Performance evaluation of floating wetland with emergent macrophytes for treatment of domestic wastewater

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Summary

Floating wetlands are considered an alternative treatment by using the root-bed to purify and remove the pollution compounds from wastewater. Studies in Australia specifically use floating treatment wetlands for stormwater, which leads to the question as to; whether this application can remove contaminants from domestic wastewaters. Therefore, the objective of this study was to evaluate the effectiveness of floating wetlands for domestic wastewater treatment using native South Australian aquatic species. The floating wetland application was investigated at the mesocosm scale during winter, when the growth rates of wetland plants are likely low. The wastewater source for this study was from the pond storing wastewater treated by the High Rate Algal Ponds (HRAPs) at the community wastewater treatment scheme at Kingston on Murray, South Australia. The major aim of the study was to compare growth parameters of four emergent aquatic macrophytes, which were; bare-twig rush *(Baumea juncea),* stiff-leaf sedge *(Cyperus vaginatus),* common sedge (*Carex tereticaulis)*, and tall sedge *(Carex appressa)* in domestic wastewater. Comparisons were made any measured changes in wastewater quality in the presence and absence of macrophytes. The results showed that all emergent wetland plants were able to survive in the wastewater environment in winter. The growth rates of the common sedge and tall sedge suggested they were the more suitable species to apply in the treatment of HRAP effluent rather than bare-twig rush and stiff-leaf sedge. There was a strong correlation between the increase in plant biomass production and nitrogen content in plant tissue, which indicated the capacity for nitrogen removal by floating wetlands. At the end of the trial, the water quality in the tanks within the floating wetland system showed a slight decrease in ammonium, BOD_5 and organic carbon. There was, however, a statistically significant increase in the removal of suspended solids and chlorophyll-*a* in the tanks containing wetland plants compared to the control tank containing wastewater only. This resulted in a notable improvement in wastewater clarity following treatment by floating wetland plants. Therefore, floating treatment wetlands are not only beneficial in wastewater treatment and water management but also provide economic value, environmental services and sustainability benefits.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed...

Date...

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Abbreviations

BOD	Biodegradable organic matter
Chl-*a*	Chlorophyll-*a*
CO_2	Carbon Dioxide
DO	Dissolved Oxygen
E. coli	*Escherichia coli*
EC	Electrical Conductivity
EPA	Environmental Protection Agency (United States)
HRAPs	High Rate Algal Ponds
HRT	Hydraulic Retention Time
IC	Inorganic Carbon
NH_3	Ammonia
NH_4	Ammonium
NO_2	Nitrite
NO_3	Nitrate
pH	Potential of Hydrogen
PO_4	Orthophosphate
TN	Total Nitrogen
TC	Total Carbon
TOC	Total Organic Carbon
TP	Total Phosphorus
SS	Suspended Solid

1. INTRODUCTION

1.1. Background

The increase in global population at unprecedented rates leads to the expansion of urbanised areas and simultaneously the increase in wastewater from residences, institutions, and commerce (World Water Assessment Programme [WWAP] 2017). United Nations Water (2015) uses two terms to define this wastewater; blackwater and greywater. Blackwater refers to toilet water, which contains human faeces and urine, while greywater refers to used wash-water from kitchens, laundries, and bathrooms. Sperling (2007) identifies the main contaminants in household wastewater as including high concentrations of suspended solids (e.g. total suspended solids, TSS), Biochemical oxygen demand (BOD), nutrients (e.g. nitrogen N and phosphorus P), pathogenic microorganisms (e.g. bacteria, viruses, protozoa and helminths), and inorganic dissolved solids in excess of that existing in natural water. Domestic and sewage wastewater is rich in nitrogen and phosphorus at high enough levels to pose environmental problems (Tjandraatmadja et al. 2010). Wastewater treatment, therefore, becomes of significance in environmental management because the discharge of untreated effluent into natural water resources leads not only to eutrophication but also to human health risks (WWAP 2017).

Carstensen, Henriksen and Heiskanen (2007) considered eutrophication as the increase in harmful algal blooms, which impacts water clarity, creates oxygen depletion, leads to dead zones in the water column, and damages aquatic organisms and habitats. Although eutrophication occurs naturally, Cloern (2001) documented that wastewater from human activities exaggerates the frequency and magnitude of this phenomenon. In Australia, rich nutrient runoff water was one of the major causes of the occurrence of blue-green algae (cyanobacteria) blooms in the Murray Darling Basin in the early 1990s, which caused many negative socio-economic and environmental impacts (Murray-Darling Basin Ministerial Council 1994, p. 5). However, the impact is not only on the environment. Cyanotoxins, which are a product of harmful cyanobacterial blooms, are a major concern to public health. Chorus and Bartram (1999) noted that short-term contact with high concentrations of cyanobacteria may cause skin and eye irritations. Long-term exposure, to hepatotoxins may impact liver health, cause diarrhoea and upset stomach, and exposure to neurotoxins may result in disorders of the nervous system (Chorus & Bartram 1999). In addition to the inland impacts, evidence from Miller et al. (2010) demonstrated high concentrations of cyanotoxins in the coastal environment, which caused the death of marine mammals, while threats to other species were also associated with areas of excessive nutrient load.

At the same time, the high concentration of pathogenic microorganisms in the wastewater can cause waterborne diseases and gastrointestinal illness to those who directly use or consume contaminated water (Tjandraatmadja et al. 2010). United Nations Water (2015) reports that poor drinking-water access is one of the main global factors causing 1.45 million people to die each year from diarrhoeal illness, of which 43% of deaths are children below five years of age. Although several waterborne disease outbreaks are recorded in developing countries, the incidence of microbial contamination in drinking water are also still found in developed countries (WWAP 2017). These harmful incidents confirm that it is important to remove the excessive nutrient concentration and pathogenic microorganisms through proper treatment systems before reusing and discharging wastewater to the environment (National Health and Medical Research Council [NRMMC] 2004).

1.2. Conventional wastewater management and its challenges

Wastewater treatment plants are necessary infrastructure constructed with the ultimate objective of managing the problems associated with wastewater impacting human health and the environment. The acceptable quality of the treated wastewater depends on the specific end use of the resource (Natural Resource Management Ministerial Council [NRMMC] 2006). For example, the water quality guideline for recycled water use in landscape irrigation (e.g. trees, shrubs, public gardens) is BOD_5 <20mg/L, SS <30 mg/L and *Escherichia coli* (*E. coli*) <1000 colony forming units (cfu)/100mL, while the guideline for commercial food crops is set at BOD_5 <20mg/L, SS <30 mg/L, and *E. coli* <100cfu/100mL (NRMMC 2006, p. 105). Environment protection (water quality) policy in the United States (Environmental Protection Agency [EPA] 2015) documents the phosphorus, nitrogen (including ammonia NH_3), and BOD_5 loading concentration from any source into receiving water bodies which are not to exceed 0.5 mg P/L, 5mg N/L and 10 mg BOD_5/L respectively (EPA 2019, p. 13). It is also noted that different states in the USA and elsewhere may document different acceptable quality in these parameters to a receiving environment. Typically, there are four main processes in conventional wastewater treatment plants: preliminary, primary, secondary, and tertiary. Each process is designed to remove different pollutants, which have been classified as shown in Table 1.1 by Sperling (2007).

Table 1.1. Wastewater treatment level, mechanism and respective contaminant removal based on those stated by Sperling (2007)

Wastewater treatment level	Treatment mechanisms	Contaminant Removed
Preliminary	Physical treatment using screen or grit chamber	Coarse solids (large material and sand)
Primary	Physical treatment using sedimentation tank	• Settleable suspended solids • Particulate Biochemical oxygen demand (BOD)
Secondary	Biological treatment processes include • Stabilisation ponds • Anaerobic reactors • Activated sludge systems • Aerobic biofilm reactors	• Particulate BOD (dissolved organic matter that was not completely removed in the primary treatment) • Soluble BOD (organic matter in the form of dissolved solids)
Tertiary	• Membranes • Disinfection with chemical production and ultraviolet radiation • Land disposal	• Nutrients • Pathogenic organism • Non-biodegradable compounds • Metals • Inorganic dissolved solid • Remaining suspended solids

Higher removal efficiencies require more treatment mechanisms, which increase both the capital and the operational cost of the wastewater treatment facility. This includes advanced technology, electricity, pumping, other constructions, and skilled human resources (United Nations Water 2015). As a result, the conventional wastewater treatment systems are more likely installed in large cities to facilitate maintenance and the most effective treatment (United Nations Water 2015). Recently, the gradual increase in urban areas and the associated increase in the volume of wastewater requires the existing wastewater treatment to work harder to maintain the reuse/disposal water quality standard. Inadequate capacity in financial and human resources is a key factor driving the failure in the operation and maintenance of the treatment system. The obvious consequence is inevitably the discharge of the non-compliant wastewater to the receiving environment, which may be reused for irrigation and washing activities (United Nations Water 2015). The situation in Kumasi and Accra (Ghana) is a case study, which illustrates the causal link between the use of untreated wastewater for irrigation and a public health problem from food production. The frequent failure of the central wastewater treatment plants pushed the health threat into the risk level where pathogenic bacteria and helminth eggs were found in vegetables sold in markets (Keraita & Drechsel 2004). Similarly, Ensink, Simmons and van der Hoek (2004) showed that there was an increase in parasitic infections among farmers who regularly used wastewater for irrigation in Faisalabad, Pakistan. These examples are clear evidence that traditional wastewater treatment systems have failed to operate where the technology selection and capacity of locals to manage wastewater treatment are not appropriate. Domestic wastewater treatment remains a concern with respect to socio-economic, environmental, and health aspects - especially in vulnerable communities. Therefore, alternative wastewater treatments are required for wastewater management in remote areas to provide a sustainable solution for future wastewater treatment.

1.3. Wastewater treatment using wetland plants

Wastewater treatment using wetland plants or aquatic macrophytes has been practised as an engineered technology to treat wastewaters for decades (Scholz & Lee 2005; Wu et al. 2015). This technology is designed according to the purifying function and capacity of plants in assimilating nutrients (N and P) through their roots. Specifically, nitrogen is a significant primary nutrient to support plant biomass and increase root and shoot production (Boyd 2015). In most species, nitrogen is contained in their dry weight at 5-10% or 50-100 mg/g (Boyd 2015). Related studies have found nitrogen content in grassland species around 1-3% in above-ground tissues and 0.5-2% in those below ground (Rooney & Yuckin 2019; Tang et al. 2018; Zhang et al. 2014). Plants can absorb nitrogen directly from water in inorganic forms of nitrate (NO_3^-) and ammonium (NH_4^+) to make protein and support plant metabolism (Boyd 2015). The preference of plants in using nitrate and ammonium can be observed from pH levels around the root zone. When ammonium (NH_4^+) is absorbed by plants, they will release protons (H^+) to maintain the plant's electrical neutrality in which H+ is acidic and in turn decreases pH level around the root zone. In contrast, when plants uptake nitrate (NO_3^-), they will release bicarbonate, which is alkaline and causes an increase in pH level. Under normal conditions when there is mixed nitrogen speciation, NH_4^+ is preferentially utilised because of less energy demand compared to the uptake of NO_3^- (Boyd 2015). However, in the case of plant uptake for nitrate, this understanding might not always be correct. Abbasi, Vasileva and Lu (2017) found that in higher nitrate concentration than NH_4^+-N, there was more removal efficiency for NO_3^--N. Also, among wetland plants, there was different preference for nitrogen speciation. Therefore, these findings illustrate that nitrogen removal efficiency in wastewater can be varied, depending on the dominant nitrogen concentration and selected plants species. Other researchers have suggested that a mixture of nitrogen speciation may be a better strategy to enhance plant growth performance (Miller & Cramer 2005).

Constructed wetlands are a well-known wastewater treatment process comprising of sediment ponds, vegetation, and treatment cells. Bendoricchio, Cin and Persson (2000) explained that this technology is similar to the activated sludge systems of the secondary level in traditional treatment plants. The treatment function is designed to perform natural filtration, settle the suspended particulate matter, and breakdown and absorb contaminants, such as organic compounds, nitrogen, phosphorus, and heavy metals (Wu et al. 2015). Such green technology has been applied to many wastewater sources, such as domestic sewage, industrial wastewater, agriculture, mining, landfill leachate, stormwater, and urban runoff (Scholz & Lee 2005; Wu et al. 2015). Not only have constructed wetlands received great attention regarding their effective performance for treating various wastewaters, but also attract attention due to their low cost of operation and maintenance, low energy consumption, environmental services (e.g. habitat provisioning), and commercial value in comparison to the conventional treatment systems (Dhote & Dixit 2009). Vymazal and Kröpfelová (2008) classified the constructed wetland into three main types depending on the water flow regime, which are; sub-surface flow (vertical and horizontal flow), surface flow, and combined or hybrid systems. Despite their environmental value, the challenges for constructed wetlands are the inconsistency of treatment quality, the association between plant selection and the capacity to remove contaminants, the cost associated with wetland construction, and the footprint from geographical modification, often resulting in them not being applied in some areas (Ilyas & Masih 2017).

Emergent macrophytes, submerged macrophytes, and free-floating macrophytes are three types of wetland plants which are classified depending on their growing zones (Vymazal & Kröpfelová 2008). According to a handbook of constructed wetlands by the United States EPA (1994), the emergent macrophytes, such as common reeds *(Phragmites)*, rushes *(Juncus)*, sedges *(Cyperus)*, and cattails *(Typha),* are often recommended because of their tolerance to a high nutrient concentration and change in the environment. Maine et al. (2007) conducted two years of experiments between different types of wetland plants in a fluctuating environment of low water levels with low pH, high electrical conductivity, and high metal concentrations. They found that emergent macrophytes were the most effective growth type, which became the dominant species in that study area as well as causing the disappearance of floating macrophytes at the end of the experiment (Maine et al. 2007). Besides their survival in challenging environments, emergent macrophytes are the most popular type of plants in constructed wastewater treatment wetland because of their superior root development and nutrient removal capacity in comparison to other types (EPA 1994). It is believed that a greater root area can increase the habitat for attached growth of microorganisms in the root zone. Wetland macrophytes are also well known to transfer photosynthetic oxygen to the rhizosphere (Colmer 2003; Miller & Cramer 2005). As a consequence of the increase in microbial activities and radial oxygen loss in the rhizosphere, nitrification/denitrification is actively performed which boosts nitrogen removal (Weragoda et al. 2012).

1.4. High rate algal ponds (HRAPs) in wastewater treatment

High rate algal ponds are an ecological engineered wastewater treatment system, which were innovated in the USA. They are an alternative treatment for conventional wastewater treatment ponds and have been deployed in many countries (Young, Taylor & Fallowfield 2017). In comparison to traditional wastewater treatment plants, HRAPs are more cost effective and easier in operation and maintenance (Craggs et al. 2014). The treatment incorporates various natural processes which purposely maximise the algal growth in the ponds in order to break down dissolved organic matter and increase the treatment efficiency (Young, Taylor & Fallowfield 2017). In some HRAPs, paddlewheels have been used to promote the aerobic treatment instead of CO_2 addition. In biological interaction, a massive algal photosynthesis during the daytime leads to the increase during the daytime dissolved oxygen which could be 200-300% supersaturation. A massive uptake of CO_2 and bicarbonate in photosynthesis in day time then causes carbon limitation and high pH levels (>9) (Craggs et al. 2014). Under the same circumstance, the dissolved oxygen from photosynthesis and the paddlewheel method is available for aerobic microorganisms (including heterotrophic bacteria) to mineralise the organic carbon and generate available CO_2 to support the algal photosynthetic activity in the system (Young, Taylor & Fallowfield 2017). In terms of nitrogen removal mechanisms, total nitrogen is decreased by being assimilated into algal/bacteria biomass. In the dissolved oxygen environment, ammonium is converted to nitrite and nitrate by biological nitrification (Chen, Ling & Blancheton 2006). During night-time, on the other hand, heterotrophic respiration causes the CO_2 concentration to rise and the pH to decrease. Dissolved oxygen (DO) also decreases in the absence of photosynthesis and due to bacterial respiration. The remaining inorganic nitrogen in the treated effluent may be considered a biological benefit especially for irrigation (Craggs et al. 2014).

