EFFECT OF OZONATION ON THE TOXICITY OF LANDFILL LEACHATE EVALUATED BY *ALLIUM CEPA* BIOASSAY

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Abstract

Landfill leachate, also known as percolate, is a complex effluent characterized by high organic load, dark coloration, and the presence of recalcitrant and potentially toxic compounds, posing significant risks to soil and water resources when inadequately treated. Among the available treatment technologies, ozonation has emerged as a promising alternative due to its ability to oxidize complex organic substances through the application of ozone (O_3). However, excessive ozone dosage may promote the formation of intermediate by-products with residual toxicity, highlighting the need for complementary biological assessment of treated effluents. This study aimed to evaluate the efficiency of ozonation in reducing the toxicity of landfill leachate using phytotoxicity assays with *Allium cepa* seeds and the Germination Index (GI%) as a biological response indicator. Leachate samples were collected from a sanitary landfill located in Candiota, southern Brazil, and subjected to ozone doses of 75, 150, and 300 mg O_3 L^{-1}. Raw and treated samples were tested

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at dilutions of 1:10, 1:50, and 1:100 (v/v), along with a distilled water control. The results demonstrated that ozonation significantly reduced phytotoxicity, with the intermediate dose of 150 mg O_3 L^{-1} combined with the 1:50 dilution presenting the highest germination index. These findings highlight the importance of optimizing ozone dosage and leachate dilution to enhance treatment efficiency and ensure greater environmental safety of the treated effluent.

Keywords: Leachate, Ozonation, Phytotoxicity, *Allium cepa*.

INTRODUCTION

Changes in consumption patterns, associated with population growth and accelerated urbanization, have intensified the generation of Municipal Solid Waste (MSW). This scenario increases challenges related to waste management and final disposal, since improper disposal may cause significant impacts on the environment and public health (Kaza et al., 2018). In this context, sanitary landfills stand out as a technically appropriate alternative for the final disposal of MSW, as they are structured with impermeabilization systems, leachate collection and drainage, and landfill gas handling and control (Youcai; Ziyang, 2017).

Sanitary landfills represent a more affordable alternative compared with incineration or composting and are intended to dispose of waste in the soil safely, preventing harm to public health and minimizing negative environmental impacts (Ma et al., 2024). To fulfill this purpose, monitoring becomes essential both during the construction and operational stages of the landfill and after its closure. Aspects such as leachate management, possible leaks in the liner system, groundwater quality, landfill gas migration, and structural stability are crucial factors to be evaluated (Meegoda; Hettiarachchi; Hettiaratchi, 2016).

Sanitary landfill leachate, also known as percolate or “chorume,” is a dark, viscous liquid with high turbidity and intense odor (Scandelai; Martins; De Syllos; Tavares, 2025). This effluent may present high concentrations of ammoniacal nitrogen, chlorides, organic matter, and difficult-to-degrade compounds such as humic substances, and, occasionally, metals (Kawahigashi et al., 2014). When not properly treated, it may cause serious damage to the environment, including contamination of soils and groundwater (Barbosa; Rocha, 2023).

Landfill leachate treatment is a technical challenge that plays an essential role in environmental management and in protecting public health (Nath; Debnath, 2022). This process is influenced by several factors, such as landfill age, the types of waste deposited, local climatic conditions, and the operational technologies used in landfill management (Mor; Ravindra, 2023). Thus, various treatment techniques have currently been developed to remove toxic contaminants present in leachate, aiming to comply with discharge regulatory standards, which are becoming increasingly restrictive (Abdelfattah; El-Shamy, 2024).

In Brazil, the National Solid Waste Policy (PNRS), established by Law No. 12,305/2010, sets forth the need for proper leachate management, emphasizing the importance of treatment and environmentally responsible destination (Galavote et al., 2022). Among the main treatment methods, biological processes (aerobic or anaerobic) and physicochemical processes stand out (Gomes; Schoenell, 2018). Among these options, biological treatments are the most widely used, mainly due to lower operational costs.

In addition to aerobic or anaerobic biological treatment carried out in Wastewater Treatment Plants (WWTPs), other alternatives for leachate treatment include internal recirculation within the landfill itself, the use of sequencing batch biological reactors, and different physicochemical methods such as flotation, coagulation, adsorption, air sparging, precipitation, ion exchange, and chemical oxidation (Abbas et al., 2009).

