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INSTITUTO DE BIOCÊNCIAS
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Dissertação de Mestrado

**Nature-based solutions: the use of a floating wetland as an alternative to
wastewater treatment**

LUANA HAINZENREDER BAUER

Porto Alegre, março 2020

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*Aos meus pais, Rogélia e Jairo
por serem bússolas na minha vida.*

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Resumo

Métodos físico-químicos convencionais usados no tratamento de efluentes são geralmente caros e o descarte destes efluentes ainda pode ser prejudicial ao meio ambiente. Deste modo, eco-tecnologias alternativas, como *wetlands* construídos (WC), são uma solução tradicionalmente utilizada. Uma variante dos sistemas de WC é o *Wetland* Flutuante Construído (WFC), que utiliza uma estrutura flutuante na qual um tapete radicular hidropônico é formado, auxiliando na biorremediação de águas poluídas. Portanto, o objetivo deste estudo foi avaliar a eficiência de um sistema de WFC na melhora de parâmetros da qualidade de águas residuais brutas. No presente estudo realizou-se três experimentos com triplicatas, utilizando o efluente do Campus do Vale da Universidade Federal do Rio Grande do Sul (UFRGS). No delineamento experimental foram considerados três tanques com macrófitas (M), *Typha domingensis*, e três tanques controles sem macrófitas (C). Os parâmetros analisados foram condutividade, pH, cor, turbidez, nutrientes (nitrogênio e fósforo), metais pesados (Zn, Cr, Cu, Pb e Cd) e ecotoxicidade. A comparação de tais parâmetros entre influentes (água residual bruta de entrada no sistema) e efluentes (pós-tratamento) foi por *teste-T* para amostras dependentes e para a comparação entre tratamentos (M vs C) *teste-T* para amostras independentes. Uma Análise de Agrupamento de duas vias foi realizada para identificar semelhanças entre as amostras brutas e pós-tratamento; e Análise de Componentes Principais (ACP) foi utilizada para identificar as principais variáveis explicativas do sistema. Como resultados, os tanques com *T. domingensis* apresentaram melhora significativa para condutividade, pH, cor, turbidez, nitrogênio e parâmetros ecotoxicológicos ($p < 0,001$) em comparação ao influente, menos fósforo. Os controles também apresentaram certa eficiência para todos os parâmetros ($p < 0,04$), que pode ser resultante de interações como processos de foto-oxidação e/ou da proliferação de algas/macrófitas flutuantes, que atuaram como agentes fitorremediadores. Dentre os metais pesados quantificados, apenas Zinco estava acima do limite de detecção do método analítico (0.012 mg/L) e não foi removido com eficiência nos tanques com macrófitas ou pelos controles. As macrófitas apresentaram ganho significativo de aproximadamente 30% de biomassa ($p < 0,001$), mas não houve crescimento das raízes em comprimento. Como conclusão geral, os resultados mostram que os WFCs têm o potencial de tratar o efluente bruto, contudo, o mesmo deve ser aplicado complementarmente com outras tecnologias de tratamento para garantir a melhora da qualidade do efluente bruto de modo efetivo.

Palavras-chave: *Wetlands* flutuantes; tratamento de águas residuais brutas; parâmetros de qualidade da água; países em desenvolvimento.

Abstract

Conventional physicochemical methods for effluent treatment are expensive and the resulting discharge can still be harmful to the environment. Thus, alternative eco-technologies such as constructed wetlands (CW) are a solution traditionally used. A variant from CW is the Floating Wetland Treatment (FWT) that uses a buoyant structure in which a hydroponic root network operates to bioremediate polluted waters. Therefore, the aim of this study was to evaluate the efficiency of a FWT system to improve the water quality parameters of a raw wastewater. The current study performed three experiments with triplicates, using effluent from the Vale Campus of the Federal University of Rio Grande do Sul (UFRGS). In the experimental design it was considered three tanks with macrophytes (M), *Typha domingensis*, and its respective non-vegetated controls (C). Water quality parameters analyzed were conductivity, pH, color, turbidity, nutrients (nitrogen and phosphorus), heavy metals (Zn, Cr, Cu, Pb, and Cd) and ecotoxicity. The comparison of these parameters between influent (raw wastewater) and effluents (after treatment) used *T-test* for dependent samples and to compare treatments (M vs C) *T-test* for independent samples. A Two-way Cluster Analysis was performed to identify similarities between the raw and post-treatment samples; and a Principal Component Analysis (PCA) was used to identify the main explanatory variables on the system. As a result, the floating mats vegetated with *T. domingensis* significantly enhanced conductivity, pH, color, turbidity, nitrogen and ecotoxicological parameters ($p < 0,001$) compared to influent, minus phosphorus. Control tanks presented some efficiency for all parameters ($p < 0.04$), which may have occurred by interactions such as photo-oxidation processes and/or the presence of algae/floating macrophyte proliferation, that might also have acted as phytoremediation agents. Among all heavy metals quantified, only Zinc was above the detection limit and it was not efficiently removed in neither macrophyte or control tanks. The macrophytes show a significant gain of approximately 30% of biomass ($p < 0,001$), but there was no growth of root's length. As an overall conclusion, FTW systems have potential to treat raw wastewater, nonetheless it has to be applied as a complementary technology in order to efficiently improve water quality of a complex effluent.

Key words: Vegetated floating wetlands; raw wastewater treatment; water quality parameters; developing countries.

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INTRODUÇÃO GERAL

O aumento da urbanização em escala global tem resultado na intensa poluição dos corpos de águas superficiais devido ao despejo de esgotos e efluentes industriais produzidos. A composição de tais descargas apresenta contaminantes orgânicos e inorgânicos que impactam negativamente a qualidade dos recursos hídricos (Bueno *et al.*, 2012). Componentes inorgânicos como metais pesados, cianeto, nitrogênio, fósforo, fenóis, sólidos suspensos, componentes orgânicos tóxicos, cor e turbidez podem caracterizar efluentes não tratados geralmente oriundos de fontes residenciais ou industriais (Chan *et al.*, 2009). A liberação destes dejetos líquidos em sua condição bruta pode resultar na degradação do corpo receptor e colocar em perigo suas mais diversas formas de vida (Nergis *et al.*, 2012; Jilani & Khan, 2013). Além disso, ecossistemas aquáticos poluídos por águas residuais não tratadas são fonte de inúmeras doenças; portanto, é fundamental que tais efluentes sejam devidamente tratados para a proteção dos recursos hídricos, que são muito utilizados no abastecimento de água potável, especialmente em países em desenvolvimento (Lam *et al.*, 2015).

No tratamento convencional de efluentes, a remoção de poluentes demanda uma série de procedimentos físicos, químicos e biológicos. O uso destes recursos na remoção de contaminantes pode acabar sendo um tanto dispendioso e, por vezes, prejudicial ao meio ambiente (Fu & Wang, 2011). Ainda que os parâmetros físico-químicos, incluindo cargas de poluentes, estejam de acordo com os limites exigidos na legislação, o efluente ainda pode apresentar toxicidade que, por vezes, resulta da interação dos constituintes remanescentes no efluente. O que ocorre é que as estações de tratamento convencionais foram projetadas no intuito de remover compostos químicos e orgânicos, tendo sua eficiência avaliada pela análise química de alguns poucos compostos estabelecidos nas legislações federal e estaduais, não sendo projetadas para remover toxicidade (Arenzon *et al.*, 2011).

Sistemas alternativos utilizando a capacidade de plantas e suas raízes ligadas a bactérias que auxiliam na absorção de nutrientes e matéria orgânica, têm sido designados para a remoção de diversos tipos de contaminantes. Tais sistemas são denominados *Wetlands Construídos* (WC) (Olguín *et al.*, 2008; Headley & Tanner, 2012; Wu *et al.*, 2015) e podem variar em sua estrutura dependendo do efluente a ser tratado. Uma dessas variações é o *Wetland Flutuante Construído*

(WFC); uma categoria de sistema alagadiço que abriga macrófitas em uma estrutura flutuante sem um sedimento de apoio para suas raízes que interagem com as comunidades ecológicas no meio em que está inserido (Pathak, 2013). A parte aérea das macrófitas permanece acima do nível d'água enquanto o sistema de raízes, que perpassa a estrutura flutuante, se desenvolve hidronicamente. Deste modo, as plantas são obrigadas a obter os nutrientes necessários para o seu crescimento diretamente da coluna d'água (Hubbard, 2010; Kadlec & Wallace, 2009). Por permitir o livre crescimento da parte radicular da planta, o extenso comprimento das raízes é o fator central para o desenvolvimento de um biofilme robusto capaz de reter pequenas partículas suspensas e nutrientes da coluna d'água (Headley & Tanner, 2012; Zhao *et al.*, 2012). O emaranhado de raízes fornece uma ampla área de superfície biologicamente ativa para transformações bioquímicas (Kyambadde *et al.*, 2004; Li *et al.*, 2009) e o desenvolvimento de uma comunidade diversa de micro-organismos que desempenham um papel essencial nos processos biológicos de degradação (Song *et al.*, 2009). Quando a água perpassa as raízes submersas, a remoção de contaminantes pode ocorrer através de vários mecanismos, que incluem a incorporação de nutrientes e poluentes à biomassa, adsorção aos biofilmes na rizosfera, a liberação extracelular de enzimas, a sedimentação de particulados, a ligação de contaminantes que os tornam indisponíveis e a floculação de material suspenso (Yeh *et al.*, 2015; Oliveira & Fernandes, 1998; Tanner & Headley, 2011).

Uma das vantagens dos WFCs é não demandar um amplo espaço físico comparado aos tradicionais WCs, já que sua operação ocorre *in situ*, e a remoção de poluentes se dá no próprio meio hídrico em que se encontra. Deste modo, não se faz necessária a abertura de uma área no solo para o plantio das macrófitas, exigindo um grande espaço para o tratamento de efluentes. Os WFCs também possuem vantagens adicionais, como baixo custo de manutenção e infraestrutura (Nichols & Lucke, 2016). Um comparativo a respeito do custo de construção e manutenção de um WC comparado a um tratamento convencional de efluente é detalhado por Kangas em seu livro “*Ecological Engennering: Principles and Practice*” (Kangas, P. C., 2003). Também por serem sistemas flutuantes nos quais as macrófitas não estão apoiadas no sedimento, existe o benefício singular de que a variação no nível de água abaixo das plantas não se apresente como um problema para o desenvolvimento das mesmas ou para o próprio sistema, já que as raízes estão hidronicamente adaptadas (Chang *et al.*, 2012). Em contrapartida, em um WC, a entrada

excessiva de efluente a ser tratado poderia levar as macrófitas utilizadas no tratamento à um estresse hídrico ou até mesmo sua morte, comprometendo o funcionamento do sistema.

No intuito de implementar um WFC, uma variedade de macrófitas emergentes se aplicam ao propósito de melhorar a qualidade da água, removendo nutrientes, poluentes e/ou metais pesados. No entanto, a escolha da espécie a ser utilizada deve levar em consideração uma série de fatores que incluem a capacidade de adaptação da macrófita ao WFC, seu caráter nativo, rápida taxa de crescimento, formação de um longo e denso sistema de raízes, possuir biomassa robusta, apresentar alta tolerância a poluentes e ser hiperacumuladora, principalmente na parte aérea (Valipour & Ahn, 2016). Sendo assim, baseado em estudos anteriores (Rigotti *et al.*, 2020; Cabo *et al.*, 2015), esta espécie foi escolhida por ser resistente a concentração excessiva de nutrientes, apresentar os requerimentos acima citados e já se encontrar disponível em área não contaminada no perímetro do Campus do Vale da Universidade Federal do Rio Grande do Sul (UFRGS).

