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Rapid tests for the toxicity evaluation of soil contaminated by lead-acid batteries manufacture

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Abstract

Lead-acid battery manipulation is one of the major sources of environmental contamination of lead in developing and underdeveloped countries. For the preliminary investigation of the soil ecotoxicological potential of a mechanical workshop with deposit of lead-acid battery tailings, the avoidance behavior response test with *Eisenia andrei* and the phytotoxicity test with *Lactuca sativa* were made. The quantification of cadmium, copper, lead, zinc, manganese, iron and arsenic, was also performed. The level of lead found in the test soil was 56 times higher than the permitted value in Brazilian legislation, but the soil only showed toxicity in the avoidance behavior response test with earthworms. None of the phytotoxicity tests showed toxicity to lettuce seeds in germination rate and seedling length. This preliminary study confirms the contamination of these sites by lead and indicates that this contamination can cause damages to the edaphic fauna, suggesting further studies in areas contaminated by tailings of lead-acid batteries.

Keywords: *Eisenia andrei*; Environmental contamination; *Lactuca sativa*; Lead.

INTRODUCTION

Although volcanic emissions and rock weathering are natural sources of lead, anthropic activities are undoubtedly the main current sources of this element (Azevedo & Chasin, 2003; Rauch & Pacyna, 2009). Automotive sources, industrial wastes, manufacture of materials made from metal alloys and lead-acid battery factories are the main anthropic sources reported for this metal (Irwin *et al.,* 1997). Obsolete technologies, mainly in developing and underdeveloped countries, have contributed to local environmental contamination by lead (Mao *et al.,* 2006).

The impact to human health from exposure to environments surrounding lead-acid battery factories is well elucidated in the literature (Van der Kuijp *et al.,* 2013), as well as the evaluation of the exposure scenario (Chen *et al.*, 2012; Liu *et al.*, 2014) and quantification of soil chemical elements (Onianwa & Fakayode, 2000) and in the air (Quiterio *et al.*, 2006). On the other hand, the extent of effects on soil organisms from this exposure has been less investigated (Chao *et al.*, 2016).

The toxic effects on soil organisms can be accessed through laboratory bioassays and conducted in two phases: a preliminary phase and a confirmatory phase. The tests chosen for the preliminary phase should be rapid, inexpensive and must provide evidence of more susceptible organisms and prevalent forms of exposure (Chen & Liu, 2006; Pereira *et al., 2013*; Santos, Salles and Campolino, 2017; Wijayawardena *et al.,* 2017*;* Omouri *et al.,* 2018 . Among these possible bioassays are the avoidance behavior response test and the phytotoxicity test with lettuce seeds (Da Silva Júnior *et al.*, 2014).

Previous studies have identified high concentrations of lead in soil (Fragomeni *et al.*, 2010; Garcia *et al.*, 2010) and

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atmospheric air (Vanz *et al.*, 2003) in the city of Rio Grande, located in Rio Grande do Sul State, Brazil. Although Rio Grande has an important industrial complex associated with petroleum refining and harbor activities, the distribution of lead through the city seems not to be related to this industrial activities (Fragomeni *et al*., 2010; Vanz *et al*., 2003). Vanz *et al.* (2003) warn that part of the contribution of environmental lead is not of industrial origin, but possibly related to fishing activities, lead-based paint or other unidentified activities. In this article, we verified if the manufacture of lead-acid batteries can contribute to the increase of environmental lead and if the soil contaminated by this activity causes damage to exposed organisms.

METHODS

Sampling

Soil samples were collected from five different points in a site of a battery storage yard of a mechanical workshop, located in Rio Grande, Rio Grande do Sul State, Brazil. The control soil consisted of a soil collected within the campus of the Federal University of Rio Grande, previously used in other studies (Da Silva Júnior *et al.*, 2014). In the laboratory, the collected soil samples were homogenized, sieved (2mm) and conditioned in trays. The grain size was measured using the wet sieving technique (Robertson *et al*., 1984). The pH value of soil BSY was evaluated using pH meter in 20 grams of soil dissolved in 20 mL of distilled water. The total organic matter content (OM) was estimated by ignition at 550° for 4 h. To evaluate the soil moisture, 10 grams of soil was placed in the oven at 105 ºC for two hours.

Preparation of elutriate

About 100g of soil was weighed and added to 200 mL of distilled water. This solution was mixed and allowed to stir for 24 hours on the shaker table. After this period, the mixture was kept at rest and the supernatant (elutriate) was removed.

