Chronic metal mixture toxicity: From data gap analysis to the development of an environmentally and regulatory relevant experimental program

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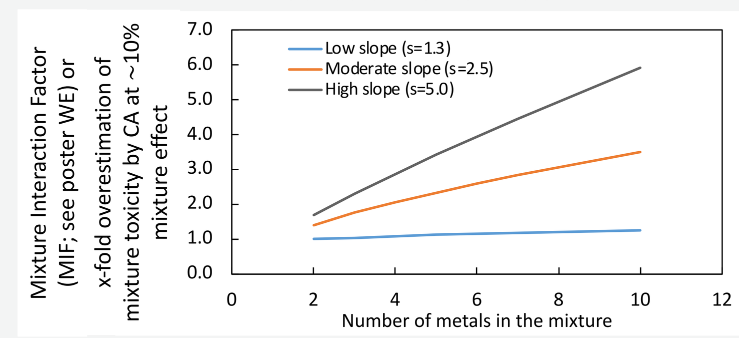
Introduction

Inorganic-Priority Contributing Substances[2]:
Ag, As, Ba, Cd, Co Cr, Cu, Hg, Mn, Ni, Pb, Se, V and Zn
I-PCS have been prioritized based on exceedances of PNEC and contribution to the overall risk pressure based on monitoring data

- The European Union Chemicals Strategy for Sustainability[1] calls to systematically integrate the issue of combined exposure into risk assessments. As such, it is important to evaluate metal mixture toxicity for environmental relevant metal combinations and at environmentally relevant concentrations.
- This study is part of the comprehensive Eurometaux Metals Environmental Exposure Data gathering program (MEED) as project 5, which focusses on metal-organic mixture toxicity.
- Previously, based on European monitoring data[2], a set of Inorganic-Priority Contributing Substances (I-PCS) have been identified to prioritize in further research.
- A recent meta-analysis[3] demonstrated the need to conduct a tesing program to complement gaps in relevant metals-mixture toxicity knowledge. Following datagaps were noted: I) limited (high-quality) data on metal mixture toxicity to aquatic organisms of anionic metals, II) limited data for mixtures combining four or more metals.

Hypothesis: overestimation of metal mixture toxicity by CA increases with number of metals in the mixture.

Theoretical calculation



Objective of this study: develop a targeted testing program that aims covering for the above data-gaps and testing hypothesis, while ensuring the environmental and regulatory relevancy of the tested metal mixture combinations.

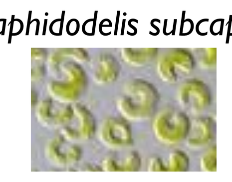
Methods

Environmental metal monitoring data: Waterbase
- Measured concentrations (i.e. reported concentration ≥ Limit of Quantification)
- Only reliable measured (>10% Detection Limit of FOREGS-database) and regulatory relevant (<1000xPNEC) concentrations

+

Species sensitivities (EC10) from REACH registration files
- for Ni, Zn, Cu, Cd and Pb EC10 bioavailability-normalized to the most sensitive 'EU-ecoregion'[4]

Daphnia magna *Raphidodelis subcapitata*



Mixture risk prioritisation based on CA using the toxic unit (TU)-approach
Step-wise approach to select environmentally relevant:
- Mixture size
- Metals
- Metal combinations
- Concentration ratios

