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Evaluation of the effect of disposal of landfill leachate in a sewage treatment plant composed of stabilization ponds

Fernanda Jukoski de Siqueira✉ & Maria Cristina de Almeida Silva

This study aimed to evaluate the combined treatment system of domestic sewage and leachate by serial stabilization ponds in a Wastewater Treatment Plant (WWTP). The WWTP had two parallel pond modules, one receiving (module II—pond M10) and the other not receiving leachate (module I—pond M5). Physical, chemical, and biological monitoring parameters collected from January 2017 to December 2021 were evaluated. High reducing efficiency for ammonium nitrogen was found, with an average of 95.7 ± 2.5%. BOD5 and COD can be considered statistically different for a 95% confidence level in the two pond modules. The phytoplankton community was composed of 46 taxa distributed in four taxonomic classes: 6 belonging to the Cyanobacteria group (13%), 22 to the Green Algae group (48%), 4 to the Diatoms group (9%), and 14 to the Phytoflagellate group (30%). No ecotoxicity was detected for the evaluated pond effluent samples. Thus, although the insertion of leachate impacted the quality of the final effluent of the ponds and the organic matter, it may not have affected the effluents' ecotoxicity. This research helps to understand the impacts of long-term leachate insertion in a real treatment system using stabilization ponds for combined treatment. In addition to the usual efficiency evaluation parameters, ecotoxicological tests and phytoplankton are also evaluated.

Keywords Combined treatment, Stabilization ponds, Quality parameters, Phytoplankton, Ecotoxicological Tests

The generation of sewage and solid waste is intrinsic to organized societies. Such waste, as well as its by-products, need to be treated to minimize the impacts of its disposal on environmental health. According to the National Basic Sanitation Survey[1], the South Region has 40.9% of municipalities with sanitary sewage services. Sewage collection by network is defined as removing sewage generated in households and establishments through closed pipes and its conduction to the sewage treatment plant or final discharge point[1].

In Brazil, most of the municipal solid waste collected was disposed of in landfills, with 46.4 million tons sent to these locations in 2022. This value means 61% of the waste collected was adequately disposed of in the country[2]. Landfill leachate is a mixture of percolated rainwater, water produced by the biodegradation of waste, and the water inherent in waste, which contains large amounts of dissolved organic matter, salts, heavy metal ions, and other organic compounds[3]. Landfill leachate can create pollution issues for soil, surface water, groundwater, and human health if not collected, treated, or discharged safely[4].

Landfill leachate treatment is necessary to avoid negative environmental impacts, but it is still challenging. The so-called combined treatment is an alternative, where leachate is mixed with domestic sewage. Based on the use of an appropriate proportion of mixture, combined treatment has shown promising results with biological treatment processes, including meeting the legal requirements for effluent discharge[5]. Combined treatment has been used in different locations to reduce the costs of implementing treatment devices in landfills. In Brazil, combined treatment is used in landfills in the states of São Paulo (Bandeirantes, São João, Vila Albertina and Santo Amaro, Tupã, Baleia, Meridiano), Minas Gerais (Salvaterra and CTR-BR040), Rio de Janeiro (Morro do Céu) and Rio Grande do Sul (Extrema), in Porto Alegre[5].

Among the biological treatments most commonly used in leachate treatment, the following stand out: anaerobic upflow sludge blanket (UASB), anaerobic filters, moving bed biofilm reactor (MBBR), and conventional activated sludge, among others[6]. In addition, stabilization ponds, considered simplified treatment systems,

Institute of Hydraulic Research of the Federal University of Rio Grande do Sul., Porto Alegre, RS Zip Code: 91501-970, Brazil. ✉email: fejukoski@hotmail.com

have shown promising results for treating landfill leachate[8,9], including for combined treatment of leachate and domestic sewage[10–13].

However, even if combined treatment is a practice that reduces the risk of contamination, leachate may contain refractory micro and macro pollutants that are not degraded after undergoing biological treatment, contributing to obtaining effluent with high toxicity[14,15]. Thus, toxicological tests can be used to evaluate the efficiency of the treatment process in removing specific pollutants and verify the ecotoxicity of treated effluents[15–17].

This work analyzed the results of the monitoring of physical, chemical, and biological parameters of the influents and effluents in two treatment systems by stabilization ponds. One received leachate (module II—pond M10) and the other did not (module I—pond M5). Thus, this work aimed to compare and determine the efficiency of the two modules' treatment, verifying the leachate's impact in this system.

Few published studies present data for the combined treatment of landfill leachate and domestic effluent in a wastewater treatment plant (WWTP) consisting of full-scale stabilization ponds. Thus, this work stands out because it is a case study that presents information from a WWTP consisting of stabilization ponds, a complex biological system monitored for an extended period.

In addition, this work also allows a better understanding of the effect of inserting landfill leachate for combined treatment with domestic sewage in this treatment modality, not only about the physical and chemical parameters typically evaluated to determine the efficiency of effluent treatment. It also proposes to assess the effect of leachate on the microbiota present in the stabilization ponds, which was also evaluated through the analysis of phytoplankton composition and toxicity-related data based on data from ecotoxicological tests using two trophic levels.