O efluente utilizado nesta pesquisa, produzido no Campus do Vale (UFRGS), possui tratamento através de sistemas individuais tipo fossa e filtro, e uma estação de tratamento coletivo que abrange parte das edificações, a Estação de Recuperação da Qualidade da Água (ERQA). O monitoramento dos sistemas individuais é inexistente e, portanto, os mesmos não podem ser considerados eficientes. A carga orgânica e elementos químicos, oriundos dos restaurantes, banheiros e laboratórios, possui potencial de degradar os corpos hídricos receptores, como o Arroio Dilúvio e, posteriormente, o Rio Guaíba. No ano de 2019, foi estimado que a produção média de efluente no Campus do Vale foi de 13.000 m³ por mês (aproximadamente 430 m³ diários) e que cerca de 20.600 pessoas frequentaram o campus. A Estação de Recuperação da Qualidade da Água (ERQA), onde foram realizadas as coletas, tem o propósito de servir como laboratório de estudos para os alunos da instituição. Contudo, é imprescindível que, até mesmo para servir de laboratório, a recuperação do efluente deva ser realizada de modo efetivo. É de responsabilidade de empreendimentos públicos e privados atender aos padrões de emissão de efluentes líquidos em águas superficiais, a fim de preservar a qualidade ambiental, de saúde pública e dos recursos naturais (CONAMA N^o 357/2005; CONAMA N^o 430/2011; CONSEMA N^o 355/2017).

Deste modo, tendo em vista a complexidade do efluente produzido dentro do Campus do Vale da UFRGS, em função de possuir elevada carga orgânica e elementos químicos (incluindo

metais pesados), esta dissertação de mestrado tem como objetivo geral a avaliação da eficiência de um sistema de WFC desenvolvido em mesocosmos na melhora dos parâmetros da qualidade da água deste efluente. Além disso, a grande maioria dos estudos, relacionados a tratamento de águas residuais com tais sistemas, focam unicamente na remoção de nutrientes e/ou metais pesados. Apenas poucos estudos incluem análises ecotoxicológicas na avaliação do melhoramento da qualidade da água (Chang *et al.*, 2012; Lutterbeck *et al.*, 2018; Tara *et al.*, 2019; Ijaz *et al.*, 2016). Portanto, os objetivos específicos desta dissertação abrangem: 1) Avaliar a melhora das características físico-químicas do efluente tratado pelo sistema de WFC; 2) quantificar a concentração de metais pesados encontrados no efluente bruto e pós-tratamento; 3) analisar a eficiência da *T. domingensis* na redução da ecotoxicidade aguda do efluente.

Os resultados deste estudo são relevantes considerando o uso de um real efluente sem tratamento prévio, o que é incomum no uso de WFCs, que são geralmente aplicados como polimento de efluentes previamente tratados. Portanto, considerando as vantagens de tais sistemas flutuantes, é importante, especialmente para países em desenvolvimento, que estudos relacionados a temática do tratamento de efluentes sejam apresentados no intuito de influenciar mudanças nos processos/tecnologias de tratamento de efluentes.

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Nature-based solutions: the use of a floating wetland as an alternative to wastewater treatment

1. Introduction

Pollution affecting water bodies in urban areas is becoming a global threat to sustainable development, especially at those places with poor infrastructure where different sources of contamination are not properly controlled and the population is still growing at a fast pace (Olguín et al., 2017). Untreated wastewater is a serious issue, notably in developing countries, where the majority is still released into surface waters in its raw form. For instance, in Brazil, as stated in the 2018 annual report “Diagnosis of water and sewage services” (SNIS, 2018), only an average of 46.3% of the sewage produced is treated. However, for many cities, sewage treatment percentage is far distant the country’s average; for example, cities in Rio Grande do Sul state, such as Caxias do Sul and Santa Cruz do Sul, present the percentage around 37% and 18%, respectively (SNIS, 2018). As a result, natural water bodies are degraded by organic, inorganic and biological pollutants and contaminants present in the raw wastewater threatening all aquatic organisms and other life-forms associated (Nergis et al. 2012; Jilani and Khan 2013). Hence, domestic and other wastewaters’ treatment prior to discharge is extremely important to protect aquatic life and human health, and this must be acknowledged by governments, regulatory agencies and local authorities (Mara, 2013). Besides, the cost to recover a polluted water body is way higher than the preceding effluent treatment.

Conventional effluent’s treatment demands a series of physical, chemical and biological procedures. Moreover, its employment removing contaminants can be expensive and, sometimes, still be further harmful to the environment (Fu and Wang, 2011), especially for treating heavy metals, that are inorganic substances not biodegradable naturally capable of bioaccumulating in the trophic chain persistently (Gall et al., 2015; Nancharaiah et al., 2015). As an alternative of wastewater treatment technology, nature-based solutions have been designed. Constructed Wetlands (CW) ecotechnologies use the potential of macrophytes and their roots linked to bacteria

to absorb nutrients and organic matter and also remove various types of pollutants (Olguín et al., 2008; Headley and Tanner, 2012; Wu et al., 2015).

A variety of CW is called Floating Treatment Wetlands (FTW), where rooted emergent macrophytes are placed in a buoyant structure with their roots growing down into the water column hydroponically (Headley and Tanner, 2008). Without establishing in the sediment, the plants are strained to uptake the nutrients required directly from the water they are inserted in (Headley et al., 2006; Vymazal, 2007; Headley and Tanner, 2008). For this reason, roots growth is crucial for the nutrient removal process. Therefore, extensive suspended roots provide a considerable surface area (biofilm) for the development of denitrifying bacteria, further creating an anaerobic condition where nitrate removal can occur through the denitrification process (Govindarajan, 2008). Besides, fine suspended particles can be captured in this root mass whether continuous in suspension as occurs in retention ponds (Headley et al., 2006). Phosphorus can be retained (through adsorption) to the biofilm and, ultimately, be uptaken by vascular macrophytes as orthophosphates (Walker et al., 2014). Because the rhizosphere works as a sorption media, besides removing nutrients, it also plays a central role in extracting pollutants from the water, such as heavy metals, pathogens, pesticides, and toxins (Chang et al., 2010). Thus, the FTW system has been employed to promote better water quality standards (Billore and Prashant, 2008; Revitt et al., 1997; Tanner and Headley, 2011; Van de Moortel et al., 2010). In this investigation, *Typha domingensis* (Southern cattail) was the native macrophyte chosen thanks to its ability to resist in extremely polluted waters (Abdel-Ghani et al. 2009) and be able to uptake excessive amounts of nutrients and pollutants with no further lethal consequences (Newman et al., 1998).

The advantages of using a floating mat include an in-situ operation that presents low cost for maintenance and infrastructure and does not demand a large physical space (Nichols and Lucke, 2016). Furthermore, because they are floating systems where macrophytes are not supported by sediment, the fluctuation in the water level below the plants does not interfere in their development or is a problem for the system itself (Chang et al., 2012; Ladislav et al., 2012). Due to this unique feature, this eco-technology has been especially exploited in studies for nutrients and pollutants removal from stormwater runoff (Borne, 2014; Chang et al., 2013; Kerr-Upal et al., 2000; Khan et al., 2013; Ladislav et al., 2015; Revitt et al., 1997; Tanner and Headley, 2008; Wang et al., 2014;

2015; White and Cousins, 2013; Winston et al., 2013). Additionally, aside from limiting eutrophic conditions through nutrient removal processes, the system's structure also reduces sunlight penetration under its domain, which helps to inhibit massive algal bloom events by preventing photosynthesis (Reinsel, 2014).

A large part of the studies related to wastewater treatments using FTWs focus on the removal of nutrients and/or heavy metals alone. Only a few studies highlight ecotoxicological outcomes for the enhancement of water quality as well (Chang et al., 2012; Lutterbeck et al., 2018; Tara et al., 2019; Ijaz et al., 2016). However, to detect the damaging effects that might be caused by wastewater in wildlife and humans, physicochemical analyses cannot warn of adverse additive, synergic, or antagonist interactions among chemicals composing the wastewater (Prasse et al., 2015). Therefore, the purposes of this study are to (1) evaluate the efficiency of FTWs to improve physicochemical parameters that are directly related to water quality; (2) quantify heavy metal concentration in raw effluent and after treatment; (3) assess the performance of *T. domingensis* to reduce acute ecotoxicity from a complex wastewater.

This research is relevant as it works with a real effluent with no previous treatment, which is uncommon in the use of FTW that are usually applied as a polishment. Thus, considering the advantages of FTWs, data using raw complex wastewater is crucial to influence changes in effluent treatment processes/technologies.

2. Material and methods

2.1. Description of the mesocosm structure

The study was carried out in a mesocosm structure placed at the Hydraulic Research Institute located at university campus of the Federal University of Rio Grande do Sul (UFRGS), in a period close to one month. Experiment 1 (E1) was performed from March 12 to March 21; experiment 2 (E2) from March 26 to April 4; and experiment 3 (E3) from April 9 to April 18 with the wastewater from the university campus. The hydraulic retention time (HRT) was 9 days and no wastewater was added to the tanks during the experiments. A 1000 L capacity water tank was used as a reservoir to homogenize 350 L of the raw wastewater collected to perform each experiment.

The raw wastewater was filtered with a sieve to remove leaves, seeds and sticks. The large tank (Fig. 1) was connected through hoses to six tanks (0.43 m x 0.62 m surface opening, 0.45 m depth each with approximately 120 L capacity) that were filled in every experiment with 60 L (tapering to 0.22 m operational water depth) of wastewater (influent). Three tanks were used to treat the wastewater with the FTW system, named macrophyte tanks (M1, M2, and M3); and the three others were used as control tanks (C1, C2, and C3) (Fig. 2). All tanks were drained and cleaned out to minimize subsequent contamination at the end of each experiment, ensuring the removal of biofilm or organic sediments attached to the wall and bottom of the tanks.

A rectangular 0.40 m x 0.50 m plastic structure made of recycled polypropylene with polyethylene floating buoys keeping it on the surface supported the plants. Each one of the three plastic structures inserted in the macrophyte tanks (M1, M2, and M3) supported 10 macrophytes in an intercalated way, allowing all plants to grow their leaves and roots in a generous space (Fig. 3). The floating wetland system in mesocosm was placed open field accounting variables such as topography, natural solar incidence, and logistics, due to periodic monitoring. All six tanks were protected from rain, preventing the effluent from being diluted, with a transparent material that allowed sunlight to pass through (Fig. 4).

2.2. Plant collection and acclimation period

The natural wetland area selected to collect the macrophyte *Typha domingensis* (Southern cattail) was located at the university campus (UFRGS) near to a native forest on one side and an academic building on the other side. In this area, water was limited to small canals in the end of November 2018 where 40 macrophyte units were collected from a muddy bottom characterized by a dense monospecific stand of *T. domingensis*. The plants were selected considering their size and healthy aspect. Each macrophyte rhizomes were carefully washed to remove any sediment/soil attached to the plant surface, their new shoots removed and the leaves pruned to 50 cm high to standardize the units. Plant species choice was according to the literature, local abundance, and previously performed mesocosm study (Rigotti et al., 2020). It is known that *T. domingensis* is characterized by being hyperaccumulator and interacts well with the microbial community, which is crucial for treating highly polluted wastewaters (Ijaz et al., 2016). The macrophytes were carried

to the experiment site right after collection and inserted in the aquatic media of the floating structure.



Fig. 1. A tank with 1000 L capacity was used to homogenize the raw wastewater collected from the Water Quality Recovery Station of university campus (UFRGS). A small sieve was used to remove sticks and leaves.

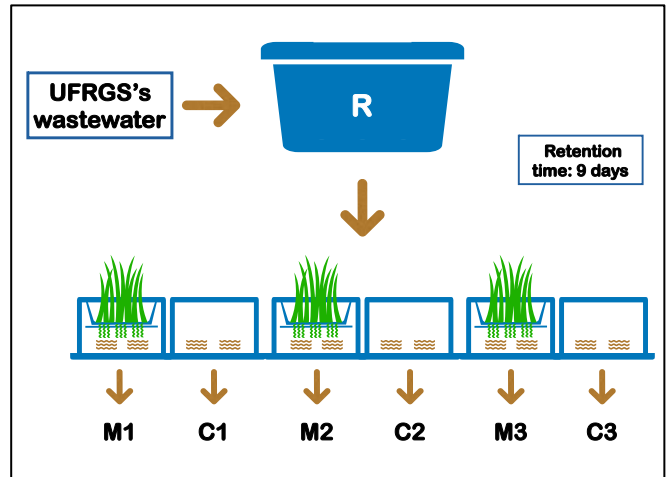


Fig. 2. Schematic representation of the mesocosm structure with macrophyte tanks represented by M1, M2, and M3 and control tanks represented by C1, C2, and C3.