1.5. Floating wetland system

Floating wetland systems are a development of wastewater treatment using plants (Weragoda et al. 2012). Besides the term 'floating wetland systems', there are many other terms which are used to refer to similar treatment systems, such as constructed floating wetlands (Benvenuti et al. 2018), artificial floatin beds, artificial floating islands (Yao et al. 2011; Yeh, Yeh & Chang 2015), and floating reedbed treatment systems (Ribadiya & Mehta 2014). In contrast to the classic wetland, which requires a large area, the floating treatment wetland is deployed on the surface of an existing wastewater pond without requiring additional topographical modification or surface area (Headley & Tanner 2006; Stewart et al. 2008; Weragoda et al. 2012). Floating treatment wetlands are engineered treatment systems with the concept of using emergent plants to remove pollution in wastewater. Headley and Tanner (2006) summarised the design of this treatment system as mimicing natural floating islands and a hydroponic plant system, which allows plant roots to develop underneath supporting matrices. The wetlands are designed to allow the shoots of plants to grow freely on the top of floating matrices while the biofilms in the roots play an important role entrapping the suspended matter and obtaining nutrition directly from the water (Figure 1.1). Figure 1.1 also depicts the flow of wastewater to the system from inlet to outlet in which the treated water is only discharged through the overflow after floating wetland plants. The free-floating macrophyte system readily adapts to the environmental fluctuations in water levels since the plants are deployed on a floating matrix (Vymazal & Kröpfelová 2008, p. 173). The technology is easier to manage in terms of harvesting and maintenance, hardly impacted by the wind, and the greater root expansion provides more suspended-solid entrapping capacity (Headley & Tanner 2012).

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Figure 1.1. The cross-section of a floating treatment wetland (Headley & Tanner 2006)

1.5.1. History of floating treatment wetland

As far back as the 1900s, artificial islands were established in Japan, USA, and China to support bird nesting, fish spawning, and other ecological habitats (Nakamura & Mueller 2008). In the early 1980s, further benefits of floating islands were identified by Lothar Bestmann, a German environmental engineer who defined this type of island in German as "*Schwimmkampen*", which means the combination of the floating object and plants (Hoeger 1988). With his design, the artificial floating wetlands could provide the benefit of bank erosion protection, water purification, biological disinfection, and landscape restoration (Hoeger 1988). The overarching focus of studies in the 1980s, considering this floating treatment wetland, was the benefits to ecological and landscape restoration of lakes, ponds, and reservoirs (Nakamura & Shimatani 1997). At that time, while the removal mechanism of nutrients in emergent, submerged, and free-floating macrophytes in the conventional treatment wetland was well-known and accepted internationally (EPA 1994; Maine et al. 2007; Vymazal & Kröpfelová 2008), the water quality control using emergent macrophytes and its mechanism of pollution removal in the soil-less environment were little understood. However, since the beginning of the 21st century, there has been further consideration to floating wetland treatment in terms of design, construction, and performance when deployed in many wastewater sources (Pavlineri, Skoulikidis & Tsihrintzis 2017). This could be because of the increased interest in their economic and sustainability advantages.

1.5.2. Floating treatment wetlands in the water/wastewater treatment

Research on the floating treatment wetland has occurred in many countries, including Japan (Nakamura & Mueller 2008; Nakamura & Shimatani 1997), China (Hu et al. 2010; Li et al. 2011; Yao et al. 2011), Sri Lanka (Weragoda et al. 2012), USA (Chang et al. 2013; Stewart et al. 2008; White & Cousins 2013), Italy (De Stefani et al. 2011), Belgium (Van de Moortel et al. 2010), Netherlands (Keizer-Vlek et al. 2014), New Zealand (Borne, Fassman & Tanner 2013; Tanner & Headley 2011), Australia (Nielsen et al. 2015; Sanicola et al. 2019; Schwammberger, Walker & Lucke 2017), and many more (Pavlineri, Skoulikidis & Tsihrintzis 2017). Through time, this technology has been applied to a broad range of water qualities and at various spatial scales (Table 1.2).

1.5.3. Potential factors influencing the performance of floating wetland systems

Many factors have the potential to effect nutrient removal efficiency of a floating wetland system besides the plant species employed. These include temperature, seasonal changes, hydraulic retention times and hydraulic loading rates in the treatment system, and the floating support matrix materials.

Table 1.2. Some floating wetland treatment studies in different wastewater types, the scale of experiments, plant species used, and citation of authors for each study

Wastewater types	Scale of study	Used plants	Authors
Natural water (lakes and reservoirs)	Pilot	*Zizania latifolia, Typha latifolia, Schoenoplectus Triangulatus, Sparganium erratum, Iris Pseudacorus, & Phragmites Australis*	Nakamura and Shimatani (1997)
	Pilot	*Phragmites australis, Carex elata, Juncus effusus, Typha latifolia, Chrysopogon zizanioides, Sparganium erectum, & Dactylis glomerata*	De Stefani et al. (2011)
	Mesocosm	*Typha angustifolia & Canna iridiflora*	(Weragoda et al. 2012)
	Mesocosm	*Canna flaccida & Juncus effusus*	White and Cousins (2013)
Airport runway runoff water	Pilot	*Typha* spp.	Revitt, Worrall and Brewer (2001)
Stormwater runoff	Microcosm	*Oenanthe javanica, Gypsophila* spp.*, Rohdea japonica, Dracaena sanderiana, Gardenia grandiflora, Gardenia prostrata & Salix babylonica*	Zhu, Li and Ketola (2011)
	Mesocosm	*Carex virgate, Cyperus ustulatus, Juncus edgariae & Schoenoplectus tabernaemontani*	Tanner and Headley (2011)
	Pilot	*Carex virgata*	Borne, Fassman and Tanner (2013)
	Pilot	*Carex appressa*	Nielsen et al. (2015)
	Mesocosm	*Chrysopogon zizanioides, Baumea juncea, Isolepis nodosa, Phragmites australis & Sarcocornia quinqueflora*	Sanicola et al. (2019)
	Real case	*Carex appressa*	Schwammberger et al. (2019)
Sewage wastewater	Mesocosm	*Canna, Cyperus, & Paspalum*	Ayaz and Saygin (1996)
	Real case	*Vetiveria zizanioi*	Ash and Truong (2003)
	Real case	*Typha domingensis*	Benvenuti et al. (2018)
	Mesocosm	*Carex* spp. *(>95%), Lythrum salicaria, Phragmites australis & Juncus effuses (<5%)*	Van de Moortel et al. (2010)
Mine drainage water	Microcosm	*Phragmites australis*	Abed, Almuktar and Scholz (2019),
Eutrophic water	Mesocosm	*Lolium perenne.*	Li et al. (2011)

- **Temperature and seasonal change**

Plant growth performance and contaminant removal efficiency may be associated with temperature and seasonal changes. Within the literature, there were a tendency was observed for researchers to conduct experiments on floating wetland treatment between Spring and Summer. To understand whether the change in temperature can impact the floating wetland system, Hu et al. (2010) conducted a microcosm-scale experiment comparing the system with aquatic plants and without plants in hypereutrophic water at different temperatures (10°C, low; 22°C, medium; and 35°C, high). Water dropwort (*Oenanthe javanica D.C.)* and watercress *(Nasturtium officinale)* were used in the study by Hu et al. (2010). The authors found that after a 4-day treatment, there was no change in nitrogen concentration in tanks in the absence of plants. In contrast, nitrite nitrogen (NO_2–N) concentration in the floating wetland system decreased from 0.23 to 0.01 mg/L at all temperatures. The higher the temperature, the more the NO_3-N concentration decreased. At a temperature of more than 22°C, total phosphorus, chlorophyll *a,* and BOD_5 were removed at 78%, 70% and 85% respectively. These findings indicated that the increase in temperature influenced nitrogen removal efficiency. They also suggested the mix of plant species in the system could enhance chemical removal capacity. Although it was unclear regarding nutrient storage in the experimental plants, enhanced microbial activities and nutrient uptake in water was claimed to be due to the presence of plants (Hu et al. 2010). Van de Moortel et al. (2010) also reported a similar influence of temperature but over a different temperature range. They found that during the two-year study period, sedges *(Carex spp)* performed the highest nutrient removal efficiency at a temperature of less than 15°C. Total nitrogen (TN), NH_4–N and P removal was 56.8%, 51% and 30.1% respectively. At temperatures of <5 °C and >15 °C, nitrogen removal relapsed. In the same study, Van de Moortel et al. (2010) also discussed whether seasonal changes could have a possible effect on treatment performance, as solar radiation and air temperature varies with season. They found the temperature under that floating wetland system changed according to the seasonal change. Also, the authors found a significant difference between the air temperature and temperature in the floating wetland system. Floating mats acted as a buffer hampering the diffusion of oxygen from the air to the water column and dampening the wide variance of temperature in the system. Therefore, in winter, the floating wetland still actively performed the removal of TN, NH_4-N and TP at 47%, 35.1%, 18.40% respectively (Van de Moortel et al. 2010). It is interesting to note that this removal efficiency was similar and even higher in some parameters in comparison to the removal in summer (Van de Moortel et al. 2010). The key learning from this finding can be that seasonal change seems not to be a key factor influencing nutrient removal in the floating wetland system compared to the water temperature and other environmental conditions. On the other hand, Zhou and Wang (2010), studied the purifying and decay phase of *Oenanthe javanica* in an ecological floating bed system. Their hypothesis was that plants provide only temporary storage of nutrients and release chemical compounds back to the environment as associated with the nutrient cycle. Their findings confirmed their hypothesis where in the purifying phase (1^{st} – 35^{th} day), the floating wetland system removed TN from 12.58 mg/L to 1.16 mg/L, NH_4-N from 9.33 mg/L to 0.31 mg/L and P from 0.68 mg/L to 0.16 mg/L. Also at plant senescence (36^{th} – 63^{rd} day), the nutrient concentration increased from 1.16 mg/L to 3.03 mg/L of TN, 0.31 mg/L to 4.52 mg/L of NH_4-N, and 0.16 mg/L to 0.32 mg/L of P. Harvesting biomass was recommended to avoid plant decay (Zhou & Wang 2010). However, Lin et al. (2002) explained that when plants were harvested, the source of carbon to support the microbial activities would be limited and impact the nitrogen removal process (Lin et al. 2002). As such, the best management for avoiding plant decay and maintaining nutrient removal remains unclear. It is possible that different plants might have different characteristics at certain temperature, seasonal periods, and at senescence.

- **Hydraulic retention time and hydraulic loading rate**

Hydraulic retention time (HRT) and the influent hydraulic loading rate may also influence nutrient removal efficiency. These factors may influence the settling of suspended particulate matter and plant metabolism associated with the breakdown and absorbance of contaminants from the wastewater (Ewemoje, Sangodoyin & Adegoke 2015; La Mora-Orozco et al. 2018). According to the constructed wetland manual of Department of Planning and Local Government of South Australia (2010), the notional retention time in the wetland for sensitive urban water in Adelaide was recommended to be 3 days but not less than 2 days. Ewemoje, Sangodoyin and Adegoke (2015) suggested a hydraulic retention time of 7 days for treating wastewater from an anaerobic lagoon using tear grass *(Coix lacryma jobi)*. The hydraulic loading rate to the experiment wetland was 19.91 m^3/m^2/day. The removal efficiency of phosphorus and TSS was up to 89.1% and 61.3% respectively (Ewemoje, Sangodoyin & Adegoke 2015). While La Mora-Orozco et al. (2018) recommend HRT of more than 10 days for piggery wastewater using cattail *(Typha sp.)* and bulrush *(Scirpus sp.).* They found that at a chemical oxygen demand (COD) concentration of 400 mg/L the average of removal efficiency for Total Kjeldahl Nitrogen (TKN), NH_3–N, and TP was 70% at a 5-day HRT and reached 85% efficiency at a 10-day

HRT. The finding from these two studies illustrates that the higher chemical loading rate requires longer hydraulic retention time to effectively perform the treatment process. However, these findings were based on the treatment performance of constructed wetlands systems which involved the use of gravel filtration. This raises the question as to whether the hydraulic retention time and the hydraulic loading rate from the above studies are relevant to a floating reedbed system. In general, there are limited published studies on examining the effectiveness of floating wetlands for wastewater treatment in different hydraulic retention times and hydraulic loading rates.

- **Floating materials**

There are records of artificial floating materials being used in the floating wetland system. A popular commercial buoyant matrix is BioHaven® Floating Matrix, which is supplied by Floating Islands International Inc., USA (Stewart et al. 2008) and Waterclean Technologies, New Zealand (Tanner & Headley 2011). This matrix was designed to support the plant standing, accommodate bacteria growth, and provide a space to support microbial activities in the system. Stewart et al. (2008) investigated the role of the buoyant matrix in water purification. They conducted a laboratory study on the nutrient removal efficiency of BioHaven mats in the absence of plants. The nutrient solution was a mix of commercial fertilizers and ammonium chloride. They found that there was microbial growth in the floating bulk (0.36 m^2 surface and 0.3 m thickness), which indicated a positive nutrient removal process. Within 16 days, they found removal rates of approximately 10,600 mg/day for nitrate, 273 mg/day for ammonium, and 428 mg/day for phosphate. Other studies such as Tanner and Headley (2011), Borne et al. (2015), Nichols et al. (2016) Lucke, Walker and Beecham (2019) and others (Ash & Truong 2003; Pavlineri, Skoulikidis & Tsihrintzis 2017) tended to apply this matrix more to supporting the plant standing and enhancing microbial activities rather than using the matrix to remove the pollution. Besides the commercial matrix, artificial floating wetlands have been made from coconut coir (Walker, Tondera & Lucke 2017), Styrofoam (Keizer-Vlek et al. 2014), Beemats (Van de Moortel et al. 2010), pontoons (Ash & Truong 2003), and floating plastic connected cables (Weragoda et al. 2012). Overall, there is a tendency to ignore that the composition of the material used for the buoyant matrix might be able to enhance the nutrient removal efficiency of the floating wetland system. Nutrient sorption by the matrix seems not to be a key focus on floating treatment wetlands systems, but instead more focus on plant tissue accumulating nutrients and water quality changes.

1.5.4. Floating treatment wetlands in Australia

In Australia, floating treatment wetlands came to the attention of researchers around two decades ago. Ash and Truong (2003) reported a brief study on the use of Vetiver grass as alternative method for Toogoolawah Sewerage Treatment in South East Queensland after the wastewater treatment plant failed to comply with new license conditions from the Environmental Protection Agency. Ash and Truong (2003) suggested this innovation, rather than upgrade the whole system at high cost. The Vetiver floating pontoons were installed on the effluent pond in combination with planting Vetiver along the pond sides. The results showed there was an association of plant installation with nutrients removed in the pond after 3 months. Ammonia was found to reduce from 1.7-1.9 mg/L to 0.07-0.57 mg/L. Total nitrogen reduced from 13-20 mg/L to 6.7-7.3 mg/L and total phosphorus decreased from 4.6-8.8 mg/L to 1.2-2.1 mg/L respectively. The seasonal fluctuation was considered the key factor causing poor growth of the plant. However, nutrient accumulation in the plant tissue was not studied. Consequently, it is not clear whether the nutrient removal was associated with the Vetiver plants at the side of the pond or by those floating in the pond as they were installed in the same number of plants.

In more recent years, floating treatment wetlands have been implemented mostly in Queensland. The studies by Nichols et al. (2016), Schwammberger, Walker and Lucke (2017), and Walker, Tondera and Lucke (2017) are a series of report on stormwater treatment at Bribie Island, Queensland. Tall sedge (*Carex appressa*) was the only emergent wetland plant used in these studies. Contaminant concentrations in the stormwater were considered low, comprising 19 - 414mg/L of TSS, 0.28-0.5mg/L of TP and 0.6 - 3.2 mg/L of TN. The finding confirmed the positive performance for nutrient removal at 80% of TSS, 53% of TP and 17% of TN. Schwammberger et al. (2019) applied this approach to two large-scale stormwater ponds at a new urban development area of south-east Queensland. About 35.2 kg of total nitrogen and 1.98 kg of total phosphorus was recorded to be removed by tall sedge over a 16-month period. The study of Sanicola et al. (2019) confirmed the effectiveness of this application in the saline environment of a stormwater pond using a greater variety of species, including *Chrysopogon zizanioides, Baumea juncea, Isolepis nodosa, Phragmites australis,* and *Sarcocornia quinqueflora*. They showed the differing abilities of plants for N and P removal, which supported the hypothesis of previous works in the literature on installing various plants for more nutrient removal effectiveness.

Overall, the studies in Australia using floating treatment wetlands are for stormwater, which leads to the question of whether this application can remove the polluted contaminants from wastewaters. The understandings of the effectiveness of floating treatment wetland in domestic sewage wastewater is considered limited. The suitability of wetland plants for use in this application requires investigation because what works in Queensland might not be suitable in Southern Australia. Moreover, there are underlying factors which are not well-defined in the justification of the treatment application; such as the influence of temperature, hydraulic retention time, loading rate, flotation materials, and other environmental conditions.

