00</td>
<td>4.48 ± 0.00</td>
<td>10 ± 3.5</td>
</tr>
<tr>
<td>Juncus microcephalus</td>
<td>stem/leaf</td>
<td>3.63 ± 0.05</td>
<td>4.25 ± 0.29</td>
<td>17 ± 0.37</td>
<td>8.10 ± 0.01</td>
<td>0.73 ± 0.01</td>
<td>-91 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>rhizome</td>
<td>3.80 ± 0.05</td>
<td>3.13 ± 0.05</td>
<td>-17 ± 0.08</td>
<td>6.30 ± 0.11</td>
<td>9.35 ± 0.00</td>
<td>48 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>root</td>
<td>2.95 ± 0.05</td>
<td>2.05 ± 0.05</td>
<td>-31 ± 0.20</td>
<td>4.10 ± 0.00</td>
<td>5.40 ± 0.00</td>
<td>32 ± 3.5</td>
</tr>
<tr>
<td>Juncus pallidus</td>
<td>stem/leaf</td>
<td>4.20 ± 0.14</td>
<td>2.88 ± 0.01</td>
<td>-31 ± 0.14</td>
<td>9.25 ± 0.04</td>
<td>6.80 ± 0.00</td>
<td>-27 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>rhizome</td>
<td>3.83 ± 0.05</td>
<td>3.40 ± 0.01</td>
<td>-12 ± 0.27</td>
<td>10.43 ± 0.07</td>
<td>9.30 ± 0.00</td>
<td>-11 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>root</td>
<td>3.25 ± 0.04</td>
<td>3.50 ± 0.00</td>
<td>7 ± 0.07</td>
<td>7.55 ± 0.00</td>
<td>5.70 ± 0.00</td>
<td>-24 ± 3.5</td>
</tr>
<tr>
<td>Juncus pauciflorus</td>
<td>stem/leaf</td>
<td>2.75 ± 0.05</td>
<td>2.98 ± 0.02</td>
<td>8 ± 0.30</td>
<td>9.28 ± 0.23</td>
<td>7.88 ± 0.00</td>
<td>-15 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>rhizome</td>
<td>3.23 ± 0.15</td>
<td>4.20 ± 0.06</td>
<td>30 ± 0.06</td>
<td>9.25 ± 0.01</td>
<td>12.00 ± 0.03</td>
<td>30 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>root</td>
<td>4.00 ± 0.14</td>
<td>3.70 ± 0.02</td>
<td>-7 ± 0.00</td>
<td>8.85 ± 0.07</td>
<td>10.23 ± 0.00</td>
<td>16 ± 3.5</td>
</tr>
<tr>
<td>Schoenoplectus validus</td>
<td>stem/leaf</td>
<td>4.63 ± 0.19</td>
<td>3.18 ± 0.02</td>
<td>-31 ± 0.14</td>
<td>11.65 ± 0.04</td>
<td>5.15 ± 0.00</td>
<td>-56 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>rhizome</td>
<td>2.48 ± 0.07</td>
<td>2.73 ± 0.19</td>
<td>10 ± 0.27</td>
<td>8.23 ± 0.04</td>
<td>9.78 ± 0.00</td>
<td>19 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>root</td>
<td>2.85 ± 0.04</td>
<td>3.20 ± 0.22</td>
<td>16 ± 0.28</td>
<td>5.15 ± 0.03</td>
<td>7.85 ± 0.00</td>
<td>52 ± 3.5</td>
</tr>
<tr>
<td>Triglochin huegelii</td>
<td>stem/leaf</td>
<td>5.35 ± 0.19</td>
<td>8.48 ± 0.05</td>
