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Performance of Different Macrophytes and Support Media in Constructed Wetlands for High Turbidity Reduction from Mine Spoil Rainwater

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Abstract: Surface runoff in mining areas transports dissolved and suspended particles into water bodies, known as mine spoil rainwater, contributing to increases in turbidity. The aim of this study was to evaluate the effectiveness of horizontal flow wetlands, free water surface (FWS), and subsurface flow (HSSF) in reducing turbidity >1500 NTU from a synthetic mine spoil rainwater. Macrophytes, support media, hydraulic retention time (HRT), and hydraulic loading rate (HLR) were analyzed. The HSSF *T. domingensis* in gravel #1 achieved a 99% reduction for 4-day HRT, with residual turbidity of 7 ± 3 NTU for 27.43 L m^{-2} d^{-1} HLR. The FWS *P. stratiotes* achieved a 99% reduction for 6-day HRT, with residual turbidity of 11 ± 5 NTU for 36.53 L m^{-2} d^{-1} HLR. *P. stratiotes* free root structures promoted interception of suspended colloidal particles, resulting in a better performance. The dense root structure of *T. domingensis* spreading through the pores of the substrate provided better efficiency than *N. humboldtiana*. However, *N. humboldtiana* proved to be promising as a native species. The use of small granulometry alkaline support media (9 to 19 mm) was highlighted. Therefore, this research proves the efficiency of constructed wetlands in reducing high turbidity and provides optimized parameters for this technology application.

Keywords: colloidal particles; hydraulic retention time; hydraulic loading rate; leaf chlorophyll; root systems; suspended solids trapping

Citation: Gomes, P.C.S.; Rochinha, I.d.S.P.; Paiva, M.H.R.d.; Santiago, A.d.F. Performance of Different Macrophytes and Support Media in Constructed Wetlands for High Turbidity Reduction from Mine Spoil Rainwater. *Resources* **2024**, *13*, 168. https://doi.org/10.3390/resources13120168

Academic Editor: Rongkui Su

Received: 1 October 2024
Revised: 1 November 2024
Accepted: 12 November 2024
Published: 28 November 2024

1. Introduction

According to the latest Mineral Summary report, global production of beneficiated iron reached 2.5 billion tons in 2019 [1]. In Brazil, mineable iron reserves total 38.4 billion tons, according to the Brazilian Mineral Yearbook, where the state of Minas Gerais stands out as the largest producer of raw iron ore (ROM), with around 363.34 million tons recorded in the Mineral Yearbook 2022 [2]. However, despite its economic importance, mineral extraction has a negative impact on the environment.

In this type of mining, waste rock and tailings also expose potential areas for sediment and erosion control [3,4], and various techniques and structures are commonly employed, including physical barriers such as brush or fabric barriers (BFB), flocculants (FL), inlet protection (IP), rock filter dams (RD), sediment barriers (SB), sediment basins (SBN), and sediment traps (ST) [5]. These approaches are suitable for sediment control by intercepting runoff and large sediment particles, contributing to erosion prevention at high-erosion sites, and an important solution for short-term issues [6]. Despite their effectiveness, these approaches have some shortcomings, including high operating and maintenance costs, the possibility of altering water flow, and the release of potentially toxic substances that promote particle aggregation.

The substantial rise in sediment load due to mining activities leads to the movement of particles to nearby water bodies, causing extensive adverse effects on the aquatic ecosystem, influencing the growth of aquatic vegetation and phytoplankton, which reduces the primary productivity and impacts higher trophic levels, leading to biodiversity loss and changes in the ecological interactions. As a result, the entire aquatic ecosystem is disestablished [7,8].

Rainfall events in eroded areas at the mining sites cause soil particle runoff from the rills and these exposed areas, transporting these pollutants to surrounding receiving areas [9]. Particles that are easily transported and settle slowly are typically silt and clay, which significantly contribute to increases turbidity [10]. The effluent resulting from this runoff at the mine site is called mine spoil rainwater, and the formation of mine spoil rainwater was considered natural. It is, therefore, incorrect to refer to this water as wastewater as it is not generated by mining processes [11].

Reducing the environmental impact of mining has become a key challenge for the sector, with a focus on promoting environmental sustainability. Sediment formation and increased water turbidity are some of the major environmental issues related to mining activities, in addition to concerns about potentially toxic elements. Santos et al. (2024) [11] reported a turbidity peak of 11,000 NTU in a watershed closely adjacent to the mining area and simulated extreme conditions with synthetic rainwaters ranging from 500 to 4000 NTU. The control and monitoring of turbidity is essential to achieve the Sustainable Development Goals (SDGs 6 and 14), which are related to clean water and sanitation, as well as aquatic life [12]. Given the need to improve their final processes and those that contribute to changing environmental quality standards, taking into account the range of social, legal, and business requirements and responsibilities, new nature-based technologies have been explored to replace the conventional ones. Conventional technologies usually provide immediate solutions, while nature-based solutions use natural processes that increase ecosystem resilience, promote biodiversity, and provide economic benefits, especially in the long term [13].

Constructed wetlands are a sustainable nature based-solution for the treatment of domestic and industrial wastewater [14]. These systems simulate natural processes through the interaction of plants, support media, and micro-organisms and have demonstrated efficiency in pollutant removal [15,16]. Due to their biological complexity, they have high operational stability and use biochemical and physical processes for water quality improvement [13,17,18]. The effluent to be treated percolates through the support media and also over vegetation, where physical (sedimentation and filtration) or biochemical (precipitation, absorption, adsorption) processes proceed to the removal of pollutants [18,19].

Horizontal flow constructed wetlands are classified according to hydraulic flow: surface flow (FWS) and horizontal subsurface flow (HSSF) [14]. FWS wetlands are effective at reducing organic pollutants and suspended solids and have a water column of less than 0.4 m [20]. HSSF wetlands, which have no visible water column, remove organic compounds, suspended solids, nitrogen, ammonia, and phosphorus through the pores in the support media and the roots of macrophytes [21,22].

Vegetated systems have the potential to treat wastewater due to the presence of aquatic macrophytes, which have been studied in studies focused on decontaminating environments with high metal concentrations [23]. Phytoremediation properties are commonly observed in macrophytes, such as those belonging to the genera *Typha*, *Pistia*, and *Nymphoides*, that have mechanisms to decrease metal concentrations through metal tolerance and accumulation [24–26]. These plants are known to use strategies such as phytoextraction, phytostabilization, phytodegradation, phytovolatilization, and rhizofiltration to remove pollutants [27]. The vegetative cover also improves the aesthetic appearance of the system, promotes the growth of aquatic microbiota, and helps to sequester and store carbon in its biomass [28–30]. In the presence of turbidity, macrophytes act as a filtering system aggregating the colloidal material in its root structures [31].

Studies show that constructed wetlands vegetated with *Pistia stratiotes* for domestic wastewater treatment achieved an efficiency of 82% for total suspended solids (TSS) [32].

Meanwhile, the macrophyte *Typha domingensis*, in the treatment of the same effluent, has achieved percentages of 43% to 61% of TSS reduction [33]. *Nymphoides humboldtiana* has not been reported in constructed wetlands. However, a study has found a better performance of this macrophyte in reducing suspended solids in a phytoremediation pond [34].

The support media play a critical role in wetland processes due to their physical and chemical properties that significantly enhance the efficiency of various pollutant removal mechanisms [35]. In particular, porous support media provide suitable hydraulic and mechanical conditions that contribute to key removal processes such as sedimentation, filtration, sorption, complexation, and precipitation while also promoting biofilm development [36]. Sedimentation and filtration are the primary mechanisms for reducing suspended solids; however, the specific characteristics of each support media can be designed to target and effectively remove specific contaminants [37]. In general, in HSSF-constructed wetlands, it is recommended to use HLRs of 20 to 200 L m^{-2} d^{-1} and HRTs of 3 to 10 days [14,21,37].

Several studies have demonstrated the potential of constructed wetlands as an effective technology for treating industrial and sanitary effluents, notably for reducing organic contaminants [38]. However, the lack of specific research on mining effluents with high turbidity limits the understanding of the effectiveness and suitability of constructed wetlands for this type of application, as well as the need for operational and design adjustments to optimize their performance under such conditions.

Therefore, the aim of this research was to evaluate the effectiveness of different types of constructed wetlands (FWS and HSSF) in the reduction of high turbidity from mine spoil rainwater. In particular, the study aims to (i) compare the FWS and HSSF types, (ii) analyze the effectiveness of different macrophytes (*P. stratiotes*, *N. humboldtiana*, and *T. domingensis*) and support media (dolomitic gravel and blast-furnace slag), (iii) determine the minimum hydraulic retention time (HRT) required to guarantee the effectiveness of the treatment and (iv) assess the possible effects of the treatment on macrophyte development.

2. Materials and Methods

2.1. Study Site and Experimental Design

The experiment was carried out in the experimental area of the Environmental Sanitation Laboratory of the Civil Engineering Department of the Federal University of Ouro Preto, in Ouro Preto, Minas Gerais. Horizontal flow constructed wetland typologies, FWS and HSSF, were chosen as they are commonly used for treating mining wastewater [39].

Three pilot cells were constructed for each line using black polypropylene plastic boxes (Uninjet/Brazil) with a capacity of 56 L and dimensions of 580 mm (length) × 400 mm (width) × 340 mm (height). The wetlands were set up on pallet structures at the site. To minimize the impact of weather and prevent variations in the applied flow rates, the experimental area was partly covered with a plastic greenhouse and ¾ PVC pipe structures. The constructed wetlands had a water column height (h) of less than 0.5 m^2 and were constructed on a pilot scale to allow a detailed assessment of each cell and of the entire system over a 6-day period of hydraulic retention time (HRT) (Figure 1).

