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Evaluation of various tests for the diagnosis of soil contamination by 2,4,5 trichlorophenol (2,4,5-TCP)

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The concentration of a toxic substance present in a soil cannot be considered as the only criteria to classify such soil as contaminated.

Abstract

The response of different types of soils to contamination with 2,4,5-triclorophenol was studied to test the validity of the concept of generic reference levels (GRL), the main criterion used to define soil contamination. Soil samples were artificially contaminated with doses of between 0 and 5000 mg $kg^{-1}$of 2,4,5-triclorophenol, and analysed by various tests. Where possible, the response of soils to the contaminant was modelled by a sigmoidal dose-response curve in order to estimate the ED_{50} values. The tests provided different responses, but only microbial biomass-C and dehydrogenase and urease activities demonstrated soil deterioration in response to contamination. The results suggest that the diagnosis of soil contamination has been greatly simplified in the legislation by the provision of a single figure for each compound, and that the GRL concept could perhaps be substituted by measurement of ED_{50} values, which better reflect the alteration of a soil due to the presence of a xenobiotic substance.

Key words: Soil contamination, ecotoxicological tests, soil enzymes, soil biochemical properties.

1. Introduction

Soil is a non renewable resource, at least on a human timescale (Fitzpatrick, 1980), and therefore preservation of soil quality is of top priority in conservation of the environment (Cihacek et al., 1996). However, unlike other resources such as air and water, for which there exist well-established quality standards that indicate clearly the extent of the degradation of the resources (Warkentin and Fletcher, 1977), no such standards exist for soil, or at least no universally accepted standards (Doran et al., 1996). It is clear that this lack of criteria has

contributed to the fact that policies regarding the protection of soil have been developed more slowly than those aimed at protecting the air or water, and many soils are therefore in an advanced state of degradation (Oldeman et al., 1991).

However, in recognition of the importance of soil as a resource, legislation has recently begun to be developed (European Directive on Contaminated Soils, 2006) with the aim of protecting soils from degradation, particularly the degradation caused by the contamination of soils exposed to toxic compounds. Despite this advance, the current legislation, possibly limited by a lack of information, places more emphasis on the amounts of toxic products to which a soil may be exposed than on quantifying the degree of deterioration as a result of the contamination. In this respect the Spanish legislation (Real Decreto 9/2005) defines Generic Reference Levels (GRL) for certain products in terms of soil use (unrestricted or for agricultural, industrial use, etc.) and considers that a soil is contaminated (for unrestricted use) when the concentration of the toxic product is 100 times higher than the GRL (Real Decreto 9/2005). The legislation also establishes that when the contamination has been produced by an unknown compound, or one for which there is no GRL, certain tests are applied to the soil to determine its state and thereby to deduce the GRL for the product. These tests, described by the OECD (2000, 2003), are based either on the estimation of the capacity for germination and root elongation of seeds planted in the contaminated soil (test 208), or on the capacity of the soil to mineralize organic nitrogen compounds (test 216) or any other organic compound, measured in this case by the emission of CO_2 (test 217).

However, despite these legislative advances in policies aimed at controlling soil degradation, the above-cited measures present several shortcomings. Thus the GRL are fixed for each substance and only depend on soil use, which means that they do not take into account soil properties that may mitigate the effect of the contaminant, and in other words all soils are considered to behave in a similar way. This is obviously an oversimplification; for example, the properties and dynamics of a soil developed on limestone, with a low content of organic matter, high pH and of clay texture are totally different from those of an acid soil, with a high content of organic matter and of sandy texture. However, both types of soils are considered in a similar way by the prevailing legislation regarding contaminated soils, by application of the GRL concept. Another deficiency in the legislation is that the methodology for carrying out the OECD tests is not fully described, which may mean that different laboratories will carry out the test in different ways, thereby complicating identification of the status of the soil. Moreover, the fact that some soil properties have not been included in the methods for diagnosing soil contamination appears to be a serious oversight. Properties such as enzymatic activities and microbial biomass are commonly considered as good indicators of soil quality (Gil-Sotres et al., 2005). These properties react rapidly in response to environmental contaminants, are easily and rapidly measured and the protocols for their determination, ggdespite a lack of consensus, are better standardized than the previously mentioned tests (Trasar-Cepeda et al., 2003). It is evident that these characteristics allow consideration of the above-mentioned biochemical properties as potentially suitable tools for the diagnosis of contaminated soils and in fact have been used as such by some researchers (Nannipieri et al., 1990; Yakovchenko et al. 1996), although other authors consider that the role as indicators of soil quality is not sufficiently validated (Dalal, 1998; Trasar-Cepeda et al., 2000).

