

Impact of the application of landfill leachate on the germination of *Senna macranthera* in different substrates

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ABSTRACT

Landfill leachate is a potential environmental pollutant. Physicochemical analyses allow identifying its elements while the use of ecotoxicological tests aims to understand the relation with the environment. The germination assay of *Senna macranthera* in different substrates (filter paper, commercial-SCM, conventional-SCV and organic-SOR) was performed with different doses of leachate. The objective was to identify the potential impact of leachate by determining the concentration able to cause inhibition in 50% of these seeds (LC50) and the values of the no observable effect concentration (NOEC) and the lowest observed effect concentration (LOEC). The LC50 and LOEC occurred in the treatments with 6.25 while NOEC was with 3.125%. The test in substrates, It was not possible to identify the LC50 in the SCM. For the SCV was with 66 and in SOR with 25%. The NOEC and LOEC for SCM and SCV were 25 and 50% and in SOR with 50 and 100%.

Keywords: Phytotoxicity; Germination; Root elongation; Reuse.

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The continuous changes in lifestyle combined with industrial and commercial growth have been accompanied by rapid increases in the production of urban and industrial waste, culminating in rising amounts of toxic elements and components in the environment, directly or indirectly affecting the quality and potential of natural resources. In this respect, the leachate generated in solid waste landfills is a polluting liquid that can harm nearby resources if it is not treated and discarded in an ecologically correct way or reused in other activities (Renou et al. 2008; Salem et al. 2008).

The reuse of wastewater and/or effluents deserves special attention because it reduces the demand for water resources, but it can have negative impacts on the environment. Reuse in agriculture aims to promote the sustainability of irrigated farming, because it saves surface water that is not polluted, maintaining environmental quality. In some cases, depending on the effluent used, it can also serve as a source of nutrition for plants. Studies have shown the importance of irrigation with effluents to supply some of the elements, mainly nitrogen, phosphorus and potassium, required by crops, increasing agricultural productivity (Kouraa et al. 2002; Hespanhol 2008; Nobre et al. 2010).

There are some limiting factors for the use of effluents on some types of agricultural crops, especially those that are consumed raw or that do not undergo any industrial processing. These crops cannot be irrigated with raw effluent. Instead, the wastewater must undergo rigorous treatment to assure water quality so as not to endanger the health of consumers. Industrial effluents, for example, due to their chemical composition, are generally unsuitable for agricultural reuse because they can contain substances toxic to humans and animals (Oliveira 2012).

Thus, among the various types of agriculture and forestry activity, the production of seedlings has potential to use leachate, since it is not directly related to food production, but rather the production of tree species for commercial plantations and reforestation. Thus, considering the need to produce seedlings of native forest species in countries such as Brazil, studies aimed at reuse and its impacts on the environment can support decision making to reduce demand on water bodies (Cromer 1980).

The reuse of landfill leachate must be carried out with care, due to its polluting potential. Additionally, it is fundamental to evaluate its biological implications and possible interactions with the environment (Costa et al. 2008; Kalcikova et al. 2011). In order to analyze the ecotoxicological effects of materials that can be used in human activities, ecotoxicity tests or bioassays should be carried out to predict the potential impact of a pollutant on the environment (Flohr et al. 2005).

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In this context, germination and seedling root development trials have been used to evaluate and quantify the toxicity of water-soluble compounds as well as mixtures of complex substances, such as industrial wastewater, soil leachate and sediments, among others (Bowers et al. 1997). These substances are submitted to acute phytotoxicity tests to evaluate both lethal effects through inhibition of seed germination and sublethal effects via stunted root development (USEPA 1996; OECD 2003).

These tests can assess either the direct impact of the substance or the indirect impact. In the first case, the test is performed using filter paper as substrate, thus ensuring that the only interference in the process is that of the substance evaluated (USEPA 1996; OECD 2003). In the second, it is possible to use soil or cultivation substrates in which the test substance is applied, and the effect of this interaction on the seed germination is analyzed (Lopes et al. 2010).

The cultivation substrate used to produce seedlings directly influences germination, since its water retention capacity, structure and aeration affects the supply of water and oxygen to the seeds. In addition, it provides physical support for the development of the seedling (Figliolia et al. 1993). According to Mondo et al. (2008), in choosing the substrate material, seed size, water requirement, sensitivity to light and ease of seedling development and evaluation should be considered.

