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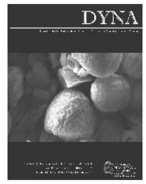

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Evaluation of toxicity from leachate lagoons sediments, using the toxicity leaching procedure - TCLP and acute toxicity tests

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Abstract
Landfills have been the most used alternative for final municipal solid wastes disposal, however, this system is the responsible of contamination problems associated with the generation of leachates, sediments and toxic gasses emissions. The sediments occur by the organic and inorganic matter precipitation from leachates dumped in retention lagoons, which can present toxic characteristics than affect the environment. The aim of this work was to evaluate the potential toxicity of leachates from a municipal landfill, using the TCLP procedure and acute toxicity assays with *Daphnia pulex* and *Poecilia reticulata* as biological indicators. The results showed that the sediment evaluated, did not exceed the stablished levels suggested by the EPA for the TCLP test, nor were they toxic to the organisms used. This results, suggests a potential agricultural use of this material in acid soils, since the nature of leachate sediments are pH alkaline and rich in nutrients and organic matter.

Keywords: acute toxicity; *Daphnia pulex*; leachates; leachate lagoon; *Poecilia reticulate*; sanitary landfill; sediments; TCLP procedure.

Evaluación de la toxicidad de sedimentos de lagunas de lixiviados, utilizando el procedimiento de lixiviación para la característica de toxicidad - TCLP y ensayos de toxicidad aguda

Resumen
Los rellenos sanitarios son la alternativa más empleada para disposición final de residuos sólidos municipales, sin embargo, generan problemas de contaminación por la generación de lixiviados, sedimentos y emisión de gases tóxicos. Los sedimentos surgen de la precipitación de los materiales orgánicos e inorgánicos de los lixiviados depositados en lagunas de almacenamiento que pueden presentar características tóxicas que afectan el ambiente. En este trabajo se evaluó la potencial toxicidad del material sedimentado de lagunas de lixiviados de un relleno sanitario municipal, utilizando el procedimiento de lixiviación para la característica de toxicidad - TCLP y ensayos de toxicidad aguda con *Daphnia pulex* y *Poecilia reticulata*. Los resultados mostraron que los sedimentos no excedieron los niveles establecidos por la EPA para TCLP, ni fueron tóxicos para los organismos empleados, lo que sugiere su potencial aprovechamiento agrícola en suelos ácidos, dado que presentan un pH alcalino y alto contenido de materia orgánica y nutrientes.

Palabras clave: *Daphnia pulex*; laguna de lixiviado; lixiviado; *Poecilia reticulata*; procedimiento de lixiviación para la característica de toxicidad – TCLP; relleno sanitario; sedimento; toxicidad aguda.

1. Introduction

Final disposition of municipal solid wastes, is generally done in landfills (LF) [1-4], this strategy has subsequent polluting problems associated to leachates, sediments and greenhouse gases (GHG) generation [3,5,6].

The production and management of leachates, is recognized as one of the major problems associated to the environmental operation of LF [2,4,5,7] and are correlated to the waste nature, water content and its compaction degree [2]. The sediments from LF, accumulated in lagoon reservoirs, are the result of deposited organic and inorganic matter precipitation from leachates dumped in retention lagoons [8-10].

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Table 1.
Maximum allowed pollutant concentration on leachates for the TCLP procedure.

Metal (mg/L)	RN* (mg/L)
Arsenic	5,0
Cadmium	1,0
Chrome	5,0
Mercury	0,2
Lead	5,0
Silver	5,0

*RN: Regulated norm. Stablish concentration as toxic.
Source: [11,12]

Toxicity assays are required since the physical-chemical characteristics of leachates and sediment solids activity may have a toxic nature for other direct application on other activities. The most appropriated toxicity techniques are the TCLP procedure and acute toxicity assays, using aquatic indicators such as *Daphnia pulex* and *Poecilia reticulata* [11].

The species used in this study were selected based on the literature [12-15] (Rivera et al., 2013, Carabalí-Rivera et al., 2017, Ramírez and Mendoza, 2013, Silva et al., 2013), the ease of obtaining and mainly, for the experiences of the Universidad del Valle in the management and use of them.