The aim of the present study is twofold. The first aim was to determine the response of soils with different characteristics (land-use, organic matter content, pH) to contamination with various doses of the same compound, 2,4,5-triclorophenol (2,4,5-TCP). This compound is included in the list of possible soil contaminants (GRL of 10 mg kg^{-1}, irrespective of soil use), and thus a soil would be considered contaminated if it contained more than 1000 mg kg^{-1} of this compound (i.e., 100 times the GRL). The second aim of the study was to determine whether the measurement of different enzyme activities, in this case of oxidoreductases (dehydrogenase, catalase) and hydrolases corresponding to the cycles of the main bioelements (phosphomonoesterase for the phosphorous cycle, urease for the nitrogen cycle and ß-glucosidase for the carbon cycle) provide more reliable information than the OECD tests in terms of diagnosing soil contamination.

2. Materials and methods

2.1. Soil sampling

A total of 22 surface horizons from Galician soils (NW Spain) dedicated to three different types of use (forest, pasture and cropland) were included in the study. Of the forest soils (all *Umbrisols*),six were developed under *climax* vegetation of Atlantic oak (oak soils) and 5 under reforested *Pinus pinaster* Ait. (pine soils). Another six soils were dedicated to pasture (*Umbrisols-Gleysols*) predominated by Lolium sp. - Trifolium sp. (pasture soils), and the remaining soils (5) were *Regosols* and degraded *Umbrisols* under a crop rotation (maize, potatoes, rapes) typically used in Galicia (cropped soils). In all cases, the surface horizon (0-20 cm) was sampled after removal of the litter layer from forest soils and of the upper densely-rooted layer from the pasture soils. In each sampling plot (approximately 1 Ha) between 15 and 20 subsamples were collected, and combined in the field to make a composite sample. The samples were transported to the laboratory in isothermic bags, and were then sieved (<4 mm) and maintained at 4 °C until analysis. An aliquot of each composite sample was air-dried (20 °C) and used for analysis of general soil properties.

2.2. General soil analysis

The pH in KCl was determined by the method of Guitián-Ojea and Carballas (1976). Total organic C was determined by oxidation with dichromate in an acid medium and total N by Kjeldahl distillation (Guitián-Ojea and Carballas, 1976). The texture was determined by the Robinson pipette method, after destruction of the organic matter with hydrogen peroxide, and with Calgon as dispersant (Guitián-Ojea and Carballas, 1976). The available P was extracted by the method described by Bowman and Cole (1978), with 0.5 M sodium bicarbonate, (soil:extractant ratio 1:50; extraction time 16 h) and the inorganic P in the extract was determined by the method of Murphy and Riley (1962), after precipitation of the organic matter by acidification to pH 1.5 with sulphuric acid.

2.3. Contamination experiments

The contaminant considered was 2,4,5-trichlorophenol, a compound that is commonly used in both the timber industry -to protect recently cut wood from fungal attack before it is removed from the forest- and in agriculture as it is a precursor in the synthesis of various herbicides (Annachhatre and Gheewala, 1996; Bollag et al., 2003). As 2,4,5-trichlorophenol is sparingly soluble in water (Czaplika, 2004), it was first mixed with quartz sand (in the proportions required to generate the doses indicated below) before being added to the soil, and this mixture was then shaken for 48 hours in a rotary shaker to achieve homogeneity (Moscoso et al., 2007). The soils were contaminated by addition to the moist soil of the sand/contaminant mixture (10 g mixture to 100 g of soil) to obtain concentrations of contaminant of 0 (D0, control soil), 100 (D1), 500 (D2), 1000 (D3) and 5000 (D4) mg kg^{-1} (i.e., 0, 10, 50, 100 and 500 times the GRL), and enough water was added to maintain the system at optimal moisture content (water held at -30.39 kPa); the mixture was then homogenized carefully. The mixtures were maintained at 20 °C for 72 hours. This contact time was selected on the basis of the results of prior experiments that indicated that the major modifications in soil properties are produced 72 hours after contamination. The control and contaminated soils were then subjected to physiological tests and tests of biological and biochemical activity, and microbial biomass was also measured.

2.4. Physiological tests

For the OECD germination and root elongation test (test 208), four Petri dishes containing 15 g of soil and 15 previously moistened garden cress seeds (*Lepidium sativum* L.) were prepared for each soil and dose of contaminant. Quantification of the germination and measurement of the root elongation was carried out 4 days after planting the seeds. The results are expressed, for each soil and dose, as a percentage of the value reached in the corresponding control (D0), for both the germination and root elongation as well as for the combined germination-elongation index, GE ,% germination × % elongation with respect to the control (Moscoso et al., 2007).