The presence of so many variables makes the study of ecotoxicity in substrates essential, especially when the objective is to evaluate the pollutant potential of reuse. Thus, the present work aimed to evaluate the direct and indirect phytotoxicity of landfill leachate application on the germination of *Senna macranthera* seeds, a Brazilian native forest species, in different growth substrates.

MATERIAL AND METHODS

MATERIALS

The tests took place in the Multidisciplinary Laboratory for Agro-environmental Technology of the Pinheiral Campus of the Federal Institute of Rio de Janeiro, located in the municipality of Pinheiral, Rio de Janeiro state.

The landfill leachate used in the study was provided by the company Haztec Technology and Environmental Planning S.A., which manages the Waste Treatment Center (WTC) located in the city of Barra Mansa, with geographic coordinates 22°35'11.52"S and 44°12'54.31"W.

The planning, compilation and maintenance of the leachate were performed according to NBR 9897 (ABNT 1987). The leachate was stored in a plastic container with 50 liter capacity and 25% of its contents were renewed monthly to avoid losing its characteristics.

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The *Senna macranthera* seeds were purchased from a supplier accredited by the National Register of Seeds and Seedlings (RENASEM). According to the georeferencelabel, the seeds were collected directly from the matrix tree with height of 8 meters in a forested area in the municipality of Cruzília, Minas Gerais state.

Three substrates for growing forest seedlings were used, called: Commercial - SCM, Conventional - SCV and Organic - SOR. The organic (SOR) and conventional (SCV) substrates were composed of the following volume ratios (v:v). The SOR was composed of organic compound (material from the decomposition of plant and animal residues) + embankment subsurface soil+ sieved charcoal (3:1:1), while SCV was composed of embankment subsurface soil+ washed sand (3:2). SCM is a commercial substrate sold by Bioplant Agrícola Ltda., developed for seedling production. Its composition is mainly pine bark, rice husks, coconut powder and vermiculite, whose proportions were not identified by the manufacturer. The substrates were physicochemically characterized according to the method recommended by the Brazilian Agricultural Research Company (EMBRAPA1997).

PHYSICAL AND CHEMICAL CHARACTERISTICS OF LANDFILL LEACHATE

The physicochemical characterization was performed according to the method of the American Public Health Association (APHA2005). The following parameters were characterized: electrical conductivity (EC), pH and total dissolved solids (TDS), through direct reading of a waterproof multiparameter PCS Testr 35; and chemical oxygen demand (COD) using the closed reflux colorimetric method of digestion of the samples with a Tecnal TE-021 dry block digester, followed by reading the samples in a Hach DR5000 spectrophotometer.

The determination of cations and anions was performed by ion chromatography. Samples of landfill leachate were diluted 1000 times and then filtered with syringes coupled to fiberglass filters with 0.22 µm pore size in order to avoid clogging with particulate matter. The filtered material was placed in vials of a Dionex ICs 3000 ion chromatography system. The apparatus was equipped with an IonPac CS16 analytical column (3.0x250mm) preceded by two pre-columns. The cation suppressor used was 300 CSRS (2.0 mm). The equipment also has an IonPac AS 23 analytical column (2.0x250 mm) preceded by a guard column and an anion suppressor (SRS 2mm). In the mobile phase testing, the cations used for detection were methyl sulfonic acid (MSA) injected into the column at a concentration of 32 mmol L⁻¹. For anions, we used solutions of carbonate/sodium bicarbonate at concentrations of 4.5 mmol L⁻¹ and 0.8 mmol L⁻¹.

For determination of the metal concentrations (Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn), samples were digested according to the USEPA procedure (1998): 20.0 mL of sample and 10.0 ml of

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The environmental conditions for both assays were: temperature (25 ± 2 °C) (MAPA 2013); photoperiod (16 hours light / 8 hours dark) and total run time of 168 hours (Linder et al. 1989). These conditions were obtained with the use of a Thelga TF34 BOD chamber, where the container dishes were prepared.

