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Improvement of constructed wetlands using *Lithothamnium calcareum*-based fertilizer for sewage treatment

Aprimoramento de *wetlands* construídos com fertilizante à base de *Lithothamnium calcareum* para tratamento de esgoto

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ABSTRACT

The universalization of water supply and sanitary sewage treatment services requires low-cost, high-efficiency solutions. Constructed wetlands (CW) stand out as a promising technology, but their effectiveness in removing nutrients, especially phosphorus, remains limited. The use of fertilizers based on the seaweed *Lithothamnium calcareum* emerges as a potential strategy to enhance this system. However, no studies have combined this seaweed with macrophytes in CW. Thus, the objective of this study was to evaluate the efficiency of CW tanks by combining different doses of *L. calcareum* fertilizer (0, 5, 15, and 35 g/L) with the cultivation of the macrophyte *Cyperus alternifolius* in wastewater treatment, through physical-chemical analyses and assessments of the plants' photosynthetic metabolism. The experiment was conducted in Araruama, RJ, over 58 days. The results showed that the highest dosage (35 g/L) yielded the best removal efficiencies: 96.7% for phosphorus, 58.5% for chemical oxygen demand (COD), and 34.5% for ammoniacal nitrogen. Additionally, stomatal conductance and photosynthetic performance indices (PI_{abs} and PI_{total}) were significantly higher in treatments with added algae, indicating improved nutritional status and photochemical activity in *C. alternifolius*. It is concluded that integrating *L. calcareum*-based fertilizer with CW planted with *C. alternifolius* accelerates pollutant removal and enhances the physiological performance of macrophytes, offering an innovative, low-cost alternative for decentralized sanitary sewage treatment.

Keywords: Ecological sanitation; Biotechnology; Phytoremediation; Macrophytes; Bioremediation.

RESUMO

A universalização dos serviços de abastecimento de água e de tratamento de esgoto sanitário demanda soluções de baixo custo e alta eficiência. Os *wetlands* construídos destacam-se como tecnologia promissora, mas sua eficácia na remoção de nutrientes, especialmente de fósforo, ainda é limitada. O uso de fertilizantes à base da alga marinha *Lithothamnium calcareum* surge como uma estratégia promissora para aprimorar esse sistema. No entanto, estudos que integrem essa alga a macrófitas em *wetlands* construídos são inexistentes. Assim, o objetivo deste trabalho foi avaliar a eficiência de tanques de *wetlands* construídos combinando diferentes dosagens de fertilizante de *L. calcareum* (0, 5, 15 e 35 g/L) com o cultivo da macrófita *Cyperus alternifolius* no tratamento de efluentes, por meio de análises físico-químicas e do metabolismo fotossintético das plantas. O experimento foi conduzido em Araruama, RJ, por 58 dias. Os resultados mostraram que a maior dosagem (35 g/L) promoveu as melhores eficiências de remoção: 96,7% para fósforo, 58,5% para demanda química de oxigênio (DQO) e 34,5% para nitrogênio amoniacal. Adicionalmente, a condutância estomática e os índices de desempenho fotossintético (PI_{abs} e PI_{total}) foram significativamente superiores nos tratamentos com adição da alga, indicando melhoria no estado nutricional e na atividade fotoquímica de *C. alternifolius.* Conclui-se que a integração do fertilizante à base de *L. calcareum* com *wetlands* construídos plantados com *C. alternifolius* acelera a remoção de poluentes e potencializa o desempenho fisiológico das macrófitas, oferecendo uma alternativa inovadora e de baixo custo para o tratamento descentralizado de esgoto sanitário.

Palavras-chave: Saneamento ecológico; Biotecnologia; Fitorremediação; Macrófitas; Biorremediação.

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Introduction

Environmental sanitation is an effective means of promoting conditions conducive to the sustainable development of societies (Taweesan et al., 2024). Nevertheless, unchecked urbanization that fails to consider integrated territorial planning practices poses complex challenges to achieving universal access to basic sanitation services (Hossain et al., 2025). In this context, nature-based solutions (NbS) are techniques that adopt an integrated approach across social, economic, and environmental dimensions, contributing to resilience to global change while being energy- and resource-efficient (Varma et al., 2022; Li et al., 2025).

In particular, constructed wetland (CW) systems emerge as a promising alternative to conventional methods due to their wastewater treatment potential, low installation costs, and environmental suitability (Gałęzowska et al., 2024; Ji et al., 2024; Ruiz et al., 2025). These systems improve upon the models found in natural wetlands by employing vegetation whose roots act as biofilters, retaining contaminants and promoting the degradation of organic matter through complex biochemical interactions (Von Sperling and Sezerino, 2018; Zhou et al., 2025).

Furthermore, the factors influencing wastewater treatment in systems such as CW include aspects derived from the complex substrate-microorganism-plant interaction (Dykes et al., 2025; Lin et al., 2025). The selection of the most suitable species for a CW system depends on the plant's tolerance to wastewater-saturated environments, its growth potential, adaptation to local conditions, and planting and maintenance costs (Amasa et al., 2025; Nabi et al., 2025).

Cyperus alternifolius is an annual aquatic plant that exhibits rapid growth and high leaf area, which may favor the uptake of nutrients such as phosphorus and nitrogen. This macrophyte plays a fundamental indirect role in wastewater treatment in CW: its root system (rhizosphere) provides attachment surfaces for the development of microbial biofilms and promotes oxygen transfer to the root zone, creating aerobic microenvironments that stimulate the activity of decomposer microorganisms (Vo et al., 2024; Hou et al., 2025). Although the plant does not directly degrade organic matter—a process carried out predominantly by bacteria and fungi associated with the filter medium and roots—the presence of *C. alternifolius* enhances system efficiency. It has been widely studied in European and Asian countries (Gholipour, 2025).