2.5. Tests of biological activity and quantification of microbial biomass

The OECD soil N mineralization test (test 216) was carried out as follows: Aliquots of soil (both control soils and the contaminated soils) were incubated for 10 days at 25 °C and optimal moisture (Leirós et al., 2000). The inorganic forms of N present before and after the incubation were extracted with 2 M KCl (ratio 1:10, 120 minutes) and determined by semi-micro distillation (Bremner, 1965), and the inorganic N produced from the mineralization of the organic forms (mineralized N) was calculated as the difference between these amounts. Soil respiration (respiration) was determined by static incubation (Guitián-Ojea and Carballas, 1976); the CO_2 produced under the same incubation conditions as for nitrogen mineralization tests was collected in 10 ml of a 0.5 M NaOH solution, which was then titrated with HCl. Microbial biomass-C (Biomass-C) was determined by the chloroform fumigation extraction method, with 0.5 M K_2SO_4 as extractant (Vance et al., 1987). The organic carbon in the extracts was estimated by oxidation with potassium dichromate. The difference in the C content of the extracts from fumigated and unfumigated samples was converted to microbial biomass-C by dividing the value

obtained by a factor (K_c) of 0.45 (Vance et al., 1987). The carbon in the extracts of unfumigated samples was estimated as a measure of labile carbon (labile C) in the soils (Milne and Haynes, 2004). Determinations were carried out in triplicate and values for each dose and soil are expressed as the percentage of the value in the corresponding control soils (*D0*).

2.6. Tests of biochemical activity

Dehydrogenase activity was determined by a modification of the method of von Mersi and Schinner (1991) described by Camiña et al. (1998), with iodonitrotetrazolium violet (INT) as substrate. Catalase activity was determined according to the method of Trasar-Cepeda et al. (1999). Acid phoshomonosterase was determined at pH 5.0 (Trasar-Cepeda and Gil-Sotres, 1987) by a modification (Saá et al., 1993) of Tabatabai and Bremner's (1969) method. ß-glucosidase was determined by the method of Eivazi and Tabatabai (1988), modified as indicated for phosphomonoesterase in Saá et al. (1993). Urease activity was determined by the method described in Nannipieri et al. (1980). All determinations were performed in triplicate and for each soil sample the average values of the three determinations (expressed on an oven-dried soil basis, 105 °C), were calculated. The results are expressed for each soil and doses as the percentage of the value in the corresponding control (D0) soils.

2.7. Calculation of ED_{50} values

In most of the soils studied and for those properties that demonstrate a clear decrease in values with increasing concentration of contaminant, it was possible to fit the variation in the property in response to the contaminant by the sigmoidal dose-response model of Haanstra et al. (1985), which follows the equation:

$$y = 100 / \{1 + \exp [b(x-c)]\}$$

where *y* is the value of the property of any dose considered, expressed as a percentage of the value corresponding to the control soil, *b* is a slope parameter related to the velocity of inhibition, *c* is the decimal log value of the ED_{50}, i.e. the dose that causes a 50% reduction in the value of the property in relation to the control soil, and *x*, is the decimal log value of the dose. This model describes a logistic curve for the property measured and the log value of the concentration of the inhibitor (Moreno et al., 2002).

2.8. Statistical analysis

Calculation of means, standard deviations, the Student´s *t* test (to calculate differences between means) and fitting to the sigmoidal model were performed with Statistica 6.0 (StatSoft®) for Windows (StatSoft Inc., 2001).

3. Results

3.1. General data

The soils were acid and of loam texture, although the former character was more noteworthy in the forest soils (oak and pine soils), particularly the oak soils, for which the mean pH in KCl was 3.70 (Table 1). The highest pH was measured in the cropped soils (mean pH 4.30). The forest soils contained the greatest amounts of organic C and total N (Table 1). Thus, the mean C content in the forest soils was 6.58-6.87%, almost double the amount in the cropped soils (3.60%), and the C content in the pasture soils was intermediate (4.23%). A similar pattern was observed for the total nitrogen. The amounts of extractable inorganic P were very low in the natural forest soils, and on average more than 30 times higher in the cropped soils (Table 1), which reflects the use of phosphate fertilizers in the latter.

3.2. Physiological tests

The soils responded similarly to the germination and root elongation tests, as well as to the combined germination-elongation index, although the intensity of the responses varied depending on the soils. The contaminant dose of 100 mg kg^{-1} barely modified the response of the soils in relation to the controls, whereas the dose of 5000 mg kg^{-1} in all cases inhibited germination and therefore elongation. The main soil responses were observed for contaminant doses of between 500 and 1000 mg kg^{-1}. For the dose of 500 mg kg^{-1} the soils that were most affected were the pasture soils, in terms of both germination and elongation, with mean values, with respect to the controls, of 51 and 34% respectively. For the dose of 1000 mg kg^{-1} the soils most affected were the cropped soil, with mean germination of 9%, mean root elongation of 6% and a mean GE value of 1%. However, it must be pointed out that the behaviour of the soils within each group was very variable, and the differences between groups were therefore generally not significant (Fig. 1).

