# Assessment of a Soil with Moderate Level of Contamination using Lettuce Seed Assay and Terrestrial Isopods Assimilation Assay

*Flavio Manoel Rodrigues DA SILVA JÚNIOR[1], Edariane Menestrino GARCIA[1], Paulo Roberto Martins BAISCH[2], Nicolai MIRLEAN[2] and Ana Luíza MUCCILLO-BAISCH[1]*

*[1]Laboratório de Ensaios Farmacológicos e Toxicológicos, Instituto de Ciências Biológicas and [2]Laboratório de Oceanografia Geológica, Instituto de Oceanografia, Universidade Federal do Rio Grande do Sul – FURG, Rio Grande, Brasil*

**Abstract**

Da Silva Júnior F.M.R., Garcia E.M., Baisch R.M., Mirlean N., Muccillo-Baisch A.L. (2013): **Assessment of a soil with moderate level of contamination using lettuce seed assay and terrestrial isopods assimilation assay**. Soil & Water Res., **8**: 56–62.

Lettuce (*Lactuca sativa*) seeds play a significant role in toxicity tests of isolated chemicals, pesticides, and environmental samples. Commonly, the main variables under study are the rate of seed germination and root elongation at the end of five days of exposure. Another organisms used in environmental assessment of soil quality are terrestrial isopods. The parameter evaluated in this assay is usually mortality rate. In this study, we suggest to use the daily number of germinated seeds and wet weight of plants, and feeding measurements (consumption rate, assimilation rate, assimilation efficiency and growth rate) in woodlice (*Armadillidium vulgare* and *Porcellio dilatatus*) to detect toxicity of moderately contaminated soil samples. The lettuce seed assay proved to be more efficient in the tested conditions, however, we do not reject the use of feeding parameters in terrestrial isopods in toxicological screening of contaminated soils.

**Keywords**: *Lactuca sativa*; short-term bioassays; soil contamination; woodlice

The use of bioassays for detecting changes caused by toxic agents has become increasingly common in diagnostic and monitoring studies. For the use in environmental evaluation, it is necessary to reunite some features like: to be standardized, simple, low cost, to have a defined endpoint, and be sensitive enough to distinguish differences among sites (Paton *et al*. 2005).

Few studies have been conducted for evaluation of soil toxicity. Most of these studies use plants and evaluate endpoint germination and growth, while there are also assessments of survival and reproduction of terrestrial invertebrates (Eom *et al*. 2007). Some of these bioassays are ruled by specific standards (USEPA 1989) and recommended in the battery for assessment of soil quality (Keddy *et al*. 1995; Whang & Freemark 1995).

Bioassays with plants have been used for evaluation of phytotoxicity of soils contaminated by various substances (Gong *et al*. 1999; Plaza *et*

*al.* 2005; Smith *et al.* 2006; Martí *et al.* 2007; Valerio *et al.* 2007). However, parameters such as germination (Keddy *et al.* 1995; Sverdrup *et al.* 2003) and growth (Robidoux *et al.* 2004) have been under debate on whether being really sensitive endpoints in phytotoxic evaluation.

Hassall *et al.* (2005) suggested the use of terrestrial isopods (woodlice) as a tool of ecotoxicological evaluation for contaminated sites. Terrestrial isopods are an important member of the invertebrate community of the soil, they are also easy to capture in the field and maintain in laboratory. Among soil invertebrates, woodlice play an important role in decomposition processes through the fragmentation of litter by their feeding activity (Zimmer *et al.* 2003).

This study aimed to investigate the ecotoxicological status of a soil with moderate levels of contamination using two approaches: (I) daily counting of germinated seeds of lettuce and fresh weight of plants after five days of experiment and (II) mortality (21 days) and feeding parameters in woodlice on the 22$^{nd}$ day of experiment.

## MATERIAL AND METHODS

**Soil samples**. Soil samples were collected from two distinct sites in Rio Grande municipality, satate of Rio Grande do Sul, southern Brazil: (*i*) soil of an industrial-urban area, under the influence of fertilizer industries via atmosphere and petroleum refining industry (contaminated), and (*ii*) soil of forested area on the Federal University of Rio Grande (Universidade Federal do Rio Grande – FURG) campus (control).

The study area (industrial-urban area of the city of Rio Grande) is located in the southern part of the coastal plain of Rio Grande do Sul State, Brazil. This area is of Holocene age, and was formed by sediment deposition resulting from marine regression. The soils are recent and evolved over udic moisture regime and thermic soil temperature regime controlled by the humid subtropical climate. These soils have sparse vegetation, mainly represented by grasses.

