

# Genotoxic and mutagenic assessment of municipal solid waste landfill leachate in the Brazilian semiarid region

Avaliação genotóxica e mutagênica do lixiviado de aterro de resíduos sólidos urbanos na região semiárida brasileira

Ketlyn Jaquelize Lima Sousa<sup>1</sup> , Gabriel Araújo Paes Freire<sup>1</sup> , Wlysses Wagner Medeiros Lins Costa<sup>2</sup> ,  
Luisa Thaynara Muricy de Souza Silva<sup>1</sup> , Libânia da Silva Ribeiro<sup>1</sup> , Veruschka Escarião Dessoles Monteiro<sup>1</sup> ,  
Marcio Camargo de Melo<sup>1</sup> 

## ABSTRACT

The monitoring of landfill leachate in the Brazilian semiarid region has traditionally been based on physicochemical parameters which, although essential, were insufficient to determine effluent toxicity and to predict complex biological risks. The objective of this study was to evaluate leachate toxicity through bioassays using *Allium cepa* (*A. cepa*). The methodology comprised physicochemical characterization (organic and inorganic parameters and metals) and toxicity bioassays with *A. cepa* using an in natura leachate sample. The phytotoxicity, genotoxicity, and mutagenicity of the effluent were assessed at different concentrations (1, 5, 10, 15, and 100%), employing control samples (positive and negative) for comparison. The results revealed an alkaline effluent with high organic loads and the presence of metals such as chromium. Biologically, the 1% concentration induced a stimulatory effect on root growth, whereas concentrations above 15% were highly phytotoxic. However, the key contribution lies in the finding that genotoxicity was observed at all concentrations, including those without apparent phytotoxic effects, and mutagenicity was detected from 10% onward. These findings establish a new analytical perspective by demonstrating that the absence of acute phytotoxicity does not ensure cellular integrity, revealing a disconnect between positive root growth and the occurrence of underlying genetic damage induced by the leachate. The climatic dynamics of the semiarid region exacerbate leachate toxicity, highlighting that the inclusion of genetic indicators in regulatory frameworks to address gaps that isolated physicochemical analyses cannot predict is urgent.

**Keywords:** effluent; phytotoxicity; genotoxicity; mutagenicity.

## RESUMO

O monitoramento do lixiviado de aterros sanitários no semiárido brasileiro baseia-se tradicionalmente em parâmetros físico-químicos que, embora fundamentais, são insuficientes para determinar a toxicidade do efluente e prever riscos biológicos complexos. O objetivo deste trabalho foi avaliar a toxicidade do lixiviado por meio de bioensaios em *Allium cepa* (*A. cepa*). A metodologia envolveu a caracterização físico-química (parâmetros orgânicos, inorgânicos e metais) e bioensaios de toxicidade com *A. cepa* em uma amostra de lixiviado *in natura*. Avaliaram-se a fitotoxicidade, genotoxicidade e mutagenicidade do efluente em diferentes concentrações (1, 5, 10, 15 e 100%), utilizando-se amostras controle (positivo e negativo) para comparação. Os resultados revelaram um efluente alcalino com altas cargas orgânicas e a presença de metais como o cromo. Biologicamente, a concentração de 1% induziu um efeito estimulante no crescimento radicular, enquanto concentrações acima de 15% foram altamente fitotóxicas. No entanto, a contribuição central reside na constatação de que a genotoxicidade foi observada em todas as concentrações, inclusive aquelas sem efeito fitotóxico aparente, e a mutagenicidade manifestou-se a partir de 10%. Esses achados consolidam uma nova perspectiva de análise ao demonstrarem que a ausência de fitotoxicidade aguda não assegura a integridade celular, revelando uma desconexão entre crescimento radicular positivo e a ocorrência de danos genéticos subjacentes induzidos pelo lixiviado. A dinâmica climática do semiárido agrava a toxicidade do lixiviado, ressaltando que a inclusão de indicadores genéticos nas normativas é urgente para suprir lacunas que as análises físico-químicas isoladas não conseguem prever.

**Palavras-chave:** efluente; fitotoxicidade; genotoxicidade; mutagenicidade.

<sup>1</sup>Universidade Federal de Campina Grande – Campina Grande (PB), Brazil.

<sup>2</sup>Universidade Federal de Pernambuco – Recife (PE), Brazil.

Corresponding author: Ketlyn Jaquelize Lima Sousa. Federal University of Campina Grande, Academic Unit of Civil Engineering – Aprígio Veloso Street, Universitário – CEP: 58428-830 – Campina Grande (PB), Brazil. E-mail: eng.ketlynsousa@gmail.com

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## Introduction

Municipal Solid Waste (MSW) undergoes a series of chemical, physical, and biological processes when disposed of in landfills, leading to the generation of by-products such as biogas and leachate. The characteristics and variability of leachate are influenced by several factors, such as climatology, the type of waste landfilled, operational and structural conditions of the landfill, moisture content of the waste, and the age of the landfill, among others (Naveen et al., 2017; Nascimento et al., 2022). These factors exert direct and indirect effects on the quantity and quality of the leachate generated.

It is important to emphasize that leachate is a highly toxic percolated liquid, and it can affect public health and the environment if not properly collected and treated. Given its complexity and elevated pollutant concentrations, the toxicity assessment of leachate should be conducted as a whole liquid contaminant (Zhu et al., 2023). Due to the presence of high concentrations of conventional pollutants, landfill leachate also contains emerging pollutants (Bhatt et al., 2022), leading to significant toxic effects. Furthermore, it exhibits carcinogenic and genotoxic properties caused by its accumulation in the ecosystem and food chain (Jolaosho, 2024).