Gautam, Kumar, and Lokhandwala (2019) also point to Advanced Oxidation Processes (AOPs) as a promising and effective process for treating leachate from hazardous-waste sanitary landfills, reducing chemical oxygen demand (COD) by up to 60% and metal content by 70%–90%, thus constituting a promising alternative to increase efficiency in treating complex leachates and to enhance effluent biodegradability. However, treatment efficiency depends on the type of AOP selected, target pollutants, and operational conditions (Deng; Zhao, 2015).

AOPs are based on the in situ generation of highly reactive species, especially hydroxyl radicals (•OH), whose high oxidation constant enables the degradation of persistent organic compounds with low biodegradability. The formation of these species may occur through the combination of ozone with hydrogen peroxide, UV radiation, or catalytic systems, increasing the ability to break aromatic structures and humic macromolecules frequently present in leachates (Pandis et al., 2022). According to Bezerra (2025), the application of AOPs, both at laboratory and industrial scales, has proven to be a promising alternative for effluent treatment, allowing more efficient removal of micropollutants through oxidation of these compounds into products of lower toxicity.

Ozone (O_3) is recognized as a highly efficient oxidizing agent, widely used in ozonation processes for direct oxidation of pollutants (Tripathi; Hussain, 2022). This technology has proven effective in removing color, odor, and microorganisms from landfill leachate, in addition to degrading specific organic compounds (Yang et al., 2021a). The mechanism of direct ozone oxidation enables rapid reactions, making it a relevant alternative to increase the biodegradability of recalcitrant compounds present in leachate (Yang et al., 2021b). Studies indicate that ozonation can achieve 80%–90% color removal and 70%–85% COD removal, depending on process conditions.

The method's efficiency is influenced by factors such as ozone dosage, contact time, and the initial quality of the water to be treated. Its main advantages include effective removal of color and odor, disinfectant capacity, and the absence of residual sludge generation (Hussain et al., 2022). However, the process has limitations such as high energy consumption required for ozone gas generation and the possibility of forming by-products during oxidation of certain pollutants (Wang; Chen, 2020). Despite these disadvantages, ozonation remains a preferred option for leachate treatment in specific applications, especially for pollutants that respond effectively to oxidation by ozone.

Although ozonation is widely recognized for high efficiency in removing color and organic matter from leachates, partial oxidation of recalcitrant compounds may result in the formation of intermediates potentially more toxic than the original precursors (Pulicharla, 2020). In this regard, simply reducing physicochemical parameters such as COD or color does not necessarily reflect an environmental improvement of the treated effluent. Studies correlating different ozone doses with direct biological response in sensitive plant bioindicators remain limited, especially in the context of sanitary landfill leachates. Therefore, this study investigated the influence of ozone dosage on the residual toxicity of leachate, using Allium cepa seeds as the test organism and the Germination Index (GI%) as an integrated indicator of phytotoxic effect, seeking to contribute to a more comprehensive environmental assessment of process efficiency.