Fig. 3. Floating structure with polyethylene floating buoys supporting ten macrophytes with a Control tank aside. Mesocosm floating wetland system filled with the raw wastewater from the Water Quality Recovery Station of university campus (UFRGS).



Fig. 4. The six tanks (Macrophytes and Controls) were protected from the rain to avoid wastewater dilution with a transparent material that allowed sunlight to reach the macrophytes used in the mesocosm floating wetland system.

Acclimation time of three months, from December 2018 to February 2019, enabled the plants to develop their roots hydroponically in a synthetic solution of nutrients composed by

$\text{Ca}(\text{NO}_3)_2$; KNO_3 ; $\text{NH}_4\text{H}_2\text{PO}_4$; MgSO_4 ; CuSO_4 ; ZnSO_4 ; MnSO_4 ; H_3BO_3 ; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$; FeDTPA . The proportion of the reagents used was adapted from the hydroponic crop solution of Furlani et al. (1999) that was based on the formulation of Hoagland & Arnon (1938) according to Resh (1996). The solution was diluted 10 times and the pH adjusted to values close to neutral pH (range of 6.5 to 7.0) using Sulfuric Acid (H_2SO_4). The water used for the solution was tap water. A synthetic solution provides better control of the environmental conditions that might vary drastically if it was used drainage waters or domestic effluents.

During the acclimation period, the plants that did not survive were replaced for healthy ones that were being acclimated in a separate tank, cultivated exclusively for this purpose. The plants were monitored three times a week and once a week the tanks were cleaned out and filled with the synthetic solution again. The acclimation period was essential for the development of the roots in length (Fig. 5).

After three months with the synthetic solution, the macrophytes were also exposed to the raw wastewater in a pilot experiment of 9 days. Considering that the university's wastewater is a complex mixture of substances with a high concentration of nutrients, pollutants and organic matter, an exposure period of the plants to it was fundamental.



Fig. 5.: Roots development in a hydroponically media during acclimation time with a synthetic solution from December 2018 to January 2019.

2.3. Plant measurements

All macrophyte units selected at the beginning of the study compose the floating wetland mats until the end of the research. The same plants were used in all three experiments, unless a

replacement was inevitable, considering great stress or the death of the macrophyte. Every macrophyte unit was identified to have its measurements monitored in all three experiments.

At the beginning of each experiment, all macrophytes leaves were pruned at 50 cm height to standardized the units. Subsequently, the plants were weighed and the root's length measured (initial time (t_0) measurements). At the end of the HRT of each experiment (9 days), the macrophytes were removed from the floating structure, carefully dried with paper towels and weighed again to verify wet biomass production and the roots measured in length to monitor growth (final time (t_9) measurements).

2.4. Raw wastewater collection and characterization

The Federal University of Rio Grande do Sul (UFRGS) wastewater at university campus can be characterized as domestic wastewater, as described by Mara (2013), with additional discharge from research laboratories. It is a complex mixture containing heavy metal elements, washing products, domestic sewage, and other chemicals. The wastewater may vary its composition considering the time of the year with an abundant flow during the semester's active academic classes ongoing and less effluent flow on summer/winter breaks. In 2019, it was estimated that the university campus had a wastewater average production of 13.000 m³ per month (430 m³ daily) and a total of approximately 20.600 people attending the campus.

The raw wastewater was transported from the Water Quality Recovery Station of university campus (Fig. 6) to the large tank in the mesocosm structure.



Fig. 6.: Water Quality Recovery Station of the university campus from where raw wastewater was collected. Picture of the Experiment 1 sampling.

The station has been inactive for the past two years and the wastewater is going to a pond without any treatment, where other domestic sewage is discharged from a nearby urban community, and subsequently flows into a constructed channel (Arroio Dilúvio) that ends up at Guaíba lake. The collection of 350 liters of wastewater and its transportation was performed three times (E1, E2, and E3) and the wastewater homogenized immediately channeled to the six small tanks of the structure.

At the end of HRT, effluent samples were collected from all macrophyte treatment and control tanks and aliquot in a 500 mL flask for physicochemical parameters analysis, 1 L flask for heavy metal analysis and 2 L in two flasks for ecotoxicological analysis. Samples used to measure nutrients (total/dissolved phosphorus and total/dissolved nitrogen) were kept in a fridge for posterior analyses performed in the same week of the collection. Color and turbidity parameters were analyzed right after the sampling procedure. For ecotoxicological tests, samples were transported in ice to the Ecotoxicology laboratory of UFRGS and frozen at -27 ± 2 °C until the day before the tests, when they were slowly defrosted until the next day at room temperature.

2.4.1. Physicochemical parameters and heavy metals analysis

A fingerprint of the raw wastewater (influent) and effluents from macrophyte and control tanks was made quantifying physicochemical parameters such as conductivity, pH, color, turbidity, total and dissolved nitrogen, and total and dissolved phosphorus as well as five heavy metals including Zinc (Z), Chromium (Cr), Copper (Cu), Lead (Pb), and Cadmium (Cd) (Table 1). Apart from heavy metals, physicochemical parameters were analyzed at the Sanitation Laboratory of Hydraulic Research Institute. The collected samples for total heavy metals were subjected to nitric acid digestion before analysis (APHA, 1998) performed at the Atomic Absorption Laboratory at the Ecology Center (UFRGS).

Ecotoxicological tests were performed only one time for each sample collected. The assays were maintained at temperature 25 ± 2 °C and photoperiod of 16/8 h light/dark cycle. All samples collected from each experiment (Influent, M1, M2, M3, C1, C2, and C3) were tested on the same day in order to use the same batch of organisms and eliminate this source of variability. Ecotoxicity tests for E1, E2, and E3 samples were performed in different periods.

Table 1. Water quality parameters, units, analytical methods and reference used to evaluate the wastewater collected at university campus - UFRGS.

Parameter	Abbreviations	Unit	Method	Reference
pH	pH	-	Potentiometric	-
Turbidity	Turb	NTU	Nephelometric/ Hach-2100N	ABNT, 1990
Color	Color	Hazen	Digimed-DM-COR	ABNT, 1988
Conductivity	Cond	$\mu\text{S cm}^{-1}$	Conductivity meter	-
Total nitrogen	TN	mg L^{-1}	TOC analyzer (SHIMADZU- TOC-VCPN) using the wet oxidation method	ABNT, 1988
Total dissolved Nitrogen	T. dissolved N	mg L^{-1}	TOC analyzer (SHIMADZU- TOC-VCPN) using the wet oxidation method	ABNT, 1988
Total phosphorus	TP	mg L^{-1}	Spectrophotometer/ Stannous Chloride Method 4500P-D	APHA, 2005
Total dissolved Phosphorus	T. dissolved P	mg L^{-1}	Spectrophotometer/ Stannous Chloride Method 4500P-D	APHA, 2005
Zinc	Zn	mg L^{-1}	Atomic Absorption Spectrometer	APHA (1998)
Chromium	Cr	mg L^{-1}	Atomic Absorption Spectrometer	APHA (1998)
Cooper	Cu	mg L^{-1}	Atomic Absorption Spectrometer	APHA (1998)
Lead	Pb	mg L^{-1}	Atomic Absorption Spectrometer	APHA (1998)
Cadmium	Cd	mg L^{-1}	Atomic Absorption Spectrometer	APHA (1998)

2.5. Ecotoxicological tests

All samples collected were submitted to ecotoxicological tests using *Danio rerio* (zebrafish) as the organism-test. The acute toxicity tests were performed at the Laboratory of Ecotoxicology at UFRGS and the standard protocol followed was an adaptation of the 48h acute toxicity test with *Pimephales promelas* by the USEPA 2000.0 (USEPA, 2002). This adaptation included modifications such as the use of *D. rerio* instead of *P. promelas* and the tested ages from 6 to 10 days post-hatching. The use of its larvae stage is justified by the fact that sensitivity is

considerably higher than the juvenile or adult stage (Stelzer et al., 2018). Larvae used in all assays were cultivated at the Laboratory of Ecotoxicology from mature wild type *D. rerio* breeding. All organisms were nurtured with *Paramecium sp.* until 2 hours prior to testing. Six concentrations (from 6.25% to 100%) plus control (dilution water) were used for all tests. Dilution water used followed ISO 12890 (ISO, 1999) protocol adjusted to the final hardness of 40 - 47 mg Ca₂₊ and pH 7.3 - 7.5. The correct dilution was verified through conductivity measurements (WTW LF 197).

2.6. Efficiency percentage

The efficiency percentage, was calculated using the following expression:

$$\text{Efficiency (\%)} = [(influentconc - effluentconc) * (influentconc) - 1] * 100$$

where influent concentration is the average of raw wastewater's parameters (conductivity, pH, color, turbidity, total and dissolved nitrogen, and total and dissolved phosphorus, ecotoxicity) measured from the three experiments; and effluent concentration represents the average of macrophyte/control samples' parameters for the three experiments.

2.7. Data analysis

The chemical and physical parameters and ecotoxicological data were submitted to a descriptive analysis with the statistical program Statistic@. A non-parametric statistical method (Trimmed Spearman-Kärber Method) proposed by Hamilton et al. (1979) was used to estimate LC₅₀ (median Lethal Concentration capable of causing toxicity to 50% of the population exposed) for ecotoxicological tests. *T-test* for Dependent Samples was performed between influent and effluent (Macrophyte and Control) to reveal significant changes after HRT. *T-test* for independent variables was performed to compare the macrophyte treatment and control to verify whether the results can be related to macrophytes action alone, also using the software Statistic@.

A *Two-way cluster analysis* was performed to identify similarities in samples before and after treatment. Also, a *Principal Component Analysis* (PCA) of abiotic variables was performed to reveal the correlation between physicochemical variables and the samples collected (influent, macrophyte and control effluents) and identify the main explanatory variables of the system. Those

analyses were performed using the software PC-ORD version 6.08 for Windows (McCune and Mefford, 2011). Wastewater samples from E1, E2 and E3 were compared considering all parameters analyzed and no significant difference was found ($p > 0.3$); therefore, all analyses considered the data for the three experiments together to achieve a robust analysis. Differences among influent, macrophyte and control effluents were deemed significant if $p < 0.05$.

3. Results and Discussion

3.1. Analytical characterization - Physicochemical scenario

A fingerprint of the influent and effluents was created quantifying 14 key properties including 8 physicochemical parameters, 5 metals and acute toxicity (LC50) (Table 2).

Table 2. Average values of physicochemical parameters, metals and acute toxicity (LC50) of the influent, control and macrophyte effluents. The data was calculated considering the three experiments performed.