TCLP is a technique stablished for the direct or indirect toxicity evaluation to human beings, it has been used to determine the organic and inorganic analytes mobility within a liquid, solid or multiphase waste and it is based on the TCLP extraction of interest compounds such as As, Cd, Cr, Hg, Pb and Ag in order to compared to the allowed concentration limits in leachates [11,16]. Table 1 shows the limit of metals of hazardous wastes stablished.

According to authors as [4,10,17,18], the sediments analysis has been only about metal content (Cu, Fe, Ni, Zn, Mn, Cd, Cr, Pb, As, Mo, V, Hg) since those can cause toxicity in water, plants, microorganism and invertebrates if direct application is done on soils [8], however, other physicochemical properties as pH, organic matter, nitrogen, phosphorus and other nutrients, could help in the evaluation of soils aimed to be fertilizer for plants.

Toxicity acute tests are applied in the evaluation of environmental toxicity and allow to classify complex wastewater as dangerous by aquatic toxicity [11]. These assays are useful as direct diagnostic tools on organisms under specific controlled conditions, and are well stablished to protect the ecosystem biota exposed to toxic substances threat.

There have been several studies using alive organism and toxicity techniques in leachate environmental impact, some of the organisms used as indicators are Green microalgae, lettuce seeds (*Lactuca sativa*), *Vibrio fischeri*, microcustaceous like *Daphnia similis*, *Daphnia magna* and *Daphnia pulex*, the crustace *Artemia salina* and fish from the species *Branchydanio rerio* and *Poecilia reticulata*. These assays have evidenced the analyzed species, including possible mutagenic and breathing problems, general activity and equilibria loose, mucous membrane secretion, indicating affection by toxic leachate compounds [12-13,19-25].

The *Daphnia* are the organisms most used for their high reproduction rate, easy handling and sensitivity to contaminants [15], the wide geographical distribution, the important role they play within the zooplankton community, the ease of laboratory culture, parthenogenetic reproduction (which ensures uniformity of response) and the short life cycle with the production of a high number of offspring, have made them ideal for the evaluation of toxicity. Also, there is extensive information about their cultivation and they are easily cultivable in the laboratory [26].

Fish have been considered as good test species for the evaluation of aquatic toxicity due to their ecological and economic importance [27]. The toxicity tests with *Poecilia reticulata* allow to classify partially a complex or non-complex waste as hazardous waste due to aquatic toxicity [11].

There are not very broad studies of sediment toxicity studies of leachate lagoons, Bert et al. [28] evaluated the toxicity effect of this solid material, using *V. fisheri bacteria*, finding mean level of toxicity.

On this study, the physicochemical characteristics of sediments from a municipal landfill leachate lagoon were determined, which were compared with the Colombian Technical Standard NTC 5167 [29] in order to compare the potential use as fertilizer and soil amendment and were evaluated the potential toxicity through TCLP test and toxicity test with *Daphnia pulex* and *Poecilia reticulat*.

2. Materials and methods

2.1. *Sediment leachate lagoon characterization vs organic products requirements as a fertilizers or soil amendments*

Leachate were collected from a cell of municipal landfill in Colombia, which are produced young, intermedium and old leachates, which are disposed on a leachate lagoon, which took four samples of sediment leachates (April 2015 to January 2016). Only in the first sampling was presented rainy weather on the landfill. Volumes of 2 kg stainless steel dredger were used for sampling. The dredger was launched twice as far as possible in all directions (left, right, and front), slowly submerged and dragged to take a homogeneous and representative samples. Sediments where stored at 4°C according to USEPA [30]. Previous to the analysis, sediments were conditioned: dried at 32°C for 1 week, milled and homogenized using a mortar and sieved at 2 mm mesh [28].

Samples sediments characterization included: pH, conductivity, organic matter content (%OM), Total Kjeldahl Nitrogen (TKN), total phosphorus (TP), cationic interchange capability (CIC), sodium (Na), potassium (K), calcium (Ca) and interchangeable magnesium (Mg), which were done according to Process Design Manual from USEPA [30]. Metal characterization was done according to Decree 4741 [12] for hazardous wastes - RESPEL (Arsenic (As), Cadmium (Cd), Cupper (Cu), Chrome (Cr), Iron (Fe), Lead (Pb), Nickel (Ni), Mercury (Hg), Silver (Ag) and Zinc (Zn)) according to APHA et al. [27].

Once sediments characterization was completed, physicochemical analysis was compared with the Colombian Technical Standard NTC 5167 [29] in order to compare the potential use as fertilizer and soil amendment.

