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Original Article

Phytotoxicity of Domestic Effluent Before and After Treatment by Stabilization Ponds

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Abstract

Phytotoxicity tests linked to physicochemical parameters can be used to determine the possible effects of releasing a polluting load into the environment, evaluating the efficiency of a given treatment and its toxicity effects. In this context, the present work sought to evaluate the phytotoxicity of domestic effluent before and after treatment by stabilization ponds, using kale seeds (*Brassica oleracea*). Toxicity tests were carried out following the methodology of the American Environmental Protection Agency (EPA, 1996) at concentrations of 100%, 80%, 40%, 20%, 10%, and 5%. The seeds were incubated in a B.O.D. incubation chamber for five days at a temperature of 25ºC ± 2ºC. After this period, germination and root elongation were analyzed. The samples were also analyzed for biochemical oxygen demand, temperature, pH, turbidity, color, total solids, ammoniacal nitrogen, and organic nitrogen. Phytotoxicity was statistically evaluated by the Relative Growth Index, T-test ($p < 0.05$) for samples at 100% concentration, and Kruskal-Wallis followed by Dunn's test ($p < 0.05$) to compare the negative control and the different concentrations. As a result, adverse effects of reduced root elongation were observed in samples of raw effluent (100%, 80%, and 20%) and treated effluent (10% and 5%), indicating a tendency toward phytotoxicity. Regarding the physicochemical parameters studied, all were within the established parameters by legislation, and no significant correlation was observed with root elongation. This may indicate the efficiency of stabilization ponds in treating domestic effluents, particularly in reducing phytotoxicity.

Keywords: *Brassica oleracea,* Effluent, Kale, Seeds, Toxicity.

INTRODUCTION

The expansion of basic sanitation is both, a fundamental right and an immediate necessity for the entire population. This expansion is crucial not only for the optimal development of the State but also because its absence or deficiency adversely impacts the environment, quality of life, and public health. It is impossible for a developing society to thrive without the provision of these essential services in both adequate quantity and quality (Silva & Heller, 2016; Santos *et al*, 2018).

Among the pillars of sanitation in Brazil, the implementation of sewage systems requires greater attention. The 2021 indicators from the National Sanitation Information System reveal that while approximately 84.2% of the population has access to a water supply network and 89.9% have household solid waste collection coverage, only 55.8% are connected to a sewage network, and of those, only 51.2% receive some form of treatment (Brazil, 2022).

Sewage treatment is an essential process for environmental preservation. It helps maintain the quality

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of water resources, prevents contamination, and promotes public health by reducing waterborne diseases. Additionally, it improves social conditions, particularly in underprivileged communities, and has positive effects on the economic system (Villagra, 2019; Obaideen *et al*., 2022).

Among the existing sewage treatment systems, stabilization ponds stand out for their cost-effective removal of undesirable constituents. This method is one of the most widely used globally. The stabilization of organic matter occurs through bacteriological oxidation and/or photosynthetic reduction by algae, depending on the type of pond, such as facultative, anaerobic, aerated, and maturation ponds (Oliveira; Azevedo, and Cavalcanti, 2021; Mahapatra; Samal, and Dash, 2022).

Stabilization lagoons represent the simplest form of sewage treatment and can be arranged in various configurations. These lagoons cover large areas and have different depths, encompassing anaerobic, aerobic, and facultative zones. The influent sewage enters at one end, undergoes a series of treatment processes, and exits at the opposite end (Von Sperling, 2017).

However, regardless of the treatment method, ensuring the quality of the effluent released into a receiving body is essential. To this end, the National Environment Council (CONAMA) Resolution No. 430 (2011) defines conditions and standards for effluent discharge, which must be assessed through physicochemical analyses and toxicity tests on organisms. This is especially important in states without monitoring regulations for effluent toxicity assessment. At the state level, Ceará has the State Environmental Council (COEMA) Resolution No. 2 (2017) to address this need.

Toxicity tests are laboratory tests conducted under specific and controlled experimental conditions. They are crucial for evaluating water quality and the pollutant load of effluents, as traditional physicochemical analyses alone are insufficient to assess the environmental risk potential of contaminants (Costa *et al*., 2008; Pimentel *et al*., 2009; Gerber *et al*., 2017; Wadaan *et al*., 2023). In this context, tests using seeds as model organisms are promising tools for evaluating effluent toxicity. These tests are cost-effective, easy to apply, and the required materials are readily available (Coutinho *et al*., 2021).