The assessment of physicochemical parameters of landfill leachate is unable to directly quantify its potentially toxic effects on living organisms (Anand and Palani, 2022). Bioassays are particularly useful for identifying the toxicity, complexity, and specific characteristics of leachate (Ghosh et al., 2017; Riegraf et al., 2021; Sousa et al., 2023). These studies frequently concentrate on acute toxicity, lethality, genotoxicity, and mutagenicity. They typically involve testing with luminescent bacteria, algae, plants, crustaceans, fish, and mammalian cells (Zhu et al., 2023; Emmanouil et al.; 2024; Tavolacci et al., 2025). Each of these tests possesses distinct attributes and assesses toxicity responses across various plant and animal species. Consequently, variations in the responses of test organisms to the contaminants in leachate may arise from the inherent differences in the physicochemical properties of the material analyzed or from its contamination levels.

Among the various toxicity tests described in the literature, the *A. cepa* test system has demonstrated significant relevance and has been utilized by numerous researchers (Srivastava et al., 2005; Leme and Marin-Morales, 2009; Klauck et al., 2013; Parvan et al., 2020; Anand and Palani, 2022). This bioindicator has proven effective in assessing toxic and mutagenic effects and is recommended as a standard for environmental monitoring (Leme and Marin-Morales, 2009; Giri et al., 2024). The *A. cepa* test system is an excellent bioindicator of cytotoxicity, genotoxicity, and mutagenicity due to its rapid root growth, high cell division rate, relatively low chromosome number ( $2n=16$ ), year-round availability, tolerance to varied cultivation conditions, ease of handling, low cost, and the lack of requirements for an ethics committee approval (Giri et al., 2024; Christudoss et al., 2025).

Bioassays using living organisms exposed to environmental pollutants can be employed to evaluate genotoxic and mutagenic effects,

which may cause DNA damage and/or result in hereditary genetic mutations. Given the issues outlined, this research aims to evaluate the toxicity of landfill leachate from the Brazil semi-arid region, investigating the genetic material of *A. cepa*. Studies addressing this methodology are scarce, especially in the Brazilian context, which has limited legislation on the subject.

## Methodology

### Study area

The landfill under investigation is situated in the Brazilian semiarid region and covers an area of 77.19 hectares, including 16.11 hectares designated as legal reserve compensation and 43.53 hectares allocated for the disposal of MSW. Operations began in June 2015, with an initial design capacity of 350 tons of MSWday<sup>-1</sup>, projected to provide a useful life of 25 years. However, during the research period, the landfill was processing an average of 682 tons day<sup>-1</sup>, receiving waste from 70 municipalities and 17 private companies across the states of Paraíba, Pernambuco, and Rio Grande do Norte.

Currently, the landfill under study includes two completed cells combined into one (cells 1 and 2) and one operational cell (cell 3). The completed cells feature a base layer lined with bentonite clay, while the operational cell, in addition to a compacted soil layer, is equipped with a high-density polyethylene geosynthetic liner for enhanced impermeabilization.

The landfill employs a herringbone drainage system in the completed cells and a grid solution in the operational cell. These systems are integrated into the base liner to channel the generated leachate to four Leachate Accumulation Ponds (LAP). These ponds serve as flow control mechanisms, where the leachate is stored and accumulated for subsequent recirculation to the cells. This process facilitates the evaporation of part of the water content in the leachate, as the evaporation rate exceeds that of precipitation in the region.

### Procedural steps

#### Step 1: sampling

The raw leachate sample was collected in December 2023, in accordance with NBR 9898:1987, and subsequently transported to the Environmental Geotechnics and Biotechnology Laboratory (LGAB), under the management of the Environmental Geotechnics Group (GGA/UFCG) at the Civil Engineering Academic Unit of the Federal University of Campina Grande (UFCG). All analyses were conducted following the protocols outlined in the Standard Methods for the Examination of Waste and Wastewater (APHA et al., 2023).

#### Step 2: physicochemical analysis

The characterization of the leachate was conducted based on the determination of the physicochemical indicators described in Table 1.

**Table 1 – Physicochemical tests performed on municipal solid waste landfill leachate.**

Parameter	Unit	Method	
<b>Inorganic</b>	pH	DIM	4500 B
	Total Alkalinity	mgCaCO <sub>3</sub> .L <sup>-1</sup>	2320 B
	TAN	mg.L <sup>-1</sup>	4500 B
	Chloride		
	VFA	mgCH <sub>3</sub> COOH.L <sup>-1</sup>	5560 C
	Sulfate	mg.L <sup>-1</sup>	4500 E
<b>Essential Metals</b>	Calcium	mg.L <sup>-1</sup>	3030 E; 3210 B
	Copper		
	Iron		
	Nickel		
	Potassium		
	Magnesium		
	Manganese		
	Zinc		
<b>Heavy Metals</b>	Aluminum	mg.L <sup>-1</sup>	3030 E; 3210 B
	Arsenic		
	Cadmium		
	Lead		
	Chrome		
	Cobalt		
	Uranium		
	Mercury		PO 098

DIM: Dimensionless; TAN: Total Ammoniacal Nitrogen; VFA: Volatile Fatty Acids. Source: APHA et al. (2023).

The physicochemical parameters were divided into three groups: (1) Inorganic; (2) Essential Metals and (3) Heavy Metals.

Metals are classified as essential or non-essential based on their concentration in comparison to their role in the metabolism of living organisms (Ahmad et al., 2021; Ahmed et al., 2024). Consequently, the vital metals identified in this research are those necessary for the growth and development of plants, whereas heavy metals are toxic to living organisms, even at low concentrations (Sevin et al., 2023; Ahmed et al., 2024).

### Step 3: biological/toxicological analysis

Biological analyses include tests using the bulb of the test organism *A. cepa* to determine the phytotoxicity, the genotoxicity, and the mutagenicity of leachate.

Biological analyses include tests using the bulb of the test organism *A. cepa* (obtained from a local market) to determine the phytotoxicity, the genotoxicity, and the mutagenicity of leachate. The analyses were conducted using five treatments (1, 5, 10, 15, and 100% leachate), in addition to control samples. Paracetamol at a concentration of 750 mg.L<sup>-1</sup> was used as the Positive Control (PC) (Sturbelle et al., 2010),

while distilled water was used as the Negative Control (NC) (Anand and Palani, 2022).