1.6. Research objectives and questions

The objective of this study was to fill the knowledge gaps regarding the effectiveness of floating wetlands for domestic wastewater treatment. These included the identification of aquatic plant species suitable for use in South Australia, and determination of the performance of this system in the winter when treatment performance is most likely to be most conservative. The floating wetland application was investigated at a mesocosm scale from April to August 2019, which was predominantly over winter in southern Australia, when wetland plants are more likely to stop growing. The wastewater source for this study was from the pond storing wastewater treated by the High Rate Algal Ponds (HRAPs) at the community wastewater treatment scheme at Kingston on Murray, South Australia.

The study aimed to compare growth parameters of different species of macrophytes in domestic wastewater and determine nitrogen removal rates. Four emergent aquatic macrophytes were used in the study, bare-twig rush *(Baumea juncea),* stiff-leaf sedge *(Cyperus vaginatus),* common sedge *(Carex tereticaulis),* and tall sedge *(Carex appressa).* The research questions were:

- What are the growth rates of the species of emergent macrophytes deployed as floating wetlands in mesocosms supplied with HRAP treated wastewater?
- How much nitrogen concentration can plants accumulate from the wastewater?
- How does the wastewater quality differ before and after the floating treatment wetland?

2. MATERIALS AND METHODS

2.1. Study area

The mesocosm experiment was performed at the community wastewater treatment site at Kingston on Murray (longitude 140° 20' E and latitude 34° 14' S), which is located 216 km northeast of Adelaide, South Australia. The wastewater treatment plant, comprising high rate algal ponds (HRAPs) was constructed in 2008. The wastewater influent to HRAPs was from the on-site septic tanks within the township, which has around 300 permanent residents (Young, Buchanan & Fallowfield 2016). The system was mainly operated by the Health and Environment Group at Flinders University of South Australia, which is a research unit based in the university's College of Science and Engineering. There were two treatment ponds which consisted of two channels each with 30m x 5m dimension (see Plate 2.1 (a)). The depth of wastewater treatment ponds was 0.3-0.5m. The rate of influent addition into the ponds was approximately 12 m^3/d (Young, Buchanan & Fallowfield 2016). At six days of theoretical hydraulic retention time (THRT), the water quality of treated wastewater effluent was 16.09±9.44 mg BOD_5/L, 22.95±12.29 mg NH_4-N /L, 15.39±4.54 mg NO_3-N /L, and 12.41±2.04 mg PO_4-P/L. The *E. coli* log_{10} reduction value was 4.19±0.75 at a depth of 0.43 m. Chlorophyll *a* concentration was $3.81^{\pm 4.32}$ mg/L (Buchanan et al. 2018). The treated wastewater effluent met the Australian reuse water guideline for non-food crop irrigation (Fallowfield et al. 2018). The effluent was stored in the storage pond (Plate 2.1 (b)) before discharging to irrigate the non-food crops near the site.

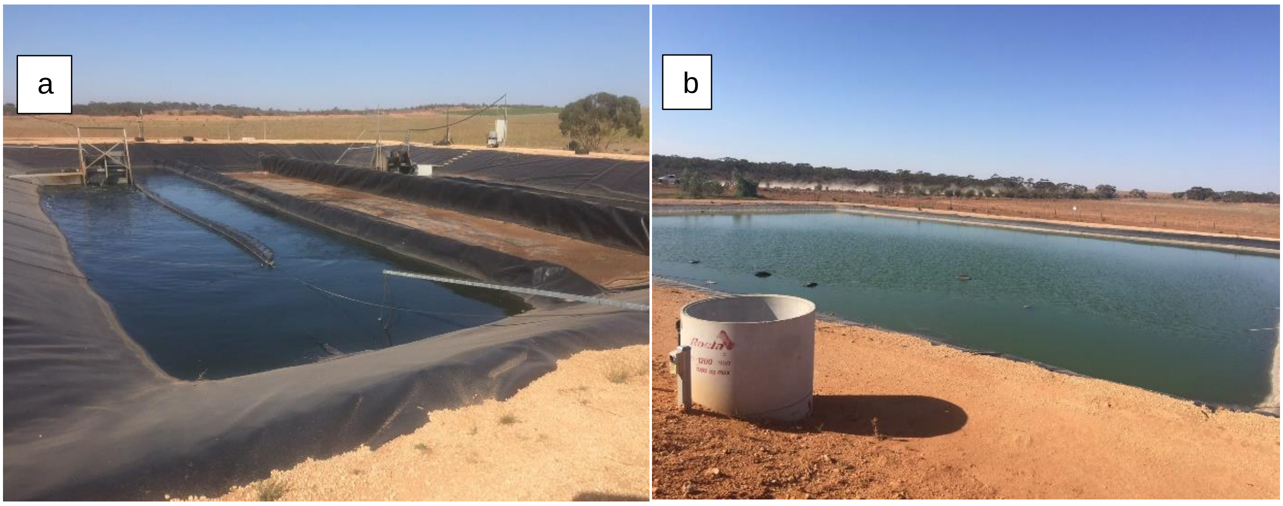


Plate 2.1. (a) a high rate algae pond and (b) the storage pond for treated wastewater effluent of HRAPs at the township of Kingston on Murray

2.2. Experimental set-up

The experiments were operated from April to August 2019 (5 months) and performed in plastic tanks. Six tanks, each 2.1m length, 1.2m width, and 1m depth were installed adjacent to the storage pond (Plate 2.2a). One tank was designed to be the control tank without floating wetland installation. The remaining five tanks were experimental tanks with floating treatment wetlands. The operational volume of wastewater was 1520 L/tank (depth of wastewater in a tank was 0.6 m). The influent used in the experiments was HRAP treated wastewater effluent obtained from the storage pond. Two submersible pumps were installed in the storage pond with each pump supplying water to three tanks operated in parallel at a flow rate of 8.4L/min to each tank, active for 30 min/d to provide a THRT of 6 days per tank. The hydraulic loading rate was 100 L/m^2/day.

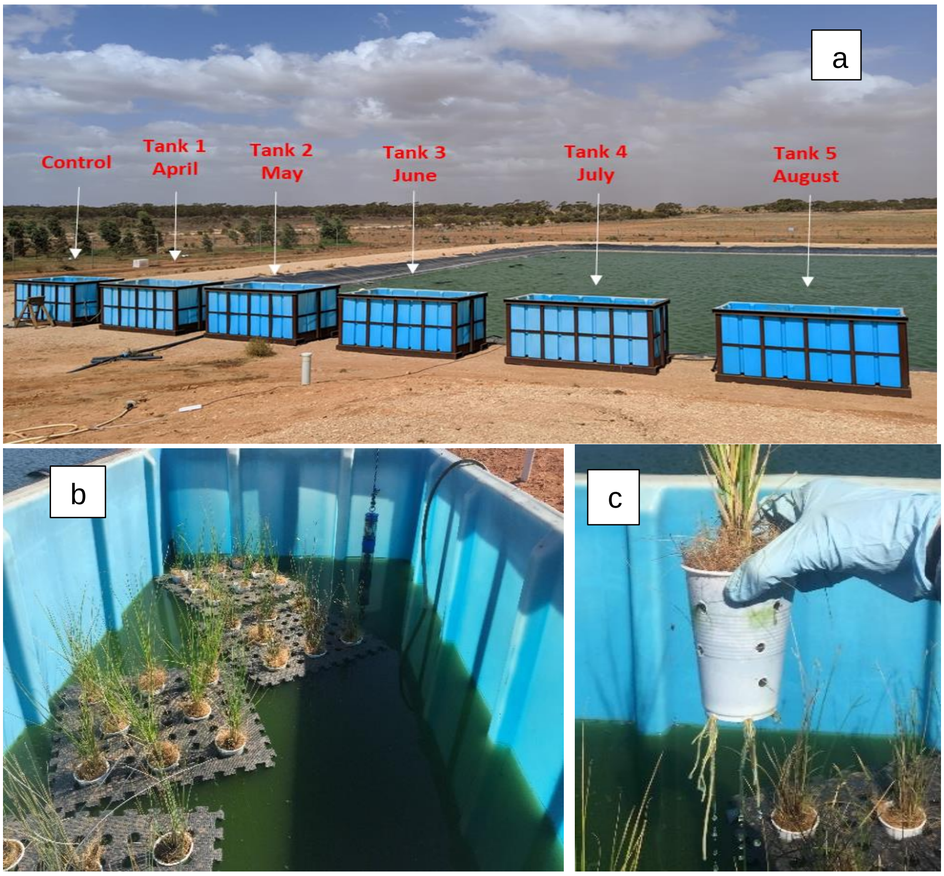


Plate 2.2. Experimental tanks (a), four floating wetland plants of different species were installed in each experimental tank (b), aerator containers using plastic cups (c)

Buoyant foam mats (0.5 m width, 0.5 m length, and 0.01 m thickness) were used to support the plants. Four floating mats were employed in each experimental tank (Plate 2.2b). Floating mats were not connected to one another but floated freely on the surface of (2.5 m^2). The floating wetland system covered around 40% of a tank surface. Each floating mat contained one plant species and was designed to support eight plant containers. The containers were specially designed to be aerator pots using plastic cups (Plate 2.2c). In a pot, there was soil and coconut coir to provide upright support of the plant. All plants in the study were installed at the same time in April. Sequential plants from each tank which represent each month then were removed and destructively tested each month from April to August of 2019.

2.3. Plant selection

The key criterion for selecting emergent macrophytes was that the species should be local to South Australia. The selected plants were also limited to those which are not nesting grasses for birds, particularly ducks or other waterfowl. Selected plants were also restricted to those with shoots less than 1-metre high. This was to avoid overturning of the floating mats when the top part of the system was heavier than the root mass. The candidate plants used in this study were bare-twig rush (*Baumea juncea*), stiff-leaf sedge (*Cyperus vaginatus*), common sedge (*Carex tereticaulis*), and tall sedge (*Carex appressa*), which were supplied by EcoDynamics Pty Ltd (SA) nursery. These plants are fast-growing emergent macrophytes which are tolerant to polluted water (Romanowski 1998).

2.4. Field monitoring

Due to the limited study equipment, a water quality multi-parameter probe (Eureka Manta Sub2 Austin, Texas, Plate 2.3a) was installed, on rotation (Table 2.1), in the tanks to continuously monitor temperature, dissolved oxygen, and pH at 4-hour intervals. The probe was located approximately 10-15 cm below the surface of the water. The probe was calibrated according to the manufacturer's instructions. Simultaneously, an automatic, refrigerated (1°C) Avalanche® Sampler (Teledyne ISCO Lincoln, Ne, Plate 2.3b), was installed to collect the wastewater samples. Wastewater (150ml) collected at 3 am and 3 pm for each of two days into the same sample bottle (total sample 600mL), representing a 2-day composite sample. Moreover, the YSI ProDSS multi-parameter meter (Xylem, USA) was used to measure conductivity and ammonium (NH_4-N) in each water sample collected from the sampler.

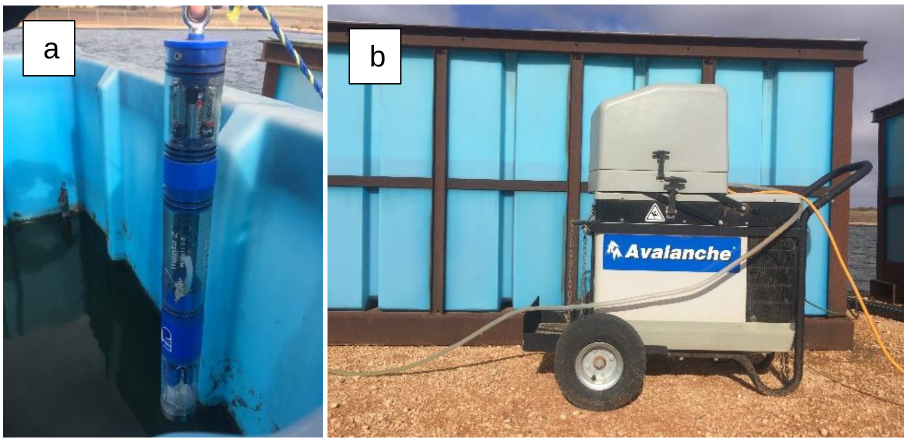


Plate 2.3. The in-situ water quality parameter (a) and the automatic water sampler (b).

Table 2.1. The rotation in the tanks of the multi-probe (Eureka Manta Sub2, Austin, Texas) water quality monitoring system from April to August 2019

Tank ID	Description	April		May		June		July		August	
		1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half
Control	Control tank	x		x		x		x		x	
Tank 1	First-month experiment		x								
Tank 2	Second-month experiment				x						
Tank 3	Third-month experiment						x				
Tank 4	Fourth-month experiment								x		
Tank 5	Fifth-month experiment										x

Note: (x) was the period over which the probe was installed in the respective tank.

2.5. Laboratory analysis

2.5.1. Plant sampling and analysis

Every month from April-August 2019, plants were brought from Kingston on Murray to the Environmental Health laboratory of Flinders University. The growth rates of emergent macrophytes were measured based on the development of roots (below-mat), shoots (above mats) and the increase in plant biomass. Nitrogen content in plant tissues was measured to determine nutrient uptake by plants from wastewater. The measurement started from the zero-month samples (plants before the experiment), 1st-month sample (April), 2nd-month sample (May), through until 5th-month sample (August). The procedure of plant measurement and analysis was based on the *Laboratory Guide for Conducting Soil Tests and Plant Analysis* by Jones (2001).

2.5.1.1. Plant growth performance

The measurement of plant height (cm, shoots) accounted only for the part exposed to full sunlight, which was from just above the growing pot to the highest part of shoots, while the length of roots (cm) was measured from the base of the shoot to the longest point of the plant roots.

The growth rate of plants was calculated using equation 1:

Relative plant growth rate (cm/day) = $\frac{H_t - H_0}{t}$ (Equation. 1)

Where: H_0 = initial height of shoot/root in zero month (cm)
H_t = height of shoot/root after planting for a certain time (cm)
t = the number of planting days (days)

2.5.1.2. Dry biomass

Before drying the plant samples, the coconut coir was removed from plant roots. Dead plant tissue was removed from shoot samples. Eight samples of the same species in each month (April-August) were combined for enough biomass volume and dried at 80-105°C for at least 24 hours before weighing the dry biomass. Due to the different residual soil content in the seedling pots, the root biomass was measured only for the extensive roots from plant pots (Plate 2.4 (a & b)). Shoot/root biomass of each species (n=8) was determined by harvesting the shoot/root materials (g) in a buoyant foam mat surface area (0.5cm x 0.5cm). The unit of dry biomass in this study was g DM/mat. Similar to the calculation of plant growth rate, the biomass growth rate was calculated using equation 2.

Relative plant biomass growth rate (g DM/mat/day) = $\frac{M_t - M_0}{t}$ (Equation. 2)

Where: M_0 = initial biomass of shoot/root in zero month (g DM/mat)
M_t = biomass of shoot/root after planting for a certain time (g DM/mat)
t = the number of planting days (days)

Moreover, the dried shoot and root samples of each species were ground (Plate 2.4c) using a laboratory blender (7011HS, Waring® Laboratory Science) and stored in a plastic sample jar at room temperature until required for the nitrogen analyses described below.

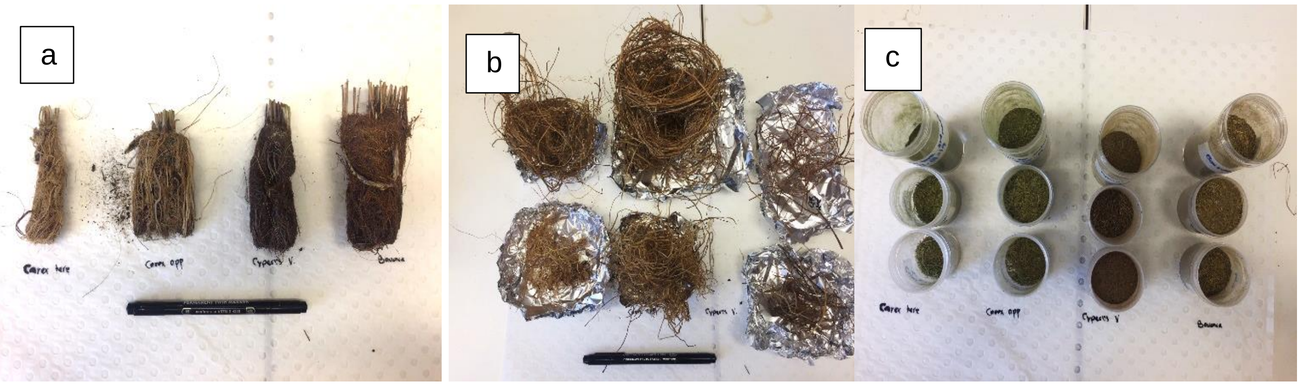


Plate 2.4. The dried plant samples showing the different residual contents of soil substrate (a), the root samples (b) and blended shoot samples of each month (c)

2.5.1.3. Plant tissue analysis

The ground plant samples were used for nitrogen analysis. Nitrogen content from above-mat and below-mat biomass were analysed separately. The nitrogen content was analysed using wet acid digestion (Jones 2001, p. 211). The TOC-L series of TOC/TN analysers (Shimadzu Corporation) was used to measure total nitrogen in digested samples. Three replicates of each sample were employed. As a control, sweet sorghum flour, with a known nitrogen content was also analysed at the same time as a control. The initial unit of nitrogen content was presented in N% / g DM. Then it was converted to mg N /g DM. The final unit of shoot/root nitrogen accumulation was in mg N/mat by multiplying shoot/root biomass of a species per mat. The total nitrogen accumulation by all floating wetlands was also all measured by the sum of plant nitrogen content in both root and shoot of all four species. The result was present in mg N/m^2. This was because a mat had 0.25 m^2 x 4 = 1 m^2. The relative nitrogen accumulation rate was calculated using equation 3.

$$\text{Plant nitrogen accumulation rate (mg N/m}^2\text{/day)} = \frac{N_t - N_0}{t} \qquad \text{(Equation. 3)}$$

Where: N_0 = initial nitrogen content in four species (shoot + root) in zero month (mg N/m^2)

N_t = nitrogen content in four species (shoot + root) after planting for a certain time (mg N/m^2)

t = the number of planting days (days)

2.5.2. Wastewater analysis

The wastewater quality in the tanks containing the floating treatment wetland was analysed in the Environmental Health laboratory of Flinders University within the day of collection from the field and compared with that of the control tank. The analytical methods were based on *Standard Methods for the Examination of Water and Wastewater* (Greenberg, Clesceri & Eaton 1992).