EFFECT ON GERMINATION AND DEVELOPMENT OF SENNA MACRANTHERA ROOTS - DIRECT IMPACT

For the evaluation of the direct impact, *Senna macranthera* seeds were used as test organisms. The seeds were pre-treated according to the Rules for Analysis of Forest Seeds - RASF (MAPA 2013), recommended to avoid contamination and break dormancy of seeds. We used the technique of immersion in 2.0% sodium hypochlorite solution of obtained from a commercial solution with 2.5% active ingredient, for 05 minutes followed by three washes in water. Then we immersed the seeds in concentrated sulfuric acid for 30 minutes, and then washed them thoroughly with running water for 10 minutes (Borges et al. 1997).

The experimental dynamics followed a completely randomized design developed in two groups: test and control. For the test group, six treatments were applied with increasing doses in a ratio of 50% of landfill leachate, as follows (Aragão & Araújo 2008; Almeida et al. 2011.): T1 = 3.125% leachate + 96.875% deionized water; T2 = 6.25% leachate + 93.75% deionized water; T3 = 12.5% leachate + 87.5% deionized water; T4 = 25% leachate + 75% deionized water; T5 = 50% leachate + 50% deionized water; T6 = 100% leachate; and control (TC) = 100% deionized water. All treatments were performed in triplicate.

In conducting the study, 20 seeds of *Senna macranthera* were divided in Petri dishes (9.5 cm in diameter) with qualitative filter paper substrate (porosity 1 µm) and moistened with 4.0 ml of the sample solutions. To ensure moisture throughout the test, the Petri dishes were wrapped with transparent parafilm and later placed in a germination chamber.

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EFFECT ON GERMINATION AND DEVELOPMENT OF SENNA MACRANTHERA ROOTS - INDIRECT IMPACT

To evaluate the indirect impact, the adapted method of Lopes et al. (2010) was applied, using 50 ml plastic containers filled with 25 g of different substrates (SCM, SCV and SOR), moistened with 13.0 ml of solution (landfill leachate + deionized water). Subsequently, 10 *Senna macranthera* seeds were sown in each substrate. Immediately after sowing, the plastic containers were covered with film (to minimize possible moisture loss) and then incubated in a growth chamber.

The experimental design, doses and test procedures followed the same protocol as for the direct impact test.

EVALUATION AND STATISTICAL ANALYSIS

At the end of the experimental period the following parameters were evaluated: number of seeds germinated and not germinated, and length of roots (USEPA 1996; OECD 2003). The root length was measured with a digital caliper.

Seeds showing a radicle protrusion were considered to be germinated (Sobrero & Ronco 2004). Tests were considered valid for germination in the control (deionized water) when the germination rate was greater than 80% (Lemos Filho et al. 1997).

For the determination of lethal effects in both tests, the LC50 was identified from comparison of the mean germination and/or graphical analysis.

The sub-lethal effects were determined by identifying the noobserved effect concentration (NOEC), which corresponds to the highest concentration/dose of toxicant that causes no statistically significant deleterious effect on the organisms during the exposure time under the test conditions; and the lowestobservedeffect concentration (LOEC), which is the lowest concentration/dose of toxicant that causes statistically significant deleterious effect on the organisms during the exposure time and test conditions (Ronco et al. 2004). NOEC and LOEC were determined by comparison between the average length of the roots.

After physical examination and obtaining the means values of variables, these were submitted to the Lilliefors test of normality and Cochran-Bartlett test to determine homogeneity. The Scott-Knott test was used to analyze the significance between treatments at 05% probability. To determine the correlation between the parameters and the concentration of pollutant (landfill leachate), we used the Pearson correlation coefficient (r) and the t-test for significance at $p < 0.05$ (Achen 1977; Aldrich 1995).

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The statistical analyses were performed using the Statistical and Genetic Analysis System - SAEG 9.1 (Ribeiro Júnior 2001).

RESULTS AND DISCUSSION

CHEMICAL AND PHYSICAL CHARACTERIZATION OF LANDFILL LEACHATE

According to the physicochemical parameters shown in Table 1, it is possible to classify the leachate as young (Kurniawan et al. 2010). The parameters nitrate, ammonium, COD, lithium, chloride and sulfate had very high levels (at least three times higher) compared to the limits in the guidelines of Brazil's National Environmental Council (CONAMA Resolution 430/2011). Given these characteristics, it cannot be discharged in water bodies, thus requiring treatment. The levels of heavy metals such as cadmium, lead and nickel were not higher than the thresholds specified by CONAMA. It is also possible to infer (Table 01) that the leachate had high salinity according to the classification of Richards (1980) based on electrical conductivity (conductivity > 2.25 dS m⁻¹), higher than the limit allowed by CONAMA.