Avoidance behavior test with *Eisenia andrei*

On a rectangular plastic container were placed 350g of test soil, and on the other side were placed 350g of control soil. Between the two soils were placed 10 adult earthworms and the plastic container was covered with plastic film with holes and maintained at 20° C in a 12h/12h photoperiod for 48 hours. After the 48 hours, a division was placed between the two soils and counted the number of earthworms in each soil. For the analysis of this test it was used the parameter mentioned by Loureiro *et al*. (2009) where soil habitat functionality is considered limiting if more than 80% of the organisms are found in the control soil. This test was performed according to ABNT NBR ISSO 17512-1:2011 (ABNT, 2011).

For this assay, the elutriate soils, prepared with dry weight soil, of the battery storage yard were diluted with distilled water in different concentrations (0%, 1%, 3%, 10%, 30%, 60% and 100%) to evaluate a dose-response curve. The assay was carried out in Petri dishes containing filter paper, where 20 seeds of *Lactuca sativa*. Commercial seeds were placed on each plate (in triplicate) with 4mL of the solution in the corresponding concentration. After 5 days at 20° C in the dark, the germination rate and seedling length were measured, according to ABNT, NBR 11: 269: 2014 (ABNT, 2014). The dry weight of the soil was obtained after drying the soil in an oven 105 ºC for two hours.

To evaluate the toxicity of the soil from the battery storage yard, test soils were mixed to control soil resulting in different concentrations (0%, 1%, 3%, 10%, 30%, 60% and 100%). The following step of the evaluation was similar to the assessment of acute soil elutriate toxicity, and the substitution of 4mL of the solution for distilled water was the only difference.

Analysis of the elements (Cd, Cu, Pb, Zn, Mn, Fe and As)

Copper and zinc were analyzed by flame atomic absorption spectrophotometry (AAS Perkin-Elmer 800), while electrochemical atomization mode with Zeeman correction was used in the analysis of chromium, nickel, lead, arsenic and cadmium in the soil. The maximum value of the relative standard deviation for the analysis of 3 replicates of an individual sample was less than 4%.

Statistical analysis

Data were presented as mean ± standard error of the mean for both avoidance behavior response test with *Eisenia andrei* and the toxicity assessment (for soil and the elutriate). The avoidance behavior response test was statistically compared using Fisher exact test, and both toxicity assessment was statistically compared using ANOVA and post-hoc Dunnet with 95% significance.

RESULTS

The granulometry and field capacity of the battery storage yard (BSY) and control soils are described in Table 1. The pH value of BSY soil was 9.73. The weight of fresh control and battery storage yard soil was 10.0126 and 10.0110, respectively, and after drying the weight was 9.9591 and 9.9228, respectively. Therefore, the control soil moisture content was 0.53% and the battery storage yard soil moisture content was 0.88%.

From the physical aspect, the soils are quite similar and this characteristic allows comparisons between them.

Concentration of metals in the soils is described in Table 2. It worth mentioning that Brazilian guidelines, reference quality values (RQV), prevention values (PV) and intervention values (IV), for the quality of the soil are presented in Table 2. RQV is the concentration of a certain substance that defines the natural quality of the soil, being determined based on statistical interpretation of physical-chemical analysis of samples of different types of soils. IV is the concentration of a given substance in soil or groundwater above which there are potential direct or indirect risks to human health, taking into account a standardized exposure scenario. Finally, PV is the concentration of the limit value of a substance in the soil, such that it is capable of sustaining its main functions. In Table 2 is possible to notice that the concentrations of most of the elements in the control and BSY soils are similar, except for lead.

The results of the avoidance behavior response test with the californian earthworm *E. andrei* are shown in Figure 1, with the fugacity of organisms exceeding 80%, indicating restricted habitat functionality, as mentioned by by Loureiro *et al*. (2009).

The phytotoxicity data of elutriate and soil exposures are summarized in Figures 2 and 3. The soil collected in the BYS did not show toxicity to lettuce seeds when tested by direct exposure or by its elutriate. Thus, although the exposure to soil contaminated by battery tailings caused the escape of the earthworms, it did not affect germination or initial growth of *Lactuca sativa* under the conditions of this study.