Materials and methods

The work was carried out at the Belém Novo WWTP (30°12′59.3"S 51°10′24.1"W), under the Municipal Department of Water and Sewage's responsibility in the municipality of Porto Alegre/RS, southern Brazil. The department made the data evaluated in this work available. The evaluated WWTP has two parallel modules of treatment by stabilization ponds, each with a capacity of 2,592 m^3/d, totaling 5,184 m^3/d of capacity. Currently, the WWTP operates with a total average flow of 2,333 m^3/d of sanitary sewage, with 1,166.5 m^3/d of each module.

Each module consists of an anaerobic pond (each with an area of 2650 m^2, depth 3.0 m, hydraulic detention time 3 d), two facultative ponds (each with an area of 8512 m^2, depth 1.5 m, hydraulic detention time 10 d), and five maturation ponds (each with an area of 3584 m^2, depth 1.0 m, hydraulic detention time 7 d (Fig. 1a). The high-rate pond currently serves as a buffer tank for receiving and storing leachate, and it is highlighted in a red rectangle in Fig. 1b. For comparison and evaluation, it was collected samples for physical, chemical, and *E.coli* characterization from 1) the raw influent (yellow circle), 2) the M5 (blue circle—module I) pond, 3) the M10 (red circle—module II) pond and 4) the final effluent collection point (white circle), which is the mixture of effluent from both modules of ponds (Fig. 1b).

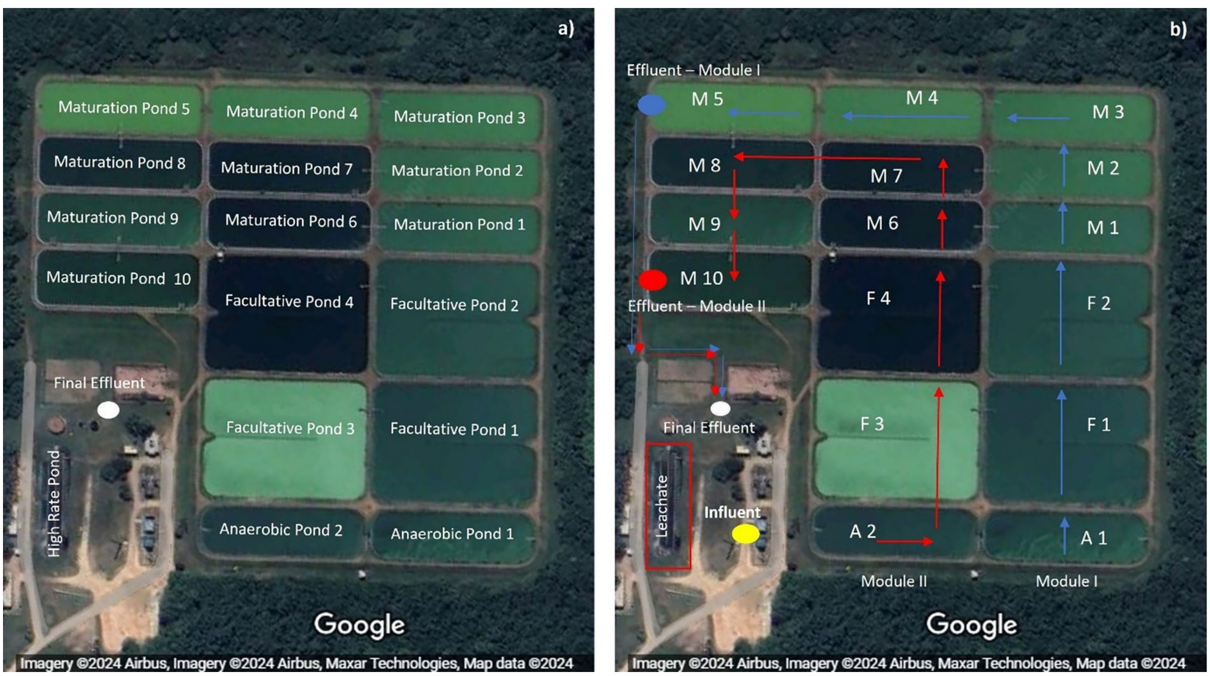

Fig. 1. View of the Belém Novo WWTP (**a**) Identification of the modules, (**b**) identification of the effluent flow represented by the arrows: in red, module II that receives the leachate and, in blue, module I that does not receive the leachate. A, anaerobic pond; F, facultative pond; M, maturation pond.

The leachate received by the WWTP comes from the Extrema Sanitary Landfill (30°12′27.6" S and 51°03′40.0" W). Its operation began in 1997, and it received a maximum load of 70 tons/day, 50 tons/day of household waste, and 20 tons/day of weeding and sweeping waste. The landfill exhausted its capacity on December 31, 2002, receiving about 820,000 tons of solid waste.

Due to the existence of the two modules, it was decided to insert the leachate in only one of the modules (module II) for combined treatment with domestic sewage, with the end of the treatment being the maturation pond number 10, which was named M10. In the other module (module I), the operation was maintained only with sewage reception, with the end of the treatment being the maturation pond number 5, M5. In this way, it was possible to compare and evaluate the effect of the insertion of leachate in the operation of module II. The percentage amount of leachate inserted in the treatment of module II during the studied period was, on average, 2.7 ± 0.4%, or 31.49 ± 4.67 m^3/d of leachate and 1,166.5 m^3/d of sewage.