Parameter	Influent	Control effluent	Macrophyte effluent
Conductivity ($\mu\text{S cm}^{-1}$)	1096 ± 34.05	841 ± 68.6	775.6 ± 84.4
pH	8.2 ± 0.03	8.2 ± 0.25	7.3 ± 0.09
Turbidity (NTU)	141 ± 33.04	75.8 ± 48.14	37.2 ± 18.27
Color (Hazen)	125.7 ± 33.36	110.6 ± 48.70	79.3 ± 4.47
Total N (mg L^{-1})	96.6 ± 4.02	55.8 ± 9.58	55.0 ± 6.04
T. dissolved N (mg L^{-1})	91.6 ± 3.03	54.3 ± 9.19	54.0 ± 6.88
Total P (mg L^{-1})	5.3 ± 0.32	5.1 ± 0.16	5.3 ± 0.32
T. dissolved P (mg L^{-1})	5.1 ± 0.25	5.0 ± 0.18	5.1 ± 0.25
Zn (mg L^{-1})	0.05 ± 0.04	0.04 ± 0.03	0.04 ± 0.03
Cr (mg L^{-1})	< 0.009	< 0.009	< 0.009
Cu (mg L^{-1})	< 0.007	< 0.007	< 0.007
Pb (mg L^{-1})	< 0.042	< 0.042	< 0.042
Cd (mg L^{-1})	< 0.006	< 0.006	< 0.006
Ecotox (LC50)	13.7 ± 2.79	30.0 ± 8.47	38.7 ± 14.52

Standard deviations are presented next to the mean value. The detection limit for Zn, Cr, Cu, Pb and Cd were 0.012, 0.009, 0.007, 0.042, and 0.006, respectively.

This mesocosm research seeks to pursue a realistic perspective of how a FTW system would perform in the recovery of a raw wastewater, with the least possible control of environmental variables. For this reason, control tanks were not covered as in previous studies (Headley and Tunner, 2008; Tanner and Headley, 2011; Lynch et al., 2015) that meant to avoid phyto-organisms proliferation that might affect nutrients and pollutants removal. Physicochemical parameters and ecotoxicological data were first submitted to a descriptive statistical analysis (Fig. 7).

The floating mats vegetated with *T. domingensis* significantly improved most of water quality parameters such as conductivity, turbidity, pH, total and dissolved nitrogen ($p < 0.001$) and color ($p = 0.003$), except total ($p = 0.05$) and dissolved phosphorus ($p = 0.91$). Likewise, ecotoxicity results presented a significant reduction ($p < 0.001$) (verified by the increase of the concentration necessary to kill half of the exposed population), showing that the use of the floating wetland system was able to enhance the wastewater quality even as a primary/only treatment.

An analysis of the control effluent also indicated a significant difference from the influent for nearly all physicochemical parameters. Conductivity, total and dissolved nitrogen ($p < 0.001$), turbidity ($p = 0.002$) and total phosphorus ($p = 0.04$) were reduced in control tanks. Ecotoxicity was also improved ($p < 0.001$) compared to the raw wastewater. Although the control tanks were expected to not present such efficiency, the results revealed an improvement of water quality parameters that can be attributed to the establishment of an unwelcome plant species (duckweed - *Lemna sp.*) and intense algae proliferation (visual observations) (Figs. 8 and 9). Thus, possibly the excessive load of nutrients in the wastewater, the absence of competition, and the open area for sunlight to reach control tanks, allowed other plants and algae to grow openly; as opposed to macrophyte treatment, in which the shoots and the floating structure shadowed the water. Therefore, the improvement of some water quality parameters in control tanks may have occurred by interactions such as photo-oxidation processes and the presence of plant and algae proliferation, which might have acted as phytoremediation agents. As stated by Yeh and collaborators (2015), being N and P key elements for algae growth, it is natural that these nutrients will be removed where algae find conditions to proliferate and, therefore, water quality is enhanced. However, it is well known that algal bloom events are not a sign of water quality; on the contrary, the elevated availability of N and P can promote a scenario where algae/duckweed multiply freely until sunlight

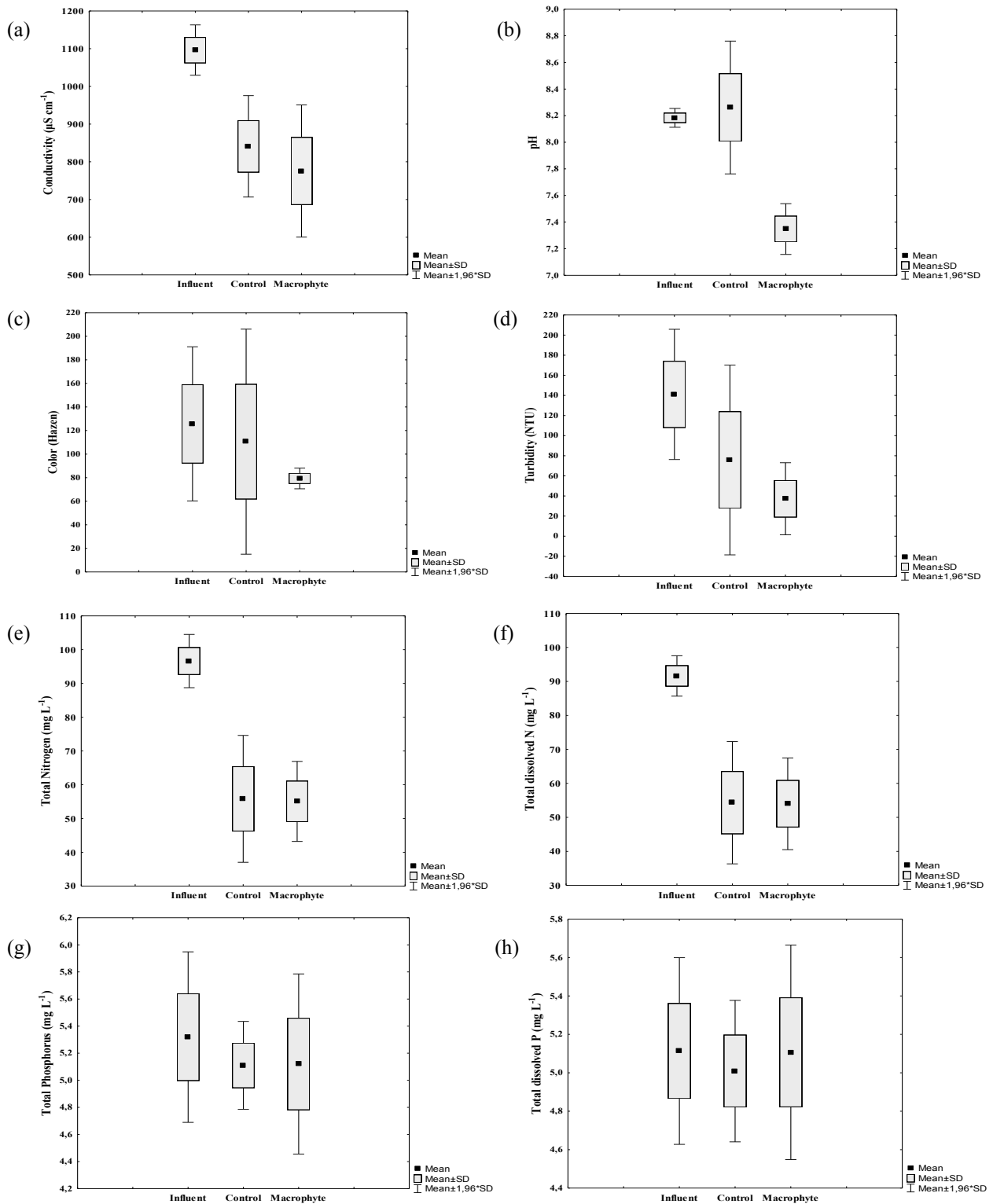


Fig. 7. Mean influent values and mean values for macrophyte and control tanks effluents for all three experiments: (a) conductivity, (b) pH, (c) color, (d) turbidity, (e) total nitrogen, (f) total dissolved N, (g) total phosphorus and (h) total dissolved P.



Fig. 8.: Proliferation of duckweed in a control tank with raw wastewater from university campus (UFRGS).

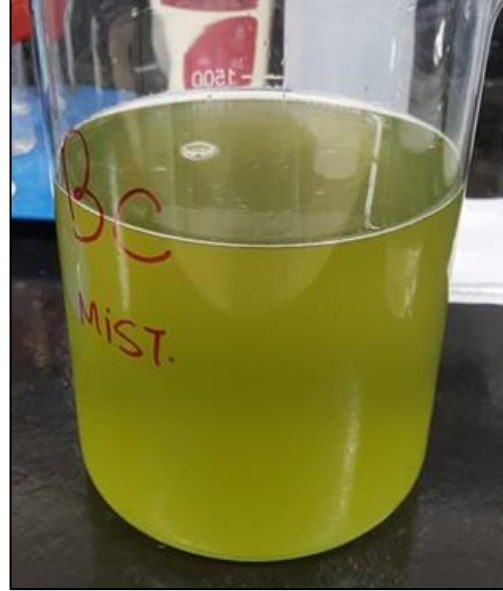


Fig. 9. Intense green color of a control effluent sample due to algal growth observed after hydraulic retention time.

is blocked through water column, preventing photosynthesis to occur and no dissolved oxygen is produced, leading to stress or death of underwater vegetation and animals (Chang et al., 2012).

As discussed in preceding studies with field-scale experiments, Paerl et al. (2014) state that for lakes, cyanobacterial blooms facilitated by hypertrophic conditions may result in food-chain disturbance and low oxygen dissolved conditions. Eutrophication is a real problem worldwide that impacts negatively ecological integrity and facilitates the loss of aquatic systems' biodiversity (Guo et al., 2014). Even in a mesocosm scale, the excessive load of nutrients in the influent (Table 2) can indicate the likelihood of an algal bloom event to occur open field, as it was already observed in the control tanks. Nine days (HRT) probably was not enough time to trigger off the drastic consequences of an algal bloom event, but in an open environment, it is a realistic scenario over a long period.

Macrophyte tanks presented limited algae and duckweed content. Competition for nutrients with macrophytes might contribute for this scenario. Also, events of excessive algae growth are unlikely to occur under the vegetated floating wetland because the structure coverage blocks the sunlight to go through the water column (Hubbard, 2010; Li et al., 2010; Yeh et al., 2015; Chen et

al., 2016), preventing algae to photosynthesize (Zirschky and Reed, 1988; Brix, 1993; Wetzel, 2001; Headley and Turner, 2008; Song et al., 2009; Hubbard, 2010; Li et al., 2010; Yeh et al., 2011). The reduction of light penetration below the floating mat also defines the nature of the bacterial community developing on root's biofilm. As discussed by Headley and Tanner (2008), non-photosynthetic microorganisms will be the majority within the roots net community, except on the edges of the floating system where sunlight marginally reaches. As a result, physicochemical parameters such as pH and dissolved oxygen might be affected as well as biogeochemical processes like nutrient cycling and pollutant removal, fundamental in the water treatment within the floating system (Headley and Tunner, 2008).

3.2. *Biological and physicochemical analysis*

3.2.1. *Macrophyte biomass production and root development*

One of the basic requirements for a macrophyte to be an efficient phytoremediation agent is to tolerate and survive in a media with elevated concentration of contaminants (Tara et al., 2019). In this research, most *T. domingensis* units presented a good adaptation to the wastewater, being only 2 of the 30 plants used in all vegetated tanks replaced throughout the three experiments. The pilot experiment with the raw wastewater used as an adaptation period (besides the one with the synthetic effluent) was extremely important to observe how the macrophytes would react and resist to an intense load of nutrients and pollutants, even already established hydroponically.

The efficiency of FTWs is advanced by macrophytes biomass production (Figge et al., 1995) and the increase of its roots surface area that supports biofilm growth and provides sorption media is the key to significantly nutrient removal (Chang et al., 2007). A significant biomass gain of approximately 30% was observed comparing the initial and final weight of the macrophytes ($p < 0.001$) (Fig. 10-a), which suggests nutrients assimilation. Shoots growth was visible in all three experiments and new sprouts germinated successfully. Headley and Tunner (2011) mentioned that aquatic macrophytes absorb around half of the nutrients in their shoots. Above-water biomass is particularly related to nitrogen and phosphorus uptake; hence it is relevant to care for temporal accumulation with the interest of maximizing the pruning strategy (Munazzam et al., 2018).