2.5.2.1. Suspended Solids (SS)

Glass microfiber filters of 1.2 µm pore size were pre-dried (24h at 105°C) and pre-weighed before triplicate aliquots of wastewater (50-100mL) were filtered. The filters were dried (24h at 105°C), weighed and the suspended solids (mg SS/L) determined by difference between pre- and post- weight. The analysis of this measurement was defined in Test 2540 D of *Standard Methods for the Examination of Water and Wastewater* (Greenberg, Clesceri & Eaton 1992). The filtrates were stored frozen (-20°C), until required for the chemical analyses described below.

2.5.2.2. Chlorophyll-*a*

Triplicate aliquot of wastewater (25mL) was filtered through glass microfiber filters of 1.2 µm pore size (mg/L). Aqueous acetone (90%v/v) was used to extract the photosynthetic pigment in the filtered filters and was analysed in UV-1800 UV/Visible Scanning Spectrophotometer (Shimadzu Cooperation). A sample blank was tested before measuring samples. The spectrophotometric method was described in Test 10200 of *Standard Methods for the Examination of Water and Wastewater* (Greenberg, Clesceri & Eaton 1992). The filtrates were stored frozen (-20°C), until required for the chemical analyses described below.

2.5.2.3. Nitrogen speciation

Triplicate aliquots of filtered wastewater (25mL) were analysed for dissolved total nitrogen (mg N/L) in a TOC-L series of TOC analyser (Shimadzu Cooperation). Ammonia (mg NH_4-N/L), NO_3-NO_2 (mg NO_x-N/L) and phosphate-ortho (mgPO_4-P/L) were analysed using SAN++ Automated Wet Chemistry Analyzer (Skalar).

2.5.2.4. Total organic carbon (TOC) and inorganic carbon (IC)

Triplicate aliquots of filtered wastewater (25mL) were analysed using the TOC-L series of TOC analyser (Shimadzu Corporation). The sample preparation and method for analysis were described in Test 5310 of *Standard Methods for the Examination of Water and Wastewater* (Greenberg, Clesceri & Eaton 1992)

2.5.2.5. Biochemical Oxygen Demand

The 5-day Biochemical Oxygen Demand (BOD_5) was determined using The OxiTop® Control system. The selected sample volume was 250 ml. The method of this analysis was described in the OxiTop® Control operating Manual (WTW 2006).

2.5.2.6. *Escherichia coli* (*E. coli*)

Escherichia. coli was enumerated using 100 mL of a water sample with a single Colilert Quanti-Tray® (IDEXX Laboratories, Inc.). The analysis was based on the manufacturer's instruction. The results were reported as the Most Probable Number (MPN) *E*. coli per 100 ml. A sample blank was analysed at the same time to ensure the quality control of sample processing.

2.6. Data analysis

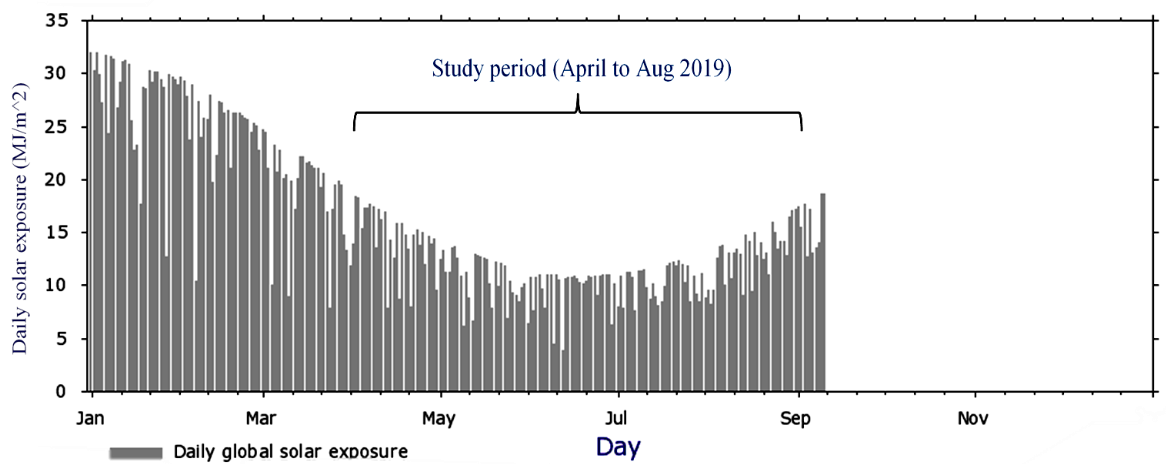
Statistical analysis was performed using Microsoft Excel. Data was presented as the mean value ± one standard deviation. ANOVA was performed to determine the difference in wastewater quality (e.g. temperature, pH, DO, SS, Chl-*a*, TOC, IC, NH_4-N, NO_x-N, PO_4-P, BOD_5, *E. coli*) and plant growth between and amongst treatments. Statistically significant difference was accepted at p <0.05. Due to low replication and combination of samples for enough biomass volume, a Bonferroni Correction was applied which based on number of tanks in the study (n = 6). Therefore, statistically significant difference for plant biomass was accepted at p = 0.008. The regression model was performed to analyse R^2 as an indication of the correlation between two variables which are; (1) the plants growth in shoots/roots, (2) the growth in shoot/root biomass and nitrogen content in shoot/root tissue, (3) The variables between temperature as the independent variable and pH and DO as dependent variables from the *in-situ* record, and (4) the concentration of suspended solids (independent) and chlorophyll-a (dependent) from the laboratory result.

3. RESULTS

3.1. Climatic conditions

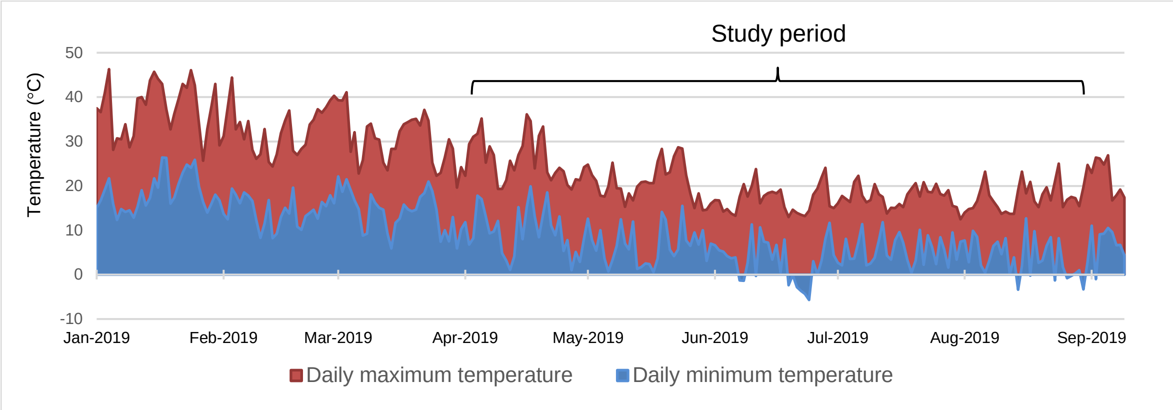
The climatic conditions at Kingston On Murray, including solar exposure and air temperature, were obtained from the Bureau of Meteorology (2019a). Over the study period, the highest daily solar exposure was in April (18.3 MJ/m^2), while the lowest was in June (3.8 MJ/m^2) (Figure 3.1). Overall, the study period was in the lower, winter range of solar exposure for the year 2019.

The air temperature at Renmark Aero (station 024048), the nearest station to Kingston on Murray, SA (Figure 3.2) showed a changing trend over time, which was similar to the daily solar exposure. The mean maximum and mean minimum air temperatures of the study period (April to August) were obviously lower than those of the earlier months (January to March). During the study period, the mean maximum air temperature was 25.9 °C in April and significantly dropped to 17°C in June. In July and August, the temperature increased slightly with mean maximum temperatures of 17.6 °C and 17.7°C respectively. The lowest mean minimum air temperature was in June (3.2 °C). The annual precipitation at Kingston On Murray in 2018 was recorded at 115.6 mm (Bureau of Meteorology 2019c). In the year 2019 from January to August, the rainfall was 61mm at Renmark Aero (Bureau of Meteorology 2019b).



Solar exposure (MJ/m^2)	Jan	Feb	Mar	Apr	May	Jun	July	Aug
Maximum daily	31.9	29.5	24.6	18.3	13.6	10.9	12.2	17.1
Minimum daily	12.6	10.3	7.7	7.8	6.1	3.8	7.5	8.1
Monthly mean	28.1	25.1	18.8	14.4	10.5	9.8	10.1	12.9

Figure 3.1. The changes in daily solar exposure 2019 (Figure) and the daily maximum, daily minimum and monthly mean solar radiation (Table; MJ/m^2) at Kingston On Murray, SA (Station 024006, Bureau of Meteorology, 2019a).



	Jan	Feb	Mar	Apr	May	Jun	July	Aug
Mean maximum temperature (°C)	37.5	32.6	30.2	25.9	20.7	17	17.6	17.7
Mean minimum temperature (°C)	18.4	14.3	14.1	9.8	6.7	3.2	5.5	3.7

Figure 3.2. The 2019 daily maximum and minimum temperature (Figure) and the 2019 monthly mean maximum and mean minimum temperature at Renmark Aero (station 024048), about 30 km away from Kingston On Murray, SA (Bureau of Meteorology 2019b).

3.2. *In situ* physico-chemical wastewater quality

The results on temperature, pH, and DO were obtained for the Control tanks (no floating treatment wetlands) in only three months in May, June, and August, while the Experimental tanks were monitored on a monthly basis before plant harvest; Tank 1 in April, Tank 2 in May, Tank 3 in June, and Tank 5 in August. Data was not obtained from Experimental Tank 4 and some months from the Control due to a field technical issue.

3.2.1. Temperature

Figure 3.3 shows the 4-hour intervals and daily mean temperature fluctuations in the Control (a) and the Experimental tanks (b). A one-way ANOVA indicated that the daily mean temperature varied significantly (p <0.05) between the Control and the Experimental tanks over the study period. Although there was only a 4 °C variance between the daily mean temperatures of the Control tank in May, June, and August, the ANOVA showed a significant difference among them (p <0.05; Table 3.1). While the daily mean temperature between the Control and Experiment tanks in May and August did not show a great difference, the daily mean temperature of the Control in June was 2 °C lower than that in Experimental Tank 3 (p = 0.026, Table 3.1&3.2). During the monitoring time, the maximum temperature was 19.79 °C recorded in Tank 1 (April), while the minimum temperature was 4.98 °C in the Control tank in June.

3.2.2. pH

The daily mean pH in both Control (a) and Experimental tanks (b) was within the range of 8 to 10 (Figure 3.4). Although the mean daily pH varied significantly ($p<0.05$) in the Control and Experimental tanks over different months, the daily mean pH of the Control and Experimental tanks with the floating wetland in the same month was not different (p = 0.43 in May, p=0.06 in June, and p = 0.66 in Aug). In the control tank, the mean monthly pH was $8.50^{\pm0.47}$ in May, $8.84^{\pm0.30}$ in June, and $9.32^{\pm0.51}$ in August increasing slightly each month (Table 3.2). The monthly mean pH in Tank 1 (April) was $9.19^{\pm0.31}$ and in Tank 2 (May) $8.58^{\pm0.27}$. The monthly mean pH value showed an increase from $8.74^{\pm0.33}$ in Tank 3 (June) to $9.26^{\pm1.15}$ in Tank 5 (August). It was obvious that the change of pH in the 4-hour intervals in Tank 5 (Figure 3.4b) was more rapid in comparison to other Experimental tanks and the Control (Figure 3.4a) which was possibly due to the electron exchange from plant uptake of NH_4^+ and NO_3^-.

3.2.3. Dissolved Oxygen (DO)

The changes in dissolved oxygen are shown in Figure 3.5a for the Control tank and Figure 3.5b for tanks with floating wetlands. In general, most tanks had a dissolved oxygen of >10 mg/L possibly as the combination of algae photosynthesis, released oxygen in wetland root zone, and wind. Similar to the daily mean temperature and pH results, the daily mean DO in tanks showed diurnal and monthly variation. The daily mean DO in the Control (Table 3.1) and tanks with floating wetland mats (Table 3.2) during the early months of the monitoring (April to June) were not significantly different (p = 0.35) with the range of 13.88 mg/L – 14.57 mg/L. However, when comparing the daily mean DO in August between the Control and Experimental tank 5, the DO in the Control was higher than the Experimental tank at $13.41^{\pm1.26}$ mg/L and $11.38^{\pm2.98}$ mg/L respectively. The DO in Tank 5 also illustrated a gradually decreasing trend (Figure 3.5b) from late August to early September.

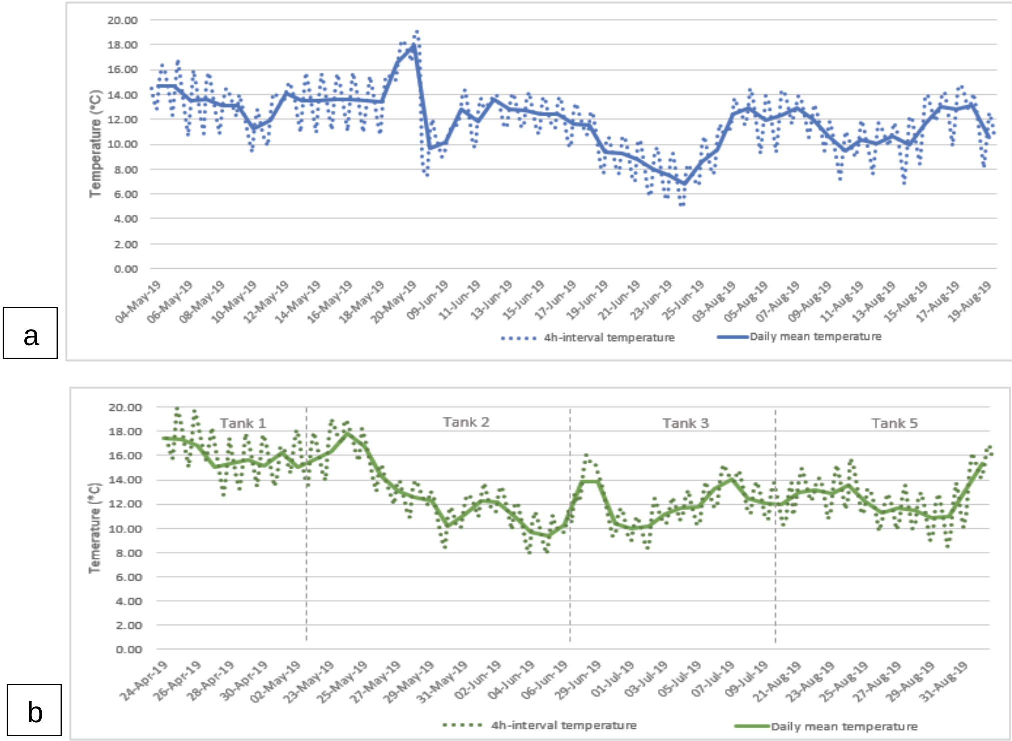


Figure 3.3. The 4-hour intervals (dots) and daily mean (solid) temperature (ºC) in the Control (a) and Experiment tanks (b) recorded by Eureka Sub2 water-quality multi-probe. The x-axis represents time/day while the y-axis displays temperature levels.