METHODOLOGY

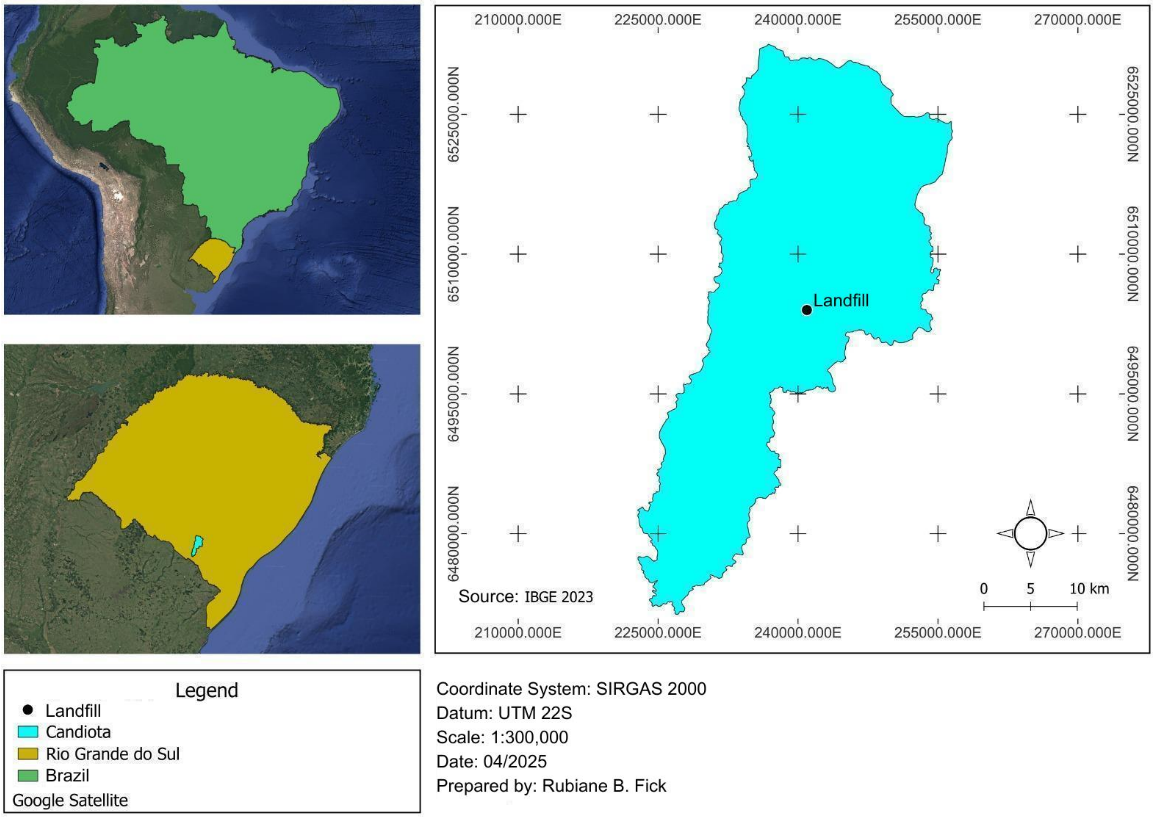
The experimental study was conducted at the Water and Effluent Analysis Laboratory, located at the Center of Engineering (CEng) of the Federal University of Pelotas (UFPel).

LOCATION

The leachate used in the experiments was collected at a sanitary landfill located in the municipality of Candiota–RS (Figure 1). The facility receives approximately 700 tons per day of MSW from 20 municipalities in the southern region of the state. It was implemented in 2011 and is currently managed by a private company.

Figure 1

Location of the sanitary landfill.



Source: Authors, 2026.

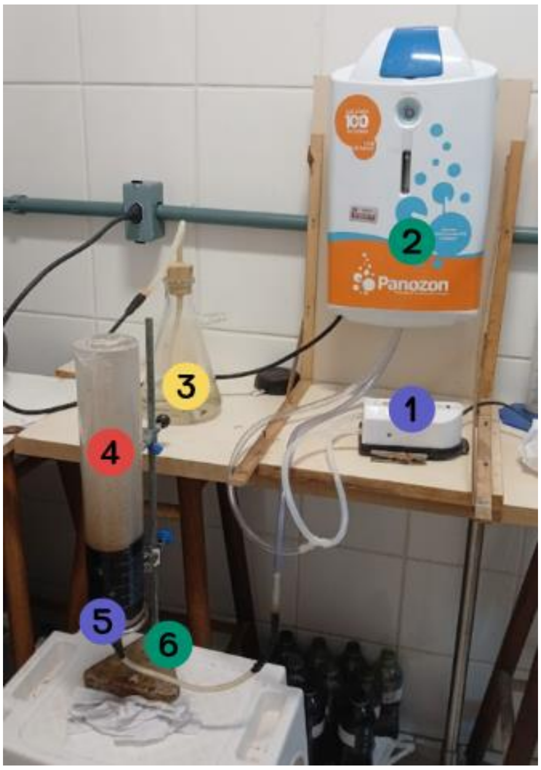
The landfill has only one stabilization pond in operation, intended for temporary storage of the effluent and its subsequent recirculation back to the landfill through a sprinkler system. Raw leachate was collected by grab sampling. After collection, the samples were stored in 20 L containers and sent to the laboratory for storage and for conducting the analyses.