Furthermore, the physicochemical parameters of the leachate were higher than allowed for class 03 water established by CONAMA Resolution 430/2011, for irrigation of tree, cereal and fodder crops (Table 01).

Table 01. Physicochemical characterization of Barra Mansa CTR landfill leachate.

Evaluated parameters	Unit	Observed values Average ¹	CONAMA 430	
			Effluent discharge ²	Use for tree crops ³
pH (water)	-----	9.0	5.0 - 9.0	6.0 - 9.0
Conductivity	dS m ⁻¹	33.3	N.D.	N.D.
COD	mg L ⁻¹	5,592	120.0	N.D.
TDS	mg L ⁻¹	23,600	N.D.	500.0
Salinity	mg L ⁻¹	16,900	N.D.	N.D.
Aluminum *	mg L ⁻¹	7,625	N.D.	0.2
Ammonium **	mg L ⁻¹	595.27	20.0	N.D.
Cadmium *	mg L ⁻¹	0.025	0.2	0.01
Calcium **	mg L ⁻¹	95.38	N.D.	N.D.
Chloride **	mg L ⁻¹	857.50	250.0	250.0
Copper *	mg L ⁻¹	0.17	1.0	0.013
Iron *	mg L ⁻¹	13.66	15.0	5.0
Lithium **	mg L ⁻¹	15.22	2.5	2.5
Magnesium **	mg L ⁻¹	15.37	N.D.	N.D.
Manganese *	mg L ⁻¹	0.31	1.0	0.5
Nickel*	mg L ⁻¹	0.48	2.0	0.025
Nitrate **	mg L ⁻¹	496.75	10.0	10.0

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Potassium **	mg L ⁻¹	694.46	N.D.	N.D.
Sodium **	mg L ⁻¹	1,172.46	N.D.	N.D.
Sulfate **	mg L ⁻¹	846.0	250.0	250.0
Zinc *	mg L ⁻¹	1.13	5.0	5.0

Source: Franco et al. (2017).

COD - chemical oxygen demand; TDS - total dissolved solids; *Read via atomic absorption; **Read via ion chromatography; N.D. - not detected; 1Mean of samples used throughout the experiment; 2Standard for effluent discharge into receiving waters; 3Standard for fresh water class 3.

Table 01 also shows that the leachate contained calcium (Ca), magnesium (Mg) and potassium (K), all considered as essential macronutrients for plant growth (Malavolta 1980). This finding can lead to studies to assess the possibility of reusing the leachate both to reduce consumption of water resources and to supply nutrients.

CHEMICAL AND PHYSICAL CHARACTERIZATION OF SUBSTRATES

In order to define the characteristics of the substrate, a commercial substrate (SCM), conventional substrate (SCV) and organic substrate (SOR) were used in experiments conducted for physicochemical characterization (Table 02).

Table 02. Physical and chemical characterization of substrates.

Substrate	pH	Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	S	H ⁺ +Al ³⁺	T	P	V	C
	H ₂ O	cmol _c .dm ⁻³					mg.dm ⁻³			%	
SCM	5.3	5.8	2.6	0.58	0.53	9.6	10.1	19.6	36.0	49	24.3
SCV	5.2	2.7	1.2	0.11	0.07	4.1	1.3	5.4	44.0	76	0.5
SOR	6.6	4.0	3.6	1.54	0.26	9.5	1.0	10.4	50.0	91	4.3

Source: The authors.

SCM = commercial substrate; SCV = conventional substrate; SOR = organic substrate. S - sum of bases (S = Ca + Mg + Na + K + Al); M ++ Al³⁺ - potential acidity; T - Value T (T = S + (M + Al)); % V - base saturation (V (%) = (SB x 100) / T); C = organic carbon.