3.3. Tests of activity and quantification of microbial biomass

The tests of activity and quantification of microbial biomass provided different results (Fig. 2). One type of response was observed in those tests in which the value of the property, relative to the control soil, increased with the amount of contaminant. This occurred with the labile carbon and the mineralized nitrogen. For the former property and for soils under pine and pasture, there was a gradual increase, so that for the 5000 mg kg^{-1} dose, mean values of respectively 167% and 195% were reached. As regards N mineralization, there was a gradual increase in this parameter with doses of contaminant of up to 1000 mg kg^{-1}, for which the amounts of mineralized N were generally more than double those corresponding to the controls. The dose of 5000 mg kg^{-1} elicited a different response, as the mean value for mineralized N in the pasture and cropped soils was lower than in the control soils, whereas for oak and pasture soils the mean value was slightly lower than that obtained with the 1000 mg kg^{-1} dose.

The other tests in this group (respiration, biomass-C) showed in contrast, decreases in the value obtained for the property as the dose increased, but at a dose of 5000 mg kg^{-1} there were

relatively high doses of the same in relation to the controls (Fig. 2). Soil respiration was scarcely modified by doses of up to 1000 mg kg^{-1} in the soils under oak, pine and pasture, and for this parameter values of close to 75% were obtained in the cropped soil. The dose of 5000 mg kg^{-1} strongly affected all soils and the mean value ranged between 32 and 53% of the respiration observed for the control soils. As regards the microbial biomass, the dose of 100 mg kg^{-1} caused a clear (although quite slight) reduction in the values for all soils, except for the soils under oak (Fig. 2). This effect increased with the dose of contaminant, and the soils most affected were the pasture soils; in these soils treatment with the dose of 5000 mg kg^{-1} resulted in a decrease in biomass-C to only 20% of that initially present. The soils in which the microbial biomass was most resistant to the contaminant were the cropped and pine soils, in which the value corresponding to the 5000 mg kg^{-1} dose was still 40% of that present in the control soils.

3.4. Tests of biochemical activity

The phosphomonoesterase activity was not affected in any of the soils at any of the doses tested, and there was only a slight reduction in the value of this activity in the pasture soils contaminated with the dose of 5000 mg kg^{-1}, which represented up to 90% of the activity in the control soils (Fig. 3). The ß-glucosidase activity was not greatly affected by the presence of the contaminant. In the cropped soils, only the dose of 5000 mg kg^{-1} generated values of up to 93% of the activity of the control soil, whereas in the other groups of soils, the values of enzyme activity corresponding to this dose of contaminant were close to 80% of the control values (Fig. 3). Catalase activity followed a similar pattern to the ß-glucosidase activity, but was more strongly affected, and for the 5000 mg kg^{-1} dose, values of 84% were obtained in the cropped soil and of between 52 and 68% in the other groups of soils. The enzyme activities most affected by the presence of contaminant were those of dehydrogenase and urease (Fig. 3). For the 5000 mg kg^{-1} dose, dehydrogenase activity was only 16 and 17% of the activity in the control soils in the pasture and cropped soils, and between 31 and 46% in the forest soils. Urease was the enzyme most affected by the contaminant; for the highest dose the urease activity in the forest soils was only 25-26% of the activity in the control soils and in the cropped and pasture soils approximately 12% of that in the control soils (Fig. 3).

3.5. ED_{50} values

As regards the physiological tests (Table 2), the mean ED_{50} values were of the same order for the four groups of soils and the deviations were relatively low (except for the GE index for the oak soils), which suggests similar behaviour independent of the type of soil, or in other words that the characteristics of the soil do not determine the effect of the contaminant on germination and root elongation. The mean ED_{50} values ranged from 400-1000 mg kg^{-1}, in other words they were very close to the threshold value (100 x GRL), in this case 1000 mg kg^{-1}, indicated by the legislation as the figure representative of a contaminated soil (Real Decreto 9/2005). The mean ED_{50} values obtained for the properties related to microbial activity (biomass-C and respiration) were much more variable, as reflected by the high values of the standard deviation obtained for each group of soils (Table 2). In addition, for the pine and cropped soils the ED_{50} values for

respiration and biomass-C were similar, although much higher than the 100 x GRL threshold value, whereas for oak and pasture soils there was a clear difference between the ED_{50} values obtained for both properties, as biomass-C was much more strongly affected than respiration by the presence of the contaminant. For the group of enzymatic activities, it was only possible to estimate the ED_{50} values for urease and dehydrogenase. The mean values obtained for each group of soils were very similar for both enzymes and for the four groups of soils (only dehydrogenase in the pasture and cropped soils showed clearly lower values), with the ED_{50} values close to one other, but higher than the threshold of 100 x GRL proposed in the legislation.