<td>58 ± 0.00</td>
<td>6.63 ± 0.00</td>
<td>5.75 ± 0.00</td>
<td>-13 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>tuber</td>
<td>3.48 ± 0.05</td>
<td>2.93 ± 0.18</td>
<td>-19 ± 0.06</td>
<td>9.60 ± 0.07</td>
<td>11.28 ± 0.00</td>
<td>17 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>root</td>
<td>5.00 ± 0.05</td>
<td>2.43 ± 0.04</td>
<td>-51 ± 0.24</td>
<td>10.03 ± 0.33</td>
<td>11.00 ± 0.00</td>
<td>9 ± 3.5</td>
</tr>
</tbody>
</table>
Table 5.39. Plant total nitrogen and total phosphorus, showing the change in nutrient levels. Standard deviations are also shown.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Tot N init mg/g</th>
<th>Tot N final mg/g</th>
<th>Tot N change mg/g</th>
<th>Tot P init mg/g</th>
<th>Tot P final mg/g</th>
<th>Tot P change mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Baumea articulata</em></td>
<td>9.39 ± 0.07</td>
<td>8.75 ± 0.01</td>
<td>-0.64 ± 0.05</td>
<td>13.20 ± 0.26</td>
<td>16.43 ± 0.02</td>
<td>3.23 ± 0.17</td>
</tr>
<tr>
<td><em>Baumea juncea</em></td>
<td>10.68 ± 0.05</td>
<td>8.33 ± 0.10</td>
<td>-2.35 ± 0.03</td>
<td>20.09 ± 0.58</td>
<td>14.01 ± 0.05</td>
<td>-6.08 ± 0.37</td>
</tr>
<tr>
<td><em>Eleocharis acuta</em></td>
<td>11.11 ± 0.03</td>
<td>11.00 ± 0.02</td>
<td>-0.11 ± 0.01</td>
<td>23.99 ± 0.25</td>
<td>19.956 ± 0.11</td>
<td>-4.03 ± 0.10</td>
</tr>
<tr>
<td><em>Juncus microcephalus</em></td>
<td>10.33 ± 0.01</td>
<td>9.43 ± 0.14</td>
<td>-0.90 ± 0.10</td>
<td>18.50 ± 0.17</td>
<td>15.49 ± 0.09</td>
<td>-3.01 ± 0.06</td>
</tr>
<tr>
<td><em>Juncus pallidus</em></td>
<td>11.38 ± 0.06</td>
<td>9.85 ± 0.05</td>
<td>-1.53 ± 0.01</td>
<td>27.20 ± 0.10</td>
<td>21.80 ± 0.04</td>
<td>-5.40 ± 0.05</td>
</tr>
<tr>
<td><em>Juncus pauciflorus</em></td>
<td>9.99 ± 0.01</td>
<td>10.90 ± 0.03</td>
<td>0.91 ± 0.02</td>
<td>27.38 ± 0.16</td>
<td>30.10 ± 0.11</td>
<td>2.73 ± 0.03</td>
</tr>
<tr>
<td><em>Schoenoplectus validus</em></td>
<td>9.98 ± 0.06</td>
<td>9.231 ± 0.11</td>
<td>-0.75 ± 0.02</td>
<td>25.03 ± 0.08</td>
<td>22.768 ± 0.01</td>
<td>-2.26 ± 0.05</td>
</tr>
<tr>
<td><em>Triglochin huegelii</em></td>
<td>13.98 ± 0.09</td>
<td>13.84 ± 0.08</td>
<td>-0.14 ± 0.02</td>
<td>26.30 ± 0.13</td>
<td>28.00 ± 0.17</td>
<td>1.70 ± 0.03</td>
</tr>
</tbody>
</table>