Data from January 2017 to December 2021 from the described monitoring points of the WWTP were evaluated. The monitoring program of the WWTP includes physical, chemical, and biological collections and analyses of the affluent sewage and the effluents of modules I and II. Data on air and water temperature, chemical oxygen demand (COD), demand (BOD_5), pH, ammonium nitrogen, total phosphorus, and *Escherichia coli*. In addition, the evaluation of total phytoplankton and toxicity using the test organisms *Vibrio fischeri* and *Daphnia similis* added further depth to the analysis. This variety of data collected demonstrates the comprehensiveness of the monitoring program and the depth of the analysis.

The methodologies used and frequencies of analysis are: pH—monthly/biweekly[18] (62 samples); BOD_5—monthly/biweekly[19] (59 samples); Monthly/biweekly COD (Method 5220 B, C, D[20]—60 samples); ammonium nitrogen—monthly/biweekly[21] (60 samples); total phosphorus—monthly/biweekly[22] (61 samples); *Escherichia coli*—monthly (Method 9223 B[20]—41 samples); Phytoplankton—monthly[55] (Method 10,200 F[20]—31 samples); *Vibrio fischeri*—semiannual[23] (6 samples); *Daphnia similis*—semiannual[24] (6 samples).

MINITAB software, version 16, was used to construct the "*Box-Plot"* graphs to visualize the data's variability. The Mann–Whitney evaluation test was also used to verify whether there is a significant difference between the values of the physical and chemical parameters of the final effluent of the M5 and M10 ponds.

The phytoplankton data were analyzed in the effluent samples of M5 and M10 ponds, from monthly analyses from May 2017 to January 2020. The data collection process was throrough, ensuring a comprehensive representation of the Green Algae class. The genera that comprise each class in the maturation ponds are also presented. The frequency of occurrence of each taxon for each maturation pond was determined by taking into account the relationship between the number of samples in which the taxon occurred and the number of samples analyzed, which was expressed as a percentage. To evaluate the frequency, we used the classification described in the work of Lobo and Leighton[25] and Albuquerque et al.[26], in which a frequency greater than 50% is considered constant, common from 10 to 50%, and rare when it is up to 10%.

The acute toxicity test was evaluated for the crude leachate, the effluent from pond M5 (sewage), pond M10 (sewage + leachate), and the final effluent from the treatment of the Belém Novo WWTP. A total of 24 toxicity analyses were performed during the study period, 6 in each sample evaluated semi-annually. The data from these tests for the two trophic levels was presented as a toxicity factor, a crucial determinant when the test is conducted with a series of sample dilutions. For the *Daphnia similis test*, this factor is expressed by the value of the dilution factor corresponding to the highest concentration of the sample in which immobility greater than 10% of the test organisms is observed. For *Vibrio Fischeri,* it is the lowest dilution factor value, where the inhibitory effect is less than 20% after an exposure period of 30 min.

Results

Porto Alegre is located at 30° S and 51° W, 22 m altitude, and has a Köppen-Geiger Cfa class (humid subtropical climate with hot summers)[27]. In the period from 2017 to 2021, in which the analyzed samples were collected, the average temperature in summer was 24.8 °C ± 0.43 and, in winter, 16.9 °C ± 1.3. However, the evaluated samples' temperature did not vary greatly, with a mean value of 21.6 °C ± 0.7. Table 1 shows the pH and *E. coli* values for raw influent and final effluent for the evaluated period.

Figures 2 and 3 show information about the efficiency of the WWTP, evaluating the raw influent and final effluent in the study period. Figure 2 presents the box diagrams illustrating the reduced efficiency of the COD (Fig. 2a) and BOD_5 (Fig. 2b) parameters in the final effluent of the WWTP. A closer look at the reduction over the study period reveals a mean reduction of COD of 14.9 ± 13.1% and BOD_5 of 47.9 ± 9.7%. Notably, the efficiency of COD reduction shows a gradual decrease, while BOD_5 reduction remains consistent in the range of 39–64%.

		2017		2018		2019		2020		2021	
		Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl
pH	Average	7.0	8.3	7.2	8.8	7.3	8.5	7.2	9.3	7.4	9.0
	Standard deviation	0.4	1.1	0.8	1.1	0.3	1.1	0.3	0.8	0.4	0.9
E.coli (MPN/100) mL)	Average	6.0×10^6	193	6.5×10^6	187	6.5×10^6	290	5.2×10^6	138	4.8×10^6	56
	Standard deviation	6.9×10^6	197	2.7×10^6	450.4	3.8×10^6	573.6	3.9×10^6	361.3	3.8×10^6	65.4

Table 1. Descriptive statistics of the records of the evaluated variables referring to the WWTP Belém Novo raw influent (Infl) and final effluent (Effl) from 2017 to 2021.

