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Biodegradation and toxicity of byproducts from the treatment of landfill leachate with hydrotalcite

Nair Conde de Almeida[a,*], Adriano Uemura de Faria[a], Valdenilson José Alves de Oliveira[a], José Silvio Govone[b], Dejanira de Franceschi de Angelis[a]

[a]*Department of Biochemistry and Microbiology – Institute of Biosciences – Universidade Estadual Paulista, UNESP, Av. 24A, nº 1515, Bela Vista, CEP 13506-900, Rio Claro, São Paulo, Brazil, Tel. +55 19 35264184; emails: ncondealmeida@yahoo.com (N.C. de Almeida), aufaria@yahoo.com.br (A.U. de Faria), zito@rc.unesp.br (V.J.A. de Oliveira), dangelis@rc.unesp.br (D.F. de Angelis)*
[b]*Center for Environmental Studies – CEA – Institute of Geosciences and Exact Sciences – Universidade Estadual Paulista, UNESP, Av. 24A, nº 1515, Bela Vista, CEP 13506-900, Rio Claro, São Paulo, Brazil, email: jsgovone@rc.unesp.br*

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ABSTRACT

Despite the toxic potential of landfill leachate, some researchers have suggested its use as fertilizer. However, high leachate concentrations can have negative impacts on the environment. Hydrotalcite has been used for the adsorption and purification of effluents. In this study, leachate in its raw and treated (sludge and leachate) forms was subjected to physicochemical, microbiological, toxicity, and biodegradability analyses. Treatment with hydrotalcite produced good results regarding the removal of conductivity (51%), turbidity (58%), biochemical oxygen demand in 5 d (95%), boron (40%), ammonia (35%), chemical oxygen demand (43%), color (70%), total coliforms, and *Escherichia coli*, but did not remove sodium or chloride and led to an increase in pH. Treatment led to a 21.63% decrease in toxicity to *Artemia* sp. and a 42% decrease in toxicity to *Lactuca sativa* seeds. The raw and treated leachate in the soil inhibited the germination and development of *L. sativa* by 12% and 5%, respectively, in comparison with the control. Landfill leachate at a concentration of 50 m^3/ha initially potentiated bacterial growth and inhibited fungal growth. The microbiota stabilized after 84 d, except in the high concentration trials, in which the inhibition of fungal growth continued. The analyses of the landfill leachate at a concentration of 200 m^3/ha revealed that repeated fertilization could make the soil unviable for planting. The raw leachate at the two concentrations tested was toxic to *Daphnia similis*. The sludge at a concentration of 2.5% stimulated the growth of *L. sativa* and increased its biomass by 42% in comparison with the control, with no negative impact on the soil microbiota or toxicity to *D. similis*. The biodegradation test showed that the inoculum increased the average daily efficiency of the process. Despite the efficient biodegradation (50% in 24 h), the toxic potential of the leachate was not eliminated. Moreover, respirometry proved not to be an effective method for the determination of the biodegradation of the sludge, since the system is influenced by the chemical characteristics of hydrotalcite.

Keywords: Landfill leachate; Hydrotalcite; Landfill leachate toxicity

1. Introduction

Despite the toxic potential of landfill leachate, some researchers have suggested its use as fertilizer. Although waste disposal in soil is the cause of controversy [1], soil is the best and safest medium for the disposal of pollutants, as it is better able to oxidize or precipitate pollutants and remove them from the food chain more safely than air or water. Another benefit of disposing waste in soil regards the possibility of its being used in the recovery of degraded areas or in agriculture as a fertilizer. With the increase in mineral

* Corresponding author.

fertilization costs, the use of byproducts of human activities has become an attractive way to improve soil conditions and reduce production costs [2].

The same characteristics that make landfill leachate a potential source of pollution are also those that make it attractive for agricultural use, such as ammoniacal nitrogen and stabilized organic matter. According to Bayer and Mielniczuk [3], organic matter determines chemical (nutrient availability, cation exchange capacity, and the complexation of micronutrients and toxic elements) and physical (enhanced particle aggregation) characteristics of the soil as well as microbiological characteristics, since organic matter is a source of carbon, energy, and nutrients for micro-organisms.

One of the main concerns regarding the release of effluents into soil is the possibility of causing contamination by heavy metals. In the case of leachate from landfills in the methanogenic phase, the concentration of heavy metals is minimized and these low levels are attributed to the fact that the pH of the leachate produced in the methanogenic phase is alkaline, which contributes to maintaining metals in their insoluble form [4]. Therefore, the concentration of metals in leachate does not constitute a limitation to the use of this product in agricultural activities. However, leachate has a high content of organic matter and dissolved minerals, such as ammoniacal nitrogen, potassium, and sodium, high concentrations of which in the soil can have negative impacts on the environment.

The use of adsorbents has been evaluated as a way to diminish the toxic potential of leachate. Hydrotalcites are double lamellar hydroxides (DLHs) with a high anion exchange capacity that have been successfully used as adsorbents for contaminants and anions, such as borates in industrial effluents [5], as well as in the removal of biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia from leachate [6]. The structure of DLHs is derived from brucite ($Mg(OH)_2$). Mg^{2+} ions are octahedrally organized by hydroxyl groups, with octahedrons sharing edges and forming neutral layers that are maintained and stacked by hydrogen bonds. DLHs have a wide variety of applications as heterogeneous catalysts [7], adsorbents [8,9], and anion exchangers [10] and are also employed in pharmaceutical products [11]. The removal of anions from a solution by DLHs usually occurs through the combination of two processes: anion exchange and adsorption [5]. An example of the removal process through anion exchange is the treatment of water for the removal of Cr(VI) compounds, phosphates, and boron [5,12].

Li and Zhao [13] used magnesium ammonium phosphate to induce N precipitation of leachate generated in Hong Kong landfills that contained a high load of ammoniacal nitrogen (2,000–5,000 mg/L), thus producing nitrogen fertilizer. The use of this fertilizer in the soil produced good results in the stimulation of plant growth.

Gillman [14] reported the use of bentonite and hydrotalcite as adsorbents to treat effluent from a confined animal feeding facility and proposed that the hydrotalcite used in the treatment could be reused as fertilizer.

There is a need for prior knowledge on the characteristics of leachate and the determination of its possible influence on the environment. This requires the evaluation of soil biodegradation through respirometric assays and toxicity analyses. Conventionally, the hazard assessment of landfill leachate is based on the identification of individual contaminants through chemical analyses. Although this procedure may determine the presence of potential contaminants, some toxic pollutants may remain undetected [15].

Assays with test organisms are indicated to evaluate the pollutant potential of landfill leachate. The toxic effect on biological systems is exerted by the combined action of all harmful substances in the sample, including those that are nontoxic by themselves, but affect the physicochemical properties of the system and, consequently, the living conditions of organisms [16]. Thus, the use of bioassays to characterize contaminants in a variety of environmental matrices, such as landfill leachates, has become a powerful screening tool for environmental toxicology [17].

The aims of this study were to (1) characterize the composition of leachate from a solid waste landfill in the city of Rio Claro (southeastern Brazil), (2) determine the efficiency of the hydrotalcite adsorbent with regard to depolluting this leachate through the analysis of physicochemical and microbiological variables, and (3) determine the toxicity and biodegradation of the byproducts of the treatment process.

2. Materials and methods

2.1. Characterization of landfill leachate from Rio Claro Treatment Plant, state of São Paulo, Brazil

The leachate from the landfill was analyzed over a 4-year period with samples collected in both the dry and rainy seasons. As no significant changes in composition were found [6], the last sample (collected July 2017) was used in this study.

The leachate was subjected to the analysis determined by Article 18 of State Decree 8468-1976 [18], which stipulates standards for effluent emission and complementary analysis for the best characterization of samples. The sample collected from the landfill was placed in ice-cooled thermal boxes with the temperature maintained between 5°C and 10°C.

2.1.1. Microbiological analysis

Microbiological analyses were performed immediately after the arrival of the samples at the laboratory.

Microbial count: Total heterotrophic bacteria were counted using the pour-plate technique in plate count agar medium with the addition of 5 ppm of actidione, following Technical Standard L. 5.201 [19]. For fungal counts, the spread-plate method was performed on a sabouraud-dextrose-agar medium with the addition of antibiotics (5 ppm of ampicillin and nalidixic acid). The plates with bacteria were kept at 35°C and those with fungi were kept at 28°C. For total coliform and *Escherichia coli* counts, the samples were diluted 100 times and analyzed using the Colilert® method.

