



ECOTOX - BRASIL

*Ecotoxicol. Environ. Contam.*, v. 9, n. 1, 2014, 29-31  
doi: 10.5132/eec.2014.01.004

**EEC**

## Acute toxicity of soil samples under the atmospheric influence of an industrial complex using Swiss mice

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(Received September 12, 2013; Accept February 24, 2014)

### Abstract

Some factors may contribute to potentiate the toxicity effects of soil contaminants on the organisms, among them is the acidification process derived from acid rain. Thus, the aim of this study was to assess the toxicity of a soil under the influence of an industrial complex through exposure of Swiss mice to soil leachates in different pH values. Soil samples were collected in Rio Grande, Brazil. At the laboratory, Swiss male mice were exposed by gavage (single dose) to soil extracts with different pH values (7.0, 5.2 and 3.6). The results showed toxic effects related to the decrease in pH and to increase in the concentration of soil extract. Therefore, the results showed that acid rain simulating solutions are able to potentiate the toxicity of the soil contaminants, and these conditions must be taken into account to environmental diagnosis in cities with occurrence of acid rain such as Rio Grande.

*Keywords:* short-term bioassays; soil contamination; rodent.

### INTRODUCTION

Soil differs from other environmental compartments due to there is not a continuous displacement, as in the case of atmospheric circulation and surface water, which may cause an increase in the residence time of the contaminants at the local level. In addition, soil can function as a filter or as a source of contaminants to other compartments requiring thus special care in their use (Da Silva Júnior & Vargas, 2007).

Some special conditions contribute to the potential toxicity of soils, including acidification. This increased sensitivity is due to increased solubility of the metallic elements in this low pH condition. However, complex mixtures may reflect this potentiation depending on the concentration of metallic elements, their degree of speciation and environmental conditions, such as the occurrence of acid rain (Pueyo *et al.*, 2003).

The municipality of Rio Grande is located near the estuary of Patos Lagoon. In this estuary, has settled an industrial park with different sectors and industries, due to this park, has already been noted that the city has favorable conditions to

the phenomenon of acid rain during most of year (Mirlean *et al.*, 2000, Da Silva Júnior *et al.*, 2013).

Although toxicological studies of drugs have been using rodents to predict damage to human health, these organisms have been little used to measure environmental damage caused by contaminated soils. Studies have been limited to investigate sub-lethal effects such as biochemical (Fouchécourt *et al.*, 1999, Budinsky *et al.*, 2008, Da Silva Júnior *et al.*, 2013), genetic (Alexander *et al.*, 2002) and histological (Garipay *et al.*, 2003) damage. This study aimed to investigate the acute toxicity of different fraction of a soil under the atmospheric influence of an industrial complex using Swiss mice.

### MATERIAL AND METHODS

#### *Soil samples*

Soil samples were collected from one sites in Rio Grande municipality, state of Rio Grande do Sul, southern Brazil,

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located in an urban area which is under the influence of fertilizer plants and a petroleum refinery via atmosphere.

### Collection and storage of surface soil

At each collection site, a simple sampling was done and soil was collected at a depth of 20 cm, using a plastic shovel. Stones and plant material were removed. In the field, the material was placed in plastic bags and transported into the laboratory. In the laboratory, the soil was separated and stored at -20 °C to biological assays and room temperature to metal analysis.

### Soil extraction

Soil samples were shaken at room temperature for 24 h with three different solvents (soil: solvent, 1:2. g mL<sup>-1</sup>): (i) distilled water, (ii) buffer solution of acetic acid and sodium hydroxide (pH 5.2), and (iii) buffer solution of acetic acid (pH 3.6). This acidic solution were prepared to simulate, respectively, the average pH of rainfall in Rio Grande (pH= 5.2) and the lowest (pH = 3.6) recorded by Mirlean *et al.* (2000).

### Acute toxicity test with mice (*Mus musculus*)

In order to evaluate acute toxicity in mice by mortality rate, the solutions of contaminated soil with different pH values were administered in mice weighing between 35 and 50 g in four different doses (357, 714, 1071, and 1428 mg kg<sup>-1</sup> body weight). These concentrations were chosen because they are close to the values of intake in humans geophagy events described by Calabrese *et al.* (1997). The control of each experiment was done only using solvent. The administration was done orally (gavage) in a single dose. Each treatment consisted of eight animals and signs of lethargy and mortality were monitored for 14 days. State of lethargy was considered when the animal was stimulated by touch and remained motionless, showing no signs of reaction.