5.6.3 Total phosphorus

Similarly, thirteen samples increased their total phosphorus concentrations, while the other eleven had reduced levels of phosphorus in their tissues. Again, it is expected that the total phosphorus content of each plant would have increased due to large increases in biomass. Generally, phosphorus increases in rhizomes (or tubers) and root parts. This was true for six of the eight plants for both rhizomes and roots. Phosphorus seems to be stored in below-ground plant parts. The highest nine results for total P were found in rhizomes, roots and tubers. The tenth highest level was recorded in the leaf and stem parts of *Juncus pauciflorus*, the plant that had the overall highest total phosphorus levels.
The range of total P measured in plant parts, as shown in Table 5.39, was from 0.7 to 12.0 mg/g. Again, these values are typically reported for wetland plants as discussed in Section 2.7.2 (Table 2.5). The highest root measurement was 11.0 mg/g in Triglochin huegelii, which was often up to twice the level of other plants. The highest leaf measurement was Schoenoplectus validus at 11.65 mg/g, which was often twice that of the other wetland plants.

Figure 5.11. Total phosphorus content of rhizome/tuber plant parts.

The highest rhizome measurement, as shown in Figure 5.11, was Juncus pauciflorus at 12.0 mg/g. Triglochin huegelii was the only plant with a
tuber and so a comparison to other plants cannot be made fairly, but the high reading of 11.28 mg/g (second highest of all phosphorus readings) suggests that it is a plant that assimilates and stores a reasonable amount of phosphorus and that its tubers perform a similar function to the rhizomes of common wetland plants.

Figure 5.12 shows that *Triglochin* has the highest weight for weight ratio of phosphorus in plant roots. When we consider total phosphorus in the whole plant (Figure 5.13), *Triglochin* has one of the highest levels. *Juncus pauciflorus*, which has the highest total P measurement, is a small reed which can tolerate permanent flooding, and thus it may be a useful plant for further research.

![Bar chart showing maximum P content mg/g for different plant species](image)

**Figure 5.12. Total phosphorus content of root plant parts.**
5.6.4 Other comments

From measurements of concentration changes, it seems that only three of the eight species had an increase in both total N and total P. *Baumea articulata* had the greatest total increase of total N and total P, followed by *Juncus pauciflorus* and then *Triglochin huegelii*. Most of the other five species had decreased levels of both total N and total P. However, as already discussed, this was not the case, as while concentrations of N and P may have decreased the overall N and P content in each plant would have increased.
The first hypothesis has not been supported as the rate of biomass change, and therefore nutrient accumulation, in *Triglochin* was not as high as in many other macrophytes. Using data on concentration changes only, *Baumea articulata* has the highest overall increase in total N and total P, presumably due to greater absorption of these nutrients. *Triglochin huegelii* has the third highest levels after *Juncus pauciflorus*.

The second hypothesis has not been supported either, even though *Triglochin huegelii* has the highest total N content in the whole plant and the highest total P in its roots. Both *Juncus pallidus* and *Juncus pauciflorus* have a slightly higher total P for the whole plant than *Triglochin huegelii*.

In conclusion, *Triglochin huegelii* does absorb and assimilate higher amounts of N and P, comparable to the other macrophytes studied. As other investigations have shown, higher nutrient removal can be expected from *Triglochin huegelii* under pond conditions where the form of the plant changes accordingly and the above-ground:below-ground ratio increases. Larger above-ground biomass and rapid increases in the number and growth of leaves ensures greater nutrient removal from greywater. Several of the macrophytes studied deserve further investigation for their possible use in wastewater treatment systems.
5.7 General Discussion

The original aims of this study were to examine the use of *Triglochin huegelii*, a submergent macrophyte from the south-west of Western Australia, for domestic greywater treatment, and to compare the effectiveness of this plant with others commonly used for nutrient removal in wastewater.

The series of investigations which were undertaken compared nutrient uptake by leaves, roots and tubers, and results have shown that *Triglochin* and other types of plants do effectively assimilate nutrients from greywater. However, unless these plants are harvested, much of this stored nutrient is recycled in the waterway as plants die and decompose, as also suggested by Hosoi *et al.* (1998).

Long term storage of nutrients, over the course of the lifetime of an artificial wetland, best occurs when they are precipitated and deposited as particulate chemical compounds or adsorbed onto particles. This is how phosphate is probably removed from wastewater. Even so, there is a difference in the amount of phosphate removed in each system. For example, all tanks in the substrate-only experiment (3 L tanks) in Investigation 4 had exceptionally high phosphate retention, either taken into plants or adsorbed onto substrate particles, while the pond system study had slightly less phosphate assimilated.
The nutrient analysis results for both *Triglochin* and *Schoenoplectus* in Investigation 1 suggest that below-ground parts (rhizomes or tubers) are storage organs, as nutrient changes did occur over the duration of the investigations as the biomass of both plants increased.