Given this, several studies have employed phytotoxicity tests to assess pollutant loads in various effluent types, using seeds as test organisms to complement traditional physicochemical analyses and to interpret potential effects on biota (Guevara *et al*., 2011; Costa, Monteiro, and Batista, 2018; Barszcz *et al*., 2019; Nogueira, Tonetti, and Leonel, 2019; Melo *et al*., 2021; Melo *et al*., 2022; Santiago, 2022).

Klauck *et al.* (2015) evaluated the phytotoxicity of landfill leachate before and after biological treatment and demonstrated that superior plant species such as lettuce (*Lactuca sativa* L.) and onion (*Allium cepa* L.) could effectively assess the toxicity of landfill leachate. This finding was supported by Almeida *et al.* (2022), who evaluated the phytotoxicity of landfill effluent using lettuce seeds (*Lactuca sativa* L.), confirming their efficacy as indicators of toxic effects and providing insights for proposing improvements and alternatives for effluent treatment plant operations.

Guevara *et al*. (2019) found, while assessing the phytotoxicity of domestic wastewater using lettuce (*Lactuca sativa* L.) and cucumber (*Cucumis sativus* L.) seeds, that such effluents did not pose a potential toxicological risk when evaluated with these species.

Oliveira (2021), in studies involving the same test organisms, demonstrated that domestic effluent presented a low acute toxicity for both species and recommended further ecotoxicological studies to evaluate any potential toxicity of domestic effluents.

In Juazeiro do Norte/Ceará, the Domestic Effluent Treatment Station ETE-Malvas is the largest and most important treatment plant in the municipality. It has been a subject of research over the years, particularly due to its discharge of treated effluents into the municipality's main river, the Salgadinho River (Lima *et al*., 2020), which is part of the Salgado River sub-basin (De Lima & Ribeiro, 2012). However, these studies have primarily focused on the physicochemical, phytoplanktonic, and microbiological characterization of the effluent and its potential impacts on the receiving water body (Freire; Santos, 2018; Faustino *et al*., 2018; Nunes *et al*., 2019; Lima *et al*., 2020; Moreira *et al*., 2024), without addressing data on toxicity in organisms.

In this context, this study aimed to evaluate the potential phytotoxic effects of raw and treated domestic effluent, processed through a system of stabilization ponds using kale seeds (*Brassica oleracea*) as test organisms exposed to different concentrations. Additionally, it was examined the physicochemical parameters and assessed the disposal conditions of this effluent.

METHODOLOGY

Sample Treatment and Collection System

Five collections were conducted to obtain samples of raw and treated domestic effluent between March and May 2022. These collections took place at the Sewage Treatment Station (STS) in Juazeiro do Norte, a city located in the state of Ceará, Brazil. The treatment system at this station consists of two anaerobic lagoons, followed by two facultative lagoons, and one maturation lagoon (Figure 1). The final effluent is discharged into the Salgadinho River through an outfall (Martins *et al*., 2019).

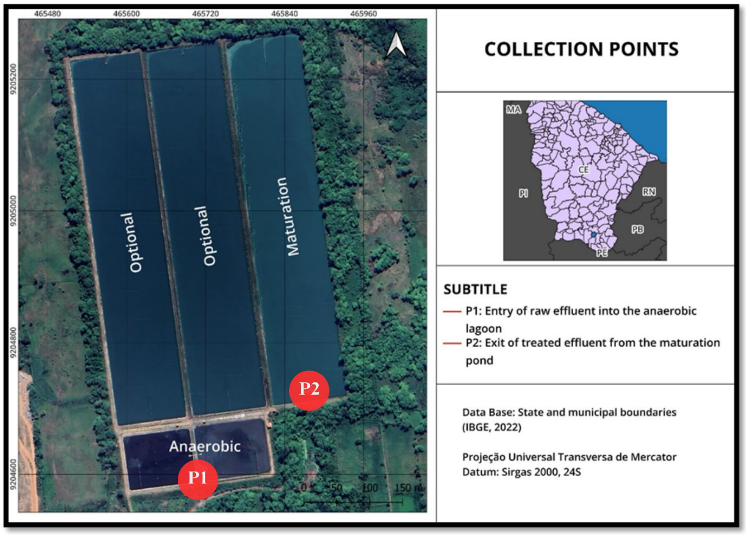

Figure 1. Sewage Treatment Station (ETE) Configuration.