2.1.2. Physicochemical analysis

Conductivity was determined using a conductivity meter (Marte®, model MD-11); pH was determined using a pH meter (Digimed®, model DM-22); and turbidity was determined using a spectrophotometer (Nanocolor®, Macherey-Nagel). Regarding color, the samples were filtered (0.45-μm

membrane filter kit) and analyzed using photometric determination (Nanocolor® spectrophotometer, Macherey-Nagel, λ 433 nm). Settleable solids were analyzed in an Imhoff cone. Chlorides were analyzed using Mohr's method [20]. Ammonia was determined using a standard ammonia ion selective electrode (Thermo Fisher Scientific MA, USA, Orion Products, Espoo, Finland). Phenols, cyanides, sulfides, sulfates, aluminum, arsenic, boron, barium, cadmium, copper, chrome, hexavalent chromium, tin, soluble iron, fluorides, manganese, mercury, nickel, silver, selenium, sodium, and zinc were determined at the São Lucas Laboratory of Environmental Analysis, Rio Claro, SP, Brazil (Table 1).

BOD_5 (BOD in 5 d) and COD were determined according to the Standard Methods [23].

2.2. Treatment with hydrotalcite (LH)

Synthetic hydrotalcite ($Mg_6Al_2(CO_3)(OH)_{16}{\cdot}4H_2O$) (Sigma-Aldrich, Sao Paulo, Brazil) was used in this experiment. The hydrotalcite was first calcined at 500°C on heating ramp of 10°C/min for 3 h in a muffle and then stored in a desiccator.

Treatment was carried out in triplicate in flasks containing 100 mL of leachate with hydrotalcite under agitation (250 rpm) on a shaker table for 30 min at a temperature of 28°C ± 2°C, followed by decantation for 2 h. The amount of hydrotalcite used was based on studies by Almeida et al. [6], who determined a concentration of 4% for the removal of boron from landfill leachate. After treatment, the leachate was filtered through 16-μm filter paper to remove residue from the adsorbent and the sludge from the treatment was oven dried at 60°C prior to the microbiological, physicochemical, and toxicity analyses.

Table 1
Parameters analyzed and methodology used at São Lucas Laboratory of Environmental Analysis

Parameters	Methods
Total metals – arsenic and antimony	Antimony and arsenic (atomic absorption, borohydride reduction) [21] Inductively coupled plasma-atomic emission spectrometry [22]
Total metals	Inductively coupled plasma-atomic emission spectrometry [22] Method: 3030E [23] Method: 3500 Cr B [23]
Dissolved metals	Method 3030E [23] Inductively coupled plasma-atomic emission spectrometry [22]
Mercury	Mercury in liquid wastes (Manual Cold-Vapor Technique) [24] Inductively coupled plasma-atomic emission spectrometry [22]
Anions	Determination of inorganic anions in drinking water by ion chromatography [25]
Cyanides	Method: 4500-Cn^-, D and E [23]
Sulfides	Method: 4500-S^{2-} D [23]

2.3. Toxicity tests

After treatment with hydrotalcite, the sludge, raw and treated leachates were subjected to toxicity tests. For the sludge tests, 10 g were placed in 90 mL of distilled, sterilized water and the flasks were shaken for 20 min. After 7 d decanting, the supernatant was used in the toxicity tests. The results of the toxicity tests (EC_{50}) with *Daphnia similis, Artemia* sp., and *Lactuca sativa* were calculated statistically using the trimmed Spearman–Karber method with the aid of the Jspear program [26].

2.3.1. Acute toxicity test with D. similis

Tests with the sludge, raw and treated leachates were conducted according to Standard NBR 12713 [27].

2.3.2. Acute toxicity test with Artemia sp.

Tests with the raw and treated leachates were conducted according to Standard L05.021/1987 [28].

2.3.3. Toxicity test with L. sativa

Tests with the raw and treated leachates were performed using concentrations of 100%, 75%, 50%, 25%, and 10% and tests with the sludge were performed using concentrations of 2.5%, 5%, and 10%. All tests were conducted in Petri dishes with filter paper in four replicates containing 10 seeds each. The germination capacity and growth of roots and stems were analyzed after 96 h.

2.3.4. Growth assay of L. sativa in soil

Seven treatments were programmed with six replicates. Vessels (8 × 8 × 9 cm; surface area: 64 cm^2) containing 300 g of soil were prepared without leachate (control) and with 200 m^3/ha of raw leachate (RL200), 50 m^3/ha of raw leachate (RL50), 200 m^3/ha of treated leachate (HL200), 50 m^3/ha of treated leached (HL50), 2.5% sludge (S2.5), and 5% sludge (S5). According to Jones et al. [1], the application rate of landfill leachate in soil is 125–250 m^3/ha per year. In this study, the concentration of 200 m^3/ha was chosen to simulate maximum annual application and the concentration of 50 m^3/ha was chosen based on the results of the respirometric experiments, in which a 10% concentration of leachate was used. The vessels with 200 m^3/ha of leachate were prepared with four waterings, with 32 mL of leachate added on the first, third, seventh, and tenth days. The vessels with 50 m^3/ha of leachate were prepared with the addition of 32 mL of leachate on the tenth day. The vessels with sludge were prepared with 7.5 g (2.5%) and 15 g (5%) mixed in approximately 3 cm of the surface layer of the soil.

The control and vessels with sludge received 32 mL of distilled water on the tenth day. Planting was performed 12 d after the addition of products, during which all vessels were kept in greenhouse at room temperature and received 32 mL of distilled water every 4 d. The quantities of water and leachate were determined considering 50% of the water retention capacity of the soil to avoid the drainage of liquids from the vessels.

After soil preparation, one of the replicates of each treatment was submitted to the initial microbiological and toxicity analysis. Ten *L. sativa* seeds were planted in each pot, totaling 50 seeds (five vessels) for each assay. The vessels were watered with 32 mL of distilled water as needed.

Plant growth was monitored for 84 d. The plants were then harvested, washed, and dried at 105°C to obtain the dry mass. Subsequently, the material was heated at 550°C in a muffle to obtain the organic matter. Due to the difficulty in separating the soil residue from the roots, the comparison of root growth was made only in terms of organic matter.

Soil was collected according to Technical Standard L.6.245 [29] from the municipality of Rio Claro in the state of São Paulo, Brazil (latitude: −22.36633347; longitude: −47.52396405). Samples were taken from the surface layer of uncontaminated sites. The soil samples were analyzed by the Campinas Soil and Fertilizer Analysis Institute Ltd., Sao Paulo, Brazil. Table 2 displays the physicochemical characteristics of the soil.

The statistical design was determined by Tukey's test [30], comparing the amount of shoot organic matter of two independent groups separately: first – control, S2.5, and S5; second – control, RL50, and HL50.

2.3.4.1. Toxicity test with microcrustacean D. similis *and initial and final microbial counts in soils*

Samples (10 g) of each soil were placed in 90 mL of distilled, sterilized water and the flasks were shaken for 20 min. After 2 h of decantation, the supernatant was submitted to the microbial count described in Section 2.1.1. Micro-organisms were quantified by counting the colony-forming units (CFUs) per g of dry soil. After 7 d of decantation, the supernatant was submitted to the toxicity test described in Section 2.3.1.

2.4. Respirometric assay

The Bartha and Pramer [31] respirometric method is a simple technique for determining the biodegradation of pollutants in soil by quantifying the CO_2 released into the system [32]. This is a low-cost, simple method for evaluating biodegradation rates of contaminants in soil. The biodegradation experiments were conducted according to the OECD [33] at a temperature of 28°C in Bartha biometer flasks (250 mL) to measure microbial CO_2 production. Triplicate flasks were prepared with 50 g of soil (described in Table 2) and water, RL, treated leachate and sludge of the treatment with and without inoculum according to the protocol displayed in Table 3 and incubated at 28°C ± 2°C in the dark. The quantities of leachate and water added to the treatments were adjusted to 70% of the water retention capacity of the soil. The test lasted 52 d for the raw and treated leachates and 129 d for the sludge.

2.4.1. Inoculum obtainment

The RL was diluted to 50% with distilled water, enriched with 1% sugarcane molasses and inoculated with the isolated micro-organisms. The flask was then shaken for 48 h at room temperature. The microbial count was performed as described in Section 2.1.1.

To estimate the initial microbial population of each test, the results from the counts listed in Table 9 were considered, multiplied by the quantities added to each Bartha flask and divided by 50 (referring to 50 g of soil). The results are displayed in Table 10.

