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A comprehensive assessment of leachate contamination at a non-operational open dumpsite: mycoflora screening, metal soil pollution indices, and ecotoxicological risks

Sidney Fernandes Sales Junior · Camille Ferreira Mannarino · Gabriel de Farias Araújo · Daniele Maia Bila · Rachel Ann Hauser-Davis · Tatiana Saint'Pierre · Gisela Lara da Costa · Manoel Marques Evangelista Oliveira · Cláudio Ernesto Taveira Parente · Fábio Veríssimo Correia · Enrico Mendes Saggioro

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Abstract The final disposal of municipal solid waste (MSW) in dumpsites is still a reality worldwide, especially in low- and middle-income countries, leading to leachate-contaminated zones. Therefore, the aim of this study was to carry out soil and leachate physicochemical, microbiological, and toxicological characterizations from a non-operational dumpsite. The L-01 pond samples presented the highest physicochemical parameters, especially chloride (Cl; 4101 ± 44.8 mg L^{-1}), electrical conductivity (EC; $10{,}452 \pm 0.1$ mS cm^{-1}), and chemical oxygen demand (COD; 760 ± 6.6 mg L^{-1}) indicating the presence of leachate, explained by its close proximity to the landfill cell. Pond L-03 presented higher parameters compared to pond L-02, except for N-ammoniacal and phosphorus levels, explained by the local geological configuration, configured as a slope from the landfill cell towards L-03. Seven filamentous and/or yeast fungi genera were identified, including the opportunistic pathogenic fungi *Candida krusei* (4 CFU) in an outcrop sample. Regarding soil samples, Br, Se, and I were present at high concentrations leading to high soil contamination ($CF \leq 6$). Pond L-02 presented the highest CF for Br (18.14 ± 18.41 mg kg^{-1}) and I (10.63 ± 3.66 mg kg^{-1}), while pond L-03 presented

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S. F. S. Junior · G. de Farias Araújo · E. M. Saggioro (✉)
Post-Graduation Program in Public Health and Environment, Sergio Arouca National School of Public Health, Oswaldo Cruz Foundation, 1480 Leopoldo Bulhões Ave, 21041-210 Rio de Janeiro, RJ, Brazil
e-mail: enrico.saggioro@ioc.fiocruz.br

S. F. S. Junior
e-mail: sidneysalesjunior@gmail.com

G. de Farias Araújo
e-mail: gabriel_gfa@hotmail.com

S. F. S. Junior · G. de Farias Araújo · R. A. Hauser-Davis · E. M. Saggioro
Environmental Health Evaluation and Promotion Laboratory, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, 4365 Brasil Ave, 21045-900 Rio de Janeiro, RJ, Brazil
e-mail: rachel.hauser.davis@gmail.com

C. F. Mannarino
Sanitation and Environment Health Department, Sergio Arouca National School of Public Health, Oswaldo Cruz Foundation, 1480 Leopoldo Bulhões Ave, 21041-210 Rio de Janeiro, RJ, Brazil
e-mail: camille.mannarino@gmail.com

D. M. Bila
Department of Sanitary and Environment Engineering, State University of Rio de Janeiro, 524 São Francisco Xavier Street, Room 5029-F, 20550-900 Rio de Janeiro, Brazil
e-mail: danielebilauerj@gmail.com

T. Saint'Pierre
Department of Chemistry, Pontifical Catholic University of Rio de Janeiro, Marquês de São Vicente Street, 225, 22541-041 Gávea, Rio de Janeiro, RJ, Brazil
e-mail: tatispierre@pucrio.com

the highest CF for Se (7.60 ± 1.33 mg kg^{-1}). The most severe lethal effect for *Artemia salina* was observed for L-03 samples (LC_{50}: 79.91%), while only samples from L-01 were toxic to *Danio rerio* (LC_{50}: 32.99%). The highest lethality for *Eisenia andrei* was observed for L-02 samples (LC_{50}: 50.30%). The applied risk characterization indicates high risk of all proposed scenarios for both aquatic (RQ 375–909) and terrestrial environments ($RQ > 1.4 \times 10^5$). These findings indicate that the investigated dumpsite is contaminated by both leachate and metals, high risks to living organisms and adjacent water resources, also potentially affecting human health.

Keywords Solid waste · Landfill leachate · Ecotoxicity · Fungi · Geo-accumulation

Introduction

About 33% of the total municipal solid waste (MSW) produced worldwide is disposed of in dumpsites (Kaza et al., 2018). These areas accumulate wastes from different sources contaminating soils, surface, and groundwater, which can directly affect ecosystems and the health of about 64 million people (Krčmar et al., 2018; Sharma et al., 2020; Uddh Söderberg et al., 2019; Vaccari et al., 2019). Therefore, global efforts are now underway to put an end to final waste disposal in dumpsites.

Leachate production is particularly high in dumpsites, as no waste compaction is carried out, and soil-covering layers are not implemented (Matern et al., 2017; Sharma et al., 2020). In this sense, the characterization and monitoring of both dumpsite and landfill leachates are paramount for environmental impact assessments (Alam et al., 2019), as well as for proposing remediation and reuse processes, including hydroponics, soil fertilization, and constructed wetlands (Kalousek et al., 2020; Singh et al., 2017). Leachate is a complex matrix and previous studies have attempted to solve several environmental problems arising from high ammonia, chloride, nutrient, and emerging and microbiological contaminant levels (Luo et al., 2020; Nika et al., 2020; Xu et al., 2018).

Regarding fungi diversity, several autochthonous strains, as well as some basidiomycetes, can be employed for leachate treatment (Islam et al., 2019; Tigini et al., 2009). This is due to the fact that fungi produce and secrete several lignocellulosic enzymes that catalyze and breakdown the complex structures of several toxic components present in leachate, such as xenobiotic compounds, humic substances, pharmaceuticals, personal care products (PPCPs), and polycyclic aromatic hydrocarbons (PAHs), thus decreasing and possibly removing the recalcitrance of this effluent (Islam et al., 2019). In addition, mycoflora monitoring is important from a pathogenic point of view, as potentially opportunistic pathogenic fungi such as *Candida krusei*, for example, may occur in leachate from dumpsites (Tigini et al., 2009; Ulfig, 1994). Contamination by metals in landfills also comprises a major challenge, with levels above limits established as safe for humans reported in several developing countries (Vaccari et al., 2019).