4. Discussion

The tests used to diagnose soil contamination provided different results for the soils considered and showed varying degrees of sensitivity to the presence of the contaminant. In this respect, the N mineralization test, despite being one of the tests explicitly included in the legislation for use in diagnosing soil contamination, was totally ineffective for this purpose because the values of mineralized N increased with the concentrations of the contaminant. The same occurred with the measurement of labile C, which indicates that this test is also ineffective for diagnosing soil contamination.

The decreases in both biomass-C and respiration with increasing amounts of contaminant show that the soil microbiota is strongly affected by the presence of the contaminant, although is not totally annihilated. The increases in labile C and mineralized N appear to be caused by the death of a portion of the soil microorganisms. The cellular contents of the dead microorganisms are spilled into the soil solution thus increasing the labile C and sources of easily mineralizable N, which explains the increasingly high values of these properties observed as the concentration of contaminant increases. If this interpretation is correct, the soil respiration test cannot be considered valid, despite the observed response to contamination. The death of a portion of the soil microorganisms will affect soil respiration by causing a decrease in microbial biomass (direct effect of the contaminant, which will cause a decrease in respiration), but the remaining microbiota will also be able to utilize the labile organic matter comprised of the cellular remains of the dead microorganisms (indirect effect, which opposes the direct effect as the presence of the labile forms of C will stimulate respiration of the surviving organisms). In other words, the soil respiration will be the result of two opposing processes, which makes it difficult to interpret the data and to diagnose the soil status.

In other words, the results obtained suggest that the soil can partially mitigate the effects of 2,4,5-trichlorophenol, thereby decreasing the toxic potential of this product in relation to the effect that this would have on the germinative potential of the seeds used in the physiological tests. On the other hand, the three hydrolases considered showed strikingly different behaviour. Thus whereas the urease activity was strongly affected, the ß-glucosidase and phosphomonoesterase activities were scarcely modified by the presence of the contaminant, which means that measurement of the latter two activities is totally ineffective for the diagnosis of soil contamination, as already indicated for labile C and nitrogen mineralization. When soil microorganisms die, microbial production of enzymes will cease and the lack of a response by phosphomonoesterase and ß-glucosidase to the contaminant indicates either that the enzymatic

activities in the soils under study correspond to enzymes stabilized on soil colloids (Burns, 1982; Nannipieri et al., 1980), or that once the fraction of these enzymes bound to the active microorganisms is released (when the cell walls are broken) the enzymes are rapidly stabilized on humic and clay colloids, thereby avoiding hydrolysis by soil proteases (Burns, 1982; Ladd and Butler, 1975). The behaviour of catalase is also noteworthy; this enzyme is theoretically linked to the presence and activity of microorganisms and should act similarly to the soil microbial biomass (García and Hernández, 1997). The fact that this activity was always maintained at higher levels than those observed in the microbial biomass reflects problems related to the method of determining this enzyme, perhaps caused by the death of the microorganisms, which results in the presence of compounds that cause the decomposition of the peroxide used in determining catalase activity (Skujins, 1976). The results showed that not all of the properties considered in the legislation governing the diagnosis of soil contamination, or properties commonly used as indicators of soil quality, may be considered as ideal for the diagnosis of the contamination generated by 2,4,5-TCP. It is therefore clear that the regulations on soil contamination with this substance have been oversimplified. Neither all the soils respond in the same way, nor do all the tests provide similar results. It is therefore almost impossible to define a soil as contaminated by use of the tools indicated in the current legislation.

The data obtained until now do not allow us to establish the factors responsible for these differences. The fact that the soils under forest vegetation are normally those least affected suggests the involvement of organic matter content and/or soil pH, which are usually higher and lower, respectively in forest soils than in soils under other types of use (Table 1). Theoretically, the effect of pH should be viewed as normal, as the dissociation *pK* of the compound is 6.92±0.02 (Severtson and Banarjee, 1996), and the higher the pH of the soil, the greater the proportion of the dissociated form, which is the form that is most toxic to microorganisms. The effect of the organic matter must be considered taking into account that both the non dissociated forms of the compound and the dissociated forms (always scarce because of the low pH of the soils used) undergo some type of fixation process on the organic surfaces (either on hydrophobic sites, for the non dissociated forms, or on positively charges sites or across cationic bridges, for the dissociated forms). This would therefore limit the presence of 2,4,5-trichlorophenol in the soil solution (Severtson and Banarjee, 1996). It is also possible that the forest soils contained a microbiota (possibly fungal, given the low pH of the soils used) that was resistant to the contaminant and had a high capacity to degrade it (Gianfreda and Rao, 2004; Reddy, 1995), although laboratory tests carried out to test this hypothesis were inconclusive (unpublished data).