Samples of the raw effluent were collected in the mixing zone at the entrance of the anaerobic lagoons (P1), and samples of the treated effluent were collected at the exit of the maturation lagoon (P2), as shown in Figure 1. The samples were collected in 2-liter bottles and transported to the Environmental and Sanitary Engineering Laboratory at the Federal Institute of Education, Science, and Technology of Ceará (IFCE) - Juazeiro do Norte campus, for immediate phytotoxicity tests and physicochemical analyses.

Physicochemical Analyses

For the physicochemical analyses of the Biochemical Oxygen Demand (BOD5), temperature, pH, turbidity, color, total solids, ammonia nitrogen, and organic nitrogen, the methodology established by Standard Methods (APHA, 2012) was followed. These analyses were carried out on raw and treated effluent samples at a concentration of 100% to evaluate the quality of the effluent released into the water body.

Phytotoxicity Tests

Kale seeds (*Brassica oleracea*) used in the research were purchased from specialized stores. All tests were conducted at concentrations of 100%, 80%, 40%, 20%, 10%, and 5% for both effluent samples. Distilled water served as the negative control, and zinc sulfate at a concentration of 1% acted as the positive control.

Phytotoxicity tests were conducted following the methodology established by the adapted United States Environmental Protection Agency (EPA) in 1996. The seeds were placed in 90 mm Petri dishes with distilled water for 30 minutes to break dormancy. Then they were transferred to Petri dishes lined with filter paper soaked with 2.5 mL of the samples at various concentrations. All tests were performed in triplicate with 10 seeds, totaling 30 seeds per concentration. The sealed Petri dishes were wrapped with aluminum land incubated in a B.O.D. incubation camera for five days (120 hours) at a temperature of 25ºC±2º. After this period, the germination was analyzed, and the root elongation was measured using a digital caliper. Negative control tests with 65% germination and root growth of at least 20 mm were considered valid.

The Germination Rate (%G) was calculated using Equation 1 (Zucconi *et al*., 1985, as cited in Alvarenga *et al*., 2006):

$$\%G = (Sg/Sn)\times 100 \qquad \text{(Equation 1)}$$

Where:

Sg = total number of germinated seeds;

Sn = total number of seeds.

Additionally, the Relative Growth Index (RGI) for rootlet elongation (mm) is calculated using Equation 2 (Alvarenga *et al*., 2006):

$$RGI = CRa/CRc \qquad \text{(Equation 2)}$$

Where:

CRa = radicle length in the sample;

CRc = radicle length in the negative control.

Toxicity effects, as determined by RGI, were categorized into three groups:

a) Inhibition of root elongation rate: $0 < RGI < 0.8$;

b) No significant effects: $0.8 \leq RGI \leq 1.2$;

c) Stimulation of elongation rate: $RGI > 1.2$.

Statistical Analysis

Phytotoxicity assessment involved comparing the results of the negative control with different tested concentrations for both raw and treated effluent samples. After conducting the Shapiro-Wilk normality test, it was found that the samples did not follow a normal distribution. Therefore, the data were subjected to Kruskal-Wallis analysis followed by Dunn's test, with a significance level of $p < 0.05$ for non-parametric samples. For the evaluation of results in raw and treated effluent samples at 100% concentration only, an unpaired T-test was conducted, with a significance level of $p < 0.05$.

In the physicochemical analyses, all parameters underwent the Spearman correlation test (since they did not follow a normal distribution by the Shapiro-Wilk test), with a significance level of $p < 0.05$. This analysis aimed to explore possible relationships between each analyzed parameter and radicle elongation for samples at 100% concentration. Statistical tests were conducted using GraphPad Prism version 8.0.1.

RESULTS AND DISCUSSION

Physicochemical Analyses

Table 1 displays the values obtained for all parameters analyzed in both raw and treated domestic effluent samples at 100% concentration.