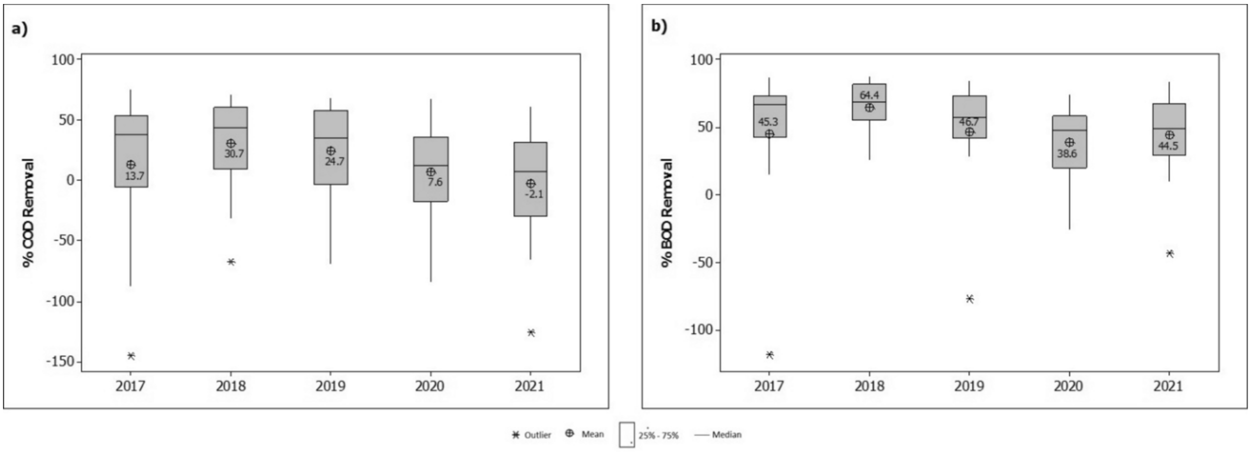

Fig. 2. Box diagrams referring to the reducing efficiencies of (**a**) COD and (**b**) BOD verified at the final effluent of Belém Novo WWTP during the study period.

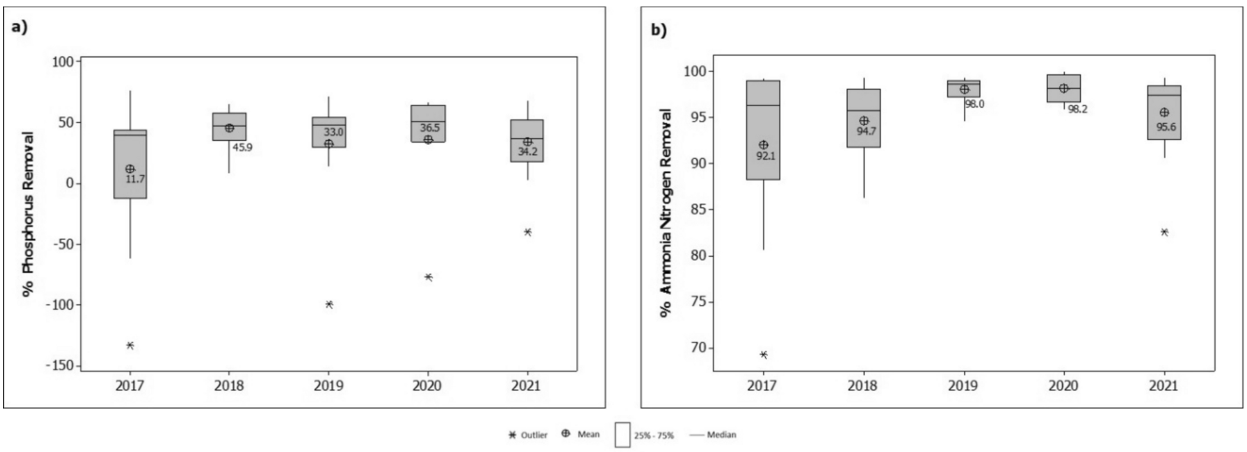

Fig. 3. Box diagrams referring to the reducing efficiencies of (**a**) phosphorus and (**b**) ammonium nitrogen in the final effluent of the WWTP during the study period.

Figure 3 shows the box diagrams referring to the treatment system's removal of nutrients, comparing the raw influent and final effluent, phosphorus (Fig. 3a), and ammonium nitrogen (Fig. 3b). Mean phosphorus reduction in the study period was 32.3 ± 12.6%, and ammonium nitrogen was 95.7 ± 2.5%.

Regarding leachate, mean COD values of 1306 ± 359.9 mg/L and N-NH_3 356 ± 56.0 mg/L were observed during the period analyzed. The characteristics of leachate and its treatability may vary with the age of the landfill. Landfills older than ten years old have a pH > 7.5; COD < 5000 mg/L; NH_3-N > 400 mg/L; BOD/COD ratio > 0.1 and low biodegradability[3,28]. Notably, the sanitary landfill that produces the leachate treated in the WWTP and evaluated in this work is considered old, both by the values of the parameters assessed and its operating time.

Table 2 shows the central trend data of the pH values and the concentrations of BOD_5, COD, ammonium nitrogen, and total phosphorus of the M5 and M10 ponds. The table also shows the results of the Mann–Whitney test, tested at a 95% confidence level ($\alpha = 0.05$). This evaluation was carried out to verify the existence of a significant difference in the effluent parameters of each pond. Although there is a high concentration of N-NH_3 in the leachate, its insertion didn't result in a significant difference between the ponds, which was verified for the organic matter parameters (COD and BOD_5). That is an important result and shows an advantage of the stabilization ponds removing ammonium nitrogen including combined effluent.

The phytoplankton community was composed of 46 taxa distributed in four taxonomic classes: 6 belonging to the Cyanobacteria group (13%), 22 to the Green Algae group (48%), 4 to the Diatoms group (9%), and 14 to the Phytoflagellates group (30%).