$$SumTU = \sum_{i} \frac{env\ conc_i}{EC10_{i,species\,j}}$$

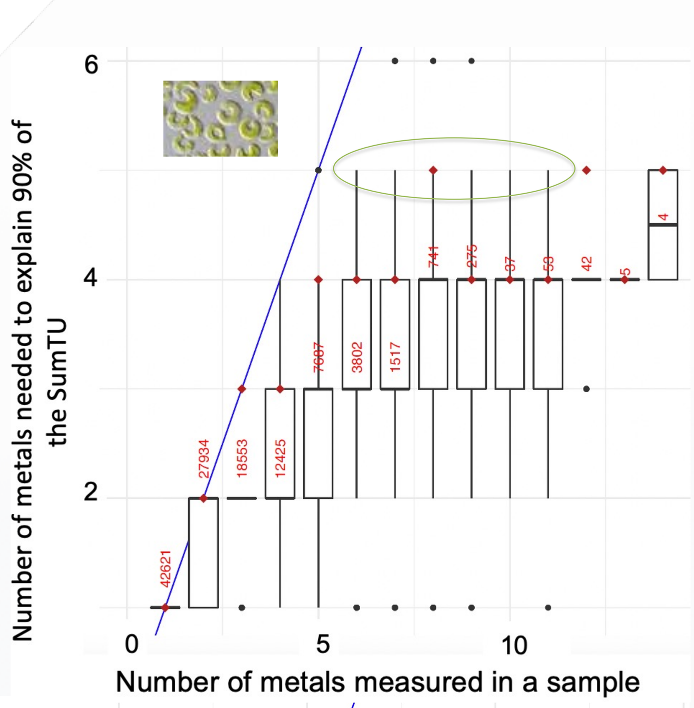
i= metal i

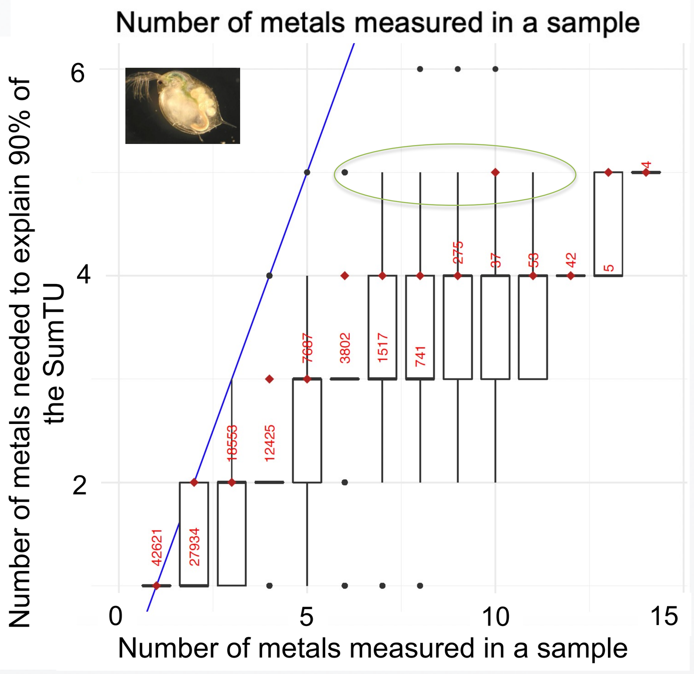
Testing program
3 test series per throphic level

Results

Step 1: How many metals contribute to mixture toxicity?

Objective: select the highest complexity that is relevant to test from an environmental perspective.