However, there was no significant expansion regarding roots' length ($p = 0.08$) over time (Fig. 10-b). *T. domingensis* roots presented an average length of around 20 cm after HRT, considering all three experiments. Before being exposed to the wastewater, at the end of the hydroponic adaptation period, the average root length was 26.1 cm. The undermined development becomes evident comparing to the previous study of Rigotti et al. (2020a), in which *T. domingensis*' roots presented a length growth through time when exposed to a synthetic solution. Although a biofilm was established and young roots development was observed during the course of the experiments, it was evident that the wastewater had an unfavorable impact on the expansion of the roots. It is also possible that macrophytes used nutrients to invest more in its aerial biomass instead of its root network, which is not ideal for floating treatment systems. Also, macrophytes characterized as fast-growing plants tend to develop a large root network when exposed to poor nutrient conditions with the purpose of reinforce root surface area available for nutrient absorption (Weragoda et al., 2012); which it was not the case since nutrient load was abundant.

Root development indeed relies on several factors including plant species and age, nutrient content, water redox status, presence of supporting rafts and, most importantly, water trophic conditions (Chen et al., 2016). According to Lammers and coworkers (2013), an elevated nutrient concentration can have a negative impact, especially to young macrophytes, when there is sulphide formation in an anoxic environment. Furthermore, root growth might be strongly diminished in waters with toxicant content (Chen et al., 2016).

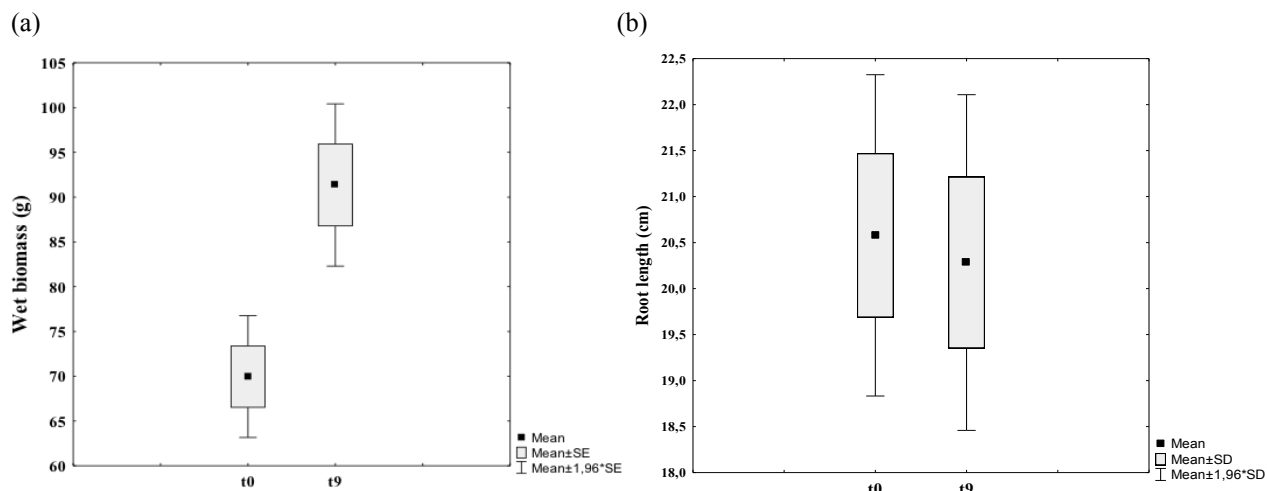


Fig. 10. Initial (t0) and final (t9) time of hydraulic retention time: (a) mean wet biomass production of *Typha domingensis* used in a floating treatment wetland system in a mesocosm scale, (b) mean root length development of all macrophytes units used in the three experiments performed exposed to the raw wastewater produced in university campus (UFRGS).

3.2.2. Nutrient removal

Hydroponically-based plants absorb nutrients to grow, resulting in the removal of nitrates and phosphates from the water column (Zimmels et al., 2006). Nutrients reduction as an action of macrophytes roots nourishing the plants was significant for total and dissolved N ($p < 0.001$), with an efficiency of up to 43.02% and 41.06%, respectively; but not for total ($p = 0.05$) and dissolved P ($p = 0.091$). These results are partially controversial to the data gathered in the literature available so far that presents efficient removal of phosphorus in FTWs (Sukias et al., 2011; Chang et al., 2012; Huang et al., 2017). Considering nutrients removal, the most vital characteristic of a FTW is to be able to develop a robust, large biofilm along the length of the macrophyte's roots. Yet, N and P are not reduced from the water column through the same mechanisms. Nonetheless, according to Chang et al. (2017), nitrification and denitrification processes are the main pathways for nitrogen removal through microbial activity. Thus, a large surface area for biofilm development is requested to enable a great number of bacteria to operate removing nitrate.

An average of total and dissolved N concentration from macrophyte tanks went from 96.6 mg/L to 55.06 mg/L and from 91.61 mg/L to 53.99 mg/L, respectively; which is relatively good,

yet characterizes a highly eutrophic effluent. Hence, it is reasonable to assume that N was removed because the biofilm existing was sufficient to shelter a considerable amount of denitrifying bacteria. As occurred for macrophyte tanks, control's effluent presented a significant reduction in total and dissolved N ($p < 0.001$) compared to influent (from 96.6 mg/L to 55.83 mg/L and from 91.61 mg/L to 54.33 mg/L, respectively), with an efficiency of 42.22% for TN and 40.69% for dissolved N. In FTWs, TN was removed up to an average of 42% by Van de Moortal et al. (2010) using floating macrophytes. As already discussed in section 3.1, the phytoremediation process took place also in control tanks due to algae and duckweed presence.

Regarding P removal, an extensive area for adsorption (retention of the particulate) is desired, considering that binding processes are the major mechanism to reduce this nutrient. Phosphorus removal processes mostly occur through physical processes, including sorption, fixation, complexation, and precipitation (Stottmeister et al., 2003; Maine et al., 2007; Chen et al., 2015; Zhang et al., 2016; Zhao, et al. 2016). Chen et al. (2016) also emphasize macrophytes uptake, sorption, and sedimentation are important routes for this nutrient removal. However, other articles highlight controversial information for this nutrient removal in FTWs. Wang et al. (2016) state that P elimination is less efficient than suspended solids or biochemical oxygen removal because mechanisms as adsorption, precipitation, microorganism's consumption, and macrophytes uptake of this nutrient are considered of minor relevance.

Total ($p = 0.05$) and dissolved P ($p = 0.21$) were not significantly removed from macrophyte's treatment, going from 5.32 mg/L to 5.12 mg/L and from 5.10 mg/L to 5.11 mg/L, respectively (Fig. 7). As discussed in the section before, even though there was a development of a biofilm in the adaptation time within University's wastewater, it was not identified a significant development of the roots' length ($p=0.08$) over time. So, considering that roots did not expand, it is possible that there was not enough surface area for the excessive quantity of P to be entrapped. A low removal rate for P was already reported in other studies. The uptake of this nutrient in FTWs by macrophytes was observed to be a nonsignificant removal pathway in another investigation treating stormwater by (Borne, 2014). Furthermore, according to Brix (1994) and Vymazal and collaborators (1998), although P is crucial for plants and bacteria growth, its contribution to biomass production is substantially inferior compared to N.

For control tanks, total ($p = 0.04$) and dissolved P ($p = 0.21$) also showed a small removal rate compared to the influent (Table 2). Algae and duckweed proliferation may have acted as phytoremediation organisms, although P remained highly concentrated. Another explanation for the insignificant P removal considering both macrophytes and control tanks in this mesocosm experiment might be related to the fact that there was no sediment at the bottom of the tanks, preventing P to find another way out. Benthic sediment usually acts as a sink for P and metals in natural wetlands and ponds (Reddy and DeLaune, 2008; Kadlec and Wallace, 2009). In fact, FTWs are systems known for their suitability in removing P because physical mechanisms such as sorption and precipitation towards the sediment with organic matter binding are favorable conditions provided by these systems (Bu and Xu, 2013; Dodkins and Mendzil, 2014). Projecting the insights of this paper on field-scale mechanisms, the presence of sediment must be a crucial P removal process since investigations worldwide had shown this nutrient removal for real ponds. As mentioned by Cerezo et al., (2001) and Dunne et al. (2012), for a long-term P deposition, organic matter accumulation works as an essential biogeochemical pathway.

The presence of bottom sediment can be extremely important for N removal as well. Most of the N in organic matter deposited in the sediments is lost via denitrification, which is an ecological cleaning mechanism converting NO_3^- to inert N_2 which is lost back in the air. The denitrification process occurs greatly in the sediments because the reaction takes place in the absence of oxygen. Hence, there is a strong desire from management to optimize denitrification as a natural way of reducing N excess. Because in this mesocosm experiment there was no sediment at the bottom of the tanks, denitrification is a reaction that must occur only at the water column and in a low rate since the effluent has to present anoxic conditions. As verified by Khadeeja et al. (2019), since nitrate is an electron acceptor for denitrifying bacteria living in the biofilm, the denitrification process takes place in anaerobic zones of root's net-work as well, being uptaken by the plants, diminishing nutrients load from the water column.

Comparing to another mesocosm study using *T. domingensis* in the removal of nutrients from a synthetic effluent using a FWT system, Rigotti et al. (2020b) showed a satisfactory TN (78%) and TP (47%) removal compared to control (TN 5% increase and 2% TP removal). For the present study, a greater nutrient removal in the macrophyte tanks might have been hindered as a

result of the absence of root length growth throughout the experiments or, plants microbial community within the rhizosphere existing were possibly affected negatively. Many previous studies (Shehzadi et al. 2014; Ijaz et al., 2015 art 31; Ijaz et al., 2016; Khadeeja et al., 2019) theorize about the importance of plant-bacterial synergism in FTWs, especially for nitrogen removal.

3.2.3. Removal performance of conductivity, pH, turbidity and color

Floating treatment wetlands using *T. domingensis* presented a good result for important water quality parameters. Conductivity, pH, turbidity ($p < 0.001$) and color ($p = 0.003$) were significantly reduced compared to the influent. In control tanks, conductivity ($p < 0.001$) and turbidity ($p = 0.001$) were also significantly reduced; but not pH ($p = 0.43$) and color ($p = 0.44$) compared to inflow (Table 2). Turbidity ($p = 0.04$) and pH ($p < 0.001$) were the only parameters that differed significantly between macrophyte and control tanks, being much more reduced under the macrophyte's phytoremediation action.

Conductivity was reduced by the floating wetland system with an efficiency of 29.26% and control tanks presented even lower efficiency of 23.29% (Table 2). In effluents tested in previous studies, conductivity was also reduced better in vegetated floating systems (13 - 20%) than in control, as verified by Ijaz et al. (2015) and corroborated by preceding findings (Lynch et al., 2015; Van de Moortel, 2008). Conductivity is a parameter related to the total of dissolved solids in a water sample. The extremely high values found in the wastewater from the University's Campus are evidence of poor water quality, which can result in the disturbance of the water body receiving this effluent. Even after macrophyte's treatment, conductivity is still on a concerning level. To understand the necessity of proper treatment, Natural Resources Conservation Service (NRCS) informs that conductivity of only 2 $\mu\text{S}\cdot\text{m}^{-1}$ (0.02 $\mu\text{S}\cdot\text{cm}^{-1}$) is safe for agricultural purposes and 4 $\mu\text{S}\cdot\text{m}^{-1}$ (0.04 $\mu\text{S}\cdot\text{cm}^{-1}$) for salt-tolerant cultivations (NRCS, 1999).