The experimental units used in this study were operated according to the parameters described in Table 1. The hydraulic retention time (HRT) was set within the range of 1 to 7 days, based on the literature related to the treatment of mining wastewater using constructed wetlands for metal reduction [40–42]. The range of the hydraulic loading rate (HRT) was also noted in the study by Singh and Chakraborty (2020) [42]. These operating conditions were specifically chosen to explore different ranges in the context of applying this technology in mining.

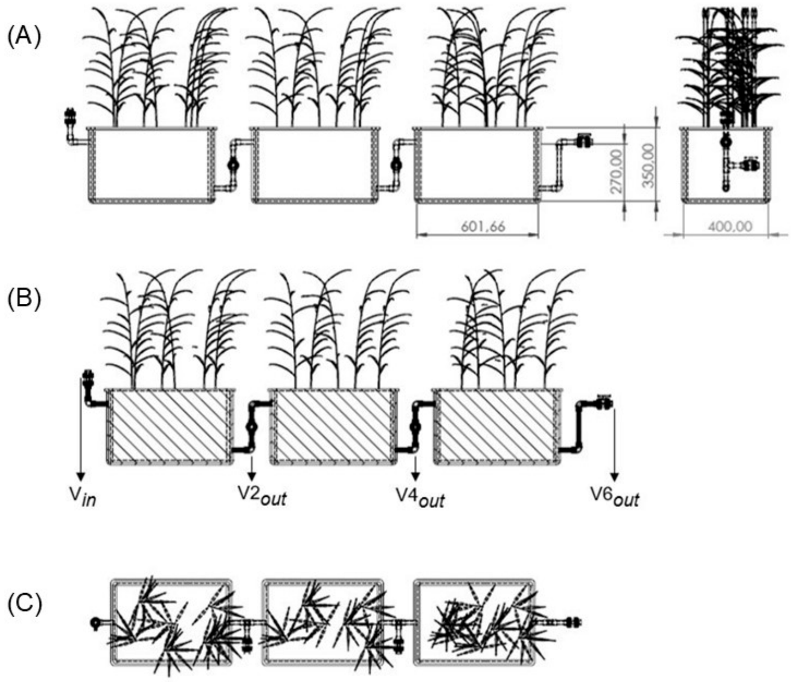

Figure 1. Layout of the constructed wetlands systems in series (mm): (**A**) FWS side view; (**B**) HSSF side view; (**C**) FWS and HSSF top view, where V_{in} indicates the inlet valve, and V_{out} are the outlet valves based on the HDT applied of $V2_{out}$ (2 days), $V4_{out}$ (4 days), and $V6_{out}$ (6 days).

Table 1. Operational parameters of the FWS and HSSF wetlands.

Unit	HRT (days)	Support Media Volume (L)	Total Volume (L)	Flow Rate (L h^{-1})	Area (m^2)	HLR (L m^{-2} d^{-1})
FWS	2	-	40	0.83	0.18	108.84
FWS	4	-	40	0.83	0.32	68.36
FWS	6	-	40	0.83	0.55	36.53
HSSF	2	20	20	0.42	0.18	54.86
HSSF	4	20	20	0.42	0.32	27.43
HSSF	6	20	20	0.42	0.55	18.27

2.2. Macrophyte Selection

The key principle for selecting species is to choose native species—those that are already adapted to the local climate and tolerant of adverse environmental conditions [13]. The species *N. humboldtiana* has been identified as native to the region and has shown good adaptation to the Iron Quadrangle and its specific characteristics, such as mineralogically enriched soils [43].

Several studies have emphasized the potential of macrophytes from the genera *Pistia* and *Typha* for use in constructed wetlands. The floating macrophyte *P. stratiotes* was chosen for its high phytoremediation potential [44–46]. *T. dominguensis* was selected for its strong phytoremediation capacity, particularly for elements such as Fe and Mn [13,15]. *P. stratiotes* was exclusively grown in the FWS wetland system due to the presence of a water column. The morphological characteristics and main traits of the macrophytes under study are detailed in Table 2.

Table 2. Morphological features of *P. stratiotes*, *N. humboldtiana*, and *T. domingensis* [47–55].

Macrophytes	***Pistia stratiotes***	***Nymphoides humboldtiana***	***Tyhpa domingensis***
Family	Araceae	Menyanthaceae	Typhaceae
Popular name	Water lettuce	Floating heart	Cattail
Origin	Native	Native	Native
Endemism	Non-endemic to Brazil	Non-endemic to Brazil	Non-endemic to Brazil
Phytogeographical area	Amazon, Caatinga, Cerrado, Atlantic Forest, Pampa, Pantanal	Amazon, Caatinga, Cerrado, Atlantic Forest, Pampa, Pantanal	Amazon, Caatinga, Cerrado, Atlantic Forest, Pampa, Pantanal
Plant type	Free-floating	Fixed or free-floating	Emergent
Roots	Adventitious and fibrous	Adventitious, fibrous	Submerged, fibrous
Stems	Absent (leaves directly from rhizome)/Stoloniferous	Submersed stoloniferous rhizome	Submerged rizhome
Leaves	Floating, oval, full-rimmed	Floating, rounded, and wavy edges	Flat with a pointed tip
Inflorescence	Single, in each sympodium	Single, floating, or on short stems	Seedheads at the top

Morphological aspects, such as root architecture, were important criteria for selecting macrophytes (Figure 2). The presence of vegetation and the distribution of fixed or free root structures in the water column influence water flow velocity and the trapping of suspended particles, thus affecting sedimentation processes [56]. *P. stratiotes* have thin, suspended roots that allow them to float and absorb nutrients directly from the water, while *N. humboldtiana* has more stable roots that grow from a submerged rhizome and anchor the plant to the wetland bottom [48,50,52]. *T. domingensis* has an extensive and dense root system, with thick roots that tightly attach the plant to the support media [51].

Figure 2. Representation of the selected macrophytes roots architecture during the cultivation period of the experiment: (**A**) *P. stratiotes*; (**B**) *N. humboldtiana*; (**C**) *T. domingensis*.

2.3. Macrophyte Cultivation and Growth

The propagules of the macrophytes studied were purchased from local businesses and cultivated at the Jorge Luiz da Silva Botanical Garden—UFOP.

Juvenile individuals of *P. stratiotes* and *N. humboldtiana* were purchased, as well as vegetative propagules (rhizomes) of approximately 30 cm in length of *T. dominguensis*. The establishment was carried out in two stages: the first (cultivation) and the second (replanting in the wetlands). Tanks of 250 and 500 L were used to grow the macrophytes, in which 25% of the total volume of the tanks was filled with dolomitic gravel, granulometry #1, in order to minimize possible future disturbance when replanting the macrophytes in the wetlands systems. Clark's (1975) [57] nutrient solution was used to nourish the propagules at a rate of 10% of the total volume of the hydroponic culture.

After 60 days of cultivation, the nutrient solution was discontinued, and the plants were watered with tap water until replanting in the treatment systems. In the second stage, young individuals (rhizome + aerial part) of the three macrophyte species were transplanted into the constructed wetland systems, following the average density of 4 propagules per square meter as established by Brix (1997) [58]. After planting, the system was fed with tap water from an underground well, free of chlorine, for about 30 days in order to adapt the macrophytes to the constructed wetland systems in the experiment [59,60].

2.4. Selection of Support Media

Several types of support media can be used in constructed wetlands. The primary criteria for selecting a substrate are its availability and cost and its physical, chemical, and biological properties for improving system performance [36]. In most cases, waste, industrial, and mineral by-products stand out as reliable alternatives for meeting the following criteria [61].

Studies on treating mining wastewater containing high metal concentrations and acidic pH have highlighted the use of limestone, which predominantly contains calcite or dolomite [40,62]. In addition, Wang et al. (2020) [36] have highlighted the low cost of this material, its medium permeability, and its high efficiency in the removal of contaminants. Similarly, blast furnace slag, a by-product of the mining industry, has a mineralogical composition of calcium aluminosilicate, medium cost, and high permeability and is suitable for the reuse of this waste [36].

Furthermore, the choice of grain size is essential because it directly impacts the system's hydraulic conductivity, porosity, and clogging [13,63]. Based on this, three different sizes of gravel and types of support media were used: blast furnace slag (diameter between 9.5 mm and 25 mm), dolomitic gravel #2 (diameter between 19 mm and 25 mm), and dolomitic gravel #1 (diameter between 9.5 mm and 19 mm).

2.5. Preparation and Characterization of Synthetic Mine Spoil Rainwater

Synthetic mine spoil rainwater (SMSR) was produced using water from the Piracicaba River, collected downstream of a mining area in the municipality of Mariana (MG) in the Iron Quadrangle region. To produce the SMSR, solid material (iron ore tailings) from an area planned for future iron ore mining, also located in the Iron Quadrangle, was mixed with the river water, as described below.

The solid material was processed by comminution. The aim was to obtain a pulp composed mainly of colloidal particles. Sieving and homogenization in a carousel provided the sample to be processed. The material obtained was then subjected to a wet grinding process in a ball mill. The mill filling factor was set at 50%, with 20% of the space occupied by grinding media (10 mm diameter steel balls), 20% solids, and 10% water. The sample was ground at a speed of 70 rpm for 60 min. Finally, wet sieving was performed using a series of three sieves (400 mesh [38 μm], 325 mesh [45 μm], and 270 mesh [53 μm]) to produce pulp, which was mixed with the river water.

The pulp obtained from wet sieving was dispersed in the Piracicaba River water in a 2000-litre agitator tank (Eco Mix 1000 1HP4P/Ecotecnologia/Brazil). The water was kept at a constant agitation speed of 102.9 rpm for the entire experiment period. Table 3 describes the main characteristics of SMSR.

Table 3. Mean concentrations characterizing the SMSR quality parameters ($n = 60$).