The phytotoxicity test involved placing one *A. cepa* bulb per treatment, in triplicate, within collection containers. The bulbs were exposed to 60 mL of control samples and leachate at varying concentrations and incubated at 25 °C for five days (120 hours), without solution renewal during the assay. The samples were incubated in the shade under moderate light conditions. On the fifth day, the number of germinated roots in the different treatments was recorded, and the root lengths were measured using a graduated ruler. Based on these observations, the Relative Seed Germination (RSG), Relative Root Growth (RRG), and Germination Index (GI) were calculated according to Equations 1, 2, and 3, respectively.

$$RSG = \frac{\text{No. of germinated roots}}{\text{No. of germinated roots in the control}} * 100 \quad (1)$$

$$RRG = \frac{\text{mean root length in the sample}}{\text{mean root length in the control}} * 100 \quad (2)$$

$$GI = \frac{RSG}{RRG} * 100 \quad (3)$$

After the phytotoxicity assay, the roots of the *A. cepa* bulbs were subjected to experimental procedures for genotoxicity and mutagenicity assessment. The roots were rinsed with distilled water and stored in Eppendorf tubes, followed by hydrolysis with 1 mol.L<sup>-1</sup> HCl for 10 minutes. Subsequently, the longest root was selected and washed with deionized water. The roots were then placed on a microscope slide, and the apical meristem (root tip) was excised and macerated into smaller fragments using a glass rod. Immediately after, a drop of 2% aceto-orcein was added and allowed to act for 2 minutes, staining the cell nuclei of *A. cepa* roots.

Subsequently, the slides were stained with a rapid panoptic kit (Lb Larboclin), adding one drop of each of the three components in the kit: triarylmethane 0.1%, xanthenes 0.1%, and thiazines 0.1%. After staining, the root was covered with a glass coverslip, excess dye was removed using filter paper, and the preparation was analyzed under a trinocular optical microscope with a 10× objective lens and a 10× ocular lens, resulting in a total magnification of 100×. A count of 500 *A. cepa* cells per slide was performed, totaling 1,500 cells per treatment. The counting of cell nuclei was carried out using ImageJ software, version 1.51.

Genotoxicity was assessed using the Chromosomal Alteration Index (CAI), as outlined in Equation 4. This index accounts for cells exhibiting chromosomal aberrations (C-metaphase, deletion, polyploidy, stickiness, bridges, and multipolarity), as well as nuclear irregularities (lobed nuclei and binucleated or trinucleated cells).

$$CAI = \frac{\text{No. of altered cells}}{\text{No. of cells observed}} * 100 \quad (4)$$

Mutagenicity was assessed using the Mutagenicity Index (MutI), as outlined in Equation 5, which involved the micronucleus analysis.

$$MutI = \frac{\text{No. of micronuclei}}{\text{No. of cells observed}} * 100 \quad (5)$$

#### Step 4: data analysis

The analysis of the results for mutagenicity and genotoxicity employed descriptive statistics to ensure data integrity and accurate interpretation, including the calculation of the arithmetic mean and standard deviation, followed by a normality test, Pearson correlation, and multivariate analysis of covariance (MANCOVA), with statistical significance set at  $p < 0.05$ , using Pillai's trace for multivariate statistics. The use of this test allowed for the evaluation of the overall effect of group factors on a set of dependent variables. This was further supplemented by the Student's *t*-test for independent samples, which enabled detailed comparisons between samples. Statistical analyses were conducted using the Jamovi software (version 2.4.11) and Excel. The experimental data were obtained from a single sampling event, which limited the application of statistical tools in the physicochemical and phytotoxicity analysis sections.

## Results and discussion

### Physicochemical analysis

The characterization of the physicochemical parameters is presented in Table 2.

The composition of landfill leachate is influenced by the characteristics of the MSW, including its composition and moisture content, as well as by seasonal factors such as rainfall and temperature (Costa et al., 2019). The high variability in leachate characteristics indicates an effluent that must be evaluated under different scenarios.

The analyzed leachate showed an alkaline pH of approximately 8.33. A pH above neutrality indicates a reduction in the organic load of the leachate, suggesting that the biological stabilization of the landfilled waste occurs in the methanogenic phase (Pohland and Harper, 1986; Kjeldsen et al., 2002; Castilhos Júnior, 2003; Abunama et al., 2018; Abdel-Shafy et al., 2024). Similar pH values, around 8.4, were observed by Siddiqi et al. (2022) in leachate from a sanitary landfill located in Muscat, Oman, which experiences climatic conditions comparable to those of the landfill studied in this research.

According to Ribeiro et al. (2021), elevated pH values in leachate indicate the consumption of Volatile Fatty Acids (VFA) within the landfill. In this study, the VFA concentration was measured at 840.00 mgCaCO<sub>3</sub>.L<sup>-1</sup>, likely due to the consumption of VFAs by anaerobic microorganisms, contributing to the increase in pH. A similar observation was made by Mallick et al. (2024), who reported a VFA concentration of 750.00 mgCaCO<sub>3</sub>.L<sup>-1</sup> in the leachate from a landfill in Virginia, United States, a value closely aligned with the findings of this study.

Another parameter influenced by pH is Total Ammoniacal Nitrogen (TAN). Naveen et al. (2017) explain that Total Alkalinity (TA) is associated with the biodegradation of organic matter, which releases

ammonia into the leachate through the dissolution and biochemical decomposition of waste. This process also affects the TAN concentrations in the leachate. In this study, the TAN and TA values were determined as 2,219.00 mg.L<sup>-1</sup> and 9,750.00 mgCaCO<sub>3</sub>.L<sup>-1</sup>, respectively. Silva et al. (2019) reported a TAN concentration of 3,048 mg.L<sup>-1</sup> in leachate from a landfill in João Pessoa, Brazil, while Zegzouti et al. (2020) documented TAN values of 6,335.21 and 6,149.00 mg.L<sup>-1</sup> for two landfills in Morocco.