These soils (of the urban and industrial area of the city of Rio Grande) are composed mainly of sand of aeolian and marine origins and they have very low concentrations of organic matter, nutrients and clay minerals. These soils have no horizons with different colour or texture, are not saturated with water, and are classified as Typic Quartzipsamments (Cunha *et al.* 1995)

**Collection and storage of surface soil**. At each collection site, surface sampling of soil was carried out 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 for biological assays and at room temperature for metal analysis.

**Soil (solubilized) extraction**. Soil samples were shaken (116 rpm) at room temperature for 24 h with mineral water (soil to solvent, 1:2 g/ml) (Da Silva Júnior *et al.* 2009).

**Acute toxicity test with lettuce (*Lactuca sativa*)**. In order to evaluate acute toxicity in lettuce seeds, contaminated and control soils were solubilized in five different concentrations (5, 15, 50, 150, and 500 mg/l of solvent). Negative control employed only the solvent and positive control a 0.02M $CuSO_4$ solution. Each concentration was tested in four replicates, using 25 seeds distributed in Petri dishes containing filter paper and 3 ml of solvent (only at the beginning) was added in the respective concentrations. No more water was added to the plants during assay.

In a second experiment, contaminated soil was mixed with control soil in six concentrations (0, 1, 3, 10, 30, and 100%) and 25 seeds were placed directly on the soil (30 g). Plates were watered with 3 ml of mineral water. This experiment was performed in triplicate.

After five days of exposure, the germination rate (classic endpoint) was assessed, in addition to the daily germination rate and wet weight of plants at the end of the experiment (new variables).

**Feeding measurements in woodlice**. The isopods used in these experiments (*Porcellio dilatatus* and *Armadillidium vulgare*) were obtained from laboratory cultures, composed of animals collected from a compost pill and brought to the laboratory where they were fed *ad libitum* with tree leaves of jambol (*Syzygium jambolanum*) and maintained at 28°C with a 16:8 h (light:dark) photoperiod for 3 months before use in experiments. Only adult animals with antennae were selected for the tests and the sexes were not distinguished. Humidity was maintained by regular spraying with distilled water and the ventilation in the boxes was ensured by periodic openings on the box cover (Ribeiro *et al.* 2001).

The animals were kept in the control and contaminated soil for 21 days in Petri plates. Each soil was

tested in six replicates (plates), with five animals in each plate. At day 20, animals were left without food to empty their gut. At day 21, the isopods were removed from the soil and the number of dead organisms was counted. The living organisms were weighed and placed on Petri plates with filter paper and food (jambol). The food offered to the animals was weighed. The faeces of the woodlice and the remaining leaf material were removed and also weighed (wet weight) (Ribeiro *et al.* 2001).

Besides the mortality rate after 21 days, the parameters measured were similar to those employed by Ribeiro *et al.* (2001): consumption rate (mg food consumed mg/animal/day), assimilation rate (mg food assimilated mg/animal/day), assimilation efficiency (percentage of assimilated food in relation to the consumed food), and growth rate (biomass gain/average initial weight). Assimilation and consumption were evaluated after weighing the pooled food, animals, faeces and the remaining food of each Petri dish. The sum was then divided by the number of living animals.

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

**Data analysis**. Results were expressed as mean ± standard deviation. For comparison of means, analysis of variance (ANOVA) was carried out whenever assumptions were met. Duncan's test was applied for comparison among groups, at 5% statistical significance using Assistat 7.6 beta.

## RESULTS

### Acute toxicity test with lettuce (*Lactuca sativa*)

Table 1 shows the germination of lettuce seeds treated with aqueous solubilized soils of the control and contaminated soils at different concentrations. The commonly used endpoint to evaluate toxicity in lettuce seeds has been the inhibition of germination after 5 days of exposure. However, this variable did not show sensitivity to reveal the toxic effects of contaminated soil. After 5 days, the germination rate did not differ between the control and any concentrations of the contaminated soil.

