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Modification of enzymatic activity in soils of contrasting pH contaminated with 2,4-dichlorophenol and 2,4,5-trichlorophenol

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ABSTRACT

According to previous studies, acidic soils may receive larger quantities of 2,4-dichlorophenol (2,4-DCP) and of 2,4,5-trichlorophenol (2,4,5-TCP) than the concentrations indicated in the prevailing legislation for defining a soil as contaminated, without any important changes in their biochemical properties. In this study, we investigated whether neutral or slightly alkaline soils behave in the same way as acidic soils in response to contamination by these compounds. For this purpose, a large number of acidic soils (pH between 4.2 and 5.9) and calcareous soils (pH between 6.5 and 8.0) were contaminated in the laboratory with different doses of 2,4-DCP (up to 10,000 times the GRL) and of 2,4,5-TCP (up to 500 times the GRL). After an incubation period of three days, the activities of several enzymes (dehydrogenase, catalase, ß-glucosidase and phosphomonoesterase) were measured in the soils. The effects of 2,4,5-TCP were much greater than those of 2,4-DCP in both the acidic and calcareous soils, regardless of the dose applied. Phosphomonoesterase and ß-glucosidase activities were scarcely affected by either of the contaminants in any of the soils, whereas the catalase activity decreased slightly. The dehydrogenase and urease activities were strongly affected in all soils and in some cases even disappeared, particularly after the application of 2,4,5-TCP. Multiple regression analysis of the percentage reductions in dehydrogenase and urease activities in relation to contaminant dose and different soil properties indicated that the reduction in enzyme activity depended, in decreasing order, on the dose of contaminant applied, total carbon content and soil pH. We suggest that the processes that regulate the toxicity of these compounds in soils are their adsorption by soil organic matter and the dissociation of the non-adsorbed compound into phenolate ions (which are toxic to microorganisms). In fact, the chlorophenols scarcely affected the biochemical properties of the soils under study because of their high organic matter contents (A horizons with total carbon contents of up to 11%). Moreover, both chlorophenols had slightly stronger effects on the calcareous soils than on the acidic soils, probably because the dissociation process was favoured at higher pH. On the other hand, the 2,4,5-TCP had stronger effects on soil biochemical properties than 2,4-DCP, which may be explained by the lower pK_a value of 2,4,5-TCP (6.9) than that of 2,4-DCP (7.9). The results show that the GRL values established by the legislation are not appropriate for either of these chlorophenol compounds.

1. Introduction

Chlorinated compounds are organic compounds widely used as bacteriological agents (Hutzinger et al., 1985). The use of some of these compounds, such as certain isomers of hexachlorocyclohexane (lindane), has been prohibited because they are toxic to various species, including humans (Willet et al., 1998). However, other compounds such as 2,4-dichlorophenol (2,4-DCP) and 2,4,5-trichlorophenol (2,4,5-TCP) are widely used as antifungal agents in silviculture to slow down the decomposition of cut timber in forests, and as precursors of different herbicides in agriculture (Hajslová et al., 1988). The presence of 2,4-DCP and 2,4,5-TCF in soils is generally due to their use as antifungal agents or to the degradation of certain herbicides and pesticides (Hutzinger et al., 1985; Czaplicka, 2004). These compounds may also be present in soil as a result of the accidental spillage of waste water generated in the paper industry, as they may be formed during the process of whitening paper (Kookana and Rogers, 1995; Cea et al., 2007). Both

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compounds are considered toxic to the environment (USEPA, 1979), and their presence in soils should therefore not surpass a certain concentration. Unfortunately, the frequent use of excessive amounts of these compounds, and of many other xenobiotic compounds, leads to contamination of soils and of the environment in general (Doran, 2002).

Although the legislation regarding soil contamination is less well established than that concerning contamination of water or the atmosphere (Oldeman et al., 1991), national governments and supranational organizations have recently made efforts to restrict the use of various compounds with the aim of protecting soils from external aggressions, including aggressions caused by the improper use of organic compounds (European Directive on Contaminated Soils, 2006). Thus, a soil can be declared contaminated, and therefore not suitable for certain uses, if the amount of any contaminant in soil exceeds 100 times the value of the concentration established by legislation. This value is denominated the generic reference level (GRL) and is defined for each compound and each soil use. For 2,4-DCP and 2,4,5-TCP, the Spanish legislation (Real Decreto 9/2005), similarly to the European legislation (European Directive on Contaminated Soils, 2006), establishes GRLs of 0.1 mg kg^{-1} for 2,4-DCP and 10 mg kg^{-1} for 2,4,5-TCP for soils, irrespective of the type of soil use. In addition, a soil is considered as contaminated if the amount of a toxic product is greater than 100 times the GRL, i.e. 10 mg kg^{-1} for 2,4-DCP, and 1000 mg kg^{-1} for 2,4,5-TCP.