Sulfate (SO<sub>4</sub><sup>2-</sup>) and chloride (Cl<sup>-</sup>) constitute a significant proportion of the anions present in landfill leachate (Qian et al., 2024). In this study, the concentrations of SO<sub>4</sub><sup>2-</sup> and Cl<sup>-</sup> were determined to be 975.00 mg.L<sup>-1</sup> and 5,998.00 mg.L<sup>-1</sup>, respectively. Naveen et al. (2017) suggest that elevated chloride levels in leachate are indicative of the presence of soluble salts within the waste. The concentrations of Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> observed in this research differed from those reported by Alemayehu et al. (2019), who measured 256.00 mg.L<sup>-1</sup> and 1,852.00 mg.L<sup>-1</sup>, respectively.

**Table 2 – Results of physicochemical analyses of municipal solid waste landfill leachate.**

Parameter	Un.	Result	MPV	
Inorganic	pH	DIM	8.33	5 a 9 <sup>1</sup>
	TA	mgCaCO <sub>3</sub> .L <sup>-1</sup>	9,750.00	- <sup>3</sup>
	TAN	mg.L <sup>-1</sup>	2,219.00	20.0 <sup>1</sup>
	Chloride	mg.L <sup>-1</sup>	5,998.00	- <sup>3</sup>
	VFA	mgCH <sub>3</sub> COOH.L <sup>-1</sup>	840.00	- <sup>3</sup>
	Sulfate	mg.L <sup>-1</sup>	975.00	500.0 <sup>2</sup>
Essential Metals	Calcium	mg.L <sup>-1</sup>	15.40	- <sup>3</sup>
	Copper	mg.L <sup>-1</sup>	<LQ	1.0 <sup>1</sup>
	Iron	mg.L <sup>-1</sup>	1.04	15.0 <sup>1</sup>
	Nickel	mg.L <sup>-1</sup>	<LQ	2.0 <sup>1</sup>
	Potassium	mg.L <sup>-1</sup>	<LQ	- <sup>3</sup>
	Magnesium	mg.L <sup>-1</sup>	70.20	- <sup>3</sup>
	Manganese	mg.L <sup>-1</sup>	0.16	1.0 <sup>1</sup>
Heavy Metals	Zinc	mg.L <sup>-1</sup>	0.33	- <sup>3</sup>
	Aluminum	mg.L <sup>-1</sup>	1.99	10.0 <sup>2</sup>
	Arsenic	mg.L <sup>-1</sup>	<LQ	0.5 <sup>1</sup>
	Cadmium	mg.L <sup>-1</sup>	<LQ	0.2 <sup>1</sup>
	Lead	mg.L <sup>-1</sup>	<LQ	0.5 <sup>1</sup>
	Chrome	mg.L <sup>-1</sup>	0.66	- <sup>3</sup>
	Cobalt	mg.L <sup>-1</sup>	<LQ	- <sup>3</sup>
	Uranium	mg.L <sup>-1</sup>	<LQ	- <sup>3</sup>
Mercury	mg.L <sup>-1</sup>	<LQ	0.01 <sup>1</sup>	

DIM: Dimensionless; MPV: Maximum Permitted Value; TA: Total Alkalinity; TAN: Total Ammoniacal Nitrogen; VFA: Volatile Fatty Acids; LQ: Limit of Quantification of the Equipment; <sup>1</sup>MPV according to Resolution No. 430/2011 of the National Environment Council (CONAMA); <sup>2</sup>MPV according to Resolution No. 02/2017 of the State Environmental Council of Ceará – Brazil (COEMA); <sup>3</sup>Does not have an MPV in Brazil.

In the context of the essential metals analyzed, elements such as Cu, Mn, Ni, and Zn were below the limit of quantification, remaining within the Maximum Permissible Value (MPV) established by CONAMA Resolution No. 430/2011 (Brasil, 2011), which defines the standards for effluent discharge in Brazil. Conversely, Fe exceeded the MPV stipulated by the same regulation. It is also worth mentioning that Brazilian legislation does not specify MPV for elements such as Ca, Mg, K, and P.

In Multaqa – Omã, Siddiqi et al. (2022) reported average concentrations of 109.32 mg.L<sup>-1</sup> for Ca, 1.83 mg.L<sup>-1</sup> for Cu, 26.47 mg.L<sup>-1</sup> for Fe, 1.16 mg.L<sup>-1</sup> for Mn, 4.28 mg.L<sup>-1</sup> for K, and 1.06 mg.L<sup>-1</sup> for P in landfill leachate, with Ni and Zn levels below the detection limit. In Barka, Oman, the same authors observed average concentrations of 34.54 mg.L<sup>-1</sup> for Ca, 2.47 mg.L<sup>-1</sup> for Cu, 16.69 mg.L<sup>-1</sup> for Fe, 0.65 mg.L<sup>-1</sup> for Mn, 4.98 mg.L<sup>-1</sup> for K, and 0.72 mg.L<sup>-1</sup> for P, with Ni and Zn concentrations below the detection limit. In Brazil, Coelho et al. (2015) reported average values of 10.44 mg.L<sup>-1</sup> for Ca, 0.10 mg.L<sup>-1</sup> for Cu, 5.07 mg.L<sup>-1</sup> for Fe, 18.38 mg.L<sup>-1</sup> for Mg, 3.66 mg.L<sup>-1</sup> for Mn, 2.08 mg.L<sup>-1</sup> for Ni, 58.55 mg.L<sup>-1</sup> for K, 4.90 mg.L<sup>-1</sup> for P, and 1.13 mg.L<sup>-1</sup> for Zn in leachate from a landfill located in Mossoró. With the exception of Zn, the concentrations of the other elements analyzed by the authors exceeded the MPV established by Brazilian regulations, with Cu falling within the toxic range of 0.1 to 1.0 mg.L<sup>-1</sup> for plants.