Table 1. Average number of germinated seeds and wet weight of seedlings exposed for five days to different concentrations of solubilized (aqueous extract) from two soil samples (control and contaminated)

| Day | Concentration (mg/kg) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 5 | 15 | 50 | 150 | 500 | positive control |
| **Control soil** | | | | | | | |
| 1 | 23.25 | 24.25 | 21.25 | 24.25 | 23.25 | 23.25 | 0 |
| 2 | 24.12 | 24.25 | 23.75 | 24.75 | 24.50 | 24.75 | 0 |
| 3 | 24.25 | 24.25 | 24.00 | 24.75 | 24.50 | 25.00 | 0 |
| 4 | 24.25 | 24.25 | 24.25 | 24.75 | 24.50 | 25.00 | 0 |
| 5 | 24.25 | 24.25 | 24.25 | 24.75 | 24.50 | 25.00 | 0 |
| Wet weight | 0.3160 | 0.2862 | 0.27497 | 0.2761 | 0.2857 | 0.2728 | 0 |
| **Contaminated soil** | | | | | | | |
| 1 | 23.25 | 23.75 | 23.75 | 23.00 | 22.50 | **20.25** | 0 |
| 2 | 24.12 | 24.25 | 24.25 | 24.25 | 24.25 | 23.75 | 0 |
| 3 | 24.25 | 24.25 | 24.50 | 24.50 | 24.25 | 23.75 | 0 |
| 4 | 24.25 | 24.25 | 24.50 | 24.50 | 24.50 | 23.75 | 0 |
| 5 | 24.25 | 24.25 | 24.50 | 24.50 | 24.50 | 23.75 | 0 |
| Humid weight | 0.3160 | 0.2946 | 0.2841 | **0.2655** | **0.2622** | **0.2524** | 0 |

Data in bold refer to results that are significantly different from the control

Table 2. Average number of germinated seeds and wet weight of seedlings exposed for five days to different concentrations of a mixture of control and contaminated urban soil

| Day | Concentration (%) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 3 | 10 | 30 | 100 |
| 1 | 20.33 | 20.67 | 18.67 | **15.33** | **15.67** | **13.67** |
| 2 | 22.33 | 22.33 | 21.33 | 22.00 | 23.33 | 22.67 |
| 3 | 23.67 | 23.33 | 2267 | 24.67 | 24.33 | 24.00 |
| 4 | 23.67 | 25.00 | 22.67 | 25.00 | 24.67 | 24.00 |
| 5 | 24.00 | 25.00 | 23.67 | 25.00 | 24.67 | 24.67 |
| Humid weight | 0.4608 | 0.4637 | 0.4808 | 0.4647 | 0.4839 | 0.4558 |

Data in bold refer to results that are significantly different from the control

The strategy to investigate the germination daily until the 5$^{th}$ day revealed significant differences between the highest tested concentration of contaminated soil and the control on day 1 of observation. The control soil showed no toxicity at all tested concentrations (Table 1).

Another endpoint used – the fresh weight of plants after five days of exposure – was also sensitive to highlight the toxic effects of the contaminated soil. The fresh weight was reduced at the highest concentrations of contaminated soil, whereas there were no changes at different concentrations of control soil (Table 1).

In the experiments with lettuce seeds exposed directly on the ground, the germination delay on the first day of exposure was more pronounced than the results obtained with the seeds exposed to soluble fraction, while by the end of five days the germination rate did not differ among any of soil concentrations. However, the wet weight did not differ among the different concentrations or between any concentration and the control (Table 2).

### Feeding measurements in woodlice

Mortality rate was between 10 and 20% for both woodlice species in the control and contaminated soil (Table 3). Feeding parameters and growth in

Table 3. Mortality and feeding parameters of *A. vulgare* and *P. dilatatus* cultured for 21 days in control and contaminated urban soil

| Parameter | Control soil | Contaminated soil |
|---|---|---|
| *A. vulgare* | | |
| Mortality (%) | 10 | 20 |
| Consumption rate | 0.28 ± 0.12 | 0.31 ± 0.12 |
| Assimilation rate | 0.20 ± 0.15 | 0.23 ± 0.11 |
| Assimilation efficiency (%) | 64.22 ± 28.19 | 73.99 ± 19.03 |
| Growth rate (%) | 12.04 ± 6.74 | 17.02 ± 11.06 |
| *P. dilatatus* | | |
| Mortality (%) | 20 | 20 |
| Consumption rate | 0.39 ± 0.09 | 0.65* ± 0.23 |
| Assimilation rate | 0.29 ± 0.07 | 0.52* ± 0.19 |
| Assimilation efficiency (%) | 76.01 ± 13.83 | 81.67 ± 13.09 |
| Growth rate (%) | 7.23 ± 11.51 | 30.36* ± 11.22 |