Parameter	Equipament (Model/Brand)	Method	Unit	Mean
Turbidity	TL2360/HACH/Germany *	Spectrophotometry	NTU	1614 ± 357
Hydrogen potential	HQ40d/HACH/Germany	Potentiometric		7 ± 0.2
Temperature	HQ40d/HACH/Germany	Thermometry	°C	20 ± 3
Dissolved Oxygen	HQ40d/HACH/Germany	Potentiometric	$Mg \cdot L^{-1}$	7
Color	DMCOR/DIGIMED/Brazil	Spectrophotometry	mg/L Pt-Co	2462 ± 502
Oxirreduction potential	HI98190/HANNA/Brazil	Potentiometric	mV	−24 ± 11
Conductivity	DM32/DIGIMED/Brazil	Potentiometric	µS/cm	66 ± 24
Total Aluminium	ICP-OES 725/ AGILENT TECHNOLOGIES/United States	Spectrophotometry	µg/L	186.4
Total Iron	ICP-OES 725/	Spectrophotometry	µg/L	362.0
Total Manganese	ICP-OES 725/	Spectrophotometry	µg/L	3.61

* Used for every turbidity analysis of the inflows and outflows of the analyzed waters.

The pulp samples were analyzed by X-ray fluorescence (Epsilon 3X/Panalytical/United Kingdom). The chemical element concentration in the pulp indicated a predominant presence of hematite (Fe_2O_3) and alumina (Al_2O_3).

The SMSR was also characterized using a laser particle analyzer (Bettersize 2000LD/ Dandong Bettersize Instruments Lt/China). The analyzer has a measuring range of 0.02–2000 µm. The D50 fraction, representing about 50% of the particles, was below 2.6 µm, while the D90 fraction, representing 90% of the particles, was below 17.0 µm. Therefore, this analysis showed that the SMSR was mainly composed of colloidal particles classified as silt (2 and 50 µm) and clay (less than 2 µm) [64].

Peristaltic pumps (DF4a/ProMinent/Germany) with a capacity of 1.5 L h^{-1} were used. The SMSR was continuously delivered to the treatments with a mean turbidity of 1614 ± 357 NTU (n = 60).

2.6. Pilot-Scale Wetlands Description

Two types of constructed wetlands were evaluated in this study: FWS and HSSF. The combinations of macrophytes (*P. stratiotes*, *N. humboldtiana*, and *T. domingensis*) and support media (dolomitic gravel #1 and #2 and blast furnace slag) were employed to design these systems.

Figure 3A shows the composition of FWS and their respective controls: FWS non-vegetated, FWS *P. stratiotes*, FWS *N. humboldtiana*, FWS *T. domingensis* fed with SMSR, and 3 control wetlands fed with tap water: FWS *P. stratiotes*, FWS *N. humboldtiana*, FWS *T. domingensis*.

The following conditions were used for HSSF (Figure 3): (B) HSSF non-vegetated and gravel #1, HSSF *N. humboldtiana* in dolomitic gravel #1, HSSF *T. domingensis* in dolomitic gravel #1; (C) HSSF non-vegetated and dolomitic gravel #2, HSSF *N. humboldtiana* in dolomitic gravel #2, HSSF *T. domingensis* in dolomitic gravel #2, and (D) HSSF non-vegetated and blast furnace slag, HSSF *N. humboldtiana* in blast furnace slag, HSSF *T. domingensis* in blast furnace slag, fed with SMSR. The controls used for this typology were HSSF *N. humboldtiana* on gravel #1, HSSF *T. domingensis* in dolomitic gravel #1, HSSF *N. humboldtiana* in dolomitic gravel #2, HSSF *T. domingensis* in dolomitic gravel #2, HSSF *N. humboldtiana* in blast furnace slag, HSSF *T. domingensis* in blast furnace slag.

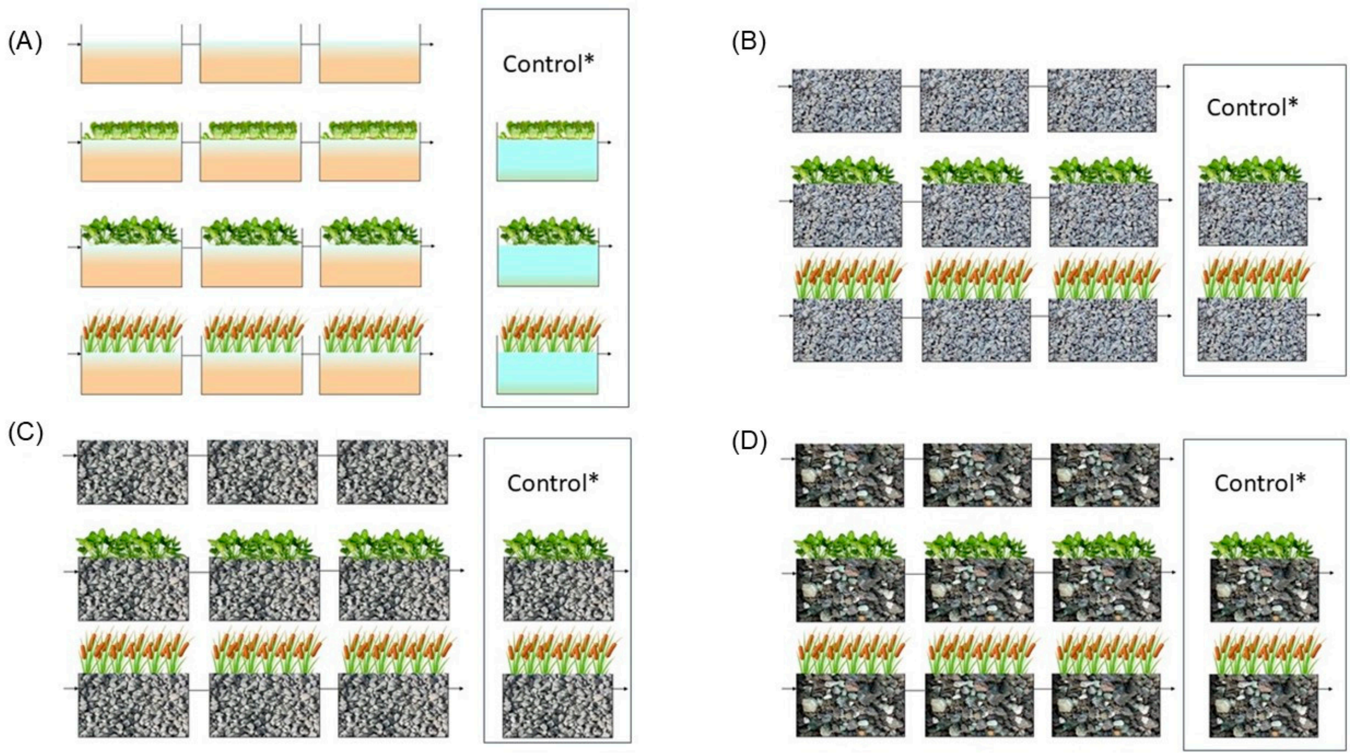

Figure 3. Setup of the FWS and HSSF wetlands: (**A**) FWS; (**B**) HSSF in dolomitic gravel #1; (**C**) HSSF in dolomitic gravel #2, and (**D**) HSSF in blast furnace slag. Control* refers to the wetlands fed with tap water.

To evaluate the impact of treatment on macrophyte physiology, control of vegetated constructed wetland systems was supplied with tap water. In the control group, two days HRT was applied at a flow rate corresponding to the treatments. These control wetlands were managed under the same climatic conditions as the experimental wetlands, and the macrophytes were similarly assessed.

The experiment was monitored for 144 days from January to July 2023 (Figure 4). Samples were collected three times a week (Mondays, Wednesdays, and Fridays) in the morning, and the sample volume was less than 5% of the total volume of each cell. Standard methods were applied for the collection [65], as described in Table 3. The samples were stored in plastic bottles and analyzed at the Environmental Sanitation Laboratory.

2.7. Determining Chlorophyll in Macrophyte Leaves

The ChlorofiLOG portable chlorophyll analyzer (CFL1030/Falker/Brazil) was used to determine the total chlorophyll content of the leaves in the treatments studied in an indirect, non-destructive way. The equipment's sensor detects the radiation reflected by the leaf and, based on these data, calculates the values corresponding to the absorbance of the chlorophylls, known as the Falker Chlorophyll Index (FCI) [66].

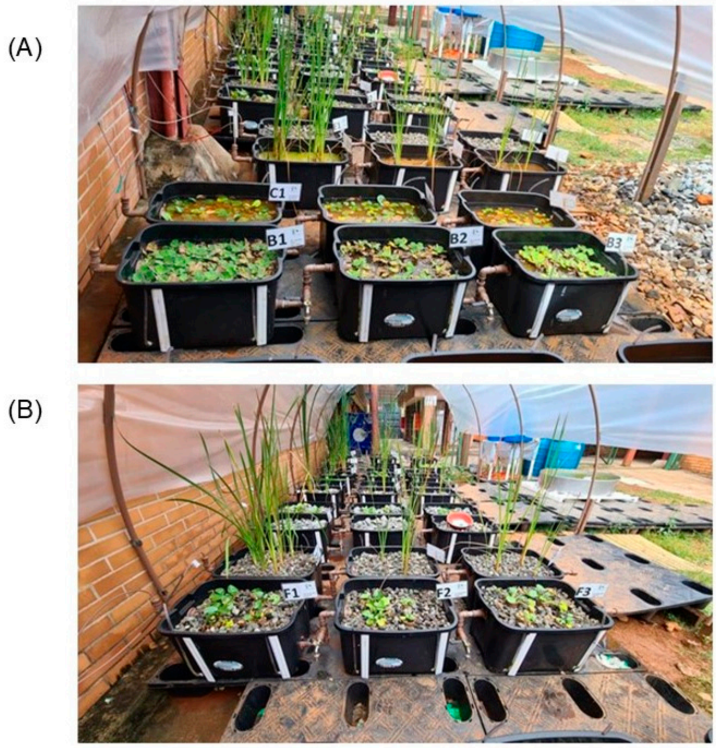

Figure 4. Photography of the pilot scale horizontal flow constructed wetlands: (**A**) FWS; (**B**) HSSF.

