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Comparison of solid-phase bioassays and ecoscores to evaluate the toxicity of contaminated soils

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The avoidance test using the soil springtail Folsomia candida is globally more sensitive to PAH contamination than acute and chronic toxicity bioassays using plants and animals but a battery of tests could reveal better in detail.

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ABSTRACT

Five bioassays (inhibition of lettuce germination and growth, earthworm mortality, inhibition of springtail population growth, avoidance by springtails) were compared, using four coke factory soils contaminated by PAHs and trace elements, before and after biotreatment. For each bioassay, several endpoints were combined in an 'ecoscore', a measure of test sensitivity. Ecoscores pooled over all tested bioassays revealed that most organisms were highly sensitive to the concentration of 3-ring PAHs. When four soils were combined, behavioural tests using the springtail *Folsomia candida* showed higher ecoscores, i.e. they were most sensitive to soil contamination. However, despite overall higher sensitivity of behavioural tests, which could be used for cheap and rapid assessment of soil toxicity, especially at low levels of contamination, some test endpoints were more sensitive than others, and this may differ from a soil to another, pointing to the need for a battery of bioassays when more itemized results are expected.

1. Introduction

Industrial activities lead to the discharge of a wide range of hazardous chemicals in soils, often far from emission sources (Jones et al., 1989; Nam et al., 2008). Soil pollutants include polycyclic aromatic hydrocarbons (PAHs) and heavy metals, known for potential adverse ecological and toxicological effects (Bispo et al., 1999; Peralta-Videa et al., 2002; Boularbah et al., 2006). Polluted soils also are a threat to ecosystem and human health (Menzie et al., 1992; Lawlor et al., 1997; Preuss et al., 2003). This threat is generally approached by quantifying the total content of pollutants in the contaminated matrices. Nevertheless this provides only limited information on pollutant bioavailability, and no information on synergetic or antagonistic interactions between pollutants (Juvonen et al., 2000), or on effects on organisms, for which only a biological approach is effective (Fernández et al., 2005). An ecotoxicological approach, using biological tests on target organisms at different trophic levels, has been recommended for a refined evaluation of environmental hazards in complement of chemical analyses (Bispo et al., 1999; Rila and Eisentraeger, 2003; Fernández et al., 2005; Plaza et al., 2005). Indeed, bioassays integrate the impact of all contaminants including those not considered or detected by chemical analyses, and they take account of additive, synergistic and antagonistic phenomena.

Direct toxic effects on survival, growth or reproduction of test organisms may reflect the ecotoxicological potential of contaminated soils (Fent, 2003). Phytotoxicity tests, such as lettuce bioassays, provide a variety of assessment endpoints such as germination and root elongation rates and enzyme activities (Ferrari et al., 1999). Soil invertebrates have also been used in ecotoxicology, in particular earthworms (Fernández et al., 2005; Eom et al., 2007), enchytraeids (Römbke, 2003), springtails (Domene et al., 2007; Eom et al., 2007) and woodlice (Jänsch et al., 2005; Loureiro et al., 2005). Springtail, earthworm and lettuce soil

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quality tests have been standardized according to ISO (1999), ISO (1993a, 1998a) and ISO (1993b, 2005a), respectively.

Based on the ability of animals to probe and flee from contaminated places (Best et al., 1978; Salminen and Sulkava, 1996; Gass et al., 2006), avoidance tests have a great potential as early screening tools in lower tier levels of ecological risk assessment, because they are robust, sensitive, cost-effective, ecologically relevant and rapid (Natal-da-Luz et al., 2004, 2008a). Avoidance tests are now currently performed with earthworms (Loureiro et al., 2005; Natal-da-Luz et al., 2008a,b; Garcia et al., 2008; De Silva and Van Gestel, 2009; Owojori and Reinecke, 2009), enchytraeids (Amorim et al., 2008; Loureiro et al., 2009; Kobetičová et al., 2009), woodlice (Loureiro et al., 2009) and springtails (Heupel, 2002; Martínez Aldaya et al., 2006; Natal-da-Luz et al., 2008a,b, 2009) and an international standard for the assessment of soil quality using earthworm avoidance tests exists (ISO, 2008a).

