HOLOS
ISSN 1807 - 1600

EVALUATION OF THE BIOREMEDIATION POTENTIAL OF *CANDIDA spp*. AND *TRICHOPHYTON spp*. FUNGI IN THE TREATMENT OF LEACHATE FROM A LANDFILL

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Submitted October 23, 2023 – Accepted December 16, 2023

DOI: 10.15628/holos.2023.16307

ABSTRACT

This study compares the efficiency in reducing the toxicity of leachate from a landfill after the application of mycoremediation processes using *Candida spp*. and *Trichophyton spp*. fungi through bioassays with *Allium cepa* seeds. Experimental units were set up for leachate treatment with varying concentrations, inoculated with the selected fungi, and incubated. After incubation, bioassays were conducted using the liquid extract to inoculate ten *Allium cepa* seeds on Petri dishes. Subsequently, macroscopic analysis indices were calculated. The results revealed that *Candida spp*. yeast showed superior toxicity reduction results in all experimental units. In conclusion, among the analyzed genera, *Candida spp*. yeast demonstrates greater efficiency and holds promise for reducing the toxicity of landfill leachate.

KEYWORDS: Mycoremediation, Bioassay, *Allium cepa*.

AVALIAÇÃO DO POTENCIAL BIORREMEDIADOR DOS FUNGOS *CANDIDA spp*. E *TRICHOPHYTON spp*. NO TRATAMENTO DE LIXIVIADO PROVENIENTE DE ATERRO SANITÁRIO

RESUMO

O presente trabalho compara a eficiência na redução da toxicidade de lixiviado proveniente de aterro sanitário após o emprego do processo de micorremediação utilizando os fungos *Candida spp*. e *Trichophyton spp*. por meio de bioensaios com semente de *Allium cepa*. Para o tratamento do lixiviado foram montadas unidades experimentais com diferentes concentrações de lixiviado que foram inoculados com os fungos selecionados e incubados. Após a incubação foi realizado os bioensaios utilizando o extrato líquido para inocular 10 sementes de *Allium cepa* em placas de Petri. Posteriormente fez-se o cálculo dos índices de análise macroscópica. A partir dos resultados, constatou-se que a levedura *Candida spp*. apresentou melhores resultados de redução da toxicidade em todas as unidades experimentais. Concluindo que entre os gêneros analisados, a levedura *Candida spp*. se mostra mais eficiente podendo ser promissora para a redução da toxicidade de lixiviado de aterro sanitário.

Palavras chave: Micorremediação, Bioensaios, *Allium cepa.*

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1 INTRODUCTION

Challenges related to municipal solid waste (MSW) begin with production and commercialization patterns, where products are manufactured with an emphasis on excessive consumption, quickly becoming ingrained in society's habits, leading to increased disposal due to obsolescence and, consequently, generating significant amounts of waste (SIQUEIRA; MORAES, 2009). Proper MSW management aims to protect public health, promote environmental quality, foster sustainability, and provide support for economic productivity. Therefore, it is of paramount importance to understand and treat the pollutants generated during MSW degradation (NASCIMENTO et al., 2015).

According to SNIS (2021), sanitary landfills are facilities with technical and operational control to prevent waste and their effluents (liquid and gaseous) from causing harm to public health and the environment. They are the primary appropriate final disposal method used in Brazil. The liquid effluent generated during waste degradation is known as leachate. Leachate is produced due to physical, chemical, and biological phenomena and is characterized by its dark color, foul odor, and high Biochemical Oxygen Demand (BOD) (PRADO, 2023). Leachate composition includes high concentrations of organic matter, inorganic macrocomponents, toxic metals, and xenobiotic organic compounds. The composition of this effluent is complex, toxic, and challenging to treat, dependent on the decomposition stages of organic matter, waste composition, environmental conditions, landfill age, and operating conditions, among other factors (ROCHA e LODI, 2022; ANDRADE, 2022; RIBEIRO, MENDES, 2018).

