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Biological and chemical assessments of zinc ageing in field soils

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Ageing effects were detected in Zn-amended field soils using ^{65}Zn isotopic dilution as a measure of lability, but not with either $CaCl_2$ extractions or a lux-marked bacterial biosensor.

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ABSTRACT

As zinc (Zn) is both an essential trace element and potential toxicant, the effects of Zn fixation in soil are of practical significance. Soil samples from four field sites amended with $ZnSO_4$ were used to investigate ageing of soluble Zn under field conditions over a 2-year period. Lability of Zn measured using ^{65}Zn radioisotope dilution showed a significant decrease over time and hence evidence of Zn fixation in three of the four soils. However, 0.01 M $CaCl_2$ extractions and toxicity measurements using a genetically modified lux-marked bacterial biosensor did not indicate a decrease in soluble/bioavailable Zn over time. This was attributed to the strong regulatory effect of abiotic properties such as pH on these latter measurements. These results also showed that Zn ageing occurred immediately after Zn spiking, emphasising the need to incubate freshly spiked soils before ecotoxicity assessments.

1. Introduction

Zinc (Zn) is an essential trace element, ubiquitous in soils, and fundamental to the healthy functioning of biological systems. It is also a potential toxicant when present at elevated concentrations. Consequently, the dynamics of Zn in soils are of widespread interest, both in relation to crop nutrient deficiencies and associated impacts on farm yield and economy (Brown et al., 1993; Alloway, 2003) and in terms of soil health and toxicity (McLaughlin et al., 2000; Warne et al., 2008a). The distribution, mobility and bioavailability of Zn in soils are controlled by a range of physico-chemical characteristics, including the nature and heterogeneity of the soil constituents, the surface charge of soil colloids, and variations in soil pH and redox status. This paper focuses on the sorption and fixation of Zn in a range of soils with differing physico-chemical characteristics. The general term 'sorption' is used throughout, due to the difficulties in differentiating between adsorption and precipitation under common experimental conditions (Sposito, 1984).

Previous research investigating the kinetics of soluble metal sorption in soils has shown that the process can essentially be divided into two steps, with an initial stage of relatively rapid sorption followed by a secondary stage that can continue over weeks, months or even years (Barrow, 1986; Smolders and Degryse, 2007). Furthermore, studies investigating metal desorption and/or chemical extractability have typically shown considerable hysteresis, with a negative correlation between desorption/extractability and the residence time of the metal in the soil (Barrow, 1986; Sparks, 1998). This gradual, ongoing process of sorption and fixation has become known as 'ageing' or 'natural attenuation'. Greater understanding of ageing could aid in the modelling and prediction of long-term changes in metal lability and bioavailability. This would be useful for environmental risk assessment and decision making (e.g. in relation to 'safe' metal loading rates) and could also enable land managers to maximise the benefits following application of trace metal fertilisers to land.

Although there is substantial evidence of Zn ageing in soils (e.g. Barrow, 1986; Ma and Uren, 1997a,b; Tye et al., 2003; Degryse et al., 2004), the mechanisms and soil properties controlling this process are only superficially understood. Moreover, much of the evidence comes from laboratory studies conducted under controlled conditions, hence leaving considerable uncertainty as to the kinetics and

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practical significance of ageing in the field. In order to understand the implications of ageing for environmental management and risk assessment this knowledge gap must be addressed, for although variations in soil properties and experimental conditions in field studies can mask effects and make data interpretation difficult, the confirmation of hypotheses under field conditions is strongly indicative of a meaningful effect. To elucidate the practical consequences of metal fixation there is also a need for more research using biological endpoints to measure the effects of ageing. To date, most of the research investigating ageing reactions in the toxic concentration range has taken a chemocentric approach, prompting Lombi et al. (2007) to highlight the importance of integrated biological and chemical assessments in revealing both the mechanisms and the effects of metal ageing.