The SCM and SOR substrates showed high values of S, which indicates the sum of all bases: 9.6 and 9.5 cmol_c dm⁻³, respectively. However, the base saturation (%) was lower for the SCM (49%), due to the high potential acidity (H⁺+ Al³⁺). The element Al³⁺, which can be toxic plants, was not identified in any of the substrates. Sodium was present in all substrates, but higher values were obtained for the SCM, followed by SOR, because they contain plant wastes (rice hulls, pine bark and coconut powder) and are enriched with fertilizers (Pimentel et al. 2011; Montemurro et al. 2010; Parizotto & Pandolfo 2009). Abad & Noguera (1998) described sodium contents of coconut husk fiber ranging from 25 -240 mg L⁻¹. According to the authors, the cause of high levels of sodium in this fiber is due to coconut crop management, because this element is considered beneficial and is commonly applied by farmers, resulting in uptake and accumulation in the plant. Therefore, it is present in parts of the plant,

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Heider Alves Franco, Sérgio Thode Filho, Daniel Vidal Pérez, Monica Regina da Costa Marques in some cases at toxic levels, as also reported by Carrijo et al. (2002). This means the sodium content of SCM can be related to the presence of coconut powder.

Oliveira et al. (2006) studied different levels of bovine manure and salinity levels in the initial growth of castor bean (*Ricinus communis* L.). They identified high sodium contents of 0.11 to 1.27 $\text{cmol}_c\text{dm}^{-3}$ after fertilization with 1:1 soil: manure ratio. This result allows inferring that the sodium identified in the SOR has a direct relationship with the presence of manure in the waste used to make the organic compound in the formulation of this substrate.

SEED GERMINATION AND ROOT ELONGATION - DIRECT IMPACT

The results of the germination and root growth of *Senna macranthera* seeds in the direct impact test of different dilutions of leachate (3.125-100%) are presented in Table 03.

Table 03. Comparison of percentages of germination and root length of *Senna macranthera* seeds submitted to the application of landfill leachate.

Parameters	Treatments						
	TC	T1	T2	T3	T4	T5	T6
Germination (%)	90.0A	73.3A	50.0B	38.3B	8.3C	0.0C	0.0C
Root length (mm)	12.7A	15.5A	8.0B	5.7C	0.8D	0.0D	0.0D

Source: The authors.

Means followed by the same capital letter in the row do not differ from each other by the Scott-Knott test at 5% probability. TC = control - deionized water; T1 = leachate dose of 3.125%; T2 = leachate dose of 6.25%; T3 = leachate dose of 12.5%; T4 = leachate dose of 25%; T5 = leachate dose of 50%; and T6 = landfill leachate dose of 100%.

According to Table 03, there was no significant difference between control treatment and treatment 3.125% (T1). However, the germination percentage decreased with increased leachate concentration. With the 6.25% dose (T2), the germination rate was 50% (LC50).

The conditions found in a substrate to germinate seeds are not always ideal. Among the various environmental factors that can influence the germination, water availability is one of the most important factors. Besides water, the salinity of the substrate significantly affects the germination response of species because it causes a reduction in water potential of the substrate (Santos et al. 1992). The osmotic potential of the external salt solutions has more negative values than those of embryonic cells, causing less capacity for water uptake by seeds, resulting in physiological drought. Furthermore, the high concentration of ions in the embryo can rise to toxic levels (Varnero et al. 2007; Vwioko & Fashemi 2005; Njoku et al. 2009). The impact of salinity on plants can be the result of low osmotic effects, leading to drought, and specific effects or ions, which can cause toxicity or nutritional

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Since landfill leachate has high salt concentration (Table 01), the deleterious effects found may be due the presence of salts, impairing germination and early growth. Similar results of salinity were also observed by Nunes et al. (2009) and Botelho & Perez (2001) in studies with *Crotalaria juncea* and *Peltophorumdubium* Taub., respectively. Still in this context, the anions nitrate, chloride and sulfate (Table 01), which are present in the leachate with high values, are very toxic and during the germination process can be easily absorbed by the seeds, causing physiological disturbances and decrease in germination potential (Lima et al. 2001). According to Santos et al. (2009), the harmful effects of salinity on beans are more evident for sodium than potassium, and among the anions, the order of harmful importance is bicarbonate > chloride > nitrate.

The highly significant negative correlation (-0.9194) of the different treatments on the germination percentage of *Senna macranthera* seeds confirms the leachate's toxicity. That is, 92% inhibition of seed germination is associated with the pollutant and 08% with random factors, such as seed vigor and oscillation in the germination potential, mainly due to the use of forest species, given that there is no controlled seed production in this sector.

In relation to the root length parameter (Table 03), it showed no significant difference between the control treatment (TC) and 3.125% treatment (T1). However, the same did not happen with the other treatments. The control group had average root length of 12.7 mm, 37% smaller than that of the plants in receiving 6.25% leachate concentration (T2).