The physicochemical characterization of landfill leachate, while providing insights into its toxic composition, does not reveal the collective impact of substances interacting within the effluent and their direct effects on the environment. To complement the analysis of leachate toxicity, it is necessary to conduct bioassays that aim to predict the potential impact of a toxic agent on the environment (FRANCO et al., 2018). This technique employs biological organisms (bioindicators) to assess the sample's conditions, for which toxicity estimation is desired. Among the organisms suitable for this purpose, higher plants, specifically *Allium cepa* (onion), offer numerous applications in monitoring and detecting contaminants, evaluating a compound's phytotoxicity through the inhibition of seed germination and root growth (SOMMAGGIO, 2016; LELES, 2017; OLIVEIRA, 2021).

Bioremediation is a technique involving the use of microorganisms to transform harmful substances into environmentally non-toxic compounds, recommended as a viable alternative for contaminant treatment within the scientific community (PENA, 2018). The selection of organisms capable of transforming target pollutants is crucial for bioremediation efficiency.

Mycoremediation is an eco-sustainable alternative that uses fungi and/or their metabolic products to remediate or reduce the pollutant potential of contaminants. This technique offers various applications in areas contaminated by toxic compounds due to fungal characteristics such as adaptability in environments with recalcitrant toxic compounds, the presence of an efficient extracellular enzymatic system, a vegetative growth form allowing direct contact with the pollutant, and low substrate specificity (BENEVIDES e MARINHO, 2015; KAO, 2023).

Some studies have connected mycoremediation with the physicochemical characteristics of leachate. These works were of great importance for this new research direction, as mycoremediation demonstrated high efficiency in the removal of organic and inorganic pollutants. The link between mycoremediation and leachate's toxic compounds began recently with the studies by Hassan et al. (2020), who evaluated fungal efficiency in removing metals and metalloids; Siracusa et al. (2020), examining *Lambertella sp*. fungus's ability to reduce total nitrogen, total organic carbon, and chloride; and Ikechi-Nwogu, Akpan (2022), using *Lentinus squarrosulus Mount* fungus to reduce leachate's physicochemical properties and microbial load.

Fungi from the *Candida* and *Trichophyton* genera have excelled in bioremediation studies. Many yeast species from *Candida spp*. produce biosurfactants and have been applied in mycoremediating petroleum, motor oil, toxic metals, and polycyclic aromatic hydrocarbons (PAHs) (SOARES et al., 2011; DURVAL, 2017). Some species of filamentous *Trichophyton spp.* have proven efficient in the bioremediation of azo textile dyes and pentachlorophenol (SANTOS, 2023).

In light of the above, this study aims to compare the results of leachate toxicity reduction from landfills following the application of mycoremediation processes using *Candida spp*. and *Trichophyton spp*. fungi through bioassays with *Allium cepa* seeds.

2 MATERIALS AND METHODS

2.1 Isolation and selection of indigenous fungi

To isolate and select indigenous fungi for the mycoremediation process, a sample of raw leachate from the landfill cells' pipelines in the municipality of Londrina, state of Parana, Brazil, located in the Maravilha district (CTR - Maravilha), was collected.

The steps for fungal selection followed the methodology of Silva et al. (2011) with adaptations. Serial dilutions were prepared by diluting the leachate in saline solution at a 1:10 ratio, resulting in dilutions of 10^{-1}, 10^{-2} and 10^{-3}. This procedure was performed in duplicate. Subsequently, using the spread plate technique, 0.1 mL of each dilution was added to Petri dishes containing Sabouraud agar supplemented with Chloramphenicol and incubated for 4 days.

Fungi with the highest incidence were selected for isolation in giant colonies on Sabouraud agar and incubated for an additional 4 days. Subsequently, fungal identification was conducted using the macroscopic and microscopic characteristics of the colonies. The identification was based on the guide for fungal identification, "Larone's Medically Important Fungi: A Guide to Identification" by Walsh et al. (2018). After the identification of the selected fungi, samples were transferred to slanted Sabouraud media in test tubes. Figure 1 illustrates a representative example of the process for isolating and selecting indigenous fungi.

