

# Determination of leachate toxicity through acute toxicity using *Daphnia pulex* and anaerobic toxicity assays

## Determinación de la toxicidad de lixiviados a través de toxicidad aguda utilizando *Daphnia pulex* y ensayos de toxicidad anaerobia

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### ABSTRACT

The municipal solid waste (MSW) of large cities, in particular the ones of developing countries, is mainly disposed in landfills (LFs), whose inadequate management generates the emission of greenhouse gases and the production of leachates with high concentrations of organic and inorganic matter and, occasionally heavy metals. In this study, the toxicity of the leachates from an intermediate-age municipal landfill was evaluated by ecotoxicity and anaerobic toxicity tests. The acute toxicity assays with *Daphnia pulex* presented a toxic unit (TU) value of 49,5%, which indicates that these leachates should not be directly discharged into water sources or percolate into the soil because they would affect the ecosystems related to these waters. According to statistical analyses, the leachate toxicity is mainly associated with the inorganic fraction, having the chlorides, calcium hardness and, calcium as the greatest influences on the toxicity. The anaerobic toxicity test showed that in the exposure stage, the methanogenic activity exceeded the control one, which suggests that the anaerobic bacteria easily adapted to the leachate. Therefore, this treatment could be an alternative to mitigate the toxicity of the studied leachates. The inhibition presented in the recovery stage, represented by a reduction of the methanogenic activity, may be because the amount of supplied substrate was not enough to fulfill the carbon and nutrient requirements of the bacterial population present.

**Keywords:** *Daphnia pulex*, leachate, municipal landfill, anaerobic toxicity.

### RESUMEN

Los residuos sólidos municipales (RSM) de las grandes urbes, en particular las de países en desarrollo, son predominantemente arrojados en rellenos sanitarios (RS), cuyo manejo inadecuado genera la emisión de gases de efecto invernadero y la producción de lixiviados con altas concentraciones de materia orgánica e inorgánica y, ocasionalmente, de metales pesados. En este estudio se evaluó la toxicidad de los lixiviados de un relleno sanitario municipal de edad intermedia, mediante ensayos de ecotoxicidad y de toxicidad anaerobia. Los ensayos de toxicidad aguda con *Daphnia pulex* presentaron valores de UT de 49,5%, lo que indica que estos no deben ser vertidos directamente a fuentes de agua ni deben percolar en el suelo, pues afectarían los ecosistemas que se sirvan de estas aguas. De acuerdo con los análisis estadísticos, la toxicidad del lixiviado está asociada principalmente a la fracción inorgánica, siendo los cloruros, la dureza cálcica y el calcio los parámetros de mayor influencia. Los ensayos de toxicidad anaerobia mostraron que en la etapa de exposición la actividad metanogénica superó a la del control, lo que sugiere que las bacterias anaerobias se adaptaron fácilmente al lixiviado y, por lo tanto, esta puede ser una alternativa de tratamiento para mitigar la toxicidad de los lixiviados estudiados. La inhibición presentada en la etapa de recuperación, representada en una reducción de la actividad metanogénica, puede deberse a que la cantidad de sustrato suministrada no fue suficiente para abastecer los requerimientos de carbono y nutrientes de la población bacteriana presente.

**Palabras clave:** *Daphnia pulex*, lixiviado, relleno sanitario municipal, toxicidad anaerobia.

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### Introduction

Municipal solid wastes (MSW) have been increasing due to factors such as population growth, elevated levels of urbanization and the massive consumption of different products (Eriksson & Bisailon, 2011). For this reason, one of

the major challenges in large urban areas, in particular the ones located in developing countries, is the management of municipal solid wastes (Moeinaddini *et al.*, 2010), with landfills being the most-used final disposal method

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worldwide (Hoorweg & Bhada-Tata, 2012). In Colombia, approximately 30 thousand tons are generated per day, and only 13% is recovered through alternative systems, so the remaining 87% goes to landfills (SSPD, 2010).

In landfills in operation for 30 years or less, at least four stages of decomposition occur: initial aerobic, acid anaerobic, initial methanogenic and stable methanogenic; however, factors such as MSW composition, climatic conditions like rainfall and infiltration, the mode of operation such as the leachate recirculation, and the implementation of aeration procedures can affect the degradation rates and times of MSW (Kjeldsen *et al.*, 2002) and leachate composition (Lee *et al.*, 2010).

