Incorporating toxicokinetics and toxicodynamics in metal bioavailability models using Enchytraeus crypticus

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Summary

Contamination of soil with metals may pose a serious threat to environmental and human health. Since bioavailability is a key factor in determining metal toxicity, the factors affecting metal bioavailability should be qualitatively and quantitatively determined. Many of these factors are related to the chemical aspects of metal speciation, and the interactions of the metal with other metals and ions present in the soil solution. Another important factor greatly affecting toxicity is time. Metal bioaccumulation and subsequent toxicity are highly determined by the exposure time, involving toxicokinetic and toxicodynamic processes.

The main goal of this thesis is therefore to take into account these modifying factors (varying soil solution chemistry, interactions of metals in mixtures, metal toxicokinetics and toxicodynamics) in developing a unified framework to predict toxicity of metals.

All experiments described in this thesis were performed with the terrestrial oligochaete worm *Enchytraeus crypticus*, using a substrate of aqueous solutions embedded in inert quartz sand. In this way, solution chemistry and therefore metal speciation could be well controlled, while at the same time allowing the worms to behave naturally

In **Chapters 2 and 3**, experiments were performed to determine environmental factors (Na^+, Mg^{2+}, K^+, Ca^{2+}, and H^+) that affect bioavailability and toxicity of nickel (Ni) to *E. crypticus* in the course of time during an exposure period of 3 weeks.

The uptake and toxicity of Ni in *E. crypticus* after different exposure times were investigated in **Chapter 2**. The body concentration and toxicity of Ni gradually increased with time and tended to reach equilibrium at longer exposure times. The dynamic accumulation process of Ni was well described by a one-compartment model using constant rate parameters for uptake and elimination. When expressed as free ion activity in the test solution, LC50 of Ni decreased approximately by a factor of 3 during an exposure period running from 4 to 21 d. However, when based on Ni concentrations in the test animals, LC50 was almost constant and independent of exposure time. Since bioavailability of metals is determined by the characteristics of the organism, the environment and the toxicant together, body concentration rather than external concentration could be a more reliable indicator of metal toxicity as it incorporates toxicokinetic processes. This study revealed that time is an important factor which should be taken into account when modeling metal toxicity. In reality, soil organisms are usually exposed to metals for much longer times than the duration of standard laboratory ecotoxicological tests. Hence, the use of chronic toxicity data to conduct risk assessment and derive environmental quality criteria is preferred.

The influence of porewater chemistry (Ca^{2+}, Mg^{2+}, Na^+, K^+ and H^+) on Ni uptake and toxicity in *E. crypticus* was determined in **Chapter 3**. The same factors (Ca^{2+}, Mg^{2+}, Na^+) were found to modify both Ni uptake and toxicity, suggesting that the way by which these cations exert their protective effects is mainly through competing with metal ions for binding sites on the surface of the organisms, leading to an inhibition of the uptake of metal ions. Ni free ion activity was calculated with the model WHAMVI to take into account the influence of water chemistry (e.g. pH, DOC) on metal speciation. By incorporating the effect of metal speciation and competitive cations, extended Langmuir and Biotic Ligand models were developed, which well described the uptake and toxicity of Ni, respectively. The ligand binding constants of Mg^{2+} and Na^+ derived from uptake and toxicity data were similar.

However, for Ca^{2+}, the binding constants derived from uptake data differed considerably from the toxicity-derived estimates. This may be explained by the possible influence of Ca^{2+} on membrane permeability, or from an interaction between Ni^{2+} and Ca^{2+} at the target sites inside the organism. Our findings show that the biotic ligand model (BLM), which was originally developed for aquatic organisms, is also applicable to terrestrial organisms, with the body surface representing the biotic ligand.