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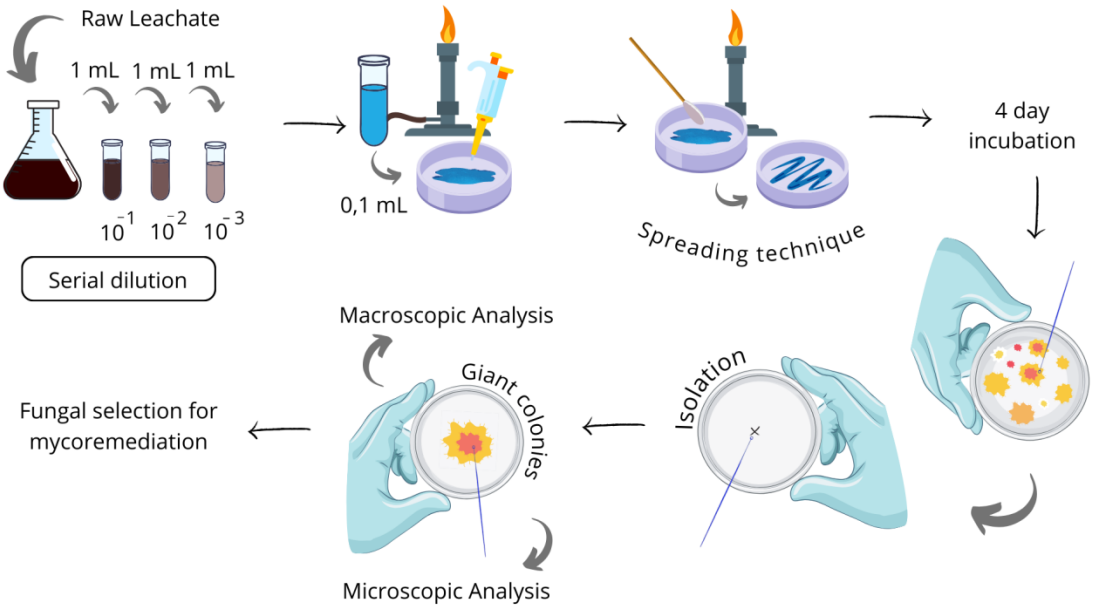

Figure 1: Schematic representation of the procedure carried out for the isolation and selection of indigenous fungi.

2.2 Inoculum preparation and mycoremediation stage

Two fungi were selected, one yeast similar to *Candida spp*. and one filamentous fungus similar to *Trichophyton spp.* Figure 2 illustrates the procedures carried out for the mycoremediation stage.

