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The influence of soil organic carbon on interactions between microbial parameters and metal concentrations at a long-term contaminated site

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HIGHLIGHTS

- Soil organic carbon affected all interactions between metals and microorganisms.
- Soil organic carbon adjustment changed correlations from positive to negative.
- Ammonium nitrate extractable metals were the most influencing fraction.
- Dehydrogenase activity was the most affected soil parameter.
- Zinc was the most toxic metal among studied metals.

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ABSTRACT

The effects of lead, zinc, cadmium, arsenic and copper deposits on soil microbial parameters were investigated at a site exposed to contamination for over 200 years. Soil samples were collected in triplicates at 121 sites differing in contamination and soil organic carbon (SOC). Microbial biomass, respiration, dehydrogenase activity and metabolic quotient were determined and correlated with total and extractable metal concentrations in soil. The goal was to analyze complex interactions between toxic metals and microbial parameters by assessing the effect of soil organic carbon in the relationships. The effect of SOC was significant in all interactions and changed the correlations between microbial parameters and metal fractions from negative to positive. In some cases, the effect of SOC was combined with that of clay and soil pH. In the final analysis, dehydrogenase activity was negatively correlated to total metal concentrations and acetic acid extractable metals, respiration and metabolic quotient were to ammonium nitrate extractable metals. Dehydrogenase activity was the most sensitive microbial parameter correlating most frequently with contamination. Total and extractable zinc was most often correlated with microbial parameters. The large data set enabled robust explanation of discrepancies in organic matter functioning occurring frequently in analyzing of contaminated soil processes.

1. Introduction

Metal and metalloid accumulation in agricultural soils may result in adverse environmental and human health impacts, including metal contamination of groundwater and accumulation in food crops (de Santiago-Martin et al., 2013). Contrary to organic pollutants, heavy metals cannot be degraded and thus constitute a persistent environmental hazard (Margesin et al., 2011). In the soil environment, the toxicity of metals depends on their bioavailability, which is considered the easily leachable and ion exchangeable fraction available for uptake by soil organisms.

Soil microorganisms participate in many soil processes and due to their short generation time and high surface-to-volume ratio they react quickly to environmental changes. Metals in elevated concentrations disturb the performance of microbial communities and a great variety of metal effects on soil microbial activities was reported in the past (Stefanowicz et al., 2010). Metals affected the growth, morphology or metabolism (Renella et al., 2007; Jiang et al., 2010), denatured proteins and destructed the integrity of cell membranes, and induced the activity of energy demanding cell detoxification metabolic pathways (Leita et al., 1995). Those physiological changes resulted in reduced microbial diversity (Jiang et al., 2010), higher ratios of microbial C to organic C and higher metabolic quotient (Renella et al., 2007). Experiments also showed deterioration of soil respiration or enzyme

Abbreviations: SOC, soil organic carbon; SMC, soil microbial C; DHA, dehydrogenase activity; TM, total metals; TM_N, ammonium nitrate extracts of metals; TM_A, acetic acid extracts of metals.

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activity, reduction of microbial biomass, and shifts in microbial community structure (Giller et al., 1998).

Microbial characteristics such as activity, biomass or diversity were suggested as suitable indicators of soil quality, health and environmental risks. The most commonly used parameters are soil microbial biomass (SMC) and respiration (e.g. Zhang et al., 2010; Niemeyer et al., 2012; Ciarkowska et al., 2014). Both of these microbial parameters are strongly influenced by organic matter content (Stefanowicz et al., 2010; Calvarro et al., 2014). Soil dehydrogenase activity (DHA) is also widely used as a measure of soils' disruption (Wolinska and Stepniewska, 2012) and was often correlated with different microbial parameters than SMC or respiration. That is possibly because dehydrogenases are a group of intracellular enzymes whose activity is associated only to viable cells (Garau et al., 2014). Similarly, DHA activity is affected by organic matter and in several recent studies analyzing organic matter (OM) effects on microbial processes in contaminated soil was determined as the most affected enzymatic activity (Ciarkowska et al., 2014; Calvarro et al., 2014).

Soil organic matter content, particle size, and pH have an important effect on heavy metal(loid)s, sorption, complexation and precipitation, which influence bioavailability. Organic matter is influencing microbial response to soil metal contamination, yet little is known about how it coincides with processes in contaminated soils (Park et al., 2011). Higher organic C levels in soil are supporting greater microbial biomass and enzyme activities because for most chemoorganotrophic microorganisms they represent sources of C and energy sources (Niemeyer et al., 2012). In addition, soil organic matter brings indirect beneficial effects to the soil microbial community by improving the soil capacity for water retention and metal complexation (Giller et al., 1998; Moreno et al., 2009). However, soil organic matter can also decrease soil pH by the introduction of humic and organic acids and increase solubility of some metal complexes. Soil pH influences metal chemistry because in high pH the surface functional groups of not only organic matter and clay particles but also iron and manganese oxyhydroxides are negatively charged and metal cations are almost completely removed from soil solution (Park et al., 2011). Similarly, precipitation is important in the presence of anions such as sulfate, carbonate, hydroxide and phosphate when the soil pH is high (Park et al., 2011).