On the other hand, *Lithothamnium calcareum* is a marine alga from the *Corallinaceae* family, representing a potential sorbent for polluting substances, with the capacity to remove nutrients such as phosphorus and metal ions such as manganese and zinc. Additionally, the adsorption capacity of *L. calcareum* contributes to the purification of landfill leachate. However, studies on wastewater treatment remain scarce (Veneu et al., 2019; Almeida et al., 2021; Veneu et al., 2023).

Despite their benefits, CWs are often associated with the need for extensive land areas, which limits their application, especially in urban regions (Soti et al., 2022; Wei et al., 2024). Preliminary studies have used *L. calcareum* only in its raw form; no studies have applied it as a fertilizer, as hypothesized in this work. It is also noteworthy that this study is innovative for integrating sustainable mechanisms not yet addressed in the literature: CW and *L. calcareum*-based fertilizer for sanitary sewage treatment.

This study aimed to evaluate the efficiency of CW tanks when combined with the application of a marine alga-based fertilizer (*L. calcareum*) and the cultivation of the macrophyte species *C. alternifolius* for wastewater treatment, through analyses of the effluent and the plants' photosynthetic metabolism. The innovative hypothesis of the study was that the *L. calcareum*-based fertilizer associated with the *C. alternifolius* species improves CW performance, enhancing nutrient removal from sanitary sewage, particularly phosphorus and ammoniacal nitrogen.

Materials and methods

The experiments were conducted in the municipality of Araruama, Rio de Janeiro.

Construction of the pilot treatment unit (cultivation tank)

To experiment, a pilot unit (cultivation tank) adapted from CW models was constructed, as shown in Figure 1.

Each section was built with internal dimensions of 1.00 m in width and 2.00 m in length; with 4 sections, this resulted in a total area of 8 m^2. The access height of 0.9 m defines a total volume of 7.2 m^3. The lower regions of the tank were subdivided to accommodate materials intended to retain and fix the rhizosphere of the crops, namely gravel 0, medium sand, and filter soil. The gravel 0 layer (nominal diameter between 2.4 mm and 12.5 mm) was dimensioned at 35 cm in the lower part of each section, followed by 20 cm of medium sand ($d10 \approx 0.25$ mm, $d60 \approx 0.75$ mm, $Cu = 3.0$; density 2.65 g/cm^3), as described by Bassani and Decezaro (2021), Kotsia et al. (2025), and Yang et al. (2025).

The upper layer, consisting of 20 cm of filter soil, comprised a mixture of 70% medium sand, 10% gravel 0, and 20% local soil (dystrophic Red Argisol, crumbled and sieved <2 mm), whose iron and aluminum oxides favor chemical nutrient adsorption (Santos et al., 2025; Yang et al., 2025). Additionally, drainage blankets (Bidim) with a grammage of 150 g/m^2 were placed between layers to promote segregation and filtration. This configuration aimed to ensure structural stability, a uniform distribution of upward flow, the prevention of dead zones, and the optimization of cycle time (Bassani and Decezaro, 2021; Matos, M.P. and Matos, A.T., 2021).