EXPERIMENTAL APPARATUS

The experimental apparatus used in the experiments is illustrated in Figure 2. The system consisted of the following components: an air compressor (1); an ozone generator (2); a gas-washing bottle (3); an ozonation column (4); a porous diffuser (5); and a universal stand with an iron ring (6).

Figure 2

Experimental apparatus used in the experiment.



Source: Authors, 2026.

For the treatments, a cylindrical glass column with a total capacity of 1000 mL (4) was used, equipped with a porous diffuser (5) for ozone introduction. A Panozon ozonizer, model 053308 P+70 (2), was used together with an air compressor (1) to supply oxygen to the column.

The gas is conveyed to the column through a silicone tube and passes through a cylindrical porous stone located inside the column. The ozone generation rate was determined by the iodometric method, adapted from APHA (2005) and Rakness et al. (1996), in order to ensure accurate quantification of the applied dosage in the assays. For the experiment, two gas-washing bottles were used, each containing 400 mL of a 2% potassium iodide solution. Ozone was bubbled through the series of bottles for 10 minutes. After this process, 10 mL of 2N sulfuric acid (H_2SO_4) were added to the solutions, which were then transferred to a 1-liter Erlenmeyer flask.

The solution was titrated with sodium thiosulfate until it reached a pale yellow color. Subsequently, approximately 5 mL of starch indicator solution were added, resulting in a bluish color.

Titration was then continued until the blue color disappeared completely. The ozone dose was calculated using Equation 1:

$$\textbf{Ozone dose (mg min}^{-1}\textbf{)} = (A + B) \times N \times 24/t \quad (1)$$

Where: A represents the titrant volume in flask A (mL), B the titrant volume in flask B (mL), N the normality of sodium thiosulfate ($Na_2S_2O_3$), and t the ozonation time (min).

Based on this analysis, an ozone generation capacity of 1.56 mg O_3/min was determined.

Based on the experimentally determined ozone generation rate, it was possible to calculate the time required to reach the previously established doses. Thus, ozonation time is obtained by the ratio between the required ozone dose and the ozone production rate of the equipment, according to Equation 2.

$$\textbf{Time (min)} = \textbf{Required } O_3 \textbf{ dose (mg) / Produced } O_3 \textbf{ rate (mg min}^{-1}\textbf{)} \quad (2)$$

Considering the required doses of 75, 150, and 300 mg O_3 L^{-1}, ozonation times of 50, 100, and 200 minutes were obtained, respectively.

EXPERIMENTAL DESIGN

In the ozonation assays, three different ozone dosages were applied (Table 1). In each assay, 250 mL of raw effluent were used, in duplicate, subjected to ozonation times of 50, 100, and 200 minutes, with 10-minute intervals after every 30 minutes of continuous application. To ensure the same conditions across different samples, duplicate tests with the same time and ozone amount were performed on the same days under similar environmental conditions.

Table 1

Relationship between time and applied ozone dosage.

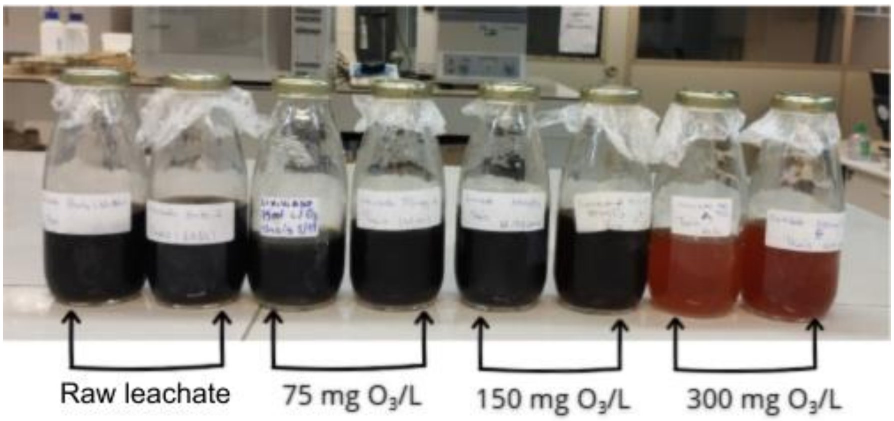
Ozonation time (min)	Ozone amount (mg O_3/min)	Applied ozone dose (mg O_3/L)
50	~1,56	75
100	~1,56	150
200	~1,56	300

Source: Authors, 2026.