2.8. Statistical Analysis

The Shapiro–Wilk test ($p < 0.05$) was used to check the normality of the data. The data collected did not follow a normal distribution. Following the methodology proposed by Mietto et al. (2015) [67] and Barco and Borin (2020) [31], the turbidity reduction percentage was calculated according to the following Equation (1):

$$\text{Reduction (\%)} = (C_{in} - C_{out})/C_{in} \times 100 \quad (1)$$

where C_{in} = affluent turbidity and C_{out} = effluent turbidity at each HRT evaluated.

In order to assess the performance of the wetland typologies, a permutational multivariate analysis of variance ($p < 0.0001$) was performed to compare the turbidity reduction rates.

The Kruskal–Wallis non-parametric test ($p < 0.001$) was used to test the significance of turbidity reduction rate between the different treatments and between the applied hydraulic detention times. The Dunn test ($p < 0.001$) was used to statistically identify significant differences. The analysis was performed using R software (4.4.0) [68].

3. Results

3.1. Constructed Wetland Typology Performance

Statistically significant differences ($p < 0.0001$) were found between the evaluated systems in the permanova analysis (Figure 5). The HSSF wetlands showed a smaller distance to the centroid (d = 0.4135) compared to the FWS wetlands (d = 0.6091) and the control (d = 1.3156). This smaller distance from the centroid reflects less dissimilarity in the turbidity reduction data, indicating that the HSSF has superior performance with less variation in turbidity reduction during treatment and greater stability.

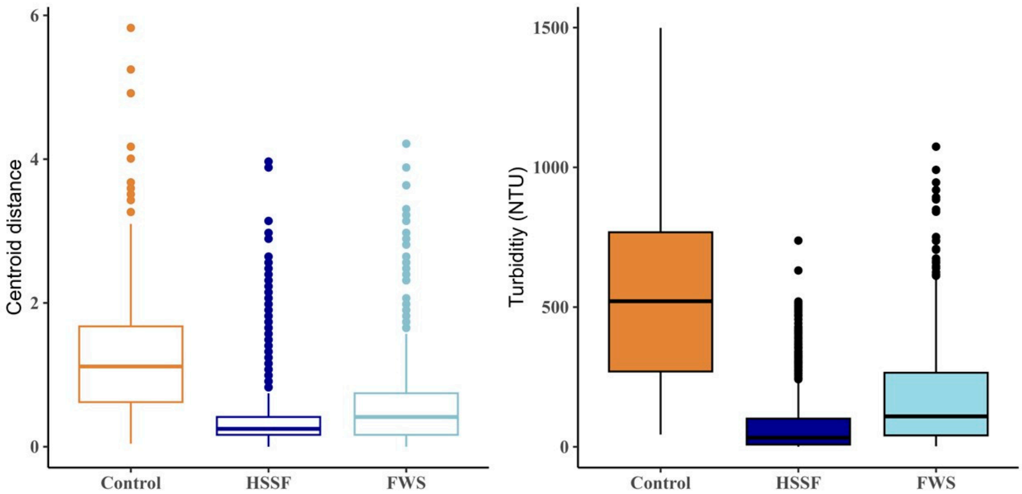

Figure 5. Assessment of turbidity reduction in constructed wetland typologies: Control (n = 174), HSSF (n = 1421), and FWS (n = 489).

Turbidity is strongly related to the presence of total and dissolved suspended solids in the effluent [14]. In terms of reduction mechanisms for suspended solids, sedimentation is the main reduction mechanism, followed by filtration and adsorption on support media and macrophytes [69–72]. The control showed lower turbidity reduction efficiency, indicating that settling ponds cannot effectively reduce turbidity in the absence of a coagulant, even at low HLR. This finding also indicates that the SMSR was suitable for the experiment, as the control exhibited measurable turbidity.

Macrophytes play a crucial role in reducing water velocity and particle sedimentation in FWS and HSSF wetlands. The stems of emergent macrophytes and the roots of free-floating macrophytes increase the contact surface for trapping suspended particles and contribute significantly to reducing turbidity [73]. Furthermore, the presence of support media within HSSF wetlands significantly contributes to the reduction of water flow velocity and significantly increases particle trapping and sedimentation. In this system, water infiltrates through the filter bed pores (support media and macrophyte roots), enhancing sedimentation and particle reduction [22]. A study by Vymazal (2019) [72] reported that HSSF wetlands reduced total suspended solids, averaging 11.2 mg·L^{-1} in 113 wetlands evaluated due to their high particle retention capacity. Hernández-Crespo et al. (2017) [74] showed that HSSF wetlands were more efficient than FWS in reducing total suspended solids and other parameters, also emphasizing that this typology requires less area to achieve output concentrations similar to FWS. These studies were both carried out in waters with high organic matter content, with averages of 364 mg·L^{-1} [72] and 65.4 to 29.9 mg·L^{-1} [74]. In this study, similar results were obtained in waters with a lower content of organic matter.

The analysis indicated that systems with a larger surface area for trapping suspended particles achieved higher performance. In particular, HSSF wetlands, incorporating both vegetation and support media, outperformed FWS wetlands, which contained only vegetation. Both systems also demonstrated superior performance than the control, a sedimentation pond that had neither vegetation nor support media.

3.2. Turbidity Reduction in FWS Wetland

The performance of FWS vegetated by different macrophytes in terms of turbidity reduction for the 2-, 4-, and 6-day HRT indicated statistical differences in treatment efficiency ($p < 0.0001$); Figure 6.

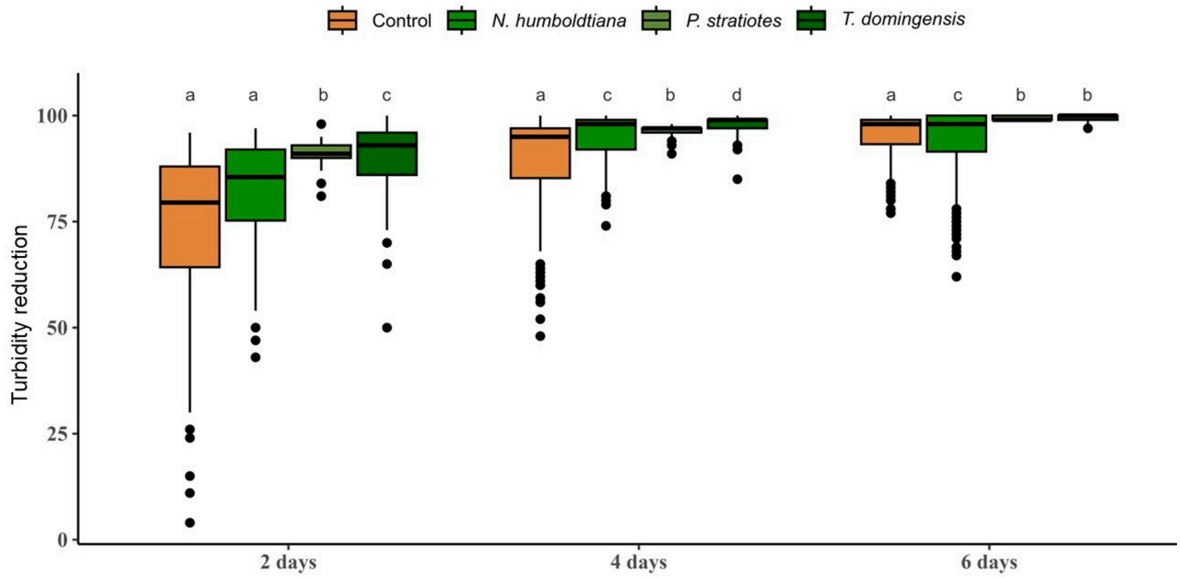

Figure 6. Performance of different support media at each HRT (2-, 4- and 6-day, $n = 60$) for FWS wetlands and the control wetland. The letters indicate statistical differences considering the vegetation species in the FWS wetlands at each HRT applied and the control wetland (Kruskal Wallis test; Dunn's test for multiple comparisons; $p < 0.001$). Comparisons between HRTs are not applied.

The FWS P. stratiotes wetland was the most effective in reducing turbidity, with reduction rates of 91%, 96%, and 99% observed for the 2-, 4- and 6-day HRT, respectively. The FWS *T. domingensis* wetland showed comparable reduction rates of 81%, 95%, and 99%, with a statistically significant difference only for 2-day HRT ($p < 0.001$).

Considering the initial turbidity of the treatments (1614 ± 357 NTU, n = 695), the mean reduction in turbidity was observed in the FWS P. stratiotes, of 166 ± 57 NTU (n = 60) for 2-day HRT. For the 6-day HRT, turbidity reduction reached an average of 11 ± 5 NTU (n = 60), while *T. domingensis* showed increased values of 26 ± 9 NTU (n = 60) (Table 4).

Table 4. Turbidity reduction in FWS wetlands for retention times of 2, 4, and 6 days for inflow turbidity (1614 ± 357 NTU, $n = 695$).