For the M5 pond (sewage) (Fig. 4a), the phytoplankton community was composed of 37 taxa distributed in the four taxonomic classes, 6 belonging to the Cyanobacteria group (16.2%), 19 to the Green Algae group (51.4%), 3 to the Diatoms group (8.1%) and 9 to the Phytoflagellates group (24.3%). For pond M10 (sewage + leachate) (Fig. 4b), the phytoplankton community was composed of 40 taxa distributed in the four taxonomic classes, 5

Parameters	Pond	*n*	Average	St. deviation	Median	*p* value
BOD_5 (mg/L)	M5	59	42.28	22.98	40.00	**0.049**
	M10	59	49.69	27.24	46.00	
COD (mg/L)	M5	60	148.45	59.24	139.00	**0.027**
	M10	60	172.25	64.94	155.00	
Ammonium Nitrogen (mg N/L)	M5	60	1.04	1.11	0.69	0.322
	M10	60	1.63	3.83	0.72	
Total phosphorus (mg/L)	M5	61	1.97	0.63	2.03	0.108
	M10	61	2.28	0.87	2.08	
pH	M5	62	9.15	1.07	9.45	0.570
	M10	62	9.00	1.05	9.10	

Table 2. Central tendency data of pH values and BOD_5, COD, ammonium nitrogen, and total phosphorus parameters of ponds M5 (module I: treatment of domestic sewage) and M10 (module II—treatment of domestic wastewater combined with landfill leachate) and the results of the Mann–Whitney test, tested at a 95% confidence level (α = 0.05). The parameters that presented a significant difference in the Mann–Whitney test, tested at a 95% confidence level (α = 0.05), were highlighted in bold and underlined.

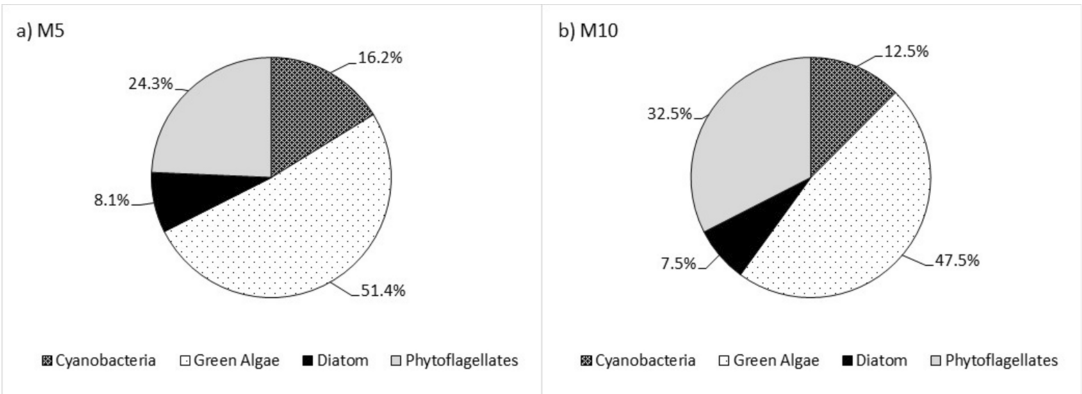

Fig. 4. Percentage contributions of the groups found in the maturation ponds to the maturation ponds (**a**) M5 (module I: domestic sewage treatment) and (**b**) M10 (module II—treatment of domestic wastewater combined with landfill leachate).

belonging to the Cyanobacteria group (12.5%), 19 to the Green Algae group (47.5%), 3 to the Diatoms group (7.5%) and 13 to the Phytoflagellates group (32.5%).

Thus, it is possible to verify a variation in the contributions of the phytoplankton communities of the two ponds. The main differences are highlighted: (1) *Cyanobacteria:* only pond M5 presented the genus *Aphanizomenon;* (2) *Green Algae:* only pond M10 presented the genera *Golenkinia, Pediastrum,* and *Polyedriopsis*; (3) *Diatoms:* only pond M5 presented the genus *Aulacoseira*. However, only pond M10 presented the genus *Navicula;* (4) *Phytoflagellates:* only M10 presented *Cryptoglena, Phacus, Peranema, Bicosoeca, and Euglena*. The genus *Goniomonas* was found only in the M5 pond. Here, the frequency results in both lagoons of the most representative phytoplankton community, green algae, will be presented.

In the present study about the green algae group, it was possible to observe that the constant genera in the two ponds were *Desmodesmus, Dictyosphaerium, Micractinium, Monoraphidium, and Scenedesmus*, as seen in Table 3. It is also noteworthy that, of the 22 genera found in the ponds, the genera *Golenkinia, Pediastrum,* and *Polyedriopsis* were not detected in the M5 pond (sewage), and the genera *Chodatella, Crucigenia,* and *Schroederia* were not found in the M10 pond (sewage + leachate). This is relevant information, which may indicate organisms that are more resistant and more susceptible to leachate, allowing for a better understanding of their contribution to effluent treatment.

The crude leachate sample presented for the acute toxicity assay with *Vibrio fischeri*, toxicity factor (TF) equal to 8, in which the inhibitory effect was less than 20% and EC_{20} equal to 19.53%. However, for the acute toxicity assay with *Daphnia similis*, a toxicity factor 32 and EC_{50} was 8.25%. These results show the importance of using more than one organism to evaluate and map the toxic effect, especially in a complex matrix such as landfill leachate. However, considering the high toxicity of the raw leachate, toxicity was not detected in the final effluent, M5 and M10 ponds samples. This result is relevant, as it shows that during the system's monitoring period, the proportion of leachate inserted may have been fundamental for the effectiveness of the treatment, including the adaptation of the organisms involved in the process.

