Quantitative modelling of the response of earthworms to metals

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Summary

Metals in soils can pose a serious threat to soil dwelling organisms, plants, and human beings. A major uncertainty in terrestrial ecological risk assessment for metals is the integrated effect of the physicochemical properties of soil on toxicity and how this allows for extrapolation of toxicity data across soils. The recognition that soil type is an important factor that determines metal toxicity, goes along with the increasing insight into bioavailability. There is a growing consensus that only a proportion of the total amount of metal in soil is available for uptake by organisms and subsequently induces toxic effects. Development of mechanistically underpinned approaches for explaining and predicting availability effects on metal toxicity has been the subject of many research efforts in terrestrial ecotoxicology.

The biotic ligand model is often used as the state-of-the-art approach to quantify the effect of metal availability on metal toxicity in aquatic systems. The biotic ligand model is a synthesis of decades of work on metal speciation, accumulation, toxicity and physiology. The BLM combines two major aspects that affect toxicity: metal speciation and cation competition. Toxicity is assumed to be proportional to the fraction of biotic ligand sites occupied by the free metal ions and competitive binding of other cations with metal ions to the biotic ligand sites can alleviate toxicity. These aspects have been used to explain, why toxicity to freshwater organisms in many cases varied greatly with water chemistry parameters such as pH, hardness, and DOC. The BLM concept is shown to be applicable to a wide range of aquatic organisms, for example fish, daphnia, and algae. In this thesis, we explored the applicability of BLM theory for soil organisms, with a special focus on earthworms (Chapter II and Chapter III). We have adopted the equilibrium partitioning theory and the porewater hypothesis in order to enable linking metal toxicity to porewater chemistry and to apply the BLM theory to soil toxicity data. It is assumed that there is a dynamic equilibrium between metals in the soil solid phase and the soil porewater and that free metal ions in soil porewater are the main toxic species for soil organisms while the base cations can mitigate toxicity through competition for binding to the biotic ligand sites.

In Chapter II, a multicomponent Freundlich model, complying with the basic assumptions of the BLM, was developed to describe the variations in Cu toxicity to earthworms (*Lumbricus rubellus*, *Aporrectodea longa*, and *Eisenia fetida*) in a range of soils of varying properties. This model has some conceptual and practical advantages over the BLM as it requires fewer parameters than the BLM and considers site heterogeneity. Only H^+ but not other cations (K^+, Ca^{2+}, Na^+, and Mg^{2+}) in soil porewater exerted a significant role against Cu^{2+} toxicity to earthworms. The Freundlich-type model in which the protective effects of H^+ were included, explained 84%, 94%, and 96% of the variation in LC50{Cu^{2+}} for *L. rubellus*, *A. longa* and *E. fetida*, respectively. Predicted values of LC50{Cu^{2+}} never differed by more than a factor of 2 from the observed values. External validation of the model showed a similar level of precision, even though toxicity data for other soil organisms and other endpoints were used. Our findings showed the possibility of extrapolating the developed models for one earthworm species to another earthworm species, and even to other soil organisms with different toxicological endpoints.

In Chapter III, we investigated the effects of Cd and Ni on the survival of earthworms (*L. rubellus* and *A. longa*) in soils. Based on empirical studies and the BLM theory, the free ion

approach was proposed as an alternative method to predict Cd and Ni toxicity to earthworms. For the two tested earthworm species, no significant influence of cations (H^+, K^+, Ca^{2+}, Na^+, and Mg^{2+}) on Cd^{2+} toxicity was seen, while Mg^{2+} was found to significantly alleviate Ni^{2+} toxicity. The free ion activity model, which is a special case of the free ion approach, sufficiently described the variability in Cd^{2+} toxicity across soils but failed in predicting Ni^{2+} toxicity. The free ion approach, in which the protective effects of Mg^{2+} were incorporated, explained 89% and 84% of the variations in LC50{Ni^{2+}} for *L. rubellus* and *A. longa*, respectively. Prediction errors were within a factor of 2. There was a lack of consistent effects from the presence of possible competing cations for different metals (i.e., H^+ for Cu^{2+}, Mg^{2+} for Ni^{2+}, and none for Cd^{2+}). We therefore suggest that metal toxicity to earthworms needs to be evaluated on a metal-specific basis.

