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An electrostatic model predicting Cu and Ni toxicity to microbial processes in soils

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ABSTRACT

Toxicity data for microorganism in soil or in soil less cultures have been described with ion competition models, however these models disregard electrostatic and osmotic effects which are known to affect ion sorption and toxicity. Using European soils with diverse characteristics, the factors that influence the toxicity of soil Cu or Ni to potential nitrification rate (PNR) and glucose-induced respiration (GIR) were evaluated based on the electrical potential (ψ_0) and ion activities ($\{M^{2+}\}_0$) at the outer surfaces of bacterial cell membranes (CMs). The zeta potentials (ζ) of bacterial (*Escherichia coli*) protoplasts, as affected by the ionic composition of the solution, were measured and used to estimate the parameters of a Gouy–Chapman–Stern (GCS) model which was then used to compute ψ_0 values. The ψ_0 values varied widely with soil type and increased markedly (became less negative) as metal salts were added. Computed ψ_0 was then used to predict the surface ion activities from the soil solution composition. The toxicity data (both PNR and GIR) were statistically related to (i) surface activities of free metal ions ($\{M^{2+}\}_0$), (ii) the ameliorative effect of surface H^+ activity ($\{H^+\}_0$), (iii) the ψ_0-influenced electrical driving force for cation uptake across CMs, and (iv) osmotic effects. This electrostatic model predicted the observed GIR and PNR with $R^2_{adj} > 0.816$ for observed vs. predicted PNR and $R^2_{adj} > 0.861$ for observed vs. predicted GIR. These predictions were generally better than those by previous models. The suggestion that metal toxicity in spiked soils is partly related to a spike-induced osmotic increase is corroborated by fitting the model to spiked soils that were or were not leached and aged to reduce the osmotic increase. The predicted soil EC50 values (in mg metal/kg soil) were within a factor of 2.5 for up to nineteen European soils with a wide range of properties.

1. Introduction

Soil microbial processes are vital functions in soil ecosystems (*e.g.* C and N cycles) and their sensitivity to metal contamination suggests that these processes need to be included in risk assessments for metals (Giller et al., 1998, 2009; Smolders et al., 2001). Numerous assays have shown that the total concentration of metals in soils required to exert an inhibitory effect vary widely and depend upon metal speciation and soil characteristics (*e.g.* pH, organic carbon, cation exchange capacity (CEC), and the ionic composition of the soil solution) (Oorts et al., 2006a,b; Smolders et al., 2004). For example, the effective concentrations of spiked Cu in soil causing 50% inhibition of potential nitrification rate (PNR; see Table 1 for a list of symbols and abbreviations) ranged from 42 to 2350 mg kg^{-1} in 19 European soils, and for glucose-induced respiration (GIR) concentrations ranged from 186 to 3660 mg kg^{-1} (Oorts et al., 2006b). Risk assessments and regulations therefore need to consider the factors that influence the metal bioavailability. Empirical relationships that relate toxicity thresholds of metals to a limited number of bulk soil properties such as CEC (Oorts et al., 2006b) and organic matter (Lighthart et al., 1983; Oorts et al., 2006b) have been reported already, although the mechanisms by which soil properties influence the toxicity of metals are not well understood.

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Table 1
Principal symbols and abbreviations.

Symbol or abbreviation	Description	Units
BR	Biological response	% of control
CM	Cell membrane	
EC50 (or EA50)	Concentration (or activity) of an ion causing a 50% reduction in the rate of a process (*e.g.* PNR or GIR)	mM or μM
ETM	Electrostatic toxicity model	
FIAM	Free ion activity model	
GCS	Gouy–Chapman–Stern	
GIR	Glucose-induced respiration	% of control
PNR	Potential nitrification rate	% of control
TBLM	Terrestrial biotic ligand model	
$[M]_{soil}$	Total concentration of metal M in soil solution	M, mM, or μM
$[M^{2+}]_b$	Concentration of metal ion M^{2+} in soil solution	M, mM, or μM
$\{M^{2+}\}_b$	Activity of metal ion M^{2+} in soil solution	M, mM, or μM
$\{M^{2+}\}_0$	Activity of metal ion M^{2+} at the CM surface	M, mM, or μM
$K_{P,I}$	Equilibrium constant for the binding of ion I at site P^0	M^{-1}
$K_{R,I}$	Equilibrium constant for the binding of ion I at site R^-	M^{-1}
P_T	Total density of binding sites P^0 at the CM surface	μmol m^{-2}
R_T	Total density of negative charges (and binding sites R^-) at the CM surface (*i.e.*, negative charges in the absence of solute binding)	μmol m^{-2}
ψ_0	Electrical potential at the CM outer surface	mV
σ (σ_0)	Surface charge density (intrinsic σ, *i.e.*, σ in the absence of solute binding) at the CM outer surface	C m^{-2}
ζ potential	Zeta potential; near-surface (plane of shear) electrical potential measured by electrophoresis	mV

Cell surfaces of bacteria are usually negatively charged (Baygents et al., 1998; Soni et al., 2008). Like plant roots, the outer surfaces of bacterial cells become less negative as pH decreases and the ionic strength of the bathing solution increases (Boonaert and Rouxhet, 2000; Bushby, 1990; Butt, 1992; Kinraide and Sweeney, 2003; Morisaki et al., 1999; Van der mei et al., 1993). For example, Kinraide and Sweeney (2003) demonstrated decreased negativity of the rhizobium (*Rhizobium leguminosarum* bv. *trifolii*) cell surface as pH declined from 6.0 to 3.5.