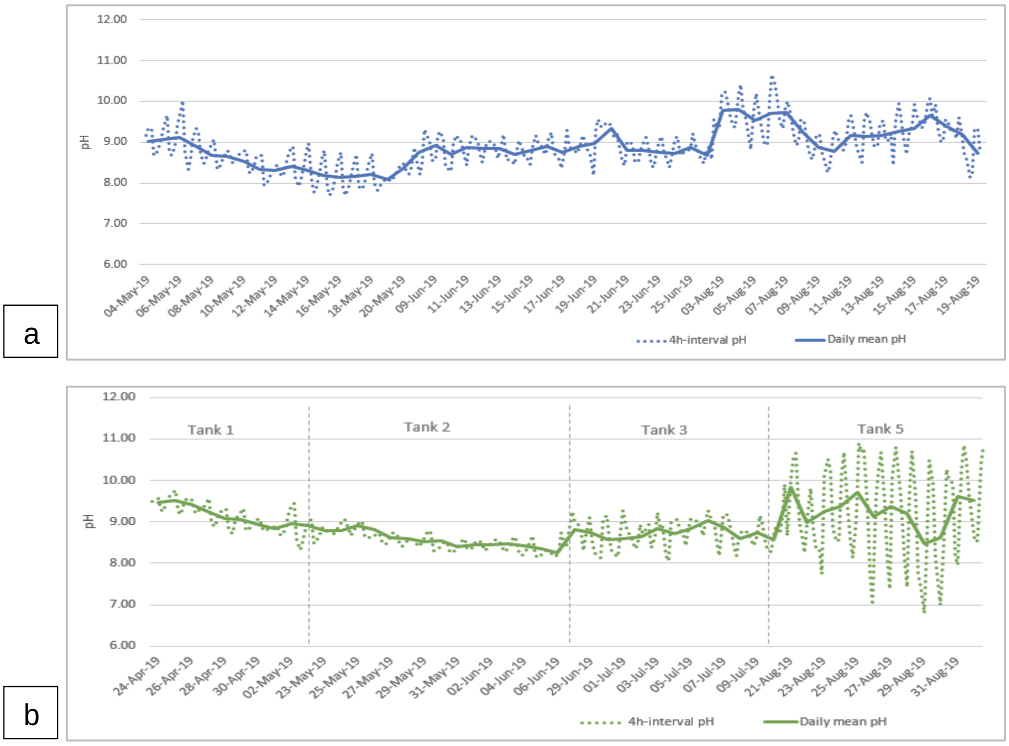


Figure 3.4. The 4-hour intervals (dots) and daily mean (solid) pH in the Control (a) and Experiment tanks (b) recorded by Eureka Sub2 water-quality multi-probe. The x-axis represents time/day while the y-axis displays pH levels

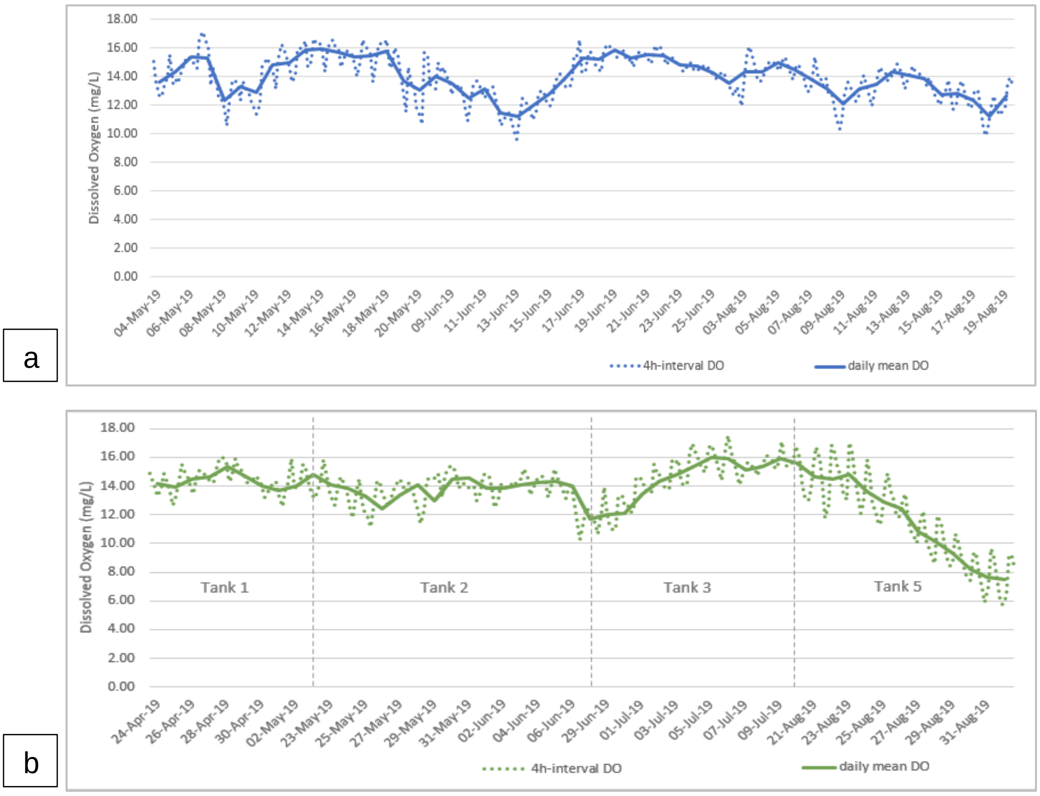


Figure 3.5. The 4-hour intervals (dots) and daily mean (solid) dissolved oxygen in the Control (a) and Experiment tanks (b) recorded by Eureka Sub2 water-quality multi-probe. The x-axis represents time/day while the y-axis displays DO levels.

Table 3.1. The mean, maximum, minimum, standard deviation (SD), and number of records (n) of temperature (°C), pH and DO (mg/L) in the Control tank in May, June and August 2019.

Parameters	**Control (May 2019)**					**Control (June 2019)**					**Control (Aug 2019)**				
	Mean	*Max*	*Min*	*SD*	*n*	*Mean*	*Max*	*Min*	*SD*	*n*	*Mean*	*Max*	*Min*	*SD*	*n*
Temp (°C)	13.87	19.12	9.39	2.07	102	10.50	14.47	4.98	2.39	114	11.58	14.84	6.88	1.82	102
pH	8.50	10.00	7.70	0.47	102	8.84	9.55	8.18	0.30	114	9.32	10.64	8.14	0.51	102
DO (mg/L)	14.57	17.13	10.61	1.55	102	13.92	16.57	9.64	1.53	114	13.41	16.14	9.91	1.26	102

Table 3.2. The mean, maximum, minimum, standard deviation (SD), and number of records (n) of temperature (°C), pH, and DO (mg/L) at the Experimental Tank 1, Tank 2, Tank 3 and Tank 5.

	Tank 1 (April 2019)					**Tank 2 (May 2019)**				
Parameters	*Mean*	*Max*	*Min*	*SD*	*n*	*Mean*	*Max*	*Min*	*SD*	*n*
Temp (°C)	16.16	19.79	12.68	1.68	48	12.79	18.94	7.80	2.79	96
pH	9.19	9.78	8.68	0.31	48	8.58	9.45	8.15	0.27	96
DO (mg/L)	14.36	15.97	12.55	0.82	48	13.88	15.89	11.13	0.95	96
	Tank 3 (June 2019)					**Tank5 (Aug 2019)**				
Parameters	*Mean*	*Max*	*Min*	*SD*	*n*	*Mean*	*Max*	*Min*	*SD*	*n*
Temp (°C)	12.06	15.95	8.25	1.80	78	12.45	16.78	8.41	1.90	72
pH	8.74	9.29	8.07	0.33	78	9.26	10.94	6.80	1.15	72
DO (mg/L)	14.42	17.47	10.27	1.75	78	11.38	16.98	5.71	2.98	72

Moreover, there were no correlation between the 4-hour interval temperature and the 4-hour interval pH and DO in the Control (F (2, 315) = 1.538, R^2 = 0.01, p>0.05) and weak correlations in Experimental tanks (F (2, 291) = 31.78, R^2 = 0.179, p<0.05).

3.3. Plant growth assessment

3.3.1. Plant growth characteristic

After five months (146 days), all emergent macrophytes survived in mesocosms supplied with HRAP treated wastewater. A general finding was that weeds were found in some pots of bare-twig rush (*Baumea juncea*) and stiff-leaf sedge *(Cyperus vaginatus)* (Plate 3.1a). The holes within the pots were shown to be inadequate for enhanced root development (Plate 3.1b). The roots were highly interwoven rather than there being many individual roots underneath the floating mat. Therefore, the roots at that stage in the experiment did not reach to the bottom of the tanks (Plate 3.1c).

Figure 3.6 shows the mean shoot height and total height (shoot + root) of the four emergent macrophytes from day zero (n=3 for each species), first-month samples in April 2019 (n=8 for each species), through until the fifth-month samples in August 2019 (n=8 for each species). The above-mat and below-mat length of plants increased over time except for the below-mat length of stiff-leaf sedge on the last month of experiment. While root length of all species started at 0 cm, the initial shoot height was slightly different with a range of 25 cm to 28 cm.

There was significant variance between the growth of shoot and root between wetland plants (p<0.05). At the end of the experiment, the tallest macrophyte among four species was the tall sedge (*Carex appressa*), which had the total height of 142 cm (i.e. 61 cm of shoot and 81 cm of root). The rate of increase by tall sedges measured over five months was 0.25 cm/day for shoots and 0.56 cm/day for roots. The second highest shoot length was the common sedge (*Carex tereticaulis*) species, which had an above-mat and below-mat length of 63 cm and 70 cm respectively. The growth rate of the shoot was 0.24 cm/day, and 0.48 cm/day for the root tissue. In comparison to other species, bare-twig rush showed the shortest growth in shoots. This species measured only 11 cm after planting for five months with a growth rate of only 0.07 cm/day. It was interesting that stiff-leaf sedges showed continual development in their roots to 35 cm length after planting for four months with the increased rate of 0.26 cm/day. Stiff-leaf sedge plants measured after five months showed inconsistent growth since the root length was only 19 cm (equivalent to 0.13 cm/day); almost a half that measured after four months growth measured in Tank 5, which was the lowest growth in root among all plants.

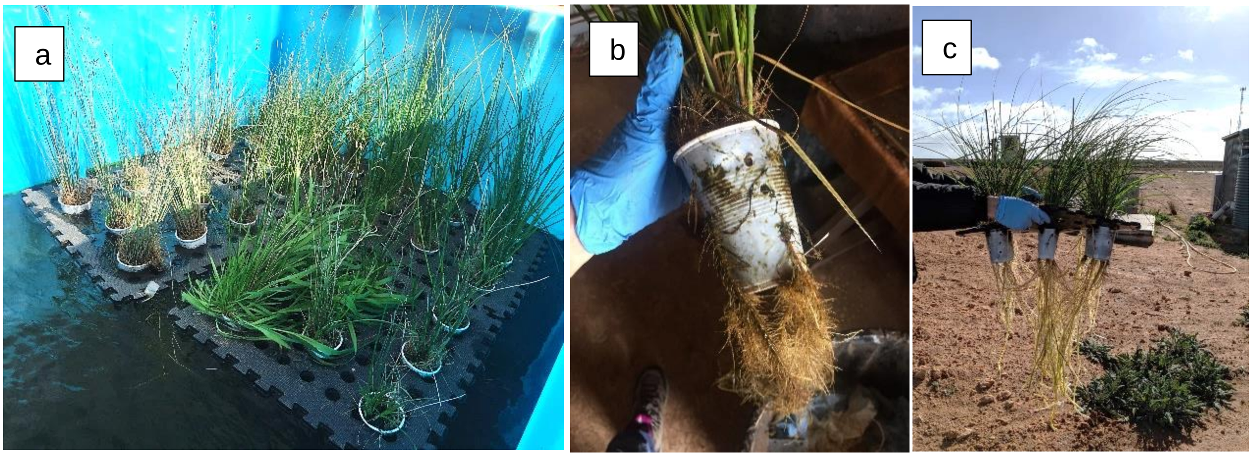


Plate 3.1. Plants growth performance shows (a) weeds found in some pots, (b) the highly dense root, (c) the interwoven of root underneath the mat of tall sedge (*Carex appressa*).

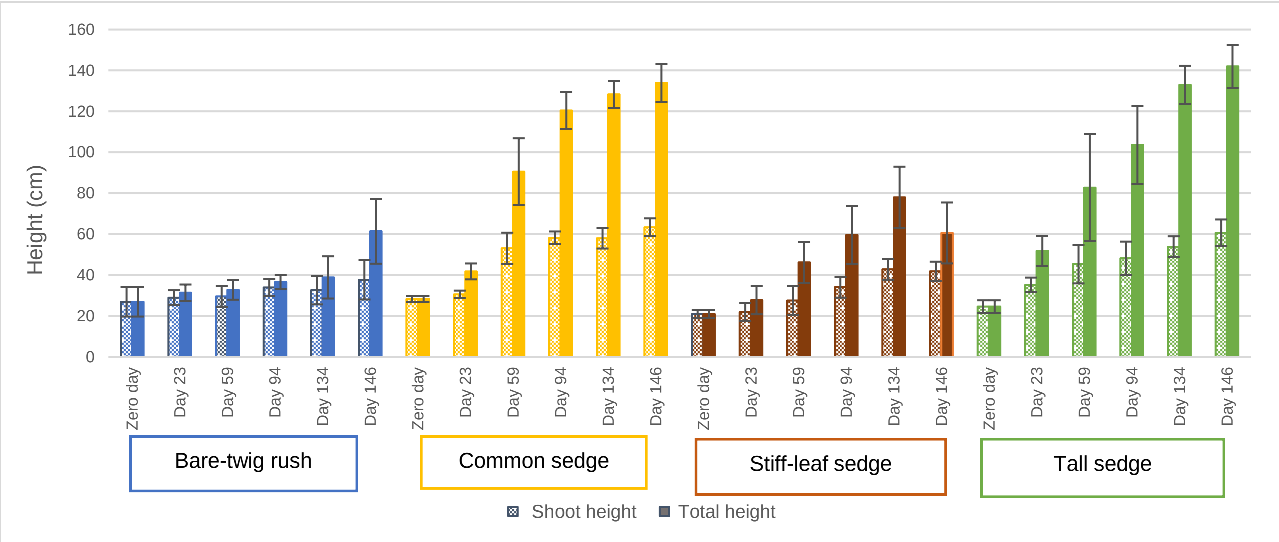


Figure 3.6. Plant growth, mean length (cm) ± one standard deviation (n=8) in which solid bars represents the whole plant (shoot and root) and pattern bars for shoot biomass of the four macrophyte species from zero-day, until the fifth month of the experiment (August).

3.3.2. Plant dry biomass

The amount of biomass of four species in different months is illustrated in Figure 3.7. Overall, shoot biomass in all species exceeded that of the roots. There were no significant differences among the mean of shoot and root biomass between four species from the zero day to the five months (n = 6, p >0.008), which identified between stiff-leaf sedges and tall sedges (p = 0.24 in shoots and p = 0.046 in roots), bare-twig rushes and tall sedges (p = 0.75 in shoots and p = 0.030 in roots), bare-twig rushes and stiff-leaf sedges (p = 0.02 in shoots and p = 0.07 in roots), bare-twig rushes and common sedges (p = 0.63 in shoots and p = 0.04 in roots), common sedges and tall sedges (p = 0.88 in shoots and p = 0.65 in roots), and common sedges and stiff-leaf sedges (p = 0.33 in shoots and p = 0.07 in roots).

It is important to note that the shoot biomass of species had different starting values, however, the below-mat biomass was the same at zero. Bare-twig rush started from the highest amount of above-mat biomass at 25.46 g dry mass/mat (a mat of 0.5m length x 0.5m width with eight plants). However, the species showed slow growth to only 35.70 g/mat after being planted for three months (94 days) and was surpassed by tall sedges and common sedges on the fourth month. Among four species that were deployed in mesocosms after five months, tall sedges had the greatest amount of above-mat and below-mat dry biomass which were 84.55 g/mat and 44.80 g/mat respectively. The growth rate of above-mat and below-mat dry biomass was 0.53 g/mat/day and 0.31 g/mat/day respectively. The second greatest macrophyte growth was common sedge. In comparison to other species, the above-mat biomass of this species started from the lowest amount at 4.18 g/mat. At the end of the experiment the above-mat biomass was 80.95 g/mat and 34.85 g/mat for below-mat dry biomass. While stiff-leaf sedge had the lowest amount of above-mat biomass after 5 months at 26.55 g/mat with the rate of 0.11 g/mat/day, bare-twig rush had the lowest amount of below-mat biomass at only 1.80 g/mat with the rate of biomass growth at 0.012 g/mat/day.