*Triglochin huegelii* can re-allocate nutrients from storage organs (roots and tubers) to growing areas (new shoots). It seems that storage occurs below-ground in sub-surface water and above-ground in deep water. The tubers may give this species greater tolerance to depth, as the size and number of tubers varies with water regime. For example, in substrate-only or subsurface conditions, there are many more tubers and small leaves. In a pond, there is less proportion of tubers and greater above-ground biomass (leaves).

Species of *Triglochin* may need less energy to mobilise resources because they can access resources from below as well as above the water, as indicated by the thin cuticle of their spongy leaves. *Triglochin huegelii* probably absorbs nitrates directly into its leaves and doesn’t solely rely on root absorption. The highest total N increase ratio occurred in the leaves. For example, plants with the highest nutrient loading in Investigation 4 (10 L tanks) demonstrated the greatest growth and nitrogen gain. *Triglochin huegelii* has the ability to store high levels of N and P - with more N in above-ground parts (leaves) and more P below-ground (roots and tubers).
The results obtained for the various parameters tested in the series of investigations had mixed results. For example, in Investigation 1 Schoenoplectus had proportionally more nitrogen and phosphorus uptake than Triglochin, whereas in Investigation 2, Triglochin had greater nitrogen gain and lower phosphorus gain than Schoenoplectus.

Slightly different results were obtained in Investigation 3. Here, tanks planted with Schoenoplectus validus reduced nitrates, phosphates and ammonium more than tanks planted with Triglochin huegelii, with both ammonium and phosphate reduction more significant (in most planted tanks) than the unplanted tanks. High levels of phosphate seem to be also removed by the control tanks. Only the tanks planted with Schoenoplectus are significantly different from the control tanks for nitrate reduction (p = 0.02). All other planted tanks had p values >0.05.

Furthermore, the hypothesis tested in Investigations 2 and 3 that "Triglochin huegelii lowers nutrients and other constituents, such as BOD, fecal coliforms and suspended solids, in greywater" was supported even though some results for planted tanks were not significantly different from the unplanted control tanks.

Some of the results from Investigations 1, 2 and 3 indicate that there may be some antagonism between Triglochin huegelii and Schoenoplectus validus. For example, phosphate reduction by Triglochin alone is statistically different from the combined plant tanks (p = 0.04) in
Investigation 1, and at other times the results are either in-between or lower than some values obtained by *Triglochin* or *Schoenoplectus* alone. It is unknown if this antagonism also occurs between *Triglochin huegelii* or *Schoenoplectus validus* and other macrophytes, but Nakai et al. (1999) have shown that inhibition of growth does occur between some macrophytes and other plants in aquatic systems.

Fecal coliforms were also greatly reduced in both planted tanks and unplanted tanks, with slightly higher reductions in planted tanks, but \( p = 0.92 \) and therefore the comparison between the control tanks and planted tanks is not statistically significant. The first three investigations generally found that *Triglochin huegelii* reduces BOD, SS, fecal coliforms, nitrates, phosphates and ammonium more than unplanted tanks, even though some results are not statistically significant.

The growth of algae in many tanks in the earlier investigations (1 and 2) has clearly affected the pH, suspended solids, nutrient levels and BOD. Generally, suspended solids were not reduced as much as expected, with large amounts of microscopic algae growing in most tanks as evidenced by the green colouration.

The shedding of this algae into the overflow pipes and its capture on the surface of the filter papers would account for the overall low suspended solids reduction. It is suspected that the nature of the suspended solids, rather than the amount, has changed. The types of substances in the
initial greywater would not be the same as that passing out the overflow pipe.