Table 1. Results of Physicochemical Analyses for Raw and Treated Domestic Effluent. (*) No BOD5 analysis was performed for the T1 test.

	pH	T (°C)	Turbidity (UNT)	Color (UC)	Total Solids (mg L^{-1})	Organic Nitrogen (mgN L^{-1})	Ammonia Nitrogen (mgN L^{-1})	BOD_5 (mg L^{-1})
				RAW				
T1	7.10	26.70	88.75	1456.67	923	3.10	57.57	*
T2	7.66	26.00	137.00	2163.33	1313	8.0	78.4	657.64
T3	7.64	24.30	180.00	2371.67	854	11.3	71.23	501.93
T4	7.71	26.80	144.50	2045	1000	13.6	79.69	494.75
T5	7.67	27.50	134.00	1763.33	1030	10.4	74.48	563.64
				TREATED				
T1	8.18	25.80	229	1570.00	560	7.10	11.54	*
T2	8.56	26.00	270	1778.33	520	9.30	6.61	96.08
T3	8.16	24.50	211	1303.33	518	6.40	8.18	122.72
T4	8.20	26.40	254	1620.00	567	10.30	12.15	147.96
T5	8.08	25.60	237	1403.33	614	12.10	12.32	139.31

From Table 1, it can be observed that the pH and temperature parameters were within the limits established by COEMA Resolution No. 2 (2017), which specifies a pH range between 5 and 9, and a temperature below 40°C. The BOD5 parameter was the only one that exceeded the recommended level of 120 mg/L for the discharge of treated effluent into the receiving body, with the exception of T2. However, the system still demonstrated BOD removal efficiency above 60% for all tests carried out. This complies with Federal Legislation, which allows the limit of 120 mg/L to be exceeded when a minimum BOD removal efficiency of 60% in the treatment system is proven (CONAMA 430, 2011).

Elevated values in the color parameter can be attributed to the presence of dissolved solids. Although this parameter is more commonly analyzed in water supply, Pereira *et al.* (2014) and Bou *et al.* (2018) explain that color in effluents can indicate the presence of metals, organic matter, plankton, and other dissolved substances. This could be a concern both during the treatment process and after the effluent is discharged.

The increase in turbidity observed in the treated effluent (Table 1) can be attributed to the presence of suspended solids, including algae, which were observed in the STS treatment system during all collections. According to Von Sperling (2017), the lagoons commonly have fluctuations in turbidity due to factors like the presence of algae, especially in facultative and maturation ponds. Suspended solids in the effluent are typically the result of algae that thrive under favorable conditions, leading to an increase in turbidity. According to Alves *et al.* (2019), the accumulation of pollutants on the surface of the lagoon harms photosynthetic activity and aquatic biodiversity by blocking the entry of sunlight.

In the raw effluent, the concentrations of total solids exhibited a maximum of 1313 mg L^{-1} and a minimum of 854 mg L^{-1}. In the treated effluent, these concentrations ranged from a maximum of 614 mg L^{-1} to a minimum of 518 mg L^{-1} (Table 1). The average removal efficiency for total solids was approximately 32.9%. Truppel (2002) and Cordero (2016), in similar studies, attribute this low efficiency in removing total solids to the constant bloom of algae and sludge release.

The presence of solids in effluents can pose a problem due to the possibility of toxic substances adhering to them, which can be harmful to the biota in receiving bodies (Vaz, 2017). According to CETESB (2019), solids in a water body can be harmful to fish and other aquatic life as they tend to settle on the riverbed. It can damage fish spawning sites. Furthermore, these solids can retain bacteria and organic waste, creating an anaerobic environment which lacks oxygen and is not favorable for the aquatic life.

In addition to the presence of solids, another critical factor is the relationship between nutrients, such as nitrogen (N) and phosphorus (P), which promote algae and cyanobacteria blooms in stabilization ponds, particularly in locations with favorable conditions for this phenomenon. These conditions include prolonged periods of intense sunlight, final effluents with an average temperature above 25°C, and an average pH around 7.5 (Ribeiro, 2007) – characteristics found in the city where the studied STS is located.

At high concentrations, the presence of these nutrients becomes a concern for the aquatic environment that can lead to significant ecological imbalances. This includes aquatic species toxicity and the induction of eutrophication, which can cause excessive growth of aquatic organisms and plants. Such excessive growth can reach levels that interfere with the intended uses of the water body (Bastos & Von Sperling, 2009; Bento, 2005).