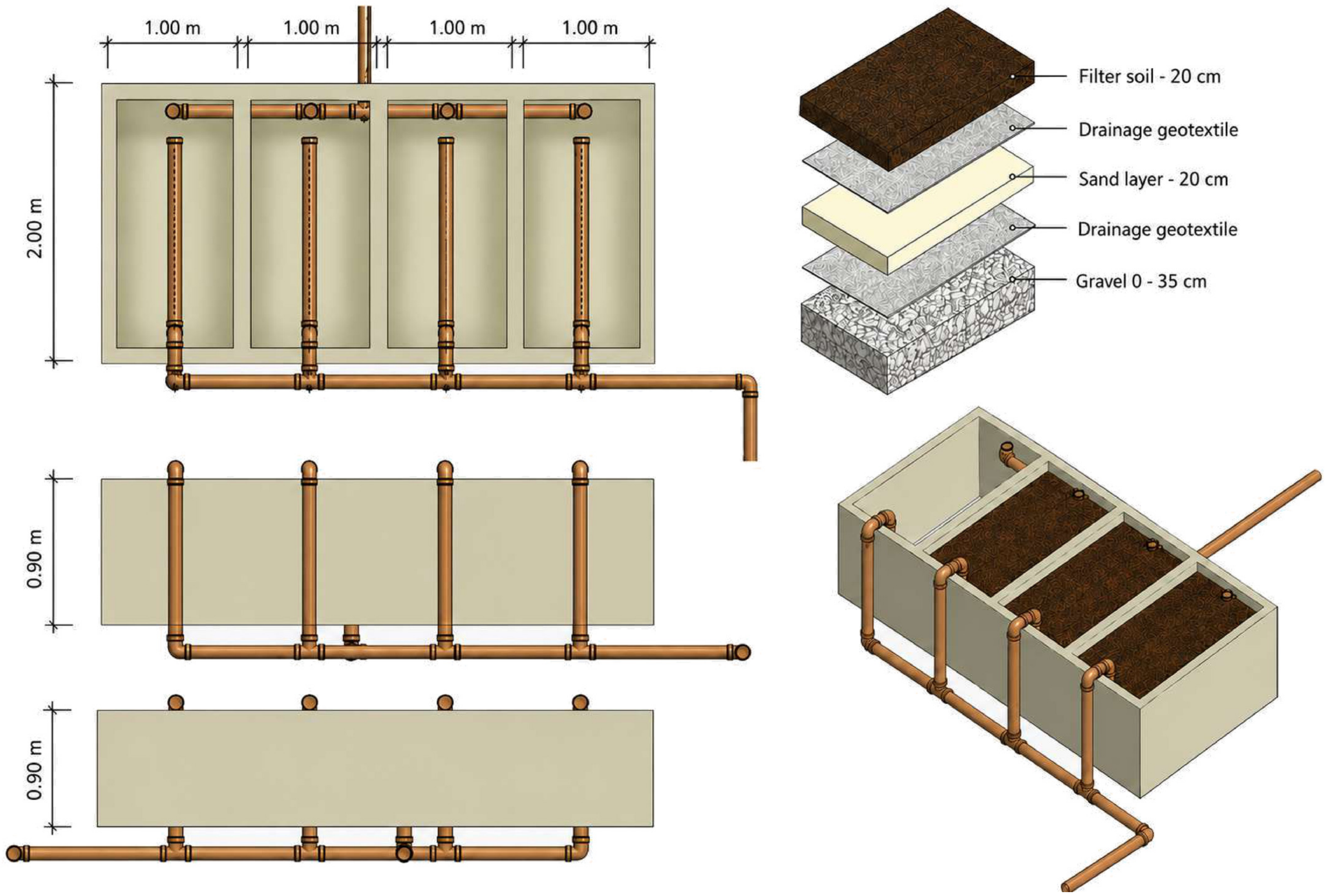

Figure 1 – Construction details of the CW tank: floor plan, rear and front elevations, and exploded isometric view indicating the filter layers.

Species used in the cultivation tank

The plants used in the experiment were selected in accordance with approaches reported in the literature and local applications in Rio de Janeiro (Kataki et al., 2021; Mohamed et al., 2025). After arranging the layers in each section, *C. alternifolius* was planted in a single row with four propagules fixed per section. Furthermore, similar plants with heights and masses of approximately 60 cm and 900 g were selected. The powdered *L. calcareum* fertilizer used throughout this study was obtained from Supramar Minerais and sold under the product name LTSupra.

Operational procedure of the pilot unit

The procedure was carried out by simultaneously operating a tank with four independent macrophyte planting sections, with differentiated *L. calcareum* soil dosages applied to assess CW functioning under treatment control (T1, T2, T3, and T4).

The experimental system consisted of an upward vertical flow CW, operated intermittently (batch mode) on sampling days. Initially, the sewage passed through a coarse screening point upstream of the study tank. Then, it was directed to a desanding box with fine screening before proceeding to treatment in the CW tank. The bed operated under unsaturated conditions between cycles, with the liquid layer restricted to a 6-hour cycle. The inflow rate per section was 120 L/day, resulting in a hydraulic loading rate (HLR) of 0.06 m^3/m^2/day. The applied organic load was 5.36 g COD/m^2/day, while nutrient loads were 0.17 g P/m^2/day and 0.94 g N-NH_4^+/m^2/day. The adopted hydraulic parameters are consistent with values reported for pilot-scale vertical-flow CWs (Bassani and Decezaro, 2021; Kotsia et al., 2025).

A key feature of the process was the differentiation of *L. calcareum* soil dosages across the macrophyte tanks. Thus, a control section was established without the product, followed by tanks with increasing amounts of calcareous algae. Accordingly, the tanks received *L. calcareum* applications at inflow rates of T1 (0 g/L), T2 (5 g/L), T3 (15 g/L), and T4 (35 g/L).

Subsequently, after manual pump activation, the effluent was directed to feed the study sections, distributed through hydraulic installations with a 100 mm PVC diameter (DN 100 mm). Each tank was fed upward. The arrangements guided independent performance control across the 4 sections. That is, each chamber was monitored through influent and effluent collection and sampling to evaluate quality parameters, with an emphasis on nutrient removal.

The filtration process of the pre-treated effluent was simulated using the established layers across the 4 sections of the tank, with gravel, medium sand, and filter soil. In terms of experimental design, the experimental phase began 30 days after planting *C. alternifolius*, on October 17, 2023, and ended on November 14, 2023, comprising 5 total operational and sampling cycles. The addition of *L. calcareum* (in particulate solid form – powder) was carried out by direct surface dispersion over the bed, subdivided over 5 minutes. Thus, surface additions of 150 g, 450 g, and 1050 g were made over sections 2, 3, and 4, respectively. After the appearance of the liquid layer over the upper layer and the beginning of effluent discharge into the upper outlet pipes, the feed pump, as well as the addition of *L. calcareum*, was interrupted.

The system was operated in batch mode (intermittent regime), characterized by point feeding of the effluent only on sampling days. Thus, the 6-hour cycle was allowed to elapse before the sampling step to evaluate treatment. Treated effluent samples were collected at the upper outlet point of each experimental section, corresponding to the overflow pipes located in the system, with sample representativeness ensured by the preceding resting period. Treatment efficiency was monitored at different dosages using physicochemical analyses of the effluent and assessments of plant functioning.

Effluent analyses

For the analysis of dissolved oxygen (DO) and pH parameters, a multiparameter probe (Hanna Instruments, model HI 98183) calibrated with pH 4.0, 7.0, and 10.0 buffer solutions was used (Dykes et al., 2025). Analyses related to chemical oxygen demand (COD) were conducted using a thermoreactor (Kotsia et al., 2025). Results for phosphorus and ammoniacal nitrogen were obtained using an ion chromatograph (Metrohm, compact model 850 Professional IC), which quantifies cations and anions simultaneously (Zhou et al., 2025). Sampling was carried out for each treatment over five cycles, at 30, 37, 44, 51, and 58 days after planting *C. alternifolius* in the respective sections.

To evaluate pollutant removal efficiency, Equation 1 was used:

$$E = \frac{C_0 - C_e}{C_0} \times 100 \quad (1)$$

Where:
E = removal efficiency (%)
C_0 = influent pollutant concentration (mg/L)
C_e = effluent pollutant concentration (mg/L)

Analyses of *Cyperus alternifolius*

At the end of the experiment, after 58 days of cultivation, 100 g of fresh mass from the macrophytes' shoots was collected from each CW tank, in triplicate per treatment. The samples were sent for analysis at the Instituto Brasileiro de Análises (IBRA) Laboratory in Sumaré/SP, which adopted the methodology of the University of São Paulo, as described by Malavolta et al. (1997). The analyses covered the following macronutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). The techniques employed were: titrimetry for N, spectrophotometry for P, and atomic absorption spectrometry for K, Ca, Mg, and S. ANOVA was applied to the results to identify parameters with significant differences ($p < 0.05$), and these were subsequently subjected to Tukey's test.