The concentrations of heavy metals, occurred below the LQ, remaining within the VPM established by CONAMA Resolution No. 430 (Brasil, 2011) for arsenic As, Cd, Pb and Hg. Al remained within the MPV specified by COEMA Resolution No. 02 (Ceará, 2017). The remaining elements do not have MPVs specified by Brazilian legislation.

### Phytotoxicity

Figure 1 illustrates the results of the phytotoxicity analysis conducted on *A. cepa* bulbs, highlighting the RRG, RSG, and GI across various leachate concentrations, as well as the control samples.

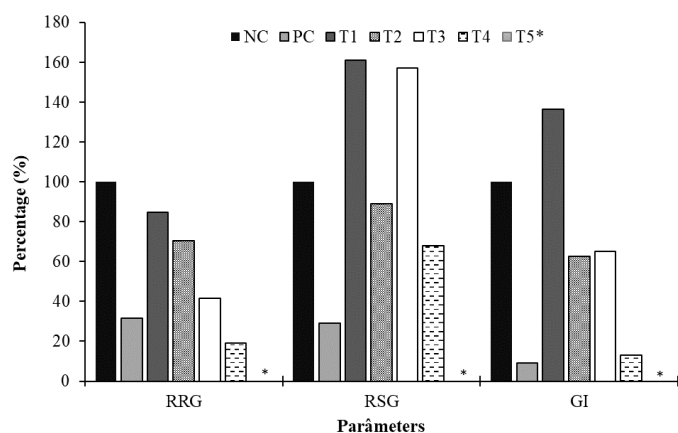


Figure 1 – Phytotoxicity effects of leachate on *A. cepa* bulbs, comparing Negative Control (NC), Positive Control (PC), and Treatments 1 to 5 (T1 to T5).

\*The absence of the T5 error bar in the graph is due to the IG value being zero.

Figure 1 illustrates that the percentages of the indicators generally decreased as the leachate concentration increased, except for T3 in the case of RSG. Notably, the 1% leachate (T1) exhibited a higher GI than the NC (blank test), likely due to the dilution of contaminants in low concentrations, which can have a stimulatory effect on plants (Zucconi et al., 1985). This stimulatory effect at low leachate concentrations was similarly observed by Silva et al. (2023) in cabbage seeds, where concentrations below 7% also led to increased growth.

As shown in Figure 1, the leachate diluted to 15% (T4) exhibited a Germination Index (GI) nearly identical to that of the Positive Control (PC), whereas the undiluted leachate resulted in no germination, thereby confirming the toxic effect of the leachate at higher concentrations.

RSG and RRG remained consistent in the NC. As leachate concentrations increased, *A. cepa* did not exhibit a consistent response pattern for RSG, while RRG showed a linear decline (Figure 2). Similarly, Gomes et al. (2018) and Silva (2022) observed higher germination indices than the relative growth index. This phenomenon occurs due to plants responding to specific combinations of light, temperature, humidity, substrate, and gas concentrations that are more favorable for seedling establishment (Baskin and Baskin, 1998; Silva, 2022). Figure 2 illustrates the root growth of *A. cepa* bulbs exposed to varying concentrations of the landfill leachate analyzed in this study.

In Figure 2, it is evident that *A. cepa* roots exhibited growth across various leachate concentrations, as well as in the NC and PC samples, except in the treatment involving undiluted leachate (T5), which highlights the toxicity of the effluent.

The toxicity of leachate is influenced by its physicochemical composition and the specific plant species exposed to the effluent (Białowiec, 2015). As defined by Zucconi et al. (1985), phytotoxicity is based on the GI and is categorized into three distinct levels: GI < 50% signifies high phytotoxicity, 50% < GI < 80% indicates moderate phytotoxicity, GI > 80% denotes the absence of phytotoxicity. Moreover, a GI ≥ 100% suggests a stimulatory effect on plant growth.

Among the leachate and control samples analyzed, high phytotoxicity was observed in the PC (with a GI of 9.19%), T4 (with a GI of 13.10%), and T5 (which exhibited no germination). Leachate dilutions of 5 and 10% (T2 and T3) demonstrated moderate phytotoxicity, with GIs of 62.73 and 65.25%, respectively. In contrast, T1 yielded a GI of 136.46%, indicating a stimulatory effect on *A. cepa* bulbs.

Zegzouti et al. (2020) also reported a stimulating effect of leachate on plant growth. Similarly, Pellenz et al. (2020) observed enhanced germination and root growth in *Lactuca sativa* (lettuce) seeds when exposed to leachate diluted to lower concentrations. Meanwhile, Vaverková et al. (2020) documented an inhibitory effect of leachate at higher concentrations on the root growth of *Sinapis alba* L. (white mustard) and *Lemna minor* L. (duckweed).