To eliminate the algal growth, tanks should have surface waters reduced to ground level, or some treatment of the water to reduce algal growth could be investigated. These include the use of floating macrophytes, such as azolla or duckweed, to shade the water and thus inhibit the growth of algae.

This led to Investigation 3 where the water level was kept below the surface and the hypothesis “That the root zone in tanks planted with Triglochin huegelli will reduce levels of nutrients in greywater more than Schoenoplectus validus” was examined.

This hypothesis was not supported. Instead, tanks with Schoenoplectus validus had a slightly higher BOD reduction than tanks with Triglochin huegelli, with the combined planted tanks having a BOD reduction in-between these two. This is typical for both pond and the sub-surface zones of the plant species.

It must be kept in mind that the emergent Schoenoplectus is a faster-growing clumping plant, whereas the submergent Triglochin is a singular plant. It is fair to say that the Schoenoplectus tanks had far greater biomass present, and studies such as this will need to consider the amount of nutrient reduction compared to plant biomass. Some indication
of this relationship was evident from Investigation 4, and this is discussed later in this chapter.

Further comparisons between *Triglochin* and *Schoenoplectus* were made when these two plants and six other emergent macrophytes were examined in Investigation 5. This study showed that, except for some plant parts in the other species in the study, *Triglochin huegelii* had the highest N and P concentrations in its tissues. Thus, the hypothesis in Investigation 5 that “*Triglochin huegelii* has the highest storage of nitrogen and phosphorus in its tissues” was only supported if you consider nutrient concentration rather than total storage. Again, only *Juncus pauciflorus* had an overall higher P content, even though Triglochin had the highest P concentration in roots and the second highest in rhizomes/tubers.

*Triglochin huegelii*, then, compares very well with other wetland macrophytes. It was the only plant with a tuber and so a direct comparison to the rhizomes of other plants cannot be made. Only *Triglochin huegelii, Juncus pauciflorus* and *Baumea articulata* all increased their N and P concentrations during the course of the study but, as discussed previously, all plants had large biomass increases so there were net N and P gains in all plants. *Juncus pauciflorus* may also have potential use in artificial wetlands for treating wastewaters as it had higher N and P concentrations than most other similar species.
Many plants had higher concentrations of N in leaves and higher concentrations of P in below-ground parts. *Schoenoplectus validus* is an exception. It had higher concentrations of both N and P in its leaves. Greenway (1997) also found that the leaves of many plants, such as *Schoenoplectus validus*, often had higher concentrations of nitrogen and phosphorus than that in roots and rhizomes.

What was unusual was that half of the plants had a decrease in total N concentration and the other half increased their N concentration over the four month experimental period. This may not seem unusual. All plant biomass increased and it is likely that while the concentration of nutrients decreased the total nutrient content in the plant would have increased. However, the significance of these wetland macrophytes to strip nutrients and store large amounts in tissues is generally poor.

The final aim of this work was to study the effect of changing water regimes on the nutrient-stripping capabilities of *Triglochin huegelii* and to examine how the plant copes with the stress of changing environmental conditions.

*Triglochin huegelii* has unique characteristics. It is polymorphic. It exhibits large variation in its characteristics which are dependent on the water regime. It can tolerate from wet soil to complete inundation. The genetic expression of its form is modified by the environment. This was very evident in Investigation 4. This was the last investigation using the 200 L tanks. Here, only *Triglochin huegelii* was used in the tanks, where
the hydraulic loading was varied. Again, tanks with *Triglochin huegelii*
reduce amounts of nitrate, ammonium and phosphate in greywater more
than the unplanted control tanks. The results for the *Triglochin* tanks
were significantly different to those of the control tanks.

Data collected on the wet and dry weights of the various plant organs, and
the concentrations of N and P in these organs (as discussed in Chapter 4
as well as in Chapter 5 (Section 5.5), show that the largest nitrogen gains
are found in the plants containing the largest biomass and kept in
conditions with the highest nutrient loading, with leaves containing the
highest total N levels, followed by tubers then roots. There is some evidence
species such as *Triglochin* require nitrogen for the production of the
amino acid proline which is produced by plants as a response to stress.
Proline was detected in *Triglochin huegelii* but the levels were generally
lower than expected. However, it was found that proline concentrations in
very small young leaves were statistically different from concentrations in
slightly larger leaves (p = 0.018). Even so, these small amounts of proline
might mean that greater amounts of other osmoprotectants could be
produced as a response to stress.