Wetland	HRT (Days)	Turbidity Reduction Rate (%)	Mean Residual Turbidity (NTU)
Control	2	47	971 ± 261
	4	72	507 ± 137
	6	87	232 ± 68
FWS *P. stratiores*	2	91	166 ± 57
	4	96	63 ± 25
	6	99	11 ± 5
FWS *T. domingensis*	2	81	335 ± 113
	4	95	99 ± 37
	6	99	26 ±9
FWS *N. humboldtiana*	2	68	586 ± 169
	4	87	237 ± 79
	6	95	88 ± 32

The research results showed that FWS *N. humboldtiana* for turbidity reduction may have some limitations. The reduction rates observed in the 2-day HRT (68%) and the control (47%) were not statistically different. Turbidity reduction rates reached 95% in the 6-day HRT, resulting in mean residual turbidity of 88 ± 32 NTU (n = 60). It was noted that residual turbidity was higher in the FWS *N. humboldtiana* than in the other FWS wetlands (Table 4).

Macrophyte structure had an effect on turbidity reduction in FWS wetlands. The best performance was observed in FWS *P. stratiotes*, a free-floating macrophyte with a fasciculated root system that forms a dense network of roots throughout the water column, assisting in trapping particles in these structures [75,76].

The second most successful macrophyte in terms of turbidity reduction was *T. domingensis*. The plant has a highly developed root system with dense and ramified growth, and the stem of the plant is typically 5 to 10 mm in diameter and remains in contact with the water column. It acts as a physical barrier to trap sediment particles. The root system is highly developed, with underground rhizomes anchoring the plant and supporting vegetative reproduction [24].

The lowest level of performance was observed for FWS *N. humboldtiana*. This fixed floating macrophyte has a well-developed root system with fine, relatively shallow roots [77]. Compared to the roots of *P. stratiotes* and *T. domingensis*, the stem of *N. humboldtiana* has fewer branches and is thinner, ranging from 1 to 3 mm in diameter, resulting in a reduced contact area. As a result, particle retention was reduced, and the performance of the wetland decreased.

Macrophytes with different morphological structures were effective in turbidity reduction. The dense root structures of *P. stratiotes* contributed a better performance than *T. domingensis*, while both were more effective than *N. humboldtiana*.

Therefore, macrophytes with denser structures provide a larger contact surface, resulting in more efficient retention of suspended particles in the water. There was a relatively high reduction in the FWS *P. stratiotes* between 2- and 4-day HRT, while *T. domingensis* showed similar trends only for 4- and 6-day HRT. Studies by Ugya and Meguellati (2022) [78] on the treatment of landfill effluent showed that *P. stratiotes* achieved 80% turbidity reduction. A study evaluating P. stratiotes for the treatment of domestic wastewater at different hydraulic retention times of 6, 12, and 24 h observed 91% turbidity reduction (15–30 NTU) in 24 h HRT [79]. The reported results are related to effluents containing high concentrations of organic matter and low turbidity. This research confirms the effectiveness of wetland technology in the treatment of mining effluent by demonstrating its ability to reduce inorganic solids. Physical retention rather than biochemical degradation is more important in this scenario.

Brazilian legislation has no direct standards for wastewater discharge to water bodies, although the classes established for the receiving water bodies must not be compromised. The water quality parameters for classifying water bodies are defined in CONAMA Resolution 357/2005 [80]. According to this resolution, the turbidity limit is less than 40 NTU for class 1 waters and less than 100 NTU for class 2 and 3 waters. Discharges to class 2 and 3 waters are also permissible. Likewise, international definitions of turbidity limits have been adopted in countries such as Australia, New Zealand, and Canada.

In Australia, according to ANZECC (2000) [81], turbidity limits for water bodies vary between 1 and 50 NTU. In New Zealand, the limit is 5.6 NTU, while in Canada, turbidity should not exceed 10% of the original value observed in the river [81,82].

HRT plays a more significant role in turbidity reduction efficiency related to these limits. For a 4-day HRT, FWS *P. stratiotes* decreased turbidity to levels suitable for class 2 and 3 waters according to CONAMA legislation 357/2005 [80]. In addition, it can be noted that the residual turbidity for a 6-day HRT is consistent with the most restrictive level of the same legislation, Class 1, and for Australian legislation [81].

Other key water quality parameters were evaluated after a 6-day HRT (Table 5). According to CONAMA legislation 357/2005 [80] that classifies water bodies, the following parameters, such as hydrogen potential (6.0–9.0) and dissolved oxygen (<6.0 $mg \cdot L^{-1}$), meet the standard limits of class 1 waters. The pH parameter was considered for the evaluation according to the ANZECC legislation (2000) [81], meeting the established limits. These results showed that the treatment was effective in terms of water quality management.

Table 5. Mean quality parameters concentration measured at the inlet and outlet of FWS wetlands (n = 60 samples).

Parameters (n = 60 Samples)	Unit		Inlet	Control	FWS *P. stratiotes*	FWS *N. humboldtiana*	FWS *T. domingensis*
Turbidity	NTU	mean	1614	230.6	11.2	87.2	25.6
		sd	357	67	5	32	9
Temperature	°C	mean	19.0	19.2	19.1	18.9	18.9
		sd	0.0	3.0	3.1	3.1	2.9
Hydrogen potential		mean	7.3	7.4	7.3	7.3	7.3
		sd	0.1	0.4	0.3	0.3	0.3
Dissolved Oxygen	mg·L^{-1}	mean	8.1	7.3	6.4	6.9	6.5
		sd	0.2	0.3	0.5	0.4	0.6

Furthermore, a granulometric analysis of the wastewater was carried out for the HRT applied. The FWS *T. domingensis* was chosen for this purpose. The residual turbidity in this system allowed particles to be identified at the instrument's detection limit. The results showed that the particle size distributions of the particles present in the sampled SMSR showed different characteristics under treatment conditions.

Particles smaller than 0.6 µm diameter were detected at 50% of the sample for 2-day HRT (Figure 7). Laser particle size analysis did not detect significant proportions of particles above the instrument's detection limit of 0.02 µm, indicating that the sample contained predominantly smaller sizes.

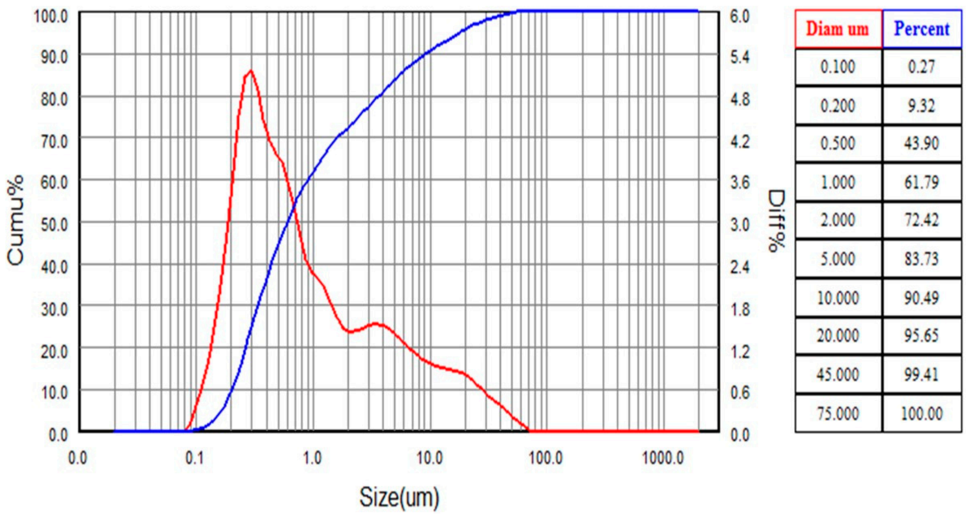

Figure 7. Particle size analysis of FWS *T. domingensis* for 2-day HRT.

This suggests that the treatment has effectively removed silt and clay particles, which are known to be the main cause of turbidity in water. It revealed that the 2-day HRT had the greatest particle retention, although there appeared to be a continuous reduction in turbidity for the 4- and 6-day HRT. The results reinforced the potential of this technology to reduce colloidal particles such as silt and clay, which can significantly impact water quality.

3.3. *Efficiency of HSSF in Turbidity Reduction*

Macrophyte and support media types, which are key components of this typology, were found to significantly influence ($p < 0.0001$) the performance of wetlands in reducing turbidity for 2-, 4-, and 6-day HRT (Figure 8).