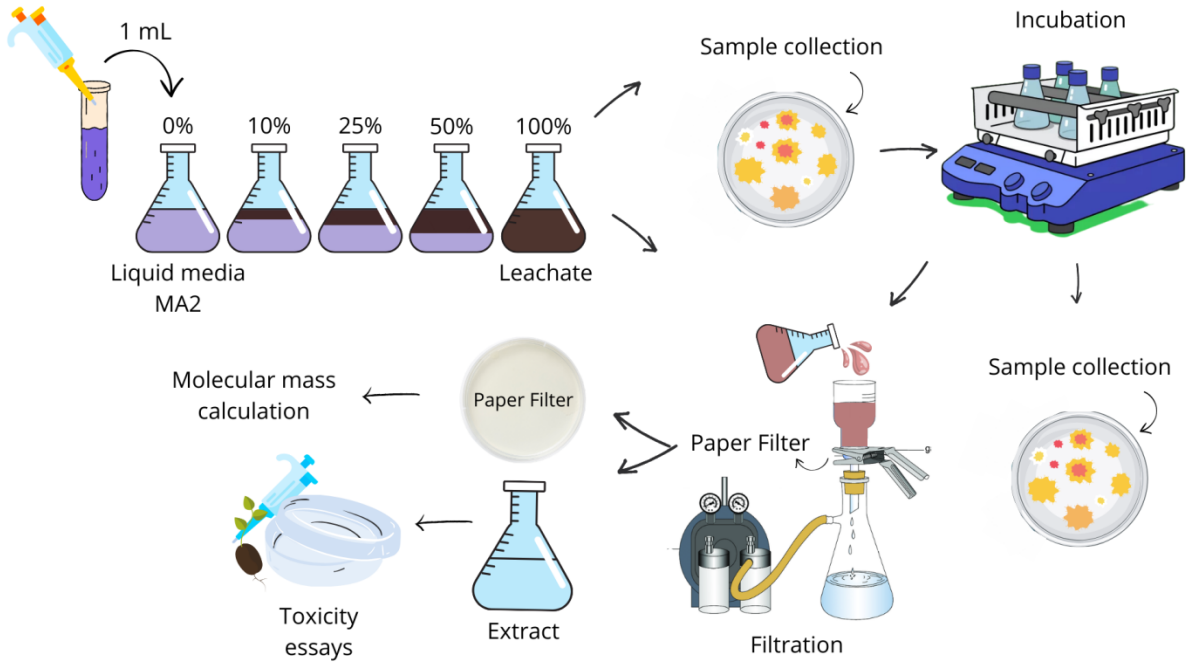

Figure 2: Procedure carried out for the assembly of experimental units (EUs) for the mycoremediation process.

For the preparation of the inoculum, a portion of fungal mass from each strain, grown in slanted test tubes, was extracted and transferred to 10 mL of liquid MA2 medium (2% Malt and 0.2% Peptone). These were then incubated on a *shaker* for 4 days.

For the mycoremediation stage, the methodology proposed by Bonassa (2021) was used with modifications. Experimental units (EUs) were set up in 250 mL Erlenmeyer flasks containing liquid medium with varying concentrations of leachate (0%, 10%, 25%, 50%, and 100%). The final volume of liquid medium in each Erlenmeyer flask was 100 mL. The 0% concentration was prepared solely with MA2 liquid medium (2% Malt and 0.2% Peptone), the 100% concentration with leachate only, and the remaining concentrations with 10 mL, 25 mL, and 50 mL of leachate supplemented with 90 mL, 75 mL, and 50 mL of MA2 liquid medium (2% Malt and 0.2% Peptone), respectively. Each EU was inoculated with 1 mL of the prepared inoculum and incubated for 7 days.

Following the incubation period, the liquid medium was filtered and used for the toxicity assays using vacuum filtration with filter paper. The filtered extracts were subjected to toxicity tests using *Allium cepa*, and the cell mass retained on the filter paper was placed in an 80°C oven to determine the dry mass. After this procedure, the samples were weighed to obtain the dry weight (cell mass).

2.3 *Allium cepa* toxicity assay

For this stage, the methodologies of Bagur-González et al. (2011), Sommaggio (2016), Silva; Tofolo (2017), and Leles (2017) were followed, using *Allium cepa* (onion) seeds as a bioindicator. After obtaining the liquid extracts, the setup of experimental units in Petri dishes was initiated. Circular filter paper was placed inside the Petri dishes, and they were exposed to ultraviolet light for 15 minutes in a laminar flow hood for sterilization.

In each properly labeled Petri dish, 10 *Allium cepa* seeds were added into the sterile filter paper along with 2 mL of the liquid extract obtained after filtration of the liquid medium. The Petri dishes were incubated in the dark for 6 days (144 hours) to allow for seed germination and radicle growth.