MSW can contain dangerous substances of organic and inorganic character, heavy metals and xenobiotic compounds (Wiszniewski *et al.*, 2006 and Aziz *et al.*, 2010). If not treated properly, these substances can cause major environmental problems, for example the emission of greenhouse gases (GHG) ( $\text{CO}_2$  and  $\text{CH}_4$ ) and the generation of leachates (Al-Wabel *et al.*, 2011), resulting from the percolation of rainfall water through the mass of the wastes, the chemical and biological reactions in the landfill cells that contain the wastes, and their own water content (Boumechhour *et al.*, 2013).

According to the operation time of the landfill, and particularly of the landfill cell from where they come from, the leachates can be classified into three groups: young (less than 5 years), intermediate (between 5-10 years) and mature (more than 10 years of operation) (Renou *et al.*, 2008 and Shouliang *et al.*, 2008). The adequate management of the leachates requires determining both their amount and composition as well as performing toxicity studies (El-Fadel *et al.*, 2002), which also allows selecting adequate treatment strategies (Shouliang *et al.*, 2008).

Ecotoxicity studies or toxicological evaluations constitute a valuable, inexpensive and effective tool for the protection, control, evaluation and classification of industrial effluents and receiving water bodies. They also allow for detecting effects that cannot be estimated by only the physicochemical characterization of the effluents (Asselman *et al.*, 2014). To evaluate the toxicity of mixtures of chemical elements in surface waters, aquatic organisms are usually used because they are characterized by a high reproduction rate, easy handling, ecological importance, easy laboratory cultivation their sensitivity to contaminants (Silva *et al.*, 2003), rapid analysis (48 hours) and low cost and test simplicity (Atwater *et al.*, 1983). The microcrustaceans *Daphnia magna* and *Daphnia pulex* have these characteristics (Barata *et al.*, 2006).

Authors including Isidori *et al.* (2003), Cho *et al.* (2009), Jemec *et al.* (2012), Ribé *et al.* (2012) and Rivera-Laguna *et al.* (2013) have performed specific studies of ecotoxicity with leachates, finding that the use of these organisms

presents significant financial and logistical advantages compared to other organisms such as fishes. In addition, the studies show that variables associated with the age and composition of the leachates, such as the pH, alkalinity, and concentration of carbonaceous and nitrogenous organic matter, heavy metals, etc., influence toxicity. In general, young leachates are more biodegradable than mature leachates (Silva *et al.*, 2003).

Pablos *et al.* (2011) conducted a study to evaluate the toxicity of MSW landfill leachates through toxicity tests, including *Daphnia magna* to make a possible correlation between physicochemical properties and toxicity results. In this study they obtained a strong to moderate correlation of 0,85 for *Daphnia*, which causes high levels of ammonia, alkalinity, chemical oxygen demand (COD) and chlorides, to be potentially toxic.

In the case of Rivera-Laguna *et al.*, (2013), they studied the influence of age on leachate toxicity using as an indicator *Daphnia pulex*, where they found that toxicity is inversely proportional to age with values Of UT (Toxic Units) of 83,1, 47,7 and 27,7 for young, intermediate and old leachate, respectively.

Anaerobic biological processes are widely used for treatment due to their low energy requirements, low generation of sludge and production of methane with high energy content; however, the methanogenic bacteria can be inhibited by toxic compounds such as sulfides, heavy metals, total ammonia nitrogen (TAN) (ammonium ion and ammonia), with TAN being the main inhibitor present in the leachates. Studies including those of Saucedo *et al.* (2007), Romero (2010), and Torres *et al.*, (2010) found that depending on its characteristics, leachate can inhibit or limit anaerobic degradation due to its high concentrations of COD, TAN and volatile fatty acids (VFAs), particularly propionic acid.

Additionally, Torres *et al.* (2010) evaluated at laboratory scale, the anaerobic toxicity of pure leachates mixed with municipal wastewaters (MWW) to establish the potential toxic effect of leachate on anaerobic digestion. In the study using pure leachate, a high reduction of the SMA was founded, identifying two types of inhibition: metabolic and physiological, while in the tests using mixtures of leachate and MWW (5 and 10%), the percentage of inhibition was substantially reduced and conditions of non-toxic substrates were reached. The results suggest the potential for application of co-treatment of leachates with MWW as a suitable strategy for the management of leachate.

