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Ecotoxicological Tools for Landfarming Soil Evaluation in a Petrochemical Complex Area*[1]

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ABSTRACT

The demand for simple and rapid bioassays in ecotoxicological evaluations is of paramount importance in order to speed up environmental monitoring programs. In this study we performed bioassays with lettuce seeds and two species of terrestrial isopods (*Armadillidium vulgare* and *Porcellio dilatatus*) for the ecotoxicological assessment of a landfarming soil from a petrochemical complex area. The solubilized content of test soil demonstrated a concentration-response type toxic effect on seed germination rate, and a delay on germination, but showed toxic effect on seedlings wet weight only at the highest concentration. Toxic effects were also observed in mortality rate and avoidance behavior of the two woodlice species. These results demonstrated the sensitiveness of the organisms studied, and highlighted the possibility to use these bioassays in environmental monitoring programs in areas contaminated with fossil fuels.

Key Words: *Armadillidium vulgare*, lettuce, *Porcellio dilatatus*, terrestrial woodlice

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The use of bioassays to detect alterations caused by toxic agents has become an important complement to the simple chemical analysis. Ideal bioassays must have some particular properties, such as being robust, standardized, cheap and simple. They must also attempt to include different ecological/biological functions, to have a clear endpoint and to allow comparisons between different sites, besides responding to environmental stress and changes in such stress (Paton *et al.*, 2005).

Ecotoxicological evaluations on soils have been performed mainly with aqueous fraction and using aquatic organisms. Studies with terrestrial species have been focused on plants and invertebrates (earthworms and springtails) (Sverdrup *et al.*, 2003; Paton *et al.*, 2005). These organisms are chosen based on the sensitiveness of their response. Such choice of organisms is normally related to the benefits of the soil and to ecosystem functions such as water storage, decomposition and nutrient cycling (Doran and Zeiss, 2000).

In this study, we performed bioassays with a vegetable species (*Lactuca sativa*, lettuce) and with two species of terrestrial invertebrates which are important to the decomposition process in the soil (*Armadillidium vulgare* and *Porcellio dilatatus*, two common Isopoda species). Germination, growth and dry or wet biomass are the parameters used in phytotoxicity evaluation of contaminated soils, which can be analyzed by planting the seeds directly in the soil or using aqueous fractions for watering the seeds (Wang and Freemark, 1995; Gong *et al.*, 1999; Henner *et al.*, 1999; Płaza *et al.*, 2005; Smith *et al.*, 2006). Lettuce is one of the most used vegetables in phytotoxicity assessment (Robidoux *et al.*, 2004; Eom *et al.*, 2007; Martí *et al.*, 2007; Valerio *et al.*, 2007).

Terrestrial invertebrates have become important organisms for monitoring polluted sites because they play important roles in decomposing organic matter and recycling nutrients. Among such invertebrates, terrestrial woodlice are particularly interesting because

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they are detritivore and accumulate heavy metals. Also, it is a group of organisms composed of species that can be very resistant or even highly sensitive (Cortet *et al.*, 2000). Mortality, growth and reproduction rates and also their avoidance behavior have been the most important endpoints used in ecotoxicological studies with woodlice (Paoletti and Hassall, 1999; Jänsch *et al.*, 2005; Loureiro *et al.*, 2005).

The objectives of the present study were to investigate the responses of different terrestrial organisms to a soil from a petrochemical area treated by landfarming with the lettuce and woodlice bioassays and to indicate the recovery status of the site.

MATERIALS AND METHODS

Study area and measurements of polycyclic aromatic hydrocarbons and heavy metals

The soil was collected from a waste treatment system SICECORS in an area under landfarming bioremediation process at Pólo Petroquímico do Sul, Triunfo, Rio Grande do Sul, Brazil, and was supplied by Companhia Petroquímica do Sul (COPESUL). This soil was at initial treatment stage (less than one year), and was contaminated with many types of pollutants, including polycyclic aromatic hydrocarbons (PAHs) and heavy metals, listed in Table I. The landfarming soil had sand 65.4%, silt 13.7%, and clay 20.9%. A control artificial soil with the same granulometry was prepared to mix with landfarming soil.

PAH analysis was performed using gas chromatography coupled to mass spectrometry (GCMS) by injecting 1 μL aliquot of the extracts, with a split/ splitless injector (1:50) and an HP-5 fused silica capillary column (60 m × 0.25 mm × 0.25 μm). Electron impact mass spectra were obtained at 1 keV of ionization energy. Helium was used as the carrier gas at a flow of 1 mL min^{-1}. Temperature was programmed from 120 to 220 °C at 5 °C min^{-1}, followed by a 10 °C min^{-1} increasing rate until it reached 280 °C. The interface temperature was 280 °C.

Copper and zinc in the soil samples were analyzed by flame atomic absorption spectrophotometry (AASPerkin-Elmer 800, Überlingen, Germany), while electrochemical atomization mode with Zeeman correction was used in chromium, nickel, lead and arsenic analysis in the soil samples.

Organisms test

Lettuce seeds from TopSeed Garden, Petrópolis, Brazil, were submerged in diluted hypochlorite (10 g L^{-1}), and rinsed in tap water before use. All lettuce experiments were initialized on the same day and they were performed with the seeds from the same batch. The woodlice *A. vulgare* and *P. dilatatus* were collected in areas of organic farming and maintained in laboratory conditions (temperature 28 °C, in the dark, and frequently moistened using water sprinklers). Only antenna-bearing adults of undistinguished sex were selected for the tests. No gravid females were used. The animals were kept in the laboratory in earthworm humus and were fed with jambul tree leaves (*Syzygium jambolanum*).

Acute toxicity test with lettuce

The soil was solubilized at six different concentrations (0, 5, 15, 50, 150 and 500 mg L^{-1}) using mineral water as solvent. The stock solution (500 g L^{-1}) was

TABLE I

Contents of polycyclic aromatic hydrocarbons (PAHs) and heavy metals in the landfarming soil from a petrochemical industrial complex area

PAH	Concentration	PAH	Concentration	Metal	Concentration
	μg kg^{-1} dry soil		μg kg^{-1} dry soil		mg kg^{-1} dry soil
Naphthalene	62.60	Benzo(a)anthracene	2 940.08	Nickel	19.49
2-Methyl naphthalene	71.87	Chrysene	5 348.95	Zinc	97.78
1-Methyl naphthalene	70.56	Benzo(b)fluoranthene	930.58	Lead	24.37
2,6-Dimethyl naphthalene	83.77	Benzo(k)fluoranthene	205.93	Copper	21.23
1,7-Dimethyl naphthalene	< 1.66	Benzo(e)pyrene	860.07	Chromium	27.95
Biphenyl	115.48	Benzo(a)pyrene	838.45	Arsenic	3.61
Acenaphthylene	5 442.47	Perylene	226.40		
Acenaphthene	118.56	Indeno(1,2,3-cd)pyrene	359.68		
Fluorene	1 056.52	Dibenzo(a,h)anthracene	188.00		
Dibenzothiophene	21.04	Benzo(g,h,i)perylene	343.62		
Phenanthrene	3 422.89	ΣTotal PAHs	29 177.97		
Anthracene	3 630.22	Σ2–3 rings PAHs	14 095.99		
Fluoranthene	1 540.49	Σ4–6 rings PAHs	13 541.49		
Pyrene	1 299.72				