Are gene expressions in the soil model fauna enchytraeid relevant as molecular biomarkers for contaminated soil risk assessment?

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Background

Soils act like sinks for chemical contamination, requiring risk assessment strategies. As their properties govern the exposure and consequently effects of contaminants on soil biota, there is a need to address the link between such parameters in risk assessment of contaminated land. When low contamination levels and confounding soil parameters hinder risk assessment, multidisciplinary approaches could benefit from the sensitivity of molecular tools.

Aim: using the model organism *Enchytraeus albidus*, we aimed to i) assess the influence of substrate properties and exposure mode on metal bioavailability, in order to ii) explore the sensitivity of gene expression to respond to the same variables.

Approach

- **Metal** contaminants: copper ($CuSO_4$; 80 mg/kg dry soil) and cadmium ($CdCl_2$; 3.5 mg/kg dry soil) and co-exposure (Cu + Cd; 80 + 3.5 mg/kg dry soil)
- **Soil** types: artificial sandy substrate and a non contaminated agricultural loamy soil
- ***Enchytraeus albidus*** exposed for 14 days and bioaccumulation measured at 0, 1, 2, 4, 7, 10 and 14 days by AAS spectroscopy
- **Modelisation** of uptake with Michaelis Menten type equations
- **Gene expression** analysed at 0, 1 and 4 days of exposure for metallothionein (*mt*) for Cu and Cd exposures

Properties of the substrates used in this study

Substrate properties	Sandy	Reference
pH (0,01 M $CaCl_2$)	7.1	6.8
Conductivity (µS)	50.2	32.5
Organic matter %	2	1.7
Composition	Sand	Silt: 56% Sand: 27 % Clay: 17%

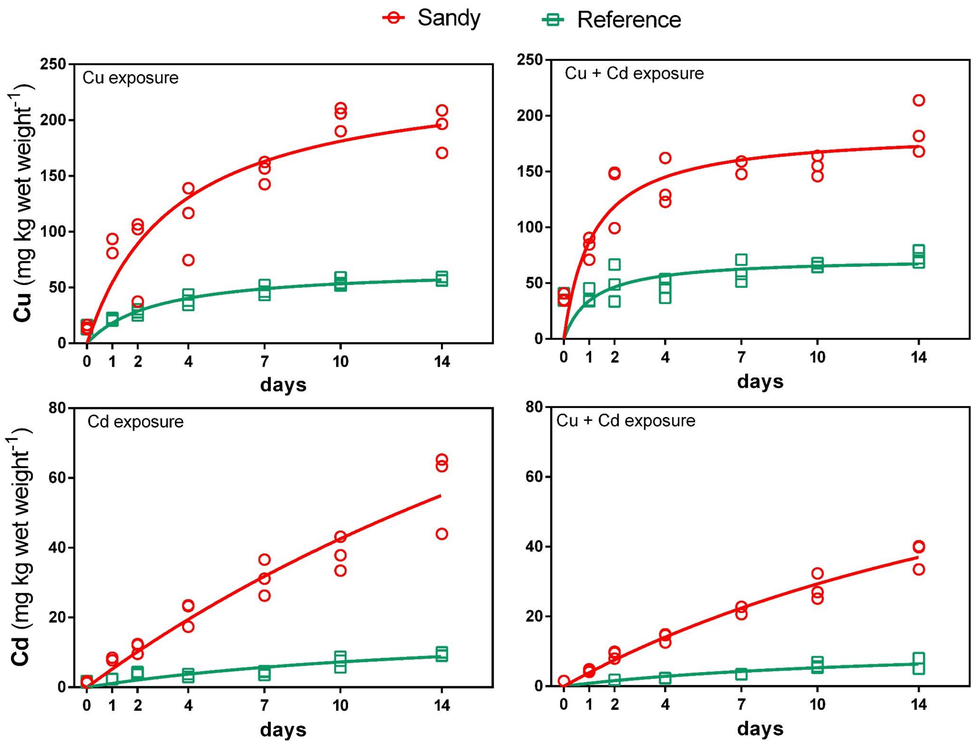

Figure 1. *E. albidus* Uptake kinetics of Cu (top) and Cd (bottom) in separate exposures (left) and in combination (right). Each graph depicts parallel exposures (n = 3) for each soil type. Lines represent non linear fit of data.