The absorption of water by the seed results in rehydration of the tissues, with consequent intensification of breathing and all other metabolic activities, culminating in the supply of energy and nutrients needed for resumption of embryonic axis growth (Carvalho & Nakagawa 2000). The water salinity potentially causes serious negative effects on water uptake and can prevent the sequence of events related to the germination (Bansal et al. 1980). Guedes et al. (2011) found that root length was impaired by increase in salinity levels in *Chorisia glaziovii* seeds.

The determination of NOEC was established in the treatment with 3.125% concentration, as the highest dose that did not cause significant effect on the length of *Senna macranthera* roots. In turn, the LOEC was established in the 6.25% treatment, because from this dose upward the sublethal effects were perceptible through the inhibition of root development. Brito-Pellegrini et al. (2006) analyzed the ecotoxicity of landfill leachate treatment after slow filtration and identified LOEC value for lettuce

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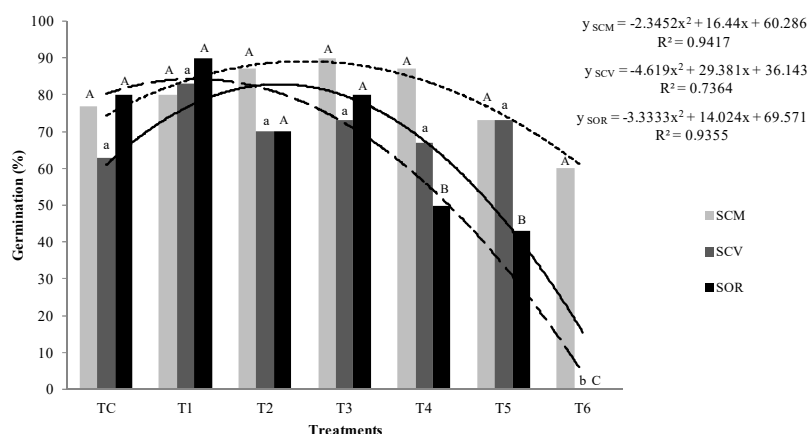
Heider Alves Franco, Sérgio Thode Filho, Daniel Vidal Pérez, Monica Regina da Costa Marques seeds (*Lactuca sativa* L.) after 16% dilution. In this test, the LOEC value became more restrictive because it was obtained from the use of only diluted raw leachate.

There was a significant negative correlation (-0.6547), i.e., 65% growth inhibition of roots associated with the pollutant and 35% random factors of the system. Thus, the higher the leachate dose, the smaller the root growth, mainly due to the high salt content of the effluent. The correlation values regarding differences in germination rate and root length are significant because a plant has different behavior in relation to salinity depending on their stage of development (Tester & Davenport 2003). The accumulation of salts in the root zone at high concentrations is sufficient to restrict water uptake by the plant. This can cause a state of water stress, with very similar symptoms to those caused by lack of water (Ayers & Westcot 1999). Furthermore, the effect on elongation of seedling roots can reflect the toxicity of soluble compounds present in low concentrations, which are insufficient to inhibit germination but can delay or inhibit root elongation, depending on the mode and site of action of the compounds (Sobrero & Ronco 2004; Tamada et al. 2012.).

SEED GERMINATION AND ROOT ELONGATION OF CROPS - INDIRECT IMPACT

The mean germination rates of *Senna macranthera* due to the introduction of landfill leachate for various substrates are shown in Figure 01. The germination was high even with high doses of leachate. This result is completely different from the direct impact test, where the high salinity of the effluent was responsible for reducing the germination potential.

Figure 01. Average germination percentage in an ecotoxicity test with *Senna macranthera* seeds submitted to landfill leachate application in commercial, conventional and organic substrates.



Source: The authors.

Means followed by the same capital letter and the same lowercase letter do not differ by the Scott-Knott test at 5% probability. TC = deionized water control; T1 = leachate dose of 3.125%; T2 = leachate dose of 6.25%; T3 = leachate dose of 12.5%; T4 = leachate dose of 25%; T5 = leachate dose of 50%; and T6 = leachate dose of 100%.