In this context, biological models have been employed to assess adverse effects and risks to humans and ecosystems resulting from exposure to dumps and leachate from landfills. In vitro cyto- and genotoxicity effects following exposure to dumpsite samples have been, for example, reported for human cells (HaCaT keratinocytes and lymphocytes) (Bakare et al., 2007; Khalil et al., 2018), as well as the induction of liver enzymes coupled to liver and bone marrow damage in exposed rats (Khalil et al., 2018). Furthermore, ecotoxicological assessments have been well described for aquatic organisms belonging to different trophic niches, such as *Danio rerio*, *Vibrio fischeri*, and *Artemia salina* (Ghosh et al., 2017; le Fol et al., 2017; Spaan et al., 2019). On the other hand, assessments concerning terrestrial organisms are still scarce. In this regard, earthworms are notable among soil invertebrates as soil toxicity bioindicators and can be employed to assess leachate environmental risks, as they

G. L. da Costa · M. M. E. Oliveira
Laboratory of Taxonomy, Biochemistry and Bioprospecting of Fungi, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, 4365 Brasil Ave, 21045-900 Rio de Janeiro, RJ, Brazil
e-mail: gisela.lcosta@gmail.com

M. M. E. Oliveira
e-mail: manoel.marques@ioc.fiocruz.br

C. E. T. Parente
Laboratório de Radioisótopos, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho s/n, Bloco G0, Sala 60, Subsolo, Rio de Janeiro 21941-902, Brazil
e-mail: claudioetparente@gmail.com

F. V. Correia
Department of Natural Sciences, Federal University of the State of Rio de Janeiro, 458 Pasteur Ave, 22290-20 Urca, Rio de Janeiro, Brazil
e-mail: fabio.correia@unirio.br

play an essential role in maintaining biological soil activity (Sales Junior et al., 2021).

In view of the above, this study aimed to evaluate the environmental health of a non-operational dump and estimate potential risks to adjacent human populations. To this end, leachate physicochemical parameters, contaminants, and mycoflora were assessed, as well as elements in soil, to enable a pollution index calculation. Subsequently, acute ecotoxicological tests were carried out with marine (*Artemia salina*), freshwater (*Danio rerio*), and terrestrial (*Eisenia andrei*) organisms. This comprehensive approach allows for overview of the environmental risks arising from the inadequate management of MSW dumps, carrying out an integrated discussion of both physicochemical and biological factors for the first time, which can consolidate a reference for the evaluation and waste management focused on environmental quality and human health.

Material and methods

Study location and leachate sampling

The non-operational dumpsite assessed in the present study is located in the metropolitan region of the state of Rio de Janeiro, southeastern Brazil (22°52′21″ S 42°49′11″ W), in a rural neighborhood. It was operational for 24 years, ending operations in 2009, although clandestine solid waste dumping took place until 2013, when the dumpsite was closed due to federal government determinations. The dumpsite originally comprised two waste massifs and three leachate accumulation ponds. During the 4 years of clandestine operations, however, the two massifs expanded to form a single large massif (Fig. 1). This dumpsite received over 100 tons of daily waste, accumulating a total volume of about 558,000 m^3 of solid material, which extends through over 47,000 m^2. The predominant soil type in the area is a cambisol originated from igneous granitic rocks containing low calcium content (Laut et al., 2019).

Initially, a potentiometric and topographic survey of the dumpsite was performed, to understand preferential groundwater and leachate flows generated throughout in the waste mass (Fig. 1). Leachate samples were collected from three leachate ponds (L-01, L-02, and L-03) in a single campaign on August 13, 2019 (comprising dry season in the Southern Hemisphere). Samples from an outcrop leachate (OL-01) located at the base of the waste mass near L-03 (Fig. 1) were also collected. All pond leachate samples were collected with the aid of stainless-steel containers, in triplicate (Brasil, 2011a), while the outcrop leachate was collected directly in 50 mL glass bottles, also in triplicate. All samples were stored at 4 °C in the dark until analysis.

Leachate characterization

Physicochemical characterizations were conducted to determine chloride, nitrite, nitrate, carbon (total, organic, and inorganic), pH, alkalinity, electrical conductivity (EC), chemical oxygen demand (COD), solids (total, dissolved, and suspended), and ammoniacal nitrogen, according to the Standard Methods for Examination of Water and Wastewater (Walter, 2018), described in the supplementary material. PAH extractions were carried out according to the USEPA 3510C protocol (1996) and quantifications were carried out following the USEPA 8270E method (2014) for naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo [b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indene[1,2,3-cd]pyrene, dibenz[a,h]anthracene, and benzo [ghi]perylene.

Mycoflora screening and identification

Aliquots (100 mL) of each leachate sample were filtered through membrane filters (0.22 μM), in duplicate, placed in Petri dishes containing SDA medium (Sabouraud Dextrose Agar Difco) dosed with antibiotics (400 mg L^{-1} chloramphenicol and 25 mg L^{-1} gentamicin) and incubated at 28 °C for 7 days for filamentous fungi screening and at 35 °C for 48 h for yeast detection. Fungal colony forming units (CFUs) were counted after 3 and 7 days, and fungal morphotypes were differentiated for pure culture isolation. Yeasts were evaluated at 24 and 48 h.

Fungi were identified by classical taxonomy according to macroscopic and microscopic characteristics associated with the chromogenic test to determine genera (Barnett & Hunter, 1998). Following the CFU counts, all filamentous fungi colonies were cultured in Potato Dextrose Agar (Difco) and yeast colonies were cultured in ChromoAgar Candida for *Candida* sp. identification.