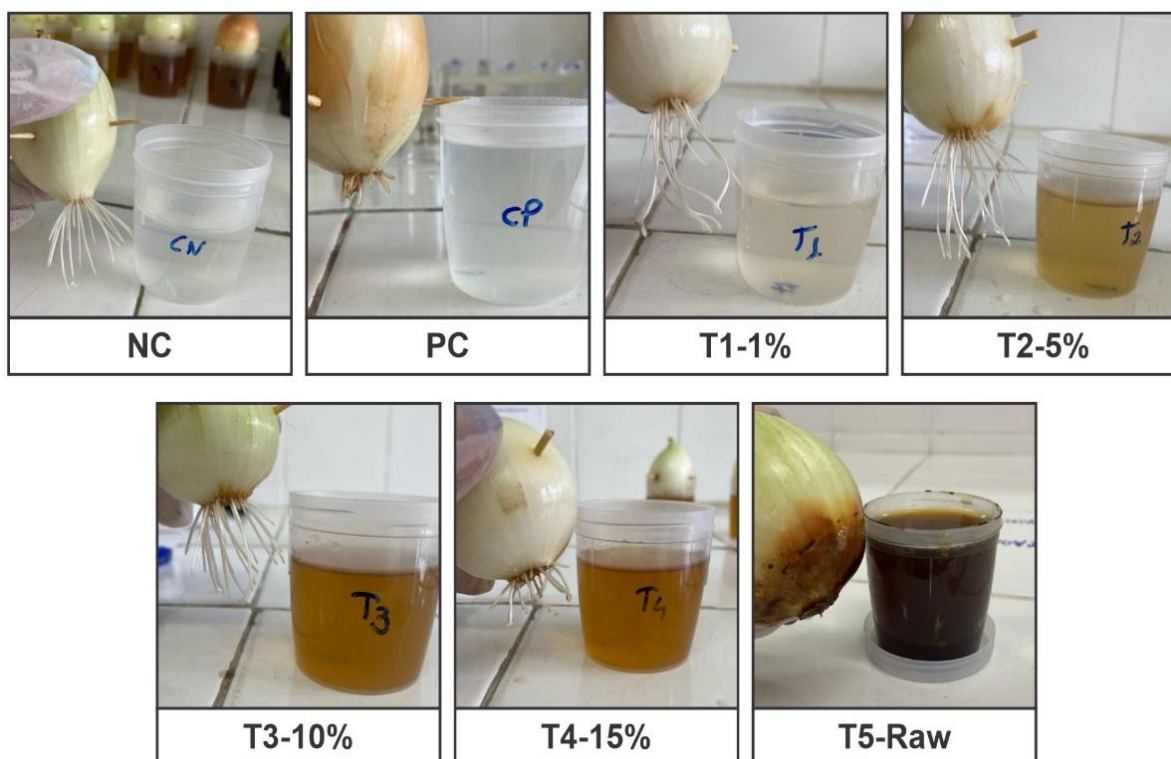


Figure 2 – Root growth of *A. cepa* bulbs under the different treatment conditions used.

Arunbabu et al. (2017) reported that lower leachate concentrations were found to enhance the germination of *Vigna unguiculata* (cowpea) seeds. The authors attributed this effect to the reduced toxicity of the leachate at lower concentrations, which results from the dilution of pollutants. This dilution allows the plants to absorb the nutrients present in the effluent more effectively.

### Genotoxicity and mutagenicity

The Multivariate Analysis of Covariance revealed a statistically significant difference between the control samples and the leachate at various concentrations ( $p=0.027$ ). However, the Univariate Test demonstrated that the GI did not significantly affect the Genotoxicity (CAI) ( $p=0.145$ ) and Mutagenicity (MutI) ( $p=0.117$ ) indices. The Box's M Test for Homogeneity of the Covariance Matrix yielded a  $p<0.001$ , confirming the homogeneity of the samples. Phytotoxicity assays revealed that landfill leachate diluted to 1% exhibited a stimulatory effect on the growth and development of *A. cepa* roots. However, the Univariate Test indicated that the germination rate did not influence the leachate's genotoxic and mutagenic potential. Despite the low phytotoxicity observed, significant DNA damage was detected in the test organism, reinforcing that phytotoxicity does not necessarily correlate with genotoxicity or mutagenicity. Further studies are needed, as a single sampling point prevents generalizations.

Table 3 presents the CAI and MutI results for the analyzed samples. These indices were not calculated for T5 (raw leachate), as the absence

of *A. cepa* root germination at this concentration precluded the analysis of cellular changes in the test organism.

The comparison between NC and PC showed a statistically significant difference ( $p<0.05$ ) for genotoxicity, indicating that the PC influenced the chromosomal alteration index. However, no significant difference ( $p>0.05$ ) was observed between NC and PC for mutagenicity; therefore, the PC did not affect the micronucleus index and was only used for the evaluation of genotoxicity.

All analyzed leachate concentrations showed a statistically significant difference ( $p<0.05$ ) compared to the NC for genotoxicity, indicating that the tested leachate concentrations are significantly different from the negative control. Therefore, genotoxicity was observed in all treatments with the different leachate concentrations.

Table 3 – Genotoxicity and mutagenicity results for the different treatment conditions used.

Sample	CAI		MutI	
	Mean±SD	p-value	Mean±SD	p-value
NC	1.67±0.30	-	2.53±1.86	-
PC	<b>9.93±4.40*</b>	0.03	8.47±9.42	0.34
T1	<b>8.27±3.64*</b>	0.03	2.73±0.61	0.87
T2	<b>13.20±6.00*</b>	0.02	6.60±2.65	0.09
T3	<b>15.87±3.56*</b>	0.002	<b>6.07±1.36*</b>	0.05
T4	<b>12.27±5.31*</b>	0.03	<b>8.40±2.69*</b>	0.04

SD: Standard Deviation; \*Significance level  $p<0.05$ .

Regarding mutagenicity, only T3 and T4 showed a statistically significant difference ( $p < 0.05$ ) compared to the NC for mutagenicity, as observed in Figure 3. Thus, only T3 and T4 induced mutagenicity in the test organisms.

The results indicate that genotoxicity, as depicted in Figure 3a, increased with the leachate concentration. Notably, T4 exhibited a lower CAI compared to T2 and T3. This could be attributed to the significantly reduced growth of *A. cepa* roots in T4, coupled with the observation that the apical meristem (root tip) appeared darkened, suggesting mitotic disruption.

Another key factor is that, although leachate at low concentrations exhibits a stimulating effect on plant growth and development, this phenomenon does not prevent alterations in the plant DNA, which can affect the germline.

It is important to recognize that DNA damage can lead to the expression of proteins that may cause pathological conditions in the organism producing them. Furthermore, if these proteins are ingested by other individuals, they have the potential to induce allergic reactions or more serious diseases.

Figure 4 illustrates cellular changes observed in the meristematic cells of *A. cepa* roots exposed to various concentrations of landfill leachate analyzed in this study.

The analysis of MutI (Figure 3b) reveals no statistically significant difference ( $p > 0.05$ ) between NC and T1 or T2, indicating that leachate concentrations of 1 and 5% were not mutagenic. However, both T3 and T4 showed a statistically significant difference ( $p < 0.05$ ) compared to NC, indicating that leachate concentrations above 10% are mutagenic. It is important to note that while not all genotoxic substances are mutagenic, all mutagenic substances are inherently genotoxic. This distinction was validated by the findings of this study, where all dilutions that showed mutagenicity also exhibited genotoxicity, though not all genotoxic samples were mutagenic.