2.4.2. Statistical analysis

The statistical design was determined by the preliminary application of Shapiro–Wilk normality test [30]. The Student's *t*-test was used for data with normal distribution and the nonparametric Wilcoxon test was used for data with nonnormal distribution [30]. The Wilcoxon test was used to evaluate the inoculum performance in terms of daily CO_2 production. The Student's *t*-test was used to evaluate the difference in biodegradation between raw and treated leachates considering the biodegradation efficiency data.

3. Results and discussion

3.1. Characterization of RL, leachate treated with HL, and sludge from treatment (S)

The analysis of the treated leachate and sludge was conducted only for the parameters with results not in compliance

Table 2
Physicochemical characteristics of soil

Macronutrients (mmolc/dm^3)							*V*%	Ratios	
K	Ca	Mg	H + Al	Al	SB	CEC	39.6	Ca/Mg	Mg/K
1.1	16	3	31	1	20.3	51.3		5.33	2.72
Micronutrients (mg/dm^3)							pH	OM	P_{res}
S	Na	Fe	Mn	Cu	Zn	B	$CaCl_2$	g/dm^3	g/dm^3
8	6	40	3.3	0.3	2.3	0.21	5.1	22	3.0
Sand									
Thick	Fine	Clay	Silt	Class			Subclass		
55.8	27.3	10.9	6.0	Sandy silt soil			Sand		

P, K, Ca, Mg: anionic + cationic exchange resin; Fe, Mn, Ca, Zn: DTPA-TEA Extractor; B: 0.125% barium chloride extractor.

Table 3
Protocol for respirometric experiment

	Treatments	Water (mL)	Inoculum (mL)	Raw leachate (mL)	Treated leachate (mL)	Sludge (g)
C	Soil (s)	8.0	–	–	–	–
Ci	s + inoculum (i)	7.5	0.5	–	–	–
RL5	s + 5% Raw	5.5	–	2.5	–	–
RL5i	s + 5% Raw + i	5.0	0.5	2.5	–	–
RL10	s + 10% Raw	3.0	–	5.0	–	–
RL10i	s + 10% Raw + i	2.5	0.5	5.0	–	–
HL5	s + 5% Treated	5.5	–	–	2.5	–
HL5i	s + 5% Treated + i	5.0	0.5	–	2.5	–
HL10	s + 10% Treated	3.0	–	–	5.0	–
HL10i	s + 10%Treated + i	2.5	0.5	–	5.0	–
S2.5	s + 2.5% Sludge	8.0	–	–	–	1.25
S2.5i	s + 2.5% Sludge + i	7.5	0.5	–	–	1.25
S5	s + 5% Sludge	8.0	–	–	–	2.5
S5i	s + 5% Sludge + i	7.5	0.5	–	–	2.5
S10	s + 10% Sludge	8.0	–	–	–	5.0
S10i	s + 10% Sludge + i	7.5	0.5	–	–	5.0

with the Brazilian environmental legislation or with values considered to impact the balance of the natural environment. The means are displayed in Tables 4–6.

3.1.1. Microbial count

The low number of heterotrophic bacteria and fungi demonstrates that the raw landfill leachate does not offer favorable conditions for microbiological growth and may hinder biological treatment based only on biodegradation. Total coliforms and *E. coli* counts were above the maximum limits permitted for discarding in class 2 rivers according to Article 11 [18]. After treatment with hydrotalcite, *E. coli* was no longer found in the leachate or sludge, indicating that this bacterium did not survive treatment. The total coliforms only appeared in the sludge. The adsorbent demonstrated good precipitation capacity, dragging the micro-organisms from the samples to the sludge (Table 4).

3.1.2. Physicochemical analysis

Among the parameters listed in Table 5, only settleable solids were in accordance with legislation. The pH of the RL was within the permitted range, but hydrotalcite alkalinized the leachate, making it necessary to adjust the pH before its disposal. Regarding the other parameters, although legislation does not determine maximum indices for the release of effluents, the values found have a high probability of causing an impact on microbiological treatment or the environment in which the leachate is discarded.

Conductivity of the RL was above value for freshwater bodies and was situated between values for saline and marine water. After treatment with hydrotalcite, conductivity decreased by approximately 40% (15 mS/cm), but was still too high for discarding in freshwater bodies, in which the mean ranges from 0 to 800 μS/cm [34].

Hydrotalcite removed about 78% of true color, but the values found were still higher (Table 4) than the maximum permitted color value of 15 U.C. in water distributed to the population [35]. According to Oliveira et al. [36], 75 PtCo/L is higher than acceptable for a class 2 river. Likewise,

Table 4
Quantification of fungi, bacteria, total coliforms, and *E. coli* of raw leachate (RL), leachate treated with hydrotalcite (HL) and sludge from treatment (S)

Parameters	RL	HL	S	MVPr
Heterotrophic bacteria (10^3 CFU/mL)	14	2.7	6.8	NA
Fungi (CFU/mL)	20	<10	<10	NA
Total coliforms (MPN/100 mL)	20,460	–	1,000	5,000
E. coli (MPN/100 mL)	240	–	–	100

MVPr: maximum value permitted for class 2 rivers by article 11 [18]; NA: Not applicable; MPN: most probable number.

Table 5
Analyses of pH, conductivity, true color, turbidity, settleable solids, COD, BOD_5, and TOC of raw landfill leachate (RL) and leachate treated with hydrotalcite (HL)

Parameters	RL	HL	MVP
PH	8.1	9.9	6–9
Conductivity (μS/cm)	25,740	15,250	NA
True color (Pt Co/L)	7,060	1,572	NA
Turbidity (NTU)	98	49	NA
Settleable solids (mL/L)	<0.1	<0.1	<1.0
COD (mg/L)	3,250	2,100	NA
BOD_5 (mg/L)	418	23.5	60
TOC (%)	0.24	0.17	NA

MVP: maximum value permitted by article 18 [18]; NA: not applicable.

hydrotalcite removed about 49% of the turbidity from the leachate, but values remained higher than 98 NTU (Table 4). Maximum turbidity permitted in the public water supply is 5.0 NTU [35].

Young landfills (<5 y) produce leachate characterized by high concentrations of BOD_5 (4,000–1,500 mg/L), COD (25,000–60,000 mg/L), and pH 4 [37]. The values displayed in Table 5 show that the leachate studied was in the methanogenic phase, with mean COD and BOD_5 around 3,250 and 418 mg/L, respectively, and pH above 8. Treatment with hydrotalcite resulted in a 94% removal rate of BOD_5, adjusting the leachate to the norm established by Ref. [18], which determines that a treatment should reduce BOD_5 by at least 80%. The BOD_5 removal by this adsorbent reached levels comparable with the rates described by Hashemi et al. [38] using filter membranes. Treatment achieved COD and TOC (total organic carbon) removal rates of 35% and 33%, respectively. Using heterogeneous photocatalysis, Chemlal et al. [39,40] obtained a 40%–92% reduction in COD at pH 5.

Table 6 displays results of the metal and nonmetal analyses. Based on Ref. [18], only boron was above the limit established by legislation. Boron is found in the composition of glass, fiberglass additives, ceramics, insecticides, and fertilizers. The appearance of this element at an amount above that expected in the leachate is likely due to the inclusion of industrial waste in the landfill [6]. Hydrotalcite removed 81% boron from the leachate. Delazari et al. [5] obtained 86% boron removal using a hydrotalcite solution of 30 mg/L. The surface of hydrotalcite has residual positive charges that are compensated by adsorbed anionic species, which, in this case, is the borate ion.

Current legislation does not establish maximum sodium or chloride values in effluents, but science demonstrates that these elements can cause an osmotic imbalance in receiving water bodies at the point of release as well as the destabilization of soil, thereby affecting plant growth. In the landfill leachate, the sodium concentration reached values higher than 1,700 mg/L. The limit established for sodium in drinking water is 200 mg/L [41]. Treatment led to a slight reduction in this index, but the value remained far beyond the desired level.

High levels of chlorides were also found in the samples (Table 6), demonstrating that treatment did not remove these substances. Souza [42] found that hydrotalcite was able to remove the anions F^-, SO_4^{-2}, and PO_4^{-3}, but not Cl^-, Br^-, or NO_3^-. Competition among anions for hydrotalcite adsorption sites leads to lower removal rates. Site preference was for the anion with the highest effective load, which is in agreement with data described by Das et al. [43] and Tong et al. [44]. Due to the complex composition of the leachate, this type of competition must also occur.