2.2. Toxicity test

In addition to the sediment physicochemical analysis, toxicity tests with TCLP procedure and acute toxicity were realized as follows:

2.2.1. Leachate procedure for the toxicity characteristics - TCLP (Section 6.1 - Resolution 0062 - IDEAM [11]).

The leachate sediments were treated as a solid waste, thus, solid procedure was applied: two solutions of acetic acid glacial at pH 4.93±0.05 (#1) and pH 2.88±0.05 (#2) were used as extraction fluids. The extraction of the samples required water addition. Depending of the final pH of the sample after extraction, the (#1) is used if pH <5, on the contrary case, the solution #2 has to be used. To select the extractive solution, 5g of the dried sediment was places in a 400 mL flask and 95.5mL of distilled water was added. A watch-glass was used to cover the flask and was stirred for 5 min and then the pH was measured. If pH <5, 3.5 mL of HCl 1N has to be added, stirred covered by the watch-glass and heated at 50°C for 10 min. The final solution was allowed to cool and then pH was measured. If pH >5, extraction solution (#2) has to be used. Later, the extractive solution volume was calculated using 25g of sample used in the extraction as follows.

$$V\ (mL) = 20 \times \text{sample mass}\ (g) \quad (1)$$

Extraction: First, 25 g of sample was weighted, then stirred in the extraction bottle with the volume of solution (#2) previously calculated. The temperature was regulated at 23°C, and stirred for 18±2 h at 30±2 rpm, and when the time process was completed, the solution was filtered. The extracted liquid was measured for pH and was stored at 4°C for later analysis. The metal concentration in the liquid volume was compared with those toxicity levels stablished in the Decree 4741 [12].

2.2.2. Acute toxicity assays using *Daphnia pulex* and fishes (*Poecilia reticula*).

It was followed the methodologies described for the IDEAM [11] and APHA [27] for the acute toxicity test in aquatic environment. The colony of this organism is located in the facilities of the Universidad del Valle - Meléndez.

The *Daphnia pulex* was obtained in Barrancabermeja in 2005, from a culture of a ecotoxicology laboratory, which were in controlled environmental conditions, while the *Poecilia reticulata* was obtained in 2014, from existing farms in Cali, under controlled conditions.

Periodically, the sensitivity tests are carried out with a toxic reference (Potassium Dichromate, $K_2Cr_2O_7$), longevity tests, reproduction and negative controls (reconstituted water) which guarantee the analytical quality of the bioassays performed. Both species are maintained in reconstituted water under environmental conditions (photoperiod: 16 light hours, darkness: 8 hours, population density: up to 12 individuals per fish tank and humidity greater than 17% [27].

On these assays, organisms were exposed to a previously prepared solution of sediment wastewater, named Wastewater Adapted Fraction - WAF, which corresponds to a part of the wastewater dilution. For this, 100 mg of sediment was added to 1L of fresh water during 7 days, followed by a sedimentation period.

While the WAF is being prepared during the 7 days, the organisms of *Daphnia pulex* and *Poecilia reticulata* are kept in the colony in transparent tanks at optimum conditions for their growth, and without disturbance. For this it is necessary: the aeration of reconstituted water (water rich in inorganic salts that give an environment conducive to *Daphnias* and *Poecilia reticulata* to live well) that is used as a culture medium and also for the preparation of WAF, until the concentration of dissolved oxygen reach saturation and have a stable pH.

The parameters required for the reconstituted water are dissolved oxygen greater than 4 mg/L O_2, pH between 7.5 and 8.5, temperature 20±2°C, alkalinity 70-90 mg $CaCO_3$/L and hardness 80-100 mg $CaCO_3$/L [27].

Each tank is cleaned every other day. The feeding of the *Daphnia pulex* colonies is based on a suspension prepared from truchina, alfalfa and yeast which is supplied every two days in each of the fish tanks. For the fish, the feeding is based on mackerel truchina which is provided every two days in each of the fish tanks.

Bioessays with Daphnia pulex: From the WAF solution, 30 mL of sample was added by triplicate to the test vessels. In each vessel, it was placed 10 *Daphnia pulex* neonates with less than 24 hours old from the colony and placed in similar environmental condition than the colony during 48 h.

After that period, mobility degree of the neonates was determined and dead organism were counted.