Macroscopic analyses were conducted after the incubation period of the *Allium cepa* seeds, following the subsequent steps: (i) Quantification of germinated seeds; (ii) Measurement of root size; (iii) Recording any potential root alterations (morphology - shape, texture, length, thickness, and color changes); (iv) Determination of macroscopic indices. Equations 1, 2, 3, 4, and 5 were used for calculating the macroscopic indices.

$$GRS = \frac{number\ of\ germinated\ seeds\ with\ sample}{number\ of\ germinated\ seeds\ in\ control} * 100 \qquad (1)$$

Where: GRS is the Relative Germination of Seeds

$$CRR = \frac{average\ radicle\ size\ with\ sample}{average\ radicle\ size\ in\ control} * 100 \qquad (2)$$

Where: CRR is the Relative Radicle Growth.

$$IG = \frac{GRS*CRR}{100} \quad (3)$$

Where: IG is the Germination Index.

$$IGN = \frac{Germ\ x - Germ\ controle}{Germ\ controle} \quad (4)$$

Where: IGN is the Normalized Residual Germination Index. Germ x is the average percentage of germinated seeds in each sample. Germ control is the percentage of germinated seeds in the control.

$$IER = \frac{Along\ x - Along\ controle}{Along\ controle} \quad (5)$$

Where: IER is the Normalized Residual Radical Elongation Index. Along x is the average length of the radicle of germinated seeds in each sample. Along controle is the average length of the radicle of germinated seeds in the control.

According to the methodology of Bagur-Gonzales et al. (2011), the IGN and IER indices are classified according to different toxicity levels, as shown in Table 1.

Table 1: Toxicity levels based on index of germination.

Index	Toxicity levels
Greater than 0	Hormesis
0 a -0.25	Low toxicity
-0.25 a -0.5	Moderate toxicity
-0.5 a -0.75	High toxicity
-0.75 a -1.0	Very high toxicity

Hormesis corresponds to low concentrations of a contaminant, not implying that it is not harmful to the organisms present in the environment.

3 RESULTS AND DISCUSSION

The leachate used in this study had a pH of 8.24 and an electrical conductivity of 24.74 µS, classifying it as stabilized leachate (DANTAS, 2021). The characteristics of the colonies analyzed for fungal identification are presented in Table 2 and Figure 3.

Table 2: Macroscopic and microscopic characteristics of selected fungal colonies.

Genera	Macroscopic Characteristics	Microscopic Characteristics
Candida	Amber color, small size, dry appearance, irregular edges, and rough surface.	Yeast - has pseudohyphae with elongated blastoconidia.
Trichophyton	White color, medium size, cottony appearance, smooth edges, and entire surface.	Filamentous fungus - has coenocytic hyphae and reproduces asexually with the presence of oval conidiophores.