Photosynthetic analyses were performed at the end of the experiment. To measure transient chlorophyll *a* fluorescence, plant leaves were dark-adapted for 30 minutes before measurement, with three replicates for each treatment. The procedure was carried out using a Handy PEA portable fluorometer (Hansatech Instruments), and the parameters were calculated according to the methodologies proposed by Strasser and Strasser (1995) and Tsimilli-Michael and Strasser (2008), which constitute the JIP-test. A list of selected JIP-test parameters is presented in Table 1.

Stomatal conductance was measured on the middle third of fully expanded leaves, with three repetitions per treatment, using a porometer (SC-1 Leaf Porometer, METER Group). Chlorophyll concentration was determined using a SPAD chlorophyll meter (SPAD-502 Plus, Chlorophyll Meter). Subsequently, the data were subjected to ANOVA and Tukey's test, with a significance level of 5%.

Results and discussion

Effluent analyses

The results of the physicochemical parameters analyzed over the five treatment cycles, conducted specifically after 30, 37, 44, 51, and 58 days after planting the *C. alternifolius* seedlings, are presented in Figure 2.

The results of the effluent analyses corroborate the treatment potential observed when using the alga *L. calcareum* in association with the macrophyte *C. alternifolius*. Studies that isolated each component indicated that *L. calcareum* acts as a promising sorbent material for nutrient removal, such as phosphorus (Marques, 2022; Veneu et al., 2023), while *C. alternifolius* plays a fundamental role in CW systems for treating a wide range of pollutants, including heavy metals, organic matter, and nutrients (Gholipour, 2025; Mohamed et al., 2025).

The results demonstrate a steady improvement in phosphorus removal efficiency with increasing *L. calcareum* dosage, consistent with previous studies conducted without macrophytes (Marques, 2022; Veneu et al., 2023). Treatment T4 (35 g/L) achieved up to

Table 1 – Summary of fluorescence parameters, extracted and derived parameters (calculated by the JIP Test) with their formulas and descriptions.

Fluorescence Parameters	Description
Ft	Fluorescence intensity at time t after the start of actinic illumination
F50 µs	Minimum fluorescence intensity at 50 µs
F100 µs and F300 µs	Fluorescence intensity at 100 and 300 µs, respectively
FJ and FI	Fluorescence intensity at the J-step (2 ms) and at the I-step (30 ms), respectively
FP (= Fm)	Maximum fluorescence at the P-step
Area	Total complementary area between the fluorescence induction curve and F_m
OEC = 1 – (FI/FJ)	Oxygen-evolving complex
Derived Parameters and OJIP Parameters	
F0 ≅ F50 µs	Minimum fluorescence when all PSII (photosystem II) reaction centers are open
Fm = FP	Maximum fluorescence when all PSII reaction centers are closed
F0/FM	Ratio between initial and maximum fluorescence of dark-adapted leaves – quantum yield of dissipation
FV/F0	Ratio between variable fluorescence and minimum fluorescence
Yields and Flux Ratios	
φP0 = TR0/ABS = 1 – F0/Fm = FV/Fm	Maximum primary photochemical quantum yield at t = 0
φE0 = φP0 * ψ0 = 1 – (FJ/FM) = ET0/ABS	Quantum yield of electron transport from QA^- to the intersystem electron acceptor chain
ψE0 = ET0/TR0 = 1 - VJ	Probability (at time 0) that a captured exciton moves an electron into the electron transport chain beyond QA^-
δR0 = RE0/ET0 = (1 - VI)/(1 - VJ)	Efficiency with which an electron can move from reduced intersystem electron acceptors to the final PSI acceptors
φR0 = RE0/ABS = φP0 × ψE0 × δR0	Quantum yield for reduction of the final PSI electron acceptors per absorbed photon
Specific Fluxes or Activities Expressed per Reaction Center (RC)	
ABS/RC = M0 × (1/VJ) × (1/φP0)	Absorption flux per RC
DI0/RC	Specific flux of excess energy dissipation per active reaction center with QA-reducing capacity
TR0/RC = M0/VJ	Trapped energy flux per RC at t = 0
ET0/RC = (M0/VJ) × ψE0 = (M0/VJ) × (1 -VJ)	Electron transport flux beyond QA^- per active reaction center
RE0/RC = (RE0/ET0) × (ET0/CR)	Specific electron flux with the capacity to reduce the final electron acceptors in the PSI electron acceptor portion per active reaction center
Performance Index	
PI_{ABS} = (RC/ABS) × (φP0/(1 - φP0)) × (ψE0/(1 – ψE0))	Performance index for energy conservation from photons absorbed by PSII up to the reduction of the intersystem electron acceptor chain
PI_{TOTAL} = (CR/ABS) × (φP0/(1 – φP0)) × (ψE0/(1 – ψE0)) × (δR0/(1 – δR0))	Total performance index for energy conservation from photons absorbed by PSII to be used for the reduction of the final PSI electron acceptor

Source: Strasser and Strasser (1995); Tsimilli-Michael and Strasser (2008).

96.7% phosphorus removal efficiency. Comparatively, Mohamed et al. (2025) reported phosphorus removal between 17.6% and 42.54% in wetlands vegetated with *C. alternifolius* operated with a hydraulic retention time of 5 days, evidencing that the addition of *L. calcareum* significantly enhances this process. Furthermore, Vijuksungsith et al. (2024) reported phosphate removal efficiencies of up to 86.3% in systems planted with the same macrophyte, values close to those obtained in the present study, but without the fertilizer addition.

Figure 2(A) also suggests that the accumulation of calcareous algae over time is phosphorus removal in the systems. Thus, over the course of the analysis process, efficiency progressively increases, reinforcing this trend. The phosphorus results comply with the limits imposed by the Operational Standard of the State Environmental Institute (RJ) – NOP INEA 45 – in all scenarios when considering discharge into lotic water bodies. However, for lentic water bodies, T4 (35 g/L) showed the best results, with three sequences below 1 mg/L (Rio de Janeiro, 2021).