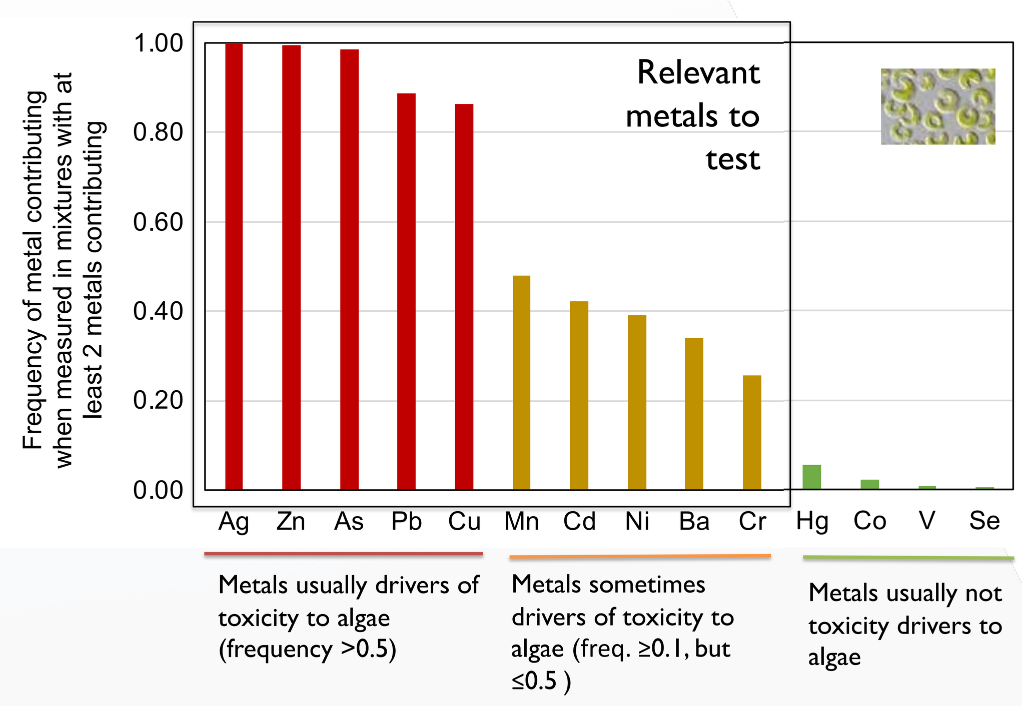
In more complex samples (≥7 measured metals) :
- 90% of the samples: 4 metals needed to explain 90% of the sumTU (red diamonds).
- Most of the samples: 5 metals needed to explain 90% of the sumTU (green elipses).

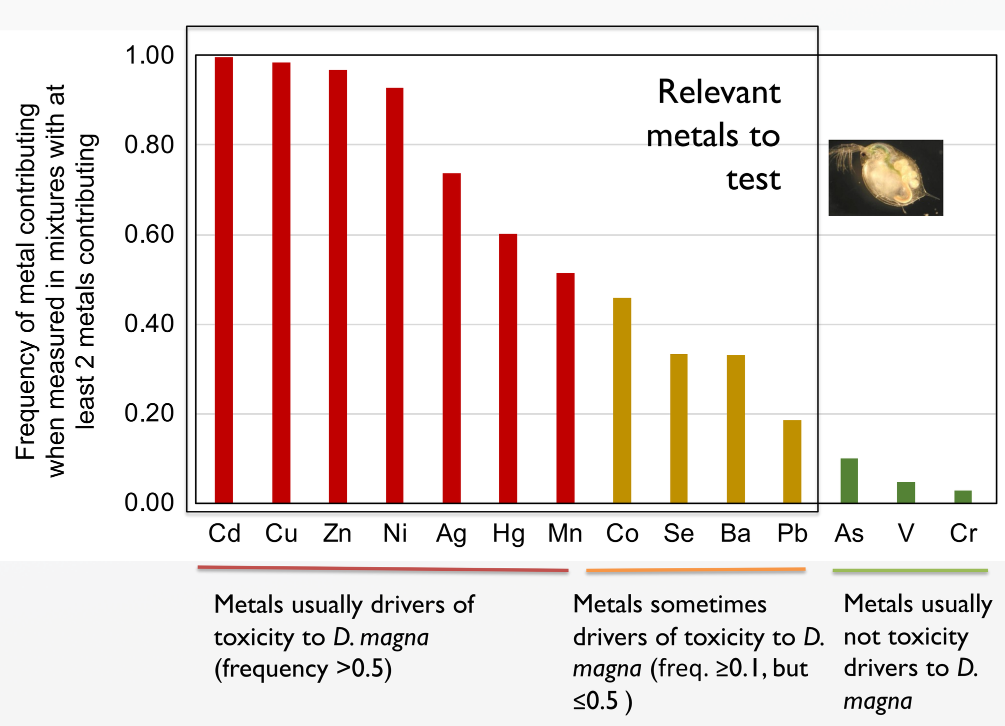
Mixtures containing up to 5 metals (=quinary) are relevant for testing both species

Step 2: Which metals drive mixture toxicity?

