Supporting Information for Validation of the Biotic Ligand Model (BLM) in Metal Mixtures: Bioaccumulation of Lead and Copper

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Theory

The BLM is an equilibrium model that is based on the assumption that metal effects will be proportional to the concentration of metal adsorbed to sensitive sites at the biological surface (1). Biouptake is assumed to result from the coupling of a rapid and reversible adsorption to membrane binding sites (transport sites or other “biotic ligands”) with an irreversible and rate-limiting internalization of carrier bound metal. For metal concentrations below saturation of the uptake sites, biological uptake fluxes are predicted to be directly proportional to the metal bound by the sensitive sites, \(\{M-R_{\text{cell}}\}\) (Equation S1). Under such conditions, the metal internalization flux, \(J_{\text{int}}\), should follow a Michaelis-Menten hyperbolic rate law (2) (Equation S2):

\[
J_{\text{int}} = k_{\text{int}} \{M-R_{\text{cell}}\} \tag{S1}
\]

\[
J_{\text{int}} = \frac{J_{\text{max}} [M^{z+}]}{K_M + [M^{z+}]} \tag{S2}
\]

where \(k_{\text{int}}\) is the internalization rate constant, \([M^{z+}]\) is the free metal ion concentration in solution, \(J_{\text{max}}\) is the metal uptake at saturation and \(K_M\) is the Michaelis-Menten constant, which represents the concentration at which transport sites are half saturated.

Under the assumption that internalization is rate-limiting, the equilibrium stability constant for the adsorption of metal to the transport sites (biotic ligand), \(K_{M-R_{\text{cell}}}\), can be obtained from the reciprocal of \(K_M\) (3). The effect of competing cations can then be described analytically using similar equilibrium considerations (Equation S3) (1):

\[
J_{\text{int}} = \frac{J_{\text{max}} [M^{z+}] K_{M-R_s}}{[M^{z+}] K_{M-R_s} + [C^{n+}] K_{C-R_s} + 1} \tag{S3}
\]
where $K_{C-Rs}$ is the affinity constant for the interaction of $C^{n+}$ with the $M^{z+}$ uptake site and $[C^{n+}]$ is its concentration. An advantage of the Michaelis-Menten approach is that the mechanistic nature of the inhibition (i.e. competitive or non competitive inhibition) can be identified from a shift in the maximum uptake fluxes of the $J_{int}$ vs. $[M^{z+}]$ curves.

Table S1 – Representative constants obtained for the binding of Cu and Pb based on a Michaelis-Menten plot of uptake fluxes.

<table>
<thead>
<tr>
<th></th>
<th>Cu</th>
<th>Pb</th>
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<tbody>
<tr>
<td>$J_{max}$ (mol cm$^{-2}$ min$^{-1}$)</td>
<td>$(8.4 \pm 0.7) \times 10^{-12}$</td>
<td>$(6.2 \pm 0.2) \times 10^{-12}$</td>
</tr>
<tr>
<td>$K_M(2)$</td>
<td>$(1.5 \pm 0.3) \times 10^{-6}$</td>
<td>$(1.2 \pm 0.1) \times 10^{-6}$</td>
</tr>
<tr>
<td>$K_{M-Recell}(=1/K_M)$ (M$^{-1}$)</td>
<td>$10^{5.8 \pm 0.1}$</td>
<td>$10^{5.9 \pm 0.1}$</td>
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Figure S1 – Schematic representation for the uptake of Cu and Pb. Low concentrations of Pb can introduce increased expression of $ctr2$, and produce feedback on the high affinity Cu transport site.
Literature Cited