The high phosphorus removal efficiency is attributed to the biogenic structure of *L. calcareum*, which is composed predominantly of

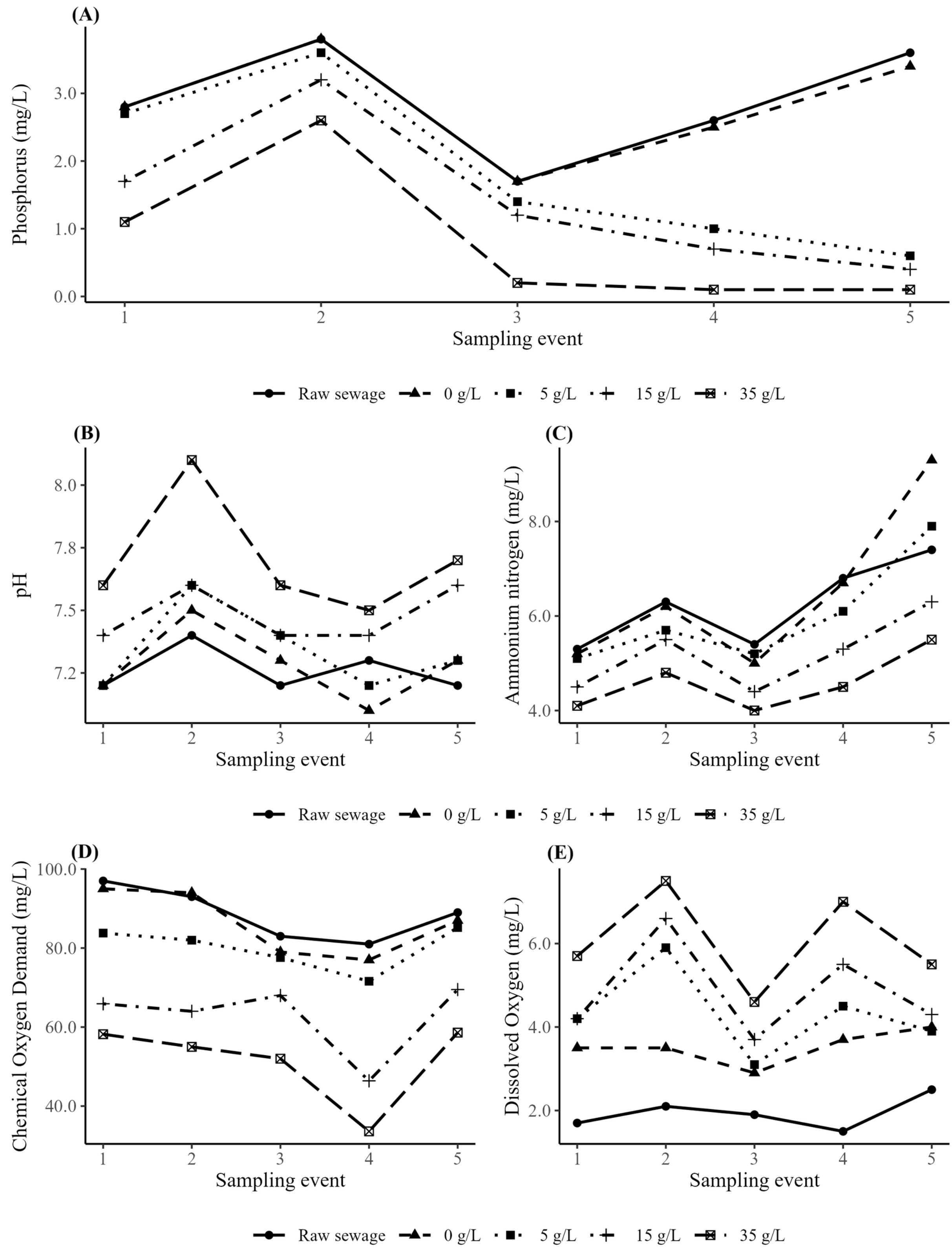

Figure 2 – Temporal variation of effluent quality parameters: (A) Phosphorus; (B) pH; (C) Ammoniacal nitrogen; (D) COD; (E) DO. Analyses were performed after periods of 30, 37, 44, 51, and 58 days following the planting of *Cyperus alternifolius*.

calcium and magnesium carbonates (Veneu et al., 2019). This composition imparts high porosity and surface area to the material, increasing the available sites for phosphate adsorption via surface chemical interactions between carbonate groups and phosphate anions (Almeida et al., 2021). Additionally, the alga's alkalinizing character favors the precipitation of sparingly soluble calcium phosphates under alkaline conditions, including hydroxyapatite-type compounds, which can deposit onto the granules' porous surface (Veneu et al., 2023). Such physicochemical retention mechanisms act synergistically and complementarily with the biological absorption carried out by the macrophyte rhizosphere, enhancing treatment efficiency and plants' physiological performance in CW systems.

Throughout the experiment, pH measurements highlight the potential of *L. calcareum* as an alkalinizing product. Regardless of the influent conditions of the raw effluent, characterized by climatic and temporal variations, a positive correlation between *L. calcareum* addition and an increase in pH is evident. Thus, across all samplings, the tank with the highest calcareous algae addition had the highest pH values, as shown in Figure 2(B). Conversely, the variations between raw sewage and T1 are subtle and indicate a low influence of *C. alternifolius* on pH reduction.

Regarding COD, the behavior shown in Figure 2(D) highlights that tanks with higher *L. calcareum* concentrations achieved better results. The maximum efficiency observed (58.5% in T4) aligns with the range reported by Nabi et al. (2025), who reported values between 43% and 57% for wastewater treatment in optimized CW. COD removal in CW is known to be influenced by the substrate type, macrophyte, and applied hydraulic load (Gholipour, 2025; Mohamed et al., 2025), and the results obtained demonstrate that *L. calcareum* addition enhances this process.