Similarly, the highest phosphorus content was found in leaves of the
largest plants, again exposed to the highest nutrient loading. Phosphorus
levels were also higher in tubers than roots.
It is clear that *Triglochin huegelii* does remove nutrients from greywater, and does so at rates comparable to many other wetland macrophytes.

It appears from evidence gathered so far that the optimum performance of *Triglochin huegelii* occurs in a pond situation. What is not established is whether changing the water regime (including the depth, duration and rate of inundation and drying phases) further enhances performance. It is clear that some macrophytes perform best under a constant water level while others (perhaps *Triglochin huegelii*) perform best under changing levels.

*Triglochin's* success in pond conditions is partly due to its leaf structure. The spongy leaf of *Triglochin huegelii* is due to the large amount of intercellular spaces, enabling good convective gas flow to below-ground regions. Microscopic cross-sections of its leaves, as shown in Plates 4.2 to 4.4 inclusive, reveal photosynthetic cells only in the top layer, some parts of the leaf having no photosynthetic cells at all and differences in structure from one part of the leaf to another.

These types of characteristics enable *Triglochin* to survive complete submersion, as oxygen is able to be absorbed from either the air or the water itself and then diffuse to lower regions below-ground. The air-filled spaces in the leaves also enable the leaves to be buoyant and float along the water's surface.
Thus, *Triglochin huegelii* has many practical applications in wastewater management, especially if the level of influent/wastewater can be controlled, thus allowing sufficient time for *Triglochin huegelii* to respond with changed structure and morphology.

This study has also found other uses for *Triglochin*. For example, *Triglochin huegelii* could have potential as a fodder source because of its high protein content (1.7 p/100 g wet weight in leaves), comparable to lucerne, and its tubers (other species of *Triglochin* are known to be eaten by Aborigines, Brand-Miller *et al.*, 1993) have potential in the growing bush tucker market.
Chapter 6  Conclusions and Recommendations

6.1 Conclusions

The treatment and then disposal of greywater can be accomplished simply by mimicking the cycling of matter in nature, using wetland plants, which are well known as both fast-growing plants and minor accumulators of nutrients, to contribute to this process. For example, these studies have shown that planted tanks, in both the pond and substrate-only systems, have, on average, more nitrate, ammonium and phosphate removal and reduction than the unplanted control tanks. In several investigations these results are statistically significant with p values < 0.05.

Evidence accumulated from these investigations has shown that the submergent *Triglochin* is an effective nutrient-stripping plant. Experimental results show that *Triglochin* is comparable in nitrogen and phosphorus uptake with *Schoenoplectus* and with many other wetland plants. The high N and P content found in both the above-ground and below-ground parts of *Triglochin* indicate the possible storage and removal potential of this species.

The implication is that instead of only planting the perimeter of lagoons, artificial wetlands and constructed basins we should be planting the bulk of the waterway with submergent species such as *Triglochin spp* which may be far more effective in stripping nutrients than emergents currently used for that purpose. Mitchell *et al.* (1995) also contend that wetland
plants can contribute to secondary effluent treatment provided that strict
human health criteria are met, and this study has shown that tanks
containing *Triglochin* can reduce fecal coliforms in wastewater.

Some plants may increase growth to maximise production while others
tended towards a more conservative strategy of nutrient accumulation.
More work will need to be undertaken to see which of the eight species
studied, including *Triglochin huetelii*, had apparent increased efficiency
of nutrient use and which ones simply were able to store additional
nutrients when required. There is some indication, for example, that
*Triglochin* may absorb high levels of nitrogen as a response to changing
water regimes and stress. Higher levels of proline are found in many
submerged plants which supports this contention.