Objective: select those metals that contribute typically to 90% of mixture contribution.

$$\text{Frequency}=\frac{\text{\# samples where metal contributes to 90\% of sumTU}}{\text{\# samples where metal has been measured}}$$





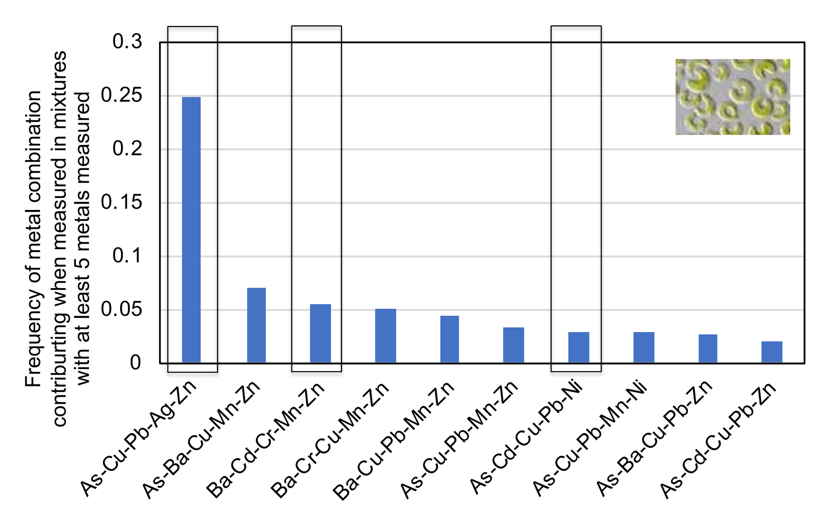
The following I-PCS are environmentally relevant toxicity drivers:
- For *R. subcapitata* Ag, As, Ba, Cd, Cu, Cr, Mn, Ni, Pb and Zn.
- For *D. magna*: Ag, Ba, Cd, Co, Cu, Hg, Mn, Ni, Pb, Se and Zn.

Given the chalenges related to ecotoxicity testing with Hg, Hg was not further considered in development of the testing program.

Step 3: What are the environmental relevant mixture combinations?

Objective: Select 3 quinary metal mixture combinations that cover all of the relevant metals (Step 2) at least once and maximum twice from the quinary metal combinations that most frequently result in a contribution to the mixture pressure

$$\text{Frequency}=\frac{\text{\# samples where all metals in the mixture combination contribute to 90\% of sumTU}}{\text{\# samples where all metals have been measured together}}$$



Selected quinary mixture combinations for *R. subcapitata* are:
- As-Cu-Pb-Ag-Zn
- Ba-Cd-Cr-Mn-Zn
- As-Cd-Cu-Pb-Ni

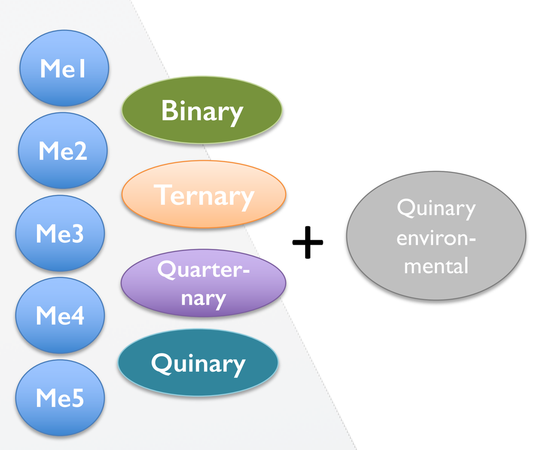
To be able to evaluate the effect of the number of metals in the mixture on the prediction performance of CA, a similar approach was used to select binary, terary and quaternary mixtures. For example, for As-Cu-Pb-Ni-Cd
- As-Cu
- As-Cu-Pb
- As-Cu-Pb-Ni
- As-Cu-Pb-Ni-Cd

For *D. magna*., the following combinations were selected:
- Cu-Cd-Zn-Ag-Ni
- Co-Cu-Mn-Ni-Zn
- Ba-Pb-Mn-Ni-Se