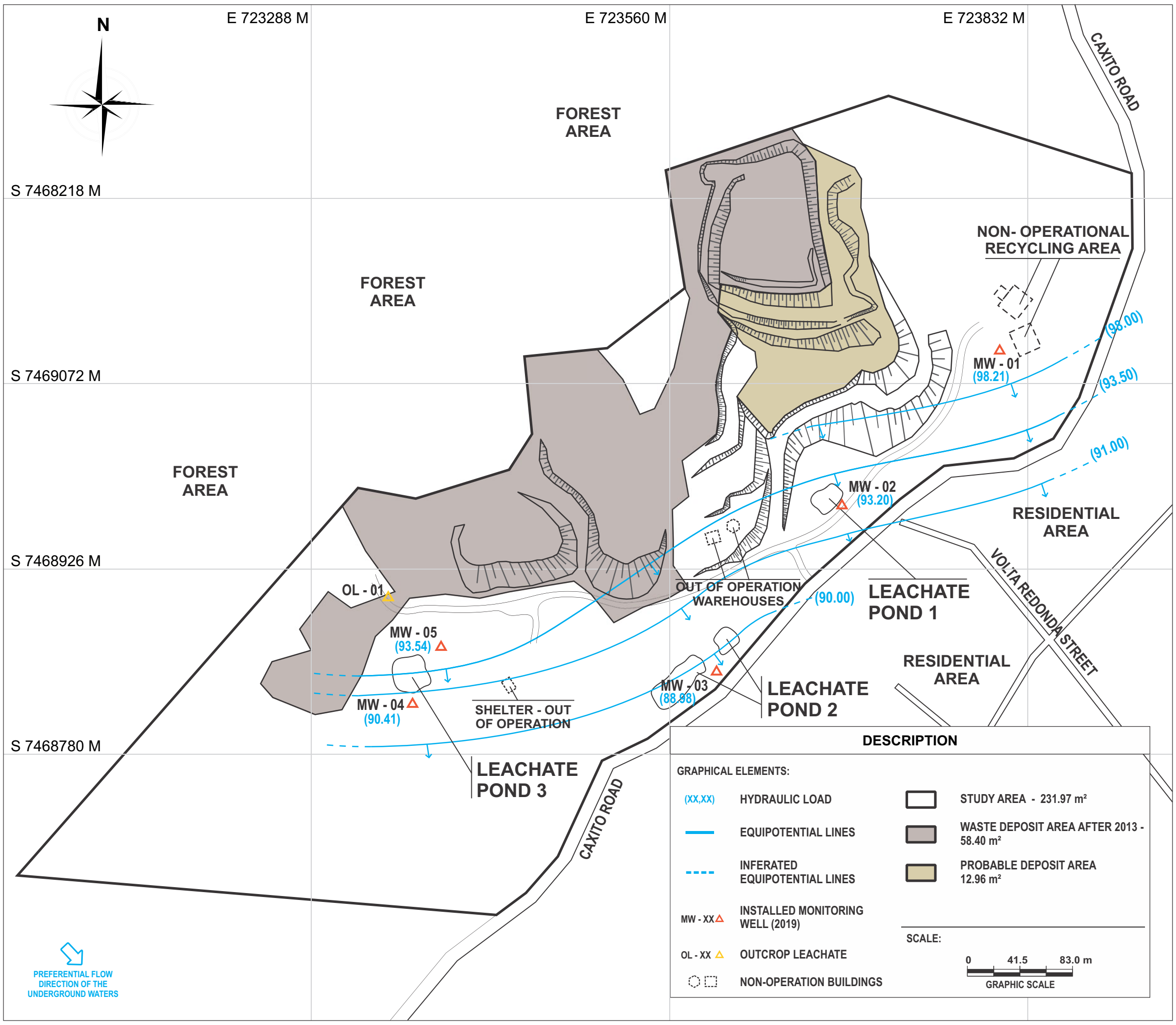

Fig. 1 Open dumpsite area indicating the solid waste masses formed due to waste disposal from 1985 to 2013, as well as the three leachate accumulation ponds, outcrop leachate (OL-01), operational structures, and potentiometry lines

Elemental soil sampling and analyses

Soil samplings followed the Manual of Sample Collection Procedures in Agricultural Areas for Environmental Quality Analysis: Soil, Water and Sediment (Filizola et al., 2006). Unwanted materials such as leaves, branches, and roots were first removed from the area and 50 mg of topsoil (0 to 5 cm) was collected using stainless-steel volumetric rings. Core samples were collected, not touching the edges of the ring, at different distances to observe how pond distance alters metal soil concentrations. Thus, soil samplings were carried out at 0, 90, 180, 270, 360, and 450 cm distance from the edge of pond L-01; 0 and 180 cm from the edge of pond L-02; and 0, 90, 180, 270, and 360 cm from the edge of pond L-03. Each soil sample was stored in sterile 50 mL polypropylene tubes until analysis. Elemental determinations ($n = 62$) were carried out by inductively coupled plasma mass spectrometry (ICP-MS) employing a NexIon 300×ICP-MS (Perkin-Elmer Sciex, Norwalk, CT, USA) under previously established instrumental conditions (Table S1). Method accuracy was verified using the SEM-2586 certified reference material for soil (Standard Reference Material 2586–Trace Elements in Soil; NIST, USA).

Metal pollution assessments

Geo-accumulation index

The geo-accumulation index (Igeo) was calculated by applying Eq. 1 to determine if metal dumpsite soil accumulation rates were above expected natural levels (Adelopo et al., 2018; Sharifi et al., 2016). The calculation was performed with the average metal concentrations between the collection distances (0 and 450 cm) determined in the soil samples. The final Igeo classifications were established according to Adelopo et al. (2018) (Table S2).

$$I_{geo} = log_2\left(\frac{C_x}{1.5 \times B_x}\right) \quad (1)$$

where C_x is the average concentration of element x in the marginal soil of each leachate pond and B_x is the mean geochemical background value of element x. The constant 1.5 is included to minimize lithological variations and anthropogenic influence effects towards the geochemical background value.

Contamination factor and contamination degree

The contamination factor (CF) and contamination degree (CD) were calculated to assess dumpsite soil (Turekian & Wedepohl, 1961) metal contamination levels (Adelopo et al., 2018; Sharifi et al., 2016), through Eqs. 2 and 3, and to determine which elements were responsible for such contamination. Therefore, the CF was calculated for each determined metal to obtain individual contamination levels. The CD was employed to assess total soil contamination levels, using the previously calculated contamination factor of each element (Sharifi et al., 2016) (Table S2).

$$CF = \frac{C_s}{B_x} \quad (2)$$

$$CD = \sum\nolimits_{n=1}^{n} CF_n \quad (3)$$

where C_s is the concentration of each element in the dumpsite soil and B_x is the mean geochemical background value of element x for the type of local soil (Turekian & Wedepohl, 1961).

Aquatic toxicity assessments

The aquatic toxicity assessment followed Brazilian legislation, which requires two organisms from different trophic levels to determine the aquatic toxicity of a certain effluent (Brasil, 2011b). *Artemia salina* assays consisted in transferring 10 mL of each leachate solutions (3.1, 6.2, 12.5, 25, 50, and 100% v/v) to six 50 mL beaker replicates containing ten organisms randomly transferred and kept in an incubator for 48 h from 23 to 27 °C in the dark with no food (Brasil, 2016a). All leachate samples were compared to a negative control group. At the end of 48 h, the lethal concentration for 50% of the exposed organisms (LC_{50}) was estimated.

Danio rerio assays followed the NBR 15,088 standard (Brasil, 2016b). All fish were acquired from a specialized farm in the adult stage (±2 cm in length) and acclimated for 7 days in dechlorinated water prior to exposure. Ten fish were exposed to each leachate sample concentration (3.1, 6.2, 12.5, 25, 50, and 100% v/v), at a maximum density of 0.54 g L^{-1} (grams of fish L^{-1}) for 48 h. Fish lethality was then assessed to estimate the LC_{50} of each leachate sample. This experiment was approved by the State University of Rio de Janeiro (UERJ) Ethics Council for the Care and Use of Experimental Animals (CEUA), under protocol no. 24/2016.

Terrestrial toxicity

This assay was performed by applying 1 mL of the sampled leachate solutions from the three leachate ponds onto 60 cm^2 of Whatman® filter paper no. 2 (Kent, England) placed inside 50 mL beakers (OECD, 1984). Leachate concentrations of 25, 50, 75, and 100% (v/v) were used and compared to a control group exposed to ultrapure water. Earthworms were exposed individually, totaling 15 organisms per leachate concentration, and kept in the dark at 20 ± 2 °C for 72 h. Lethality was assessed at 24, 48, and 72 h to estimate the LC_{50} of each leachate sample. Lethality was established as lack of responses following a mild mechanical stimulus with a small spatula at the front end of the exposed earthworms (ASTM, 2012).