To make decisions to adequately manage these wastes, the potential toxicity of the leachates generated in an intermediate-age municipal landfill was evaluated in this study, performing ecotoxicity studies with the microcrustacean *Daphnia pulex* as an indicator species. In addition, anaerobic toxicity tests were performed to

evaluate the potential application of this technology as a treatment alternative of the evaluated leachates.

## Materials and Methods

### Leachate characterization

The studied leachates were collected from a regional landfill of intermediate age (5–10 years); the characterization considered the measurement of the following parameters: pH, content of organic matter (chemical oxygen demand (COD) and biochemical oxygen demand (BOD<sub>5</sub>)), dissolved organic carbon (DOC) and volatile fatty acids (VFAs); content of solids (total (TS) and volatile (TVS)), suspended solids (total (TSS) and volatile (VSS)); inorganic macro-components (conductivity; total and bicarbonate alkalinity; total, calcium and magnesium hardness; calcium; magnesium; chlorides; sulfates; total Kjeldahl nitrogen (TKN), ammonia and organic; nitrates and orthophosphates); metals and metalloids (Fe, Mn, Cd, Pb, Ni, Al) and xenobiotics (phenols, HAPs and BTX - benzene, toluene and xylene), according to the techniques established in APHA *et al.* (2012).

Taking into account that one of the main toxic agents present in the leachates is ammonia nitrogen (Saucedo *et al.*, 2007; Rivera-Laguna *et al.*, 2013) and that the toxicity is due to the non-ionized form (free ammonia or NH<sub>3</sub>), Equation (1) (Anthonisen *et al.*, 1976) was used to relate sample pH, room temperature and ammonia nitrogen concentration.

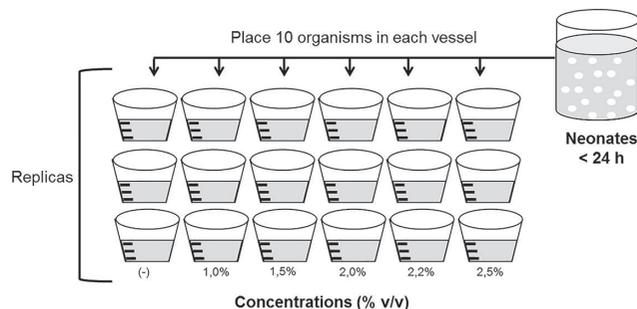
$$NH_3 \left( \frac{mg}{L} \right) = \frac{17}{14} \times \frac{N_{Ammoniacal} \times 10^{pH}}{\frac{K_b}{K_w} + 10^{pH}} \quad (1)$$

Here,  $K_b/K_w$  is the ratio between the dissociation constants of ammonia and water with respect to the temperature (°C) of the medium, which is equivalent to  $e^{(6,344/273 + °C)}$ .

### Toxicity tests

Two types of studies were performed: acute toxicity assays, or ecotoxicity tests, and anaerobic toxicity assays by specific methanogenic activity (SMA) tests.

**Toxicity tests with *Daphnia pulex*:** In the ecotoxicity tests, *Daphnia pulex* was used as an indicator organism, establishing concentration responses under controlled conditions according to APHA *et al.* (2012) and using lethal concentration (LC<sub>50</sub>) as an evaluation parameter, expressed as toxic units (TU). Five concentrations were used, each one in triplicate, exposing 10 newborns less than 24 hours after birth in each test vessel (Figure 1) for a period of 48 hours. The newborns were obtained from adult females previously selected and isolated in 1-L glass aquariums, with enough provision of nutrients and dilution water.



