Supplementary material

Cd and Pb removal from contaminated environment by metal resistant bacterium *Cupriavidus metallidurans* CH34: importance of the complexation and competition effects

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Viability of bacterial cells exposed to Cd or Pb

Fig. S1.   Viability of the bacterial cells, expressed as colony forming units (CFU) per millilitre pre-exposed for 30 min to increasing Cd or Pb concentrations in 10^{-2} M MES, pH = 5.0, and then grown in mineral salts medium 284 on Agar. Mean ± standard deviation from three replicates.

Calibration of ion-exchange technique

Table S1. Chemical composition of the five different calibration solutions (prepared and used at three different pHs: 6.1, 5.3 and 3.8) for the IET-measured metal concentration in the water extracts from soil

Solution	1	2	3	4	5
MES or Na-acetate (M)	1×10^{-2}	1×10^{-2}	1×10^{-2}	1×10^{-2}	1×10^{-2}
Na (M)	1×10^{-2}	1×10^{-2}	1×10^{-2}	1×10^{-2}	1×10^{-2}
Ca (M)	3×10^{-4}	8×10^{-4}	2×10^{-3}	4×10^{-3}	8×10^{-3}
Mg (M)	2×10^{-4}	4×10^{-4}	8×10^{-4}	1×10^{-3}	2×10^{-3}
K (M)	2×10^{-4}	2×10^{-4}	2×10^{-4}	2×10^{-4}	2×10^{-4}
Cd (M)	1×10^{-7}	1×10^{-7}	1×10^{-7}	1×10^{-7}	1×10^{-7}
Pb (M)	7×10^{-7}	7×10^{-7}	7×10^{-7}	7×10^{-7}	7×10^{-7}
Zn (M)	1×10^{-5}	1×10^{-5}	1×10^{-5}	1×10^{-5}	1×10^{-5}
Cu (M)	1×10^{-5}	1×10^{-5}	1×10^{-5}	1×10^{-5}	1×10^{-5}

Fig. S2. Dependence of the conditional partition coefficients of Cd, Zn, Pb or Cu (λ'_M) on the partition coefficient of Ca (λ'_{Ca}) at pH of 3.8, 5.3 and 6.1. Contact time of 20 min. The conditional partition coefficients of Cd, Zn or Cu (λ'_{Cd}, λ'_{Zn} and λ'_{Cu}) were linearly dependent on the partition coefficient of Ca (λ'_{Ca}) at constant pH. The correlations were used to determine λ'_{Cd}, λ'_{Zn} and λ'_{Cu} in the water extracts from soil (Table 1) according to the corresponding λ'_{Ca} at each pH separately. The dependence of the partition coefficient of Pb (λ'_{Pb}) on the λ'_{Ca} was described by a hyperbolic function.

Determination of the conditional binding constants of the competitor ions

The conditional binding constants of the competitor ions corresponding to the binding with the transporter sites (K_{Ci-Rs}) and the effective conditional binding constant of competitor ion with bacteria (K_{Ci-R}) were determined following the previously provided procedure.[1] The K_{Ci-Rs} was calculated by using the ratio of the intracellular metal in the absence of competitor $\{M\}_{int}$ to that in the presence of competing ions $\{M\}_{int,C}$ (Eqn S1), valid under the condition where $K_{M-Rs}[M]$ is much smaller than unity. Similarly, K_{Ci-R} was calculated by using the ratio of the adsorbed metal in the absence of competitor $\{M\}_{ads}$ to that in the presence of competing ions $\{M\}_{ads,C}$ (Eqn S2), valid under the condition where $K_{M-R}[M]$ is much smaller than unity. The determined binding constants will be further used in the prediction of Cd uptake in soil solutions.

$$\frac{\{M\}_{int}}{\{M\}_{int,C}} = 1 + K_{Ci-Rs}[C_i] \quad (S1)$$

$$\frac{\{M\}_{ads}}{\{M\}_{ads,C}} = 1 + K_{Ci-R}[C_i] \quad (S2)$$

Comparison of the IET-measured and Visual MINTEQ calculated Cd and Pb free ion concentrations in the presence of NTA, EHA and PPHA

Fig. S3. IET-measured Cd and Pb concentrations *versus* free metal ion concentrations calculated by Visual MINTEQ 2.53 at pH 5.0. Solid line represents linear regression fit of all data in the plot; black dash lines represent confidence interval and red dash lines give predicted interval for *P* < 0.05, confidence level 95%. Fit was performed using Sigma plot 11.

Adsorption of humic acids on bacterium *C. metallidurans*

Fig. S4. Amount of humic acids adsorbed onto bacterial cells (mg g^{-1} DW) *v.* bulk HA concentration at pH 5.0 PPHA and EHA. Solid lines represent Langmuir isotherm. Error bars represent standard deviation on duplicate experiments.

Metal speciation in soil solutions

Fig. S5. IET-measured Cd, Pb, Zn and Cu concentrations *versus* free ion concentrations estimated by Visual MINTEQ 2.53. In Visual MINTEQ the complexation of metals by the dissolved organic matter was taken into account by the NICA–Donnan model. Generic humic acid model parameters were used. Solid line represents linear regression fit of all data in the plot; black dash lines represent confidence interval and red dash lines give predicted interval for $P < 0.05$, confidence level 95%. Fit was performed using Sigma plot 11.

References

[1]   V. I. Slaveykova, K. Dedieu, N. Parthasarathy, R. Hajdu, Effect of competing ions and complexing organic substances on the cadmium uptake by the soil bacterium *Sinorhizobium meliloti. Environ. Toxicol. Chem.* **2009**, *28*, 741. doi:10.1897/08-379R.1.