Risk characterization

The risk quotient (RQ) compares the exposure to contaminants (dose) and biological responses (effect), establishing a preliminary risk assessment (European commission, 2013). The RQ is calculated through the following equation.

$$RQ = \frac{PEC}{PNEC} \quad (4)$$

where *PEC* is the predicted environmental concentration of dumpsite (or landfill) leachate and *PNEC* is the predicted no-effect leachate concentrations on tested organisms. The predicted environmental leachate concentrations were based on previous studies proposing laboratory and pilot-scale remediation tests, including hydroponics, constructed wetlands, and soil fertilization. Considering the short-term toxicity results, PNECs were calculated by the ratio between the lowest LC_{50} for each organism (numerator) and the assessment factor of 1000 (denominator) (European Commission, 2003). Risk quotient > 1 indicate a "high risk," as environmental concentrations (PEC) exceeded safety values (PNEC).

Statistical analyses

The LC_{50} for each leachate sample were determined by the Spearman–Karber test (Hamilton et al., 1977), at a confidence limit equal to or greater than 95% ($p \leq 0.05$), employing the GraphPad Prism version 5.00 software.

Results and discussion

Dumpsite and leachate characterization assessments

Potentiometric calculations (Fig. 1) indicated a groundwater flow trend in the direction of the waste masses (southeast) towards the leachate ponds. Therefore, the leachate produced in the landfill cell acquires a preferential flow from the massifs towards the ponds, specifically from L-01 to L-03.

No PAH was quantified (LQ 0.01 mg L^{-1}) in the pond samples. However, leachate samples from L-01 and L-03 presented the highest physicochemical parameters (Table 1), higher at L-01, probably due to cell proximity. L-01 and L-03 receive greater leachate inputs than L-02, confirmed by higher COD, chloride (Cl^-) and EC values (Table 1).

High Cl^- (4101 mg L^{-1}) and EC (10,452 mS cm^{-1}) levels were detected in L-01 when compared to other leachates sampled in Nigeria (Cl^-: 269 mg L^{-1}; EC: 199 µS cm^{-1}) (Oyelami et al., 2013), Lebanon (Cl^-: 115–775 mg L^{-1}; EC: 1796–40,300 µS cm^{-1}) (Khalil et al., 2018), and Brazil (Cl^-: 3913 mg L^{-1}; EC: 25.5 µS cm^{-1}) (Sales Junior et al., 2021). This indicates constant leachate production given the presence of uncovered waste under aerobic conditions (Fauziah et al., 2013; Tchobanoglous & Kreith, 2002). Low ammoniacal nitrogen (< 0.4–68 mg L^{-1}) and COD (53–720 mg L^{-1}) levels also corroborate a constant supply of newly formed leachate, as ammonia nitrogen tends to increase over time due to the hydrolysis and fermentation of nitrogenous fractions from biodegradable substrates, usually ranging from 500 to 2000 mg L^{-1} (Kjeldsen et al., 2002).

COD values (82–760 mg L^{-1}) were below those observed in leachates from Croatia (673–1653 mg L^{-1}) (Ančić et al., 2020), Greece (1537.5 mg L^{-1}) (Genethliou et al., 2021), and Nigeria (28.4–80.1 mg L^{-1}) (Ikem et al., 2002), indicating once again the initial formation of leachate, as this parameter is characteristic of mature leachate (Ko et al., 2016). These findings indicate that this dump is still biologically active and far from stabilized.

Leachate production and migration in dumpsite soil are diffuse pollution sources to groundwater, potentially altering water parameters and making it unsuitable for human consumption (Oyelami et al., 2013). In this regard, surrounding areas near a dumpsite in Nigeria presented groundwater with Cl^-, pH, and Mn values above World Health Organization (WHO) limits of 250 mg L^{-1}, 6.5–8.5, and 0.1 mg L^{-1}, respectively (Oyelami et al., 2013). In another assessment, Sabahi et al. (2009) identified that one out of five groundwater monitoring wells in a dumpsite in Yemen contained Mg concentrations above WHO limits (50 mg L^{-1}), two wells presented EC levels and Na^+ concentrations above established limits (1400 mS cm^{-1} and 50 mg L^{-1}, respectively), and three wells presented total solids (TS), Cl^-, and Ca concentrations above maximum limits (500, 250, and 75 mg L^{-1}, respectively). The detected contamination was, thus, attributed to leachate percolating throughout the dumpsite soil (Sabahi et al., 2009).

Table 1 Physicochemical characterization of leachate samples from leachate ponds (L-01, L-02, and L-03) in a non-operational open dumpsite in southeastern Brazil

	Alkalinity ($mg\ L^{-1}\ CaCO_3$)	Ammoniacal nitrogen ($mg\ L^{-1}$)	Total inorganic carbon ($mg\ L^{-1}$)	Total organic carbon ($mg\ L^{-1}$)	Total carbon ($mg\ L^{-1}$)	Total nitrogen ($mg\ L^{-1}$)	Chloride ($mg\ L^{-1}$)
L-01	291 ± 0.3	68 ± 0.1	192 ± 0.4	285 ± 0.6	477 ± 0.6	76 ± 0.1	4101 ± 44.8
L-02	46 ± 0.9	2.5 ± 0.1	32 ± 0.1	74 ± 0.13	106 ± 0.14	6.3 ± 0.02	495 ± 5.9
L-03	89 ± 1.2	< 0.4	61 ± 0.3	96 ± 0.1	157 ± 0.1	6.7 ± 0.01	750 ± 4.5
	Conductivity ($mS\ cm^{-1}$)	Apparent color ($mg\ Pt\ L^{-1}$)	Real color ($mg\ Pt\ L^{-1}$)	Chemical oxygen demand ($mg\ L^{-1}$)	Total hardness ($mg\ L^{-1}\ CaCO_3$)	Phosphorus ($mg\ L^{-1}$)	pH (25 °C)
L-01	10,452 ± 0.1	5078 ± 47.1	504 ± 2.3	760 ± 6.6	736 ± 0.0	2.2 ± 0.03	8.7
L-02	1621 ± 2.9	268 ± 3.0	209 ± 2.1	82 ± 0.0	111 ± 1.29	1.1 ± 0.01	8.1
L-03	2787 ± 1.0	512 ± 2.9	275 ± 39.6	137 ± 2.9	232 ± 4.6	0.2 ± 0.01	9.2
	Nitrate ($mg\ L^{-1}$)	Nitrite ($mg\ L^{-1}$)	Sulfate ($mg\ L^{-1}$)	Total solids ($mg\ L^{-1}$)	Fixed total solids ($mg\ L^{-1}$)	Total volatile solids ($mg\ L^{-1}$)	Turbidity (UNT)
L-01	< 1.5	< 2	15 ± 0.2	6251 ± 18.1	5254 ± 38.2	997 ± 25.8	131 ± 0.6
L-02	< 1.5	< 2	< 6	888 ± 7.0	765 ± 6.81	123 ± 0.58	8 ± 2.7
L-03	< 1.5	< 2	129 ± 0.7	1627 ± 24.9	1343 ± 16.9	284 ± 11.8	21 ± 3.7
	Total dissolved solids ($mg\ L^{-1}$)	Fixed dissolved solids ($mg\ L^{-1}$)	Volatile dissolved solids ($mg\ L^{-1}$)	Total suspended solids ($mg\ L^{-1}$)	Fixed suspended solids ($mg\ L^{-1}$)	Volatile suspended solids ($mg\ L^{-1}$)	PAH ($mg\ L^{-1}$)
L-01	5842 ± 39.9	5072 ± 33.2	770 ± 72.7	409 ± 23.7	182 ± 33.2	228 ± 93.9	< LQ[a]
L-02	845 ± 0.67	726 ± 0.23	119 ± 0.13	43 ± 0.8	39 ± 0.5	4 ± 0.2	< LQ[a]
L-03	1568 ± 1.7	1293 ± 0.58	274 ± 0.79	59.2 ± 0.7	49.7 ± 0.7	9.6 ± 0.3	< LQ[a]