**Figure 1.** Mounting procedure of the toxicity bioassay with *Daphnia pulex*.

In addition, the concentration corresponding to the LC<sub>50</sub> obtained from the sensitivity test with the reference toxic compound K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, a positive control, at 24 h, and the blank test or negative control prepared with reconstituted water were included (Rivera-Laguna *et al.*, 2013). When the adequate ranges were obtained, several bioassays were performed, which allowed for calculating the LC<sub>50</sub> expressed in TUs for the leachate, where TUs are toxic units calculated according to the following Equation (2) (Pivato and Gaspari, 2006).

$$TU = \frac{100}{\%LC_{50}} \quad (2)$$

To analyze and to interpret the obtained results, PROBIT software version 1.5 was used, implemented by the United States Environmental Protection Agency (USEPA, 1990). In addition, from the obtained LC<sub>50</sub> values, a statistical correlation analysis of these results was performed with the physicochemical characteristics of the leachates, using the Pearson correlation coefficient for the data that complied with the normality assumption and using the Spearman coefficient for the parameters that did not comply with the normality assumption, to measure the degree of association of the data.

**Anaerobic toxicity tests:** To perform the tests, the liquid volume displacement method was used in 500-mL reactors operated at a temperature of  $30 \pm 2^\circ\text{C}$  in a special conditioned room with temperature control. Figure 2 shows the experimental setup for the toxicity tests.

**Inoculum:** The inoculum was obtained from a UASB reactor from an industrial wastewater treatment plant with a concentration of TSS of  $48,4 \text{ g L}^{-1}$  and of VSS of  $40,3 \text{ g L}^{-1}$  (VSS/TSS 0.83), a stability of  $30 \text{ mL CH}_4 \text{ g}^{-1} \text{ VSS}^{-1} \text{ d}^{-1}$  and an SMA of 0,45 and 0,40  $\text{g COD}_{\text{CH}_4} \text{ g}^{-1} \text{ VSS}^{-1} \text{ d}^{-1}$ . The inoculum concentration for the tests was  $1,5 \text{ g VSS L}^{-1}$ .

**Substrate:** The substrate was the leachate collected from an intermediate-age municipal landfill, and two solutions of VFAs were used with mixtures of acetate:propionate:butyrate (C2:C3:C4) in proportions of 55:30:15 and 73:23:4 (Torres *et al.*, 2010) and were added in an equivalent concentration of  $4000 \text{ g L}^{-1}$  COD. The tests consisted of an exposure stage

(first feed) and a recovery stage (second feed), performed in triplicate. For the analysis, the values of the replicates were averaged. In the exposure stage, the treatments were fed with VFAs, macro- and micronutrients, and leachates; and in the recovery stage, only with VFAs and macro- and micronutrients (Torres *et al.*, 2010).

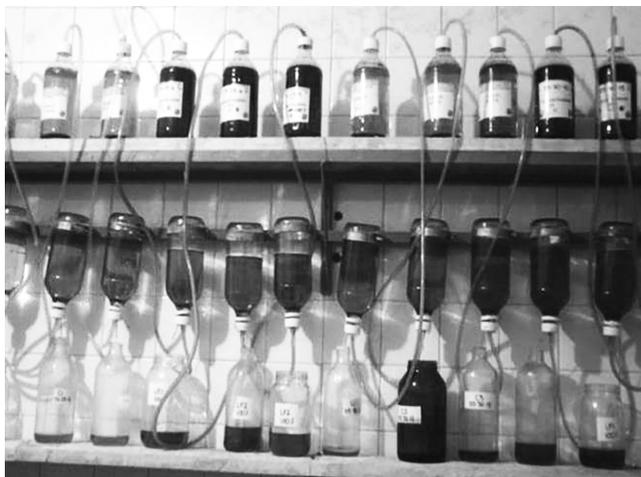


Figure 2. Anaerobic toxicity experimental set-up with the leachate.

Table 1 presents the added concentrations of substrate and VFAs in each reactor.

Table 1. Characteristics of the leachate toxicity tests

Reactor	VFA COD (g L <sup>-1</sup> )	Leachate COD (g L <sup>-1</sup> )	Total COD (COD <sub>VFA</sub> + COD <sub>LEACHATE</sub> ) (g L <sup>-1</sup> )
Control	4,0	0,000	4,000
Treatments	4,0	6,190	10,190

By recording the methane volumes accumulated over time, the SMA of each reactor was determined, and the ratio between the SMA of the treatment and the control Equation (3) and the inhibition percentage Equation (4) were calculated.

$$\%SMA = \frac{SMA_{Treatment}}{SMA_{Control}} \times 100 \quad (3)$$

$$\%Inhibition = 100 - \%SMA \quad (4)$$

## Results and Discussion

### Leachate characterization

Table 2 shows the physicochemical characteristics of the leachate. In general, it presents characteristics of an intermediate-age leachate with an age of 5 to 10 years (Renou *et al.*, 2008; Shouliang *et al.*, 2008 and Torres *et al.*, 2014).