Several studies compared some toxicity and avoidance endpoints (Greenslade and Vaughan, 2003; Loureiro et al., 2005; Martínez Aldaya et al., 2006), but a comparison between tests commonly used for the biological assessment of soil quality is clearly lacking, and studies using a battery of soil and aquatic test organisms did not include avoidance endpoints (Fernández et al., 2005; Pandard et al., 2006; Domene et al., 2008). Such a comparison should be valid both for scaling toxicity and behavioural tests according to their sensitivity as early screening tools, and for pooling them in a bulk index of soil toxicity.

The reported work evaluates the toxicity of contaminated soils by comparing a variety of solid-phase bioassays applied to PAH-contaminated soils issued from former coke sites in northern France. Studied soils differed by their PAH content and the presence or not of a mixed pollution by heavy metals and/or cyanides. The aims of our study were: (1) to characterize contaminated soils using ecotoxicological (including behavioural endpoints) and chemical analyses, (2) to estimate the likely relationships between pollutants and toxicity responses, (3) to compare the sensitivity of toxicity tests representing different trophic and toxicity levels with the *Folsomia candida* (Collembola) avoidance test. Toxicity tests relied on the germination and growth of the lettuce *Lactuca sativa* (Asteraceae) and on the survival and reproduction of the springtail *F. candida* (Isotomidae) and the earthworm *Eisenia fetida* (Lumbricidae). Two alternative hypotheses were (1) either a test or a group of tests gives a better response to all soils and thus could be used preferentially as a sensitive indicator of soil quality, its performance being measured by an 'ecoscore', or (2) each test or group of tests exhibits a specific response and as a consequence is not enough to assess soil quality, in which case several bioassays are necessary.

2. Materials and methods

2.1. Soil samples

Experiments were carried out on PAH-co1ntaminated soils from three industrial sites located in the North of France, the main activity of which was the distillation of coal tar. Soil 1 was fairly polluted with a mixture of PAHs, cyanides and heavy metals. Soil 2 was recovered after 18 months of windrow biotreatment. Despite bioremediation, this soil was still characterized by a high content of PAHs, cyanides and heavy metals. Contrary to Soil 2, Soil 3 was only polluted by PAHs, with a concentration similar to that of Soil 2. In the same site a windrow biotreatment was applied to this soil and Soil 3T was sampled after six months of biotreatment (Lors et al., 2009). After biotreatment, Soil 3T showed a PAHs concentration lower than that of Soil 1.

Unpolluted soils were also sampled in the three studied sites in uncontaminated areas (Table 2), which were used as controls in the avoidance test and as a matrix of dilution in toxicity bioassays. Previous chemical and ecotoxicological analyses were performed on control soils, which did not reveal any toxicity.

2.2. Chemical analyses

Soil pH_{water} was measured using a Consort® C83 pH-meter fitted with a glass electrode corrected for temperature and a Schott® box with Ingold® combined electrodes, according to ISO (2005b). Total organic carbon concentration was obtained from total carbon and inorganic carbon contents, determined with a TOC-5000A Shimatzu® analyser, according to ISO (1995a). Total organic nitrogen concentration was determined by the Kjeldahl method, according to ISO (1995b). Total phosphorus as well as metals (As, Cd, Co, Cr, Cu, Ni, Pb, Zn) were dosed by Inductive Coupled Plasma Atomic Emission Spectrometry (ICP-AES) in a 138 Ultrace Jobin Yvon® analyser after hot hydrofluoric and perchloric acid digestion of solid phase, according to ISO (2001, 2008b).