[a]Analytical limit of quantitation: 0.01 $mg\ L^{-1}$

Mycoflora screening and identification

Seven filamentous fungi and/or yeast genera were identified (Fig. 2) in the dumpsite leachate samples (Table 2). The total fungal leachate load was of 48.5 CFU 100 mL^{-1} per sampling point (L-01 = 12 CFU; L-02 = 17 CFU; L-03 = 68 CFU). The *Cladosporium*, *Penicillum*, and *Paecilomyces* genera were the most frequently isolated (Table 2). Opportunistic pathogenic fungi comprising members of the *Candida*, *Aspergillus*, *Fusarium*, *Cladosporium*, *Paecilomyces*, and *Penicillium* genera were also identified (Tigini et al., 2009; Ulfig, 1994), corroborating with Tigni et al. (2014), who also isolated fungi belonging to the *Aspergillus* and *Candida* genera from landfill leachate and wastewater samples from Italy.

Fungal isolation was higher at pond L-03 and at the outcrop leachate OL-01. It is important to note the pathogenic *Candida krusei* yeast were also detected by Amani et al. (2018) at a landfill leachate treatment plant in Borj Chakir, Tunisia, and comprised the second most frequent species (43.24%) detected in the leachate. In another study, *C. krusei* was also isolated from waters from polluted rivers and was positively correlated to sewage contamination parameters (Medeiros et al., 2012). However, the *Candida* sp. virulence profile isolated from landfill leachate is, so far, largely unknown, with Amani et al. (2018) reporting potentially dangerous amphotericin B-resistant *Candida* sp. in leachate samples. In view of this, the implementation of *Candida* yeast detection assays in leachate characterization and monitoring efforts may become a relevant measure, aiming to avoid the natural selection of strains resistant to antifungal agents available as a therapeutic arsenal.

Soil elements

Except for Ba, all 62 determined elements (Table S3) were below the guideline values for soil quality established in Brazil (Brasil, 2009). Most elements were homogeneously distributed in the soil samples obtained from the margin

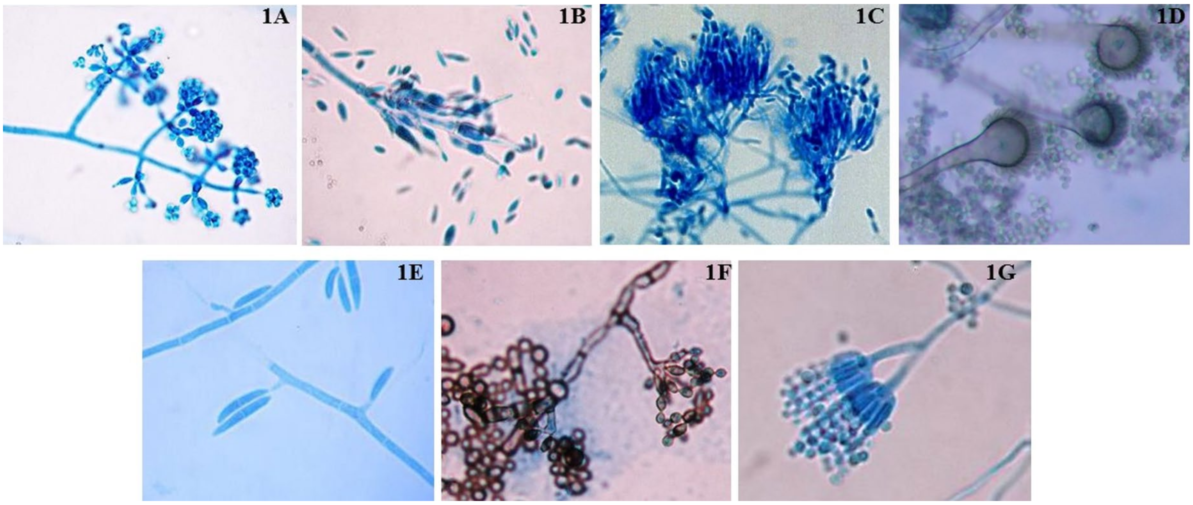

Fig. 2 Fungi isolated from leachate from a non-operational open dumpsite in Southeastern Brazil. **1A** *Trichoderma* sp., **1B** *Paecilomyces* sp., **1C** *Metarhizium* sp., **1D** *Aspergillus* sp., **1E** *Fusarium* sp., **1F** *Cladosporium* sp., **1G** *Penicillium* sp

of pond L-01, except for Cu, Mn, Zn, Br, and Sr (Fig. 3), which increased with increasing distances from the pond margin. The distribution of these elements may be associated with groundwater flow trend through the dumpsite soil, generating a metal carryover process by rainwater and groundwater leaching (Adelekan & Alawode, 2011).

The opposite was observed in marginal L-02 soil, with element concentrations decreasing with increasing distance from the pond (Table S3). This indicates that the increase in elemental concentrations with pond proximity occurs through soil adsorption in L-02, and not by the groundwater flow (Fig. 1). On the other hand, Ca, Zn, Br, and Sr concentrations increased with increasing distance from L-02 (Fig. 3), as observed at L-01 for the same elements, indicating rainwater runoff and leaching to groundwater.

Table 2 Fungal microbiota determined in samples from an outcrop leachate OL-01 and leachate ponds L-01, L-02, and L-03 from the assessed non-operational open dumpsite in southeastern Brazil

Samples	Colony-forming unit (CFU)	Fungi
OL-01	71	*Cladosporium* sp.
	20	*Cladosporium* sp.
	01	*Penicillium* sp.
	01	*Fusarium* sp.
	04	*Candida krusei*
L-01	12	*Paecilomyces* sp.
L-02	04	*Cladosporium* sp.
	13	*Trichoderma* sp.
L-03	09	*Aspergillus* sp.
	01	*Metarhizium* sp.
	13	*Penicillium* sp.
	01	*Paecilomyces* sp.
	44	*Cladosporium* sp.