DISCUSSION

Several studies (Loureiro *et al.*, 2005; Sisinno *et al.*, 2006) have used ecotoxicological analyzes with terrestrial organisms to show the sensitivity of these animals when exposed to soils contaminated by chemical substances. The study carried out by Gao *et al.* (2016) shows that Oligoqueta, *Eisenia fetida*, has a fugacity behavior when in contact with soils contaminated by metals, among them Zn and Pb, being able to detect harmful substances and escape from these environments, thus demonstrating a good indicator for soil quality.

Electronic waste is composed of a variety of metals such as copper, aluminum, iron, zinc, nickel, lead, cadmium and mercury, and is often deposited with various types of plastic and ceramic materials (Damasceno *et al.*, 2015). Waste of electric and electronic equipment are often disposed with organic and household garbage being destined to landfills or dumps, what results in a process of compostaging of organic matter along with electronic residues. Several studies have addressed the contamination of landfills by heavy metals from electronic waste such as batteries, demonstrating a growing concern about the potential negative environmental impact of this kind of waste (Li *et al.*, 2009; KIDDEE *et al.*, 2013).

The high concentration of lead in the evaluated soil is evident in Table 2, where BSY soil showed approximately 276 times more lead in its composition when compared to the control soil. Thus, it is possible to affirm the contamination by lead in these places where there is storage of lead-acid batteries.

Studies such as Machado *et al*. (2011) reported the concentrations of different metals in a controlled and inactive (for more than 30 years) dump. They observed that there is a tendency to decrease the Zn, Cu, Pb and Cr levels in the lower layers of the soil, even though they have concentrations above the reference values in the superficial layer. This reduction of

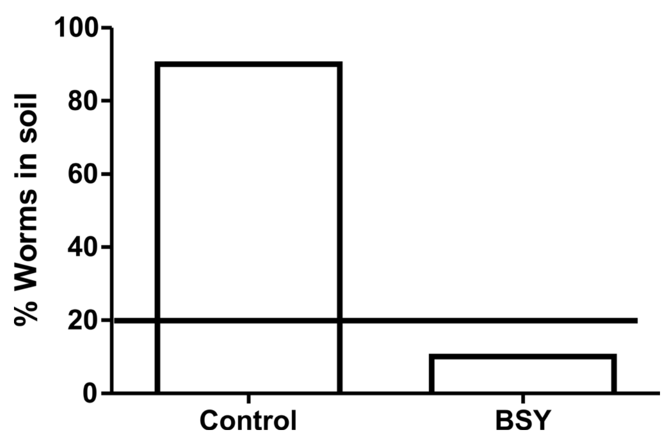

Figure 1. Results of avoidance behavior response test with earthworms exposed to soil contaminated by battery tailings, values in means ± SD. There was a statistical difference between the groups through the Fisher exact test.

Table 1. Physicochemical properties, granulometry and water retention capacity (WRC) of soil samples collected.

Soil	Type soil	pH	Soil moisture (%)	Organic matter (%)	Gravel (%)	Sand (%)	Silt (%)	**Clay (%)**	**WRC***
BSY	Spodosols	9.73	0.88	4.69	4	78	7	11	48.3
Control	Spodosols	6.02	0.53	4.20	0	85	7	8	50

* WRC in mL of water per 100 g of soil

Table 2. Concentration of metallic elements (mg Kg^{-1}) in soil samples and Brazilian guiding values (Conama, 2009).

Soil	**Cd**	**Cu**	**Pb**	**Zn**	**Mn**	**Fe**	**As**
BSY	294,08	33,99	10117,08	135,97	80,78	5274,60	2970,706
Control	558,0	31,64	36,70	102,45	NM	NM	2280,0
RQV	360	37	27	33	-	-	NI
PV	400	9	18	31	-	-	NI
IV	1300	60	72	300	-	-	15000