Bioassays with fishes (Poecilia reticulata): The bioassay procedure using fish was similar to the used with *Daphnias*, previously described. A sample of 400 mL was added in a test vessel and were added from 8 to 10 fish with less than 24 hours old from the colony. After 96 h, it was evaluated the mobility of alevins, determining the number of dead organism. For both acute toxicity tests, the pH has to be between 7.5 and 8.6, and Dissolved Oxygen (DO) over 3mg/L at the end of the exposition. According to IDEAM [11], only if the number of dead organism is superior to 50%, there is ecotoxicity from the leachates. No toxicity is considered if organisms alive are superior to 50%.

On Table 2, it is presented some characteristics of the bioassays done with each biologic indicator. Fig. 1 shows the procedure applied to each biological assay.

Table 2.
Description of bioassay's characteristics for each biologic indicator.

Set-up characteristics	***Daphnia pulex***	***Poecilia reticulate***
Volume per concentration of WAF (mL)	30	400
Organisms	Neonates with less 24h of new-born	Alevins with less than 24h of new-born
Number of organism	10 neonates	From 8 to 10 alevins
Vessel Volume	50 mL	600 Ml
Duration of the essay (Hours)	48	96

Source: The authors

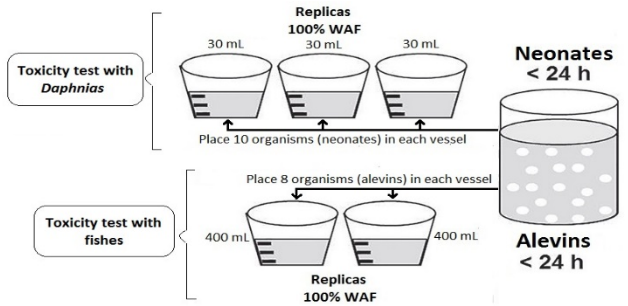

Figure 1. Procedure for the toxicity bioessay using *Daphnia Pulex* and fish (*Poecilia reticulata*)
Source: The authors

3. Results and discussion

3.1. Leachate lagoon sediments characterization vs organic products requirements for being used as fertilizers or soil amendment

On Table 3, it is presented the results of the physicochemical characterization of leachate lagoon sediments. It can be seen a pH close to 8.0 (8.19 - 9.21 units).

These values are related to the alkaline pH of the leachate that is disposed on the lagoon where the sediments were taken (7.77 - 8.60) [31]. This characteristic is associated to carbonates and bicarbonates content, which are the most predominant at this conditions. In addition, this alkalinity is desired for the application on soils as liming agent [32].

The high conductivity is related to the content of calcium, magnesium and sodium ions, which are directly related with conductivity. The carbonates and bicarbonates are salts related to the pH, hardness and alkalinity, and the greater the pH is increased, the more concentrated they are due to precipitation. In the other hand, high salt content in this leachate is associated to the solid wastes decomposition. [12,13].

The interchangeable basic ions are important for plant development and nutrient availability. The values of the sediment CIC indicates a medium nutritive reservoir in the sediment according to the classification mention by de Fernández et al. [33].

These sediments also presented good levels of organic matter and nutrients (N and P), which are vital for growing and developing of organisms. Besides, alkaline pH could favor the efficiently as amendment enhancing the soil of physical, chemical and biological of acid soils by decreasing the Fe and Al phosphorous fixation. Thus, it not only may reduce acidity but increase the soil fertility [32].

Soil fertility is associated to the C/N and C/P ratio, which are important for the plant nutrient harnessing [34]. According to Villarroel [35], the value of the C/N ratio, indicates that sediment has enough nitrogen to ensure normal plant growth without adding fertilizant [33]. Thus, leachate sediment can be considered rich in this nutrient [33]. The high content of P, is associated to the alkaline pH (values > 7.5 favors the precipitation of calcium phosphates [35]). In that way, the C/P ratio indicates the microbial activity can be enhanced and released to be used by plants [34].

Table 3.
Leachate lagoon sediments physicochemical characterization.