Another uncertainty in ecological risk assessment is species-specific responses. It has become apparent that the taxonomy-based approach is inappropriate in estimating metal toxicity. This is because taxonomically related species do not necessarily show similar sensitivity to metals. Instead, each species may possess different combinations of traits (characteristics) to deal with metal stress.

In Chapter IV, accumulation and toxicity of Cu, Cd, Ni, and Zn in three earthworm species (*L. rubellus*, *A. longa* and *E. fetida*) were examined. At the same exposure concentration, internal concentrations followed the order: *L. rubellus* > *E. fetida* > *A. longa* for Cu and Ni, *L. rubellus* ≈ *E. fetida* ≈ *A. longa* for Cd, and *L. rubellus* > *A. longa* > *E. fetida* for Zn. The concentrations of Cu, Cd, and Zn in *E. fetida* generally levelled off at high exposure concentrations but not for the other two species. *A. longa* showed a high capability of regulating internal Ni concentrations. For all the four metals tested *L. rubellus* was the most sensitive species, followed by *A. longa* and E. *fetida*. Based on traits theory, a set of traits (such as soil habitat, mobility, ratio of surface area to mass) can be related to the metal accumulation ranking of the earthworm species tested. Differences in sensitivity between species might be explained using the following traits: soil habitat, calcium glands activity, ratio of surface area to mass, and immune-competent cells. However, implementing the traits theory for quantitatively explaining all the observed accumulation patterns and sensitivity of different earthworm species was shown to be difficult. More research is needed in this respect to build up solid relationships between species-specific responses and traits, enabling cross-species extrapolation of accumulation and toxicity data.

In soil, metals are typically present as mixtures because of their natural origin as mixtures in parent rocks and because of diverse pollution sources. Interactions of mixture components can occur at different toxicological levels, making it difficult to predict toxicity of metal mixtures. Classical mixture models (CA or IA) with additivity as the basis ignore these interactions. This may cause overestimation or underestimation of the actual risks.

In Chapter V, individual and binary mixture effects of Cd and Zn on the survival of the earthworm *Aporrectodea caliginosa* were investigated. An increase in Cd concentration decreased Zn toxicity. Similarly, the addition of Zn over a certain concentration range (100 to 1000 mg/kg) decreased Cd toxicity. Beyond a critical Zn concentration of around 1000 mg/kg, Cd toxicity dramatically increased. The partitioning of Cd and Zn between the soil solid phase and the porewater was affected neither by the concentrations nor by the presence of the other metal, suggesting no interactions at the exposure level. Interactions of Cd and Zn

occurred at the organism level, limiting the predictive ability of the simple concentration addition model. MIXTOX modelling showed that mixture interactions were mainly antagonistic and the magnitude of antagonism depended on the relative concentrations of Cd and Zn, and on the concentration magnitudes for the whole ranges of concentrations tested. These findings highlight the importance of identifying the relative influence of various interactions from external exposure to internal assimilation in assessing metal toxicity.

To conclude, our studies present a clear indication of the importance of bioavailability when modelling metal toxicity in soil and suggest the applicability of the BLM theory to soil organisms. The developed bioavailability models (the Freundlich-type model and the free ion approach) in this thesis provide a mechanistic framework for linking soil porewater chemistry to metal toxicity in soils. The traits-based approaches assist in explaining differences in metal accumulation and toxicity between earthworm species. Integration of effect models and traits-based approaches may offer a means to facilitate extrapolation of accumulation and toxicity data across species. With respect to the mixture effects, our study clearly shows the importance of identifying interactions at relevant levels and of incorporating bioavailability effects in modelling mixture toxicity. This can be especially helpful to explain differences in interaction patterns that occur between different soil types. Ultimately, the findings in this thesis well fit into the broader picture of soil metal risk assessment.