The average pH measured in macrophyte tanks was reduced to 7.35 from 8.18 in the inflow, with a removal reduction efficiency of 10.15%. Control tanks, on the other hand, presented a slight pH increase to 8.26. The reduction of this parameter in vegetated treatments is documented in the literature (Ijaz et al., 2015; Strosnider et al., 2017, Sullivan et al., 2019) and might be related to carbon dioxide (CO_2) release during root's respiration and acidic root exudates liberation

(Bezbaruah and Zhang, 2004; Sooknah and Wilkie, 2004; Iamchaturapatr et al., 2007; Borne et al., 2013b; Van de Moortel et al., 2010; Pedersen et al., 2013; White and Cousins, 2013; Lynch et al., 2015). Moreover, the decomposition of organic matter by microbial activity also releases organic acids (Ijaz et al., 2015; Zhai et al., 2013; White and Cousins, 2013; Bezbaruah and Zhang, 2004; Sooknah and Wilkie, 2004; Iamchaturapatr et al., 2007; Lynch et al., 2015) and chemical reactions occurring in the treatment area beneath the vegetated structure could also be a source of acidification (Neori et al., 2000; Headley and Tanner, 2012). Vymazal (2007) and Luo et al. (2010) states that this parameter should be in a range of 6.5 to 8.5. Control tanks that present algae and duckweed proliferation might have pH parameter rise due to photosynthesis and its consequent consumption of dissolved CO₂ (Reid and Mosley, 2016).

Turbidity (Nephelometric Turbidity Units - NTU) is related to the clearness of the water regarding the total suspended solids (TSS) in it. This parameter is critical because it determines the amount of light penetration able to enter the water column, and hence, the euphotic zone. Considering the improvement of turbidity, there was a significant reduction of fine suspended particles in the FWT ($p < 0.001$) as well as in control tanks ($p = 0.001$) (Table 2). Even though, effluent from macrophyte tanks presented this parameter significantly inferior ($p = 0.04$) compared to the unvegetated tanks, as already verified by others (Headley and Tunner, 2008). In the current experiment, the removal efficiency of turbidity in macrophyte tanks was 73.6% and control tanks 46.3%, compared to the influent. Significant removal of this parameter in both macrophyte and control media was observed in other research (Abed et al., 2017) and this fact might be explained by the settling process occurring in control tanks that were not disturbed by environmental external conditions.

Since this mesocosm study was devoid of sediment, fine particles that would not be effectively entrapped in a bottom soil through settlement, as in real ponds, can be majority removed within macrophytes root network (Karnchanawong and Sanjitt, 1995). As corroborated by Yu et al. (2008) and Headley and Tunner (2008), the plant rhizosphere and its biofilm have great potential to capture fine particulates within their root net. This information was indeed tested by Tanner (2011) who proved that a non-vegetated floating structure with artificial roots and soil did not differ significantly from the controls regarding turbidity reduction, on the contrary of a planted FTW

system after 3 and 7 days, that presented a great decrease of this parameter. Therefore, a pond with floating plant mats showed 41% more reduction in TSS than a pond without any FTW system. This indicates that plants do play an important role in the removal of fine suspended particulates beyond the physical process of sedimentation dominant in unvegetated controls. Moreover, a mesocosm experiment performed by Tanner and Headley (2011) tested a synthetic stormwater with fine particles of clay and the initial turbidity (10.2 NTU) in the vegetated floating wetland was more efficiently reduced (57% - 67%) than in unplanted control (23%), considering a HRT of 7 days. In addition, as reported by Chen et al. (2012), physical binding with roots and microorganisms by fine suspended solids is facilitated when the water depth is low, as occurs in this experiment (22 cm depth), which enhances turbidity reduction. The results from this investigation and previous studies make clear that the presence of a FTW will provide a substantial reduction of turbidity better than an unvegetated one.

The reduction in wastewater's color after macrophyte treatment was significant ($p = 0.004$) compared to the influent, with an efficiency of 36.87%. Control tanks did not present a significant difference ($p = 0.43$) (Fig. 7), as expected. The color went from 125.7 Hazen to 79.3 Hazen in vegetated tanks, while in control effluent was 110.5 Hazen. Color is a parameter related to humic substances, originated by bacterial degradation and chemical processing of organic matter derived from plants and animals. That is why DOC - Dissolved Organic Carbon - is positively correlated with the color of wastewater (Tara et al., 2018). Thus, vegetated floating mats improve water quality by reducing dissolved organic compounds. As the organic matter content increases, so does microbial activities, consuming more oxygen from the water column, which characterizes a hazardous environment to a biodiverse community. Depending on the features of aquatic humic substances, color can vary from light yellow to black, in which the intensity of the color, molecular weight and carbon content increase (Stevenson, 1982). This parameter probably remained elevated in control tanks as a result of algae proliferation, which may have increased organic degradation.

Daily mean insolation registered 5.9 hours and mean temperature of the period ranged from a minimum of 18.6 °C to a maximum of 28.0 °C (INMET, 2019). Although the water temperature was not measured, elevated air temperature confirms that the experiment occurred in the summer. Wetland operation is highly impacted by air temperature and solar radiation (Nelson, et al., 2009).

According to Afzal et al. (2011), nutrient removal is more effective during summer and minimally in winter.

3.2.4. Metal analysis

The wastewater used in this research produced by the university campus has majorly the characteristics of domestic sewage. Therefore, it should not present high concentrations of heavy metals, like industrial effluents. Still, it was chosen to analyze metals such as Cu, Zn, Cr, Pb, and Cd because this wastewater is also composed of residues generated in the laboratories. Thus, toxic elements are possibly present considering that are various chemical analyses being carried out within the academic institution. Also, the chosen metals were included by Rezanian et al. (2016) within the recommended ones that are generally present in heavy metal discharges. Metal accumulation through the trophic chain and contamination of all-life forms are a vital health hazard that deserves careful attention as these inorganic substances are not biodegradable and can remain in the biota permanently (Gall et al., 2015; Nancharaiah et al., 2015).

From all the heavy metals analyzed, Zn was the only one that could be identified above the detection limit (Table 2). The influent presented an average concentration of 0.054 mg/L. This concentration cannot be characterized by being highly toxic, considering that for a 48h ecotoxicity test with a freshwater fish (*Gambusia affinis*), Taylor (1978) showed that only up to 0.116 mg/L of Zinc toxic effects could be observed. Also, according to CONSEMA No 355 (2017), “State Council for the Environment” of the Rio Grande do Sul state Resolution, liquid effluents from polluting sources can only be released into surface water bodies, directly or indirectly, meeting the emission standards for Total Zinc of 2 mg/L. However, even though Zinc concentration found in university campus wastewater is below the required limit stated by legislation, it is not possible to guarantee that the found value will not present any biological hazard.

Effluent from both floating mats ($p = 0.47$) and control tanks ($p = 0.55$) did not present a significant Zn removal (Table 2; Fig. 11-a). Most previous studies using FTW systems to reduce Zn (and other heavy metals) present the opposite response testing artificial stormwater (Headley and Tunner, 2007), raw sewage (Van de Moortel et al., 2010) and natural ponds (Borne et al., 2013). The removal of metals in FTWs can occur through a series of mechanisms including plant,

bacteria, and algae absorption, metal sulfides agglomeration, adsorption, entrapment into the roots net biofilm (Kadlec and Wallace, 2008), and further precipitation on the bottom sediment (Borne and Fassman, 2011). As important for nutrient removal, a large root surface area available to sorption is also desired for heavy metal capture. Roots development with robust biofilms is essential to catch suspended particles associated with heavy metals and organic exudates might act as flocculants forming larger agglomerates of dissolved metals resulting in their easier entrapment in the root network or their sedimentation (Headley and Tanner, 2006; 2011; Schwab et al., 2005; Wase and Forster, 1997). It is believed that the rhizosphere community is able to speed the process of a variety of trace metals precipitation in wastewater (Vainshtein et al., 2003; Miretzky et al., 2004; Afzal, Yousaf, et al., 2013; Sessitsch et al., 2013). Therefore, it is possible that because the root system did not present a vigorous growth in this study (Fig. 10-b), Zn removal was not efficient in macrophyte tanks. This limited heavy metal removal from poor biofilm development was already reported by other researchers (Stephenson and Lester, 1987; Santos et al., 2010).

Another reason for the inefficient Zn removal might be related to the absence of bottom sediment. For this metal, precipitation was observed to present a major role in its distribution, while biological mechanisms and secondary sedimentation affect more Cu, Cd and Ni (Santarsiero et al., 1998). Retention ponds are largely employed to control pollution from urban storm-waters precisely because it has the structure to provide particulate-associated metal complexation and immobilization through settlement, decreasing dissolved heavy metals concentration (Dechesne, 2002).

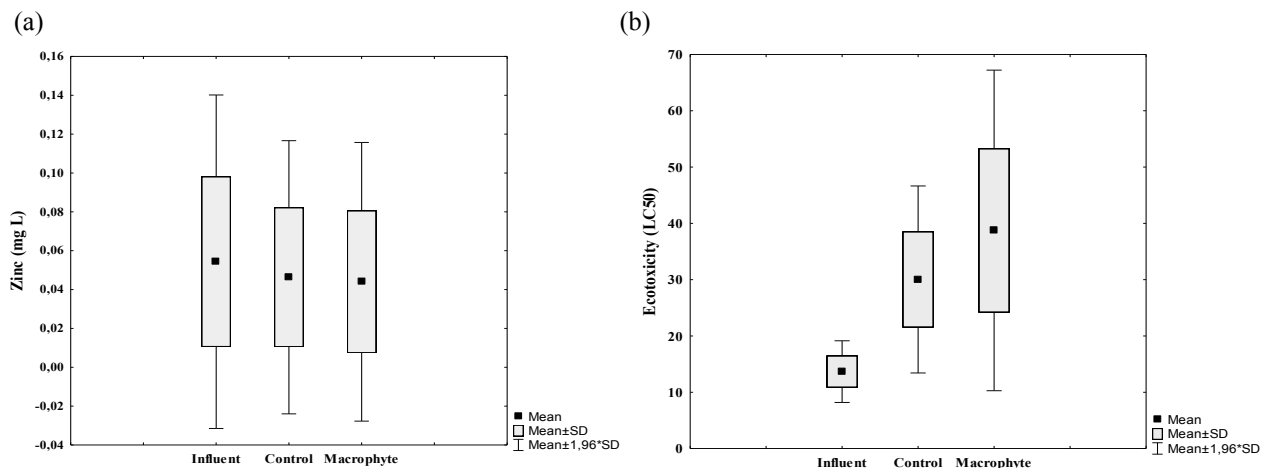


Fig. 11: Mean influent values and mean values for macrophyte and control effluents for (a) Zinc and (b) Ecotoxicity.

3.3. Ecotoxicity assessment

Acute toxicity identification is an important warning that effluent's treatment must be effective to protect the aquatic systems exposed. Ecotoxicology tests are capable to evaluate the toxicity of the whole effluent and it allows to understand the effects of different compounds acting together (Oliveira; Arend; Gerber, 2011). Also, it is important to acknowledge that, even when an effluent meets physicochemical parameters standards, it can still present ecotoxicity.

Both macrophyte treatment and control tanks witnessed a significant reduction in wastewater acute toxicity ($p < 0.001$). After *T. domingensis* treatment, acute toxicity was efficiently reduced up to 64.71% (Table 2; Fig. 11-b). This reduction may be explained as a result of the physicochemical parameters enhancement since there was N, conductivity, pH, turbidity and color reduction in vegetated tanks. Despite that, the results support evidence that the FTW system alone with *T. domingensis* is not enough to promote a full recovery of the water quality, as already confirmed by Ijaz et al. (2016) in an investigation with sewage and industrial effluent. However, a possible improvement of this scenario has been discussed in previous findings certifying that the combination of plants with bacteria may improve FTWs efficiency regard wastewater toxicity removal (Tanner and Headley, 2011; Ijaz et al., 2016; Rehman et al., 2018).

Control tanks also showed an efficiency of 54.5% (Fig. 11; Table 2). Considering some quality parameters improvement, this result is in congruence with the establishment of phytoremediation organisms in these tanks. Nonetheless, although the excessive proliferation of algae and duckweed may assist to remove acute toxicity, it can also lead to the eutrophic scenario mentioned in section 3.1., which might result in oxygen depletion and threatening of ecological biodiversity, in a real context.