Given the lower their ED_{50} values, the physiological tests appear to be more sensitive than the tests based on soil biochemical properties, whether properties related to the microbiota or to the enzymatic activities. From the point of view of a rigorous protection of the ecosystem and given this greater sensitivity to the presence of the product, physiological tests are perhaps preferable to those based on soil properties, although results obtained by our research group (unpublished data) indicate that physiological tests produce highly variable results. In addition, the fact that similar results were obtained for very different types of soils suggests that the physiological tests reflect the presence of the contaminant but not its effect on soil functioning, in other words they are incapable of demonstrating soil deterioration.

Some of the tests based on biochemical and microbial properties, such as biomass-C, dehydrogenase and urease activities, show some advantages over the physiological tests. On one hand these properties are affected to different extents depending on the soil characteristics and the amount of contaminant, and are therefore capable of reflecting the degree of deterioration that each soil may suffer. On the other hand, the ED_{50} values obtained for these tests, although usually higher, were very close to the 100 x GRL threshold value currently accepted as defining a soil as contaminated. In terms of establishing a method for diagnosing soil contamination, this coincidence will perhaps allow the GRL concept to be replaced with that of ED_{50}, and the degree of soil contamination to be determined, not by the presence of the contaminant (as in the prevailing legislation by the GRL), but rather by the soil status in relation to a control soil, not affect by the contaminant. Acceptance of this method of diagnosing soil contamination would involve the need to establish the key soil property or properties to be considered.

5. Conclusions

The results clearly show that defining soil contamination in terms of the presence of a particular quantity of contaminant is an oversimplification, as each soil deteriorates to a different degree in response to the same amount of a contaminating substance. Calculation of ED_{50} values appears to be more appropriate than GRL values for defining the status of a contaminated soil, as the ED_{50} values reflect not only the presence of the contaminant but also the status of the affected soil. Measurement of some biochemical and microbial properties of soils appears more appropriate for the diagnosis of soil contamination than the OECD tests, and should therefore be considered in protocols for the diagnosis of soil contamination. Further investigations should be carried out to determine which properties are the most appropriate for inclusion in future legislation regarding the diagnosis of soil contamination.

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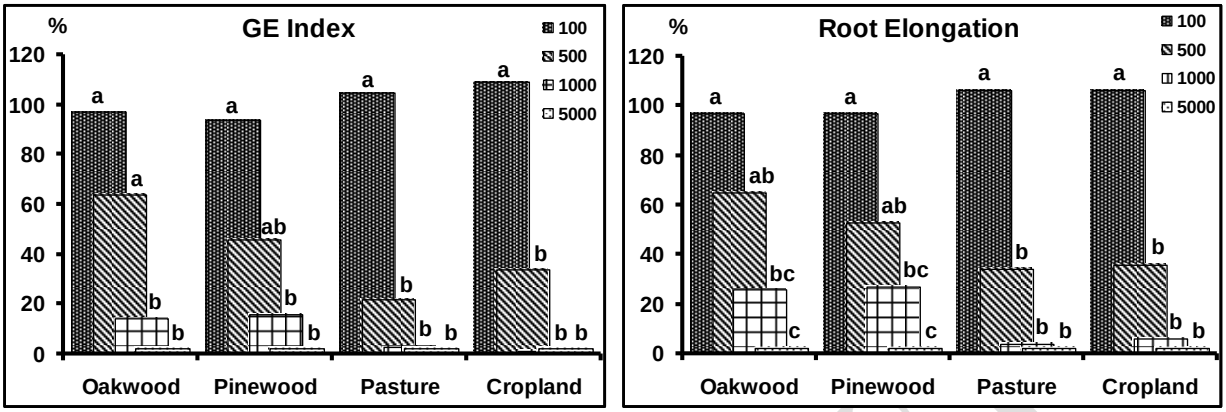

Fig. 1. Mean values for root elongation and the germination-elongation index (values expressed as a percentage of the value obtained for the control soil, D0). The same lower case letters above the bars indicate that for the soils of the same use the effect of dose is not significantly different ($p<0.05$). Dose values in mg kg^{-1}.