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According to Figure 01, there was no significant difference between the leachate doses for the SCM, so it was not possible to obtain the LC50 for this substrate, i.e., there was no observable indirect deleterious effect of applying leachate on germination in commercial substrate. This result suggests that the organic carbon content of 24.3% (Table 02) in the SCM substrate can contribute to the sodium adsorption and salinity as a whole (Freire & Freire 2007). Some authors state that the use of biofertilizer (rich in organic matter) in saline environments can partly mitigate effects of soil salinity due to the potential buffering by organic matter (Landgraf et al. 1999; Bezerra et al. 2010; Cavalcante et al. 2010; Ould Ahmed & Moritani 2010; Andrade et al. 2012).

According to Baldotto & Baldotto (2014), humified organic matter in the soil plays a fundamental role by buffering concentrations of protons and metal cations. Surface reactions such as those involved in ion exchange form the chemical basis for this buffering capacity (Ulrich & Sumner 1991), which may have contributed to the reduction of the toxic potential of the leachate used in this study, both by reducing the salinity and buffering the concentrations of ions. Humic acids, constituents of the organic matter, can react with several substances in the soil, such as pesticides. The humic substances, both in the form of individual colloids and interaction of mineral surfaces, can react with these compounds, and in some cases detoxify them considerably (Schwarzenbach et al. 1990; Martin-Neto et al. 1994). Other studies also point to the potential of humic acid to reduce the harmful effects of salinity stress on plants (Batista et al. 2012).

Analyzing the SCV germination (Figure 01), significant differences were observed only in the T6 treatment (100% leachate), where there was complete inhibition of germination. It was not possible to identify the LC50 for SAs from the doses tested, so we performed the calculation of dose-response probability using probit analysis (Bliss 1934a, 1934b), which revealed that the LC50 occurs at a dose of 66%.

The germination results for SOR showed no difference between the control and treatments T1, T2 and T3. However, at doses higher than 25% the germination rate declined. This concentration was defined as that corresponding to LC50, highlighting, however, that there was no statistically significant difference between the 25% and 50% doses.

Thus, it can be concluded that the landfill leachate associated with SCM caused no toxicity to the seeds, whereas for the SCV and SOR substrates, the LC50 values were respectively 66% and 25%. The doses corresponding to LC50 in this study corroborate those of Cheng & Chu (2007), in *Brassica chinensis* and *Lolium perenne*, where the authors found that these values ranged from 3% to 46% of leachate from four different landfills.

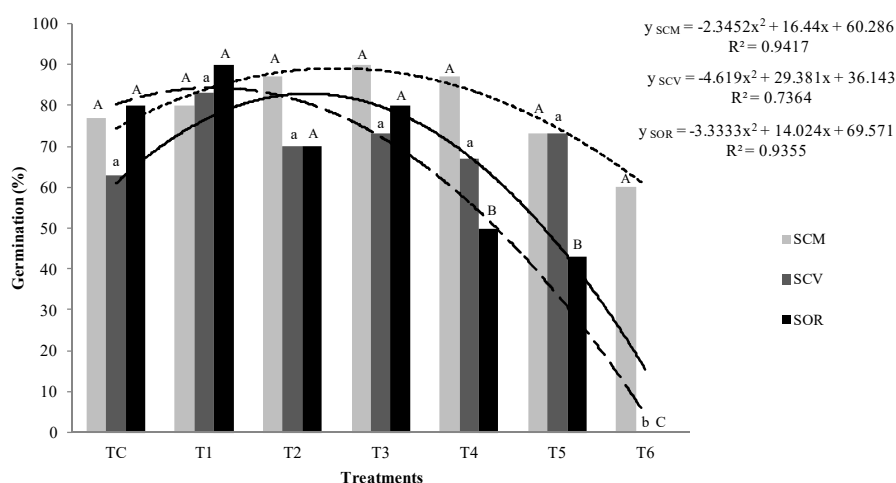
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The germination rates in SCV and SOR were similar, but very different than the SCM substrate. The low organic carbon content of SCV and SOR (Table 02) may have contributed to the reduction of the capacity of substrates to adsorb ions in solution, increasing the deleterious effects of salinity (Freire & Freire 2007; Bezerra et al. 2010; Cavalcante et al. 2010; Ould Ahmed & Moritani 2010).