The high levels of ammonia demonstrate that the leachate is in an intermediate phase of aging, acquiring characteristics of the methanogenic phase, in which ammoniacal nitrogen levels are between 3,000 and 5,000 mg/L [37]. The hydrotalcite removed ammonia by about 12%.

As hydrotalcite has magnesium and aluminum in its constitution, additional analyses were performed to determine whether excessive amounts of these two elements would be added to the leachate. However, neither element had high indices after treatment (Table 6).

Table 6
Chemical analysis of metals and nonmetals in raw landfill leachate (RL), treated with hydrotalcite (HL) and residual sludge from the treatment (S)

Metals	RL (mg/L)	HL (mg/L)	S (mg/kg)	MVP (mg/L)	MVPr (mg/L)
Arsenic	0.043	–	–	0.2	0.1
Aluminum	0.383	0.325	28,444	NA	NA
Barium	0.178	–	–	5.0	1.0
Boron	**10.63**	**1.966**	**43**	**5**	**5**
Cadmium	<0.001	–	–	0.2	0.01
Lead	<0.005	–	–	0.5	0.1
Copper	0.017	–	–	1	1
Chrome	0.160	–	–	5.0	0.05
Hexavalent chromium	<0.100	–	–	0.1	0.1
Tin	0.025	–	–	4.0	2.0
Soluble iron	1.304	–	–	15	15
Fluoride	<10.000	–	–	10	10
Manganese	0.178	–	–	1	1
Magnesium	61	89	160,288	NA	NA
Mercury	<0.0002	–	–	0.01	0.002
Nickel	0.163	–	–	2	2
Potassium	875	978	3,433	NA	NA
Silver	<0.005	–	–	0.02	0.02
Sodium	**1,719**	**1,531**	**3,812**	**NA**	**NA**
Selenium	<0.005	–	–	0.02	0.01
Zinc	0.234	–	–	5	5
Nonmetals	LN	LH	S	MVP	MVPr
Chlorides	**2,617**	**2,696**	**–**	**NA**	**NA**
Cyanide	<0.020	–	–	0.2	0.2
Sulfide	0.447	–	–	NA	NA
Sulfate	<50.000	–	–	NA	NA
Phenol	<0.05	–	–	0.5	0.001
Phosphorus	9.3	0.307	580	NA	NA
Ammonia	**2,267**	**2,003**	**–**	**NA**	**0.5**

MVP: maximum value permitted by article 18 [18]; MVPr: maximum value permitted for class 2 rivers by article 11 [18]; NA: not applicable; –: not quantified. The bold is to highlight the parameters that are in quantity above the desired ones, being able to impact the environment.

In the characterization of the sludge from the treatment with hydrotalcite, high amounts of nitrogen, magnesium, phosphorus, and potassium were found, which suggests its use as a fertilizer (Table 6). However, the amounts of sodium and aluminum may be limiting factors and toxicity experiments are therefore required.

3.2. Toxicity tests of raw landfill leachate, leachate treated with hydrotalcite, and sludge from treatment

3.2.1. Acute toxicity test with D. similis *and* Artemia *sp.*

Bioindicator tests are important tools for the evaluation of the toxicity of a given material. To determine toxicity in

Table 7
Toxicity test with *D. similis* and *Artemia* sp. with raw landfill leachate (RL), leachate treated with hydrotalcite (HL), and sludge from treatment (S)

	RL	HL	S
D. similis EC_{50}	1.23	1.78	NT
Artemia sp. EC_{50}	19.56	23.79	NA

EC_{50}: minimum concentration capable of causing harmful effect on 50% of test organisms; NT: not toxic; NA: not applicable.

this study, the pH of the treated leachate was adjusted to 7.9 with the use of phosphoric acid, since *D. similis* would not withstand the pH 9.9 resulting from the treatment. The introduction of phosphoric acid caused the precipitation of salts, probably ammonium phosphates. Li and Zhao [13] used $MgCl_2{\cdot}6H_2O^+/Na_2HPO_4{\cdot}12H_2O$ to precipitate NH_4^+ –N from the leachate of a landfill in Hong Kong, obtaining a recovery rate of 92% of NH_4^+ –N in the precipitate. The results of the acute toxicity tests showed that, despite the adsorbent efficiency in the removal of important substances, the decrease in toxicity to *D. similis* was not effective. However, trials with *Artemia* sp. showed lower levels of toxicity (Table 7). *D. similis* is a freshwater microcrustacean and therefore cannot tolerate high levels of conductivity and salinity, which certainly influenced the survival of these organisms. Thus, it was not possible to detect an effective difference in toxicity between the treated and RLs. In contrast, *Artemia* sp. are marine organisms capable of tolerating the high concentrations of chloride ions found in most leachates and are adequate for the determination of toxicity due to sources other than chlorides [45]. Therefore, the high conductivity and high amount of chlorides in the landfill leachate did not alter the survival of this microcrustacean. Treatment with hydrotalcite removed 21.63% toxicity of the landfill leachate (Table 7). The toxicity of the landfill leachate in the methanogenic phase is probably due to the high concentration of ammonia. Svensson et al. [45] filtered a leachate sample through an ion exchange membrane to remove ammonia and heavy metals and found that toxicity was eliminated, but when the samples were filtered through activated carbon

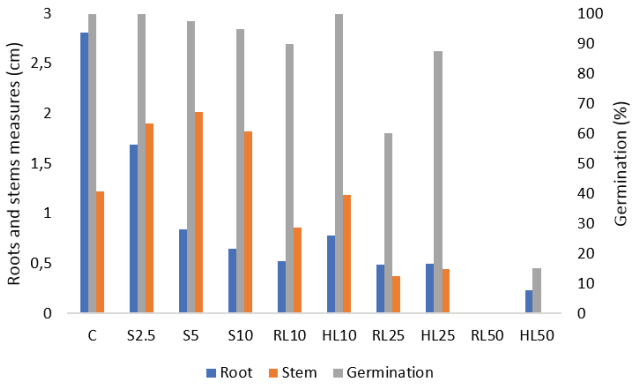

Fig. 1. *L. sativa* root and stem measurements and germination after 96 h of growth in soil with water (C), 10%, 25%, and 50% raw landfill leachate (RL10, RL25, and RL50) and leachate treated with hydrotalcite (HL10, HL25, and HL50) and 2.5%, 5%, and 10% sludge from treatment (S2.5, S5, and S10).

to remove the organic fraction, most of the toxicity persisted. This led to the conclusion that the toxicity was mainly due to ammonia, since the concentration of heavy metal was not high enough to induce toxicity. In this study, the sludge demonstrated no toxicity to *D. similis*.

3.2.2. *Toxicity test with* L. sativa

Fig. 1 shows that the percentage of germination in the sludge tests remained high, with levels close to that found in the control. However, significant differences were found regarding root and stem development. The roots in the treatments with 2.5%, 5%, and 10% sludge were approximately 40%, 70%, and 75% smaller than the control, respectively, whereas stem development surpassed that of the control by 55%, 64%, and 45%, respectively. The availability of nutrients alters root architecture. In poorer soils, root development is greater due to the nutrient demands, whereas less root development is found in richer soils [46]. Thus, it can be concluded that the sludge concentrates important nutrients for plant development and demonstrated no toxicity under these circumstances.

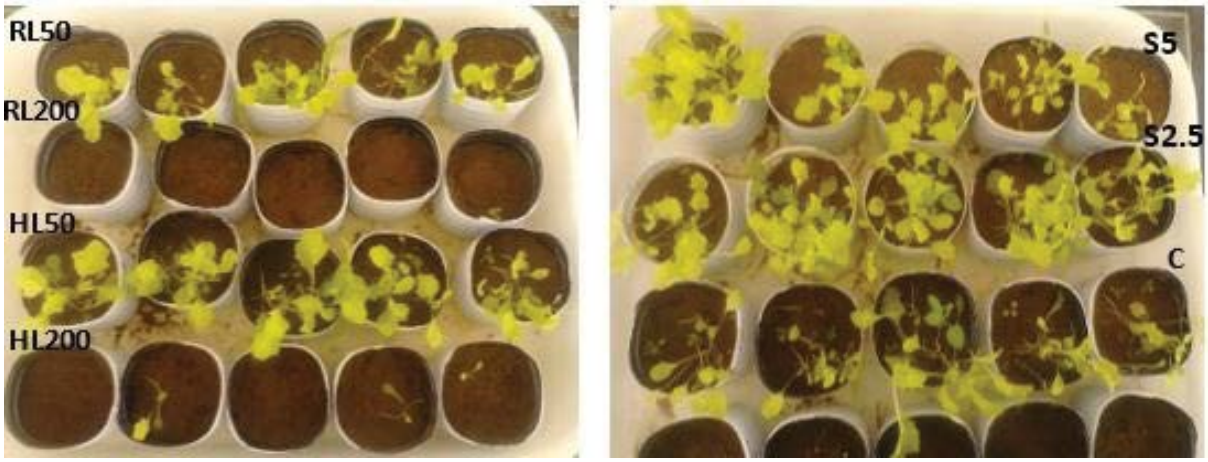

Fig. 2. Vessels 42 d after planting.