Limited information is available particularly on interactions between enzyme activities and soil properties that include chronic pollution with Zn, Pb and Cd (Ciarkowska et al., 2014). Field variability of environmental factors is high so specific procedures need to be developed to enable determination of hidden processes. Consequently, the aim of this study was to estimate the effects caused by soil organic matter, pH and clay content on the response of microbial characteristics to concentrations of total and bioavailable metals in a long time contaminated field site. That was achieved by comparing correlations between metal concentrations and microbial parameters with effects caused by soil organic carbon, clay content and pH. The results demonstrated that organic matter changed significantly most of the relationships but clay content and pH also participated in covering reactions of microorganisms to metals.

2. Material and methods

2.1. Sites and sampling

The sampling area (about 5 km^2) was located in the vicinity of a lead smelter in Příbram, Czech Republic (Supplementary Fig. 1). The smelter has been operating since 1786. The primary smelting of lead ores ceased in 1972 and was replaced by industry processing of secondary lead sources such as car batteries and other lead-bearing materials. Since 1982 the contamination with heavy metals decreased 300–500 times due to the building of a 160 m stack equipped with a 98% efficient dust separator (Kalac et al., 1991; Riuwerts and Farago, 1996). Lead emissions were 780 kg per year in 2007. The average rainfall at the area is 600–650 mm y^{-1} and average annual temperature 7.3 °C (149 days have temperatures higher than 10 °C, winter days with 0 °C or lower temperatures are 83). Prevailing winds are from south-west and north-west. Soils were classified as Cambisols (IUSS/ISRIC/FAO, 2006).

A total of 121 sites were selected at the studied area surrounding the smelter (Supplementary Fig. 1). Among them, 87 sites were located on arable land and 34 sites were on grassland. The ratio reflected the distribution of cultivation practices in the area. Grassland sites were mostly found closer to the smelter than arable land sites. At each site, soil samples were collected in triplicate after harvest in the second half of September 2008. Soil cores were taken from the depth of approximately −15 cm. The soil was mixed, larger debris was removed, and soils were passed through a 2-mm plastic sieve. For microbiological analysis the soils were stored moist for no longer than 2 days at 4 °C.

2.2. Soil organic carbon (SOC)

Soil organic carbon was determined according to Sims and Haby (1971) with slight modifications: 1 g of air dried soil was mixed with 10 ml conc. H_2SO_4 and 10 ml 0.34 M $K_2Cr_2O_7$. The soil suspension was adjusted to 100 ml with distilled water after 30 min. The absorbance was determined photometrically at 600 nm.

2.3. Soil microbial C (SMC)

Soil microbial C was determined by fumigation-extraction and calculated using the relationship: SMC = 2.64 Ec, where Ec is the difference between organic C extracted from the fumigated and non-fumigated samples, both expressed as μg C g^{-1} oven dry soil, 2.64 is the constant suggested by Vance et al. (1987).

2.4. Respiratory activity, metabolic quotient qCO_2 and dehydrogenase activity

Three soil subsamples of 100 g were incubated in 1 l tightly closed plastic containers containing 5 ml 1 M NaOH. The CO_2–C evolved was determined after absorption into 1 M NaOH and precipitation with $BaCl_2$. Excess NaOH was titrated with 1 M HCl by an automatic titrator Titrino 716 (Metrohm AG, Switzerland). Metabolic quotient (qCO_2), expressed in units of CO_2–C evolved per unit of microbial C per hour was calculated according to Anderson and Domsch (1990). Dehydrogenase activity (DHA) was determined according to Nannipieri et al. (1990).

2.5. Total and extractable metal concentrations

Soil samples were air-dried at 20 °C, ground in a ceramic mortar and passed through a 2-mm plastic sieve. Total concentrations (TM_{tot}) of As, Cd, Cu, Pb and Zn were determined in subsamples of 0.5 g after digestion with 8 ml of concentrated HNO_3, 5 ml of HCl, and 2 ml of concentrated HF. The mixture was heated in an Ethos 1, microwave-assisted wet digestion system, (MLS GmbH, Germany) for 33 min at 210 °C. After cooling, digests were transferred to 50 ml Teflon® vessels and evaporated to constant weight at 160 °C. The digest was dissolved in 3 ml of a concentrated HNO_3 and HCl mixture (1:3), transferred to a glass tube, brought to volume 25 ml by deionized water, and kept at 21 °C until the measurement. A certified reference material RM 7001 Light Sandy Soil (Analytika s.r.o., Czech Republic) was used as a quality reference of the results. The soluble and exchangeable fractions of metals were determined in ammonium nitrate extracts (TM_N) after extraction for 2 h (10 g of soil and 25 ml 1 M NH_4NO_3) and centrifugation for 10 min at 3000 rpm (Pruess et al., 1991); and in acetic acid extracts (TM_A) with 1 g of soil and 40 ml 0.43 M solution of CH_3COOH after 5 h of shaking and subsequent centrifugation for 10 min at 3000 rpm (Quevauviller et al., 1993). Each extraction was carried out in triplicate, all chemicals were of analytical reagent grade purity. Blank samples representing 5% of the total number of extracts were prepared using exactly the same procedure for given fraction as for soil extracts. Metal