3.4. Multivariable analysis - Cluster and PCA

Cluster analysis was used to identify the similarity groups among all samples collected during the entire experiment period. It yielded a dendrogram (Fig. 12), grouping all 21 wastewater samples (3 influent samples; 9 macrophyte treatment effluents; and 9 control effluents) from the three experiments performed. The cluster A (1Inf, 2Inf, 3Inf) is the most dissimilar group consisting of all three influent samples. The group shows the highest values of conductivity, TN,

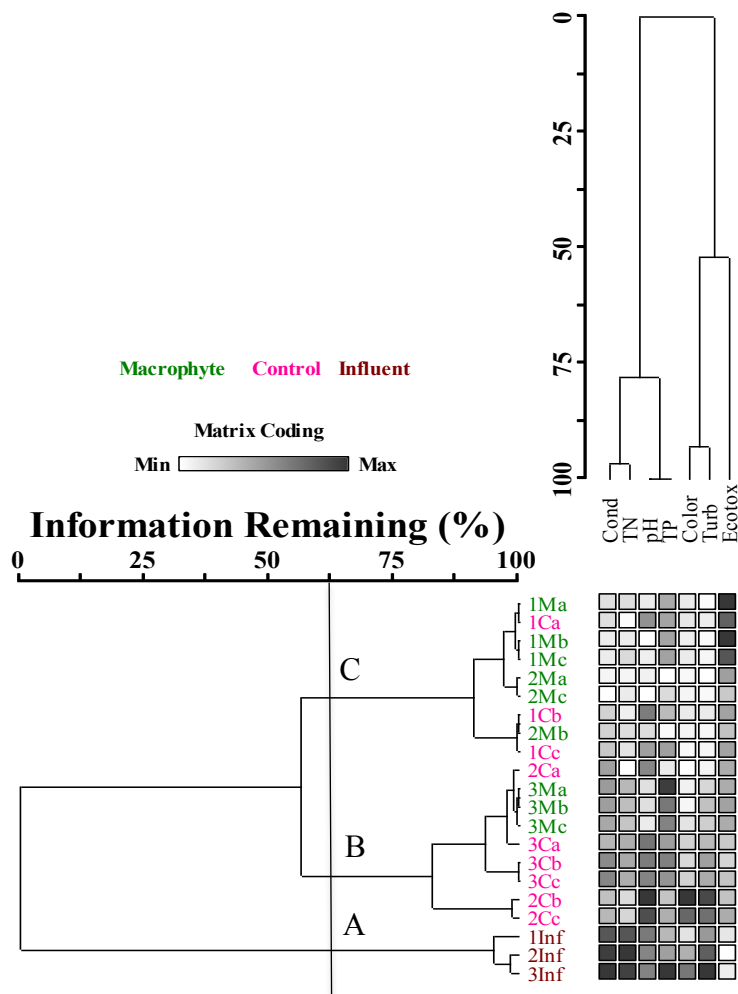


Fig. 12: Two-way cluster dendrogram of the samples collected throughout the three experiments (1, 2, 3 represent E1, E2, and E3; M (Macrophyte), C (Control), Inf (Influent); and a, b, c are the three tanks used for vegetated and non-vegetated treatment). Similarity among physicochemical parameters measured (conductivity (Cond), total nitrogen (TN), pH, total phosphorus (TP), color, turbidity (Turb) and ecotoxicity (Ecotox)) are correlated to the samples.

pH, and turbidity, and the lowest value for LC₅₀, confirming the unsafe water quality discussed of the raw wastewater so far. The cluster B is a mix compound of the control samples from E2 and E3, plus macrophyte samples from E3. Control tanks presented the greatest pH values, considering that there was an increase in pH in those samples. Color and turbidity are strong for control tanks of E2 as a result of algae proliferation. Moreover, dissolved organic matter and photosynthetic organisms are able to absorb light, increasing turbidity. Huang et al. (2017) had also verified the increase in turbidity due to algae blooms. Also, macrophyte tanks from E3 are included in this cluster due to the elevated concentration of TP, which is completely dissimilar to cluster C. The last cluster was composed of macrophyte tanks from E1 and E2 in addition to control tanks from E1. The highest values of LC₅₀ compared to the other samples indicate that ecotoxicity was most

reduced in the tanks with macrophytes. Also, the samples in this group are very similar considering the lowest values of turbidity, color, conductivity, TN, and pH, compared to the other clusters. The control samples from E1 showed low values of color probably because no algae bloom was predominant in this first experiment.

The Principal Component Analysis (PCA) of physicochemical parameters revealed the significance on the first axis (59.7%, $p = 0.001$), identifying the poor water quality as a result of the high turbidity ($r = -0.953$) and conductivity ($r = -0.886$) (Fig. 13), considering influent samples aligned to those parameters, in addition to the high concentration of total nitrogen ($r = -0.840$). Control tanks of the second and third experiment were more correlated to color ($r = -0.700$) and turbidity ($r = -0.953$), when it was observed an elevated algae proliferation. Control tanks of the first experiment showed good ecotoxicity ($r = -0.756$) removal as well as most macrophyte tanks (Fig. 7). On the second axis, Pearson and Kendall correlations were not significant.

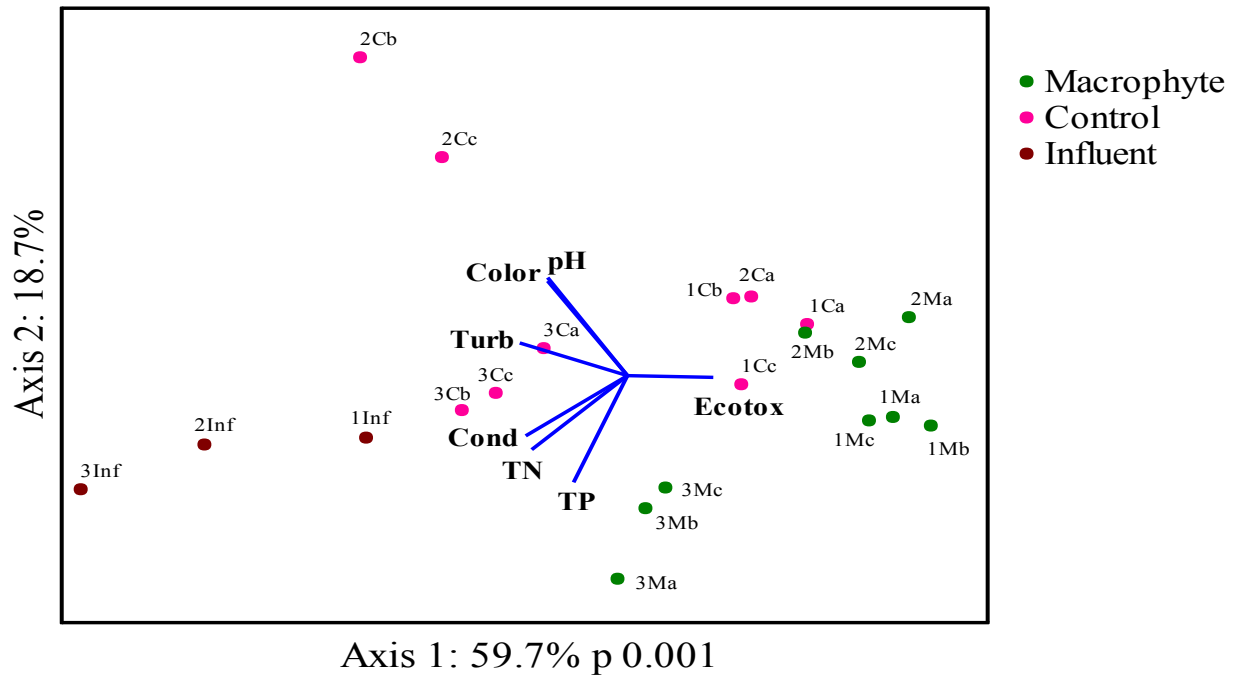


Fig. 13. Principal component analysis (PCA) showing samples distribution (1, 2, 3 are the experiments numbers; M (Macrophyte), C (Control), Inf (Influent); and a, b, c are the three tanks used for vegetated and non-vegetated treatment) correlated with physicochemical parameters: pH, color, turbidity (Turb), conductivity (Cond), total nitrogen (TN), total phosphorus (TP) and ecotoxicity (Ecotox).

3.5. Legislation limits: how close/far are we?

The wastewater produced in the university campus (UFRGS) is discharged in its raw form into a pond that receives more domestic effluent from a nearby urban community. The effluent then goes to a constructed canal (Arroio Dilúvio) and enters the Guaíba lake without any treatment. Monitoring the water quality parameters of the effluents produced is crucial to preserve the entire aquatic ecosystems. The physicochemical tests of raw wastewater in this research revealed TN, and TP contents to be above the threshold allowed by CONSEMA N^o 355 (2017) (Table 3).

Table 3. Average physicochemical characteristics of the raw wastewater (influent) and after macrophyte treatment and maximal allowed standards for sewage discharge according to State Council for the Environment of the Rio Grande do Sul state Resolution (CONSEMA N^o 355 - 2017).

Parameter	Influent	Macrophyte	CONSEMA 355/2017
Conductivity ($\mu\text{S cm}^{-1}$)	1096 \pm 34.05	775.6 \pm 84.4	n.r.
pH	8.2 \pm 0.03	7.3 \pm 0.09	6 - 9
Turbidity (NTU)	141 \pm 33.04	37.2 \pm 18.27	n.r.
Color (Hazen)	125.7 \pm 33.36	79.3 \pm 4.47	=*
TN (mg L^{-1})	96.6 \pm 4.02	55.0 \pm 6.04	20 mg L^{-1}
Total dissolved N (mg L^{-1})	91.6 \pm 3.03	54.0 \pm 6.88	n.r.
TP (mg L^{-1})	5.3 \pm 0.32	5.3 \pm 0.32	3 mg L^{-1}
Total dissolved P (mg L^{-1})	5.1 \pm 0.25	5.1 \pm 0.25	n.r.
Zn (mg L^{-1})	0.05 \pm 0.04	0.04 \pm 0.03	2 mg L^{-1}
Cr (mg L^{-1})	< 0.009	< 0.009	0.5 mg L^{-1}
Cu (mg L^{-1})	< 0.007	< 0.007	0.5 mg L^{-1}
Pb (mg L^{-1})	< 0.042	< 0.042	0.2 mg L^{-1}
Cd (mg L^{-1})	< 0.006	< 0.006	0.1 mg L^{-1}

Standard deviations are presented next to the mean value. The detection limit for Zn, Cr, Cu, Pb and Cd were 0.012, 0.009, 0.007, 0.042, and 0.006, respectively.

n.r.: does not require monitoring.

=*: equal to that of the receiving water body.

Even after macrophyte's treatment, the mean value of TN and TP was still much higher than the limit requested. Conductivity and turbidity are not specified in the resolution, although as discussed in *section 3.1.3*, conductivity is way above the ideal mark even for agricultural use or salt-tolerant cultivations; and although turbidity does not require monitoring, the legislation establishes the concentration of 125 mg/L for a flow rate between 100 and 500 m³ per day of TSS.

For ecotoxicity criteria, the results of ecotoxicological tests must be accepted by the environmental agency, carried out in the effluent. pH values were positively buffered through FTW tanks, even though already in the range of 6 and 9 in the raw samples. Thus, as much as the use of a FTW may help to improve water quality parameters of the wastewater produced, there is still much to be done to achieve legislation's requirements.

4. Conclusion

This research states the applicability of FTWs to improve quality parameters of a complex wastewater effluent considering a mesocosm-scale treatment. It is clear that vegetated mats with *T. domingensis* improved physicochemical and ecotoxicity parameters better than control tanks. Also, the fast eutrophication in the unvegetated tanks proved that this scenario is a realist prognosis of what can happen in the field. As stated by Headley and Tunner (2012), the inclusion of FTW systems might help to prevent eutrophic conditions and, subsequently, algal bloom, which may harm all aquatic communities. However, it is crucial to acknowledge that the effectiveness of mats vegetated with *T. domingensis* working as stand-alone ecotechnology is not enough to completely recover water quality standards of highly polluted wastewaters. The significant improvement in the university's wastewater is insufficient to discharge this effluent freely in a receiving water body. Therefore, a first treatment must be conducted for raw wastewaters in order to enhance the macrophytes phytoremediation potential. A combination with other mechanisms such as aeration, bacteria inoculation and the insertion of more artificial structures to expand sorption media is also a good alternative to enhance FTWs efficacy.