Regarding pond L-03, apart from Na, which decreased with increasing distance from the margin, higher metal concentrations were observed between 90 and 270 cm from the pond margin, although no relevant increase or decrease in relation to distance from the pond margin was noted. The marginal soil at pond L-03 presented the lowest elemental concentrations when compared to L-01 and L-02, except for B and Mn, which were higher in L-03 (Fig. 3).

Element disposals in open dumpsites are a concern in MSW management, as these are persistent compounds that can remain in both dumpsites and landfills for about 150 years (Adelopo et al., 2018; Vaccari et al., 2018). In this sense, Mama et al. (2021) detected high metal concentrations in soil samples from two dumpsites in Nigeria, ranging from 0.1 to 88.9 mg kg^{-1} in the topsoil (0 to 15 cm) and between 0.06 and 83.1 mg kg^{-1} in the subsoil (> 15 cm).

Metals in dumpsite leachate and soil can present harmful potential to adjacent water resources (Mama et al., 2021; Oyelami et al., 2013). Groundwater around two

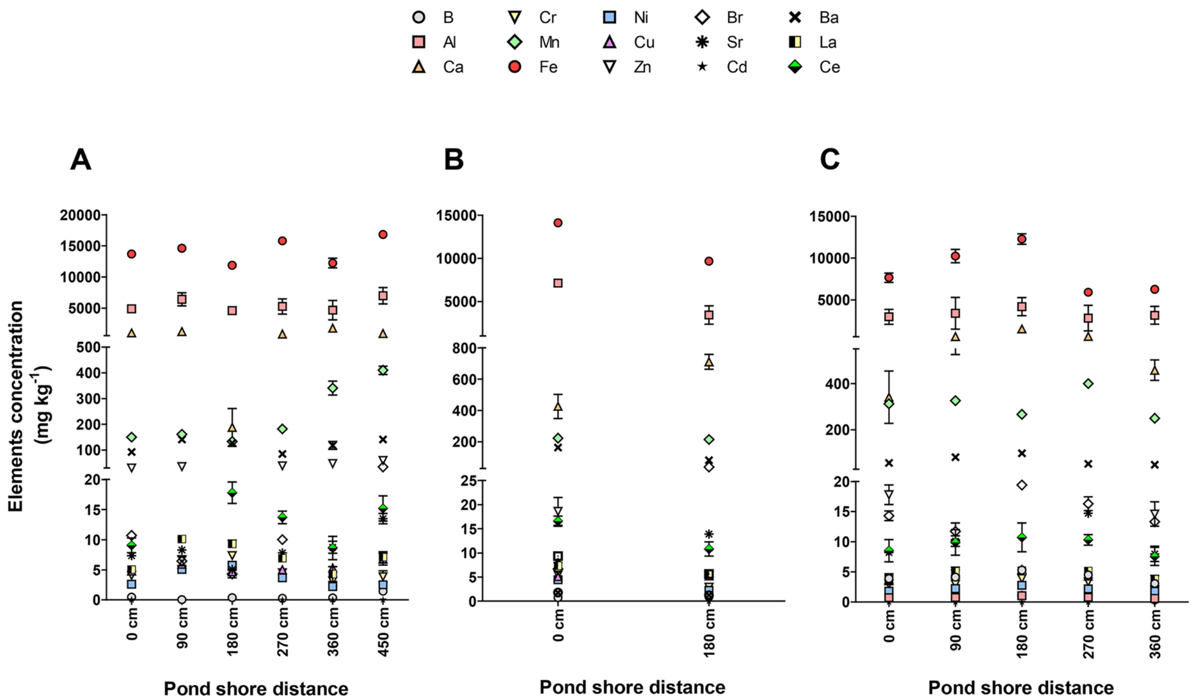

Fig. 3 Elements determined in marginal soil samples from leachate ponds L-01 (**A**), L-02 (**B**), and L-03 (**C**), per sampling distance, in the assessed non-operational open dumpsite in southeastern Brazil. Data are expressed as mg kg^{-1} of soil. The error bars presented in each symbol represent the standard deviation of samples

dumpsites in Nigeria, for example, contained Al, Cd, Cr, Fe, Ni, and Pb above WHO standards for drinking water (Ikem et al., 2002), and seven elements in groundwater near four dumpsites in India were above local potability standards, indicating high metal pollution (Sharma et al., 2020). In addition, decreased contamination levels were observed with increasing distance from the dumpsites, demonstrating a direct influence on the quality of water resources (Sharma et al., 2020).

Elements in dumpsite soil may be a result of leachate soil percolation (Khalil et al., 2018; Oyelami et al., 2013). Considering that the soil is the first environmental compartment impacted by leachate in dumpsites, concerns regarding harmful potential to terrestrial organisms have emerged in recent years (Baderna et al., 2019; Ghosh et al., 2017), as several metals are toxic even at low concentrations (Krewski et al., 2019a, b; Yuan et al., 2019). In the present study, dumpsite soil samples presented high Ca, Fe, and Mg concentrations, indicating potential oxidative risks to exposed organisms. Furthermore, Cr, Ni, Mn, Fe, and Zn also display the potential to induce cellular DNA damage, mutations, and cancer (Bakare et al., 2007; Li et al., 2010), further demonstrating harmful potential to terrestrial species and the need for further assessments in this regard.

Fourteen lanthanides were detected (Table S3). These elements, alongside Sc and Y, form the rare earth metal group (Doulgeridou et al., 2020). The most abundant rare earth elements were La, Ce, Nd, and Gd (Fig. 3). Rare earth metals are found naturally concentrated in mammal liver, lungs, and brain (Doulgeridou et al., 2020). However, the inadequate disposal of technological products in landfills can increase their concentrations in exposed organisms, altering cellular homeostasis (Doulgeridou et al., 2020). Reports in this regard indicate that concentrations between 300 and 600 mg kg^{-1} day^{-1} of Ce, can lead to genotoxicity in rats exposed orally for 28 days (Kumari et al., 2014), while gadolinium can induce neural cell death and increased reactive oxygen species production in rat cells exposed in vitro to 20 μmol L^{-1} Gd for 20 min (Xia et al., 2011). Furthermore, neural cell toxicity and antioxidant system alterations in rats exposed to La and Nd at 20 mg kg^{-1} for 14 days have also been reported (Zhao et al., 2011).

Metal pollution assessment

Geo-accumulation index

Most of the elements determined in the dumpsite soil samples (55 out of 62) presented an average geo-accumulation index (Igeo) below zero (Table S4), indicating no accumulation. However, moderate and high accumulation levels were observed for Co, Br, Se, I, and Au, with Igeo values ranging from 1.50 to 3.07 in the marginal soil of at least one of the three leachate ponds (Table 3). Br, Se, and I were noted as the most critical elements in all marginal pond soils. Bromine exhibited the highest accumulation at L-02, followed by I at L-02 and Se at L-03.