The experiment reported in this paper used soil samples from four Australian field trials, each with a wide range of Zn loadings as $ZnSO_4$, to investigate the ageing of soluble Zn under field conditions over a two-year period. Both chemical and biological based assessments were used to investigate Zn ageing, including: 0.01 M $CaCl_2$ extractions; isotopic dilution with ^{65}Zn (E_a- and E_e-values); and the acute toxicity response of a genetically modified lux-marked bacterial biosensor (*Escherichia coli* HB101 pUCD607). Lux biosensors present a novel approach for investigating the effects of Zn ageing, and this study is one of the first to use the method for this application (see Paton et al., 1997 for further information on the use of microbial biosensors for soil toxicity testing). The use of the lux biosensor to measure changes in acute toxicity has a major advantage over more traditional microbial ecotoxicity endpoints (e.g. substrate induced respiration or nitrification) as the lux-marked bacteria are not indigenous to the soils and results thus cannot be confounded by adaptation of the soil microbial community.

2. Materials and methods

2.1. Experimental design, field sites, and field-aged soil samples

The field trials used in this study were established as part of the Australian National Biosolids Research Program (NBRP), a large-scale research program designed to investigate the potential benefits and risks of recycling biosolids to agricultural land (McLaughlin et al., 2006; Broos et al., 2007; Warne et al., 2008a,b). Metal-salt trials (cadmium (Cd), copper (Cu) and Zn) were also established at many of the NBRP field sites and the work presented here used $ZnSO_4$-amended samples from four of these sites, chosen to cover a range of soil properties. Samples that had been spiked (11 $ZnSO_4$ treatments per site) and aged in the field for up to two years were compared with freshly spiked samples established using 'control' soils from the same four sites. Two field replicates from each Zn treatment were analysed for the field-aged soils, and two experimental replicates for the matching freshly spiked treatments. Field-aged samples were collected after the first (T1) and second (T2) crop harvests at each field site. Hence, depending on the site, T1 samples were aged in the field for 7–12 months, and T2 samples for 17–24 months after addition of soluble Zn^{2+}. For a detailed description of the NBRP field trial establishment and subsequent soil sampling, preparation and storage refer to Broos et al. (2007).

2.2. Preparation of freshly spiked soil samples

Freshly spiked (T0) samples were prepared in the laboratory using control soils from each of the four field sites to give approximately the same range of Zn concentrations as were present in the field samples (T1 and T2). For each Zn treatment duplicate 150 g soil samples were placed in plastic containers. The appropriate aliquot (ranging from 40 μL to 4500 μL) of $ZnSO_4$ stock solution (containing 659.6 g $ZnSO_4 \cdot 7H_2O$ L^{-1}) was diluted with deionised (DI) water to give a spiking solution volume of 30 mL per sample, then added to the soil and mixed thoroughly by hand. After a 16-hour equilibration period, the samples were leached with several pore volumes of 'Artificial Rain Water' (ARW) containing 10^{-3} M $CaCl_2$ and 5×10^{-4} M K_2SO_4 (Broos et al., 2004) until the electrical conductivity (EC) of the leachate was <2 mS m^{-1}. The leached samples were air-dried at 40 °C and sieved (2 mm).

2.3. Chemical and physical characterisation of soils

Soil pH was measured using 1:5 soil:0.01 M $CaCl_2$ extracts (pH_{CaCl_2}) and EC was measured in 1:5 soil:water extracts. Clay content (<0.002 mm) was determined by the pipette method. Organic carbon (OC) was determined as the difference between total carbon and carbonate carbon, with total carbon concentration measured by ignition with a Leco CNS elemental analyser and carbonate carbon determined by measuring pressure increases after addition of HCl to the soil in closed containers (Sherrod et al., 2002).

Soil samples (2.5 g) were extracted for determination of exchangeable cation concentrations and cation exchange capacity (CEC) (1 M NH_4Cl, pH 7.0) using a mechanical leaching device based on the method of Rayment and Higginson (1992). Samples were analysed for Ca^{2+}, Mg^{2+}, Na^+ and K^+ using a GBC 906AA Atomic Absorption Spectrophotometer, and for NH_4^+ using an Alpkem segmented flow autoanalyser. Pre-treatment to remove carbonates was not required.