Parameter	Units	Raw sediment				Maximum allowed limit for biosolid and amendments
		1	2	3	4	
pH	Units	8.19	8.87	8.71	9.21	-
Conductivity	mS cm^{-1}	13.19	16.51	15.87	19.9	Declared [b]
CIC	meq 100 g^{-1}	18.2	17.7	18.7	18.3	≥ 30[b]
Na interchan.	meq 100 g^{-1}	104	27.2	78.1	10.6	-
K interchan.	meq 100 g^{-1}	67.9	37.6	60.2	29.5	-
Ca interchan.	meq 100 g^{-1}	274	390	431	340	-
Mg interchan.	meq 100 g^{-1}	234	429	545	452	-
% OM	%	25.1	22.6	26.3	26.6	≥ 15[b]
NTK	%	0.93	0.70	0.76	1.13	-
P_T	%	0.76	0.23	0.63	0.99	-
C/N	-	26.9	32.3	34.6	23.5	-
C/P	-	33.0	98.3	41.7	26.9	-
Metals						
As	mg Kg^{-1}	3.46	2.21	2.16	3.42	41[a,b]
Cd	mg Kg^{-1}	0.60	0.30	0.38	0.22	39[a,b]
Cr	mg Kg^{-1}	223	179	174	157	1200[b]
Cu	mg Kg^{-1}	76.7	31.3	28.1	25.7	1500[a]
Fe	mg Kg^{-1}	3,994	3,781	3,901	3,807	NR
Pb	mg Kg^{-1}	13.0	<9.00	<9.00	<9.00	300[a,b]
Ni	mg Kg^{-1}	62.2	49.9	50.7	36.3	420[a,b]
Hg	mg Kg^{-1}	<0.49	<0.49	<0.49	<0.49	17[a,b]
Ag	mg Kg^{-1}	<3.28	<3.28	<3.28	<3.28	NR
Zn	mg Kg^{-1}	400	244	262	207	2800[a]

[a] Norm 503 (EPA, 1994) for bio solids [36], [b] Norma 5167 (2004) (Amendments and liming agent for soils) [29], NR: Not Regulated
Source: The authors

Regarding the metals, the alkaline characteristics of the sediment may favor the precipitation of heavy metals (As, Cd, Cr, Cu, Fe, Ni and Zn). Other metals determined in this study such as Pb, Hg and Ag, were under the detection limit, and are according to low metal concentration of leachates found by Aulestia [37], thus, no significant amount of them was found in the sediment. In general, the metal content was similar in studies done for sediments of leachate lagoons from landfills according done by Øygard et al. [17], Öman and Junestedt [18] and Al-Wabel et al. [4]. All of them are according to the maximum limit allowed by national and international regulations.

That means, these sediments are acceptable to be employed as a potential organic amendment. According the organic product requirements as fertilizers, amendments or liming agent, the physicochemical characterization of solid indicates that the sediment leachate availated, satisfies the majority of the stablished parameters, in particular by the organic matter, nutrients and heavy metal content [29].

3.2. Toxicity test

3.2.1. Leaching procedure for the toxicity characteristic - TCLP

In the studies of toxicity using the leaching procedure for the toxicity characteristic - TCLP, the metal concentration reported in Table 4 is low and did not exceeded the regulatory level stablished by the EPA [11,16].

These results are according to the fact that municipal landfills are a final disposal for municipal solid wastes and not for hazardous wastes.

Once the time of the test finished for all the four samples, the maximum immobility evaluated was 3%, which is 1 dead per 30 exposed organisms. Even though, some values correspond to 0% where no dead organism was presented. Since the dead percentage was lower than 50% in all samples evaluated, the sediment can be classified as a no ecotoxic waste for aquatic environment with *Daphnia pulex* [11].

3.2.2. Toxicity test with with *Daphnia pulex*

On Table 5 it is show the resulted for the ecotoxicity for the sediments using *Daphnia pulex*.

3.2.3. Fish toxicity test (*Poecilia reticulata*).

The Table 6 shows the results for the ecotoxicity for the samples using *Poecilia reticulata*.

The results show that with samples 1, 2 and 4 it was not presented organism dead (0% immobility).

Table 4.
Results of the leaching procedure toxicity characteristic - TCLP for the sediment samples.

