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Impact of repeated single-metal and multi-metal pollution events on soil quality

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HIGHLIGHTS

- Single- and multi-metal repeated pollution events negatively affected soil quality.
- Acid phosphatase was decreased by repeated Cu, Zn and multi-metal treatments.
- β-glucosidase activity increased after repeated Pb pollution events.
- Fungal gene abundance decreased in all repeated single-metal treatments.
- Relative metal bioavailability increased with successive pollution events.

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ABSTRACT

Most frequently, soil metal pollution results from the occurrence of repeated single-metal and, above all, multi-metal pollution events, with concomitant adverse consequences for soil quality. Therefore, in this study, we evaluated the impact of repeated single-metal and multi-metal (Cd, Pb, Cu, Zn) pollution events on soil quality, as reflected by the values of a variety of soil microbial parameters with potential as bioindicators of soil functioning. Specifically, parameters of microbial activity (potentially mineralizable nitrogen, β-glucosidase and acid phosphatase activity) and biomass (fungal and bacterial gene abundance by RT-qPCR) were determined, in the artificially metal-polluted soil samples, at regular intervals over a period of 26 weeks. Similarly, we studied the evolution over time of $CaCl_2$-extractable metal fractions, in order to estimate metal bioavailability in soil. Different metals showed different values of bioavailability and relative bioavailability ($[metal]_{bio}/[metal]_{tot}$) in soil throughout the experiment, under both repeated single-metal and multi-metal pollution events. Both repeated Zn-pollution and multi-metal pollution events led to a significant reduction in the values of acid phosphatase activity, and bacterial and fungal gene abundance, reflecting the negative impact of these repeated events on soil microbial activity and biomass, and, hence, soil quality.

1. Introduction

Soil is a heterogeneous, dynamic, complex living ecosystem that represents a unique balance between physical, chemical and biological properties and whose condition is vital to the proper function of terrestrial ecosystems (Doran and Parkin, 1996). Regrettably, due to a variety of anthropogenic activities (including smelting, mining and agricultural activities), soil pollution with toxic metals is currently an environmental problem of great concern worldwide (Gómez-Sagasti et al., 2012). Indeed, metal pollution is negatively affecting soil quality at a global scale with deleterious effects on the valuable ecosystem services provided by the soil ecosystem (Jeffery et al., 2010).

Soil quality has been defined as "the capacity of soil to perform its functions" (Doran and Parkin, 1996) and, more recently, as "the capacity of a given soil to sustainably perform its ecological processes, functions and ecosystem services at a level similar to that of a reference soil, without causing an adverse impact on the proper functioning of surrounding ecosystems or human health" (Garbisu et al., 2011). Accordingly, it is imperative to have a reliable set of indicators of soil quality which must provide information on the impact of disturbances on soil functioning (Karlen et al., 1997). Traditionally, soil physicochemical parameters have been used as indicators of soil quality; nonetheless, microbial parameters are increasingly being used as bioindicators of soil quality owing to their rapid response, sensitivity, ecological

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relevance, and capacity to provide information that integrates many environmental factors (Epelde et al., 2009a). Indeed, microbial communities play a key role in many soil processes (*e.g.*, organic matter decomposition, nutrient cycling) and the delivery of essential soil ecosystem services (Jeffery et al., 2010). After all, soil microorganisms have intimate relations with their surroundings due to their high surface to volume ratio, and are, to a great extent, responsible for the health of the soil ecosystem (Nannipieri et al., 2003).

On the other hand, the capacity of a given soil to recover from disturbances (its resilience) can be assessed by monitoring soil microbial activities (Nannipieri et al., 2003). Hence, microbial parameters reflecting the biomass, activity and diversity of soil microbial communities have been frequently used as bioindicators not only of the impact of metals on soil quality (Rusk et al., 2004; Kao et al., 2006) but also to evaluate the effectiveness of soil metal remediation processes (Epelde et al., 2009a,b).

Yet, many of these studies were carried out in soils artificially polluted through a single-metal pollution event. By contrast, most frequently, soil metal pollution results from the occurrence of repeated single-metal and, above all, multi-metal pollution events (Mertens et al., 2006). On the other hand, van Bruggen and Semenov (2000) emphasized the importance of monitoring soil microbial responses at regular intervals after the application of stresses.

The aim of this work was to assess the impact of repeated single-metal and multi-metal (Cd, Pb, Cu, Zn) pollution events on soil quality, through the study of the evolution over time of a variety of soil microbial parameters with potential as bioindicators of soil functioning. Similarly, the evolution over time of $CaCl_2$-extractable metal (Cd, Pb, Cu, Zn) fractions in the polluted soil samples was also determined as an estimation of metal bioavailability and, hence, toxicity.