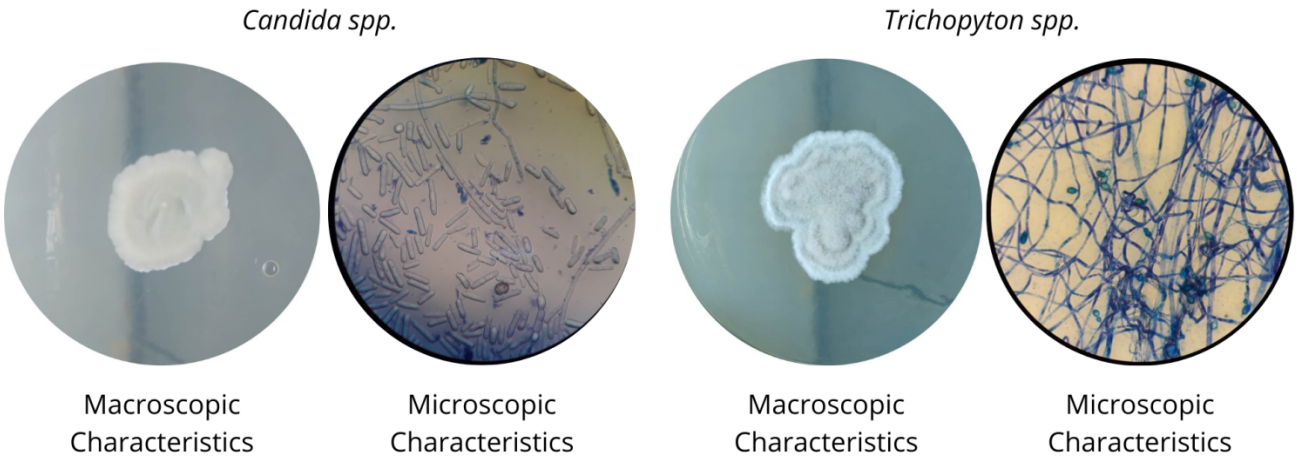

Figure 3: Macroscopic and microscopic characteristics of selected fungal colonies.

According to Tramontini (2013), fungi that form pseudohyphae exhibit differentiated radial growth that allows for greater contact with pollutants, potentially accelerating the degradation of organic matter and consequently reducing compounds in the environment.

During the experiment, the yeast similar to the *Candida* genus exhibited robust fungal growth. Its cell mass peaked in EU-10%, indicating that the fungus was effective in degrading the organic matter in the leachate. In EU-50%, it had the lowest cell mass value, suggesting that high leachate concentrations inhibit its growth. Quantifying EU-100% for this genus was not possible. The filamentous fungus *Trichophyton spp*. displayed a high cell mass value in EU-0%, indicating that the environment was conducive to fungal development. In EU-100%, it had the lowest cell mass value, indicating that high leachate concentrations hinder the growth of the filamentous fungus. Figure 4 illustrates the cell masses of each fungus in the EUs.

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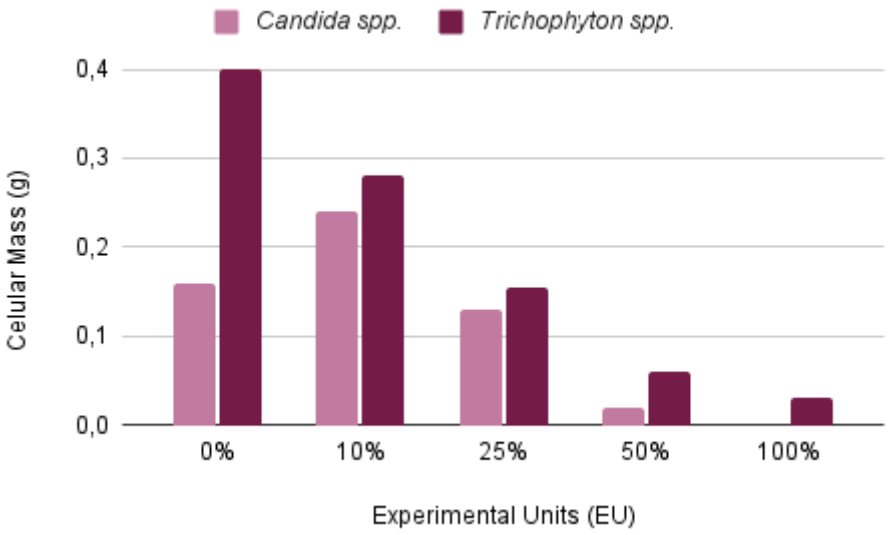

Figure 4: Comparison of cell mass after the mycoremediation process between the *Candida* and *Trichophyton* genera.

Analyzing Figure 4, it is evident that the fungus *Trichophyton spp*. has a higher cell mass than the yeast *Candida spp*. in all EUs. Due to structural differences, where filamentous fungi are multicellular (forming mycelium) and yeasts are unicellular, the cell mass of filamentous fungi is greater than that of yeasts. This difference is not indicative of better efficiency or development. Figure 4 illustrates how different leachate concentrations influence the fungal growth of each genus, with higher effluent concentrations leading to lower fungal cell mass.

