Mixture toxicity of Cu, Ni, Cd and Zn to barley can conservatively be predicted by the concentration addition model

Versieren Liske[1], De Schamphelaere Karel[2], Blust Ronny[3] and Smolders Erik[1]

[1]Division Soil and Water Management, KULeuven, Belgium
[2] Laboratory of Environmental Toxicology and Aquatic Ecology, UGent, Belgium

[3]Laboratory for Ecophysiology, Biochemistry and Toxicology, University of Antwerp, Belgium

E-mail contact: liske.versieren@ees.kuleuven.be

1. Introduction

Soil and water metal contamination more often occurs with a mixture of different metals than with a single metal. Risk-evaluation, however, is almost always based on the effects of single contaminants [1]. The current approach in risk-evaluation is only justified if the exposure to mixtures does not bear the risk of an increased toxicity relative to the toxicity at single exposure to any of the mixture components. Thus, there is a need to understand how metals act together in producing combination effects, especially at low effect concentrations of the individual metals. This study aims to investigate and predict toxicity and interactions of multiple metal mixtures (Cu, Ni, Cd and Zn) to barley root elongation in nutrient solutions at metal concentrations individually causing low level effects. Plant metal concentrations were measured in order to find possible tissue load based explanations for these interactions.

2. Materials and methods

Two barley root elongation tests (5 days) to assess Cu, Ni, Cd and Zn mixture toxicity and interactions were performed in nutrient solutions with metal activities buffered by a solid resin [2]. In experiment 1, a design consisting of 62 treatments was chosen to assess mixture toxicity and interactions of Cu, Ni, Cd and Zn. Emphasis was on combinations of the 4 metals at low effect concentrations of the 4 metals. A second experiment with a similar design, consisting of 51 treatments, was set up to more sensitively test the metal interactions observed in experiment 1 and to confirm suggested hypotheses of experiment 1.

Dose-response curves of the single metals were fitted using a best fit approach with JMP, using a log-logistic model: $y = \frac{max}{1+\left(\frac{C}{EC_{50}}\right)^{\beta}}$, where y is the root elongation as % of the root elongation in the control treatment.

To *predict mixture toxicity*, two basic models: the concentration-addition (CA) model and independent action (IA) model were used, based on single metal toxicity data. The predicted yield was then plotted to observed yield for all mixture treatments to have a first impression on possible interactions, and to evaluate predictions using this component-based approach. Global *interactive effects*, i.e. general departures from additivity, were evaluated by the method developed by Jonker et al. [3] using R.

3. Results and discussion

3.1. Experiment 1: metal mixture toxicity and interactions

The measured RRE is compared with the predictions by the CA and the IA model using single metal data and the IA model performs better (root mean squared error RMSE = 24) in predicting RRE than the CA model (RMSE= 27) (Figure 1). The CA model, however, is more conservative than the IA model as the CA model generally predicts a higher toxicity than the IA model. More in detail, in some of the mixture treatments, deviations between observed RRE and predictions with both reference models are observed; this is an indication of interactions between the metals.

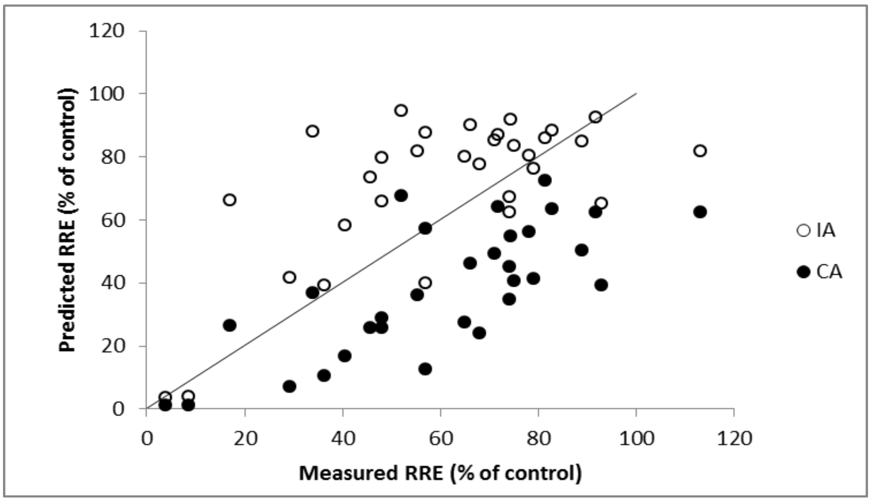

Figure 1: Comparison of the measured relative root elongation (RRE: 100%=uninhibited control) with the predictions by the CA and the IA model (exp.1)

Most observed antagonistic interactions occur at elevated Zn concentrations. In addition, mixtures with elevated Zn + Cd concentrations generally lead to antagonistic interactions to the CA model. In some treatments synergistic interactions are observed, and the hypothesis is that these synergistic interactions are mainly due to Ni + Cu interactions, and only occur at low Zn concentrations. Global interactive metal mixture effects were assessed by the Jonker method using all mixture data (whole mixture approach). The results indicate perfect independent action. In addition, using the CA concept, the Jonker model indicates general antagonistic interactions.

3.2. Experiment 2: metal mixture toxicity and interactions

The results of experiment 1 were confirmed as the IA model performs better (RMSE=15) in predicting RRE than the CA model (RMSE=25). The CA model, is again more conservative than the IA model and almost no synergistic interactions to the CA model are observed. In addition, general metal mixture interactions were assessed by the Jonker method using all mixture data (whole mixture approach). In experiment 2, the results indicate general antagonistic interactions to both the CA ($p<0.01$) and the IA ($p<0.01$) model. The results of the binary Zn + Cd mixtures confirm the suggested hypothesis of exp. 1 that elevated Zn + Cd concentrations generally lead to antagonistic interactions. Binary Cu + Cd and ternary Cu + Ni + Cd mixtures generally produce additive or even small synergistic effects, whereas when extra Zn is added these interactions shift to additivity or antagonism. This confirms the hypothesis of exp. 1 that Zn has a protective effect on multiple metal toxicity as most observed antagonistic interactions in multiple metal mixtures occur at elevated Zn concentrations .

4. Conclusions

In terms of risk-assessment, the CA model using a component based approach is generally conservative and can be used as a conservative model to predict metal mixture toxicity to barley. The few cases of synergism were found at low Zn and not at high Zn supply, suggesting protective effects of Zn It should be noted, that mixture toxicity cannot be neglected even under antagonistic conditions since a mixture of different metals has a larger effect than the effect of the most toxic metal in that mixture. In addition, a mixture of different metals at concentrations singly causing only slightly toxic effects can lead to significant mixture effects.

5. References

[1] Backhaus T, Faust M (2012) Predictive Environmental Risk Assessment of Chemical Mixtures: A Conceptual Framework. Environ. Sci. Technol. 46 (5):2564-2573. doi:10.1021/es2034125

[2] Versieren L, Smets E, Smolders E (2013) A resin buffered method for controlling metal speciation in nutrient solutions for plant toxicity tests. Plant Soil 373 (1-2):257-267. doi:10.1007/s11104-013-1797-7

[3] Jonker MJ, Scendsen C, Bedaux JJM, Bongers M, Kammenga JE. (2005) Significance testing of synergistic/antagonistic, dose level-dependent, or dose ratio-dependent in mixture dose-response analysis. Environ Toxicol. Chem. 24:2701-2713

Acknowledgement - The authors thank the Fund for Scientific Research - Flanders (FWO) for financial support (project FWOG0460.12)