### Quantification metals in the extracts

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

## RESULTS AND DISCUSSION

The results of metals quantification in the extracts evaluated are described in Table 1. The concentrations of chromium, nickel and lead exceeded the maximum allowable limits for groundwater according to Brazilian standards (CONAMA, 2009). Considering the acid extracts in addition to Cr, Ni and Pb, arsenic also exceeded legal limits. The concentration of

some elements such as arsenic, cadmium, copper and zinc in acid extracts increased.

Table 2 shows the mortality associated to three treatments, at different concentrations of contaminated soil. No effect was observed in the conventional treatment (distilled water), whereas in the alternative treatments (simulating the average pH of rainfall and acid rain in Rio Grande) effects were observed at higher concentrations. In the treatment simulating the average pH of rainfall, the highest concentration of soil (1428 mg kg<sup>-1</sup>) resulted in lethargic effects on animals, while the two highest concentrations of soil (1071 and 1428 mg kg<sup>-1</sup>) for the treatment simulating the effects of acid rain caused mortality (25 and 37.5% respectively).

The short-term bioassay with mice has not been applied to assess toxicity of contaminated soils. This assay was proposed to evaluate the toxicity of contaminated soil in three different situations, dissolved in solvents with different pH conditions: distilled water and two acidic solutions with pH of 5.2 and 3.6. Although none of the treatments have induced a high mortality rate of animals, the most pronounced results were obtained from treatments of soil dissolved in acid.

These two acid solvents were chosen to simulate leaching events in soil under normal (pH 5.2) and acidic (pH 3.6) rainfall conditions. These pH values were taken from Mirlean *et al.* (2000) for rainfall in Rio Grande. In fact, acidic conditions may increase the bioavailability of toxic compounds such as heavy metals (Pueyo *et al.*, 2003, Da Silva Júnior *et al.*, 2009).

Table 1 - Metal concentration (µg L<sup>-1</sup>) in three inorganic fractions (extraction) of the contaminated urban soil.

Samples	As	Cd	Cr	Cu	Ni	Pb	Zn
Contaminated soil (aqueous fraction)	5.058	1.740	<b>101.180</b>	183.360	<b>73.860</b>	<b>116.400</b>	203.000
Contaminated soil (pH5.2 fraction)	<b>12.510</b>	3.404	<b>63.300</b>	235.600	<b>68.520</b>	<b>48.200</b>	899.400
Contaminated soil (pH3.6 fraction)	<b>15.654</b>	4.294	<b>57.700</b>	286.800	<b>87.680</b>	<b>56.800</b>	497.400
Brazilian standards to groundwater <sup>1</sup>	10	5	50	2000	20	10	1050

\*letargic effects

Table 2 - Mortality and lethargy of mice exposed by gavage (single dose) at five concentrations of three fractions of contaminated urban soil extracts.

Concentration (mg kg <sup>-1</sup> )	Aqueous fraction	pH 5.2 fraction	pH 3.6 fraction
0	0	0	0
357	0	0	0
714	0	0	0*
1071	0	0	25%*
1428	0	0*	37,5%*

Acidic extract of soil has already shown a greater mutagenic character than aqueous extract of the same soil sample (Da Silva Júnior *et al.*, 2009). The results of this study highlight the risks for human populations and other organisms when exposed to contaminated soil after rain events and acid rain.

The increased toxicity observed in the test with mice was associated with the increased acidity in the fraction extracted of the soil. Among the analyzed elements, arsenic, cadmium, zinc and copper showed clear increase with the decrease in pH of the extract. So, among the elements analyzed, these elements are candidates as the highest contributors to the toxicity of the studied soil samples.

### CONCLUSION

The use of acute toxicity test in mice was useful for predicting the effect of events of acid rain potentiate the toxicity of soils with moderate level of contamination.

#### Acknowledgements

The authors thank to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, for the Doctoral (F.M.R. da Silva Júnior) and Master (E. M. Garcia) scholarships.

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