Diss. ETH No.16834

Estimation of critical limits for cadmium and lead in Swiss forest soils

A dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH

for the degree of
Doctor of Sciences

Presented by

ANNA LAZZARO

Diploma di Laurea in Scienze Naturali, Universita' degli Studi di Milano

Born 21.07.1976

Citizen of Italy

Accepted on the recommendation of

Prof. Dr. Rainer Schulin, examiner

Dr. Beat Frey, co-examiner

Dr. Franco Widmer, co-examiner

2006

Summary

Atmospheric deposition and accumulation of toxic heavy metals such as Cd and Pb in soil is an environmental risk for terrestrial ecosystems. Regulations of emissions and deposition rates of such heavy metals involve the definition of threshold concentrations, the critical limits, below which no adverse effect is caused on the environment. Currently, existing preliminary critical limits of Cd and Pb have been estimated from extrapolation of total background concentrations in soils. Critical limits based on real observed toxicological effects are lacking. Moreover, they need to be related to the concept of bioavailability. Bioavailability and toxicity of heavy metals to soil organisms is related to their chemical speciation and partitioning between the solid and the soil solution phase, and may vary strongly with changing soil properties and soil types.

The objective of this thesis was to estimate toxicity threshold values for Cd and Pb for Swiss forest soils differing in physico-chemical properties, based on the analysis of an important component of soil, the soil microbial communities. The study was based on laboratory incubations of forest soils treated with increasing amounts of Cd or Pb. Toxic effects of Cd and Pb on microbial community activity and on the structure of the soil bacterial communities were related to different estimations of Cd and Pb bioavailability, which included experimental measurements as well as Cd and Pb speciation modeling. In addition, important bacterial groups involved in reactions to Cd were identified in one of the soils studied.

In a first part of the study, several ecotoxicological indices (enzymatic activities, basal respiration, T-RFLP fingerprinting of the bacterial 16S rDNA) and predictors of Cd bioavailability (water-extractions, Lakanen extractions, free Cd^{2+} predictions) were assessed on seven forest soils of different physico-chemical characteristics. The objective was to identify the ecotoxicological indicators and the measurements of Cd bioavailability which could best explain Cd toxicity in forest soils. Acidic and sandy soils (pH < 5.8, clay < 50%, sand > 50%) responded to the Cd treatments with strong changes in the structure of the bacterial communities. In particular, it was noticed that several operational taxonomic units (OTUs), which represent bacterial groups in the T-RFLP profiles, increased to up to 10% in relative abundance in the Cd-contaminated samples. Calcareous soils (pH > 6.7, clay > 50%) did not show

marked changes in the bacterial communities in relation to the Cd treatments but showed inhibition of basal respiration. Both basal respiration and bacterial community fingerprinting are therefore useful indicators of Cd toxicity in forest soils. The enzymatic assays were instead very variable among the soils and did not reveal significant trends or reactions to the Cd treatments. Water-extracts and free ion estimations were better related to the toxicity data than total amounts or Lakanen extractions.

In a second part of the study, the same laboratory incubation setup was applied to assess the effects of Pb on microbial communities of forest soils in a time course. The objective was to investigate how Pb solubility may change over time and how this can influence microbial community activities and structures. Moreover, soil stability (functional resistance and resilience) in relation to the Pb application was compared among different soils. It was observed that after 90 days of incubation, water-extractable Pb increased significantly in some of the soils, in parallel with an increase in water-extractable DOC. This suggests that the increase of DOC could have caused a release of Pb in the form of soluble Pb-organic matter complexes. In this experiment, basal respiration could not represent Pb toxicity, and showed no or variable responses to the Pb treatments, among the soils. We thus hypothesized that basal respiration was probably more affected by an initial application of a C supply (dry milled leaf material) to maintain the microbial communities throughout the length of the experiment. T-RFLP fingerprinting, however, revealed significant changes in bacterial community structures of some soils, which tended to become stronger with time.

The third part of the study aimed at characterizing bacterial groups, which appeared dominating in the short term (30 days of incubation) the T-RFLP profiles of a Cd-treated soil. Through molecular cloning, the T-RFs which were most abundant in the Cd-contaminated sample could be isolated and sequenced. The major bacterial groups that were found to sustain high levels of bioavailable Cd belonged to the genus *Burkholderia* and to the class *Actinomycetes,* close to the genus *Streptomyces.* Such groups may provide good indicators for Cd contamination in forest soils.

Finally, we used the NOEC data derived from our microcosm experiments to estimate threshold Cd and Pb concentrations for the different soils. We derived critical limits in relation to water extracts, which provided the best predictors of Cd

and Pb bioavailability. The lowest NOECs were found in two calcareous clayey soils (pH > 6.7, clay > 50%). This suggests that, despite the lower water-solubility of Cd and Pb, the microbial communities of such soils may be more sensitive to Cd and Pb contamination than more acidic soils.

The effect-based critical limits for water extracts estimated in this work exceeded official guideline concentrations for total Pb and Cd in the soil solution from 2-10-fold for Pb to 10-fold or even more for Cd. These results reflect the differences occurring between different soil types, and the different approaches used for the derivation of critical limits. To take into account such differences, divisions of the concentrations by a safety factor is needed. For example, following the application of a safety factor of 10, the critical limits suggested in this thesis are of 1.2 µg Cd l^{-1} and 1.8 µg Pb l^{-1}. In future strategies for the regulation of deposition of Cd and Pb, effects of such metals on a broader range of terrestrial environments must be considered to reduce data extrapolation and the use of safety factors.

In conclusion, this study contributed to the understanding of the importance of soil properties in influencing Cd and Pb bioavailability and consequent toxicity responses of the microbial communities. Laboratory incubations permitted to compare and evaluate simultaneously bacterial community composition, indices of ecotoxicity and of Cd and Pb bioavailability in different forest soils, to obtain effect concentrations as a basis for future strategies for the regulation of deposition of Cd and Pb in Swiss forest soils.