Concentrations of the 16 PAHs of the US-EPA list (Verschueren, 2001) were measured using High Performance Liquid Chromatography (HPLC) in a 2690 HPLC Waters® analyser fitted with an ultraviolet inverted phase C 18 Supelco® column (length 250 mm, internal diameter 2.1 m) coupled to a 996 Waters® UV photodiode array detector according to ISO (1998b), after extraction by dichloromethane/acetone (50/50 v/v) using the Accelerated Solvent Extractor Dionex® ASE 200. Total cyanides were determined according to ISO (2003). All chemical analyses were done in triplicate.

2.3. Toxicological analyses

Toxicity results were the responses of test organisms according to concentration of soil samples in test media (%, w/w). NOEC was the highest effective concentration at which no significant effect was detected, while EC_{10}, EC_{20} and EC_{50} were the calculated concentrations at which the measured endpoint was reduced to 10%, 20% and 50% of the control value, respectively. Toxic effects were also calculated as percent inhibition at the highest concentration of the contaminated soil and as Toxic Units or TU ($= 100/EC_{50}$). In mortality tests (endpoint survival), results were expressed as lethal concentrations reducing survival by 10%, 20% and 50% (LC_{10}, LC_{20} and LC_{50}, respectively) compared to controls.

Phytotoxicity tests were conducted according to ISO (1993b, 2005a), using only *L. sativa* (lettuce). Tests were carried out in a chamber at 20 ± 2 °C under constant illumination (4000–7000 lx), with a 16:8 day–night light cycle. Assays were conducted in plastic pots (diameter 11 cm, height 10 cm) containing 200 g of contaminated substrate moistened at 70–80% water-holding capacity. The moisture level was maintained constant by adding distilled water every day. Twenty seeds were placed at the surface of the test medium. Five concentrations of the contaminated soil were tested: 100%, 60%, 35%, 20% and 10%, w/w. For each concentration, analyses were done in triplicate. Seedling emergence (%) was determined after seven days of exposure. Seedling wet and dry biomasses were measured after 14 days of exposure. Results were expressed as percent lettuce germination and growth in comparison with controls.

Acute toxicity tests with the earthworm *E. fetida* were carried out according to ISO (1993a). The survival of adult earthworms was determined after 14 days of exposure. Ten individuals were placed in a glass jar containing 500 g of wet soil at 70–80% (w/w) moisture. Various concentrations of the studied soil in the control soil were tested in the range 1–100%. For each tested concentration, four replicate cultures were done. The jars were exposed in an environmental chamber at 20 ± 1 °C under a 16:8 (400–800 lx) day–night light cycle. Results were expressed as percent mortality in comparison with controls.

The springtail reproduction test was conducted according to ISO (1999), modified according to Martínez Aldaya et al. (2006). Population growth responses were assessed by introducing 10 parthenogenetic females of *F. candida* into each of five rearing chambers (crystal polystyrene boxes 45 mm diameter, 25 mm height), fifth-filled with the control soil or with the polluted soil at 0.35%, 1%, 5%, 10%, 50% and 100% concentration. A small amount of dry cattle dung powder was added above the soil substrate before animals were introduced, then boxes were incubated at 20 °C in darkness during 40 days. At the end of the experiment, the whole population was collected, using forceps and flotation.

Table 1
Main physicochemical characteristics of the four studied soils.

	Texture	Moisture (%)	pH_{water}	Total carbon (%)	Total organic carbon (%)	Total organic nitrogen (mg kg^{-1})	Total phosphorus (mg kg^{-1})
Soil 1	Silty sand	9.0 ± 0.2	8.1 ± 0.02	9.7 ± 0.2	9.2	1300	770
Soil 2	Sand	18.9 ± 0.6	7.8 ± 0.03	44.3 ± 1.4	44.2	5600	1900
Soil 3	Sand	17.4 ± 0.1	7.9 ± 0.02	11.2 ± 0.6	9.0 ± 0.5	1700	620
Soil 3T	Sand	16.3 ± 0.3	8.3 ± 0.01	8.6 ± 0.2	5.7 ± 0.1	2088	670

Means of three replicated measures followed by standard deviations. Concentrations are expressed on a dry soil basis.