Quantifying the toxicity of mixtures of metals and metal-based nanoparticles to higher plants

Liu, Y.

Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/35907 holds various files of this Leiden University dissertation

Author: Yang Liu
Title: Quantifying the toxicity of mixtures of metals and metal-based nanoparticles to higher plants
Issue Date: 2015-10-20

Summary

Metals are widely present in oceans and in the crust of the earth. Some of them provide organisms on this planet with the necessary nutrients to sustain proper functioning. However, excessive quantities of metals can be toxic. The physicochemical properties of water and soil in the natural environment, such as hardness, pH and dissolved organic carbon, may affect the bioaccumulation of metals and complicate their risk assessment in aquatic and terrestrial ecosystems. Integrating these factors in the development of mechanistic models allows the variability in predicting bioavailability and toxicity of metals to be reduced.

The study presented in Chapter 2 investigated the influence of Ca^{2+}, Mg^{2+}, K^+, Na^+ and pH on the acute toxicity of Ni and Cd to butter-head lettuce seedlings (*Lactuca sativa* L.). It was shown that only Mg^{2+} and not H^+, K^+, Na^+, and Ca^{2+}, exerts a significant alleviative effect on the toxicity of Ni to lettuce, whereas no significant influence of any of these common cations was observed on the effect of Cd on the root growth of lettuce. Based on the biotic ligand model (BLM), the competition of Mg^{2+} with Ni^{2+} for binding sites at the biotic ligand at the water-organism interface was incorporated in the prediction of Ni toxicity. This greatly improved the predictive power (R^2=0.80) in assessing the toxic effects of Ni at varying concentrations of Mg^{2+} in the solution, compared to the total metal model (TMM) (R^2=0.49) and the free ion activity model (FIAM) (R^2=0.60). As regards Cd, since the overall variations of IC50$\{Cd^{2+}\}$ at the different concentrations of H^+, K^+, Na^+, Ca^{2+}, Mg^{2+} in the solution were rather small, the TMM performed just as well as the FIAM in explaining the inhibition of root elongation of lettuce by Cd. We therefore suggest that mechanistically underpinned models for assessing the toxicity of metals to higher plants should be generated on a metal-specific basis according to the toxicological data.

Another toxicity-modifying factor, namely mixture effects, also plays an important role in the assessment of adverse effects of metals on terrestrial plants. Humans and other organisms living in the natural environment are often exposed to a variety of substances. Toxicity of metal mixtures to organisms may deviate significantly

from the added effects of individual metals because of interactions. Hence, researchers are constantly improving the accuracy of methods for toxicity assessments for multiple metals.

Chapter 3 reports on a study to investigate the joint toxicity of binary metal mixtures, i.e. Cu-Cd, Ni-Cd, and Cu-Ni, using concentration addition (CA) based models and independent action (IA) based models. Inhibition of the root elongation of lettuce by Cu-Cd, Ni-Cd and Cu-Ni mixtures was quantified by statistical software, i.e. the MixTox model. The toxicity of these binary metal mixtures was equally well predicted by the CA-based and IA-based models. Statistically significant deviations from additivity were often found in the toxicity modeling of these three metal mixtures, and the deviation patterns were variable for specific combinations and for different base models. To examine whether these statistically significant deviations were reproducible, other datasets derived from independent experiments using Ni-Cd and Cu-Ni mixtures were used as input in the same mixture models. However, the deviations patterns were found to be inconsistent or even contradictory across various ranges of metal concentrations and different base models. We therefore recommended that a statistically significant deviation from a standard model must be interpreted with caution, and does not necessarily reflect a biologically relevant interaction. Finding statistically significant deviations may be a starting point for further measurements and modeling to improve the understanding of non-additive interactions occurring inside organisms.

In Chapter 4, the biotic ligand model (BLM) was extended to predict the overall toxicity of Cu-Ni, Cu-Zn, and Cu-Ag mixtures to lettuce (*Lactuca sativa* L.) in three approaches based on the concept of additivity, namely the toxic unit approach (TU), the toxic equivalency factor approach (TEF) and the approach of determining the fraction of the total number of biotic ligand sites bound by metal ions in mixtures (f_{mix}). The impacts of environmental chemistry and ion-ion interactions can be incorporated in the assessment of both bioavailability and toxicity of metal mixtures by combining the BLM with toxicity indexes. Using the method of bootstrapping, the predictive capabilities of these non-nested BLM-based approaches for each combination were compared, and the best fitted model was found to be dependent on the specific composition of the mixtures. This finding may be attributable to

diverse physiological properties of individual metals with regard to higher plants, and different underlying mechanisms of metal mixtures in lettuce.

Engineered metal-based nanoparticles (NPs) are a new source of environmental contamination, but the information concerning their release, fate and toxicity is limited. It is therefore difficult to assess the potential effects of metal-based NPs in the environment.

The study presented in Chapter 5 systematically evaluated the combined effects and mutual impacts of Cu NPs and ZnO NPs, using six nested combinations, namely mixtures of $Cu(NO_3)_2$-$Zn(NO_3)_2$, $Cu(NO_3)_2$-Cu NPs, $Zn(NO_3)_2$-ZnO NPs, $Cu(NO_3)_2$-ZnO NPs, $Zn(NO_3)_2$-Cu NPs, and Cu NPs-ZnO NPs. No substantial differences were found in the aggregation or agglomeration of Cu NPs or ZnO NPs and their mixtures with nitrates. More than 80% of the variability in the combined effects of mixtures of $Zn(NO_3)_2$-ZnO NPs and Cu NPs-ZnO NPs was explained by the independent action (IA) model. The variations left in toxicity modeling of Cu NPs and ZnO NPs can be explained by small antagonistic effects found among dissolved metal species as well as non-dissolved particulate fractions of NPs. These results demonstrated that mutual impacts of soluble metals and non-dissolved particles can affect the combined toxicity of Cu NPs and ZnO NPs, which cannot be easily explained by a simple combination of $Cu(NO_3)_2$ and $Zn(NO_3)_2$.

In conclusion, our study emphasizes the importance of two toxicity-modifying factors (the composition of the surrounding exposure media and mixture effects) in the assessment of toxic effects of metals and metal-based NPs on higher plants. Based on the affinity of metals for binding sites on the biotic ligand at the water-organism interface, the mechanistic models we developed provide better links with the toxicity of metal mixtures. We also recommend that finding a statistically significant deviation from additivity can be the starting point for further mechanistic research concerning toxicologically relevant interactions between substances, instead of the endpoint of research used so far. As an extension of the research discussed in the third chapter of this thesis, the commonly known model for the toxicity of mixtures was proven to be suitable for preliminarily assessing the

effects of metal-based NPs on terrestrial organisms. The experimental design of nested combinations helps establish a more realistic exposure scenario for the environment and makes it possible to identify where and how chemical-chemical interactions occur with metal-based NPs. Consequently, our findings enrich the rapidly evolving field of toxicology regarding metals and metal-based NPs.