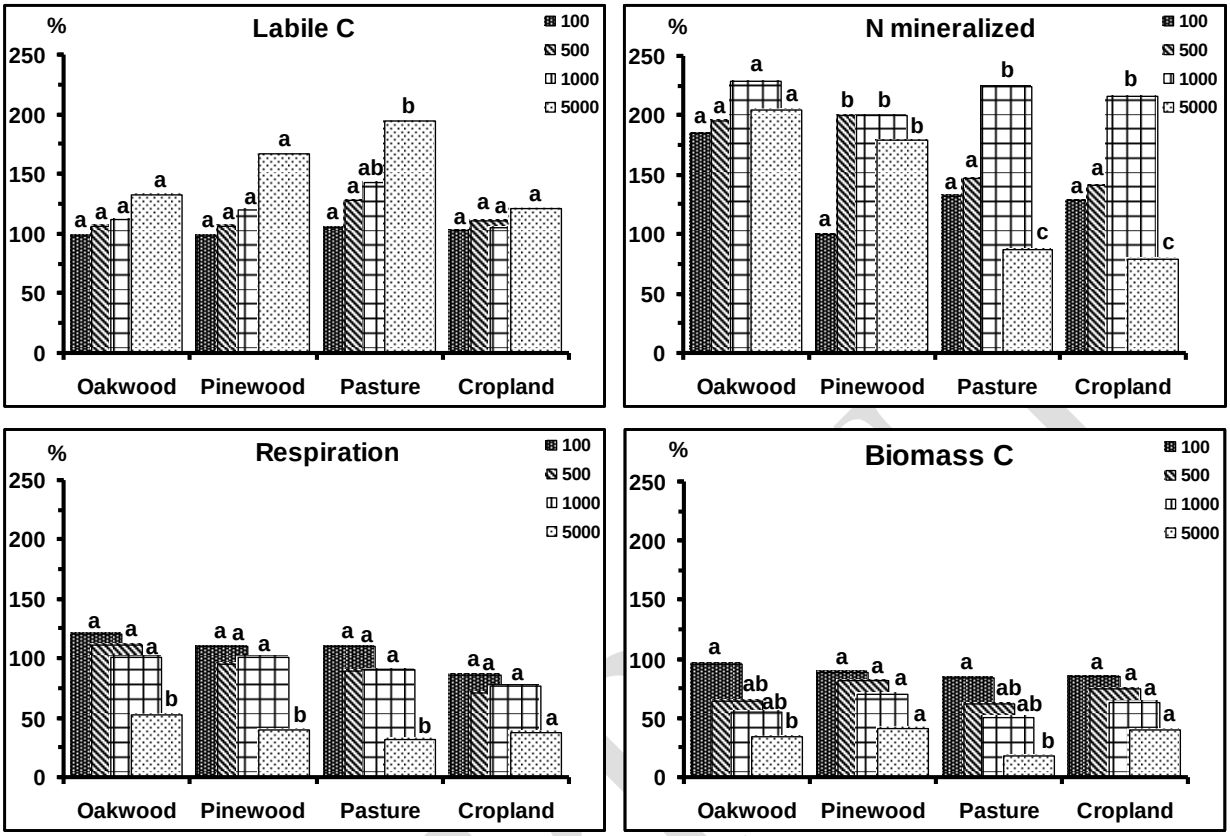

Fig. 2. Mean values obtained in the microbial activity tests (values for each dose are expressed as a percentage of the value corresponding to dose D0). The same lower case letters above the bars indicate that for the soils of the same use the effect of the dose in not significantly different ($p<0.05$). Dose values in mg kg^{-1}.