Table 1. Toxicokinetics parameters derived from Michaelis- Menten type fit of saturation uptake data: maximal uptake concentration (C_{max}) and rate constant (k) for the tested exposure conditions.

Soil	Cu		Cu+Cd → Cu		Cd		Cu+Cd → Cd	
	k	C_{max}	k	C_{max}	k	C_{max}	k	C_{max}
Sandy	3.5	243.8	1.2	186.4	38.1	205.1	26.01	105.7
Reference	2.7	67.7	1.1	72.6	16.7	18.9	13.6	12.5

Bioaccumulation

Whatever the substrate, the type of metal (essential vs. xenobiotic) dictates the uptake profiles. No steady state was reached over 14 days when *E. albidus* was exposed to Cd, while in the case of Cu a plateau was observed around 7 – 10 days. Different metal homeostasis and detoxification mechanisms are likely responsible for the different handling in Cu and Cd body burdens.

Exposure medium properties deeply influence bioaccumulation. Bioavailability of both metal was greater in a sandy substrate, compared to a loamy soil. For Cu, a steady state was reached sooner in the reference soil, with lower C_{max} compared to sand. Similarly, Cd bioaccumulation was faster (k reference < k sand) in the case of exposure in reference soil.

Single metal bioaccumulation is lower in the case of co-exposure. This was more pronounced in the case of sand where for Cu a plateau is reached sooner, and Cd body burdens are remarkably lower when a mix of contaminants is present. This trend is less pronounced in the reference soil.

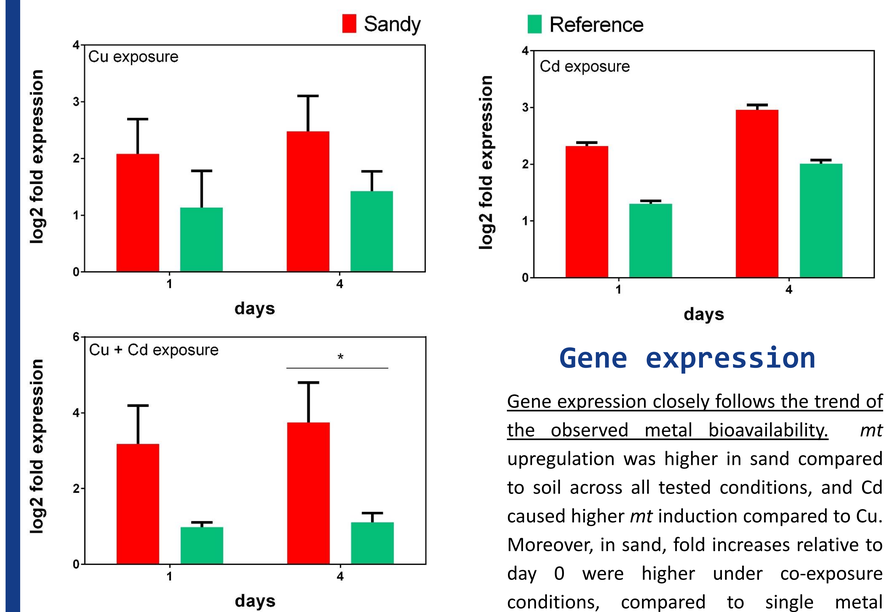

Figure 2. *mt* expression in *E. albidus* after 1 and 4 days exposures. Values are log2 fold changes (mean ± SE)

Gene expression

Gene expression closely follows the trend of the observed metal bioavailability. *mt* upregulation was higher in sand compared to soil across all tested conditions, and Cd caused higher *mt* induction compared to Cu. Moreover, in sand, fold increases relative to day 0 were higher under co-exposure conditions, compared to single metal experiments.

Overview

- Substrate and contaminant type drive metal bioaccumulation, and *mt* expression reflects well the observed level of exposure
- Gene expression represents a sensitive and relevant sublethal endpoint to low contminated soils
- Such molecular tools would meaningfully inform multidisciplinary risk assessement strategies

Ongoing and future work

- Parallel experiment involving organic contaminants (PAHs) is underway
- Evaluation of sublethal endpoints in *E. albidus* (reproduction, effect biomarkers)
- Validation through exposure to contaminated natural soils, collected *in situ,* and the application of a Triad approach implemented with such molecular tools

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