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Microbial properties for the derivation of critical risk limits in cadmium contaminated soil

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ABSTRACT

Soil microbial communities play essential roles in soil functioning. Therefore, soil microbial properties are increasingly being used as indicators of soil quality and ecological risk in contaminated soils. In this study, a variety of microbial parameters and indexes reflecting the activity, biomass and diversity of soil microbial communities were determined in two soils artificially contaminated with a gradient of cadmium (from 0 to 1000 mg Cd kg^{-1} dry weight soil). From the soil microbial properties whose values decreased at increasing Cd concentrations following an exponential pattern, IC50 (IC = inhibitory concentration) values for Cd contamination, as well as ecological thresholds, were calculated in order to determine their suitability for the derivation of critical risk limits. The most cautious IC50 value obtained for the two soils of this study was 254 mg Cd kg^{-1} dry weight soil. However, taking into account the hump-shaped exponential decay pattern observed as well as the calculated ecological thresholds, the safest risk limit was established at 44 mg Cd kg^{-1} dry weight soil (from data of the dehydrogenase/WSOC index), a value close to the risk limits established by legislation in many European countries. It was concluded that, although certainly relevant from an ecological point of view, before using soil microbial properties to derive critical risk limits, it is essential to reach an agreement regarding which specific microbial properties to use and to establish different risk limits for specific soil types.

1. Introduction

Metals are natural components of the soil ecosystem (Smith, 2009). Nonetheless, they often appear at high concentrations entailing a risk for human and environmental health. These high metal concentrations might be caused by a variety of reasons such as burning of fossil fuels, mining and smelting of metalliferous ores, downwash from power lines, municipal wastes, fertilizer and pesticide use, etc. (Garbisu and Alkorta, 2001). In particular, cadmium (Cd) is one of the most hazardous metal contaminants in soil that can easily enter the food chain through root absorption (Oliver, 1997). Moreover, Cd is located in the seventh position of the Priority List of Hazardous Substances (ATSDR, 2011).

In soil, total metal concentration does not provide enough information regarding the potential environmental impact of metal contaminants (McLaughlin et al., 2000; Giller et al., 2009). Indeed, a major factor governing the toxicity of metals in soils is their bioavailability. Chemical analyses have traditionally been used for the estimation of the biologically available fraction of metals in soils, but the transfer of results obtained in non-biological systems to biological ones is certainly questionable (Alkorta et al., 2006).

On the other hand, metal contamination can negatively affect soil microbial communities (Sandaa et al., 2001; Burges et al., 2015). Then, microbial properties reflecting the activity, biomass and diversity of soil microbial communities are increasingly being used as indicators of metal contamination, owing to their high sensitivity to metal-induced stress (Epelde et al., 2009; Moreno et al., 2009; Pardo et al., 2014), together with their quick response to disturbances, ecological relevance and capacity to provide information that integrates many environmental factors (Schloter et al., 2003; Bloem et al., 2006a; Garbisu et al., 2011). Microbial properties can be interpreted as individual parameters, simple indexes (*e.g.*, specific enzyme activities, fungi to bacteria ratio, etc.) and complex indexes (*e.g.*, soil quality indexes; Bastida et al., 2008).

Several European countries have implemented ecological risk assessment methodologies and defined soil screening values (*e.g.*, Austria, Finland, Germany, Netherlands, Spain), and many more are on their way (Carlon, 2007). In order to set soil screening values, the species sensitivity distribution (Aldenberg et al., 2002), based on ecotoxicological databases (the Dutch e-tox database developed by RIVM is a common reference for EU countries), is determined.

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Additionally, values of total concentration of soil contaminants are taken into account. From these data, the potentially affected fraction (% PAF) of species is calculated (Urzelai et al., 2000). Unfortunately, for the same contaminant, ecotoxicological values from different databases can easily differ by more than one order of magnitude. On the other hand, there is no general agreement on the acceptable level of incremental risk, but 50% PAF is the most common value. Then, here, we propose that ecological risk limits can similarly be inferred from IC50 values (concentration of a contaminant that inhibits 50% of a specific biological process) derived from data on the values of soil microbial properties obtained when soils are exposed to a gradient of a specific contaminant. When it comes to establishing ecologically relevant risk limits, parameters related to the microbial communities living in the specific soil under study are more appropriate than assays performed on model organisms that very often have little to do with the soil ecosystem.

Finally, the effect of contaminants on soil quality does not necessarily follow a linear pattern, but it can, instead, show ecological thresholds or breakpoints, defined as "a point or zone of abrupt change in ecological relationships" (Huggett, 2005; Groffman et al., 2006). When ecosystem resilience is sufficiently degraded or lost by the action of disturbances, the ecosystem has a high risk of shifting from a desirable state to an undesirable state (Sasaki et al., 2015). Ecological thresholds represent the point where even small changes in environmental conditions associated with disturbances lead to a switch between ecosystem states (Standish et al., 2014). The threshold concept has applications in multiple fields of ecology, including the analysis of shifts in ecosystem state, the determination of critical loads and the evaluation of the effects of extrinsic factors (Groffman et al., 2006). In any case, statistical problems associated with the precise quantification of thresholds have caused some to question the legitimacy of the threshold concept (Slob, 1999), particularly when applied to complex systems, like ecosystems (Van Straalen, 1997). However, ideally, ecological thresholds should be taken into consideration when establishing critical contaminant loads and, concomitantly, ecological risk limits.