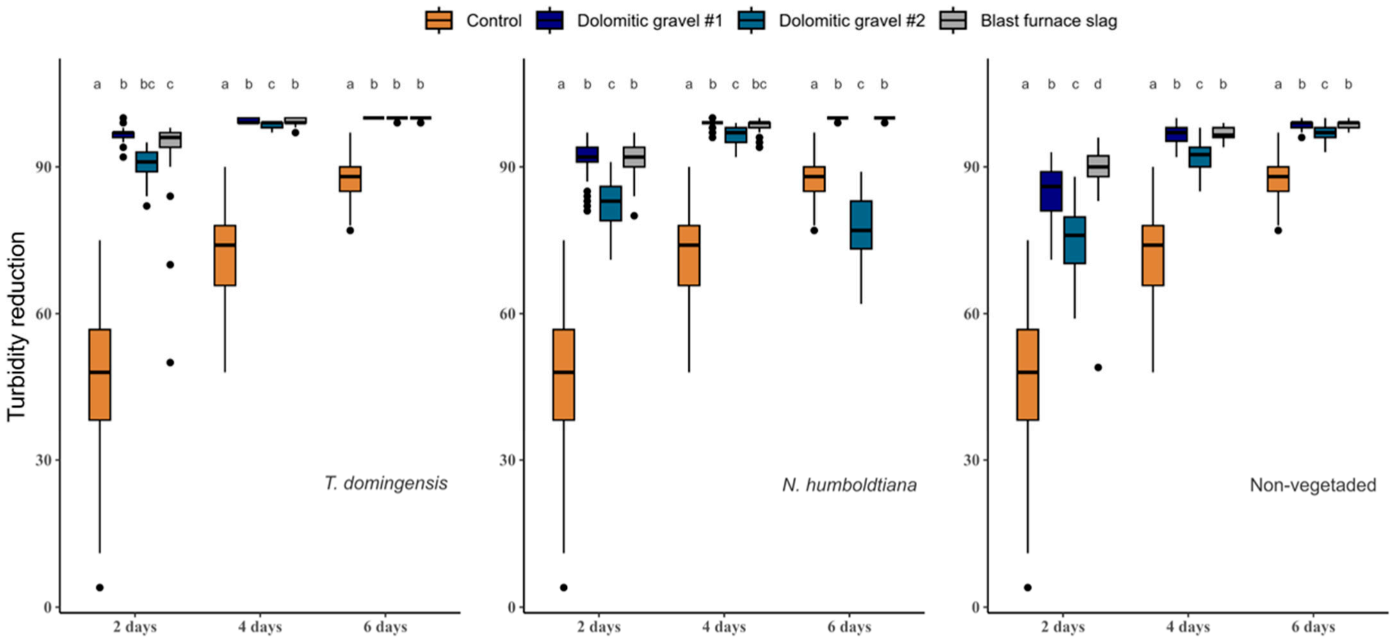

Figure 8. Performance of different support media at each HRT (2-, 4- and 6-day, *n* = 60) for HSSF with *T. domingensis*, *N. humboldtiana* and non-vegetaded wetlands and the control wetland. The letters indicate statistical differences considering the support media at each HRT and the control (Kruskal Wallis test; Dunn's test for multiple comparisons; $p < 0.001$). Comparisons between HSSF vegetation types or HRTs are not applied.

For turbidity reduction observed in the HSSF *T. domingensis* in dolomitic gravel #1, higher values of 97%, 99%, and 100% reduction were observer at HRT of 2, 4, and 6 days, respectively (Figure 8). HSSF *T. domingensis* in blast furnace slag showed similar reduction rates of 94%, 99%, and 100% for the evaluated HRT. A reduction rate of 91% for 2-day HRT was the lowest reduction rate observed for HSSF *T. domingensis* in dolomitic gravel #2. The assessment of residual turbidity (n = 60) for the HRT also demonstrated the performance of the HSSF *T. domingensis* in the dolomitic gravel #1 (Table 6). The lowest mean residual turbidity of 45 ± 18 NTU for 2-day HRT indicated higher efficiency, followed by 7 ± 3 NTU for 4-day HRT and 2 ± 1 NTU for 6-day HRT. Greater residual turbidity of 91 ± 111 NTU for the 2-day HRT, 12 ± 8 NTU for the 4-day HRT, and 3 ± 2 NTU for the 6-day HRT was observed for HSSF *T. domingensis* in blast-furnace slag. The HSSF *T. domingensis* in dolomitic gravel #2 showed another increase in the residual turbidity for the HRT of 2 and 4 days, averaging 135 ± 43 and 22 ± 9 NTU, and 3 ± 1 NTU for 6 days.

Similar turbidity reduction rates for HSSF *N. humboldtiana* in both dolomitic gravel #1 and blast furnace slag were observed. It was 91% for the HRT of 2-day, 99% and 98% for the 4-day, and 100% for the 6-day of HRT. Lower performance was observed in the HSSF *N. humboldtiana* in dolomitic gravel #2; the reduction rates were 82%, 97%, and 78% for the HRT of 2, 4, and 6 days (Figure 8).

Additionally, the residual turbidity showed better efficiency of HSSF *T. domingensis* in dolomitic gravel #1 than HSSF *N. humboldtiana* in the support media evaluated. Even though the HSSF *N. humboldtiana* also achieves suitable residual turbidity values. The HSSF *N. humboldtiana* in dolomitic gravel #1 reduced the turbidity to 128 ± 57 NTU, 17 ± 11 NTU, and 3 ± 2 NTU for 2-, 4-, and 6-day HRT (n = 60), respectively. HSSF *N. humboldtiana* in slag also presented decreases of 135 ± 54 NTU, 26 ± 20 NTU, and 5 ± 5 NTU for the HRT evaluated. Higher turbidity was detected for HSSF *N. humboldtiana* in dolomitic gravel #2 of 272 ± 66 NTU, 53 ± 28 NTU, and 13 ± 10 NTU (Table 6). These suggest that a small support media grain size is suitable for reducing turbidity.

Table 6. Turbidity reduction in FWS wetlands for retention times of 2, 4, and 6 days for inflow turbidity (1614 ± 357 NTU, *n* = 695).

Wetland	Support Media	HRT (Days)	Turbidity Reduction Rate (%)	Mean Residual Turbidity (NTU)
HSSF *T. domingensis*	Dolomitic gravel #1	2	97	45 ± 18
		4	99	7 ± 3
		6	100	2 ± 1
	Blast furnace slag	2	94	91 ± 111
		4	99	12 ± 8
		6	100	3 ± 2
	Dolomitic gravel #2	2	91	135 ± 77
		4	98	53 ± 25
		6	100	21 ± 13
HSSF *N. humboldtiana*	Dolomitic gravel #1	2	91	128 ± 57
		4	99	17 ± 11
		6	100	3 ± 2
	Blast furnace slag	2	91	135 ± 54
		4	98	26 ± 20
		6	100	5 ± 5
	Dolomitic gravel #2	2	82	272 ± 66
		4	97	53 ± 97
		6	78	13 ± 10
HSSF Non-vegetated	Dolomitic gravel #1	2	84	233 ± 84
		4	97	53 ± 25
		6	99	21 ± 13
	Blast furnace slag	2	89	162 ± 82
		4	97	50 ± 23
		6	99	22 ± 12
	Dolomitic gravel #2	2	75	370 ± 93
		4	92	118 ± 44
		6	97	48 ± 22

Turbidity reduction rates in HSSF non-vegetated showed that the presence of macrophytes is important for improving wetlands performance. Significant variations in turbidity reduction rates were observed, particularly for 2-day HRT (84%). For 4-and 6- day HRT, the rates of 97% and 99% HSSF in dolomitic gravel #1 were recorded (Figure 8). The same reduction was observed for HSSF in blast furnace slag with rates of 89%, 97%, and 99% at the same HRT and of 75%, 92%, and 99% for HSSF in dolomitic gravel #2.

HSSF-non-vegetated had a significant decrease in performance, as observed in the residual turbidity. Higher values of 233 ± 77 NTU, 53 ± 25 NTU, and 21 ± 13 NTU were reported for HSSF in dolomitic gravel #1 (n = 60). For HSSF in blast furnace slag, the NTU values were 162 ± 82, 50 ± 23, and 22 ± 12 (n = 60), and for HSSF in dolomitic gravel #2, the turbidity values were even higher at 370 ± 93, 118 ± 44, and 48 ± 22 NTU (n = 60), as shown in Table 5.

The HSSF systems were notable for their performance for 4-day HRT. The proposed HSSF *T. domingensis* in dolomitic gravel #1 proved to be effective for 4-day HRT, obtaining residual turbidity similar to that observed in Class I rivers, according to the CONAMA Resolution 357/2005 [80]. This turbidity level is also lower than the limits set by international legislation in Australia and New Zealand [81] and Canada [82].

The evaluated water quality parameters after a 6-day HRT are presented in Table 7. The analyses showed that hydrogen potential and dissolved oxygen were in accordance with the limits established by the Brazilian legislation CONAMA 357/2005 [80] and the

Australian and New Zealand legislation ANZECC legislation (2000) [81], reinforcing the treatment potential to the water quality management.

Table 7. Mean quality parameters concentration measured at the inlet and outlet of HSSF wetlands (n = 60 samples).

Parameters (n = 60 samples)	**Unit**		**Inlet**	**HSSF**		
				Dolomitic gravel #1		
				Non-vegetated	***N. humboldtiana***	***T. domingensis***
Turbidity	NTU	mean	1614	21.9	4.1	1.6
		sd	357	13	3	1
Temperature	°C	mean	19.0	19.3	19.2	19.5
		sd	0.0	3.0	3.0	2.9
Hydrogen potential		mean	7.3	7.8	7.6	7.6
		sd	0.1	0.2	0.2	0.2
Dissolved Oxygen	$mg \cdot L^{-1}$	mean	8.1	7.1	6.8	6.7
		sd	0.2	0.4	0.4	0.5
Parameters (n = 60 samples)	**Unit**		**Inlet**	**HSSF**		
				Blast furnace slag		
				Non-vegetated	***N. humboldtiana***	***T. domingensis***
Turbidity	NTU	mean	1614	23.0	5.6	3.8
		sd	357	12	5	3
Temperature	°C	mean	19.0	19.0	19.1	19.1
		sd	0.0	3.0	2.9	3.0
Hydrogen potential		mean	7.3	8.7	8.2	8.1
		sd	0.1	0.2	0.3	0.2
Dissolved Oxygen	$mg \cdot L^{-1}$	mean	8.1	6.3	6.3	6.5
		sd	0.2	0.5	0.5	0.5
Parameters (n = 60 samples)	**Unit**		**Inlet**	**HSSF**		
				Dolomitic gravel #2		
				Non-vegetated	***N. humboldtiana***	***T. domingensis***
Turbidity	NTU	mean	1614	50.8	14.0	3.5
		sd	357	24	10	1
Temperature	°C	mean	19.0	19.1	19.1	19.1
		sd	0.0	3.0	3.0	3.0
Hydrogen potential		mean	7.3	8.1	7.8	7.6
		sd	0.1	0.2	0.2	0.2
Dissolved Oxygen	$mg \cdot L^{-1}$	mean	8.1	7.1	6.9	6.4
		sd	0.2	0.3	0.4	0.5

This study fills an important gap by evaluating the effectiveness of wetlands in treating high turbidity (>1500 NTU), although several studies report wetland effectiveness on another mining effluent. Studies of synthetic acid mine drainage with a maximum initial turbidity of 68 NTU have reported reduction rates of up to 48% [83] and 67% [84]. The study by Gaballah et al. (2022) [85] on mining lake water found a maximum reduction rate of 94% at an initial turbidity of 40 NTU. The majority of the studies were conducted on *Typha* species. The results presented here indicated that the HSSF wetlands technology could achieve even higher performance at higher turbidity levels.