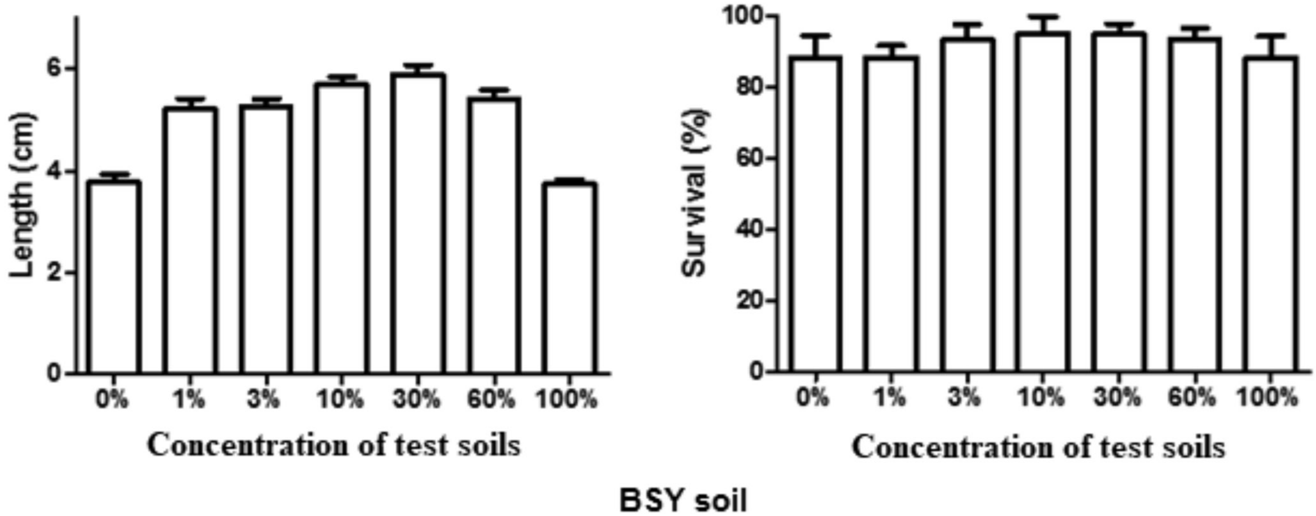

Figure 2. Survival and growth of lettuce seedlings exposed to the test soils from BSY, values in means ± SD. No significant differences were found using ANOVA and post-hoc Dunnet with 5% significance.

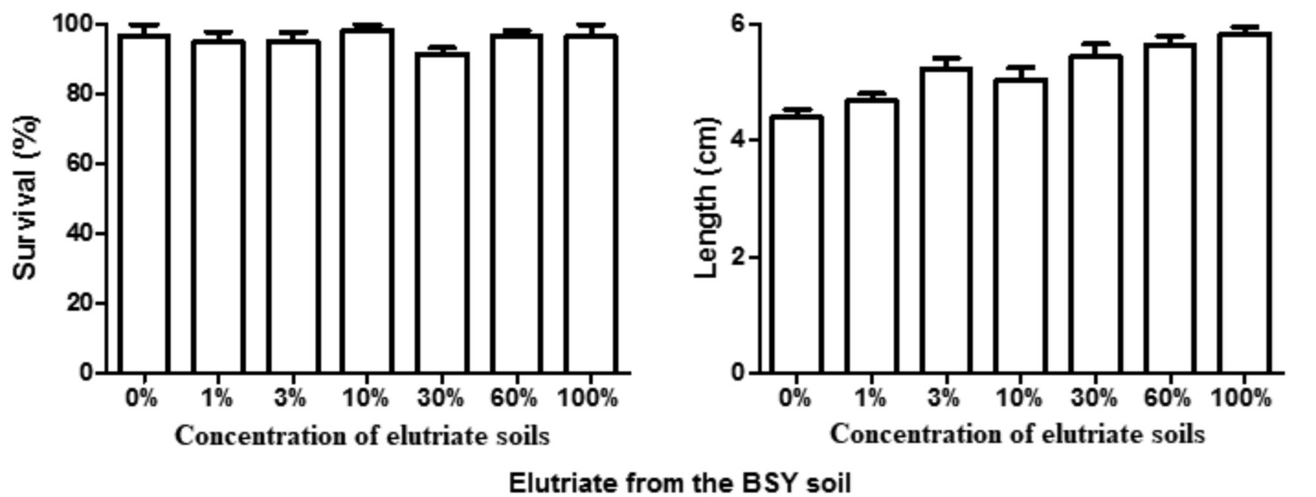

Figure 3. Survival and growth of lettuce seedlings exposed to the elutriate from the test soils from BSY, values in means ± SD. No significant differences were found using ANOVA and post-hoc Dunnet with 5% significance.

metals can occur due to factors such as the retention of metal ions in the mass of garbage mixed with the soil, the adsorption of the metals in the soil particles and the precipitation of the metals in the stable forms (Cavallet *et al*., 2013).

The kinetics and the presence of heavy metals in the soil, mainly in old dumps, depends on the time of deposition of the residue, degradation and composition of the material, besides taking into account the climatic conditions and mineralogical compositions of the soil (Fontes & Gomes, 2003). The pH values also have a strong influence on the adsorption and mobility of heavy metals (Wowk & Melo, 2005), affecting the concentration in the soil solution. In acidic conditions, the adsorption is more significant in the control of the bioavailability of metals, whereas the solubility or precipitation and complexation reactions have a greater influence in neutral or alkaline conditions (Elliott *et al*., 1986).