Table 2. Physicochemical characterization of the leachate

Parameter	Range		Standard of discharge
pH	7,55	8,49	6,00 to 9,00*
<b>Organic matter</b>			
COD (mg L <sup>-1</sup> )	3,673	6,638	2,000*
DOC (mg L <sup>-1</sup> )	1,240	5,196	-
BOD <sub>5</sub> (mg L <sup>-1</sup> )	496	1,957	-
BOD <sub>5</sub> /DOC	0,14	0,32	-
VFAs (meq L <sup>-1</sup> )	13	100	-
<b>Solids</b>			
TS (mg L <sup>-1</sup> )	10,596	17,950	-
TVS (mg L <sup>-1</sup> )	2,390	8,134	-
TSS (mg L <sup>-1</sup> )	80	393	400*
VSS (mg L <sup>-1</sup> )	25	217	-
TDS (mg L <sup>-1</sup> )	10,473	17,775	-
<b>Macro-inorganic components</b>			
Conductivity (mS cm <sup>-1</sup> )	16,2	25,2	-
Total alkalinity (mg L <sup>-1</sup> CaCO <sub>3</sub> )	4,626	12,042	200**
HCO <sub>3</sub> <sup>-</sup> alkalinity (mg L <sup>-1</sup> CaCO <sub>3</sub> )	4,518	10,746	-
Total hardness (mg L <sup>-1</sup> CaCO <sub>3</sub> )	866	1,862	-
Calcium hardness (mg L <sup>-1</sup> CaCO <sub>3</sub> )	265	625	-
Magnesium hardness (mg L <sup>-1</sup> CaCO <sub>3</sub> )	337	1,470	-
Ca <sup>2+</sup> (mg L <sup>-1</sup> )	106	250	-
Mg <sup>2+</sup> (mg L <sup>-1</sup> )	95,1	357	-
Chloride Cl <sup>-</sup> (mg L <sup>-1</sup> )	1,398	3,099	500*
Sulfates SO <sub>4</sub> <sup>=</sup> (mg L <sup>-1</sup> )	<2,7	218	600*
TKN (mg L <sup>-1</sup> )	1,204	2,072	-
NH <sub>3</sub> -N (mg L <sup>-1</sup> )	728	1,848	-
NH <sub>3</sub> (mg L <sup>-1</sup> )	33,5	204	-
NH <sub>4</sub> <sup>+</sup> (mg L <sup>-1</sup> )	597	1,503	-
N <sub>org</sub> (mg L <sup>-1</sup> )	28	1,232	-
NO <sub>3</sub> <sup>-</sup> -N (mg L <sup>-1</sup> )	134,6	325,2	-
Orthophosphates (mg L <sup>-1</sup> )	8,4	31,7	-
<b>Metals and metalloids</b>			
Fe (mg L <sup>-1</sup> )	5,783		-
Mn (mg L <sup>-1</sup> )	0,397		-
Cd (mg L <sup>-1</sup> )	<0,041		0,05*
Pb (mg L <sup>-1</sup> )	<0,053		0,20*
Ni (mg L <sup>-1</sup> )	0,287	0,300	0,50*
Zn (mg L <sup>-1</sup> )	0,307	0,406	3,00*
Al (mg L <sup>-1</sup> )	<0,644		3,00*
<b>Xenobiotics</b>			
Phenols (mg L <sup>-1</sup> )	0,023	6,68	0,20*
BTEX (mg L <sup>-1</sup> )	<0,0001		-
HAPs (mg L <sup>-1</sup> )	<0,0001		-

\*Art 14 Resolution 0631 (2015) that rule the Decree 3930 (2010)

\*\*Art 7 Resolution 2115 (2007)

The alkaline pH (7,55-8,49) is related to the high content of bicarbonates and low content of VFAs. Because the

landfill is of intermediate age, it is beginning or is in the methanogenic stage, transforming the VFAs into methane and carbon dioxide (Renou *et al.*, 2008; Kheradmand *et al.*, 2010; Rivera-Laguna *et al.*, 2013). However, although the content of organic matter, expressed as COD, is high, the BOD<sub>5</sub>/COD ratio is low, which indicates the decreased biodegradability of the leachates with age and coincides with what has been found by authors including Fátima *et al.* (2012) and Ramírez-Sosa *et al.*, (2013).