*Triglochin* has other characteristics which enhance its potential as a plant
which should be considered for wastewater treatment. For example,
*Triglochin* shows no seasonal senescence, with growth occurring
throughout the winter period, and because of its high protein content,
comparable to lucerne, it could have future use as a fodder source (and is
edible by humans too).

All of this suggests that *Triglochin huetelii* is an ideal candidate for use in
wastewater treatment systems, particularly in situations that require the
use of ponds, or where the levels of wastewater varies seasonally or where
other species experience seasonal senescence due to adverse climatic
conditions.

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6.2 Future Research and Recommendations

6.2.1 Recommendations for further research

Many other opportunities exist to further explore the use of plants for greywater treatment. Some of these suggestions are briefly described below:

- further work on *Triglochin huegelii*. Long-term studies could be undertaken on the expected nutrient removal per plant or per unit of treatment area. The amount of nitrogen and phosphorus removal per hectare and/or per person equivalent in a household could be investigated.

Furthermore, the changes in morphology in *Triglochin huegelii* and any implications for wastewater treatment, taking into account the seasonal changes in water regime, need investigating. Part of this study could include the effect of individual leaf removal (prior to their natural death and replacement) on the viability of the plant. The harvesting of leaves for compost production or fodder could be investigated.

- research into other endemic macrophytes, such as *Juncus pauciflorus*, *Juncus pallidus* and *Baumea articulata*, which showed potential as nutrient accumulators. These types of plants are good candidates for the biological treatment of wastewater as they have high levels of nitrogen and phosphorus in their tissues, sometimes as high or higher than *Triglochin huegelii*.
more research needs to be undertaken with *Triglochin huegelii* in polyculture conditions. It seems that inhibiting agents may be produced by some local macrophytes which would hinder the effectiveness of *Triglochin huegelii* as a nutrient-stripping mechanism in wastewater treatment. *Triglochin huegelii* itself might produce such chemicals. Various combinations of plants could be examined for the presence of allelopathic reagents.

biochemical studies on *Triglochin huegelii*. Other *Triglochin* species are known to contain high levels of proline as a response to salinity. Other chemicals, such as the sugar alcohols mannitol and pinnitol, and the amino acids glycine and betaine, are known to be produced as a response to stress in other plants. This study has shown that *Triglochin huegelii* does produce free proline, but not in the expected amounts. Further investigations could be undertaken to examine whether *Triglochin huegelii* also produces other chemicals, besides proline, and whether changes in water regime (as stress) triggers their production. These studies may indicate whether *Triglochin huegelii* (and other *Triglochin* species) absorb high levels of nitrate (and contain high levels of total N as protein) as a response to stress conditions typically experienced by these plants. More studies could also be undertaken to determine the amounts of proline produced by *Triglochin huegelii* under different water regimes.
6.2.2 Possible domestic on-site treatment system

The optimal greywater treatment system for any domestic situation would be an on-site treatment and disposal system using a tank which could be set up as a pond or subsurface flow. Either way, plants such as *Triglochin huegelii* could be used to help reduce nutrients in the wastewater.

The design discussed below could fill a niche in the marketplace. It could enable people to become more responsible for their own wastewater by providing the necessary mechanisms and processes to treat domestic greywater.

The optimum trench configuration would be a free-standing trench, one metre wide, five metres long and 900 mm high as shown in Figure 6.1. The trench is essentially a pond with soil mix (of sand and gravel/stone) lining the bottom 400 mm and water above for another 400 mm, leaving 100 mm or so at the top to prevent excess water loss by spillage during loading and to accommodate excess water addition during rainfall.

The trench should contain a series of baffles about 400 mm apart, the placement and heights of which will enable water movement in a vertical flow system. Vertical flow systems have shown the capability to oxidise ammonium ions as well as BOD much more efficiently than horizontal flow systems. It can be expected that the up and down movement of water in vertical flow will enhance aeration, allowing some loss of ammonia directly to the air and the conversion of ammonium to nitrate, which can
then be acted upon by the plants and micro-organisms present in the system. High levels of oxygen will help kill pathogenic, enteric organisms and increase the general levels of beneficial organisms in the wastewater treatment process.