For ammoniacal nitrogen (NH_4^+-N), fertilizer application led to efficient removal, particularly in the tank with the highest dosage (T4), as shown in Figure 2(C). Overall, results demonstrate improved performance in the presence of calcareous algae. From this perspective, treatment T4 (35 g/L) achieved an efficiency of up to 34.5% in the fourth cycle. Comparatively, Gholipour (2025) reported NH_4^+-N removal between 66.7% and 96.2% in wetlands vegetated with *C. alternifolius*. The moderate efficiency observed in the present study may be attributed to the intermittent feeding regime and the relatively short operating period (58 days), which is insufficient for the complete maturation of the nitrifying community in the biofilm (Hou et al., 2025; Vymazal, 2025).

The National Environmental Council (CONAMA) Resolution No. 430/2011 establishes a maximum value of 20.0 mg/L for total ammoniacal nitrogen in effluents discharged into receiving water bodies (Brasil, 2011). NOP-INEA 45 (Rio de Janeiro, 2021) adopts similar criteria, being more restrictive for lentic water bodies, with a limit of 10 mg/L. In this context, only treatments with intermediate (T3) and high (T4) dosages consistently met the established standards.

The increase in fertilizer seems to have contributed to greater photosynthetic activity of the macrophytes. This process, by consuming carbon dioxide (CO_2) and releasing oxygen, explains the positive impact observed in DO levels, as indicated in Figure 2(E). The pH elevation also recorded in this treatment is a direct consequence of CO_2 removal from the water by photosynthesis, not a cause for the increase in DO. Thus, the data set suggests that the addition of *L. calcareum* created a more favorable microenvironment for photosynthetic activity, resulting in greater oxygenation of the water column (Marques, 2022; Veneu et al., 2023). Therefore, the elevation in pH and the increase in DO in treatments with higher *L. calcareum* dosage suggest phosphate precipitation and a microenvironment more favorable to organic matter oxidation, which supports the observed phosphorus removal and COD reduction (Mohamed et al., 2025; Nabi et al., 2025).

The results obtained for phosphorus (96.7%), COD (58.5%), and NH_4^+-N (34.5%) removal demonstrate that *L. calcareum* addition enhances the performance of CW vegetated with *C. alternifolius*. The high phosphorus removal efficiency is directly associated with the alga's adsorption capacity and the chemical precipitation induced by pH increase, as demonstrated by Veneu et al. (2023). The moderate removal of ammoniacal nitrogen reflects the need for more time to establish nitrifying communities in the biofilm, a widely recognized aspect in the literature (Hou et al., 2025; Vymazal, 2025). The COD removal efficiency, although lower than that reported in studies with lower organic loads (Basílico et al., 2024; Amasa et al., 2025), evidences the synergistic role between the fertilizer and the macrophyte in organic matter degradation.

Cyperus alternifolius analyses

The basic nutrient analyses of *Cyperus alternifolius* plant tissues are shown in Figure 3.

In particular, N, P, and K are considered primary macronutrients, while Ca, Mg, and S are secondary macronutrients. Nitrogen is directly associated with protein formation and with the synthesis of essential plant compounds such as chlorophyll and alkaloids. Conversely, phosphorus contributes to plant respiration and energy production. Furthermore, calcium contributes to the structural strengthening of plant organs and plays a role in the maintenance of roots and leaves (Benites et al., 2010; Raza et al., 2022).

Nitrogen concentrations were significantly higher in macrophytes cultivated with elevated levels of *L. calcareum*, with the lowest nitrogen result observed in the control tank. In comparison, phosphorus showed more pronounced accumulation in plants without fertilizer addition; however, the differences were small, and no statistically significant differences were observed between treatments ($p > 0.05$). Regarding calcium, the changes corroborate a direct association between higher calcareous algal abundance and increased metal