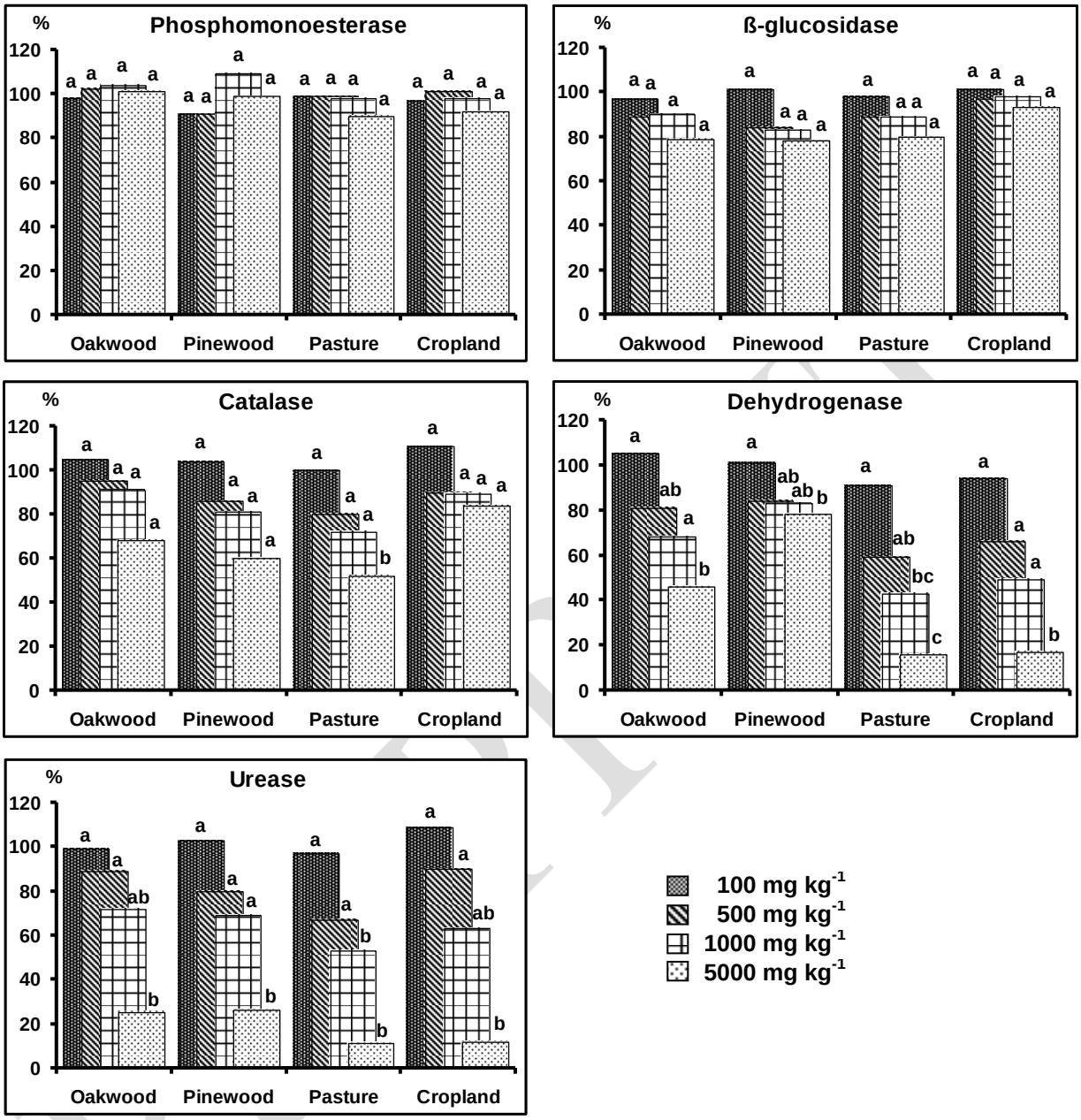

Fig. 3. Mean values of enzyme activities (values are expressed as percentages of the values obtained for the control soil, D0). The same lower case letters above the bars indicate that for the soils of the same use the effect of the dose is not significantly different ($p<0.05$). Dose values in mg kg^{-1}.

Table 1. Mean values of some general properties for each of the groups of soil under study.

	pH KCl	Total C %	Total N %	C/N	Inorganic P* (mg kg^{-1})	Texture
Oakwood	*A*3.69±0.30	*A*6.58±2.72	*A*0.32±0.12	A21±6	*A*6±2	loam
Pinewood	*B*3.93±0.08	*A*6.87±3.19	*A*0.36±0.19	A20±5	*A*4±10	loam
Pasture	*BC*4.17±0.52	*B*4.23±1.26	*AB*0.25±0.14	A19±9	*A*106±183	loam
Cropland	*C*4.29±0.07	*B*3.60±0.33	*B*0.18±0.06	A22±9	*A*186±136	loam

For each property, the capital letters preceding the values indicate significant differences among the four groups of soils ($p>0.05$).

Table 2. Mean ED_{50} values obtained for the 4 groups of soils with the tests used (GE index, combined germination-elongation index).

	Elongation	**GE index**	**Respiration**	**Biomass-C**	**Urease**	**Dehydro**
Oakwood	*A*705±300*a*	*A*1100±950*a*	*A*5070±1622*b*	*A*865±1450*a*	*A*1550±1905*a*	*AB*1545±974*a*
Pinewood	*AB*629±271*a*	*A*630±270*a*	*AB*4467±3220*bc*	*A*4620±5710*ac*	*A*1680±1600*ac*	*A*1860±840*bc*
Pasture	*B*424±138*a*	*A*415±125*a*	*B*3250±955*bc*	*A*1410±1795*ac*	*A*1450±1565*ac*	*B*835±360*a*
Cropland	*AB*464±90*a*	*A*630±275*ac*	*A*B3015±2630*b*	*A*2500±1960*b*	*A*1615±825bc	*B*1025±310*b*

The capital letters indicate that the results of a test differ among the four groups of soils. Different lower case letters indicate that the results of the tests differ within each group of soils ($p < 0.05$).