In this context, organic nitrogen concentrations in this system ranged from 13.6 mgN L^{-1} to 3.1 mgN L^{-1} for the raw effluent and from 12.1 mgN L^{-1} to 6.4 mgN L^{-1} in the treated effluent (Table 1). These maximum values are similar to those reported by Martins *et al*. (2019) when analyzing the effluent discharge from the same STS as in this study.

Given that nitrogen is one of the most limiting elements for the plant development and it can be directly incorporated into organic compounds, its presence in domestic effluents, along with phosphorus and potassium, can influence the growth of various species (Cuba *et al*., 2015).

Regarding phosphorus, Araújo (1993) has shown cases where the removal ranged from 41% to 54% in various stabilization pond systems. Von Sperling (2017) adds that the most significant reduction of phosphorus can occurs through the precipitation of phosphates, in the form of hydroxyapatite or struvite, at high pH levels as above 8. This phenomenon can potentially take place in the lakes within the scope of this study, considering the pH range observed, which is approximately 8.2 for the treated effluent (Table 1).

Furthermore, the obtained pH range can lead significant environmental implications, as it indicates that ammonia is primarily in its ionized form ($NH4^+$), which is less toxic. In it own free form (NH_3), ammonia can be harmful to biota, potentially causing toxic effects (Von Sperling, 2014).

Phytotoxicity Tests: Relative Growth Index

In all conducted tests, the germination rate exceeded 65%, and the root growth was above the recommended levels, validating these tests as per EPA (1996) guidelines.

To assess the toxicity of the raw and treated effluent and simulate conditions that might be encountered in the receiving body, the Relative Growth Index (RGI) was calculated for various concentrations (Table 2).

Table 2. Relative Growth Index (RGI) of kale seeds exposed to samples of raw and treated domestic effluent at different concentrations. (*) Indicates values below the recommended thresholds, and (**) Indicates values above the recommended thresholds.

	RAW					
	100%	**80%**	**40%**	**20%**	**10%**	**5%**
T1	0.771*	0.849	0.829	0.755*	0.942	0.975
T2	0.624*	0.923	0.905	0.910	0.819	0.806
T3	0.748*	0.947	1.040	1.038	1.040	0.872
T4	0.819	0.750*	1.097	1.081	1.039	1.185
T5	1.092	1.042	1.379**	1.287**	1.448**	1.234**
	TREATED					
	100%	**80%**	**40%**	**20%**	**10%**	**5%**
T1	0.978	1.038	0.851	0.647*	0.781*	0.708*
T2	0.882	0.885	0.868	0.836	0.474*	0.584*
T3	0.859	0.965	1.041	0.897	0.773*	0.834
T4	0.943	1.175	1.212**	1.013	0.664*	0.978
T5	1.294**	1.337**	1.172	0.833	1.146	1.103

According to Table 2, when comparing the RGI separately for the raw and 100% treated effluent samples, improvements in root elongation are observed. The raw effluent samples presented RGI < 0.8 in three (T1, T2, T3) out of the five tests performed, indicating an inhibition of root elongation. In contrast, the treated effluent samples showed four tests (T1, T2, T3, T4) within the range without significant effect (0.8 ≤ RGI ≤ 1.2), and one test (T5) showed a stimulation in root elongation rate (RGI > 1.2). However, different effects were observed in the tests at certain concentrations for both samples.

The same reduction in root elongation observed for the raw effluent at 100% (T1, T2, T3) was also observed in the T4 test at 80% concentration and in the T1 test at 20% concentration. In the raw effluent, an RGI > 1.2 was only observed for T5 at concentrations of 40% to 5%, indicating growth stimulation (Table 2).

Vaz (2017) explains that the presence of toxic substances adhered to suspended solids can lead to phytotoxic effects. In this context, Santiago (2022) states that the principle of toxicity, manifested specifically at certain concentrations when analyzing raw effluent, could be associated with the presence of polluting substances or agents in the samples. These pollutants were not analyzed or quantified in the physicochemical analysis of the present study; however, the presence of total and suspended solids was confirmed, the latter through turbidity analysis (Table 1). Alternatively, it may be concurrent with the presence of organic and inorganic loads, and the reduction of which correlates with the decrease in phytotoxicity (Young *et al*., 2012).