Figure 3 shows the visual variation in the color of raw leachate after applying different ozone doses, evidencing the effect of increasing O_3 dose on removal of compounds responsible for effluent color.

Figure 3

Visual appearance of raw leachate and after application of different ozone doses.



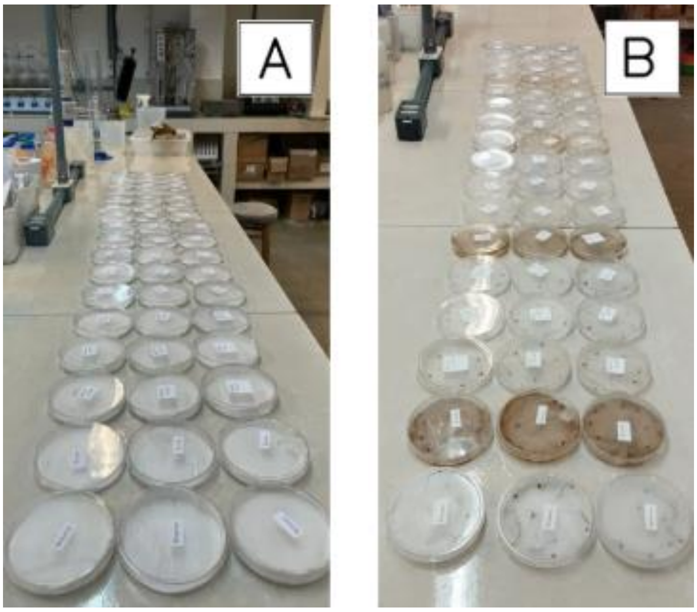
Source: Authors, 2026.

PHYTOTOXICITY ANALYSIS

For the phytotoxicity test, raw and treated samples, as well as the distilled-water control (Blank), were applied in Petri dishes (Ø 80 mm) containing a double layer of coffee filter paper, in triplicate, with Allium cepa seeds. Untreated seeds (without chemical pest control), Isla brand, were used, acquired from the website https://www.isla.com.br/. Next, the filter papers were moistened with the volume defined in the preliminary test (5 mL), at concentrations (v/v) of 1:10, 1:50, and 1:100 (Figure 4). Subsequently, the dishes were wrapped with plastic film to reduce internal moisture loss.

Figure 4

Stages of the phytotoxicity assays: A) Petri dishes with filter paper and seeds; B) Dishes with samples wrapped with plastic film.

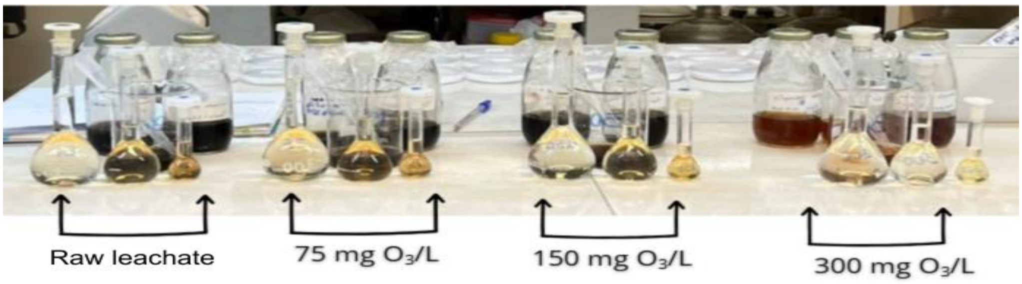


Source: Authors, 2026.

Dilutions were performed with the objective of reducing interference from the high initial color of the leachate, allowing better visualization of the changes promoted by ozonation (Figure 5).

Figure 5

Visual appearance of raw leachate and ozonated samples with different ozone doses, after dilution.



Source: Authors, 2026.

The dishes were placed in a B.O.D.-type incubator and kept incubated for seven days under controlled temperature (25 ± 2 °C), under a photoperiod regime of 8 hours of light and 16 hours of darkness. At the end of the incubation period, the number of germinated seeds and root length were evaluated with the aid of the ImageJ application.