Regarding solids, the predominance of dissolved solids (DS) is observed, which indicates that the chemical components are dissolved as ions and salts. According to Rivera *et al.*, (2013), in addition to conductivity, hardness and alkalinity indicate the presence of ions in the leachate in the form of dissolved salts composed of calcium, magnesium, chlorides, orthophosphates, sulfates, carbonates and bicarbonates, which are also related to the DS content.

Some inorganic matter is composed of nitrogen, with ammonia nitrogen in the greatest proportion, as the deamination of the amino acids and the destruction of organic compounds occurs first (Kulikowska and Klimiuk, 2008 and Rivera-Laguna *et al.*, 2013). With time, nitrates start to increase by the stabilization of the cells, and ammonia nitrogen content decreases (Rivera-Laguna *et al.*, 2013) due to the oxidation process. As with heavy metals and alkalinity, nitrogen compounds present in leachates have been widely studied because they are related to the generation of potentially inhibitory or toxic effects (Olivero *et al.*, 2008; Pablos *et al.*, 2011).

Regarding metals, metalloids and xenobiotic compounds, there is no quantifiable presence of heavy metals, BTX or HAPs, possible because the residues come from domestic wastes that contain low levels of these contaminants and because the dissolution processes, precipitation, adsorption, dilution, volatilization and other factors influence the landfill (Kulikowska and Klimiuk, 2008 and Rivera-Laguna *et al.*, 2013). In some cases, the phenol content exceeds the maximum permissible limit, which suggests the need to control this parameter to prevent it from negatively affecting the environment.

In general, characteristics of leachate indicate that it should not be discharged into water bodies without previous treatment because it represents a risk for aquatic ecosystems.

### Toxicity tests

*Toxicity tests with Daphnia pulex:* The results obtained in the toxicity tests with *Daphnia pulex* are shown in Table 3.

The LC<sub>50</sub> (2,02% V/V) and TU (49,5%) values are inversely proportional and indicate that the leachate is toxic to the evaluated indicator species, even at a low concentration of

leachate, which is in agreement with Rivera-Laguna *et al.* (2013) for an intermediate leachate (TU 47.7)

**Table 3.** Data obtained for the toxicity tests with *Daphnia pulex*

Results	Value
Number of bioassays	21
Average LC <sub>50</sub> (%V/V)	2,02
Average TU (%)	49,5
Standard deviation	0,30
CV (%)	15

Toxicity values can be associated with the content of organic (Isidori *et al.*, 2003) or inorganic (hardness, conductivity, alkalinity and chlorides) matter (Rivera-Laguna *et al.*, 2013). In this case, even though the studied leachate has a high concentration of ammonia nitrogen, the predominant form is the ammonium species NH<sub>4</sub><sup>+</sup>, which cannot be associated with toxicity. Ammonia (which is the non-ionized form) is the toxic form because it permeates easily through biological membranes due to its high solubility in lipids, causing damage to the respiratory surface (Rivera-Laguna *et al.*, 2013).

*Statistical Analysis – Correlation analysis:* Table 4 shows the results of the correlations of the LC<sub>50</sub> with the physicochemical characteristics of the leachates, obtained by determining the Pearson coefficient for the parameters that comply with the assumption of normality and the nonparametric Spearman coefficient for the parameters that do not comply with the assumption of normality.

The results show that the leachate toxicity can be mainly attributed to the content of inorganic matter, expressed in terms of chlorides content (correlation coefficient: -0,886), calcium hardness and calcium content (correlation coefficient: -0,783). This agrees with the findings of Isidori *et al.* (2003), who found that divalent cations affect leachate toxicity, and with Öman *et al.* (2008), who found that high concentrations of chlorides are dangerous for freshwater organisms, including *Daphnia pulex*.

The results of the ecotoxicity tests and the correlation analysis performed confirm that leachate cannot be discharged into water sources without previous treatment because it can alter and affect aquatic ecosystems.