Figure 3.8 illustrates the coefficient of determination using exponential and linear fit function in examining the relationship between the plant growth and biomass in above and below mats for four species. The coefficient of determination values on the graphs were close to 1 for all species (n = 6, R^2>0.70, p<0.05) which indicated a high strongly positive correlation between the plant growth and biomass. The relationship means the increase in height of plants was associated with the increase in biomass.

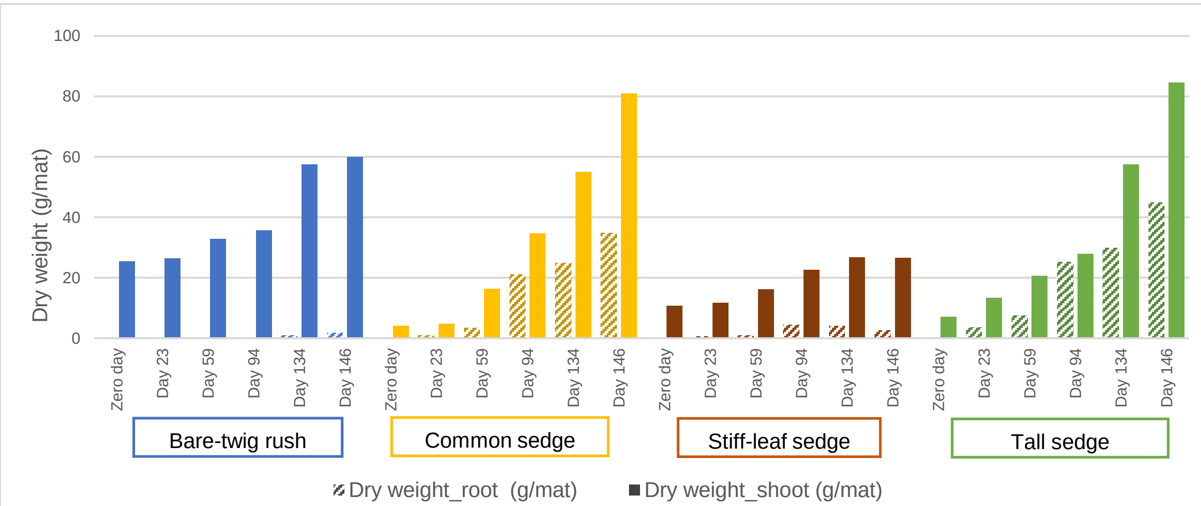


Figure 3.7. The relative dry biomass which solid bars represents the mass of above mat and pattern bars for below-mat biomass of four macrophyte species from zero-day, first-month experiment (April) until fifth-month experiment (August).

3.3.3. Plant nitrogen content

Figure 3.9a shows the nitrogen content (%/DM g) in shoot tissues of four species at day zero, first-month sample (23 days), second-month sample (59 days) and third-month sample (94 days). Figure 3.9b illustrated the nitrogen content in root dry mass of three species including tall sedge, common sedge, stiff-leaf sedge on April 2019 or the first month of experiment. There was no bare-twig rush because the root of that species did not develop. However, there was no result for nitrogen content in shoots of plants in July and August and in root tissues in May to August due to an analysis issue in the laboratory. The variance of nitrogen content was found in the range of 0.56-3.56% or 5.6-35.6 mg N/ DM g for above-mat dry mass and 0.9-1.42 % or 9-14.2 mg N/g for below-mat dry mass. The highest nitrogen content in the above-mat tissue was found in common sedges and tall sedges for below mat tissue. The lowest nitrogen content was found in bare-twig rushes for both roots and shoots. There was no significant difference in nitrogen content between all species (p >0.05). The significant positive correlation between the increase in biomass and the increase in nitrogen content in shoots of all four species was found at (R^2 >0.75, p <0.05, n = 4).

The relative increasing rate of nitrogen content (mg N/mat) in shoots of different species in a mat (e.g. eight plants per mat) are illustrated in Figure 3.10. Overall, nitrogen accumulation in the above-mat tissue of all species showed an increasing trend in each day, especially common sedges (b) and tall sedges (d) after day 25. Over 94 days of monitoring, total nitrogen accumulation in shoots of wetland plants was 3098.73 mg N/m^2 in which the sum of 1,234.71 mg N/ common sedge mats, 958.75 mg N/ tall sedge mats, 612.51 mg N/ stiff-leaf sedge mats, and 292.76 mg N/ bare-twig rush mats. The combined rate of increase in nitrogen content by four emergent macrophytes (e.g. sum of N content in shoots and roots) in the first month (Day 23) was 9.5 mg N/m^2/day. Although there was no data for the nitrogen content in root for day 94, the rate of nitrogen accumulation in shoots of four species combined was 29.39 mg/m^2/day which was three times higher than the first month rate. Specifically, the rate of N accumulation in above-mat tissue for common sedges, tall sedges, stiff-leaf sedges, and bare-twig rushes was 12.64 mg N/mat/day, 9.57 mg N/mat/day, 5.57 mg N/mat/day, and 1.60 mg N/mat/day respectively.

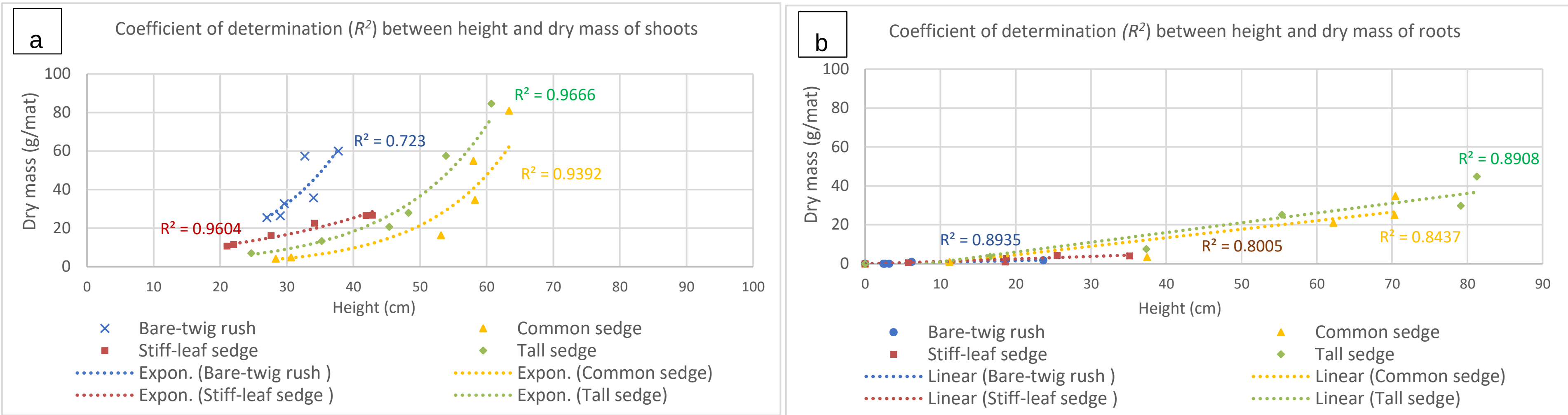


Figure 3.8. The R^2 using exponential fit function (a) to examine the relationship between the shoot height and shoot dry biomass (n=6) and linear fit function (b) for examine the relationship between the root length and its dry biomass (n=6) for four species.

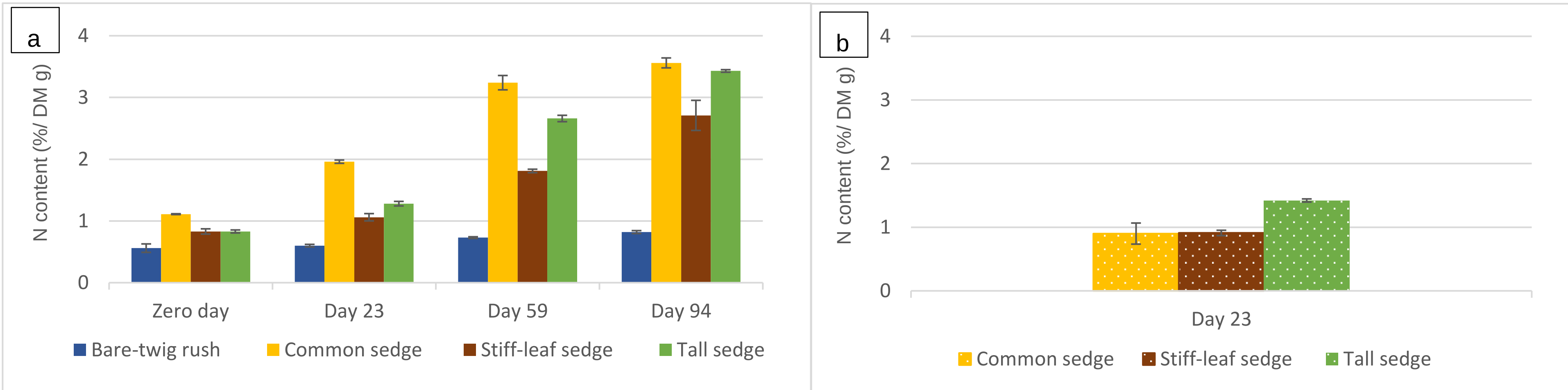


Figure 3.9. The bar graph (a) presents the average nitrogen content with standard deviation (n=3) in above-mat plant tissue of four species in the zero-day, first-month (April), second-month (May) and third-month (June) sample and the Figure (b) shows nitrogen content with standard deviation (n=3) with in three species on May 2019

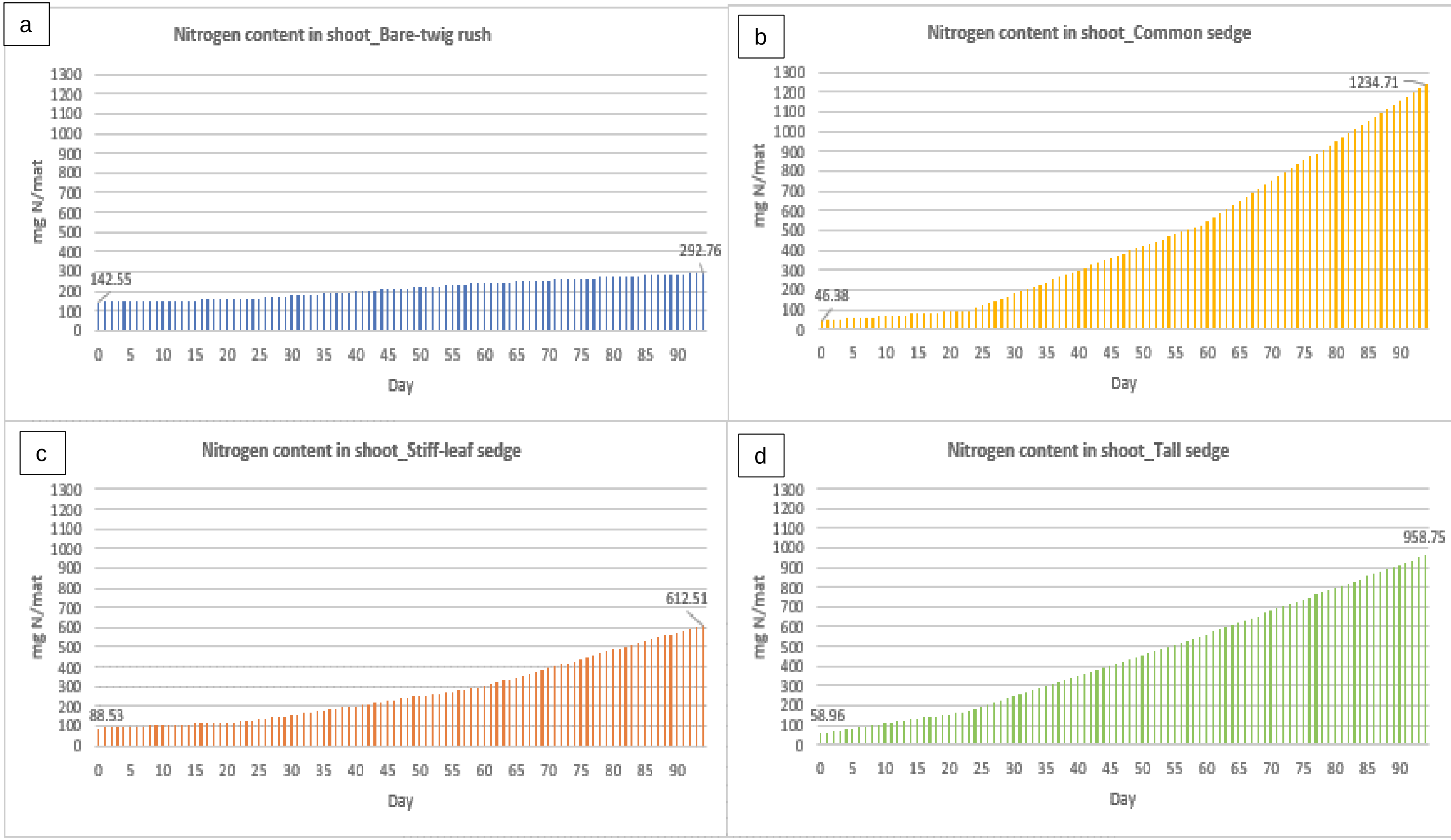


Figure 3.10. The relative increase in nitrogen accumulation in above-mat tissues of different wetland species from day 0-94.

3.4. Water quality

Water samples from the Control tank were collected only in April, May, and June. While the samples from the floating wetland system were collected in April for Tank 1, late May to early June for Tank 2, July for Tank 3, early to mid-August for Tank 4, and late August to early September for Tank 5. In general, there were improvements in terms of water clarity in the Experimental tank and the Control. The initial appearance of water in April was cloudy-green (Plate 3.2a). The massive organic biomass was observed on the wall of experimental tanks after monitoring for two months. However, the water from June to August became clearer, simultaneously with the reduction of biomass on the wall, which allowed sunlight to shine through to the bottom of Experimental Tank 5 (Plate 3.3a).

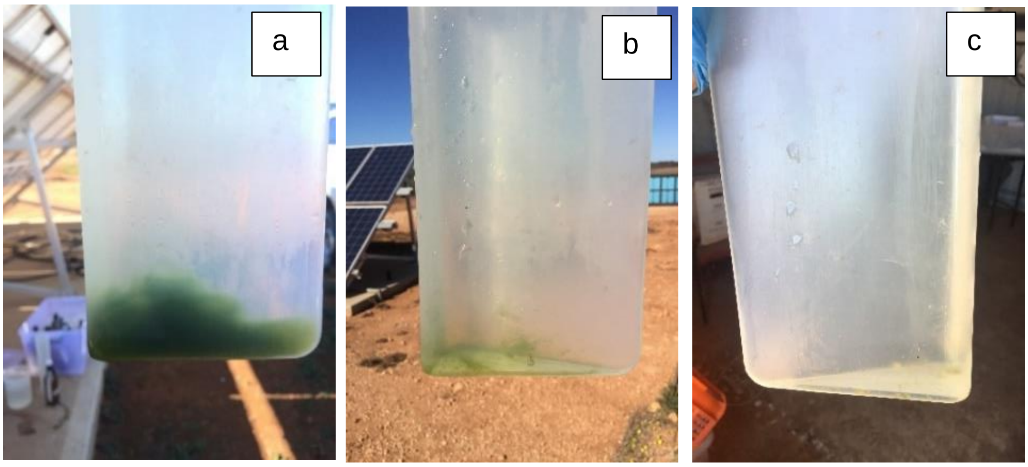


Plate 3.2. Wastewater samples floating wetland Tank 2 (a), Tank 4 (b), and Tank 5 (c) showing algal reduction

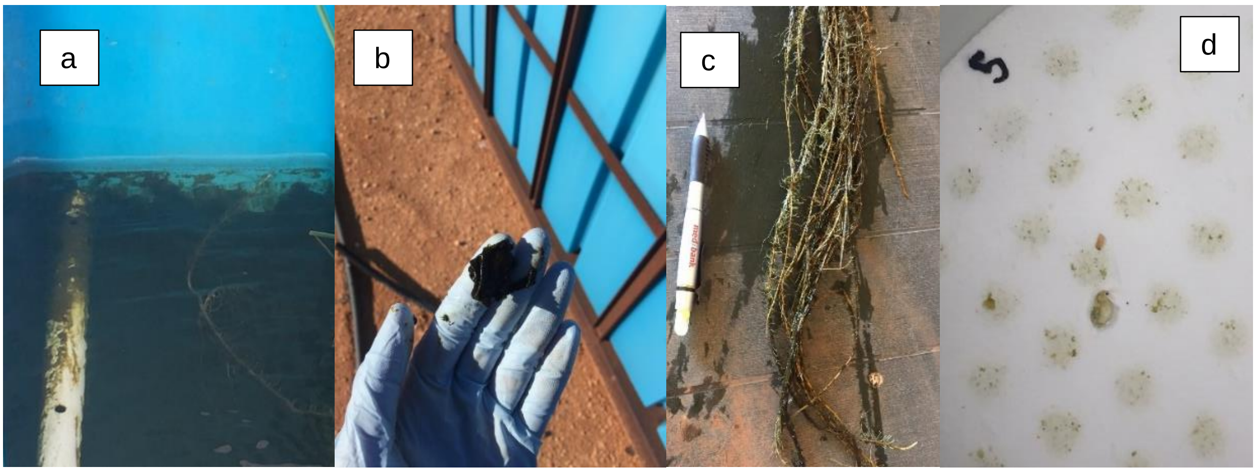


Plate 3.3. The clarity of wastewater in the Experimental tank 5 (a), algal biomass on the tank wall found in both Control and Experimental tank in June (b), evidence of root attached biomass from wastewater (c), and zooplankton population were unexpectedly found in all tanks in August (d).