The Germination Index (GI%) was determined according to Equation 3 adapted from Mendes et al. (2016):

$$GI\,(\%) = (Gm / Gc) \times (Lm / Lc) \times 100 \tag{3}$$

Where: GI%: seed germination index expressed as a percentage; Gm: number of germinated seeds under exposure to the leachate sample; Lm: root elongation of seeds under exposure to the leachate sample (cm); Gc: number of germinated seeds under exposure to distilled water (control); Lc: root elongation of seeds under exposure to distilled water/control (cm).

RESULTS AND DISCUSSION

The Germination Index (GI%) was used as a comparative parameter among raw samples, samples treated with the applied ozone doses, and the control, enabling identification of the most favorable conditions for attenuating leachate toxicity. The results obtained are presented in Table 2.

Table 2

Results of the phytotoxicity test

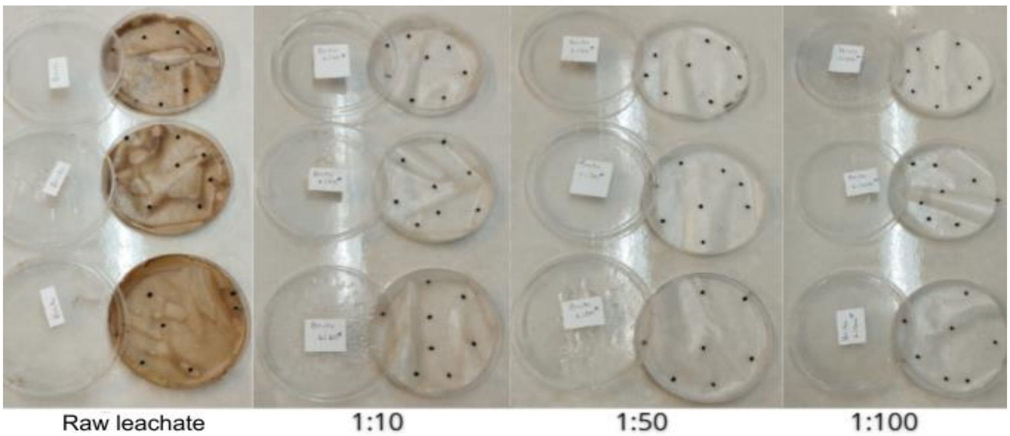
Samples	Dilution (v/v)	Germination Index (%)
Blank	N/D*	100
Raw	N/D*	0
	1:10	0
	1:50	0
	1:100	0
75 mg O3/L	N/D*	0
	1:10	5,46
	1:50	57,43
	1:100	58,41
150 mg O3/L	N/D*	0
	1:10	27,16
	1:50	87,62
	1:100	35,41
300 mg O3/L	N/D*	0
	1:10	19,15
	1:50	42,52
	1:100	61,01

*N/D: no dilution
Source: Authors, 2026.

Analysis of the results shows that raw leachate exhibited high toxicity, since no germination occurred in any of the tested dilutions (Figure 5). According to Kopliku (2015), leachate from municipal solid waste landfills may contain genotoxic and mutagenic substances capable of impairing root growth of Allium cepa, reinforcing the need for prior treatment.

Figure 5

Germinated seeds in the raw leachate sample.



Source: Authors, 2026.