Only Ba (49.07–163.95 mg kg^{-1}) was detected above the established limits (150 mg kg^{-1}), set as a contamination prevention concentration (Brasil, 2009). The Igeo for Co, Br, Se, I, and Au, however, were still moderate to high (Table 3), even at concentrations below established limits (Brasil, 2009). This highlights the harmful potential of non-operational dumpsites, where high waste degradation leads to greater metal availability and accumulation (Adelopo et al., 2018). This has also been reported in the topsoil of a Nigerian closed landfill for Co (Igeo: 0.24 to 0.84) and Se (Igeo: 0.20 to 0.23) (Adelopo et al., 2018), similar to the present study, where Igeo values 2- to sixfold higher for Co and tenfold higher for Se were observed, demonstrating high accumulation.

Metal soil accumulation may result in harmful potential to humans and the environment. In the present study, Br, Se, and I were considered the most critical in terms of soil accumulation. Bromine phytotoxicity has been reported in the literature, reducing *Triticum aestivum* and *Hordeum vulgare* pigmentation, in addition to bioaccumulation (Shtangeeva et al., 2021), which can pose risks following consumption (Bratec et al., 2019). Furthermore, decreased shoot growth in *Hordeum vulgare* exposed to iodine has been observed from 5 mg kg^{-1} and decreased root growth, from 25 mg kg^{-1} (Duborská et al., 2018). Regarding Se, LC_{50} values have been reported as 1.52 μg·cm^{-2} estimated in an acute contact test employing *Eisenia fetida* earthworms and as 65.36 mg kg^{-1} in an acute soil test for the same species (Yue et al., 2021), and an LC_{50} value estimated in the acute soil test was much higher than the highest Se concentration determined in the soil of the dumpsite investigated herein (4.06 mg kg^{-1}), indeed. However, moderate to high geo-accumulation levels can result in long-term issues, as this element can bioaccumulate in exposed organisms (Yue et al., 2021).

Table 3 Geo-accumulation indices (Igeo) and contamination factors (CF) in marginal soil samples from leachate ponds L-01, L-02, and L-03, in the assessed non-operational open dumpsite in southeastern Brazil

Element:	**S**	**Cr**	**Mn**	**Co**	**Ni**	**Zn**	**Br**	**Se**	**I**	**Ba**	**Au**
L-01 (mean ± SD)											
Igeo	0.30 ± 0.42	−0.39 ± 0.48	−1.49 ± 0.68	1.50 ± 0.37	−0.98 ± 0.57	−0.61 ± 0.43	2.56 ± 1.11	2.08 ± 0.48	2.52 ± 0.75	−3.45 ± 0.31	0.74 ± 0.00
CF	1.91 ± 0.57	1.20 ± 0.42	0.59 ± 0.30	4.37 ± 1.03	0.81 ± 0.33	1.02 ± 0.31	11.36 ± 8.82	6.67 ± 2.42	9.56 ± 4.52	0.14 ± 0.03	1.25 ± 1.37
L-02 (mean ± SD)											
Igeo	0.53 ± 0.65	−0.63 ± 0.63	−1.41 ± 0.04	1.75 ± 0.01	−1.10 ± 0.70	−1.38 ± 0.38	3.07 ± 1.84	2.19 ± 0.56	2.78 ± 0.51	−3.43 ± 0.69	–
CF	2.28 ± 0.99	1.02 ± 0.43	0.56 ± 0.01	5.05 ± 0.04	0.74 ± 0.35	0.58 ± 0.15	18.14 ± 18.41	7.10 ± 2.69	10.63 ± 3.66	0.15 ± 0.07	–
L-03 (mean ± SD)											
Igeo	0.35 ± 0.17	−1.06 ± 0.37	−0.93 ± 0.27	1.81 ± 0.17	−1.68 ± 0.26	−1.52 ± 0.32	2.92 ± 0.28	2.32 ± 0.24	2.41 ± 0.29	−4.26 ± 0.44	2.53 ± 1.12
CF	1.93 ± 0.23	0.74 ± 0.17	0.80 ± 0.15	5.31 ± 0.67	0.47 ± 0.09	0.53 ± 0.11	11.54 ± 2.30	7.60 ± 1.33	8.11 ± 1.68	0.08 ± 0.03	4.00 ± 6.52

CF and CD

Most elements (50 out of 62) exhibited a CF lower than 1 (Table S4). Of the remaining 12 elements, eight were classified as presenting a moderate contamination degree, one as a considerable contamination degree, and three as a high degree of contamination.

The L-01 soil samples contained most elements (11) exhibiting a moderate or high degree of contamination (Table 3), followed by L-02 (eight elements) and L-03 (eight elements). Therefore, the marginal soil of the three ponds was characterized as highly contaminated by metals, mainly S, Co, Br, Se, and I. The CD, however, indicated that L-02 presented the highest contamination (CD = 46.92) compared to L-01 (CD = 41.79) and L-03 (CD = 40.57), due to the higher CF calculated for S, Co, Br, Se, and I. Thus, even though L-02 soil contained a lower number of metals with higher CF compared to moderate CF at L-01, a higher metal contamination degree was noted, with Br, Se, and I noted as the most critical elements. The same has been reported in two Nigerian landfills for Zn, Cd, Cu, Pb, and Ag, with a very high contamination factor, even when present at concentrations lower than those permitted by local guidelines (Adelopo et al., 2018). The CD values for both landfills indicated a very high degree of contamination (CD > 28) (Adelopo et al., 2018). Another study carried out in a Malaysian dumpsite reported significant contamination by As and Cd (CF ≥ 03) (Hussein et al., 2021). In Iran, Cr, Zn, Mn, and Ni were classified as moderate ($1 \leq CF < 3$) and Pb and Cu as considerable ($3 \leq CF < 6$), leading to a considerable degree of contamination (CD = 15.3) (Sharifi et al., 2016).

In general, the elemental concentrations detected in the marginal pond soil samples and the calculated indices reflect the observed metal contamination (Sharifi et al., 2016). In addition, the results demonstrate that final MSW disposal without applying any pollution prevention technologies can significantly impact the environment due to metal soil accumulation. In this sense, several literature reports regarding soil metal contamination in dumps and landfills are available and continuous monitoring should be conducted in this regard.

Aquatic and terrestrial toxicity

Artemia salina toxicity evaluations indicated higher toxicity for the L-03 sample compared to L-02 after 24 h of exposure (Table 4). No lethal effect was observed for *Artemia salina* exposed to the L-01 sample. A lethal effect was observed for *Danio rerio* only for the L-01 sample following 96 h exposure, with an estimated LC_{50} of 32.99% v/v (Table 4). Regarding *Eisenia andrei*, a lethal effect was observed for all pond samples after 72 h of exposure in an acute contact test, with LC_{50} of 56.64, 50.30, and 58.47% v/v for L-01, L-02, and L-03, respectively (Table 4).