Because of the electrical potential at the outer surfaces of bacterial cell outer membranes (ψ_0), the concentrations or activities of ions at the cell membrane (CM) surface differ significantly from those in the cell-bathing medium. The ψ_0 is often sufficiently negative to enrich cations and deplete anions at the CM surface by more than 10-fold relative to the bulk-phase medium. Cations in the bulk medium, such as Al^{3+}, Ni^{2+}, Ca^{2+}, Mg^{2+}, and H^+, reduce the negativity of ψ_0 by charge screening and ionic binding, thereby reducing the surface activity of cations such as Cu^{2+} and Ni^{2+}. It is also likely that the values for ψ_0 of microorganisms in the soil range widely, given large variations in soil pH and solution concentrations of cations (Wolt, 1994). ψ_0 is difficult to measure directly, but the zeta (ζ) potential, the electrical potential at the hydrodynamic plane of shear (located at a small distance from the CM surface), can be determined by electrophoretic mobility. In addition, a Gouy–Chapman–Stern (GCS) model is available to calculate ψ_0 in response to the solution ionic composition (Kinraide et al., 1998; Kinraide and Wang, 2010; Yermiyahu and Kinraide, 2005).

The quantitative description, and prediction, of adverse effects on microbial processes in soils is less advanced than those for plants or invertebrates, due to a number of limitations. First of all, variations in soil properties affect the composition and metal sensitivity of the indigenous microbial community (Mertens et al., 2010). Consequently, different toxicity responses among soil microbial communities result not only from differences in metal bioavailability but also from differences in the sensitivity of the microbial community tested. The latter may be, in part, attributable to the variation in cell-surface negativity for bacteria which, in plants, has been reported to link the variation in salinity and Al sensitivity (Jozefaciuk and Szatanik-Kloc, 2004; Wagatsuma and Akiba, 1989; Yermiyahu et al., 1999). Secondly, the surface charge density (σ) of bacteria, as influenced by the ionic composition of the soil solution, has an important impact on the adhesion and mobility of bacteria (Baygents et al., 1998; Morisaki et al., 1999) and, consequently, influences the exposure and the sensitivity to soluble metals.

Spiking soils with soluble metal salts increases the ionic strength (*i.e.*, increases the soluble Ca, Mg, and the corresponding osmotic stress) and decreases the pH. All of these factors affect metal bioavailability to microbial processes. An increase in the osmotic potential results in inhibition of microbial processes, especially for nitrification (Li and Huang, 2008; Rysgaard et al., 1999). Indeed, the apparent decrease in toxicity with the leaching and aging of metal salt-amended soils may be partially attributed to the leaching of ions and the concomitant reduction in osmolarity. The effects of leaching and aging have been analyzed in terms of the changes in speciation and osmotic effects (Oorts et al., 2007), but have not yet been modeled (Buekers et al., 2010). Separation of these multiple toxic effects in soils should help to increase the ecological relevance of laboratory toxicological tests.

The objectives of this study therefore are to 1) verify that a GCS model computes reasonable ψ_0 values for bacterial protoplasts that are at least proportional to measured ζ potentials, 2) estimate metal (Cu and Ni) toxicity toward microbial processes (PNR and GIR) in order to elucidate mechanisms by which soil properties affect the toxicity of metals, giving particular consideration to membrane electrical characteristics (both surface potential and transmembrane potential differences) and ion activities at the membrane surface, 3) isolate osmotic effects from other effects that influence microbial processes, and 4) develop electrostatic toxicity models (ETMs) for metal toxicity to soil microbes to predict the critical soil metal concentrations for soils with a wide range of properties.

2. Materials and methods

2.1. Determination of ζ potentials of bacterial protoplasts

Escherichia coli (DH-5α) is an engineered strain of this gram-negative, rod-shaped bacteria and its cell wall contains a thin peptidoglycan layer adjacent to the cytoplasmic membrane (Ishii et al., 2006). *E. coli* was cultured in 500-mL Erlenmeyer flasks containing 200 mL LB medium (Tryptone 2.0 g L^{-1}, Yeast Extract 1.0 g L^{-1}, NaCl 1.0 g L^{-1}). The pH was adjusted to 7.2 with 1 M NaOH or HCl, and the medium was autoclaved at 121 °C for 20 min. The culture was grown at 25 °C and continuously shaken at 200 rpm in the dark. After 16 h of growth, and during the logarithmic growth phase, cells were harvested by centrifugation at 3500 *g* for 20 min at 4 °C. The pellet was suspended in 0.01 M Tris buffer (pH 7.0) and centrifugally washed three times. The pellet was re-suspended in 0.01 M hyper-osmotic Tris buffer (pH 7.0) containing 0.5 M sucrose and then heated at 37 °C for 5 min. A lysozyme (Sigma-Aldrich) solution at 37 °C was added with a final concentration of 0.5 g L^{-1}. The cells were incubated at 150 rpm for 20 min at 37 °C. An EDTA solution was added with a final concentration of 0.01 M and incubated for an additional 15 min. The rate of protoplast formation