Acquired metal tolerance of a soil microbial community used as an early warning for *In Situ* stress in a terrestrial ecosystem

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Abstract

Trace metals are present in the soil matrix in different forms, and this obscures the relationship between the amounts of metals, their biological availability and effects. The most direct approach to analyse trace metal induced community changes, is the determination of the trace metal tolerance of whole bacterial communities (Bååth et al., 1998; Holtan-Hartvik et al., 2002). This method involves shor- term trace metal exposure of suspension of extracted soil bacteria, during which the growth rate is determined by measuring the rate of [^{3}H] thymidine or [^{14}C] leucine incorporation (Bååth 1992). This allows a proximate assessment of LC_{50} concentrations (i.e. the metal concentration which inhibits 50% of the activity), which is a measure of the overall metal tolerance of the community. A greater LC_{50} value indicates increased metal tolerance, which is a strong indicator that the available trace metals in the soil has reached effectual amounts in that parts of the community has already been damaged. Studies of the microbial communities in soils can uncover changes in the microbial community, indicating whether or not the community has adapted to high trace metal concentrations and other environmental stresses.

Trace metal contamination occurs in many soils and it is important to assess metal mobility and environmental risk. The main physico-chemical processes controlling solubility and speciation of trace metals in soil solution are all reasonably well described and incorporated in mechanistic models such as the Windermere Humic Aqueous Model, WHAM (Tipping 1998). Model simulations may provide information about physico-chemical forms of the dissolved metals, which is relevant for assessing reactivity and potential availability to living organisms.

In the present paper, we used acquired Zn- and Cd tolerance of the soil microbial community as a reporter of its exposure, and compared it with chemical determination of Zn and Cd in ten soils differing in pH, organic matter content, texture, vegetation-/cultivation history and metal contamination. The tolerance was measured as LC_{50} (i.e. the metal concentration which inhibits 50% of the activity) in suspensions of extracted soil bacteria, by measuring the incorporation rate of [^{3}H] thymidine at different metal concentrations. Chemical determination of Cd and Zn in soils included total concentrations by aqua regia extractions (AR), and total concentrations in extracted pore water (PW). In addition was the "effective concentration" (C_E) determined using the Diffusion Gradients in Thins films method (DGT). The free ion activity of Cd and Zn in pore water was calculated using WHAM/ModelVI. The LC_{50} values correlated total metal concentration in with PW (r^2 = 0.90 for Cd and r^2 =0.97 for Zn), the FIA (r^2 = 0.95 for Cd and r^2 =0.98 for Zn) and with the C_E (0.90 for Cd and 0.98 for Zn). These correlations were better compared to the correlation with AR (0.72 for Cd and 0.82 for Zn). After excluding a single extremely contaminated soil from the analysis, the correlation of LC_{50} with AR was much poorer (r^2 =0.03 (ns) for Cd and r^2=0.48 for Zn), whereas correlations remained significant for both PW (0.90 for Cd and 0.87 for Zn), the FIA (r^2 = 0.86 for Cd and r^2 =0.82 for Zn) and C_E (0.54 for Cd and 0.84 for Zn. In conclusion, the FIA and PW concentration of Cd and Zn appeared to be the best predictor of trace metal exposure for the soil microorganisms withdrawn from soil solution of different soils, as determined by the LC_{50} values

References

Bååth, E., 1992. Thymidine incorporation into macromolecules of bacteria extracted from soil by homogenization centrifugation. Soil Biology & Biochemistry 24, 1157-1165.

Bååth, E., Frostegård, Å., Díaz-Raviña, M., Tunlid, A., 1998. Microbial community-based measurements to estimate heavy metal effects in soil: The use of phospholipid fatty acid patterns and bacterial community tolerance. AMBIO 27, 58-61.

Holtan-Hartvik, L., Bechman, H., Høyås, T. R., Linjordet, R., Bakken, L. R., 2002. Heavy metals tolerance of soil denitrifying communities: $N_{2O\ dynamics}$. Soil Biology and Biochemistry 34, 1181-1190.

Tipping, E., 1998. Humic ion-binding Model VI: An improved description of the interactions of protons and metal ions with humic substances. Aquatic Geochemistry 4, 3-48.