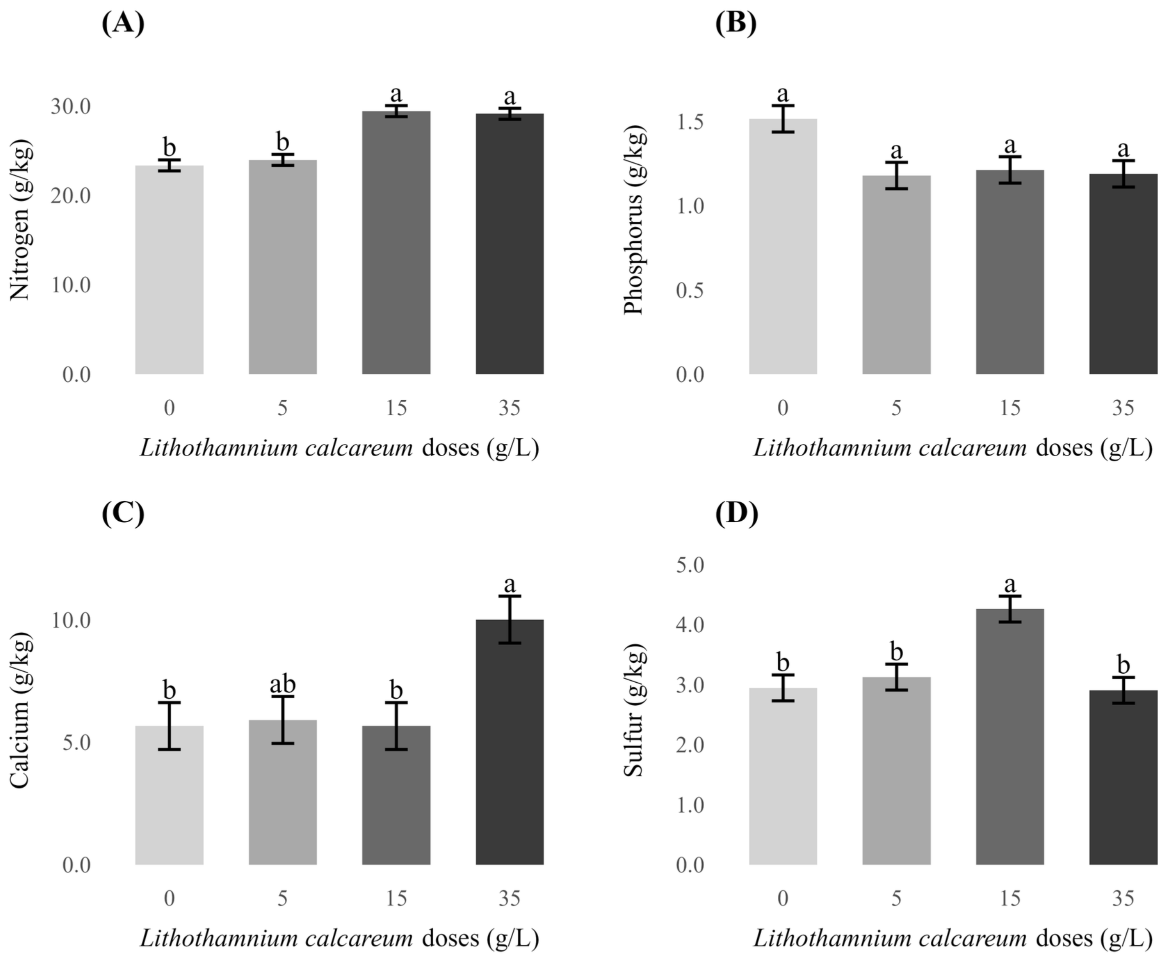

Figure 3 – Variation in macronutrient content in the shoots of *Cyperus alternifolius* cultivated under different dosages of *Lithothamnium calcareum*: (A) Nitrogen, (B) Phosphorus, (C) Calcium, (D) Sulfur. Plants at 58 days after planting of seedlings in the CW.

concentration, which can be explained by the composition of *L. calcareum*, as also identified by Oliveira et al. (2025). For sulfur, a notably higher presence was observed in plants with high fertilizer addition, with the tank receiving 15 g/L showing the most pronounced contribution to this parameter.

This behavior can be explained by the role of sulfur as a secondary macronutrient, essential for the synthesis of amino acids such as cysteine and methionine, and for the formation of coenzymes and the regulation of plant metabolism (Kataki et al., 2021). The greater availability of sulfate in the soil, possibly stimulated by the chemical conditions created by *L. calcareum* application, may have favored its absorption and assimilation by the plants. In a study on CW, Mesacasa et al. (2025) demonstrated that the presence of macrophytes influences sulfur dynamics in the rhizosphere, elevating sulfate concentrations and promoting its cycling, which may have contributed to the observed results.

According to SPAD index measurements, which measure leaf chlorophyll levels based on the amount of green identified by the equipment, the average values ranged between 53 and 58, regardless of the applied *L. calcareum* doses. The leaves of *C. alternifolius* in treatments T1, T2, T3, and T4 showed no statistically significant differences in chlorophyll concentration among themselves ($p > 0.05$). Therefore, variations in *L. calcareum* dosages demonstrated no influence on photochemical performance (Leonardo et al., 2013; Taiz et al., 2017).

Tanks T2 and T4, with 5 g/L and 35 g/L of *L. calcareum*, expressed the best results for stomatal conductance, as presented in Figure 4.

Stomatal conductance, a measure of stomatal opening for gas exchange, exhibited distinct dynamics across treatments. Notably, the lowest expression was observed in the control tank, which did not contain calcareous algae. Furthermore, given that greater soil nutrient availability is positively associated with stomatal conductance, as observed by Faralli et al. (2019), the results support the possibility that

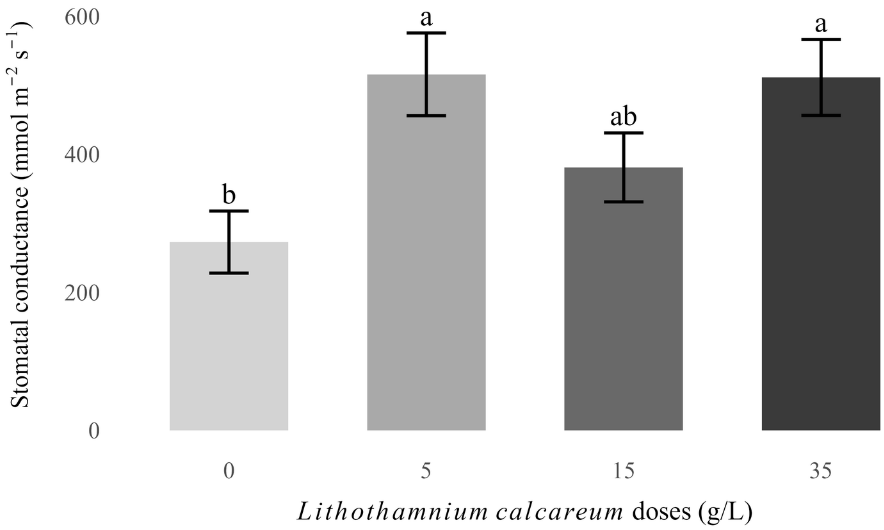

Figure 4 – Stomatal conductance of *Cyperus alternifolius* under different dosages of *Lithothamnium calcareum*. Equal letters do not differ statistically from each other according to Tukey's test at the 5% level. Plants at 58 days after planting of seedlings in the CW.

L. calcareum provides higher nutrient levels than scenarios without fertilizer application.

The results of transient chlorophyll *a* fluorescence under different dosages of *Lithothamnium calcareum* are presented in Figure 5.