The methods used for the determination of dithionite–citrate extractable Fe and acid ammonium oxalate extractable Fe were those of Blakemore et al. (1987). Total Zn and Fe concentrations were determined by aqua regia digestion. Elemental analysis was by inductively coupled plasma-optical emission spectroscopy (Spectroflame Modula ICP-OES).

2.4. 0.01 M $CaCl_2$ extractions

For the 0.01 M $CaCl_2$ extractions, 5 g samples of soil were equilibrated for 16 hours with 25 mL of 0.01 M $CaCl_2$ on an end-over-end shaker. The samples were then centrifuged at 2200*g* for 15 minutes and an aliquot was used for pH measurement (completed within 2 hours of extraction). The remaining sample volume was filtered (0.45 μm) and acidified to pH 1 with 5 M HNO_3, before being analysed for Zn by ICP-OES.

2.5. Isotopic dilution with ^{65}Zn (E-values)

Isotopic dilution with ^{65}Zn was used to monitor changes in the labile Zn fraction over time. The procedure was based on the method of Young et al. (2000) but 0.1 M $CaCl_2$ was used instead of 0.1 M $Ca(NO_3)_2$ and all solutions were filtered (0.45 μm) before analysis. The activity of the ^{65}Zn spike was adjusted for different soil types (20–40 kBq ^{65}Zn per sample) to ensure that a suitable gamma-counting rate would be obtained. Analysis of Zn concentrations in solutions was by ICP-OES and a Wallac 1480 Wizard™ 3″ Automatic Gamma Counter was used for radio-assay.

The labile or 'isotopically available' Zn fraction (henceforth referred to as the E_a-value) was calculated using Equation (1) (Hamon et al., 2002a):

$$E_a = \frac{Zn_{sol}}{Zn^*_{sol}} \times R \times \frac{V}{W} \tag{1}$$

where E_a is the concentration of labile Zn in the soil (mg kg^{-1}), Zn_{sol} is the concentration of non-radioactive Zn in solution (mg L^{-1}), Zn^*_{sol} is the concentration of radioisotope remaining in solution after the 3 days equilibration time (Bq mL^{-1}), R is the total amount of ^{65}Zn added to the sample (Bq mL^{-1}), V is the solution volume (L) and W is the mass of the soil sample (kg).

All E_a-values calculated for this experiment were corrected for background Zn and the lability results are thus expressed in terms of the percentage of total added Zn. This was calculated using Equation (2):

$$\%E\left(Zn^{added}\right) = \frac{E_a - E_a^{control}}{Zn^{total} - Zn^{control\ total}} \times 100 \tag{2}$$

where E_a is the sample E_a-value, $E_a^{control}$ is the mean E_a-value for the matching control soil, Zn^{total} is the total Zn concentration of the sample, and $Zn^{control\ total}$ is the mean total Zn concentration for the matching control soil. All values are expressed as mg kg^{-1}.

The 'isotopically exchangeable' Zn fraction (E_e-value) was also calculated for each sample. This fraction represents Zn on soil surfaces that are in equilibrium with the soil solution (Hamon et al., 2002b). The E_e-value differs from the E_a-value by excluding the solution Zn component as shown in Equation (3) (with all terms expressed in mg kg^{-1}).

$$E_e = E_a - Zn_{sol} \tag{3}$$

2.6. *E. coli* HB101 pUCD607 lux biosensor assay

The lux biosensor was applied as an acute toxicity assay to all samples. The sample solutions used in the assays were 1:1 soil:water extracts (25 g soil: 25 mL DI water, equilibrated for 4 hours on an end-over-end shaker, centrifuged at 2500*g* for 45 minutes and filtered (0.45 μm)). Duplicate 900 μL aliquots from each sample solution were transferred into 5 mL luminometer sample tubes for the bioassay. The remaining solution was divided into 2 subsamples, with one aliquot acidified in preparation for ICP-OES analysis and the other frozen (−19 °C) until analysed for dissolved organic carbon (DOC) using a Scalar Formacs HT TOC Analyser.