Genera	Frequencies in the ponds			
	M5		M10	
	% Found	Classification	% Found	Classification
Golenkinia sp.	Not detected	Not detected	3%	Rare
Pediastrum sp.	Not detected	Not detected	3%	Rare
Polyedriopsis sp.	Not detected	Not detected	6%	Rare
Scenedesmus sp. z o.o	77%	Constant	90%	Constant
Monoraphidium sp.	74%	Constant	74%	Constant
Desmodesmus sp. z o.o	71%	Constant	77%	Constant
Dictyosphaerium sp.	68%	Constant	74%	Constant
Micractinium sp.	61%	Constant	65%	Constant
Actinastrum sp.	45%	Common	55%	Constant
Tetrahedron sp.	19%	Common	29%	Common
Chlorella sp.	10%	Rare	10%	Rare
Coelastrum sp.	10%	Rare	13%	Common
Coronastrum sp.	10%	Rare	13%	Common
Oocystis sp.	10%	Rare	16%	Common
Didymogenes sp.	6%	Rare	19%	Common
Chodatella sp.	3%	Rare	Not detected	Not detected
Closterium sp.	3%	Rare	3%	Rare
Crucigenia sp.	3%	Rare	Not detected	Not detected
Crucigeniella sp.	3%	Rare	6%	Rare
Kirchneriella sp.	3%	Rare	3%	Rare
Pseudokirchneriella sp.	3%	Rare	3%	Rare
Schroederia sp.	3%	Rare	Not detected	Not detected

Table 3. Composition and frequency of the green algae group in the two maturation ponds: M5 (module I: sewage treatment) and M10 (module II: treatment of domestic wastewater combined with landfill leachate).

Discussion

The first evaluation is regarding the WWTP's total efficiency, analyzing the monitoring data collected regarding the raw and final effluent (mixture of effluents from the two modules). The typical composition of raw domestic sewage considered weak is COD of 339 mg/L, BOD of 133 mg/L, and total phosphorus of 3.7 mg/L[29]. The composition of the sewage influent to the Belém Novo WWTP presented, in the five years of monitoring adopted in the present study, average values of the parameters close to those described by the authors above, with COD of 201 ± 11.6 mg/L; BOD_5 of 120 ± 13.4 mg/L and total phosphorus of 3.7 ± 0.3 mg/L.

The efficiency ranges reported by Jordão and Pessoa[30] for facultative ponds followed by maturation ponds are BOD_5 80–85%, COD 70–83%, ammonium nitrogen 40–80%, and Phosphorus > 50%. However, the reducing efficiency of COD was 14.9 ± 13.1% and of BOD_5 was 47.9 ± 9.7%; in other words, the low efficiency of COD and BOD_5 reducing in the ponds stands out. This result is believed to be due to the presence of phytoplankton in the final effluent. In 2021, BOD_5 analysis was performed with a filtered sample (removing suspended phytoplankton), obtaining an average BOD_5 value of 17.2 ± 10.4 mg/L. Thus, the average reducing efficiency of this parameter with the filtered sample was 80.8 ± 14.9%, and with the unfiltered sample, it was 44.5 ± 29.6%. Similar data were described by Ali et al.[31], which evaluated the performance of a stabilization pond used for domestic sewage treatment in a WWTP located in Egypt. The authors found COD and BOD_5 reduced efficiency values by 25%. It is important to evaluate the inclusion of complementary treatment to remove phytoplankton from the effluent and improve its quality before it is sent to the receiving source.

Notably, in the WWTP evaluated, there is no mechanism for removing phytoplankton from the effluent; these organisms are sent to the receiving source. According to Barroso Júnior et al.[32], in stabilization ponds, conditions are provided for the development of microalgae, which may be present in treated sewage. Thus, if not removed, they can increase BOD_5 and COD values in the final effluent. In addition to organic matter, according to the same author, the non-reducing of microalgae from the liquid also leads to a decrease in nutrient-reducing efficiency from the final effluent, for phosphorus between 25 and 55% and total nitrogen of 40–90%.

The treatment by ponds showed a high reduction of ammonium nitrogen. The reduction of ammonium nitrogen in stabilization ponds can occur due to three factors[32]: (1) volatilization through the elevation of pH by photosynthesis; (2) nitrification reactions in the presence of dissolved oxygen above 1.5 mg/L and denitrification; and (3) assimilation by microalgae, being transformed into organic nitrogen. It is believed that the three factors described above contributed to the high ammonium nitrogen-reducing efficiency. As can be seen in Table 2, the pH values during the entire monitoring period averaged 9.0 or more for the final effluent. In addition to this parameter, the presence of phytoplankton was found in the ponds, which, in addition to assimilating the nutrient, also provide dissolved oxygen in the effluent through photosynthesis, favoring the occurrence of nitrification.

Arashiro et al.[33] found a similar result using high-rate algal ponds to treat urban wastewater. The results showed up to 90% efficiency in ammonium nitrogen reduction. Nitrogen and ammonium are removed in algal ponds mainly through incorporation into algal biomass and pH-dependent ammonium nitrogen volatilization[34].