In Table 3, the calculated indices based on the results of the toxicity tests after the mycoremediation process are presented.

Table 3: Macroscopic indices calculated from the results of toxicity tests for experimental units (EUs).

	Candida spp					*Trichophyton spp*				
Experimental Units	GRS (%)	IG (%)	CRR (%)	IGN (%)	IER (%)	GRS (%)	IG (%)	CRR (%)	IGN (%)	IER (%)
0%	100,00	100,00	100,00	0,00	0,00	100,00	100,00	100,00	0,00	0,00
10%	106,32	165,92	156,06	0,66	0,56	71,43	42,37	59,32	-0,58	-0,41
25%	87,55	159,54	182,22	0,60	0,82	21,43	2,19	10,23	-0,98	-0,90
50%	18,76	14,89	79,37	-0,85	-0,21	0,00	0,00	0,00	-1,00	-1,00
100%	6,25	4,25	68,03	-0,96	-0,32	0,00	0,00	0,00	-1,00	-1,00

(GRS) = Relative Seed Germination; (CRR) = Relative Radicle Growth; (IG) = Germination Index; (IGN) = Normalized Residual Germination Index; (IER) = Normalized Residual Radical Elongation Index.

Hormesis | Low Toxicity | Moderate Toxicity | High Toxicity | Very High Toxicity

According to the macroscopic indices presented in Table 3, it can be observed that for the yeast *Candida spp*., the GRS value for EU-10% is higher than EU-0%, indicating that the germination percentage in the EU with a 10% leachate concentration is greater than in the leachate-free EU (0%). EU-25% also shows good GRS values. EU-50% and EU-100% have the

lowest GRS values, as well as IG. EU-10% and EU-25% have higher CRR indices than EU-0%, indicating better root growth at leachate concentrations of 10% and 25% compared to the leachate-free concentration. EU-50% and EU-100% have lower CRR values than EU-0%, but they are still positive results. Analyzing the GRS, IG, and CRR indices for EU-50% and EU-100%, there is an indication that a high leachate concentration inhibited seed germination but not their growth. In other words, *Allium cepa* seeds that managed to germinate in these concentrations exhibited good root growth.

The IGN and IER indices classify EU-10% and EU-25% as hormesis (low pollutant concentrations, not implying that they are not harmful to the organisms in the environment). EU-50% and EU-100% are categorized as very high toxicity by the IGN index and low and moderate toxicity, respectively, by the IER index.

The yeast of the *Candida* genus is employed to treat various pollutants, such as dyes, oil, wastewater, polycyclic aromatic hydrocarbons (PAHs), and toxic metals (SOARES et al., 2011; DURVAL, 2017). Authors Camargo and Corso (2002) used some species of the *Candida* genus for the biosorption of the amaranth dye, achieving a reduction of the dye to over 90% for the *Candida catenulata* and *Candida Kefyr* species. The work carried out by authors Amorim et al. (2014) suggests that *Candida lipolytica* yeast is an effective remediation agent for motor oil and environmental pollutants generated by the petroleum industry. Author Marinho (2009) studied the efficiency of *Candida oleophila* yeast in removing organic load, total polyphenol content, and toxicity of wastewater from olive oil production, with removal rates of 50, 83, and greater than 50%, respectively, revealing that the yeast has good depurative and toxicity reduction capacities. These studies support the present study, highlighting the versatility and high remediation potential of *Candida spp*. yeast.

Studies show that some species of *Trichophyton spp*. are efficient remediators of azo dyes and pentachlorophenol, although this fungal genus is still underexplored (SANTOS, 2023).

Table 3 reveals that for the filamentous fungus *Trichophyton spp*., the GRS index shows good results in EU-10% and a moderate result in the CRR index, indicating that despite seed germination in this medium, there may have been inhibition of root growth. EU-50% and EU-100% do not show seed germination, indicating that the medium remains highly toxic.