As shown in **Chapters 2** and **3**, bioavailability and toxicity of metals are dependent on both exposure time and characteristics of the exposure solution. Existing BLMs were often developed for equilibrium conditions with fixed exposure times, neglecting the effect of time on metal uptake or toxicity. In **Chapter 4**, we show that the developed acute BLM cannot be used for accurately predicting chronic metal toxicity; the acute BLM underestimates chronic Ni toxicity to *E. crypticus*. In this chapter therefore two generic bioavailability models were developed based on a static BLM to take into account the influence of time by incorporating time-dependency in the parameters. In model b the fraction of binding sites occupied causing 50% mortality (f_{50}) was allowed to vary; in model c the binding 'constants' of the metal and competitive cations (K_{MeBL} and K_{CBL}) were variable. With these generic models, toxicity of Ni to *E. crypticus* was successfully described for varying exposure times and water chemistries. In previous studies, the stability binding constants were supposed to be metal- and species-specific and can only be derived under equilibrium conditions. As such chemical and biological equilibrium might not be reached upon short exposure times, the future development of BLMs should be cautious when using binding constants obtained in acute toxicity tests to predict chronic metal toxicity.

Metals are commonly present in mixtures in mining, industrial and domestic effluents, hence, risk assessment should take into account mixture toxicity. For this purpose, in **Chapters 5 and 6**, the dynamic toxicity of Ni and Co mixtures to *E. crypticus* was evaluated, using the same exposure media as described above and again following a toxicokinetics and toxicodynamics approach.

In **Chapter 5**, the Concentration Addition (CA) model was used to predict the toxic effect of metal mixtures. Deviations from concentration addition indicated that interactions occurred between Ni and Co. In order to better understand the underlying mechanism causing this finding, the interactions were determined at different levels. The interaction patterns at different exposure times were determined with the MIXTOX model, relating toxicity to metal free ion activities in solution and body concentrations in the test animals, respectively. When based on free ion activity, the interaction was mainly antagonistic and varied with exposure time, while when based on body concentrations no deviation from concentration addition was found. This suggests that interaction between Ni and Co mainly occurred during uptake, probably through competition for binding or uptake sites on the biotic ligand. This hypothesis is further supported by reduced Ni body concentrations in the animals in the presence of Co. Moreover, the use of body concentrations for predicting toxicity of metal mixtures could reduce the time dependence of the interaction pattern by taking into account toxicokinetic processes. The variation in toxicodynamic processes should be considered as well in future studies for establishing more accurate environmental quality criteria.

In **Chapter 6**, a newly developed WHAM-F_{TOX} model, assuming humic acid as a surrogate of non-specific biotic ligand sites, was applied to test its feasibility for delineating

the dynamic uptake and toxicity of metal mixtures. This assumption was confirmed by our findings that uptake of Ni and Co in *E. crypticus* linearly correlated with the amount of metal binding to humic acid, which was calculated with the WHAMVI speciation model taking into account the chemical composition of the test solutions. Single and mixture toxicity at different exposure times was well fitted by the WHAM-F_{TOX} model. Metal-specific accumulation kinetics (slow/fast) led to more/less time dependence of metal toxicity. This could explain why the estimated toxicity coefficient of Ni was time-dependent, while this was not the case for Co. The WHAM-F_{TOX} model shows to be a promising tool for predicting metal toxicity, which is mainly based on pure chemistry. Nevertheless, the biological aspect plays an important role in regulating metal uptake and metal detoxification, which cannot be ignored in the development of a mechanistic model.

In summary, our research showed that kinetic and dynamic processes must be included in the analysis of bioaccumulation and toxicity of metals. Traditional toxicological studies with fixed exposure times are of limited meaning for the risk assessment of toxicants with long-term exposure. In our research, the toxicity of metals was always related to two exposure levels: environmental concentrations and body concentrations in the test organisms. In this way it was shown that metal bioavailability and toxicity are affected mainly through the competitive interactions between ions during metal uptake. The developed bioavailability models, incorporating the effect of exposure time and exposure conditions, successfully predicted toxicity of metals, both single and in mixtures. The results of this thesis contribute to improving our mechanistic understanding of the uptake and toxicity of metals and to better define their risks in the environment.