Together with the acid component, part of the adverse effects investigated may be related to the presence of lead in high concentrations in the tested soil. This element present in the batteries causes toxic effects in plants and animals (Sharma & Dubey, 2005).

Earthworms are annelids that have a beneficial effect on the functioning, maintenance and sustainability of the soil. Lavelle *et al*. (2006) highlighted some of the main functions of these organisms, as the movement of soil particles, resulting in the improvement of the structure and physical quality of the soil, and in the decomposition of organic matter (humus formation). These animals are considered bioindicators and good biomarkers of soil contamination, being exposed to contaminants through the epidermis and feeding. They are also used to monitor and verify the effectiveness of soil remediation procedures. In addition, these organisms are used in tests mainly for their characteristic to bioaccumulate and biomagnify contaminants (Castellanos & Hernandez, 2007).

The Figure 1 shows a high difference between the numbers of earthworms in the control soil and the BSY soil. This should occur mostly by the high concentration of lead, causing the worms to avoid being in the BSY soil and thus remain in the control soil.

Earthworms are a good model to investigate the ecotoxicity of contaminated soils, as explained in NIVA *et al*. (2016). The avoidance behavior response test with earthworms is widely used to assess soil quality, considering that soils in which the earthworms escape have limited functionality (Loureiro *et al*., 2009). Ecologically, this escape can mean local extinction of the species and consequently, loss of the ecological role exerted by earthworms in the soil.

In another approach, as described by Sharma *et al*. (2005), phytotoxicity assays was used to evaluate lead toxicity in different plant species in which the treatments tested influenced the activity of different enzymes with great metabolic importance for the process of photosynthesis. However, changes in the development of the plant exposed have an intrinsic relationship with many factors, such as the nature of the compounds, concentrations involved, sorption kinetics and product mobility (Jjemba, 2002).

In addition, we must take into account the sensitivity of each plant species to different pollutants. Being so, plants such as lettuce have been widely used as organisms for phytotoxicity testing due to their high sensitivity to metals (Eom *et al*., 2007).

Exposure to lead and its uptake during germination also varies from species to species due to differences in seed structure. This happens because metal exposure occurs only after the tegument rupture, where the toxicant is rapidly absorbed and accumulated in the meristematic regions of the roots and the hypocotyl, which may compromise plant development. Therefore, the tegument is considered a protection of the seed in the process of absorption of lead.

In our study the absence of phytotoxicity in the initial germination of *L. sativa* may be related to the intrinsic protection presented by this species, as was observed by Pereira *et al*. (2013), where the seeds of *L. sativa* presented a great tolerance to lead, probably due the protection and resistance of the tegument structure. Because to the resistance and high absorption of heavy metals, mainly lead, *L. sativa* contains a great phytoremediation potential, as demonstrated by Priebe *et al*. (2015). However, is important to notice that in a study made by Silva, Santos & Guilherme (2015), root growth was inhibited by concentrations higher than 1 mM of lead.

It is estimated that each year 330 thousand tons of lead are released into the atmosphere, 20% of which ends up being dispersed through the air. Air is considered the main route of dispersion of this metal, being able to be carried by winds and rains, accumulating in soil and water (Capellini *et al.*, 2013). According to Braga & Krusche (1999), the average of winds speed in the city of Rio Grande is approximately 10.2 km/h, which is an average of the sum of all directions of the winds, and the average of the highest velocity peak was 20.6 km/h for southbound winds. Thus, it is possible that the high concentration of lead in soil (Garcia *et al.*, 2010) and atmospheric air (Vanz *et al*., 2003) in this city is directly related to the storage sites of lead-acid batteries, because after a certain time these lead particles can be carried by the wind to other parts of the city, distributing itself along the soil of the region.

CONCLUSION

In the present study, both biological models were useful to predict damages due to exposure to battery rejects in the soil and are configured as tools to be incorporated in the evaluation of soil quality in cases of lead contamination. Although these results are preliminary, these are important data regarding the resistance of *L. sativa*, under the conditions tested on soil contaminated by battery tailings. Other parameters and experimental conditions should be used to evaluate the existence of sublethal effects on growth, development and bioaccumulation in plants, including *L. sativa*, using soil contaminated by battery rejects.

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