Results are expressed as the mean of the parameter and standard deviation; *indicate significant differences between control and contaminated soil

Table 4. Metal concentration in soil samples and soil aqueous extracts

| | As | Cd | Cr | Cu | Ni | Pb | Zn |
|---|---|---|---|---|---|---|---|
| **Soil samples** (mg/kg) | | | | | | | |
| Control soil | 2.28 | 0.558 | 95.50 | 31.64 | 19.66 | 36.70 | 102.45 |
| Contaminated soil | 8.71 | 0.902 | 185.83 | 88.91 | 39.75 | 179.03 | 504.56 |
| Soil prevention levels[1] | 15 | 1.3 | 75 | 60 | 30 | 72 | 300 |
| Soil investigation levels[1] (residential soil) | 55 | 8 | 300 | 400 | 100 | 300 | 1000 |
| **Soil extract samples** (µg/l) | | | | | | | |
| Control soil | 0.367 | – | 28.30 | 55.71 | 12.79 | 8.075 | 77.92 |
| Contaminated soil | 2.529 | 0.87 | 50.59 | 91.68 | 36.93 | 58.20 | 101.50 |
| Brazilian standards to groundwater[1] | 10 | 5 | 50 | 2000 | 20 | 10 | 1050 |

[1]CONAMA (2009)

*A. vulgare* were not significantly different between the control and contaminated soils, while some parameters in *P. dilatatus* were statistically distinct between both soils. However, the contaminated soil improves feeding measurements in *P. dilatatus* in the following parameters: consumption rate, assimilation rate and growth rate (Table 3).

### Metal quantification

Among the metallic elements analysed, the concentration of Cr, Cu, Pb and Zn in contaminated soil was above prevention levels laid down by Brazilian legislation, but no evaluated metal exceeded the investigation levels for residential soils (CONAMA 2009), characterizing this soil as moderately contaminated. In the control soil, only Cr had levels above the level of prevention, while none of the elements exceeded the investigation levels for residential soils (Table 4).

On the other hand, the Cr, Ni and Pb levels in the aqueous extract of contaminated soil were above the Brazilians limits allowed for groundwater while in the control soil extract none of the elements exceeded the Brazilians levels (CONAMA 2009) (Table 4).

## DISCUSSION

This study evaluated the toxicity of a soil located in an urban-industrial zone, where the major input of contaminants from industries originates from atmospheric dispersion. Soil contamination under these conditions requires a sensitive endpoint for visualization of environmental damage, especially in cases where the element concentrations are at levels permitted by legislation. For this study, bioassays with three species were used for assessing toxicity: acute toxicity bioassay with lettuce seeds and toxicity tests with woodlice (*Armadillidium vulgare* and *Porcellio dilatatus*).

In the bioassay with lettuce seeds, the number of seeds germinated after five days of exposure is commonly used as a classical endpoint. If only this endpoint was taken into account, the soil shows no toxicity for the experimental model, neither for the solubilized test nor seeds exposed directly to soil. However, when other endpoints were added (daily number of germinated seeds and fresh weight of plants after five days of exposure) toxicity was observed in the sample of contaminated soil and absence of toxicity in the control soil sample, which confirmed the sensitivity of these other endpoints.

Studies evaluating the phytotoxicity of soils have been recommended for assessment of soil quality (Keddy *et al.* 1995; Whang & Freemark 1995). However, some studies have shown the low sensitivity of seed germination to predict phytotoxicity of soil samples (Sverdrup *et al.* 2003; Smith *et al.* 2006), including the toxicity test with lettuce seeds (Eom *et al.* 2007). Robidoux *et al.* (2004) and Marti *et al.* (2007) used parameters related to growth, and they also found low sensitivity of the assay with lettuce to predict toxicity of soil samples. These results underline the importance of finding endpoints sensitive in bioassay with lettuce, including soils with low or moderate levels of contamination.

Our strategy to evaluate the toxicity of the solubilized soil and the effect of the seeds directly on the soil was useful, considering that each form of exposure showed different sensitivity to the two alternative endpoints: assessment of the solubilized presented wet weight as an important endpoint, while seeds deposited directly on the ground revealed a pronounced delay in germination at the highest concentrations of contaminated soil. This delay in germination was previously reported by Valerio *et al*. (2007) working with soluble elements of soil. In this study, the soluble fraction also induced germination delay, but it was more pronounced in the study with the seeds deposited directly on the ground.