Step 4: Selection of metal mixture concentration ratios

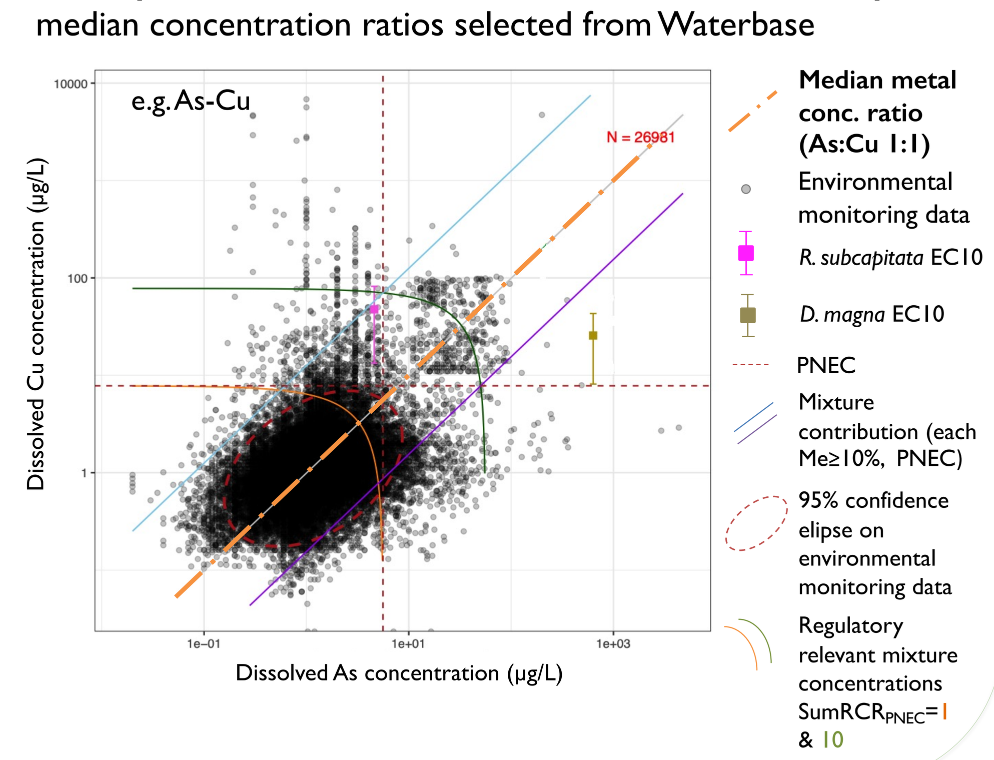
Objective: Select those metal mixture concentration ratios that allows to evaluate our hypothesis while also evaluate metal mixture toxicity at environmentally and regulatory relevant concentrations

Testing of CA hypothesis
at equitoxic ratios



'smart mixture design'= singles (5 metals/rays) + equitoxic metal mixtures (4 rays) + environmental relevant concentration ratios (1 ray) **all tested simultaneously** (10 rays in total)

Quinary environmental realistic concentration ratio ray
median concentration ratios selected from Waterbase



Conclusions

- A testing program focusing on metal mixture toxicity to *R. subcapitata* and *D. magna* was designed using a stepwise approach that integrated European monitoring data and species sensitivities from REACH registration dossiers. The followed approach ensured the environmental relevancy of the experimental program by selecting the most relevant mixture size, metals, metal combinations and metal concentration (ratios) to be tested.
- The outcome of the experimental project will contribute to the discussions on the implementation of combined exposure into environmental risk assessment.
- The testing program is currently ongoing. The first results are presented in poster WE343.

Acknowledgements: The authors wish to thank the Metals Environmental Exposure Data (MEED) Program for sponsoring this research.

References: [1] European Comission. 2021. EU Chemicals Strategy for Sustainability. https://environment.ec.europa.eu/strategy/chemicals-strategy_en; [2]ARCHE 2022, I-PCS identification and MAF level 2 refinements. Final report prepared for Eurometaux.; [3] ARCHE 2023. Quantitative reappraisal of chronic metal mixture toxicity to aquatic organisms. Draft report prepared for MEED/Eurometaux. [4]ECHA. 2008. Technical Guidance on Environmental Risk Assessment.