2. Materials and methods

2.1. Soil characterization and experimental design

A microcosm study was carried out with soil collected from the upper 20 cm layer of a natural grassland located in Derio (Basque Country, northern Spain). Immediately after collection, the soil was air-dried at 25 °C for 24 h and sieved to <2 mm. Soil physico-chemical parameters were determined according to standard methods (MAPA, 1994). The soil was a clay loam, with a water holding capacity (WHC) of 71.5%, a pH of 5.2, an organic matter (OM) content of 4.12%, a total nitrogen (N) content of 0.23%, a C/N ratio of 10.4, a phosphorus (P) content of 26.4 mg kg^{-1}, and an electrical conductivity of 0.08 dS m^{-1}.

Prior to metal treatment application, the soil was preconditioned in 0.5 L plastic pots (with holes to allow aeration) for 2 weeks under the following experimental conditions: darkness, 20 °C and 60% WHC. Then, soil portions of 250 g of dry weight (DW) soil were individually polluted (by mechanical mixing in plastic trays) with Cd (36 mg kg^{-1} DW) as $CdCl_2$, Pb (660 mg kg^{-1} - DW) as $PbCl_2$, Cu (500 mg kg^{-1} DW) as CuCl, Zn (1680 mg kg^{-1} DW) as $ZnCl_2$, and a combination of all of them (*i.e.*, multi-metal treatment): 36 Cd + 660 Pb + 500 Cu + 1680 Zn (in mg kg^{-1} DW). These four metals were chosen here due to their environmental relevance; on the other hand, the choice of metal concentrations was based on the decision of doubling the Reference Critical Values reported for the protection of ecosystems in the Basque Country (*i.e.*, 18, 330, 250 and 830 mg kg^{-1} for Cd, Pb, Cu and Zn, respectively) (IHOBE, 1998). The same soil was used as control (without metal application). All six treatments (*i.e.*, Cd, Pb, Cu, Zn, multi-metal, control) were run in triplicate.

Then, soil samples were incubated in 0.5 L plastic pots for 26 weeks under the same experimental conditions described above. Throughout the 26-week incubation period, WHC was held constant at 60% by adding distilled water. After 9 and 18 weeks of incubation, a second and third metal pollution event, respectively, was applied to all soil samples using the same treatments mentioned above. Then, the impact of these repeated (3 times: at weeks 0, 9 and 18) single-metal and multi-metal pollution events on soil quality was assessed through the study of the evolution over time of a variety of soil microbial parameters with potential as bioindicators of soil functioning.

2.2. Soil metal bioavailability

For the determination of soil metal bioavailability, the soil was sampled 4 weeks after each one of the three pollution events. $CaCl_2$-extractable (0.01 M) metal fractions in soil, as an indicator of metal bioavailability (Novozamsky et al., 1992; Houba et al., 2000), were determined as described by Houba et al. (2000). Cadmium, Pb, Cu and Zn concentrations in the extracts were analysed by flame atomic absorption spectrometry (Varian).

2.3. Soil microbial parameters

For the determination of soil microbial parameters, 10 g of FW (fresh weigh) soil was sampled, from the experimental pots, 1 d, 1 week, 4 weeks and 8 weeks after each one of the three pollution events (*i.e.*, 3 pollution events x 4 sampling times = a total of 12 samples). Soil microbial parameters were also determined twice (after 1 and 2 weeks) during the 2-week preconditioning period, confirming their stability before the soil was distributed into portions prior to treatment application (data not shown). Before each sampling, soil was mixed thoroughly with a spatula in order to ensure the homogeneity of the sample.

β-glucosidase and acid phosphatase activities were determined according to Dick (1997) and Taylor et al. (2002). Potentially mineralizable nitrogen, N_{min}, an indicator of the capacity of the soil to supply plant-available nitrogen, was measured as described by Powers (1980).

Soil samples for DNA analysis were stored fresh at −20 °C. DNA was extracted from soil samples (0.25 g of DW soil) using Power Soil™ DNA Isolation Kit (MO BIO Laboratories, California, USA) according to the manufacturer's specifications. Real-time qPCR (RT-qPCR) was carried out for measurements of bacterial and fungal gene copy abundance as described in Epelde et al. (2014). The primers used to assess 16S rRNA gene fragments for total bacteria were Ba519F and Ba907R; the primers used to assess 18S rRNA gene fragments for total fungi were Fung5f and FF390r (Lueders et al., 2004a,b).

2.4. Statistical analysis

Differences among treatments for values of $CaCl_2$-extractable metal concentrations and soil microbial parameters were analysed with one-way ANOVA using Microsoft Stat View Software (SAS Institute, 1998). Tukey–Kramer test was used to establish the significance of the differences among means. The Soil Quality Index (SQI) was determined from the values of all soil microbial parameters according to Bloem et al. (2006):

$$SQI = 10^{\log m - \frac{\sum_{i=1}^{n} |\log m - \log n_i|}{n}}$$

where m is the reference (mean value of control non-polluted soil, set to 100%) and n are the measured values as percentages of the reference.

Multivariate analyses were applied to explore the relationships between experimental factors and response variables using Canoco 5 (ter Braak and Šmilauer, 2012). First, the temporal trends of the