Various studies carried out by our research group have shown that Galician soils, which generally have acid surface horizons (pH between 4.2 and 5.9) and high organic matter contents (values of total carbon between 5% and 12%), are capable of receiving much higher concentrations of 2,4-DCP and 2,4,5-TCP than the levels indicated in the prevailing legislation for diagnosing a soil as contaminated, without any apparent effects on the biochemical, biological or physiological properties and, therefore, without any loss of soil quality (Moscoso et al., 2007; Bello et al., 2008, 2011). Similar results have recently been obtained by Martí et al. (2011) for soils in the Mediterranean area, although some properties of these soils, such as the total C content, are very different from those of Galician soils. In other words, it is difficult to demonstrate the loss of quality in soils that receive such high quantities of chlorophenols, as the indicators listed in the legislation for determining deterioration of soil quality do not always change or respond in a similar way to the presence of these compounds (Bello et al., 2008). Nevertheless, although the responses of biochemical properties such as respiration and nitrogen mineralization are rather inconsistent and increase or decrease randomly, the changes in enzyme activities are generally more consistent and usually decrease in response to increasing doses of chlorophenols (Moscoso et al., 2007; Bello et al., 2008).

Moreover, as observed by other researchers working on diverse chlorophenols (He et al., 2006; Cea et al., 2007), contamination by 2,4-DCP and 2,4,5-TCP generally has less effect on the biochemical properties of acidic soils with high contents of organic matter than on the biochemical properties of other types of soils (Moscoso et al., 2007; Bello et al., 2008). These observations were established in studies involving large numbers of soils, always of pH (in water) lower than 6. Despite the relatively narrow range of pH in these soils (between 4.2 and 5.9), it was observed that as the pH increased, the effect produced by the chlorophenols generally increased. This suggests that soil pH may be an important factor in determining soil deterioration in response to contamination, although the effect may be masked by other edaphic properties (Bello et al., 2008).

Taking the latter into account, and as only acidic to strongly acidic soils have been considered in previous studies, the objectives of the present study were: a) to investigate the behaviour of different oxidoreductases and hydrolytic enzymes in neutral to slightly alkaline soils in response to contamination with 2,4-DCP and 2,4,5-TCP; b) to compare the degree to which the properties are affected in these soils and in acidic soils with similar characteristics; c) to further our knowledge of the mechanisms of action of these chlorophenols in soil, and d) to shed some light on the reason why the GRLs indicated in the prevailing legislation are in many instances not applicable to soils.

2. Materials and methods

2.1. Soils

Thirteen neutral and slightly alkaline soils developed over limestone (calcareous soils), and 17 moderate and strongly acidic soils developed over different siliceous parent materials (acidic soils) were sampled at sites distributed throughout Galicia (NW Spain) and under different types of use (forest, pasture, crop). The calcareous soils are mainly Leptosols, Phaseozems and Luvisols, whereas the acidic are Umbrisols and Regosols (ISSS Working Group R.B., 1998). At each site, 10–15 samples of the A horizon (0–10 cm) were collected at random and pooled in the field to produce a composite sample. The samples were transported in isothermal bags to the laboratory where they were sieved (<4 mm). A sub-sample from each site was air-dried to determine general soil properties, and the remainder was stored at 4 °C until analysis of different enzymatic activities and for the experiment involving contamination of soils with 2,4-DCP and 2,4,5-TCP. The enzymatic analysis of the soil samples and preparation of the soil contamination experiment were carried out within one week of obtaining the soil samples.

2.2. Soil contamination

Triplicate aliquots of the fresh soils were artificially contaminated with four different doses of 2,4-DCP (0, 100, 500 and 1000 mg kg^{-1}, i.e. 0, 1000, 5000 and 10,000 times the GRL established by the Spanish legislation) or with 5 doses of 2,4,5-TCP (0, 100, 500, 1000 and 5000 mg kg^{-1}, equivalent to 0, 10, 50, 100 and 500 times the GRL for this compound). As both chlorophenols are sparingly soluble in water (Czaplicka, 2004), they were first mixed with quartz sand (in the proportions required to generate the doses indicated), and the mixtures were then shaken for 48 h in a rotary shaker to achieve homogeneity (Moscoso et al., 2007). The soils were contaminated by addition of the sand/contaminant mixture to the moist soil in a proportion of 10% (10 g of the mixture was added to an amount of moist soil equivalent to 100 g of oven-dried soil) to obtain the above-indicated concentrations of contaminant. Distilled water was added to maintain the system at field capacity (Gardner, 1986), i.e. the amount of water retained by the soil layers at a −33 kPa (1/3 bar) matrix potential, which corresponds to the water potential traditionally used to afford optimal moisture conditions in mineralization experiments (Leirós et al., 1999). After addition of the water, the mixtures were homogenized carefully and maintained at 20 °C for 72 h. This contact time was selected on the basis of the results of prior experiments that indicated that the major modifications to soil properties occur 72 h after contamination (Bello et al., 2008). The control soils were mixed with sand only (the same amount added for spiking soil samples). At the end of incubation period (72 h), the soils were analyzed to determine the enzymatic activities.

2.3. Analysis of soil physical and chemical properties

The following properties were determined by the methods described by Guitián and Carballas (1976): pH in water (1:2.5,