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Metal toxicity assessment in soils using enzymatic activity: Can water be used as a surrogate buffer?

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ABSTRACT

Ecotoxicological tests based on soil enzyme activity are widely used to assess the terrestrial ecotoxicology of metals in soils. However, several standard enzymatic methods use buffers that may alter the chemical pseudoequilibrium of soils and affect metal speciation, and, in turn, the metal effects on enzymes and enzyme kinetics. Researchers have suggested the use of H_2O as a solvent rather than chemical buffers, but opponents are concerned about pH fluctuations during incubation and the resulting difficulty in comparing enzymatic studies. Enzyme assays were conducted on 10 pairs of Zn-contaminated soils to evaluate 1) the buffer effect on Zn lability 2) the pH fluctuation during enzymatic assays conducted in water and 3) the comparison of enzymatic results obtained using chemical buffers versus water. Four standard enzymatic methods covering the major biogeochemical cycles were targeted: arylsulfatase (acetate pH 5.8), urease (borate pH 10), acid phosphatase (modified universal buffer pH 6.5) and protease (THAM pH 8.1). Furthermore, deionized water was tested in parallel as a surrogate solvent for these four methods. With the exception of the acetate buffer, the tested solvents did not significantly change the labile Zn concentration in the soil samples. The pH slightly fluctuated by +0.57 pH unit, corresponding to the intrinsic variability of soils. Enzymatic methods using buffers showed similar results compared to those using water, except for urease. These observations suggest that enzymatic methods setting alkaline conditions should be used with caution and that H_2O could be used as surrogate solvent in this context.

1. Introduction

The ecotoxicological assessment of metal-contaminated field-collected soils based on the estimated aggregated activity of several soil enzymes appears to be a suitable approach to develop routine terrestrial ecotoxicological tests (Epelde et al., 2008). Soil enzymatic activity (EA) reflects the rate-limiting step of biogeochemical decomposition required for microbial processes and is reproducible, affordable and easy to execute (German et al., 2011). Aggregating the results of many enzyme activities covering the C, P, N, and S cycles yields unique quantitative indices of soil functional diversity that could be used as bioindicators for soil health and metal toxicity (Bastida et al., 2008). For example, an assessment of the functional diversity of metal-contaminated soils using a modified Shannon's diversity index adapted for soil enzymes (acid and alkaline phosphatase, urease, arylsulfatase and β-glucosidase) showed that the presence of metal significantly affects the index (Epelde et al., 2008). Sunray plots were also used as schematic representations of enzymatic activities in metal-contaminated soils (dehydrogenase, urease, acid and alkaline phosphatase, arylsulfatase and β-glucosidase) compared to an uncontaminated soil (Hinojosa et al., 2004; Bastida et al., 2008). Biochemical analyses of metal-contaminated field-collected soils constitute a powerful approach to integrate the *in situ* partitioning of trace elements, thus considering metal speciation and bioavailability at pseudoequilibrium—two critical concepts for the assessment of metal toxicity in ecosystem studies (Sauvé, 2001). The labile (reactive) and free metal concentrations are the most widely-accepted metal fractions estimating metal bioavailability. The labile metal in the soil solution includes the free metal and metal ion-pair (mainly inorganic but also some organic ligands) showing a rapid dissociation–association kinetics (Nolan et al., 2005).

Most of the soil enzymatic activity measurement methods recommend running assays in soils using optimal parameters such

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as pH and temperature in order to measure maximum potential activity (Dick, 2011). This procedure is widely used since it makes it possible to compare the enzymatic results of several studies and ensures reproducible assay conditions. The choice of buffer in routine operational protocols depends on several factors, including the buffering capacity of the enzyme's optimal pH value and the absence of buffer inhibition or interference with the enzyme or soil component (Tabatabai, 1994).

Soil enzymatic activity assessments were also conducted in buffer at a pH value close to the actual soil pH value, generating non-optimal but more realistic *in situ* results (Sinsabaugh et al., 2000; Yavitt et al., 2004; Li et al., 2009). Alternatively, soil enzyme activity was measured in equivalent volumes of H_2O instead of buffer to better assess enzyme activities under field conditions (Kandeler and Gerber, 1988; Kandeler et al., 1996; Schinner et al., 1996; Caldwell et al., 1999; Taylor et al., 2002; Chaperon and Sauvé, 2007; Dussault et al., 2008; Chaer et al., 2009). This approach has been criticized mainly because the enzyme assays conducted in H_2O could be less reproducible due to possible pH fluctuations occurring during the enzymatic assay. Indeed, these pH fluctuations may alter the affinity of the enzymes for given substrates as well as the solubility of the enzymes, substrates and cofactors, thus modifying the kinetics of pH-dependent enzymatic reactions (Tabatabai and Dick, 2002; Turner, 2010).

However, the use of a single pH optimal value for a specific enzyme activity seems elusive because the optimal soil enzyme pH depends on the tested soils (Malcolm, 1983; Turner, 2010), and specific activities (e.g. phosphomonoesterase, β-glucosidase) may have different optima. For example, the urease pH ranges between 6–7 and 9–10 depending on the tested soils, suggesting that the use of a single pH buffer is questionable when a potential EA is intended (Alef and Nannipieri, 1995). Moreover, studies comparing soil enzymatic activity in buffer or H_2O have not resulted in clear trends or significant pH fluctuations in unbuffered soil enzyme assays since they were carried out using few soils (Ross, 1987; Kandeler and Gerber, 1988; Taylor et al., 2002).