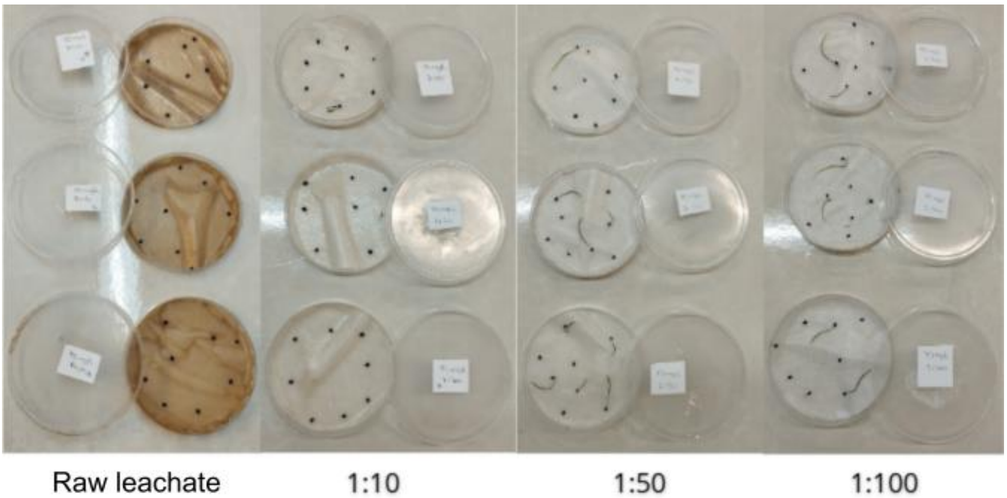
Studies based on plant bioassays indicate that sanitary landfill leachates have a complex composition, containing potentially toxic substances such as recalcitrant organic compounds and heavy metals. These contaminants may compromise essential stages of germination and early seedling development by interfering with water uptake, inducing oxidative stress, and affecting cell division in root meristems of Allium cepa (Klauck; Rodrigues; Silva, 2015).

Furthermore, the toxicity of raw leachate was also evidenced in ecotoxicological assays conducted with Allium cepa and the fish Leporinus obtusidens, in which a significant reduction in root growth and mitotic index was observed in bulbs exposed to 100% leachate concentration, indicating relevant genotoxic effects (Klauck; Rodrigues; Silva, 2013). These results reinforce that the presence of persistent compounds in leachate, even after treatment processes, may maintain expressive phytotoxic effects, justifying the need for complementary or combined treatment alternatives before final disposal or reuse.

Application of 75 mg O_3 L^{-1} promoted partial toxicity reduction, with a significant increase in germination index in the 1:50 (57.43%) and 1:100 (58.41%) dilutions, although still below the control (Figure 6).

Figure 6

Germinated seeds at the 75 mg O_3 L^{-1} dose.



Source: Authors, 2026.

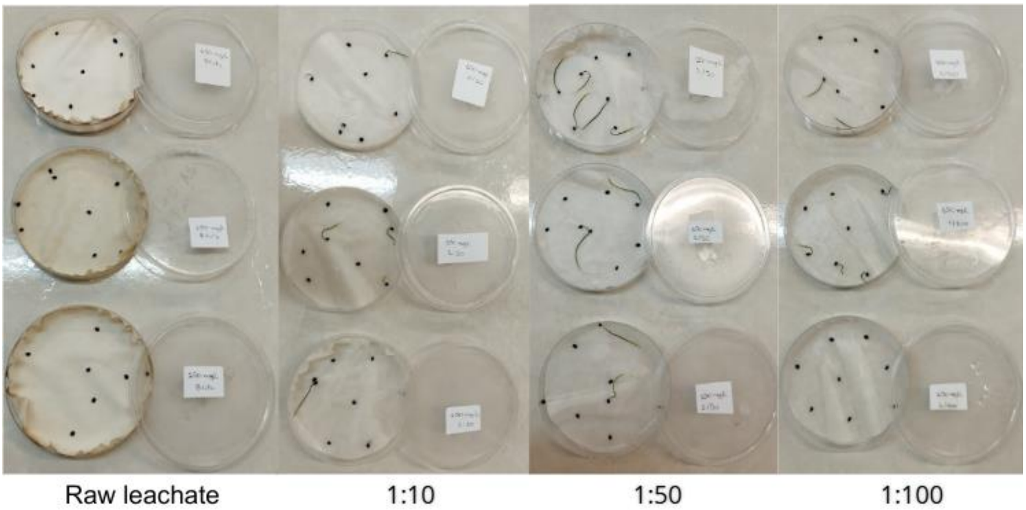
The partial reduction in toxicity observed may be attributed to ozone's ability to oxidize complex organic compounds, which largely account for the toxicity and low biodegradability of this effluent. Recent studies show that combining ozone with metallic catalysts, such as systems with 5Å zeolite–Fe–Zn, can also significantly improve leachate biodegradability (BOD_5/COD ratio), increasing from 0.14 to 0.58. As well as converting initially non-biodegradable leachate into an easily biodegradable fraction, this reinforces that ozonation can fragment recalcitrant and potentially toxic organic molecules into less aggressive intermediates more susceptible to biological degradation (Javaid et al., 2025).