Fig. 3. *L. sativa* development in S2.5, S5, RL50, HL50 and control treatments at 84 d.

Germination was inhibited in the tests with the raw (RL) and treated (HL) leachate at concentrations 75% and 100%, demonstrating the toxic potential of these substances. At concentrations below 50%, the treated leachate performed better than the RL, with 28% greater germination in the HL25 assay compared to the RL25, but root and stem growth was about 82% lower compared to the control. In the HL10 assay, no inhibition of germination occurred and stem growth was about 27% greater compared with the RL10 treatment, approaching the stem measurements found in the control. At concentrations 10% and 25%, the difference in the level of toxicity between treated and RL was evidenced by the results regarding germination and stem development (Fig. 1). The EC_{50} was 25.22 for the RL and 35.78 for the treated leachate, which is an improvement of approximately 42% in terms of *L. sativa* seed germination after treatment.

3.2.3. *Growth assay of* L. sativa *in soil*

L. sativa germination in the control was 92%. In contrast, no germination was found in the vessels with 200 m^3/ha RL. Only three seeds germinated in the vessels with 200 m^3/ha treated leachate about 20 d later than all other treatments and did not develop normally (Fig. 2). This demonstrates that repeated fertilization with landfill leachate can saturate the soil and render it unviable for planting. Bowman et al. [47] and Williamson [48] noted soil salinity problems and vegetation scorching during long-term field trials (>1 year) involving the application of leachate to grassland. Moreover, any disturbance caused by pollutants in the soil may impair major microbiological factors and biochemical processes related to biogeochemical cycles [4,49].

With 50 m^3/ha (RL50 and HL50), the germination rate was 54% and the treated leachate test produced larger specimens with wider leaves compared to the RL (Fig. 3).

The vessels with S2.5 sludge exceeded the control, with a germination rate of 96%. Fig. 3 shows better plant development with 2.5% sludge in the vessels. With 5% sludge, germination decreased to 64% and the plants did not develop with the same vigor as in the S2.5 treatment.

The mean dry weight and organic matter of each treatment are displayed in Fig. 4. The S2.5 treatment potentiated plant growth, with the roots and shoots (stem and leaves) developing about 100% and 45% more, respectively, than in the control. Gillman [14] found that hydrotalcite used in the treatment of swine breeding effluent adsorbs macronutrients and micronutrients. When used as fertilizer, the release of these nutrients is gradual, which helps avoid leaching losses and stimulate plant growth. This gradual release also prevents excess phosphates from being carried to the surface of water bodies and inducing algal blooms. Increasing the amount of sludge to 5% resulted in the inhibition of growth, which demonstrates that a previous evaluation of the application conditions of the sludge is required for each type of plant and soil, as excess nutrients also inhibit plant growth. Moreover, this sludge contains sodium, which can destabilize the osmotic balance of the soil. Tukey's test [30] demonstrated significant differences among the control, S2.5, and S5 treatments ($p < 0.01$).

Although the treated leachate performed better than the RL as fertilizer for the development of *L. sativa*, the 50 m^3/ha treatment did not provide an increase in productivity, as a reduction of about 0.9% in the organic matter was found in comparison with the control (Fig. 4). This indicates that long-term sustainable leachate irrigation schemes are likely to work at below-optimal rates of biomass production [1]. Tukey's test [30] demonstrated significant differences ($p < 0.01$) between the HL50 and RL50 treatments as well as between the RL50 and control treatments, whereas the difference between HL50 and control treatments was nonsignificant.

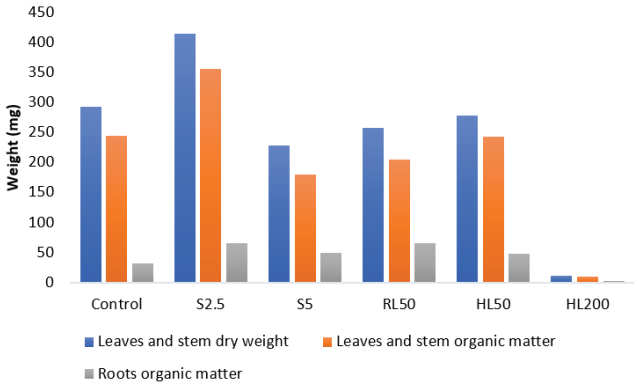

Fig. 4. Dry weight of shoots (stem and leaves), organic matter of shoots and roots of assays with 2.5% and 5% sludge (S2.5 and S5), 50 and 200 m^3/ha of treated leachate (HL50 and HL200) and 50% raw leachate (RL50) after 84 d.

Table 8
Initial and final microbial soil counts in treatments: control (C), 200 and 50 m³/ha of raw leachate (RL200 and RL50), 200 and 50 m³/ha of hydrotalcite treated leachate (HL200 and HL50) and 2.5% and 5% sludge of treatment (S2.5 and S5)

	Heterotrophic bacteria (10^5 CFU/mL)		Fungi (10^3 CFU/mL)		Total coliforms (MPN/100 mL)		*E. coli* (MPN/100 mL)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Control	7.0	4.2	61.5	35.9	–	–	–	–
RL200	2.6	3.6	12.1	2.59	7,300	5,400	–	–
RL50	13.2	4.8	23.0	31.0	200	200	–	–
HL200	16.1	4.9	2.8	2.8	–	–	–	–
HL50	139.0	5.2	2.5	12.7	–	–	–	–
S2.5	19.0	3.9	2.0	14.9	250	200	–	–
S5	23.0	4.0	2.3	22.8	550	500	–	–

3.2.3.1. Microbial count of vessels

The initial count (Table 8) revealed that a high concentration of RL caused a decrease in the amount of bacteria in the soil, whereas increases were found in the other samples. The HL50 treatment stimulated growth, with a 100-fold increase in the number of bacteria in the soil. All products added to the soil caused a decrease in the amount of fungi, especially the HL200 and HL50 treatments. Pattison et al. [50] found the inhibition of the growth of arbuscular mycorrhizal fungi in soil under the action of landfill leachate. Chen et al. [51] found that fungi developed better in soil at acidic pH. Therefore, the high pH of the samples may have rendered the medium unsuitable for fungal growth. The RL and the