Regarding the sensitivity of the wet weight as endpoint for evaluation of toxicity of soil samples, Eom *et al*. (2007) found that wet weight was a more sensitive parameter than dry weight to investigate soil toxicity in lettuce grown directly on the ground. In this study, the wet weight of plants growing directly on the ground showed no differences between the control and the highest concentrations of contaminated soil. However, the fresh weight was a sensitive endpoint for plants grown in the solubilized treatment, decreasing weight as the concentration of contaminated soil on soluble increased. Phytotoxicity of soil under the influence of atmospheric dispersion of pollutants investigated by bioassay with lettuce seeds could be related to concentrations of metallic elements found in soil and its extracts

Regarding the soil invertebrates, Odendaal and Reinecke (2004) commented that little attention was paid to the toxic effects of environmental mixtures on non-target organisms and that this strategy involved more realistic models for the understanding of environmental toxicity. For both woodlouse species, there was no difference between mortality rates from animals exposed to control and contaminated soil.

The feeding-related parameters have been reported as a promising tool for the investigation of sub-lethal toxicity in terrestrial isopods exposed to metals (Drobne & Hopkin 1995; Drobne 1997). In the present study, these parameters were not affected by metals present in the urban soil under the influence of atmospheric dispersion of pollutants. The short exposure period (21 days) could be one reason for this absence of toxic effect in woodlice (Ribeiro *et al*. 2001). Another explanation also approached by Ribeiro *et al*. (2001) is a narrow line between lethal and sub-lethal effects in woodlice. This can be possible because the woodlice can trap certain metals in granules in their internal organs (Donker 1992; Paoletti & Hassall 1999), not suffering the toxic effects of the metals. The contaminated urban soil may not contain the toxic concentrations of metals for the species studied, or metals in the mixture have antagonistic effects.

Bioassays used in the present study showed distinct responses for assessment of an urban soil with moderate level of contamination. Some researches affirm to be unsatisfactory to use a single ecotoxicity test for soil quality assessment, revelling a disproportionate effect on different organisms (Paton *et al*. 2005; Lors *et al*. 2010). Our results reaffirm the view of using different organisms to investigate the soil quality, mainly in soil with moderate or low levels of contamination. Even at moderate concentrations, the mixture of contaminants in the soil seems to exert phytotoxic action in lettuce seeds while not displaying toxicity to woodlice. Although the concentration of metals in contaminated soil and its extract appears to contribute to phytotoxicity, we cannot discard the negative contribution of organics, especially petroleum hydrocarbons arising industrial and vehicle emissions.

## CONCLUSION

The alternative parameters analysed in the lettuce seed bioassay proved to be sufficiently sensitive for evaluation of acute toxicity of soil extracts with moderate level of contamination. On the other hand, the terrestrial isopod assimilation assay did not show any differences between both soils evaluated. However, this bioassay can be tested in other contamination conditions.

***Acknowledgements.*** The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, for the Doctorate (F.M.R. da Silva Júnior) and Master's (E.M. Garcia) scholarships.

### References

CONAMA (2009): Standard 420. Disposes on Criteria and Guidelines Values of Soil Quality Concerning the Presence of Chemical Substances and Establishes Guidelines for Environmental Management of Areas Contaminated by These Substances as a Result of Anthropogenic Activities. Conselho Nacional do Meio Ambiente, Brasília. (in Portuguese)

Cunha N.G., Silveira R.J.C., Severo C.R.S. (1995): Study from soils of Rio Grande Municipality. Universitária/ UFPel; EMBRAPA/CPACT. Documentos 16/96. Pelotas. (in Portuguese)

Da Silva Júnior F.M.R., Rocha J.A.V., Vargas V.M.F. (2009): Extraction parameters in the mutagenicity assay of soil samples. Science of the Total Environment, **407**: 6017–6023.

Donker M.H. (1992): Energy reserves and distribution of metals in populations of the isopod *Porcellio scaber* from metal contaminated sites. Functional Ecology, **6**: 445–454.

Drobne D. (1997): Terrestrial isopods – a good choice for toxicity testing of pollutants in the terrestrial environment. Environmental Toxicology and Chemistry, **16**: 1159–1164.

Drobne D., Hopkin S.P. (1995): The toxicity of zinc to terrestrial isopods in a standard laboratory test. Ecotoxicology and Environmental Safety, **31**: 1–6.

Eom I.C., Rast C., Veber A.M., Vasseur P. (2007): Ecotoxicity of a polycyclic aromatic hydrocarbon (PAH)-contaminated soil. Ecotoxicology and Environmental Safety, **67**: 190–205.