The aim of this work was to follow the evolution of soil microbial properties and derived indexes in two soils with different physicochemical characteristics along an experimental Cd-contamination gradient. Then, IC values and ecological thresholds were calculated to analyse their relevance for soil quality evaluation and ecological risk assessment.

To the extent of our knowledge, this is the first time that the calculation of IC values and ecological thresholds has been attempted in a Cd contaminated soil using so many soil microbial parameters and indexes.

2. Materials and methods

2.1. Soil sampling and characterization

Two soils with different physicochemical characteristics were collected in the province of Bizkaia (Basque Country, northern Spain). The here-called "DERIO" and "OROZKO" soils were collected from a natural grassland located in the town of Derio (latitude 43°17′; longitude 2°52′) and a mountainous grazing area in the town of Orozko (latitude 43°6′; longitude 2°56′), respectively. Soils were sampled (upper 0–30 cm), sieved to <2 mm, air-dried at 30 °C, and subjected to physicochemical characterization according to standard methods (MAPA, 1994). Pseudototal metal concentrations in soil samples were determined using flame atomic absorption spectrometry (AAS) following acid digestion with a mixture of HNO_3/$HClO_4$ (Zhao et al., 1994).

2.2. Cadmium treatments

In order to obtain a Cd-contamination gradient (*i.e.*, 0, 1, 2.5, 5, 10, 100, 500 and 1000 mg Cd kg^{-1} dry weight—DW soil), soils were artificially contaminated in plastic trays (each tray contained 1.6 kg DW soil) with a $CdCl_2$ solution. This process was performed at 50% water holding capacity (WHC). Initially, the soil in each tray was divided in four portions which were then separately contaminated by manually mixing the $CdCl_2$ solution with the soil. Subsequently, the four soil portions were thoroughly mixed together to ensure a homogeneous distribution of the metal. In this respect, the soil was divided again in four portions and the homogenization was checked by measuring pseudototal Cd concentration in each portion. Following this procedure, we obtained a very homogenous distribution of the metal in the soil matrix: to give an example, in the soil contaminated with 1000 mg Cd kg^{-1} DW soil, the standard error was only 6 mg Cd kg^{-1} DW soil. Therefore, for each soil type (DERIO, OROZKO), eight different treatments (*i.e.*, 0, 1, 2.5, 5, 10, 100, 500 and 1000 mg Cd kg^{-1} DW soil) were established. Treatments were conducted in triplicate.

Previous studies (Si et al., 2006; Khodaverdiloo et al., 2012) have shown that wetting and drying cycles can accelerate metal ageing processes. Thus, five wetting-drying cycles were applied to the Cd-contaminated soils in the plastic trays. In each cycle, soils were saturated with deionized water (up to 100% WHC) and then allowed to dry (air-drying) at room temperature until the soils appeared completely dry by means of visual inspection. After each cycle, extractable Cd concentrations were determined. Extractable Cd concentrations were determined using three extractants: water, 0.01 M $CaCl_2$, and a low-molecular-weight organic acid solution (see below). In total, during these five cycles, Cd-contaminated soils were incubated at room temperature for five weeks.

Once extractable Cd concentrations were stabilized, each soil was again homogenized by manually mixing and then split into three 150 g (fresh weight) sub-samples which were placed in 500 ml pots. Holes were made in every pot to avoid anoxic conditions. Then, pots were incubated for another five weeks in a growth chamber under the following constant conditions: temperature = 25 °C, relative humidity = 60%, darkness. Water holding capacity was kept at 50% throughout the 5-week incubation period.

2.3. Soil chemical parameters

Throughout both incubation periods (*i.e.*, in the trays after each wetting-drying cycle and in the pots at the end of the 5-week incubation period), soil samples were taken from the trays or pots to determine pseudototal Cd concentrations as well as three different extractable Cd concentrations: water-extractable, 0.01 M $CaCl_2$-extractable, and extractable with a low-molecular-weight organic acid solution. Pseudototal Cd concentrations in soil were determined using flame atomic absorption spectrometry (AAS) following acid digestion with a mixture of HNO_3/$HClO_4$ (Zhao et al., 1994). Water-extractable Cd concentrations in soil were measured after extraction of 4 g DW soil with 50 ml deionized water in a horizontal shaker at 200 rpm during 16 h (Hernández-Allica et al., 2006). The $CaCl_2$-extractable (0.01 M $CaCl_2$) fraction of Cd was determined according to Houba et al. (2000). A low-molecular-weight organic acid (LMWOA: acetic acid, lactic acid, citric acid, malic acid and formic acid in a total concentration of 0.01 M) solution was also used as extractant following Feng et al. (2005). Methods used for the determination of extractable concentrations as indicators of metal bioavailability in soil should simulate real field conditions as much as possible; then this LMWOA extractant was used here to simulate rhizosphere conditions. Cadmium concentration in all extracts was analyzed by AAS.