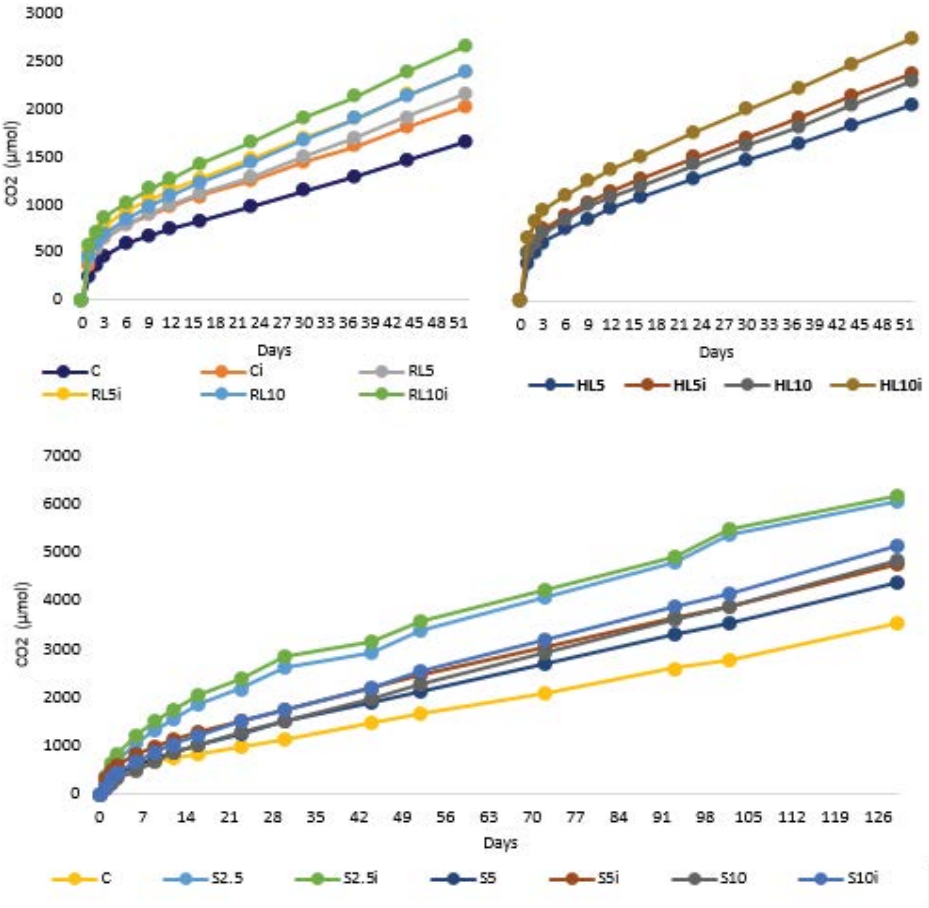

Fig. 5. Cumulated CO_2 production of soil (C), soil with inoculum (Ci), 5% and 10% raw leachate without and with inoculum (RL5, RL5i, RL10, and RL10i), leachate treated with hydrotalcite without and with inoculum (HL5, HL5i, HL10, and HL10i) and 2.5%, 5%, and 10% sludge without and with inoculum (S2.5, S2.5i, S5, S5i, S10, and S10i).

sludge introduced total coliforms that were not detected in the control and remained in the final count. *E. coli* was not found in any assay. The final count (Table 8) indicates that stabilization of the bacterial population in the soil occurred after 84 d and the count in all trials was around 10^5 CFU/mL, which is very close to that of the control. The final count of fungi (around 10^4 CFU/mL) shows that the soil conditions returned to being propitious to fungal growth after 84 d. The two exceptions were the RL200 and HL200 treatments, where the inhibitory effect remained.

The soil in the S2.5, SL5, HL50, and HL200 vessels exhibited no toxicity, whereas the soil in the RL50 and RL200 vessels was toxic to *D. similis*. This demonstrates that the use of leachate at these concentrations without prior treatment can cause harm to the environment.

3.3. Respirometric assay

Fig. 5 shows that CO_2 production was intensified in all experiments in which the inoculum was introduced. In the comparison of the control trials without (C) and with inoculum (Ci), the introduction of the inoculum increased CO_2 production by 23% in 52 d. This shows that the inoculum produced from the leachate has a greater capacity than the autochthonous micro-organisms in the soil to biodegrade the organic matter available in the environment.

The results of the Wilcoxon test [30] for the evaluation of the positive influence of the inoculum on biodegradation was significant for all assays: C and Ci ($p = 0.0022$), RL5 and RL5i ($p = 0.006$), RL10 and RL10i ($p = 0.0029$), HL5 and HL5i ($p = 0.0029$), HL10 and HL10i ($p = 0.0022$), and S2.5 and S2.5i ($p = 0.0007$).

Table 9 shows the microbial counts used to estimate the initial amount of micro-organisms in the respirometric experiment. The initial and final counts are displayed in Table 10.

All tests with the raw and treated leachates demonstrated good biodegradation efficiency. About 50% biodegradation was achieved in all leachate tests in only 24 h (Fig. 6) and all tests reached efficiency above 100%, which lends support to the hypothesis that the micro-organisms in the leachate were able to biodegrade the organic compounds in the soil as well as those in the leachate itself. As old landfill leachate has very recalcitrant organic matter, the micro-organisms that live under these conditions develop mechanisms and metabolic pathways capable of ensuring their survival. When these micro-organisms come into contact with organic matter in

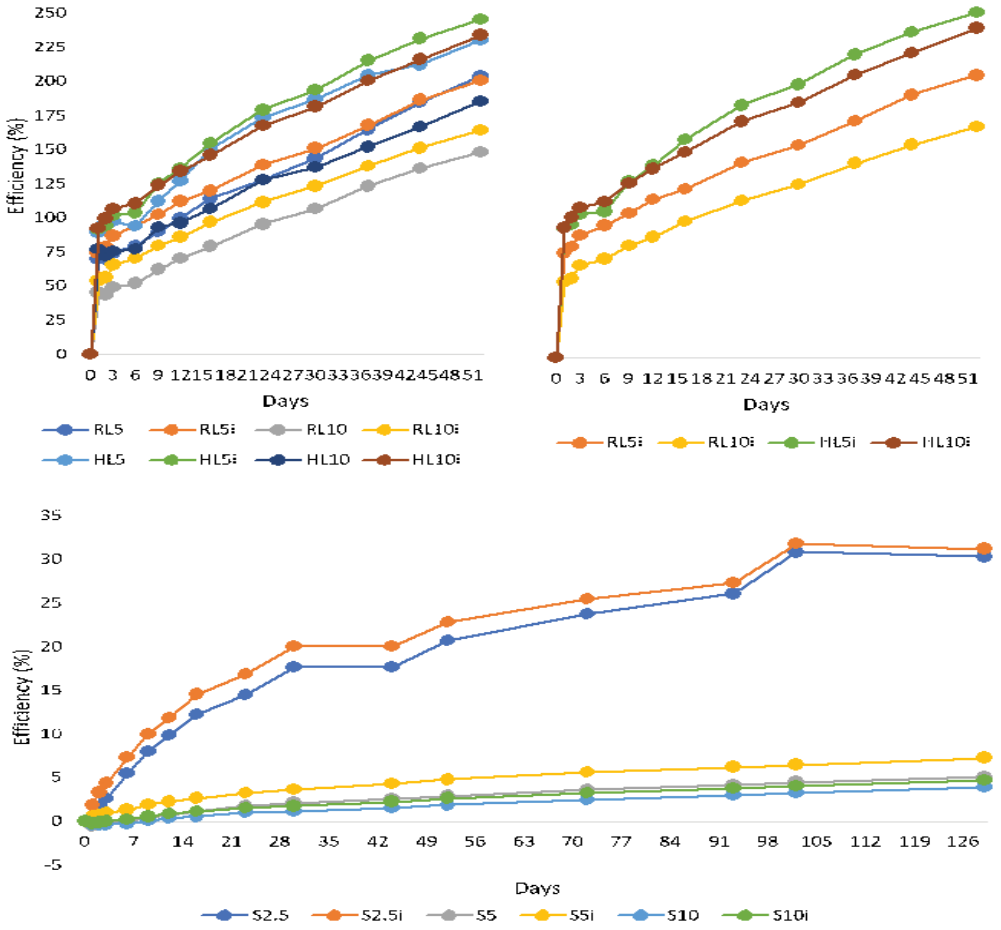

Fig. 6. Efficiency of biodegradation of 5% and 10% raw leachate without and with inoculum (RL5, RL5i, RL10, and RL10i), treated leached without and with inoculum (HL5, HL5i, HL10, and HL10i), and 2.5%, 5%, and 10% sludge without and with inoculum (S2.5, S2.5i, S5, S5i, S10, and S10i).

Table 9
Microbial counts in soil, inoculum, sludge (S), raw (RL), and treated (HL) leachates

	Heterotrophic bacteria	Fungi
Soil (CFU/g dry soil)	1.25×10^5	1.00×10^3
Inoculum (CFU/mL)	2.45×10^8	1.75×10^5
S (CFU/g)	6.80×10^3	<10
RL (CFU/mL)	1.40×10^4	20
HL (CFU/mL)	2.74×10^3	<10

Table 10
Initial and final microbial counts of control without and with (C and Ci), 5% and 10% raw leachate without and with inoculum (RL5, RL5i, RL10, and RL10i), leachate treated with hydrotalcite without and with inoculum (HL5, HL5i, HL10, and HL10i), and 2.5%, 5%, and 10% sludge without and with inoculum (S2.5, S2.5i, S5, S5i, S10, and S10i)

	Heterotrophic bacteria (10^5 CFU/g)		Fungi (10^3 CFU/g)	
	Initial	Final	Initial	Final
C	1.25	3.30	1.00	9.70
Ci	25.75	26.00	2.75	8.00
RL5	1.25	3.30	1.00	3.75
RL5i	25.75	17.50	2.75	3.55
RL10	1.26	4.50	1.00	3.60
RL10i	25.76	13.00	2.75	3.75
HL5	1.25	3.30	1.00	3.70
HL5i	25.75	24.00	2.75	2.60
HL10	1.25	3.40	1.00	1.90
HL10i	25.75	22.00	2.75	3.80
S2.5	1.25	6.0	1.00	7.30
S2.5i	25.75	54.00	2.75	13.00
S5	1.26	4.40	1.00	4.20
S5i	25.76	30.00	2.75	4.60
S10	1.26	5.40	1.00	4.60
S10i	25.76	29.00	2.75	3.60

soil, they are better able to metabolize it than native micro-organisms. This indicates the possibility of introducing landfill leachate in areas contaminated with recalcitrant material in order to improve biodegradation and the bioremediation of such areas. Campos et al. [52] used soil with landfill leachate to aid in the degradation of polycaprolactone and polypropylene blend films.