Methods measuring soil enzyme activity have been set up using uncontaminated soils. When using the soil enzyme activity for the ecotoxicological assessment of metal contaminated soils, the use of buffered assays may modify the metal speciation and interactions between metal species with enzymes and substrates (Tabatabai and Dick, 2002; Chaperon and Sauvé, 2007). In such cases, enzymatic results are not representative of the *in situ* speciation and potentially inhibitory effects of metals. Furthermore, the chemical composition and buffer concentration could be suitable for some soils but inadequate for others because the chemical pool of a soil-buffer-metal combination may vary significantly between soils, unequally affecting the kinetics of the enzymatic reaction (Speir and Ross, 2002). To date, such considerations remain theoretical because, to the authors' knowledge, no study has analyzed the effect of chemical buffers on soil solution chemistry and metal speciation and lability.

The overall aim of this study was to evaluate the possibility of using water instead of chemical buffers as a solvent when determining the EA in metal-contaminated field-collected soils. The specific objectives of this study were to assess: (i) the effect of buffers on soil metal speciation using field-collected non-spiked soils, (ii) the pH fluctuation of soil samples during enzymatic assays conducted in H_2O and (iii) the correlation or divergence between the enzymatic assays conducted in buffers compared to those conducted in H_2O. Zinc was chosen for this study because several monometallic contaminated field-collected soils of various Zn concentrations were available and also because Zn is a co-factor of several soil hydrolytic enzymes with inhibitory effects at higher concentrations (Smolders et al., 2003).

2. Materials and methods

2.1. Soil sampling and physicochemical characterization

Zn-contaminated top soils (0–15 cm) from Québec (Canada) were sampled under galvanized pylons and sieved in the field to <2 mm. Fifteen pairs of soils, in each case a contaminated soil and an uncontaminated reference soil (with similar soil characteristics), were sampled on different sites in a 300-km radius around Montréal to create a pool of field soils available for subsequent analyses. The contaminated soil was sampled in the center of the soil surface under the pylon, and the reference soil was sampled at 25 ± 5 m from the pylon. Visual inspection (texture, color) was done in the field in order to sample a reference soil with physicochemical properties similar to those of contaminated soil. Laboratory analyses were carried out to select the 10 pairs with the closest physicochemical properties within each pair (except for total Zn concentration) prior to the biological analyses (see Table 1). Field-moist sieved soils were stored at 4 °C in an inert plastic covered box.

The soil pH was measured with a pH-meter and glass electrode (Accumet, model 25) in the supernatant of a soil slurry prepared by shaking 10 g of soil and 20 ml of 0.01 M KNO_3 solution (extra pure grade) with a vortex mixer for 1 min and waiting 1 h for the soil suspension to settle (Carter and Gregorich, 2008; Stephan et al., 2008). The buffer capacity was analyzed through pH measurement after the addition of solutions of increasing nitric acid concentrations (Zagury, 1997). The total carbon was analyzed using the combustion method (CEAEQ, 2006) and the total organic carbon (TOC) using the Walkley-Black wet-acid oxidation method with dichromate solution (Carter and Gregorich, 2008). The texture (% sand, % silt, % clay) was determined by hydrometric analysis preceded by the pre-treatment of soil organic matter and Fe and Al sesquioxides (Carter and Gregorich, 2008). Total metal was extracted by total digestion: 1 g of sieved-ground dry soil was mixed with 15 ml HNO_3 (70% v/v, environmental grade) and gently boiled on a heating plate for 60 min in a Teflon covered beaker. This step was repeated. When the mixture was cooled, 20 ml $HClO_4$ (70% v/v, environmental grade) and 1 ml HF (48% v/v, environmental grade) were added to the beaker, and the soil digest was gently boiled on a heating plate until the acid mixture became clear and white fumes appeared. All the soil samples, extractions and analyses were done in triplicate, except for the texture analysis (only one sample per soil). The total metal content (Al, Mn, Co, Ba, Ni, Cu, Pb, Cd, Zn) of the diluted filtrate was then analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Varian ICP-820M, Montréal, QC Canada) (Martin et al., 1994). The total concentrations for Al, Mn, Co, Ba, Ni, Cu, Pb and Cd were at the geological background levels, meaning that all the soils were only contaminated by Zn.

2.2. Labile Zn concentration based on the solvent

Labile Zn concentration was measured in soil extraction experiments using deionized water (H_2O) or buffers used for the enzyme activities in order to determine eventual changes in Zn speciation based on the solvent used during the enzymatic assay. Buffers with various pH and chemical compositions associated with four soil hydrolases involved in nutrient biogeochemical cycles were used in this study: buffers related to arylsulfatase (acetate pH 5.8), acid phosphatase (modified universal buffer MUB pH 6.5), urease (borate pH 10) and protease (trishydroxymethyl-aminomethane TRIS pH 8.1) and deionized water as a solvent surrogate were used for each soil tested. The ten selected Zn-contaminated field soils were analyzed; reference soils were also tested but all their labile