The IGN and IER indices classify EU-25%, EU-50%, and EU-100% as very high toxicity, while EU-10% is categorized as high toxicity by the IGN index and moderate toxicity by the IER index. Figure 5 presents a comparison of the toxicity levels between the EUs inoculated with *Candida* yeast and those inoculated with the filamentous fungus *Trichophyton*.

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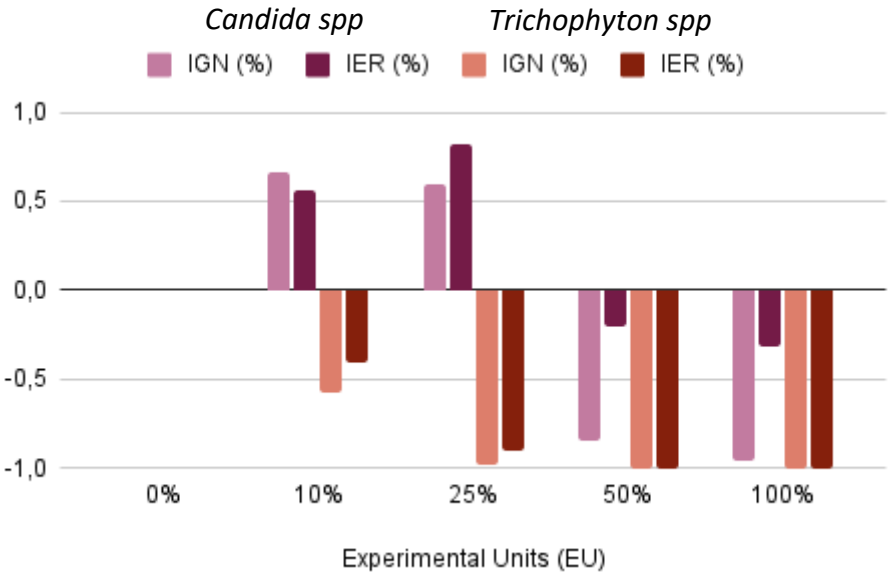

Figure 5: Comparative analysis of toxicity levels in experimental units (EU) inoculated with fungi of the *Candida* and *Trichophyton* genera after the mycoremediation process.

Based on the presented macroscopic indices and the analysis in Figure 5, it is evident that the yeast *Candida spp*. delivers better results in all experimental units, indicating that yeast is more efficient as a remediating organism for the toxicity of leachate from a sanitary landfill than the filamentous fungus of the *Trichophyton spp*.

4 CONCLUSION

Through the analysis of fungal cell mass, it is evident that the yeast *Candida spp*. was able to thrive in the EU-10%. Fungal growth was observed in different leachate concentrations, with higher leachate concentrations leading to reduced fungal cell mass, indicating that high leachate concentrations influence the fungal growth of the genera studied in this work.

The toxicity test with *Allium cepa* (onion) showed that the yeast was able to reduce leachate toxicity to hormesis levels at lower leachate concentrations. Comparing both fungi, the yeast of the *Candida* genus proved to be more efficient in remediating the toxicity of leachate from a sanitary landfill than the filamentous fungus *Trichophyton spp.*

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HOW TO CITE THIS ARTICLE:

Aranega Pesenti, M. E., Marques, T. A., Campos, V. A., Urata, S. L., & Prates, K. V. M. C. AVALIAÇÃO DO POTENCIAL BIORREMEDIADOR DOS FUNGOS CANDIDA spp. E TRICHOPHYTON spp. NO TRATAMENTO DE LIXIVIADO PROVENIENTE DE ATERRO SANITÁRIO. HOLOS, 5(39). Recuperado de https://www2.ifrn.edu.br/ojs/index.php/HOLOS/article/view/16307

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Editor: Francinaide de Lima Silva Nascimento
***Ad Hoc* Reviewer:** Francisco Lledo dos Santos e Patrícia Martins Diniz

Submitted October 23, 2023
Accepted December 16, 2023
Published December 18, 2023