Gong P., Wilke B.M., Fleischmann S. (1999): Soil-based phytotoxicity of 2,4,6-trinitrotoluene (TNT) to terrestrial higher plants. Archives of Environmental Contamination and Toxicology, **36**: 152–157.

Hassall M., Zimmer M., Loureiro S. (2005): Questions and possible new directions for research into the biology of terrestrial isopods. European Journal of Soil Biology, **41**: 57–61.

Keddy C.J., Greene J.C., Bonnell M.A. (1995): Review of whole-organism bioassays: soil, freshwater sediment and freshwater assessment in Canada. Ecotoxicology and Environmental Safety, **30**: 221–251.

Lors C., Ponge J-F., Aldaya M.M., Daminot D. (2010): Comparison of solid-phase bioassays and ecoscores to evaluate the toxicity of contaminated soils. Environmental Pollution, **158**: 2640–2647.

Martí E., Sierra J., Sanchez M., Cruañas R., Garau M.A. (2007): Ecotoxicological tests assessment of soils polluted by chromium(VI) or pentachlorophenol. Science of the Total Environment, **378**: 53–57.

Odendaal J.P., Reinecke A.J. (2004): Effect of metal mixtures (Cd and Zn) on body weight in terrestrial isopods. Archives of Environmental Contamination and Toxicology, **46**: 377–384.

Paoletti M.G., Hassall M. (1999): Woodlice (Isopoda: Oniscidea): their potential for assessing sustainability and use as bioindicators. Agriculture, Ecosystems & Environment, **74**: 157–165.

Paton G.I., Killham K., Weitz H.J., Semple K.T. (2005): Biological tools for the assessment of contaminated land: applied soil ecotoxicology. Soil Use Management, **21**: 487–499.

Plaza G., Nalecz-Jawecki G., Ulfig K., Brigmon R.L. (2005): Assessment of genotoxic activity of petroleum hydrocarbon-bioremediated soil. Ecotoxicology and Environmental Safety, **62**: 415–420.

Ribeiro S., Souza J.P., Nogueira A.J.A., Soares A.M.V.M. (2001): Effect of endosulfan and parathion on energy reserves and physiological parameters of the terrestrial isopod *Porcellio dilatatus*. Ecotoxicology and Environmental Safety, **49**: 131–138.

Robidoux P.Y., Gong P., Sarrazin M., Bardai G., Paquet L., Hawari J., Dubois C., Sunahara G.I. (2004): Toxicity assessment of contaminated soils from an antitank firing range. Ecotoxicology and Environmental Safety, **58**, 300–313.

Smith M.J., Flowers T.H., Duncan H.J., Alder J. (2006): Effects of polycyclic aromatic hydrocarbons on germination and subsequent growth of grasses and legumes in freshly contaminated soil and soil with aged PAHs residues. Environmental Pollution, **141**: 519–525.

Sverdrup L.E., Krogh P.H., Nielsen T., Kjaer C., Stenersen J. (2003): Toxicity of eight polycyclic aromatic compounds to red clover (*Trifolium pratense*), ryegrass (*Lolium perenne*), and mustard (*Sinapsis alba*). Chemosphere, **53**: 993–1003.

USEPA (1989): Protocol for Short Term Toxicity Screening of Hazardous Waste Sites. EPA, Chicago.

Valerio M.E., García J.F., Peinado F.M. (2007): Determination of phytotoxicity of soluble elements in soils, based on a bioassay with lettuce (*Lactuca sativa* L.). Science of the Total Environment, **378**: 63–66.

Whang W., Freemark K. (1995): The use of plants for environmental monitoring and assessment. Ecotoxicology and Environmental Safety, **30**: 289–301.

Zimmer M., Kautz G., Topp W. (2003): Leaf litter-colonizing microbiota: supplementary food source or indicator of food quality for *Porcellio scaber* (Isopoda: Oniscidea). European Journal of Soil Biology, **39**: 209–216.

Received for publication June 4, 2012
Accepted after corrections November 21, 2012

*Corresponding author:*

Flavio Manoel Rodrigues da Silva Júnior, Universidade Federal do Rio Grande do Sul – FURG, Instituto de Ciências Biológicas, Laboratório de Ensaios Farmacológicos e Toxicológicos, Campus Carreiros, CEP 96203-900, Rio Grande, Brasil
e-mail: f.m.r.silvajunior@gmail.com