Complementarily, hybrid processes such as electrocatalytic ozonation have shown high efficiency in removing pollutants from raw leachate, with reductions above 89% in Chemical Oxygen Demand (COD), Total Organic Carbon (TOC), and five-day Biochemical Oxygen Demand (BOD_5) under optimized conditions, demonstrating that associating ozone with catalysts and additional energy sources can intensify oxidation and degradation of toxic compounds (Mehralian et al., 2024).

Application of 150 mg O_3 L^{-1} resulted in the best performance among the evaluated conditions, especially at the 1:50 dilution, in which the germination index reached 87.62%. This behavior suggests that the intermediate dose was sufficient to promote oxidation of recalcitrant organic compounds and humic substances responsible for toxicity, without favoring significant formation of cumulative reactive intermediates. The observed biological response indicates a balance between oxidative degradation and minimization of toxic by-products, constituting a more efficient operational condition from the ecotoxicological standpoint (Figure 7).

Figure 7

Germinated seeds at the 150 mg O_3 L^{-1} dose.

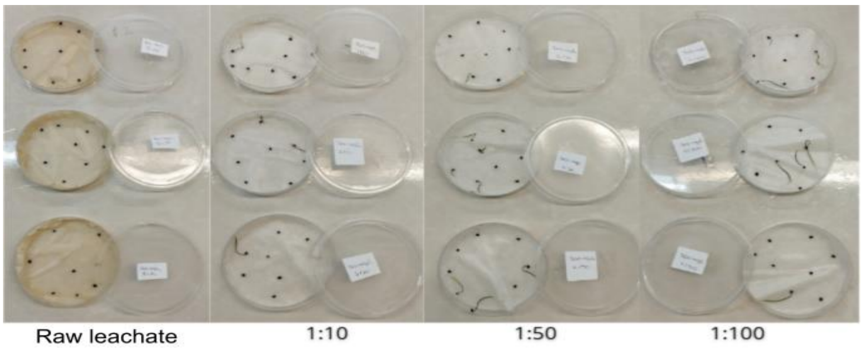


Source: Authors, 2026.

However, during ozonation, radical formation may generate toxic by-products, reinforcing the importance of toxicity tests throughout treatment (Campos, 2024). At the highest dose (300 mg O_3 L^{-1}), a reduction in germination index was observed compared with the intermediate dose, indicating a possible adverse effect associated with ozone overdosing. Intensified oxidation may have promoted the formation of intermediate compounds such as aldehydes, low-molecular-weight organic acids, or partially oxidized derivatives, which may present higher bioavailability and acute toxicity to plant organisms. These results reinforce that indiscriminately increasing oxidant dose does not necessarily imply greater environmental efficiency; optimizing the process is essential (Figure 8).

Figure 8

Germinated seeds at the 300 mg O_3 L^{-1} dose.



Source: Authors, 2026.

This result is consistent with the study by Bastos et al. (2021), who investigated ozonation of sanitary landfill leachate in the state of Rio de Janeiro and found that the best process performance was achieved under high pH and lower ozone dosage conditions, demonstrating that increasing dose does not always translate into greater efficiency. Thus, ozonation effectiveness depends not only on effluent characteristics—such as organic matter composition, presence of recalcitrant substances, and potential for by-product formation—but also on treatment aspects, such as proper definition of ozone dose and leachate dilution for subsequent uses.

CONCLUSION

The ozonation process demonstrated high efficiency in attenuating the phytotoxicity of sanitary landfill leachate, with the intermediate dose of 150 mg O_3 L^{-1} combined with the 1:50 (v/v) dilution showing the best biological performance. The results indicate that controlled ozone application can promote oxidation of potentially toxic organic compounds, increasing the environmental safety of the treated effluent.

It should be emphasized, however, that defining the optimal dose is fundamental to avoid the formation of undesirable oxidative by-products. Thus, ozonation constitutes a promising technology for complementary leachate treatment, potentially enabling its controlled recirculation within the landfill itself or restricted agricultural application, provided that current environmental and agronomic criteria are observed.

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