In this experiment, efficiency decreased when the amount of leachate was increased (Fig. 6). The increase in initial carbon did not promote a proportional increase in biodegradation. Thus, the tests with concentrations of 5% obtained better results compared to tests with concentrations of 10%, although both reached good biodegradation levels.

The biodegradation efficiency of the treated leachate (HL5 and HL5i) was, on average, 25% better than the RL (RL5 and RL5i). When the concentration was increased to 10%, the biodegradation efficiency of the treated leachate was, on average, 48% better compared with the RL under the same condition. This result was expected since the treated leachate introduced less organic matter into the system and had fewer toxic products. The Student's *t*-test [30] revealed significant improvements in the biodegradability of the treated leachate compared with the RL in all the trials ($p < 0.0001$).

In the sludge tests, negative results were found regarding the biodegradation efficiency of S5, S10, and S10i. Moreover, the 5% and 10% sludge tests did not obtain good results, with a maximum efficiency of 7.2% in the S5i assay at the end of 129 d. The tests with 2.5% sludge showed an improvement in biodegradation efficiency, reaching about 30% at 102 d (Fig. 6). These findings suggest that the sludge caused the inhibition of microbial activity, but the microbial counts (Table 10) showed an increase in soil microbiota with the addition of the sludge.

The sludge from the treatment was basically hydrotalcite mixed with the leachate components that it was able to adsorb. Researchers, such as Reijers et al. [53], have shown that this adsorbent is very reactive and has a considerable ability to bind to CO_2. Therefore, as the biodegradation occurred, the CO_2 produced may have been recaptured by the hydrotalcite, making it impossible to react with KOH in the trap of Bartha flasks, thereby impeding its precise quantification.

4. Conclusions

Hydrotalcite achieved good removal levels with regard to color, turbidity, COD, BOD_5, TOC, boron, total coliforms, and *E. coli*, but was less effective at removing ammonia and conductivity and was unable to decrease chloride and sodium levels. The use of hydrotalcite made the leachate alkaline, making necessary to adjust the pH before its disposal.

Treatment with hydrotalcite lowered the toxicity to *Artemia* sp. by 21.63%, with an EC_{50} of 23.59. The high conductivity and salinity influenced the survival of *D. similis* and it was therefore not possible to identify the difference in toxicity between the raw and treated leachate for this microcrustacean.

The *L. sativa* seed germination tests revealed that treatment with hydrotalcite lowered the toxicity by 42%, with an EC_{50} of 35.78.

The use of leachate in the soil had an inhibitive effect on *L. sativa* germination and development, which was about 12% less in the vessels with RL and 5% less in the vessels with treated leachate compared to the control. The use of 200 m^3/ha leachate in the soil resulted in almost total inhibition of germination, demonstrating that repeated applications of landfill leachate can saturate the soil and make it unviable for planting.

With regard to soil micro-organisms, the leachate initially potentiated bacterial growth and inhibited fungal growth. However, stabilization of the microbiota occurred after 84 d, when the counts were close to those found in the control, except in the high concentration assays (RL200 and HL200), in which the fungal inhibition persisted. The toxicity tests of percolated soil from the vessels showed that both concentrations of the RL were toxic to *D. similis*.

The addition of 2.5% sludge to the soil stimulated the growth of *L. sativa* and increased plant biomass by 42% in comparison to the control, with no negative impact on the

soil microbiota and no toxicity to *D. similis*. However, the use of 5% sludge was not favorable to *L. sativa* development.

The biodegradation test with 5% leachate showed that the inoculum introduced encouraged biodegradation, increasing the average daily efficiency of the process by 6% for the treated leachate and 9% for the RL.

Although all the leachate tests achieved good results with regard to biodegradation, reaching 50% in only 24 h, the experiment with *L. sativa* showed that the addition of leachate to soil can inhibit germination and plant development, demonstrating the toxic potential of the leachate.

The respirometry experiment did not prove to be an efficient technique for the determination of the biodegradation of the sludge, since the system is influenced by the chemical characteristics of hydrotalcite.

Although the results obtained show a decrease in the toxicity of the treated leachate and good results in relation to the use of 2.5% sludge in soil, the introduction of these products in the environment must be studied strenuously with regard to various aspects. There are many parameters to take into account in order to ensure the safety of the use of this waste product as a fertilizer. This is a preliminary work, which can serve as a basis for further study.

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References

[1] D.L. Jones, K.L. Williamson, A.G. Owen, Phytoremediation of landfill leachate, Waste Manage., 26 (2006) 825–837.
[2] M.J. Tedesco, P.A. Selbach, C. Gianello, F.D.O. Camargo, Resíduos orgânicos no solo e os impactos no ambiente, Fundamentos da matéria orgânica do solo: ecossistemas tropicais e subtropicais, 2 (1999) 113–136.
[3] Agronômicas, Informações, C. Bayer, J. Mielniczuk, Dinâmica e função da matéria orgânica, Culturas de cobertura isoladas e/ou consorciadas na produção de massa seca, produtividade de milho e soja, atributos químicos e matéria orgânica do solo, 34.6 (2015) 16.
[4] C.A. Santos, L.C. Panchoni, D. Bini, B.H. Kuwano, K.B. Carmo, S.M.C.P. Silva, A.M. Martines, G. Andrade, D.S. Andrade, E.J.B.N. Cardoso, W. Zangaro, M.A. Nogueira. Land application of municipal landfill leachate: fate of ions and ammonia volatilization, J. Environ. Qual., 42 (2013) 523–531.
[5] T. Delazare, L.P. Ferreira, N.F. Ribeiro, M.M. Souza, J.C. Campos, L. Yokoyama, Removal of boron from oilfield wastewater via adsorption with synthetic layered double hydroxides, J. Environ. Sci. Health, Part A, 49 (2014) 923–932.
[6] N.C. Almeida, V.J.A. Oliveira, D.F. Angelis, Comparison of the adsorptive action of hydrotalcite, *Moringa oleifera* and activated carbon in the treatment of landfill leachate, Desal. Wat. Treat., 90 (2017) 220–230.
[7] A. Béres, I. Pálinkó, I. Kiricsi, J.B. Nagy, Y. Kiyozumi, F. Mizukami, Layered double hydroxides and their pillared derivatives – materials for solid base catalysis; synthesis and characterization, Appl. Catal., A, 182 (1999) 237–247.
[8] N.K. Lazaridis, D.D. Asouhidou, Kinetics of sorptive removal of chromium (VI) from aqueous solutions by calcined Mg–Al–CO_3 hydrotalcite, Water Res., 37 (2003) 2875–2882.
[9] Y. Seida, Y. Nakano, Removal of phosphate by layered double hydroxides containing iron, Water Res., 36 (2002) 1306–1312.
[10] L.D. Conceição, S.B. Pergher, C.C. Moro, L.C. Oliveira, Magnetic composites based on hydrotalcites for removal of anionic contaminants in water, Quim. Nova, 30 (2007) 1077–1081.
[11] L. Costa, C. Ladeira, A. Colli Badino, Overproduction of clavulanic acid by extractive fermentation, Electron. J. Biotechnol., 18 (2015) 154–160.
[12] T. Wajima, Removal of boron from geothermal water using hydrotalcite, Toxicol. Environ. Chem., 92 (2010) 879–884.
[13] X.Z. Li, Q.L. Zhao, Recovery of ammonium-nitrogen from landfill leachate as a multi-nutrient fertilizer, Ecol. Eng., 20 (2003) 171–181.
[14] G.P. Gillman, Charged clays: an environmental solution, Appl. Clay Sci., 53 (2011) 361–365.
[15] P. Ghosh, I.S. Thakur, A. Kaushik, Bioassays for toxicological risk assessment of landfill leachate: a review, Ecotoxicol. Environ. Saf., 141 (2017) 259–270.
[16] A. Ronco, M.C.D. Baez, Y.P. Granados, Conceptos Generales, G.C. Morales, Ed., EnsayosToxicológicos y Métodos de Evaluación de Calidad de Agua: estandarización, intercalibración, resultados y aplicaciones, IMTA, Mexico, 2004, 142 pages.
[17] B.M. Wilke, F. Riepert, C. Koch, T. Kühne, Ecotoxicological characterization of hazardous wastes, Ecotoxicol. Environ. Saf., 70 (2008) 283–293.
[18] Estado de São Paulo, Decreto Estadual nº 8468, de 8 de setembro de 1976: aprova o regulamento da lei nº 997, de 31 de maio de 1976, que dispõe sobre a prevenção e controle da poluição do meio ambiente, Diário Oficial do Estado de São Paulo, São Paulo (SP).
[19] CETESB, Companhia Ambiental de São Paulo, Norma técnica L. 5.201 – Bactérias Heterotróficas – Contagem em placas: método de ensaio, São Paulo, 1986, p. 26.
[20] Titrations. info., Mohr Method, Available at: http://www.titrations.info/precipitation-titration-argentometry-chlorides-Mohr, Accessed November 2016.
[21] USEPA, Method 7062: Antimony and Arsenic (Atomic Absorption, Borohydride Reduction), Part of Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, Revision 00, 1994, Available at: https://www.epa.gov/sites/production/files/2015-12/documents/7062.pdf.
[22] USEPA, Method 6010C (SW-846): Inductively Coupled Plasma-Atomic Emission Spectrometry, Revision 3, 2007, Available at: https://www.epa.gov/homeland-security-research/epa-method-6010c-sw-846-inductively-coupled-plasma-atomic-emission.
[23] APHA SMEWW, American Public Health Association, American Water Works Association, Water Environment Federation, Standard Methods for the Examination of Water and Wastewater, 22nd revised ed., 2012.
[24] USEPA, Method 7470A (SW-846): Mercury in Liquid Waste (Manual Cold-Vapor Technique), Revision 1, 1994, Available at: https://www.epa.gov/homeland-security-research/epa-method-7470a-sw-846-mercury-liquid-wastes-manual-cold-vapor-technique.
[25] USEPA, Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography, Revision 1.0, 1997, Available at: https://www.epa.gov/homeland-security-research/epa-method-3001-revision-10-determination-inorganic-anions-drinking-water.
[26] M.A. Hamilton, R.C. Russo, R.V. Thurston, Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays, Environ. Sci. Technol., 11 (1977) 714–719.
[27] ABNT, Associação Brasileira de Normas Técnicas, NBR 12713:2016 Ecotoxicologia Aquática – Toxicidade aguda – Método de ensaio com *Daphnia* spp. (Crustacea, Cladocera), Rio de Janeiro, 2016, p. 21.
[28] CETESB, Companhia Ambiental de São Paulo, Norma técnica L. 05.021 – Água do Mar – Teste de toxicidade aguda com *Artemia*, São Paulo, 1987, p. 15.
[29] CETESB, Companhia Ambiental Do Estado De São Paulo, Norma técnica L. 6.245 – Coleta e preparação de amostras, Procedimentos, São Paulo, 1984, p. 25.
[30] J.H. Zar, Biostatistical Analysis, 5th ed., Pearson Prentice Hall, USA, 2010, 994 pages.

