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Suitability of the OCDE tests to estimate contamination with 2,4-dichlorophenol of soils from Galicia (NW Spain)

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Abstract

The objective of the present study was to verify whether the *generic reference levels* (GRL) for soils contaminated with 2,4-dichlorophenol (2,4 DCP), established by Spanish legislation and published in the *Real Decreto 9/2005*, are accurate for Galician soils. For this, the surface horizons of seven soils under different types of land use were experimentally contaminated with different doses (between 0 and 10,000 times the GRL) of 2,4 DCP, and were then were subject to OECD toxicity test numbers 208 (root emergence and elongation) and 216 (soil nitrogen mineralization). The results obtained for the nitrogen mineralization test were difficult to interpret because they varied among soils, whereas the results of the root germination and elongation test were more coherent — the values decreased with increasing doses of contaminant added to the soil. The results suggest that the root elongation test reflects the effect of this contaminant more clearly than the soil nitrogen mineralization test. Nevertheless, considering that in the lowest quality soils (i.e. agricultural soils containing a very low level of organic matter) contaminant doses of up to 1000 times the GRL did not affect root germination and elongation, it is clear that the GRL indicated in the relevant legislation are very low for the soils under study and that the threshold should be established taking into account the soil characteristics.

Keywords: Polluted soils; Generic reference levels; OECD tests 208 and 216; 2,4-dichlorophenol

1. Introduction

The Spanish Ministry of the Environment (*Ministerio de Medio Ambiente*) has recently published a Royal Decree (*Real Decreto* 9/2005: BOE 18 enero, 2005) that outlines potentially contaminating activities and establishes the criteria and standards by which a soil can be declared as contaminated. The *Real Decreto*, which to a certain extent completes Law 10/98 on Waste Products, (*Ley 10/98 de Residuos*) outlines the regulations for the so-called generic reference levels (GRL — a basic parameter used to evaluate soil contamination by certain substances), describes the same for groups of substances, and specifies the criteria for calculating generic reference levels for those substances not included in the corresponding annexes.

Although the decree is a notable improvement in terms of legislation on contaminated soil, its application to specific cases raises some questions. Treatment of all soils in the same way, without taking into consideration their different physical, chemical and biochemical characteristics, may affect the response to the contaminant, without even considering generic levels. Furthermore,

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the methodology for demonstrating the degradation suffered by a soil as a result of the presence of a contaminant is scarcely mentioned and at most different OECD toxicity tests are cited as diagnostic methods: OECD test 208 (germination and root elongation), test 216 (mineralization of soil N) and test 217 (mineralization of soil C). These tests are described by the OECD itself very briefly (OECD/OCDE, 2000, 2003), which leads to them being carried out in different ways in different laboratories, thereby complicating interpretation of the results.

With the aim of finding out the problems associated with the above-mentioned decree, we studied the effects of 2,4-dichlorophenol (2,4 DCP) on some soils from Galicia. The compound was selected because of its widespread use as a feedstock for the manufacture of certain methyl compounds used in mothproofing, antiseptics and seed disinfectants (Spectrum Laboratories Inc., 2006) and because of the lack of published data on its effects on soil.

2. Materials and methods

The contaminant used was 2,4-dichlorophenol, for which the generic reference level (GRL) has been fixed for soils with unlimited use at 0.1 μg g^{-1} In other words a soil would be considered contaminated if it contained more than 10 μg g^{-1} of this compound (100 times the GRL). The compound was mixed with washed quartz sand before being added to the soils, because of its low solubility in water. The mixture of quartz sand and contaminant was placed in a rotary shaker for 48 h before use to achieve a homogeneous mixture. A total of 7 Galician soils subject to different types of land use, and with different properties, were used in the study (Table 1). The soils were: oak 1, oak 2 (under oakwood); pine 1, pine 2 (under pinewood); eucalyptus (under *E. globulus* L. vegetation); pasture (under pasture) and vegetable (under vegetable crops). All soils were Umbrisols, except the soil under vegetable crops, which was classified as an Aric Anthrosol (ISSS Working Group RB, 1998).

The soils were contaminated by adding the sand/contaminant mixture (5 g of sand to 100 g dry soil) to achieve contaminant levels of 0, 10, 100, 500 and 1000 μg g^{-1} (i.e. 0, 100, 1000, 5000 and 100,000 times the GRL) and enough water was added to maintain optimal moisture conditions. After 72 h, aliquots of soil were subject to OECD tests 208 (germination and root elongation) and 216 (soil N mineralization) (OECD/OCDE, 2000, 2003).

For toxicity test 208, four Petri dishes containing 15 g of soil and 15 garden cress (*Lepidium sativum* L.) seeds (previously soaked in water) were prepared for each soil and each dose of contaminant, i.e. a total of 60 determinations per soil. Quantification of germination and measurement of root elongation were carried out 5 days after sowing the seed. For OECD toxicity test 216, experiments were prepared in which aliquots of the soils were incubated for 10 days at 25 °C and optimal moisture content (Leirós et al., 2000). Inorganic forms of N present before and after incubation were extracted with 2 M KCl (ratio 1:10, for 2 h) and determined according to Bremner (1965), by semi-micro distillation.

The methods described by Guitián and Carballas (1976) were used to determine the following soil properties: pH in water (1:2.5, soil:water ratio), pH in 1 M KCl (1:2.5, soil:solution ratio); total C content (by potassium dichromate oxidation); total N content (by Kjeldahl digestion) and particle size distribution (with a Robinson pipette and Calgon as dispersant).

Differences in means were tested by analysis of variance (ANOVA), with SPSS 12.0 (SPSS®, SPSS Inc., 1989–2003).

Table 1
General properties of the soils used

Soil	pH water	pH KCl	Total C (%)	Total N (%)	C/N	Texture
Oak 1	4.25	3.46	8.27	0.434	19	Sandy-loam
Oak 2	4.95	3.95	4.86	0.399	12	Loam
Pine 1	4.32	3.52	7.25	0.423	17	Sandy-loam
Pine 2	4.67	3.97	5.02	0.392	12	Loam
Eucalyptus	4.59	3.97	5.24	0.388	13	Sandy-loam
Pasture	5.30	4.47	7.28	0.493	15	Loam
Vegetable	6.12	5.34	1.84	0.206	9	Sand

3. Results

3.1. General soil data

The soils are acid, except the vegetable soil, which was almost neutral (Table 1). The total carbon content ranged between 1.84% in the vegetable soil and 8.27% in the surface horizon of the Umbrisols developed under oakwood (Oak 1). The soil textures were loam, sandy-loam and sand, with the sand fraction predominating in all cases. The C/N ratio greatly varied (9–19) among soils and the lowest value was found in the vegetable soil.

3.2. Germination test

In general, doses of up to 500 μg g^{-1} of the contaminant had no effect on germination of the seeds used,