Leme and Marin-Morales (2009) note that the aneugenic and clastogenic effects identified by the *A. cepa* test system may be attributed

to the presence of metals in the samples. For instance, chromium and certain chromium compounds exhibit clastogenic effects, directly interacting with DNA and inducing chromosomal alterations (Liu et al., 1992; Leme and Marin-Morales, 2009; Klauck et al., 2017; Alias et al., 2020). Liu et al. (1995) also emphasize the effects of other metals, including magnesium, cobalt, and mercury. However, as Steinkellner et al. (1998) have observed, the *A. cepa* test is less sensitive to metals such as arsenic, cadmium, lead, copper, and zinc.

Anand and Palani (2022) investigated the toxicity of leachates from both active and closed landfills, revealing that metals such as chromium, lead, and nickel caused the most significant DNA damage in *A. cepa*. According to the authors, this DNA damage results from excessive production of reactive oxygen species and the disruption of the bonds between the nitrogenous bases of DNA.

The genotoxic and mutagenic potential of leachate, even at low concentrations, has been documented in various bioassays. For instance, Zegzouti et al. (2020) investigated the genotoxicity and mutagenicity of raw and treated leachate from a landfill in Morocco using *Vicia faba* (faba bean) assays. The study revealed that raw leachate concentrations exceeding 10% led to mitotic disruption, precluding micronuclei (MN) scoring. The results for lower leachate concentrations showed a significant increase in MN frequency, with a progressive rise in micronuclei correlating with higher leachate concentrations. These findings align with the results observed in the present study.

Klauck et al. (2013) examined leachate from a landfill in Rio Grande do Sul, Brazil, and reported a decrease in root growth of *A. cepa* attributable to the effluent. Although significant frequencies of chromosomal alterations were not observed in the bioindicator across different leachate concentrations, the study did report a higher incidence of total chromosomal anomalies at elevated leachate concentrations.

Srivastava et al. (2005) and Gamoñ et al. (2019) demonstrated that leachate diluted to concentrations of 10 and 6.25% can induce mitotic and chromosomal anomalies, as well as alterations in DNA, in the meristematic cells of *A. cepa*.

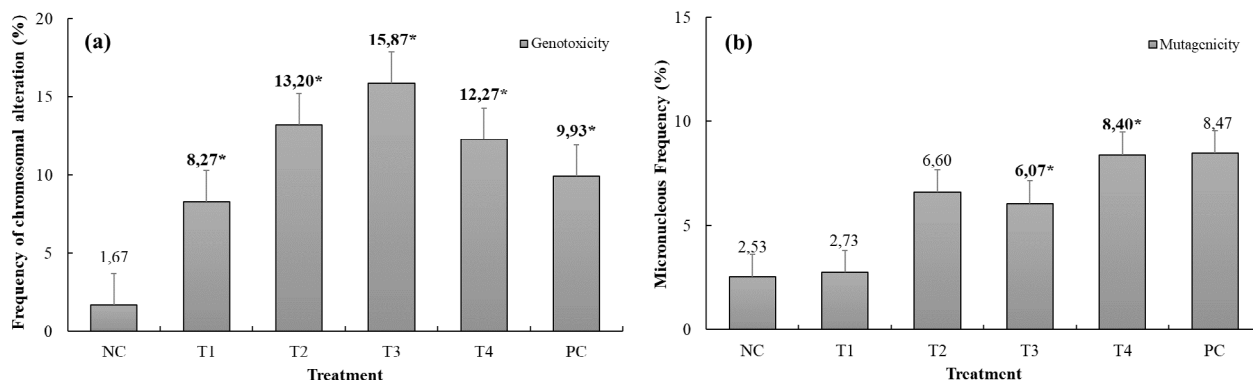


Figure 3 – Descriptive graph of (a) CAI and (b) MutI results for the different treatment conditions used.

\*Significance level  $p < 0.05$ .

The genotoxicity and mutagenicity observed in raw leachate may be attributed to its physicochemical composition, such as elevated concentrations of COD and  $\text{NH}_4^+$  (Zegzouti et al., 2020). Sang and Li (2004) demonstrated a correlation between genotoxicity and COD in leachate. Generally, the genotoxicity of leachate can be associated with the presence of organic compounds and heavy metals in the effluent, which induce the formation of free radicals and oxidative stress (Curtis et al., 1988; Sang and Li, 2004).

The physicochemical characterization of the leachate in this study revealed high concentrations of organic compounds, including  $\text{BOD}_5$ , COD, and VFA. Additionally, elevated nitrogen levels in the forms of total ammoniacal nitrogen, nitrite, and nitrate were detected. The leachate also contained significant concentrations of metals such as calcium, iron, magnesium, manganese, zinc, aluminum, and chromium (Table 2). Notably, chromium was present at levels exceeding those established by Brazilian regulations and has been shown to induce chromosomal alterations and cause DNA damage.

Based on the aforementioned findings, it can be concluded that the observed genotoxicity and mutagenicity of the leachate in this study are attributable to the high concentrations of toxic compounds identified through the physicochemical characterization of the effluent.

The integrated analysis of phytotoxicity, genotoxicity, and mutagenicity data demonstrated that there is no direct correlation between phytotoxic effects and the potential for DNA damage. The 1% leachate concentration stimulated root growth ( $\text{GI} > 100\%$ ) but also induced a significant increase in the Chromosomal Alteration Index ( $\text{CAI} = 8.27\%$ ), at a level comparable to the positive control. This result indicates that the stimulatory effect observed at low concentrations, possibly associated with the presence of nutrients, does not prevent the effects of genotoxic agents. Compounds such as heavy metals and recalcitrant organic pollutants identified during physicochemical characterization, act directly on cell division and cause damage that is not reflected in vegetative growth.