[31] R. Bartha, D. Pramer, Features of a flask and method for measuring the persistence and biological effects of pesticides in soil, Soil Sci., 100 (1965) 68–70.
[32] A.P. Mariano, S.H.R. Crivelaro, D.F. Angelis, D.M. Bonotto, The use of vinasse as an amendment to ex-situ bioremediation of soil and groundwater contaminated with diesel oil, Braz. Arch. Biol. Technol, 52 (2009) 1043–1055.
[33] OECD, Guideline for the Testing of Chemicals, *Test No. 307: Aerobic and Anaerobic Transformation in Soil*, OECD Publishing, Paris, 2002.
[34] AGSOLVE, Como e porque medir a Condutividade Elétrica com sondas muiltiparâmetros, Available at: https://www.agsolve.com.br/noticias/como-e-porque-medir-a-condutividade-eletrica-ce-com-sondas-muiltiparametros, Accessed November 2016.
[35] SABESP, Companhia de saneamento básico de São Paulo, Qualidade de água, Available at: http://site.sabesp.com.br/site/interna/Default.aspx?secaoId=40, Accessed November 2016.
[36] C.N. Oliveira, V.P. Campos, Y.D. Pinto Medeiros, Avaliação e identificação de parâmetros importantes para a qualidade de corpos d'água no semiárido baiano. Estudo de caso: bacia hidrográfica do rio Salitre, Quím. Nova, 33 (2010) 1059–1066.
[37] C. Amor, E. De Torres-Socías, J.A. Peres, M.I. Maldonado, I. Oller, S. Malato, M.S. Lucas, Mature landfill leachate treatment by coagulation/flocculation combined with Fenton and solar photo-Fenton processes, J. Hazard. Mater., 286 (2015) 261–268.
[38] H. Hashemi, Y. Hajizadeh, M.M. Amin, B. Bina, A. Ebrahimi, A. Khodabakhshi, A. Ebrahimi, H.R. Pourzamani, Macropollutants removal from compost leachate using membrane separation process, Desal. Wat. Treat., 57 (2016) 7149–7154.
[39] R. Chemlal, N. Abdi, N. Drouiche, H. Lounici, A. Pauss, N. Mameri. Rehabilitation of Oued Smar landfill into a recreation park: treatment of the contaminated waters, Ecol. Eng., 51 (2013) 244–248.
[40] R. Chemlal, L. Azzouz, R. Kernani, N. Abdi, H. Lounici, H. Grib, N. Mameri, N. Drouiche, Combination of advanced oxidation and biological processes for the landfill leachate treatment, Ecol. Eng., 73 (2014) 281–289.
[41] WHO – World Health Organization, Guidelines for Drinking-Water Quality: Recommendations, World Health Organization, 2004.
[42] L.G. Souza, Estudo da remoção de ânions de soluções aquosas por meio de sorção em hidróxidos duplos lamelares, Dissertação de mestrado, Universidade Federal do Rio de Janeiro, Escola de Química, 2008, 132 pages.
[43] J. Das, B.S. Patra, N. Baliarsingh, K.M. Parida, Adsorption of phosphate by layered double hydroxides in aqueous solutions, Appl. Clay Sci., 32 (2006) 252–260.
[44] X. Tong, Z. Yang, P. Xu, Y. Li, X. Niu, Nitrate adsorption from aqueous solutions by calcined ternary Mg-Al-Fe hydrotalcite, Water Sci. Technol., 75 (2017) 2194–2203.
[45] B.M. Svensson, L. Mathiasson, L. Mårtensson, S. Bergström, *Artemia salina* as test organism for assessment of acute toxicity of leachate water from landfills, Environ. Monit. Assess., 102 (2005) 309–321.
[46] R.F.H. Giehl, N.V. Wirén, Root nutrient foraging, Plant Physiol., 166 (2014) 509–517.
[47] M.S. Bowman, T.S. Clune, B.G. Sutton, Sustainable management of landfill leachate by irrigation, Water, Air, Soil Pollut., 134 (2002) 81–96.
[48] K. Williamson, Soil-Vegetation Based Remediation Studies of Landfill Leachate, Diss. University of Wales, Bangor, 2001.
[49] A.S. Nakatani, A.M. Martines, M.A. Nogueira, D.S. Fagotti, A.G. Oliveira, D. Bini, J.P. Sousa, E.J.B.N. Cardoso, Changes in the genetic structure of bactéria and microbial activity in an agricultural soil amended with tannery sludge, Soil Biol. Biochem., 43 (2011) 106–114.
[50] G.S.B.G. Pattinson, B.G. Sutton, P.A. McGee, Leachate from a Waste Disposal Centre reduces the initiation of arbuscular mycorrhiza, and spread of hyphae in soil, Plant Soil, 227 (2000) 35–45.
[51] H. Chen, N.V. Mothapo, W. Shi, Soil moisture and pH control relative contributions of fungi and bacteria to N_2O production, Microb. Ecol., 69 (2015) 180–191.
[52] A. Campos, J.C. Marconato, S.M. Martins-Franchetti, The influence of soil and landfill leachate microorganisms in the degradation of PVC/PCL films cast from DMF. Polímeros, 22 (2012) 220–227.
[53] H.T.J. Reijers, S.E. Valster-Schiermeier, P.D. Cobden, R.W. van den Brink, Hydrotalcite as CO2 sorbent for sorption-enhanced steam reforming of methane, Ind. Eng. Chem. Res., 45 (2006) 2522–2530.