In the context of HSSF, vegetation plays a crucial role in reducing suspended particles through its submerged structures, such as rhizomes, roots, and stems, by trapping and retaining suspended solids, including colloidal particles that do not settle naturally [86,87]. The surface of macrophyte tissues is catalytic and promotes the adsorption of particles, as well as the oxygenation of water, by releasing O_2 from the roots into the rhizosphere, creating aerobic conditions in the root zone and facilitating the precipitation of metals commonly found in mining effluents [15,87]. The presence of macrophytes also improves the hydrodynamics of the system. It makes the flow more homogeneous, reducing velocity and stratification and increasing the contact area [39,88]. The role of macrophytes was demonstrated in these results. Vegetated HSSF wetlands showed better performance compared to non-vegetated HSSFs at the lowest HRT.

Sanchez-Ramos et al. (2017) [89] obtained a variety of turbidity reduction rates in an effluent with a high organic load in HSSF wetland filled with gravel #0 (diameter of 4.8 to 9.5 mm), vegetated with *Eleocharis* sp. (77%), *Typha* sp. (55%), *Eleocharis* sp., and *Typha* sp. jointly (43%), as well as a HSSF non-vegetated (68%). The greater performance of *T. domingensis* was also observed for reducing turbidity associated with inorganic loading, mainly in the HSSF wetlands with smaller granulometry of the support media, as dolomitic gravel #1 and blast-furnace slag.

The efficiency of *T. domingensis* in reducing inorganic turbidity can be attributed to its phytoremediation potential due to its widespread use in treating this type of effluent [25,90–93]. Studies on the use of *N. humboldtiana* for mining effluent are still limited. A very promising potential for expanding the use of *N. humboldtiana* was observed. Not only did the macrophyte show a significant improvement in the reduction of turbidity in the HSSF, but it also showed excellent adaptability when cultivated in dolomitic gravel #1 and blast-furnace slag, with reduction rates similar to those observed for *T. domingensis* for 4-day HRT. The use of native plants is recommended, taking into account potential ecosystem disturbance caused by the introduction of new species.

Another important component of HSSF is the support media. Smaller grain sizes provide a greater specific surface area for contact with the effluent, resulting in better particle trapping and turbidity reduction [63,93,94]. For constructed wetlands, it is recommended to use support media with grain size between 8 and 16 mm [95,96]. This research also achieved better performances at the wetlands composed of smaller grain sizes, such as dolomitic gravel #1 (9.5 and 19 mm) and blast furnace slag (9 and 25 mm), compared to dolomitic gravel #2 (19 and 25 mm). Although the performance of dolomitic gravel #2 was considered good, the predominance of smaller particles in dolomitic gravel #1 and blast furnace slag ensured better system performance. However, further research into the clogging process is recommended.

These support media are classified as alkaline and are strongly recommended for treating effluents with high metal loads, such as those commonly found in mining effluents [97]. The availability of hydroxyl ions helps to neutralize the pH, which facilitates metal adsorption and precipitation processes for suspended particles [36,39]. Thus, the reduction in colloidal and particulate material reduces effluent turbidity and can also be effective in removing metals, which is also enhanced in vegetated wetlands [87,98].

Overall, these results highlighted the potential of *T. domingensis* and the effectiveness of small granulometry support media, such as dolomitic gravel and blast furnace slag, in reducing turbidity. The HSSF wetlands proved to be effective at a hydraulic loading rate of 27.43 L m^{-2} d^{-1} for 4-day HRT. Vegetated HSSF wetlands enabled the application of lower HRT achieving high reduction rates, thus optimizing this parameter. Therefore, this implies the need for smaller wetlands area, which is one of the main challenges of this technology.

N. humboldtiana has great potential to be used in places where it is considered native, avoiding the introduction of new species in the environment. Therefore, a significant contribution to efficiency and effectiveness could be achieved by increasing the use of this macrophyte.

3.4. Leaf Chlorophyll in Response to Environmental Stress

The total leaf chlorophyll (TChl) index of macrophytes showed statistical differences between the treatment with SMSR and the control with tap water ($p < 0.0001$). Chlorophyll, a common plant pigment, is essential for understanding physiological processes and environmental influences on plant health. The ClorofiLOG 1030 portable chlorophyll meter (Falker) provides a leaf chlorophyll index, which is equivalent to the statistical leaf chlorophyll index from laboratory analysis [99]. The Falker total leaf chlorophyll index is used as a bioindicator in this study. Changes in chlorophyll levels give an indication of the photosynthetic capability of plants, their nutritional status and the stress conditions they are exposed to [100]. Macrophytes grown in tap water are used as controls in this analysis.

The TChl index for *P. stratiotes* (FWS) showed statistical differences between these values according to the HRT applied and the control. The average TChl index (n = 17) with for 2-day HRT (31.2 ± 4.4) showed significant differences compared to the retention times at 4 days (22.7 ± 4.4), at 6 days (21.2 ± 3.4) and at the control 2-day HRT (23.0 ± 2.4), as shown in Figure 9.

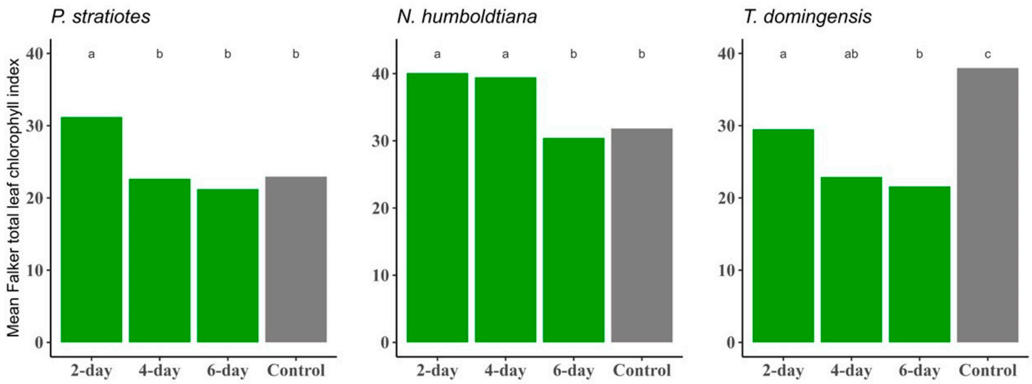

Figure 9. Mean Falker total leaf chlorophyll index (TChl) of *P. stratiotes*, *N. humboldtiana*, and *T. domingensis* in FWS wetlands at the HRT applied of 2, 4, and 6 days (n = 17). The letters indicate statistical differences considering the FWS wetlands macrophyte's and the control vegetation at each HRT (Kruskal Wallis test; Dunn's test for multiple comparisons; $p < 0.001$). Comparisons between FWS wetlands vegetation species are not applied.

The mean TChl index in *N. humboldtiana* (FWS) showed statistical differences between the 2-day HRT (40.1 ± 7.3 TChl, n = 17) and 4-day (39.4 ± 7.4 TChl, n = 17) wetlands. For 6-day HRT, the mean TChl index was 30.4 ± 4.9 (n = 17), which was similar to that observed for the control (31.8 ± 8.3 TChl, n = 17) at the HRT of 2 days (Figure 9).

For *T. domingensis* in FWS (n = 17), the mean TChl index for the 2-day retention time of 29.5 ± 7.2, for the 4-day 22.9 ± 7.8 and for the 6-day 21.6 ± 6.3 were considered significantly different from that observed in the control, 38.0 ± 7.3 (2-day HRT). The 2-day TChl was similar to the 4-day, and only the 4-day HRT was similar to the 6-day HRT, considering the retention times applied.

These results show that the highest TChl levels have been observed in the first retention times, especially with the 2-day HRT. For *P. stratiotes* and *N. humboldtiana*, these levels were even higher than those recorded in the respective controls, suggesting a positive adaptation response to the environmental conditions of the experiment. The reduction in TChl content occurred in systems with HRT of 4 and 6 days. This suggests that these longer retention times may have affected the integrity of the macrophytes. It is possible that the reduction in turbidity at these times affected the availability of nutrients to the macrophytes, and as a result, there was a reduction in TChl.

In the HSSF, the TChl indices of *N. humboldtiana* and *T. domingensis* differed significantly in the HRT applied and in the respective controls (Figure 10). There were no significant differences in the mean TChl content of *N. humboldiana* cultivated at the HSSF in blast furnace slag (50.3 ± 8.1 TChl, n = 17), in dolomitic gravel #1 (36.9 ± 4.0 TChl, n = 17), and in dolomitic gravel #2 (38.7 ± 5. 4 TChl, n = 17) with 2 days HRT and their respective controls in blast furnace slag (65.9 ± 5.8 TChl, n = 17), in dolomitic gravel #1 (34.6 ± 4.6 TChl, n = 17) and in dolomitic gravel #2 (48.6 ± 6.5 TChl, n = 17). Cultivation on the HSSF in blast furnace slag showed a significant reduction in TChl content at 4 days HRT (33.7 ± 4.8 TChl, n = 17) and 6 days (33.2 ± 9.1 TChl, n = 17) compared to the control and 2 days. For the HSSF in dolomitic gravel #1, there was a significant increase in TChl content for 4-day HRT (46.7 ± 4.9 TChl), but for 6-day (32.4 ± 4.9 TChl), the average reduction reached a value similar to that observed in the control and after 2 days. For the HSSF in dolomitic gravel #2, there was no statistical difference between the TChl reduction between the 2-, 4- and 6-day HRT. However, the TChl reduction for 4-day (37.2 ± 6.1) and for 6-day (33.5 ± 7.3) was significant compared to the control.