Table 4 Mean lethal concentrations (LC_{50}) for *Artemia salina*, *Danio rerio*, and *Eisenia andrei* samples from leachate ponds L-01, L-02, and L-03 from the assessed non-operational open dumpsite in southeastern Brazil

Species	LC_{50} (%)		
	L-01	L-02	L-03
Artemia salina (24 h)	> 100	87.06	79.91
Danio rerio (96 h)	32.99	> 100	> 100
Eisenia andrei (72 h)	56.64	50.30	58.47

Although leachate lethality in aquatic species is recognized in the literature, toxicity values are variable. Leachate sample from Sweden was reported as toxic to *Artemia* sp., with an estimated LC_{50} of 75% (v/v) (Svensson et al., 2005), while an LC_{50} of 18.3 ± 4.4% was noted following exposure to a leachate from Colombia (Olivero-Verbel et al., 2008). Leachate toxicity towards *Artemia* sp. can vary throughout the year, as leachate composition is altered according to seasonal conditions, with LC_{50} of 62.1, 72.3, 86.8, 95.2, 54.6, and 76.4% calculated for February, April, June, August, October, and December, respectively (Tsarpali et al., 2012).

Danio rerio has also been reported as sensitive to leachate exposure, with reported LC_{50} between 2.2 and 5.7% (Sisinno et al., 2000) and between 15 and 35% (Costa et al., 2019), both following exposure to Brazilian leachate. The leachate toxicity observed herein towards *Artemia salina* and *Danio rerio* may be attributed to high organic matter and NH_3 and NH_4^+ leachate loads, as well as to the presence of metals (Borba et al., 2019).

Regarding earthworm toxicity, exposure to a Brazilian landfill leachate resulted in lethal effects following both an acute contact test ($LC_{50} = 8.0 \pm 0.3\%$) and an acute soil test ($LC_{50} = 31.4 \pm 1.3\%$), attributed to estrogenic activity (660 ± 50 ng L^{-1}) and high ammoniacal nitrogen (2398 mg L^{-1}) and metal levels (Sales Junior et al., 2021). In addition, leachate exposure also led to an escape effect with loss of habitat ($EC_{50} = 31.6 \pm 6.8$ mL kg^{-1}) and to negative reproductive effects, reducing cocoon production and juvenile numbers (Sales Junior et al., 2021).

Risk characterization

Risk characterization is established by quantifying the RQ (European Commission, 2003). Considering short-term toxicity to aquatic organisms, $PNEC_{water}$ values were 0.80 mL L^{-1} for *A. salina* and 0.33 mL L^{-1} for *D. rerio*. Although leachate is a complex matrix, it is possible to estimate environmental concentrations in aquatic environment (PEC_{water}) derived from studies that proposed its application for remediation and reuse. In a study that evaluated the phytoremediation potential of two fiber hemp plant varieties (*Cannabis sativa* L.) through hydroponic cultivation alongside landfill leachate, strong adverse effects on leaf and root growth were observed even at the lowest dose of 100 mL L^{-1} (Kalousek et al., 2020). In another approach, growth inhibition in hydroponic cultivation of the same species was reported at an even lower concentration from 6.48 mL L^{-1} of landfill leachate (Vaverková et al., 2019). A high environmental risk (RQ > 1) for aquatic organisms can be estimated (RQ = 8–20) from this lower concentration. More critical RQ values can be estimated from remediation proposals with satisfactory results. A hydroponic system with cattail (*Typha latifolia*) treated with landfill leachate (300 mL L^{-1}) demonstrated high efficiency for nutrient (nitrogen and phosphorus) and salinity (Na^+ and Cl^-) removal (Xu et al., 2018). Although this study is aimed at remediation, the inclusion of a toxicological approach results in extreme risks to the aquatic environment, of RQ 375–909. In fact, environmental risks are many times underestimated, as pilot-scale studies, constructed wetlands, and soil fertilization are extensively tested and applied for leachate remediation and reuse for biomass production (Bakhshoodeh et al., 2020; Bhagwat et al., 2018; Cretescu et al., 2013; Singh et al., 2017).

Regarding terrestrial organisms, the $PNEC_{soil}$ based on the acute test (72 h) was 0.84 L ha^{-1}. Sales Junior et al. (2021) performed the same test (filter paper contact) and reported a time- and dose-dependent relationship with earthworms exposed to landfill leachate, resulting in low estimated $PNEC_{soil}$ of 0.23 L ha^{-1} (48 h) and 0.13 L ha^{-1} (72 h). On the other hand, a higher $PNEC_{soil}$ 108 L ha^{-1} can be estimated based on an acute soil test performed by Sales Junior et al. (2021), indicating that soil attenuates leachate toxicity. Regarding PEC_{soil}, Jones et al. (2006) in a study on phytoremediation of landfill leachate proposed soil irrigation with 685 L ha^{-1} day^{-1} as a safety limit for a period of 10 years. Other approaches aiming at phytoremediation through landfill leachate discharges on anthropogenic soils and wheat (*Triticum aestivum*) fertilization resulted in high leachate loads in soils of PEC_{soil} 117,400 L ha^{-1} (Cretescu et al., 2013) and 150,000 L ha^{-1} (Singh et al., 2017). In all proposed scenarios, the estimated $PNEC_{soil}$ and PEC_{soil} result in high risks (RQ > 1) for terrestrial organisms.

Conclusions

Physicochemical, microbiological, and ecotoxicological assessments at a closed dumpsite were conducted in the same study for the first time in Brazil. This assessment demonstrated that MSW disposal in dumpsites creates a significant contamination zone by leachate, as no environmental protection practices are applied to these locations. Leachate mycoflora screening was confirmed herein as a valuable tool in environmental impact assessments, preventing the emergence of new fungal species resistant to antifungal agents, as observed in recent candidiasis cases caused by *C. auris*.

High geo-accumulation indices calculated for Co, Br, Se, I, and Au pointed to these elements as the main responsible for soil contamination. Therefore, future studies should assess contamination at different soil depths and in groundwater. Leachate was lethal for *Artemia salina*, *Danio rerio*, and *Eisenia andrei* for at least one leachate pond sample, indicating variations in toxicological responses and highlighting the importance of a multispecies ecotoxicological approach. Furthermore, the risk characterization applied herein resulted in high environmental risks in all proposed scenarios.

Future studies should consider ecotoxicological assessments at sub-lethal leachate concentrations in order to assess chronic effects. Specifically for terrestrial organisms, exposure to native soil from the investigated dumpsite should also be carried out. Investigations on the endocrine activity of leachate samples are also noteworthy, as many studies report the presence of endocrine disruptor compounds in leachate.

Author contribution SFSJ: investigation, formal analysis, writing — original draft; CFM: data curation, writing — review and editing; GdeFA: data curation, formal analysis; DMB: investigation; RAH-D: formal analysis, methodology, data curation; TS'P: data curation, formal analysis; GLdaC: data curation, formal analysis; MMEO: data curation, formal analysis; CETP: writing — review and editing, data curation, formal analysis; FVC: writing — review and editing, supervision; EMS: conceptualization, resources, writing — review and editing, supervision, project administration, funding acquisition.

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Availability of data and materials Not applicable.

Declarations

Ethics approval and consent to participate All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

Competing interests The authors declare no competing interests.

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