3.4.1. Nitrogen speciation concentration

Figure 3.11 shows nitrogen concentration of NH_4-N (from YSI ProDSS multi-parameter meter, mg N/L) and NO_3-NO_2 (from laboratory analysis, mg NO_x-N/L) in the Control tank (on the left) and Experimental tanks with floating wetland mats (on the right). Total Nitrogen (TN) and ammonium (mg NH_4-N/L) from laboratory analysis were excluded from the results because of incomparable data. In the beginning of the observations, there was obviously a higher NH_4-N concentration than NO_3-NO_2 in both the Control and Experimental tanks. There were, however, no significant differences in April in either the NH_4-N or NO_3-NO_2 between the Control tank and the Experimental tanks (p >0.05). The mean NH_4-N concentration in the Control tank showed small changes between months; 10.16±0.97 mg NH_4-N /L in April, 11.96±4.05 mg NH_4-N /L in May, and 8.00±1.50 mg NH_4-N /L in June. The average concentration of NO_x-N/L showed an increasing trend from 2.00±0.27 mg NO_x-N/L /L in April, 4.96±0.38 mg NO_x-N/L /L in May, and 8.15±0.99 mg NO_x-N/L /L in June due to biological nitrification. Nitrogen speciation in tanks with floating wetlands showed a similar slight decrease in NH_4-N and an increase in NO_x-N/L concentration over time. In Tank 1 (April) there was 12.05±2.88 mg NH_4-N /L and 3.70±0.56 mg NO_x-N/L initially. At the end of the experiment in August (Tank 5), the NH_4-N concentration was lower (7.61±0.77 mg NH_4-N/L) and the concentration of NO_x was higher (5.62±1.62 NO_x-N/L) than in April.

3.4.2. Total Organic Carbon and Inorganic Carbon

Figure 3.12 shows the concentration of total organic carbon (TOC) and inorganic carbon (IC) in the Control (a) and the five Experimental tanks (b). The trends in Control and Experimental tanks were similar over the duration of the study; there was more TOC than IC at the start and slightly higher of IC than TOC at the end of this study. While TOC in the Control started at 83.65±10.10 mg TOC/L, TOC in the Experimental Tank 1 (April) was 71.22±7.47 mg TOC/L. TOC in both control and experimental tanks showed a gradual decrease in the following month and remained constant at 32.63±2.59 mg TOC/L in the Control tank in June and 29.88±1.80 mg TOC/L in the experimental Tank 3 in late June to early July. The mean concentration of inorganic carbon showed a small variation in both Control and Experimental tanks over the monitoring period. In the Control, IC was 38.41±3.44 mg IC/L in April, 42.03± 4.60 mg IC/L in May and 30.23±6.57 mg IC/L in June. In the experimental tanks, IC was 46.53±0.33 mg IC/L in Tank 1, 38.53±2.14 mg IC/L in Tank 2, 29.51±2.24 mg IC/L in Tank 3, 26.76±3.20 mg IC/L in Tank 4, and 29.01±9.80 mg IC /L in Tank 5. Overall, there was no significant difference between IC concentration in the Control and Experimental tanks ($p > 0.05$). However, TOC between Experimental tanks was found to be significantly different ($p = 0.0013$).

3.4.3. Suspended Solids and Chlorophyll *a*

Figure 3.14 illustrates the changes in suspended solids (SS) and chlorophyll-*a* (Chl-*a*) concentration over time for the Control (a) and Experimental tanks (b). The results of these parameters were statistically significantly different ($p < 0.05$) between the Control and Experimental tanks. The mean concentration of SS in the Control tank slightly decreased from 235.71±39.52 mg SS/L in April to 130±33.67 mg SS/L in June. Similarly, the chlorophyll-*a* concentration decreased from 2.36±0.44 mg Chl-*a* /L in April to 1.65±0.24 mg Chl-*a*/L in August. The concentration of SS and Chl-*a* in the floating wetland tanks showed a more remarkable decrease than those in the Control. In Tank1 in April, SS and Chl-*a* were 180±8.16 mg/L and 1.91±0.12 mg/L respectively. These parameters greatly decreased to 62.86±19.76 mg SS/L and 0.77±0.16 mg Chl-*a*/L in July and 0.18±0.12 mg/L for Chl-*a* in August (Tank 5). Plate 3.2 shows the different concentrations of green pigment which were associated with algae and Chl-*a* in tanks with floating wetland mats. Often the increase in the concentration of suspended solids can have two major impacts from organic and inorganic particles (Boyd 2015). A strong positive correlation between the concentration of suspended solids (SS) and chlorophyll-*a* (Chl-*a,* Figure 3.13) was found in both Control (Figure 3.13, left) and Experimental tanks (Figure 3.13, right). However, the significant relationship between these parameters was also found stronger in the Experimental tanks ($R^2 = 0.81$, $p < 0.001$) versus the Control tanks ($R^2 = 0.57$, $p < 0.001$). These findings indicated the organic particle or algal concentration was the main contributor to the SS in the system. Plate 3.3 illustrates algal biomass stuck on the tank walls which also could be responsible for SS and Chl-*a* in tanks reducing over time.

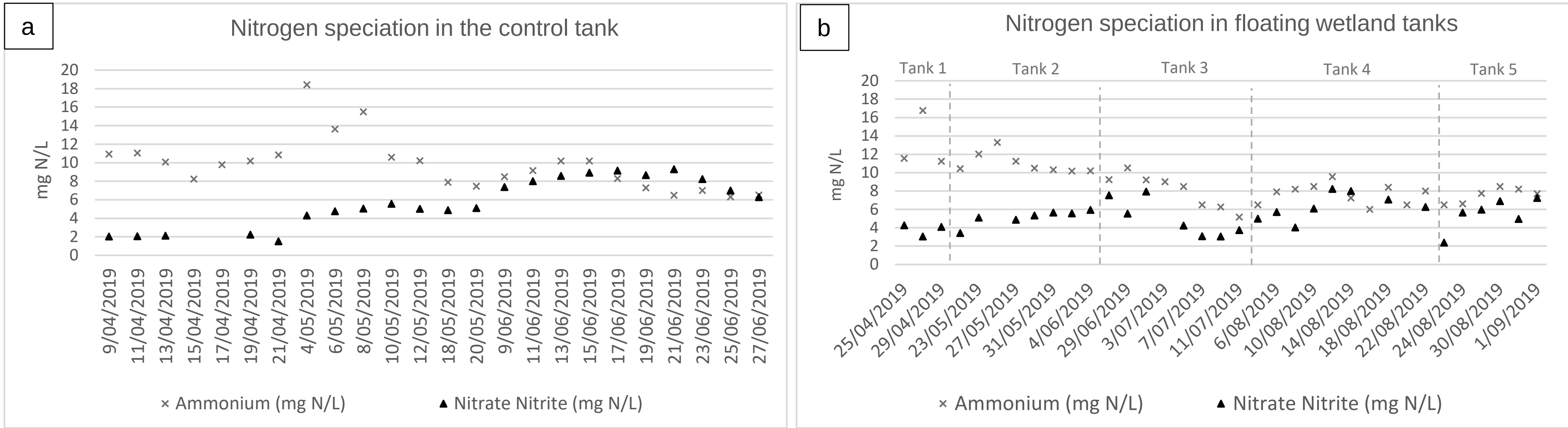


Figure 3.11. NH_4-N (mg/L) and mg NO_2-N + mg NO_3-N/L concentration for the Control (a) and Experimental tanks (b).

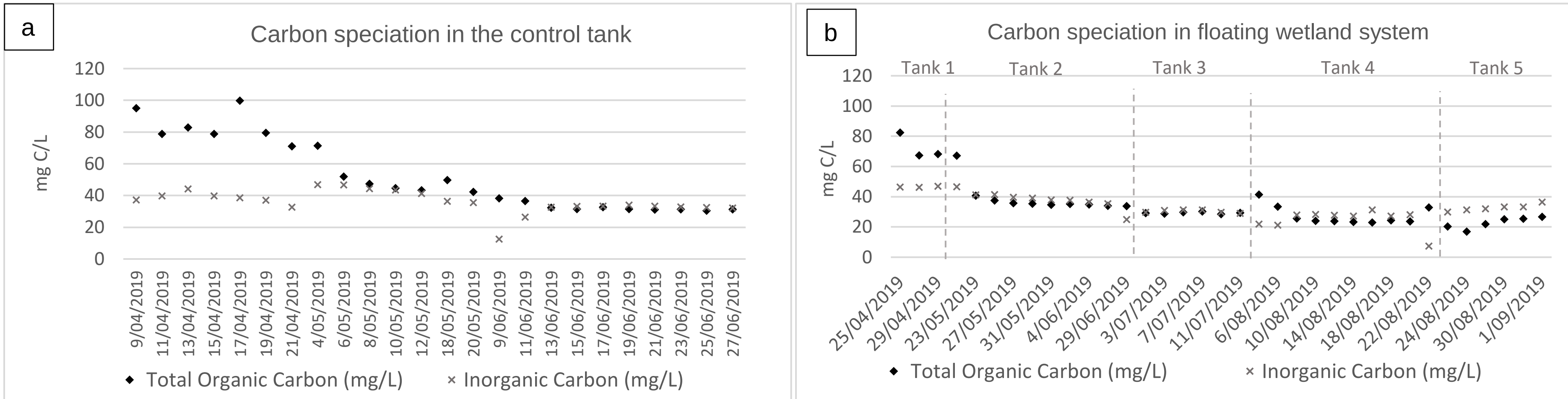


Figure 3.12. Total Organic Carbon (mg TOC/L) and Inorganic Carbon (mg IC/L) concentration over time for the Control (a) and Experimental tanks (b)

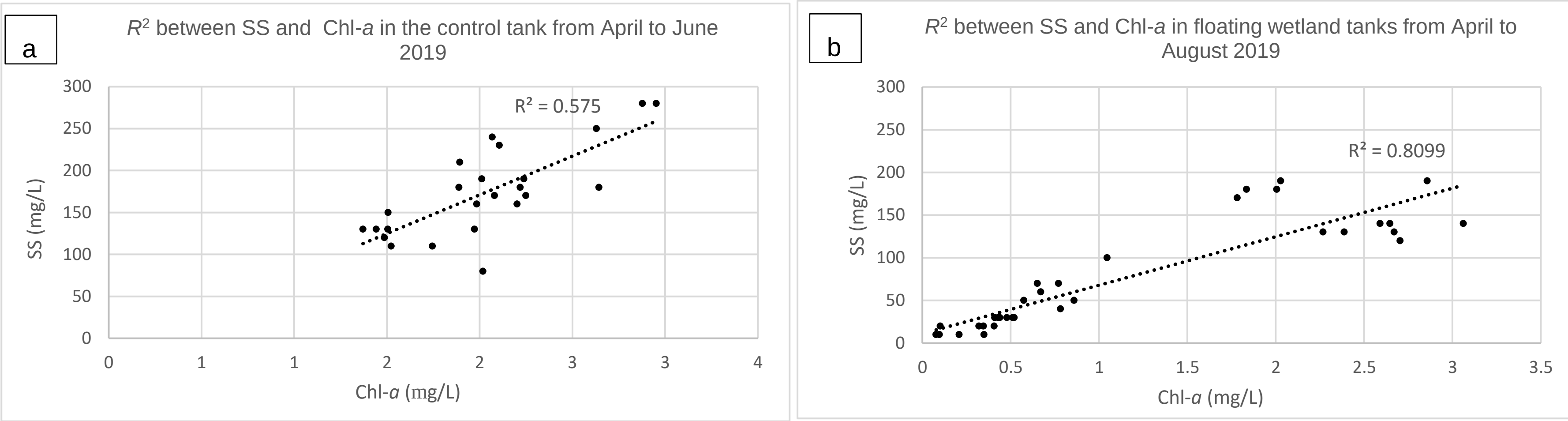


Figure 3.13. The correlation of linear regression between suspended solids and chlorophyll-*a* for the control (a) and experimental tanks (b).

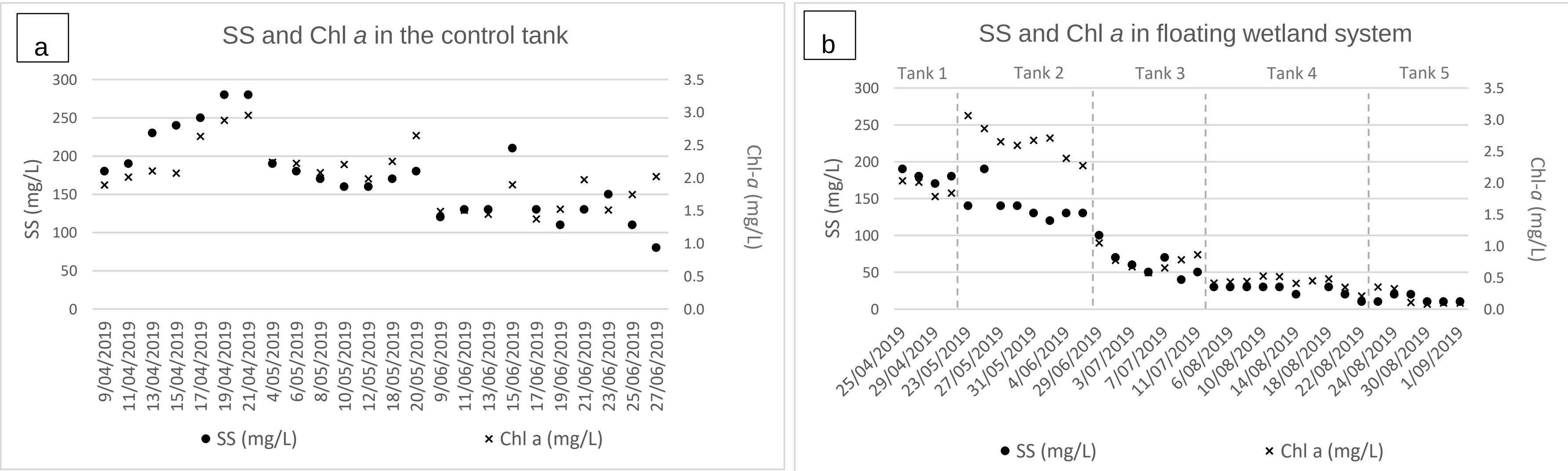


Figure 3.14. Variance of suspended solids and chlorophyll-*a* concentration over time for the Control (a) and Experimental tanks (b)

3.4.4. Other wastewater parameters

Table 3.3 shows wastewater electrical conductivity (EC), BOD_5, *E. coli* and PO_4-P in the Control and Experimental tanks between April to August 2019. There was no statistically significant difference in any of these parameters between the Control and Experimental tanks (p >0.05). Both control and floating wetland tanks showed a decrease in the mean of EC, BOD_5 and *E. coli* from the beginning to the end of the monitoring. In the control tank, EC decreased from 1601.57±19.73 µs/cm in April to 1368.9±43.01 µs/cm in June. While in the floating wetland tanks EC dropped from 1587.75±19.97 µs/cm in Tank 1 to 1143.86±282.45 µs/cm in Tank 5. BOD_5 in the control decreased from 143±12.73 mg/L in April to 58.7±6.66 mg/L in June. Tanks with floating wetlands showed a lower BOD_5 concentration at 133.67±13.50 BOD_5 mg/L in Tank 1 (14.8±0.99 BOD_5 mg/L) in Tank 5. *Escherichia coli* bacteria were found from the control at 556 MPN *E. coli* /100ml in May and 500.4 MPN *E. coli* /100ml in the Experimental tank 1 (April) to 135 MPN *E. coli* /100ml in June for the Control and 59.1 MPN *E. coli* /100ml in Tank 5 (August). The concentration of PO_4-P showed fluctuations between 3 to 5 mg P/L throughout the duration of the experiment.