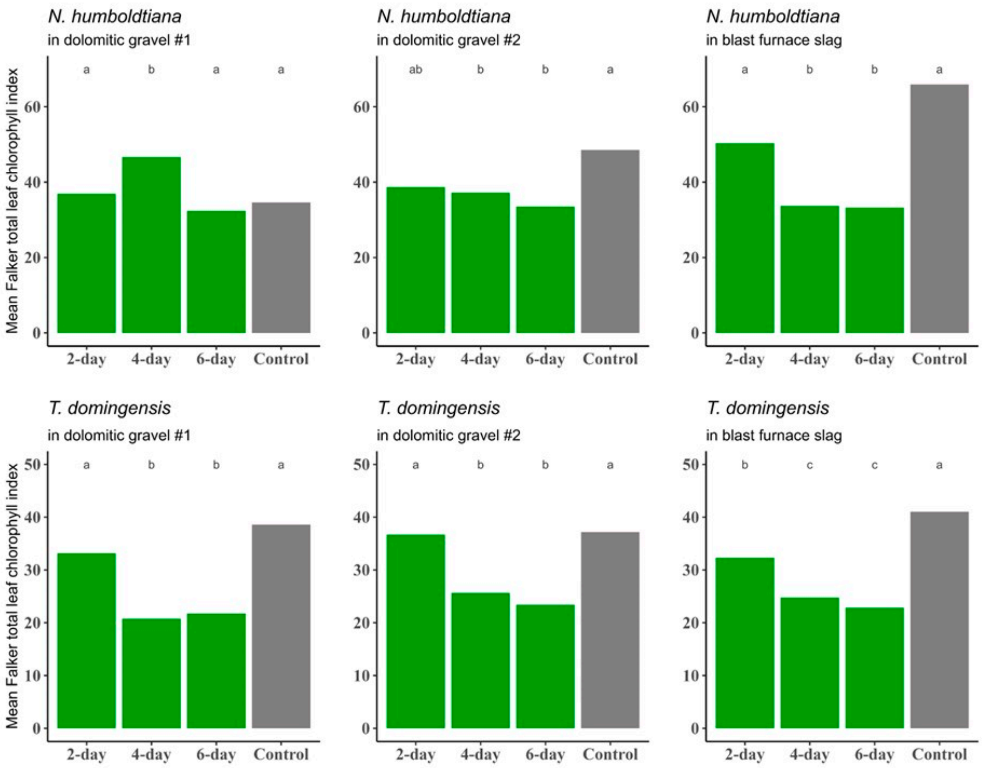

Figure 10. Mean Falker total leaf chlorophyll index (TChl) of *N. humboldtiana* and *T. domingensis* in HSSF with different support media and at the HRTs applied of 2-, 4-, and 6- days (n = 17). The letters indicated statistical differences considering the HSSF wetland macrophyte's and the control vegetation at each HRT (Kruskal Wallis test; Dunn's test for multiple comparisons; $p < 0.001$). Comparisons between HSSF wetlands vegetation species are not applied.

T. domingensis cultivated at the HSSF in blast furnace slag showed the highest TChl content in the control (41.0 ± 5.1 TChl), which was statistically different over the retention times evaluated. The TChl content for 2-day (32.3 ± 8.9 TChl, n = 17) was significantly different from that observed for 4-day (24.8 ± 5.9 TChl, n = 17) and for 6-day (22.9 ± 4.4 TChl, n = 17); also, a decrease in these values can be observed. Similarities were observed between the pattern of this species cultivated at the HSSF in dolomitic gravel #1 and in dolomitic gravel #2. Leaves' chlorophyll content in dolomitic gravel #1 (33.2 ± 6.4 TChl, n = 17) and dolomitic gravel #2 (36.7 ± 11.6 TChl, n = 17) HSSF wetlands at 2 days was similar to the respective controls in dolomitic gravel #1 (38.6 ± 8.1 TChl, n = 17) and in dolomitic gravel #2 (37.2 ± 8.3 TChl, n = 17). The reduction in TChl content was significant for *T. domingensis* at the HSSF in dolomitic gravel #1 (20.8 ± 4.4 TChl, n = 17) and in dolomitic gravel #2 (25.7 ± 6.0 TChl, n = 17) at 4 days when compared to the control at 2 days of HRT. The TChl values found in these macrophytes in dolomitic gravel #1 (21.8 ± 4.5 TChl, n = 17) and dolomitic gravel #2 (23.4 ± 3.5 TChl, n = 17) at 6 days of HRT were not different from those at 4 days.

As shown by the FWS macrophyte, the leaves chlorophyll content of *N. humboldtiana* and *T. dominguensis* at the HSSF wetlands was also higher for the 2 days. A decrease in TChl was observed with increasing retention times. For *T. dominguensis*, which was the most effective species for turbidity reduction in HSSF, this TChl levels decrease was more pronounced.

High turbidity is characterized by a high concentration of suspended particles in the water, so the reduction in these particles also results in the reduction in essential nutrients for macrophytes [101,102]. It will be reflected by the leaf chlorophyll, where higher levels indicate the nutritional status of macrophytes [103]. Studies show that plants with optimal chlorophyll levels are more resistant to environmental stresses such as drought and nutrient deficiency and benefit from their antioxidant properties; on the other hand, low chlorophyll levels may be an indication of lower photosynthetic efficiency and growth inhibition [104–106].

Therefore, there was a reduction in TChl, which can be explained by the effective reduction in turbidity and, thus, the reduction in available nutrients, which affected macrophyte development and may have affected performance at 4- and 6-day retention times. In general, the macrophytes proved to be resilient and remained adapted to the treatments even under stressful conditions.

This study demonstrates the effectiveness of constructed wetland systems in reducing high turbidity in mine spoil rainwater, providing a promising alternative for treating this water. By identifying key parameters such as HRT, HLR, and turbidity reduction rates, the research contributes to the understanding of the factors that influence wetland performance. In addition, different options for achieving high reduction rates are identified by evaluating different constructed wetland components, such as macrophytes and support media. These findings will enable the natural-based technology to be improved and applied on a real scale, promoting a sustainable and environmentally efficient solution for the treatment of water contaminated by mining activities.

4. Conclusions

This study confirmed the effectiveness of the different typologies of constructed wetlands (FWS and HSSF) in reducing high turbidity (<1500 NTU) from spoiled mine rainwater. The better performance was reported for HSSF wetlands for a retention time of 4 days and for the FWS wetlands of 6 days. These wetlands were capable of reducing the turbidity at 99%, achieving residual turbidities of 7 ± 3 NTU and 11 ± 5 for HSSF and FWS, respectively.

In addition, this study found that HSSF allows higher hydraulic loading rates, ensuring high efficiencies in turbidity reduction, suggesting important perspectives for the full-scale application of this technology. This technology offers economic benefits by reducing operating costs and improving water quality, which could further promote the use of constructed wetlands in these industries.

The wetlands elements evaluated showed that the FWS *P. stratiotes* performed better than FWS *T. domingensis,* and both were more efficient than FWS *N. humboldtiana*. The HSSF *T. domingensis* showed higher efficiency on all support media tested (dolomitic gravel #1 > blast furnace slag > dolomitic gravel #2). The outstanding performance of the native macrophyte *N. humboldtiana* in turbidity reduction in HSSF with the same materials should be highlighted. In the HSSF non-vegetated wetland, the blast furnace slag outperformed dolomitic gravel #1 and dolomitic gravel #2, suggesting that small granulometries are more suitable for wetlands performance.

Macrophytes proved to be resilient and adapted to the wetlands even under adverse conditions provided by the SMSR, as shown by the analysis of the total leaf chlorophyll index. The reduction in the leaf chlorophyll levels was attributed to the application of longer retention times and lower nutrient availability throughout the treatment, but no effect on macrophyte development or wetland performance was observed.

Moreover, this research has also demonstrated the potential of constructed wetlands to reduce silt and clay particles, major causes of turbidity, showing significant benefits for water quality and aquatic ecosystem conservation. Optimization of hydraulic retention times, selection of plant species, and support media are essential to maximize the efficiency of these systems.

This study was conducted on a pilot scale and, therefore, has some limitations that should be taken into account. Further studies should consider that different scales may affect the dynamics presented here, and possible adjustments in configuration and operational parameters should be explored.

Although the results provide valuable insight into the potential of this technology, further studies are recommended to validate and optimize the results presented in this experiment. In addition, the evaluation of support media clogging and the cultivation of new macrophyte species are strongly recommended, also including the evaluation of hybrid constructed wetland systems.

Author Contributions: P.C.S.G.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, validation, visualization, writing—original draft, review and editing. I.d.S.P.R.: data curation, writing—original draft, review and editing. M.H.R.d.P.: writing—original draft, review and editing. A.d.F.S.: conceptualization, funding acquisition, investigation, methodology, project administration, resources, supervision, writing—original draft, review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financed in part by the Coordination for the Improvement of Higher Education Personnel—CAPES—Finance Code 001, by the Federal University of Ouro Preto and the Postgraduate Program in Environmental Engineering, and by VALE S/A through its Partnership for Research, Development, and Innovation with the Federal University of Ouro Preto.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author due to further analysis of the complementary database.

Acknowledgments: The authors express their gratitude to the Coordination for the Improvement of Higher Education Personnel—CAPES, the Federal University of Ouro Preto and Postgraduate Program in Environmental Engineering. We would like to thank the Department of Civil Engineering and the SPRT project members for their guidance and support throughout this project.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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