The root length results for the different treatments are shown in Figure 02. Statistical calculations between control and test groups, by the Scott-Knott test ($p < 0.05$), were performed. Significant differences were observed starting at the 50% dose (T5) for SCM and SCV substrates, while for SOR this occurred only at the 100% dose (T6).

Figure 02. Mean root length in an ecotoxicity test with *Senna macranthera* seeds submitted to landfill leachate application in commercial, conventional and organic substrates.



Source: The authors.

Means followed by the same capital letter and the same lowercase letter do not differ by the Scott-Knott test at 5% probability. TC = deionized water control; T1 = leachate dose of 3.125%; T2 = leachate dose of 6.25%; T3 = leachate dose of 12.5%; T4 = leachate dose of 25%; T5 = leachate dose of 50%; and T6 = leachate dose of 100%.

The NOEC and LOEC values for the SCM and SCV substrates were observed in treatments with 25% and 50% respectively, while for the SOR substrate this occurred in treatments with 50% and 100%, indicating that sublethal effects (LOEC) occur in high doses 50% and 100%.

The root length values were also influenced by salinity of the leachate, since the reduction in water absorption by the seed delays basic biochemical and physiological processes, so the seedlings have stunted development (Silva et al. 2007). Also, according to those authors it is well established that the growth is reduced by increased salinity as a result of metabolic changes induced by salts.

For both parameters (Figure 01 and Figure 02) investigated in the indirect impact test, the behavior followed a quadratic profile, with an initial increase in the average parameter followed by

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Heider Alves Franco, Sérgio Thode Filho, Daniel Vidal Pérez, Monica Regina da Costa Marques reduction with higher doses. Such behavior is explained by Fonseca & Perez (2001), who indicate that tolerance to salt stress can result from mechanisms that control the uptake and allocation of salts in the plant, osmotic adjustment and other physiological processes, whose results are mainly expressed in germination percentage and speed (Oliveira et al. 1998; Sivritepe et al. 2003).

CONCLUSIONS

A deleterious effect was observed of landfill leachate on the germination of *Senna macranthera* seeds in different substrates, with high variability.

Comparing the two methods for germination, germination rate and root length presented different behaviors after the introduction of the pollutant. The test in Petri dishes with filter paper substrate was more restrictive, from the standpoint of ecotoxicology and reuse. The inhibition of germination was observed in 50% of the seeds as well as reduction in root length using a leachate dose of 6.25%.

Moreover, the germination test in cultivation substrate allows introduction of much greater leachate dose. The LOEC value occurred with concentration of 50%. This fact can be attributed to the physical characteristic of the substrate, which ensures distribution of the pollutant and less direct contact with the seed and also because of the substrate's chemical constitution. In this respect the commercial substrate (SCM) has higher organic matter content compared to the other two substrates tested. We believe this characteristic was responsible for alleviating the acute lethal effect on seed germination, since this was not possible to determine from the LC50 dose tested in SCM.

Thus, according to principles of ecotoxicity, *Senna macranthera* seeds have high tolerance to landfill leachate, at doses up to 50% in cultivation substrates.

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Impacto da aplicação do lixiviado de aterro na germinação de *Senna macranthera* em diferentes substratos

RESUMO

O lixiviado de aterro é um potencial poluente ambiental. Análises físico-químicas permitem identificar seus elementos enquanto o uso de testes ecotoxicológicos visa compreender a relação com o ambiente. O ensaio de germinação de *Senna macranthera* em diferentes substratos (papel de filtro, comercial-SCM, convencional-SCV e orgânica-SOR) foi realizada com diferentes doses de lixiviado. O objetivo foi

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Heider Alves Franco, Sérgio Thode Filho, Daniel Vidal Pérez, Monica Regina da Costa Marques identificar o impacto potencial do lixiviado via determinação da concentração capaz de causar inibição em 50% do sementes (CL50) e os valores da concentração de efeito não observável (CENO) e a concentração de efeitos observados (CEO). A CL50 e a CEO ocorreu nos tratamentos com 6,25% enquanto a CENO ocorreu com 3,125%. Não foi possível identificar o CL50 no SCM. Para o SCV ocorreu com 66% e em SOR com 25%. A CENO e CEO para SCM e SCV foram 25% e 50% e em SOR com 50% e 100%.

Palavras-Chave: Fitotoxicidade; Germinação; Crescimento radicular; Reúso.

Submission: 15/05/2018

Acceptance: 28/11/2019