*Toxicity test with anaerobic bacteria:* Figure 3 and Table 5 show the results of the toxicity tests. These results show that in the exposure to the toxic compound (leachate) stage, there was greater bacterial activity than in the control, while in the recovery stage (without the toxic compound), there was an inhibition, represented by the decrease of the SMA.

Even though the behavior was similar in both of the evaluated cases, with different C2:C3:C4 ratios of VFAs, the inhibition percentages varied, showing the importance of

knowing the composition of leachates in terms of VFA type because they influence anaerobic biodegradability degree due to their complexity (Field, 1995).

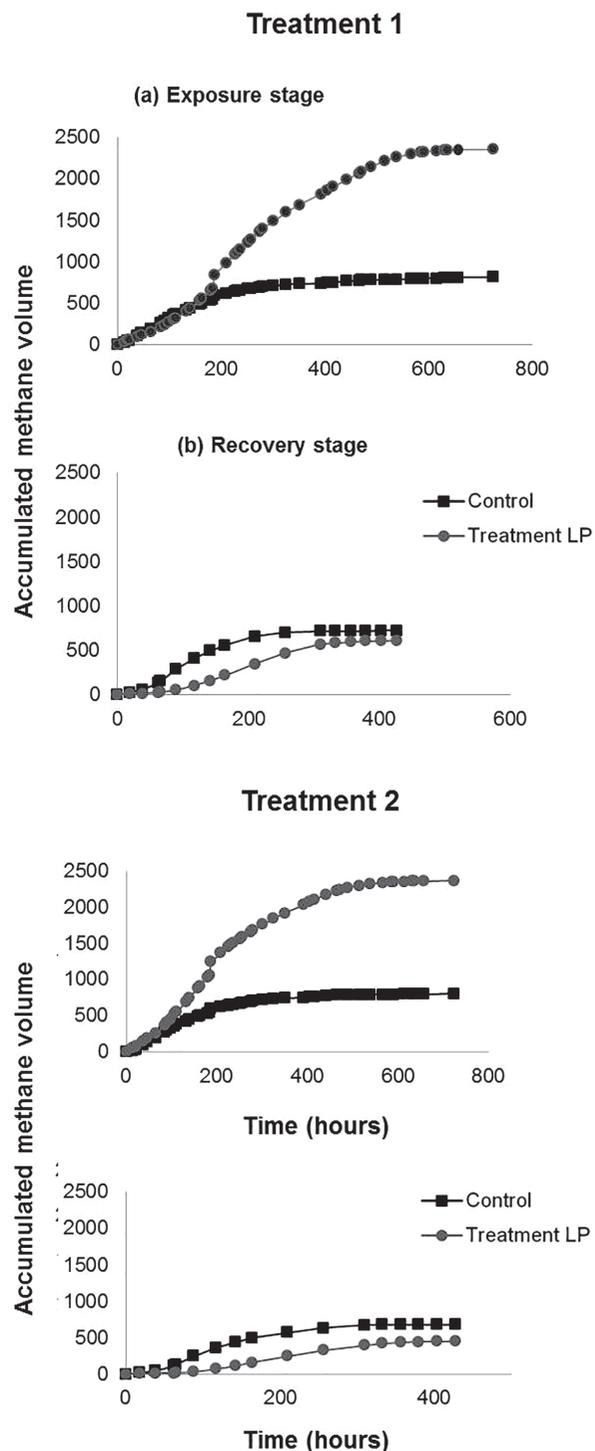
**Table 4.** Correlation results of the leachate toxicity with the physico-chemical parameters using Pearson and Spearman correlation coefficients