In regions such as the Brazilian semiarid, where legislation is restricted to physicochemical parameters, leachate that appears to exhibit low toxicity may, in reality, be introducing genotoxic agents into water bodies and the soil. The results obtained in this research therefore demonstrate that the application of phytotoxicity, genotoxicity, and mutagenicity assays is not only feasible but necessary for monitoring landfill leachates and other toxic effluents. It is also recommended that environmental agencies incorporate these tests into their monitoring protocols for enterprises that produce liquid effluents. These assays are practical, straightforward, and yield reliable results, as evidenced by Parvan et al. (2020), Sousa (2024) and Christudoss et al. (2025).

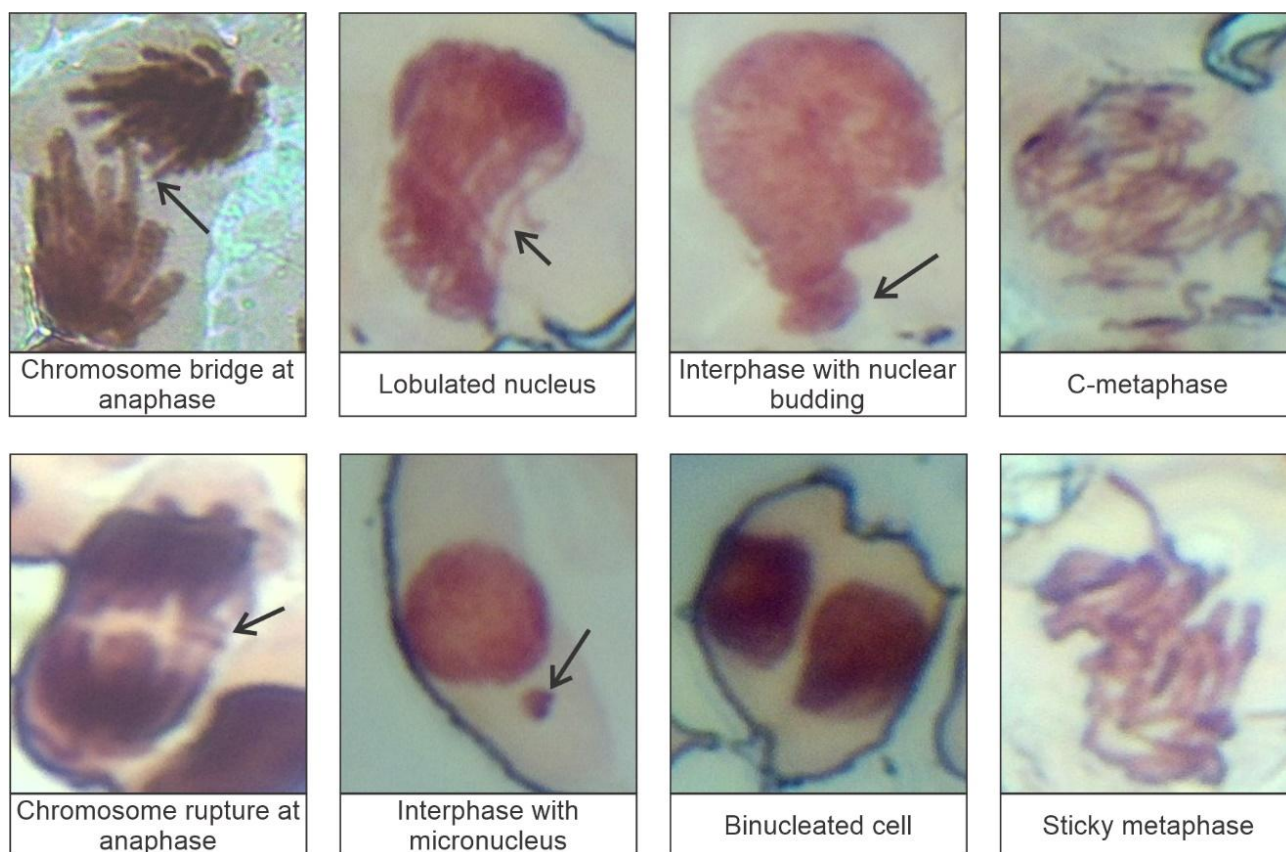


Figure 4 – Cellular alterations in *A. cepa* cells observed in this study.

## Conclusion

This study demonstrated that landfill leachate from the Brazilian semiarid region exhibits genotoxic and mutagenic potential even at low concentrations, including those that stimulate root growth in *Allium cepa*. This latent toxicity, operating independently of visible phytotoxic effects, indicates that the absence of growth inhibition does not ensure genetic integrity, thereby requiring monitoring protocols to incorporate specific bioassays for the detection of DNA damage.

The research demonstrated that the lack of phytotoxicity is not evidence of environmental safety, since genotoxic and mutagenic damage may occur underlyingly even in the presence of stimulated root growth. These findings are particularly relevant for semiarid climates, where the water balance favours evaporation and the consequent accumulation of pollutants. This scenario intensifies the risk associated

with leachate toxicity, rendering local biota more vulnerable to genotoxic and mutagenic effects.

These findings establish a technical basis supporting the revision of environmental monitoring strategies. The inclusion of genotoxicity bioassays is advocated to complement physicochemical analyses and is essential to address regulatory deficiencies in Brazil, ensuring comprehensive ecosystem protection in light of the climatic particularities of the semiarid region.

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## Authors' Contributions

**Sousa**, K. J. L.: concept, data curation, formal analysis, research, methodology, writing – first draft; **Freire**, G. A. P.: data curation, validation, visualization, writing – first draft; **Costa**, W. W. M. L.: data curation, supervision, validation, writing – first draft; **Silva**, L. T. M. S.: data curation, supervision, validation, writing – first draft; **Ribeiro**, L. S.: supervision, writing – review and editing; **Monteiro**, V. E. D.: supervision, writing – review and editing; **Melo**, M. C.: supervision, writing – review and editing.

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