Parameter	Unit	Raw sediment				RT (mglL)
		1	2	3	4	
Arsenic	mg L^{-1}	0.043	0.025	0.031	0.022	5.0
Cadmium	mg L^{-1}	<0.081	<0.081	<0.081	<0.081	1.0
Chromium	mg L^{-1}	0.049	0.038	0.047	<0.156	5.0
Mercury	mg L^{-1}	<0.001	<0.001	<0.001	<0.001	0.2
Lead	mg L^{-1}	<0.450	<0.450	<0.450	<0.450	5.0
Silver	mg L^{-1}	<0.164	<0.164	<0.164	<0.164	5.0
Nickel	mg L^{-1}	0.334	0.401	0.387	0.144	-

*RT: Regulative Norm. Determined concentration by the EPA to be considered as hazardous
Source: IDEAM (2011) [11]

Table 5.
Ecotoxicity results for the sediments samples using *Daphnia pulex*.

Sediment	pH	OD	% dead or immobility			Total newborn exposed by essay	Ecotoxic Yes/ No
			Essay 1	Essay 2	Essay 3		
1	7,54	7,03	3	0	3	30	NO
2	8,20	7,20	0	0	3	30	NO
3	8,18	7,13	0	3	0	30	NO
4	8,13	7,25	0	0	3	30	NO

Source: The authors

Table 6.
Results of ecotoxitity for the sediment samples using *Poecilia reticulata*

Sediment	pH	DO	%Immobility or dead			Total alevins exposed	% dead average	Ecotoxic Yes/ No
			Essay 1	Essay 2	Essay 3			
1	7,53	7,11	0	0	0	16	0	NO
2	8,15	7,18	0	0	0	16	0	NO
3	8,01	7,21	0	6	0	16	2	NO
4	8,13	7,17	0	0	0	16	0	NO

Source: The authors

However, with sample 3 it was presented an essay with 6% of immobility which implies, that from 16 organism exposed there was 1, dead alevin. However, this percentage of dead of 6% is lower that 50%, which is the limit to stablish toxicity for the evaluated specie, then the sediment of leachate, can be classified too as a no ecotoxic by fishes [11].

The toxic contribution of excreta from *Poecilia reticulata*, may be negligible in the assays due to aspects such as:

1) The tests have very strict controls regarding reconstituted water, where the tests are carried out in triplicate with the controls and the treatments and if any effect is detected it will be detected in them.
2) The guppies are ovoviviparous fish, that is, the females develop the eggs inside them until they are mature and have already consumed their yolk sac completely. The females ovulate every three days and light approximately every twenty-eight days. When they light, the fry leave the belly of fully developed mothers, falling first to the bottom for immediately after swimming, the fry are therefore already completely independent, that is, they are born fully capable of taking care of their own needs and ensuring their existence. This indicates that they do not have much food requirement to subsist so their excreta are in very little quantity, so that the environment contaminates them. They can survive up to 5 days. [38-40].
3) The excreta are not considerable, then they would not reach a high level of toxicity to affect the organisms [41].
4) All aquariums have their respective filter. Every week the filters are washed and *chaetostoma* fish are also available that contribute to their cleaning.

The results found in this study, are the opposite in comparison with other toxicity studies done with leachates using *Daphnia pulex*, which evidenced toxicity with the same test [12,13]. In that cases, the organic [42] and inorganic matter was found to be toxic, also the hardness, conductivity, alkalinity and chlorides for being in high concentration [12]. Other studies using *Daphnia magna* as indicator also reported that the leachates in landfills in Finland and Lituania are toxic [43-45]. The same situation was found in the toxicity test with fishes (*Poecilia reticulata*) in a study done in the India, in which it was determine severe toxicity for organism [22].

According to Øygard et al. [17] the differences in the toxicity test for the leachates and the sediments of leachates, like the ones performed in this study, are that the presence of metals in sediments are joined to iron oxides and have a low solubility. Thus, the low bioavailability probably causes a low effect on ecotoxic tests. In that way, leachate sediments resulted in no ecotoxic for life organisms or in the TCLP test.

4. Conclusions

The physicochemical characterization of the sediment from leachates of this municipal landfill, shows a high organic matter, nitrogen and phosphorous content, which suggests a potential agricultural use. The high pH also indicates potential for using these leachate sediments as liming agent in acid soils, this is material rich in nutrients and organic matter.

According to the Leaching procedure for the toxicity characteristic - TCLP and ecotoxicity using *Daphnia pulex* y *Poecilia reticulata* as biological indicators, the sediments from the leachate lagoon did not present toxic characteristics for the used organisms. In that order, sediment could be valorized not only as liming agent but also as amendment for soils. However, further experimental verification is recommended before using as agriculture complement.

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