Table 3.3. The mean ± standard deviation and number of samples (n) of electrical conductivity, BOD_5, *E. coli* and PO_4-P from different tanks between April to August 2019.

Tank ID	**EC (µs/cm)**	**BOD_5 (mg/L)**	***E. coli* (MPN/100ml)**	**PO_4-P (mg P/L)**
Control (9-21 Apr)	1601.57±19.73 n = 7	143±12.73 n=2		3.53±0.52 n=6
Control (4-20 May)	1441.71±297.09 n=7	62.03±21.71 n=3	556 n=1	4.06±0.33 n=6
Control (9-27 Jun)	1368.9±43.01 n=10	58.7±6.66 n=3	135 n=1	4.08±0.93 n=9
Tank 1 (25 Apr-1 May)	1587.75±19.97 n=4	133.67±13.50 n=3	500.4 n=1	3.68±0.37 n=2
Tank 2 (23 May-6 Jun)	1450.5±34.53 n=8	37.13±7.77 n=3	464 n=1	4.09±0.27 n=8
Tank 3 (29 Jun–11 Jul)	1350.86±46.83 n=7	30.07±4.04 n=3	59.1 n=1	3.78±1.27 n=6
Tank 4 (4 -20 Aug)		14.8±0.99 n=2		5.16±0.57 n=7
Tank 5 (22 Aug-1 Sep)	1143.86±282.45 n=7		35.5 n=1	

4. DISCUSSIONS

4.1. Plant growth evaluation

The novel aspect of this research was the trialling of native emergent macrophytes for the further treatment of wastewater from an HRAP in South Australia. The results showed that the tall sedge *(Carex appressa)* and the common sedge *(Carex tereticaulis)* were the best and fittest candidates for growth in wastewater as they produced dense shoot growth and fibrous network of roots across the five months, whereas *Buamea* and *Cyperus* species were slow growing and suffered weed attacks. To the author's knowledge, there is no similar comparative study on the growth performance of common sedge *(Carex tereticaulis)* and *Cyperus* species. Sanicola et al. (2019) reported on the plant biomass growth of bare twig rush *(Baumea juncea)* in a floating wetland system for treatment in the saline water. Unlike the result of this study, the growth in shoot and root biomass increased around three times and four times respectively after 12 weeks (Sanicola et al. 2019). In contrast, the biomass of both shoot and root reported in my study was almost unchanged after 94 days. Although tall sedge *(Carex appressa)* was reported as being widely used in treatment of stormwater in Queensland (Table 1.2), the plant growth performance was not monitored. Nichols et al. (2016), Schwammberger, Walker and Lucke (2017), and Walker, Tondera and Lucke (2017) focused more on the performance of this species in removing pollution. The other species I used are indigenous in South Australia and, consequently, there are no comparative quantitative growth studies from other locations interstate.

The change from autumn to winter did not significantly impact the growth of plants in the wastewater environment, which was similar to the findings of Van de Moortel et al. (2010). Notwithstanding that the experimental period of this study was in the lowest range of annual solar radiation and air temperature, the significant growth of *Carex* species suggests that they did not have a state of dormancy during winter but continually grew. In a natural condition, some plants go dormant in winter because of the extreme low temperature, extreme drought, inadequate sunlight for photosynthesis, or nutrient deficiency (Stein & Hook 2005). However, living in the hydroponic system with an HRAP treated wastewater supply, wetland plants were supplied with sufficient nitrogen. Moreover, the air temperature during this period did not significantly impact the wastewater temperature or the temperature in the root zone. Low temperature in the plant root zone at a certain point could limit the maintenance of health and production of the plants (Stein &

Hook 2005). Many studies have considered the impact of low temperature on plant growth in various species. They have reported that below the optimum temperatures in the root zone for different species could negatively impact the ability of the roots to take up nutrients, which can then affect the stomatal conductivity and overall plant growth (Alvarez-Uria & Körner 2007; Hood & Mills 1994; Wang et al. 2016).

In my study at Kingston on Murray, during the month with the coldest air temperature (e.g. mean minimum 3.2°C in June), the presence of floating mats in the Experimental tanks resulted in a higher mean minimum wastewater temperature (7.58°C) than that of the Control (4.98°C). These results confirmed that the benefit of buoyant floating mats extends beyond merely supporting emergent macrophytes to include insulating the wastewater from changes in air temperature, which was similar to the result of Van de Moortel et al. (2010). The finding also suggested that the emergent macrophytes appeared tolerant to the water temperature of 7°C. Apart from the temperature, they were also able to tolerate the high pH environment. Often when pH is relatively high, NH_3-N can become toxic to plants (Miller & Cramer 2005). However, the availability of NO_3-N in the wastewater supplied in this study may provide a benefit to plants, which can selectively use NO_3-N to balance the living environment when there is NH_3 toxicity. Miller and Cramer (2005), Hachiya and Sakakibara (2016), and Abbasi, Vasileva and Lu (2017) support the hypothesis that the availability of both nitrate and ammonium in the environment plays a crucial role for optimum plant growth. Therefore, the pH level in this study was not considered as having a significant impact on the growth of emergent macrophytes in the experimental system but more on the availability of various nitrogen species.

It was not possible to determine any preferential uptake of nitrate or ammonium from the analysis of wastewater quality. The increase in nitrogen content in the shoot tissues over time in all plants suggests that nitrogen was taken up from the wastewater. The nitrogen content in shoot tissues of tall sedges and common sedges (3.43% and 3.56% respectively) after 96 days' growth was slightly higher than the 3% reported for natural grasslands and wetlands (Rooney & Yuckin 2019; Tang et al. 2018). The nitrogen in roots (<2%) was similar to that reported by Tang et al. (2018) and Rooney and Yuckin (2019). A supportive argument for the higher nitrogen content was because plants grew in a rich nitrogen environment. Mohidin et al. (2015) reported that the nitrogen content increased from 2 to 5% with the use of fertilizer in high nitrogen concentration. The plants were in the process of active growth, which demands nitrogen for biomass production. The nitrogen content reported here is comparable with other studies. Nitrogen removal rates by the plants increased from 9.5 mg N/m^2 /day in the first month to 29.4 mg N/m^2/day in the third month. The absence of some plant tissue nitrogen data, however, limits interpretation of nitrogen uptake rates. The results suggest that plants with high growth rates (e.g. *Carex* species) should be used for better nitrogen removal.

Even though there has been no consensus on the perfect design for floating wetlands for treatment, the design from White and Cousins (2013) was adapted for this study and enabled plant development. The aerator containers using plastic cups supported the plant. The root expansion, however, may have been limited by the holes within the pots being too small. The coconut fibre was considered difficult to remove from around the plant prior to the growth assessment at the end of the experiment and its use should be discontinued. The rapid growth of the plants suggested that they were relatively wind resistant.

4.2. Effects on wastewater quality

There were some difficulties in comparing the difference in wastewater quality between the Experimental and Control tanks as the results were not collected concurrently. Furthermore, the missing water quality data, associated with equipment failure, for July and August in the Control tanks confounds the judgement on whether the tanks with floating plants showed better water quality results than the Control tanks without plants.

However, it was quite clear at the beginning of the experiment that there was no significant difference between the wastewater quality of the Control and Experimental tanks (especially Tank 1 when plant roots did not develop much). The daily mean pH and DO in both the Control and Experimental tanks (Table 3.1& 3.2) fluctuated throughout the day. During the daytime pH was elevated (>8) due to the consumption of CO_2 in the system by photosynthesising algae and to a lesser extent due to the activity of autotrophic nitrifying bacteria. The elevated daytime pH may also increase ammonia volatilisation (Craggs et al. 2014). During the daytime, dissolved oxygen, a by-product of algal photosynthesis, also increased (>10 mg/L). During the night time, heterotrophic respiration in the absence of photosynthesis caused the CO_2 concentration to rise and the pH to decrease. Although the data were limited, it suggests that nitrification occurred in both Control and Experimental tank 1. A high BOD_5 concentration was found at more than 130 mg/L in both tanks. The existing heterotrophic bacteria from the HRAPs system was assumed to still be active in mineralising organic carbon and nitrogenous waste. The CO_2 as a by-product was a carbon source in the system to support the microalgal growth, which generated dissolved organic carbon back in the cycle (Sperling 2007), which was demonstrated by the concentration increasing to 99.65 mg TOC/L in the Control and 82.40 mg TOC/L in the Experimental tank 1. The increase in organic biomass on the tank walls could be considered the product of organic compound mineralisation in the tanks.

It is important to note that the Experimental tanks contained four different wetland plant species in which each species responded differently to the wastewater environment, as reflected in the dissimilar relative growth rates. The changes in wastewater quality then obviously cannot be ascribed to any one species, as the water quality changes are the result of the equilibrium between water and all the plants' roots. There are four aspects for consideration regarding the change in water quality in Experimental tanks. First is the interaction between plant uptakes of nitrogenous compounds. It is known that when the plant uptakes NH_4+/NO_3- in the root-zone, a net acidification/alkalization occurs which influences the change of pH (Boyd 2015; Miller & Cramer 2005). The pH could alter rapidly, especially in a soilless environment, from the electron exchange process in the root zone (Miller & Cramer 2005). The rapid change of pH in a 4-hour interval in August may be an indicator of this process. While difficult to determine any preferable uptake of NH_4^+/NO_3^- it was tentatively inferred that the plants preferred using NH_4^+ rather than NO_3^-, as the concentration of NO_x^-N increased slightly from April to August. However, this may reflect the difference in the relative rates of production and uptake of NO_3.

Secondly, the presence of floating wetlands could influence nitrification/ denitrification processes, which was associated with the nitrogen removal in the system. Roots of macrophytes are known to produce photosynthetic oxygen in the daytime and respire at night (Sukias et al. 2010). The aerobic nitrification process likely decreases NH_4-N nitrogen during the daytime. Weragoda et al. (2012) reported greater removal of NH_4-N than NO_3-N in the floating wetland system because this system provided more aerobic versus anaerobic environments. In my study, NH_4-N decreased and NO_x-N slightly increased in Experimental tanks in April to August, which supports the likelihood of nitrification by which the NH_4^+ is oxidised to NO_2^- and NO_3. Sukias et al. (2010) documented that the denitrification process at night could also occur under the floating wetland matrix from the respiration in the plant root itself and the heterotrophic microbial populations attached in the root biofilm. The nitrogen speciation could then be removed under reduction from nitrate to organic nitrogen (Sperling 2007) resulting in the overall lower NO_x-N concentration in the floating wetland tanks versus the control in the same month (i.e. 4.59 mg NO_x-N /L in the Experimental tank 3 in late June to July and 8.15 mg NO_x-N /L in the Control in June).

Thirdly, although the microbial population was not monitored in this study, a slight decrease in BOD_5 and TOC could result from the microorganism activities in floating wetland plant roots. Hu et al. (2010) reported that in a floating wetland system the dissolved oxygen could be limited due to the competition between the respiration of nitrifying bacteria populations and the microorganism removing BOD_5 in root biofilms. The decreasing DO trend in Experimental tank 5 in August in my study could be an indicator for this oxygen competition. The reduction of BOD_5 from 133.67 mg/L in April to 14.8 mg BOD_5/L in July in the Experimental tanks could result in a decreased amount of dissolved oxygen required to support aerobic microbial population in oxidizing organic matter. Hu et al. (2010) also suggested the presence of floating wetland can decrease organic matter in the study environment because the heterotrophic microbial populations under the floating mats dominantly use organic carbon as a carbon source. This process could be reflected in the decrease from 82.40 mg TOC/L in the Experimental tank 1 to 24.13 mg TOC/L in Experimental tank 5. As such, it is possible that the greater coverage of root mass increased the surface area for microorganism activities, which contributed to the low concentration of BOD_5 and TOC in the wastewater.

The seasonal zooplankton in the storage lagoon after HRAPs treatment was found in late August. In a stabilised environment, a microalgal consumer like zooplankton naturally develops according to the natural food cycle (Canovas et al. 1996; Muylaert et al. 2003). Therefore, further research is suggested to study this aspect because the macrophytes can support the development of this microflora, which benefit the reduction in organic biomass and naturally improve water quality (Muylaert et al. 2003). In regard to the slight reduction of *E. coli* and EC, the data were insufficient to determine the effect of floating wetlands. The PO_4-P fluctuated between 3 to 5 mg P/L over the 5-month period of monitoring; however, the role of plant uptake cannot be determined as plant phosphorous contents were not analysed. Headley and Tanner (2012) reported a similar increase in dissolved reactive phosphorus in their floating wetland experiment and suggested the cause was P desorption and organic matter and/or soil media leaching from the floating matrix. The change of PO_4-P reported in my study could be due to similar causes. Therefore, future research should consider the fate of phosphorous in floating wetlands.

Finally, the presence of floating wetlands can be considered as a key driver for the reduction of suspended solids and chlorophyll-*a,* which resulted in the greater water clarity at the end of the experiment. The statistically significant difference in these parameters between the Control and Experimental tanks confirmed the improvement in tanks with floating wetlands compared to the Control tank. Although, this study did not monitor the settlement/entrapment on plant roots at different hydraulic retention times, the results after three months clearly showed greater reduction of SS and chlorophyll-*a* in floating wetland tanks (62.86 mg SS/L and 0.77 mg Chl-*a*/L in Tank 3) compared with the Control tank (130 mg SS/L and 1.65 mg Chl-*a*/L in June). Moreover, these findings suggest that the 6-day hydraulic retention time used in this study was suitable to allow suspended particulate matter from the supplied wastewater (hydraulic rate of 252 L/day/tank) to settle and/or be entrapped on plant roots.

4.3. Limitations

Despite the evident improvement in water clarity, the result of water improvement in terms of water chemistry is not clear. Firstly, the influent and effluent from the tanks were not monitored concurrently, confounding interpretation of any change in water quality, especially in nitrogen removal, due to the presence of plants. The limited time in designing the experimental set-up and monitoring is responsible for some uncertainties. The study provides limited data regarding nitrogen accumulation by the plants. Therefore, the nitrogen removal by floating wetland plants of South Australia requires further research. Secondly, the interaction between the plant root and microbial activities remains to be determined. Even though there is evidence that SS and Chl-*a* concentrations gradually decreased, more so in Experimental tanks with plants, the mechanism(s) remain unclear. There are no data to support how much the phytoplanktonic biomass was attached to the wetland plant root. The seasonal development of zooplankton could have an impact on water quality, especially regarding the reduction of the phytoplankton population in the tanks. Therefore, monitoring of the seasonal difference in the plant uptake of nitrogen in association with the seasonal microflora development is suggested for future studies. Furthermore, studies are suggested to measure N, C, and P content in plant tissue, especially in roots and in the matrix, as nutrient sorption by the floating matrix was found to have occurred in some studies. Other factors requiring further investigation are hydraulic retention time, the density of the plants, and the role of specific plant species in removing nitrogen, as these aspects may have the potential to affect nutrient removal efficiency of a floating wetland system.

5. CONCLUSION

In this study, emergent macrophytes, especially the common sedge *(Carex tereticaulis),* and tall sedge *(Carex appressa),* were able to grow in wastewater pre-treated by an HRAP even though there was a low range of solar exposure during the 5-month study. Due to the presence of floating mats, the seasonal temperature in winter did not greatly influence the water temperature in the Experimental tanks, and the low temperature did not negatively impact the growth of these species. While the actual amount of nitrogen removal by the floating wetland system for treatment of domestic wastewater was not determined, the increase in plant biomass production showed the correlation with the increase in nitrogen content in plant tissue. This demonstrated that the plants absorbed nitrogenous compounds from wastewater. Although the effect of floating wetlands on wastewater quality was inconclusive, the slight decrease in NH_4-N, BOD_5, TOC and remarkable decrease in SS and Chl-*a* in the tank with floating wetland plants confirmed that the presence of living plants played a key role in improving the water clarity.

Overall, this research has shown that the floating wetland system can be a suitable and practical treatment of effluent water from high rate algal ponds because of the healthy growth performance, the potential capacity of nitrogen removal by plant uptake, the entrapment of suspended solids on plant roots, and the improvement in water clarity. However, the complexity in physical, chemical, and biological aspects of this application cannot be adequately explored in a short time period of monitoring, such as was undertaken in this study. Therefore, further studies are recommended to assess more thoroughly the effectiveness of the floating wetland system by comparing influent and effluent water quality, observing the change in water quality at different hydraulic retention times, studying the relationship between the microbial community and water quality, and comparing the different nutrient removal efficiencies among the candidate wetland species in different seasons over a longer period of monitoring.

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