The development of a robust root net-work is the key to a successful FTW system. Microorganisms growth on the biofilm present along the roots form the essential community that will be in charge of most improvement mechanisms of water quality. This finding resonates with

several studies cited throughout this article. Besides preserve for a healthy bacterial ecosystem, aerial biomass should also be well attended. Considering *T. domingensis* being a species that develop long above-water biomass, harvesting is an essential practice that prevents nutrients return into the water column, in case of leaves decomposing. Furthermore, plants harvested can be re-utilized and turn into biogas, bio-fertilizer, recycled as a biomaterial or serve as food for animals and humans (Yeh et al., 2015), without heavy metals incorporated into their biomass.

Ecotoxicity was reduced from both macrophyte and control effluents, but more efficiently from the FTW systems. It is important to conclude that even for extremely polluted wastewaters and all contaminants' synergic or antagonist interactions, the system was able to provide a media that helped the improvement of this parameter.

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CONSIDERAÇÕES FINAIS

O uso de *Wetlands* Flutuantes Construídos (WFC) em mesocosmos no tratamento do efluente bruto produzido no Campus do Vale (UFRGS) mostrou que a maioria dos parâmetros de avaliação da qualidade abordados neste estudo foram melhorados em comparação ao controle (sem presença da vegetação). Dentre os parâmetros físico-químicos avaliados, incluindo pH, condutividade, turbidez, cor, nitrogênio total (NT) e dissolvido e fósforo total (PT) e dissolvido, o tratamento com as macrófitas foi capaz de reduzir significativamente a concentração de quase todos os parâmetros, com exceção do fósforo. Dentre todos os metais quantificados (Zn, Cr, Cu, Pb e Cd), zinco foi o único elemento encontrado acima do limite de detecção do método analítico e não teve sua concentração reduzida pelo tratamento com as macrófitas ou após o tempo de retenção no controle. A ecotoxicidade, também avaliada neste estudo, foi removida significativamente.

Os tanques-controle apresentaram significativa eficiência na redução da concentração de diversos parâmetros; resultado que contradiz a hipótese na qual, na ausência da macrófita *T. domingensis*, não haveria melhora significativa da água residual bruta. Contudo, os tanques sem a estrutura flutuante com macrófitas tiveram uma proliferação abundante da macrófita flutuante *Lemna sp.* e algas microscópicas. Tendo em vista que o efluente continha alta concentração de nutrientes; estava mais exposto a radiação solar devido a ausência da estrutura flutuante; e não possuía outros organismos previamente competindo por ela, reações de foto-oxidação e o intenso acúmulo de algas e macrófitas agindo como agentes fitorremediadores fez com que tal efluente também tivesse suas propriedades melhoradas. Tendo em vista que este experimento também busca prever como a presença ou ausência de WFCs atuaria no ambiente real, a proliferação de algas sem um sistema com macrófitas operando, é um cenário consideravelmente realista. Portanto, é importante salientar que *blooms* de algas não são um sinal de qualidade da água; pelo contrário, elas podem formar um tapete que bloqueia a luz solar de alcançar plantas submersas, impedindo o processo de fotossíntese de ocorrer e levando à depleção de oxigênio na coluna d'água. Assim, efluentes com elevada carga de nutrientes devem ser apropriadamente tratados para evitar tais eventos, que resultam na redução da biodiversidade aquática e deterioração da qualidade da água.

A quantificação da biomassa úmida e a medida do comprimento das raízes durante o experimento foi crucial para a compreensão do funcionamento dos sistemas de WFCs. Todas as macrófitas foram identificadas e monitoradas ao longo dos três experimentos realizados, comparando suas medidas iniciais e finais a cada tempo de retenção. Foi observado um ganho significativo de biomassa de aproximadamente 30% comparando o peso inicial e final das macrófitas ($p < 0,001$), sugerindo assimilação de nutrientes. Headley & Tunner (2011) mencionaram que as macrófitas aquáticas absorvem cerca de metade dos nutrientes em suas folhas. A biomassa aérea está particularmente relacionada à captação de nutrientes; portanto, é relevante avaliar sua acumulação ao longo do tempo no intuito de maximizar a estratégia de poda (Munazzam *et al.*, 2018).

No entanto, não houve expansão significativa do comprimento das raízes ($p = 0,08$) ao longo do tempo. Embora um biofilme tenha se estabelecido e o crescimento de novas raízes tenha sido observado durante os experimentos, foi evidente que o efluente causou um impacto negativo na expansão das raízes. Também é possível que as macrófitas tenham investido mais em sua biomassa aérea ao invés de em sua rede radicular, o que não é ideal para os sistemas de tratamento flutuantes, visto que segundo Headley & Tanner (2008), o atributo mais significativo que determina se um sistema WFC é adequado para uso é o crescimento de extensas raízes.

O sistema de WFC deste estudo não atuou de forma efetiva na remoção de fósforo e do metal pesado Zn. Com relação à remoção de P, uma extensa área de adsorção (retenção de particulados) pelas raízes é desejada, considerando que os processos físicos de ligação são o principal mecanismo para reduzir esse nutriente. Deste modo, tendo em vista que as raízes não se expandiram ao longo do estudo, não houve área de superfície suficiente para que a quantidade excessiva de P fosse adsorvida. Em estudos de tratamento de águas pluviais, observou-se que a assimilação desse nutriente nas WFCs pelas macrófitas é uma via de remoção não significativa (Borne, 2014). Além disso, de acordo com Brix (1994) e Vymazal *et al.*, (1998), embora P seja crucial para o crescimento de plantas e bactérias, sua contribuição para a produção de biomassa é substancialmente inferior em relação ao N.

Os controles também não apresentaram remoção de fósforo. Portanto, outra explicação para a remoção insignificante de P, considerando ambos tanques controle e com macrófitas, pode estar

relacionada à ausência de sedimento, impedindo a saída deste nutriente por outra via. Sedimentos geralmente atuam como uma fonte de P e metais em áreas úmidas e lagoas naturais (Reddy & DeLaune, 2008; Kadlec & Wallace, 2009). De fato, os WFCs são sistemas conhecidos por sua aptidão na remoção de P porque mecanismos como sorção e precipitação pela junção à matéria orgânica são condições favoráveis fornecidas por esses sistemas (Bu & Xu, 2013; Dodkins & Mendzil, 2014). Como mencionado por Cerezo *et al.*, (2001) e Dunne *et al.* (2012), para uma deposição de P a longo prazo, o acúmulo de matéria orgânica funciona como uma via biogeoquímica essencial.

Importante não apenas para a remoção de nutrientes, uma grande área de superfície radicular é importante para a captura de metais pesados. O desenvolvimento de raízes com biofilmes robustos é essencial para capturar partículas suspensas associadas com metais e exsudatos orgânicos que podem agir como floculantes formando aglomerados maiores de metais dissolvidos, resultando em seu aprisionamento na rede radicular ou em sua precipitação (Headley & Tanner, 2006; 2011; Schwab *et al.*, 2005; Wase & Forster, 1997). Como o sistema radicular não apresentou um crescimento vigoroso neste estudo, a remoção de Zn não foi eficiente em tanques com macrófitas. A remoção limitada de metais pesados devido ao baixo desenvolvimento do biofilme já foi relatada por outros pesquisadores (Stephenson & Lester, 1987; Santos *et al.*, 2010).

Outro motivo para a remoção ineficiente de Zn pode estar também relacionado à ausência de sedimento. Para este metal, observou-se que a precipitação apresenta um papel importante em sua distribuição, enquanto mecanismos biológicos e sedimentação secundária afetam mais Cu, Cd e Ni (Santarsiero *et al.*, 1998). As lagoas de retenção são amplamente empregadas para controlar a poluição das águas pluviais urbanas, precisamente porque possuem estrutura para proporcionar complexação e imobilização de metais associados a partículas por meio de assentamentos (Dechesne, 2002).

Os tanques de tratamento com macrófitas e os controles testemunharam uma redução significativa na ecotoxicidade da água bruta residual ($p < 0,001$). Essa redução pode ser explicada como resultado do melhoramento de vários dos parâmetros físico-químicos analisados, uma vez que houve redução das concentrações de nitrogênio, condutividade, pH, turbidez e cor em tanques com vegetação. Apesar disso, os resultados corroboram com evidências de que o sistema de WFC

sozinho com *T. domingensis* não é suficiente para promover uma recuperação completa da qualidade da água dentro de padrões de tratamento de efluentes, como já confirmado por Ijaz et al. (2016) em uma investigação com esgoto doméstico e efluente industrial. No entanto, uma possível melhoria desse cenário foi discutida em outros artigos, sugerindo a combinação de plantas com bactérias pode melhorar a eficiência dos WFCs em relação à remoção da toxicidade de águas residuais (Tanner & Headley, 2011; Ijaz et al., 2016; Rehman et al., 2018). Os tanques de controle também mostraram uma eficiência na redução da toxicidade considerando a melhora de alguns parâmetros na qualidade da água, resultante do estabelecimento de organismos fitorremediadores nesses tanques.

As águas residuais produzidas no Campus da Vale (UFRGS) são liberadas em sua forma bruta em um lago que recebe mais efluentes domésticos de uma comunidade urbana próxima. O efluente então se encaminha para o canal construído Arroio Dilúvio, desaguardando finalmente no lago Guaíba. O monitoramento dos parâmetros de qualidade de águas residuais é fundamental para que o ecossistema aquático seja preservado. Os resultados das análises físico-químicas desta pesquisa revelaram que os parâmetros cor, TN e TP do efluente bruto estão acima do limite permitido pelo Conselho Estadual do Meio Ambiente (CONSEMA) No 355 (2017). Mesmo após o tratamento com macrófita, o valor médio de TN e TP ainda era muito superior ao limite solicitado. A condutividade não é especificada na resolução, embora a mesma esteja muito acima do considerado ideal até para uso agrícola ou cultivos tolerantes à sal (NRCS, 1999); e, embora a turbidez não exija monitoramento, a legislação estabelece a concentração de 125 mg/L de sólidos suspensos totais para uma vazão entre 100 e 500 m³ por dia. Para os critérios de ecotoxicidade, os resultados dos ensaios ecotoxicológicos devem ser aceitos pelo órgão ambiental. Os valores de pH foram tamponados positivamente nos tanques com macrófitas, embora já estivessem na faixa desejada (entre 6 e 9) no efluente bruto.

Os resultados deste estudo afirmam a aplicabilidade dos sistemas de WFCs como uma maneira ecológica de melhorar os parâmetros de qualidade da água de águas residuais, considerando um experimento em mesocosmos. Foi observado o efeito eficaz da macrófita *T. domingensis* na redução de concentrações dos parâmetros físico-químicos e na ecotoxicidade em comparação aos resultados obtidos nos tanques controle. Conforme declarado por Headley &

Tunner (2012), a inclusão de sistemas de WFC pode ajudar a prevenir condições eutróficas e, posteriormente, a proliferação de algas, que podem prejudicar todas as comunidades aquáticas. No entanto, é crucial reconhecer que a eficácia dos sistemas flutuantes com macrófitas atuando como tratamento exclusivo não é suficiente para o enquadramento adequado dentro dos padrões de qualidade de águas residuais altamente poluídas. Portanto, um primeiro tratamento deve ser realizado no efluente bruto, a fim de aumentar o potencial de fitorremediação das macrófitas. Uma combinação com outros mecanismos, como aeração, inoculação de bactérias e inserção de mais estruturas artificiais para expandir o meio de sorção, também é uma boa alternativa para aumentar a eficácia dos FTWs.

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