Regarding phosphorus reduction, according to Von Sperling[35], the dependence on high pH values is more significant than nitrogen because, for considerable phosphorus precipitation, the pH must be at least 9.0 since the most critical reduction can occur through phosphate precipitation. Wang et al.[36] relate that a high pH condition provides the phosphate to precipitate for two main reasons: (1) The saturation index of typical phosphorus precipitates would elevate along with increasing pH value because phosphate would be deprotonated, and with more hydroxyl ions, the components of hydroxyapatite would be available under high pH; (2) The solubility of phosphorus precipitates is lower under high pH conditions, which benefits the deposition these minerals.

Furthermore, phosphorus reduction in ponds can also occur through phosphorus accumulation in algal biomass[31,37]. In addition to nitrogen, phytoplankton may have contributed to removing total phosphorus through cellular assimilation. Young, Taylor, and Fallowfield[34] reported an extensive range of total phosphorus reduction using ponds in the literature, from 10.48 to 97.2%, with a median of 42.7%.

According to Leite et al.[11], the factors that can interfere with bacterial decay along the stabilization pond system are (1) temperature; (2) wind direction and speed; (3) the intensity of sunlight; (4) geometry of the ponds; (5) high concentrations of dissolved oxygen; (6) variations in pH magnitudes and (7) the phenomenon of predation. From the evaluation in Table 1, it is noteworthy that *Escherichia coli* reduction in the present study was above 99.9% in the entire period analyzed. Exposure to solar radiation is the most critical factor for pathogen reduction in effluent treatment systems by stabilization ponds[38].

From the analysis of the monitoring data of the M5 and M10 ponds in the period evaluated in the present study, shown in Table 2, it is possible to highlight the mean pH values of 9 or more in the analyzed period for both ponds. The pH of pond effluent increases during the day due to solar radiation and consequent photosynthesis of microalgae[32]. Wallace, Champagne, and Hall[39] explain that this occurs because carbonates and bicarbonates from the effluent are consumed to produce carbon dioxide, a process that contributes to the accumulation of hydroxyl ions in the medium.

The final effluent from both ponds evaluated meets the discharge standards described in the operating license of the WWTP: COD < 180 mg/L; BOD_5 60 < mg/L; ammonium nitrogen < 20 mg/L. According to the state regulation to effluent discharge[52], the maximum allowed value for thermotolerant coliform, for the WWTP flow rate, is 10^4 MPN/100 mL. Considering the proportion of *E. coli*/thermotolerant coliform of 0,6[53], the values shown in Table 1 also demonstrated this accordance. The only parameter that would not agree would be total phosphorus, which the license sets at a value of less than 2.0 mg/L.

It was generally verified that the final effluent of the M10 pond, module II, presented higher concentrations of BOD_5, COD, ammonium nitrogen, and total phosphorus parameters. However, from the evaluation in Table 2, there was no significant difference between the concentrations of ammonium nitrogen and total phosphorus and the pH values in both ponds. For the BOD_5 and COD parameters, the effluents from ponds M5 and M10 are considered statistically different for a 95% confidence level (p value < 0.05). In this way, the effect of the insertion of leachate in the final effluent of the ponds is verified, which impacted the reduction of organic matter. This fact may be due to the presence of recalcitrant compounds.

Using a biological process for leachate treatment can result in low COD and BOD_5-reducing efficiencies, mainly due to refractory organic compounds[40]. Mature landfills, also called stabilized, anaerobic conditions, predominate, and the BOD/COD ratio is less than 0.1[41], also considered low biodegradability in the leachate from old landfills[42]. It is believed that most of the biodegradable organic matter is decomposed in the stabilization stage, but the non-biodegradable organic matter remains unchanged. Consequently, the BOD/COD ratio decreases with time, presenting more difficulty in biodegrading and recalcitrance when subjected to biological treatment. For comparison, the raw influent`s average BOD/COD ratio was 0.59 in the analyzed period. This average ratio for M10 effluent pond (sewage + leachate), was 0.28, and for M5 effluent pond (sewage) was 0.30.

Rigotto et al.[10] verified this due to the significantly higher organic matter concentration and the predominant presence of humic and fulvic acids in landfill leachate. Even its small proportion inserted in the treatment system (2.7%, in this work) compared to sanitary sewage undeniably exerts a prevailing impact on the overall humic profile of the sample, represented by organic matter parameters. The recalcitrance associated with humic compounds was cited in the literature[43,44], because it is mainly composed of a series of heterogeneous polymeric organic, identified as one of the most difficult biodegradable fractions in the dissolved organic matter.

Algae and bacteria are essential for the effluent treatment of the pond to function correctly. The bacteria break down the complex organic compounds present, converting them into simple compounds, making them possible for algae to use. Algae, on the other hand, produce the oxygen necessary for aerobic bacteria to survive. The reactions of biodegradation and mineralization of the effluent by the bacterium, as well as the synthesis of new organic compounds in the form of algal biomass, can result in effluent containing a high content of total suspended solids, which contributes to turbidity[45]**,** high content of organic matter, nitrogen, and phosphorus, which can be used as a substrate for bacterial growth[47,48]. Thus, phytoplankton act in symbiosis with aerobic bacteria in the biological treatment of sewage in stabilization ponds. The predominance of several phytoplankton species depends on their ability to adapt to existing environmental conditions[46]. Furthermore, the frequency of some phytoplankton genera in both ponds may indicate the organisms' more excellent resistance or susceptibility to leachate.