Furthermore, inadequate nutrient content can have limiting effects on plant growth (Sfredo & Borkert, 2004; Kirkby & Romheld, 2007). Therefore, in the present study, the effluent containing an excess or deficiency of nutrients, due to serial dilutions in the samples from each test, may have influenced the development of seed radicles in samples with raw effluent.

In samples with treated effluent, an RGI>1.2 is observed at concentrations of 100% (T5), 80% (T5), and 40% (T4), indicating stimulation in root elongation. However, concentrations of 20% (T1), 10% (T1, T2, T3, T4), and 5% (T1 and T2) show an RGI < 0.8, indicating a reduction in the root elongation (Table 2).

Similar studies have shown root elongation stimulation when treated domestic effluent was with seeds of pepper (*Capsicum Annum* L.) (Melo *et al*., 2022), cucumber (*Cucumis sativus* L.) (Guevara *et al*., 2019), and wheat (*Triticum aestivum* L.) (Nogueira; Tonetti, and Leonel, 2019).

Melo *et al*. (2021) pointed out in a study with domestic sewage that in toxicity tests, treated sewage samples had 16.6% GI <80% and 69.4% GI >120%, indicating the presence of substances that stimulate the germination of lettuce seeds.

This phenomenon may be related to the presence of nutrients such as nitrogen and phosphorus in the lake samples, as these are components typically present in domestic sewage and are essential for the initial development of plants (Cuba *et al*., 2015; Von Sperling, 2017; Oliveira, 2021). The presence of nitrogen, in particular, is observed in both the treated and raw effluent in the present study (Table 1), which may have contributed to root growth.

Nevertheless & Reyes (2015) reported that in samples from a river containing domestic effluent, cases were observed in which undiluted samples stimulated root development. As the concentration decreased with dilutions, growth inhibition increased, tending to increase the value of phytotoxicity, especially at points with low concentrations of organic matter. This phenomenon possibly occurred with the inhibitory effects in the treated effluent at the lowest concentrations (20%, 10%, and 5%) in the present study, due to the decrease in organic matter and nutrients resulting from the dilutions performed.

Phytotoxicity Tests: Statistical Analysis

To evaluate the efficiency of the treatment, an unpaired T-test with a significance level of $p<0.05$ was used to assess the relationship between root growth in raw and treated effluent samples at a 100% concentration (Figure 2).

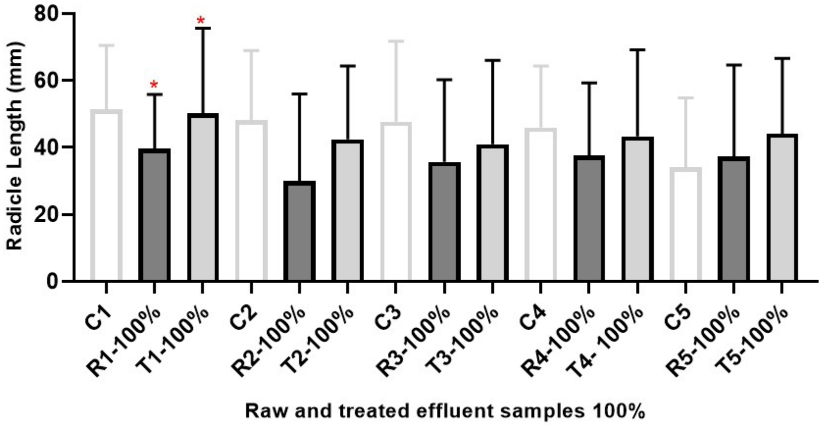

Figure 2. Radicle Length (mm) of Kale Seeds Exposed to the Raw and Treated Effluent Samples at 100% with an Unpaired T-Test Considering the Five Tests. (*) Indicates a statistically significant difference ($p<0.05$); (C) Negative Control, (B) Raw Effluent, (T) Treated Effluent.

Figure 2 shows that Test 1 presented a statistically significant difference ($p < 0.05$) when comparing the raw and treated effluent samples (R1 vs. T1). Furthermore, it was observed that there was an increase in the length of the rootlets in all five tests for the treated effluent when comparing the average length of the rootlets (mm) to that of the raw effluent. This root elongation phenomenon is similar to the one observed in the RGI calculation (Table 2) for samples at 100% concentration of the treated effluent.