Parameter	Normality test	Correlation Coefficient	
	Shapiro-wilks – p-value	Coefficient	P-value
LC <sub>50</sub>	0,128	–	–
pH	0,487	-0,365	0,421
Total alkalinity	0,056	0,357	0,432 *
VFAs	0,618	0,066	0,888
COD	0,293	0,546	0,205
BOD <sub>5</sub>	0,002	0,286	0,535 *
TKN	0,219	0,007	0,988
NH <sub>3</sub> -N	0,609	0,739	0,058
NH <sub>3</sub>	0,378	0,046	0,922
NH <sub>4</sub> <sup>+</sup>	0,247	0,741	0,057
N-N <sub>org</sub>	0,026	-0,541	0,210 *
Conductivity	0,810	0,571	0,181
Total hardness	0,378	0,254	0,582
Calcium hardness	0,284	-0,783	0,037
Magnesium hardness	0,224	0,368	0,417
Ca <sup>+2</sup>	0,277	-0,783	0,037
Mg <sup>+2</sup>	0,220	0,367	0,417
TS	0,583	0,313	0,494
TVS	0,548	0,309	0,500
TSS	0,0002	0,071	0,879 *
VSS	0,0001	-0,179	0,702 *
TDS	0,898	0,304	0,508
LC <sub>50</sub>	0,068		
Chloride	0,404	-0,886	0,0188 *
NO <sub>3</sub> <sup>-</sup> -N	0,182	0,464	0,3542 *
PO <sub>4</sub> <sup>3-</sup>	0,021	0,257	0,6228 *

\* The Spearman correlation coefficient was used due to the failure to comply with the normality assumption for LC<sub>50</sub> or some of the physicochemical parameters. In the remainder of the tests, the Pearson correlation coefficient was used.

The observed SMA decrease during the recovery stage can most likely be attributed to two factors: i) the treatment with the toxic compound (leachate) had a greater amount of organic matter than the control one (treatment: 10,190 g COD L<sup>-1</sup>, control: 4 g COD L<sup>-1</sup>) and provided a greater amount of substrate for the bacterial community, allowing a greater production of methane; ii) the bacteria adapted

to the substrate in the exposure stage, which allowed the degradation of the organic matter, leading to population growth and thus to greater methane production activity (Fernández *et al.*, 2002 and Chen *et al.*, 2008). In the recovery stage, the bacterial population had less access to organic matter, which could provoke competition for the substrate and a possible self-destruction process in the bacteria, which was reflected in their inhibition.



**Figure 3.** Curves of methane production in the exposure and recovery stages for the two studied treatments.

**Table 5.** Results obtained in the anaerobic toxicity test for the two treatments.

Test	Sample	Vfa ratio C2:C3:C4	Exposure stage			Recovery stage		
			SMA	% SMA	% Inhibition	SAM	% SMA	% Inhibition
1	Control	55:30:15	0,336	128	No Inhibition	0,514	88	12
	Treatment		0,431			0,454		
2	Control	73:23:04	0,352	153	No Inhibition	0,458	73	27
	Treatment		0,539			0,336		

It is emphasized that, although UT are very high, indicating high toxicity of the leaching, the anaerobic bacteria are capable of adapting the leaching to such a point that in the recovery stage produce quantities of methane similar to control. Thus, in the stage of exposure, the anaerobic organisms were able to transform the organic matter, which is reflected in the behavior of the methane production curves of the treatments, in which an adaptation of the sludge to the leachate was presented and, therefore, a higher production of methane compared to the controls. In the recovery stage, it is observed that the sludge was not affected by the leachate, producing almost the same amount of methane as the control.

It is also observed that the adaptation time of the sludge to the leachate in the exposure stage was 200 hours, which is equivalent approximately to 8 days. On the other hand, in the recovery stage, the adaptation time is lower, close to 90 hours, which is equivalent to approximately 4 days, when methane production starts, achieving a similar production to that of the control. This indicates that in more precise experiments and, on a larger scale, a greater biodegradability of the leachate could be achieved because the sludge adapted to the waste, showing no inhibition and achieving almost the same methane production of the control with smaller adaptation times.

These results show the treatment potential of the evaluated leachate by anaerobic processes because the microorganisms manage to adapt to this substrate.

## Conclusions

- The physicochemical characteristics of the leachate, the results of the toxicity tests with *Daphnia pulex* and the correlation analyses performed show that the leachates are toxic by an aquatic mechanism, with 49,5% TU and the leachate inorganic fraction (mainly chlorides, calcium hardness and calcium) being the parameters that are most associated with its toxicity.
- Thus, this type of waste should not be directly discharged into surface or underground waters, as it would affect the fauna, flora and ecosystems that exist in these waters.

- The anaerobic toxicity assays showed the potential of this process for the treatment of the evaluated leachates because the methanogenic bacteria easily adapt to the supplied substrate. These results suggest the need to evaluate this anaerobic treatment at a greater scale to determine the adequate design and operation conditions of the system.

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