When comparing the probability that a captured exciton moves an electron in the electron transport chain after Quinone A (ψE0), there is similarity across all treatments; however, greater oscillation occurs for T2 and T3, achieving results superior to T1. Regarding the quantum yield of electron transport from Quinone A (QA) to the intersystem electron acceptor chain (φE0), T2 showed the most significant increase relative to the control, surpassing it by 22%. The increase in both parameters demonstrates the efficiency of PSII, which, according to Baker and Rosenqvist (2004), is associated with CO_2 assimilation in response to soil nutrient stocks, also evidenced by the stomatal conductance data. This is also corroborated by the high bioavailability of trace elements (such as iron, manganese, and zinc) adsorbed onto the cell walls of the calcareous alga (Veneu et al., 2023; Oliveira et al., 2025).

The quantum yield for reduction of the final PSI (photosystem I) electron acceptors per absorbed photon (φR0) and the absorption flux per reaction center (ABS/RC) showed no considerable variations among the different scenarios with *L. calcareum* addition. Furthermore, energy dissipation per reaction center (DI0/RC) presented lower values than the control, which means that *L. calcareum* addition demonstrates a positive influence on the cultivation and photosynthetic relationships of the macrophyte. For T4 and T3, the DI0/RC reaction was reduced by 34%, whereas for T2, results differed by 13% from the control T1.

The parameter responsible for electron transport at the beginning of the photosynthetic process (ET0/RC) is 7% higher in T3 than in the control. The parameter responsible for the reduction of the final electron acceptor on the PSI electron acceptor side (RE0/RC) is lower in all tanks with *L. calcareum* addition. According to the performance indices, the performance index (PI_{abs}) linked to energy conservation of photons absorbed by photosystem II, up to the reduction of the intersystem electron acceptor chain, showed a value for T3 that was 100% higher than the control. In comparison, T4 exceeded it by 47%, and T2 by 31%, indicating improved *C. alternifolius* cultivation and the macrophyte's robustness in converting light energy into biomass under the effect of the fertilizer (Bezerra et al., 2022). Likewise, the total performance index, which measures performance up to the final PSI electron acceptors (PI_{total}), showed rates above the normalized value in both T2 and T4, with even higher values in treatment T2, while T3 showed no variation.

Therefore, unlike studies that focused only on evaluating pollutant removal from sanitary effluents in CWs (Soti et al., 2022; Vo et al., 2024) or that used *Lithothamnium calcareum* as a biosorbent for metals in plant-free environments (Veneu et al., 2019; Almeida et al., 2021; Veneu et al., 2023), this study evidenced the positive impact of the ecological integration between an *L. calcareum*-based fertilizer and CW, indicating the acceleration of phosphorus, ammoniacal nitrogen, and COD removal processes from sanitary sewage, with the hypothesis being reinforced by the physiological results of the plants.

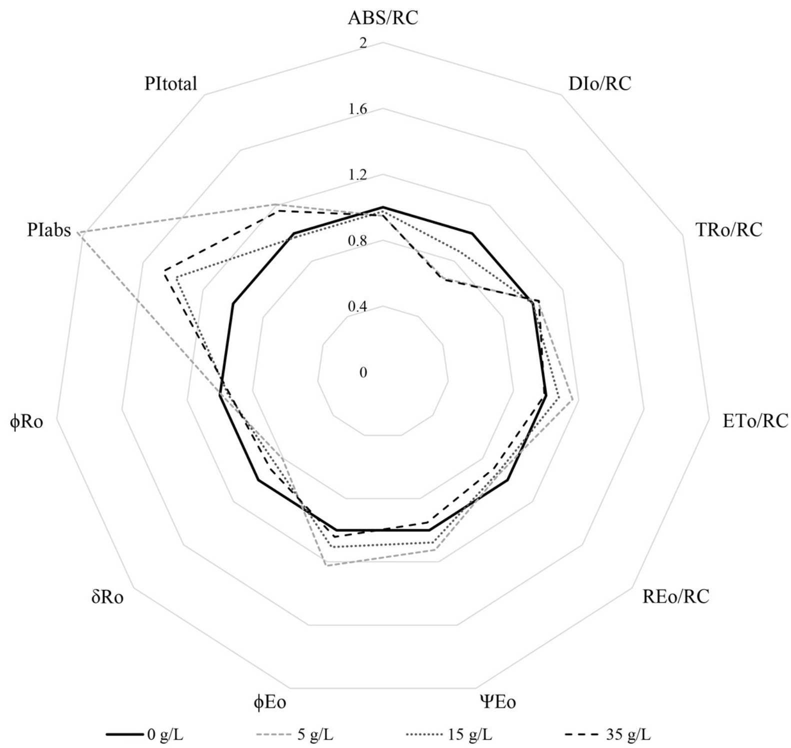

Figure 5 – Transient chlorophyll a fluorescence of *Cyperus alternifolius* when cultivated under different dosages of *Lithothamnium calcareum* at 58 days after planting of seedlings in the CW.

Conclusions

The results demonstrate that the 35 g/L dosage of the *Lithothamnium calcareum*-based fertilizer (treatment T4) was the most efficient for wastewater treatment, contributing to both the photosynthetic performance of the macrophytes and the removal of pollutants from sanitary sewage. The combination of the ecological strategies employed achieved excellent results, with efficiencies of up to 96.7% for phosphorus removal, 58.5% for COD, and 34.5% for ammoniacal nitrogen (NH_4^+-N), and evidenced the fertilizer's alkalinizing characteristic, as indicated by the pH values obtained.

Stomatal conductance data suggest that the *L. calcareum*-based fertilizer contributed to making greater quantities of nutrients available to the macrophyte *Cyperus alternifolius*. Furthermore, transient chlorophyll *a* fluorescence analyses indicated that the PI_{abs} and PI_{total} performance indices exceeded those of the control by 47% and 20%, respectively, reinforcing the potential of ecological integration between the fertilizer and CW for sanitary wastewater treatment.

Although the 58-day experimental period was sufficient to observe significant differences between treatments and achieve stable removal efficiencies, it is recognized that complete maturation of the microbial biofilm in CW systems may require longer periods. Future studies with extended monitoring (> 90 days) are recommended to assess the system's resilience to seasonal and long-term variations.

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