Therefore, it can be inferred that there may have been a reduction in the toxicity of the effluent for the kale seeds during treatment using the lagoon system, as indicated by the induction of radicle growth when exposed to the treated effluent. Oliveira (2021) reports similar findings, where there was a reduction in the phytotoxicity of effluent treated by lagoons, particularly in effluents from the facultative lagoon and maturation lagoon, for lettuce (*Lactuca sativa* L.) and cucumber (*Cucumis sativus* L.) seeds.

Furthermore, the positive results from the treatment of effluents in lagoons, in terms of reducing phytotoxicity, have been observed in various studies for different test organisms (Barros *et al*., 2006; Costa & Castilhos, 2012; Oliveira, 2021).

To more comprehensively evaluate the effects of phytotoxicity at different concentrations, statistical tests were carried out on samples of raw effluent (Figure 3) and treated effluent (Figure 4).

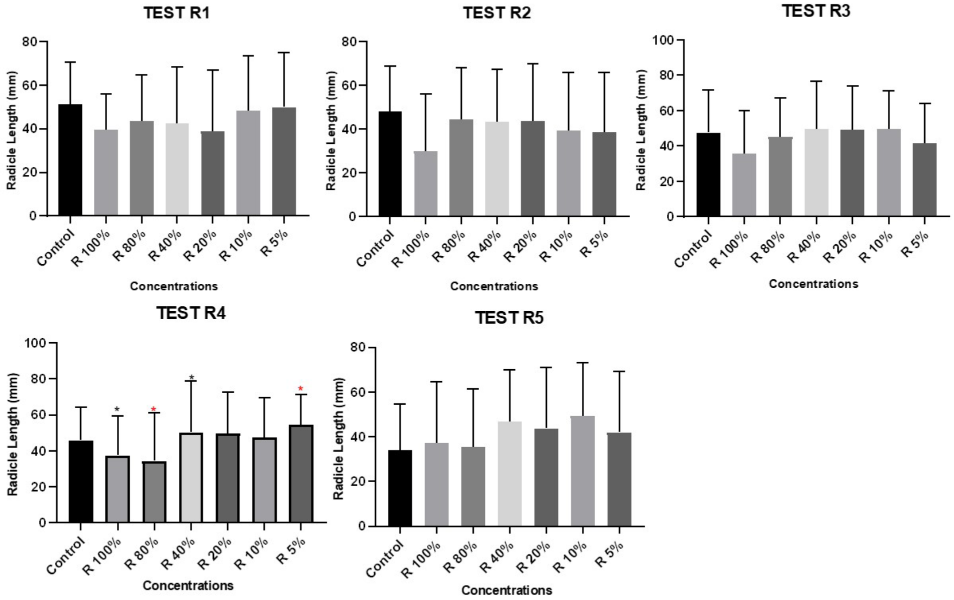

Figure 3. Radicle Length (mm) of Kale Seeds for Raw Effluent at Different Concentrations with Kruskal-Wallis Test Followed by Dunn's for the 5 Tests Performed. (*) Indicates Significant Difference for $P < 0.05$ for 100% and 40%. (*) difference between 80% e 5%. (B) Raw Effluent Sample.

For raw effluent samples (Figure 3), only Test R4 showed a significant difference ($p < 0.05$) between the 100% and 40% concentrations, and between the 80% and 5% concentrations. This indicates that, for this test, under these conditions, the highest concentrations (100% and 80%) resulted in a reduction in the elongation of kale rootlets. This may be attributed to the hormesis effect, as reported by Silva *et al*. (2020), which occurs when the presence of a substance in high concentrations causes inhibitory effects, whereas in low concentrations, it can lead to stimulatory reactions.

In organisms exposed to samples at different concentrations of raw effluent, the radicle length did not show a significant difference ($p < 0.05$) when compared to the negative control. This result is similar to findings reported by Cunha (2011) using lettuce and by Guevara *et al*. (2019) using cucumber in their analysis of phytotoxicity with raw domestic effluent, where no phytotoxic effects were observed. For the treated effluent, adverse effects on root elongation were observed in statistical tests (Figure 4).