The baffles will need holes at 400 mm from the bottom (initially plugged) so that they will not impede water flow through the trench if the water regime is changed to subsurface flow.

**Elevation**

![Diagram of pond system]

- Inflow
- Water level
- Baffle spacing = 400 mm
- Outflow
- Trench width = 1000 mm, length = 5000 mm

*Figure 6.1. Domestic wastewater treatment tank. Total Pond volume = 4 m³. Soil/gravel/stone volume = 2 m³. At a loading rate of 200 L/day the detention time could be expected to be about 20 days.*

While this design is seen as the optimum, the costs of production of these units may be prohibitive. Tanks made of fibreglass would cost over $2000 each while fibrocement (Hardie planking) tanks about $800 each. The only other alternative is to dig a trench with a backhoe and line the trench with
a geotextile and a PVC membrane. This is not seen as appropriate because of the following reasons:

- too much variation in the size (width) of trenches.
- non-uniformity of sides and shape causing differential flow patterns.
- difficulty in placing and securing baffles to increase flow length.
- cost of machine hire, including removal and stockpiling soil - total soil dug/removed in excess of 50 m$^3$. Machine hire about $350 per day.

The only other requirement for a tank is a suitable site. This tank system requires flat ground (a horizontal site area) with no tree cover, as shading and organic matter falling into the ponds will jeopardise treatment efficiency.

The treated wastewater would leave the tank after a few weeks via an outlet pipe to a disposal area. Ideally, this would be a subsurface trench in a garden area where water could move through the soil and be absorbed by vegetation or eventually re-enter the atmosphere through some combination of evaporation from the soil and transpiration by plants.
7 References


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Appendix 1

Description and Contents of CD

Files are Microsoft Excel. The following files are found on the attached CD.

Here is a short description of the contents of each file.

Investigation 1

- Expt 1 - totals for N and P values for each tank system.
- Expt 1data - T tests for differences between means.
- Ex1Drym - N and P concentrations in plants.

Investigation 2

- Ex2rawdata - all results/measurements for SS, BOD, fecal coliforms, nitrate, ammonium and phosphate concentrations for each trial (8).
- Expt2 - SS and ammonium results.
- Expt2data - SS, ammonium, BOD, fecal coliforms and nitrate ANOVA and t tests.

Investigation 3

- Ex3rawdata - SS, BOD, nitrate, ammonium and phosphate concentrations for each trial (6).
- Expt3data - % reduction of BOD, nitrate, ammonium and phosphate ANOVA and t tests.
- Expt3 - % reduction of BOD, nitrate, ammonium and phosphate for correlation and regression analysis.
Investigation 4

- Expt4nitrogen - raw data for N concentration changes in tanks.
- Expt4phosphorus - raw data for P concentration changes in tanks, and ANOVA.
- finalno3nh4 - ANOVA nitrate and ammonium averages between tank systems.
- Expt4 - ANOVA for nitrate and ammonium concentration changes in tanks.
- Expt4data - N and P changes in concentration in leaves, roots and tubers, ANOVA and t tests for ammonium and nitrate
- Expt 4weights - plant dry weights, AG:BG ratios, TN:TP ratios (Table 4.6), t tests for AG:BG ratios, ANOVA for TN:TP ratios
- Ex4Biomass - initial and final dry mass gains/losses for each tank, wet weight changes. Regression analysis for biomass gain and TP uptake.

Investigation 5

- Macrophytes - raw data of initial and final TN and TP, and nitrate and phosphate concentrations in plant tissues.

Other Excel files

- Proline - concentration in leaves, ANOVA and t tests.
- Prolinedata - ANOVA and t tests for comparison between young leaves - short and long.
- NPproline - N and P concentrations of emergent and submergent leaves (Table 4.11).