The algae assimilate the nutrients in the effluents, retaining various chemical compounds in the biomass, as *Scenedesmus obliquus*[49]. Souza et al.[50] evaluated the effect of landfill leachate, submitted to secondary treatment in a stabilization pond, on the cultivation of *Scenedesmus* sp. The authors found that *Scenedesmus* sp. removed metals from the 80% mixture by biosorption, suggesting that the microalgae can also be used to remediate effluents with high polluting potential.

According to Marttinen et al.[51], the acute toxicity of leachate is attributed to high ammonium nitrogen concentration. Pivato and Gaspari[16] found that ecotoxicological laboratory tests have confirmed that the toxicity, evaluating *Vibrio fischeri*, of the leachate depends strongly on the ammonium nitrogen concentration, and the toxicity was considerably lower in sustainable landfills where the ammonium nitrogen was degraded. Costa et al.[54] also found strong correlations between the ammonium nitrogen concentration in leachate and the toxicity of *Daphnia similis*.

About ammonium nitrogen, it stands out that the mean concentration of leachate is 356 ± 56.0 mg/L. These values can be attributed to the high toxicity in the crude leachate sample, with *Vibrio fischeri* EC_{50} equal 19.53% and *Daphnia similis* EC_{50} at 8.25%. Similar results were found by Costa et al.[17], who detected high toxicity of the leachate using *Daphnia magna*, with EC_{50} values in the range of 3.44 to 7.33%. Although the result of the crude leachate, no toxicity was found in the effluents of both pond modules in this work. It is highlighted that the mean ammonium nitrogen in the effluent of pond M5 was 1.04 mg N/L and 1.63 mg N/L for M10.

Furthermore, due to several parameters, the landfill's age determines the leachate composition[41]. However, even though the addition of leachate had an impact on the organic matter content of the final effluent when comparing the two pond modules, the presence of recalcitrant compounds in the effluent from the M10 pond, associated with the added percentage of 2.7%, did not influence its toxicity, as it did not was found in tests carried out with *Vibrio fischeri* and *Daphnia similis*.

Conclusion

Considering the results of the present study, with a full-scale ETE and long-term monitoring operation, the stabilization pond system may be a simple and low-cost alternative for the combined treatment of leachate and sewage. Thus, advances have been made in evaluating the efficiency of this treatment technology in this specific condition. Therefore, it is worth noting that 2.7% of leachate classified as old was assessed in the present study. The WWTP had an average COD and BOD_5 reduction of $14.9 \pm 13.1\%$ and $47.9 \pm 9.7\%$, respectively. The low efficiency of organic matter removal can be attributed to the presence of phytoplankton in the final effluent.

Notably, this study evaluated two stabilization pond modules, in which the module completed by pond M5 was used for domestic sewage treatment. In the module completed by pond M10, the combined treatment of domestic sewage and leachate was carried out. Comparing the effluents of these two ponds, only the parameters related to organic matter (BOD_5 and COD) showed a statistical difference for a 95% confidence level (p value < 0.05). Thus, even if the addition of leachate affected both systems' organic matter, this should not have influenced their ecotoxicity. Although there is a high concentration of $N\text{-}NH_3$ in the raw leachate, its insertion did not affect the treatment system since no significant difference was observed in this parameter in the two evaluated ponds. This result may be related to the stabilization ponds' high efficiency in removing ammonium nitrogen (values above 95%). This may also have contributed to the absence of ecotoxicity in ponds M5, M10, and the final effluent of the WWTP.

The phytoplankton community of the ponds was composed of 46 taxa distributed in four taxonomic classes: 6 belonging to the Cyanobacteria group (13%), 22 to the Green Algae group (48%), 4 to the Diatoms group (9%) and 14 to the Phytoflagellate group (30%). The genera considered constant, about their frequency, in the two maturation ponds (M5 and M10) were: (1) cyanobacteria: *Merismopedia*; (2) green algae: *Scenedesmus, Monoraphidium, Dictyosphaerium, Micractinium*; (3) phytoflagellates: *Pteromonas*. The predominance of green algae in both modules indicates the adaptation of phytoplankton to the presence of leachate, which may also have contributed positively to the treatment. The frequency of occurrence of some phytoplankton genera in both lagoons may indicate the more excellent resistance or susceptibility of the organisms to the presence of leachate, information that is highly relevant and allows for progress in understanding the role of these organisms in the treatment, given the inherent complexity of stabilization ponds.

The importance of evaluating the inclusion of complementary treatment in the ponds is highlighted, considering the impact of the leachate on the organic matter present in the final effluent of the leachate-receiving system and the removal of excess phosphorus, which was observed in the effluent of both ponds modules. However, it is also emphasized that more information needs to be evaluated to ensure the safety of the combined treatment in stabilization ponds, such as heavy metals in the effluent.

Data availability

The authors declare that the data supporting the findings of this study are available in the article. If raw data files in another format are required, they are available from the corresponding author upon reasonable request.

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Author contributions
F. J. S: Tabulation and data analysis. Discussion of results. M. C. A. S: Discussion of results. All authors reviewed the manuscript.

Competing interests
The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to F.J.d.S.