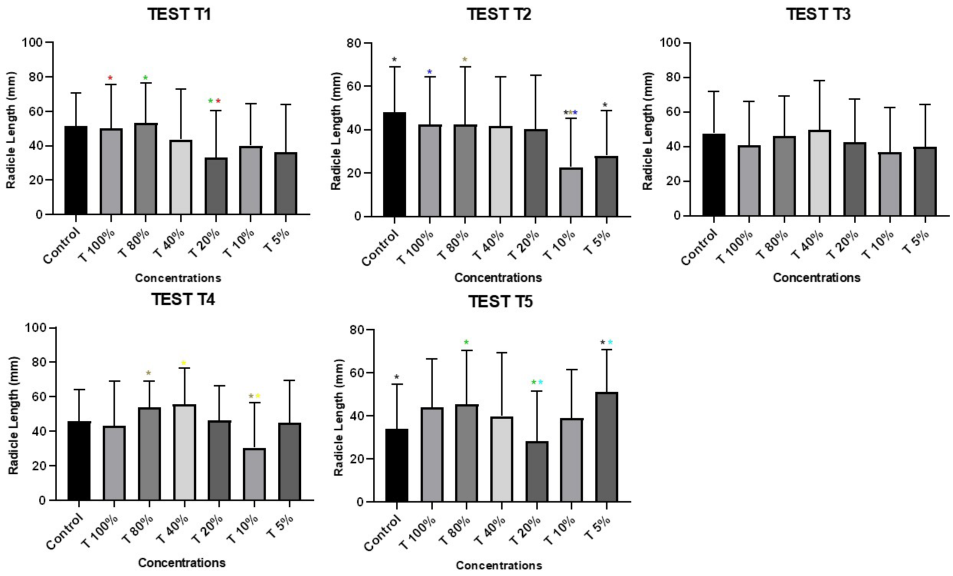

Figure 4. Radicle length (mm) of kale seeds for effluent treated at different concentrations with Kruskal-Wallis Test followed by Dunn's for the five tests performed. (*) Indicates significant difference for P < 0.05, control, 10% and 5%. (*) difference 100% and 20%. (*) difference 100% and 10%. (*) difference 80% and 20%. (*) difference 80% and 10%. (*) difference 40% and 10%. (*) difference 20% and 5%. (T) Treated Effluent Sample.

All tests showed some significant differences ($p < 0.05$), except for T3. In the T2 and T5 tests, a significant difference was observed compared to the control for the 5% concentration, and the T2 test also showed a difference at the 10% concentration.

For tests T1, T2, T4, and T5, statistical differences were observed between concentrations, with a tendency towards a reduction in root elongation at the lowest concentrations (5%, 10%, and 20%) compared to the highest (80% and 100%). In this context, for the treated effluent, there is a potential phytotoxic effect indicated by the reduction in radicle length for kale at concentrations of 10% and 5%.

This phenomenon, as previously reported, can occur due to the high organic load in domestic wastewater, where the effect of the dissolved nutrients can mask the presence of unquantified phytotoxic pollutants. These effects become evident when the organic load decreases through the process of dilution (Reyes, 2015; Vaz, 2017). Sánchez *et al*. (2007) also report cases where toxicity increased, rather than decreased, after treatment, as BOD values were reduced, showing a correlation with COD and nitrogen compounds.

In the present study, however, the evaluation using the Spearman correlation test for raw and treated effluents (100%) did not show a significant correlation ($p > 0.05$) between the physicochemical parameters and root elongation.

CONCLUSION

The toxicity of domestic wastewater was evaluated before and after treatment by stabilization ponds using kale seeds. The study showed varying levels of toxicity, with some samples exhibiting a reduction in root elongation while others showed a stimulation. The decrease in elongation could be attributed to the decrease in organic matter during dilution or the presence of contaminants not analyzed in the study. Conversely, the stimulation of elongation can be attributed to the presence of organic matter, which is typically found in domestic effluent and is essential for plant growth. These findings indicate the effectiveness of stabilization ponds in treating domestic wastewater, particularly in terms of reducing phytotoxicity.

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AUTHOR CONTRIBUTIONS

AMSS: Experimental development, article writing, data processing. **LPF:** Review and translation. **GMA:** Review. **JSA:** Experimental development, article writing review.

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