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PREDICTING THE RELATIVE TOXICITY OF METAL IONS USING ION CHARACTERISTICS: MICROTOX® BIOLUMINESCENCE ASSAY

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Abstract—Quantitative structure–activity relationships have been used to predict the relative toxicity of organic compounds. Although not as common, ion characteristics have also proven useful for predicting the relative toxicity of metal ions. The purpose of this study was to determine if the relative toxicity of metal ions using the Microtox® bioassay was predictable using ion characteristics. Median effect concentrations (EC50s) were determined for 20 metals in a $NaNO_3$ medium, which reflected freshwater speciation conditions, using the Microtox bacterial assay. The log of EC50 values was modeled using several ion characteristics, and Akaike's Information Criterion was calculated to determine which ion characteristics provided the best fit. Whether modeling total ion (unspeciated) or free ion (speciated) EC50 values, the one variable which best modeled EC50s was the softness index (σ_p, i.e., [coordinate bond energy of the metal fluoride − coordinate bond energy of the metal iodide]/[coordinate bond energy of the metal fluoride]), while a combination of $\chi_m^2 r$ (χ_m = electronegativity, r = Pauling ionic radius) and $|\log K_{OH}|$ (absolute value of the log of the first hydrolysis constant, K_{OH} for $M^{n+} + H_2O \rightarrow MOH^{n-1} + H^+$) was the best two-variable model. Other variables, including ΔE_0 and $\chi_m^2 r$ (one-variable models) and ($AN/\Delta IP$, ΔE_0) and ($\chi_m^2 r$, Z^2/r) (two-variable models), also gave adequate fits. Modeling with speciated (free ion) versus unspeciated (total ion) EC50 values did not improve fits. Modeling mono-, di-, and trivalent metal ions separately improved the models. We conclude that ion characteristics can be used to predict the relative toxicity of metal ions whether in freshwater ($NaNO_3$ medium) or saltwater (NaCl medium) speciation conditions and that this approach can be applied to metal ions varying widely in both valence and binding tendencies.

Keywords—Metal Toxicity Bioactivity Microtox® Bacteria

INTRODUCTION

Quantitative structure–activity relationships (QSARs) have been used extensively to predict the bioactivity of classes of organic compounds. These relationships, first developed in pharmacology, are based on surrogate or indirect measures of molecular qualities (e.g., lipophilicity using octanol/water partition coefficients [K_{ow}]). In contrast, similar relationships for inorganic compounds are rare in the literature but, if developed, could prove useful for predicting intermetal trends in bioactivity.

Ion characteristics of inorganic species can be used to predict the relative toxicity or sublethal effects of metal ions [1–5]. Many of these characteristics reflect the binding tendencies of metals to ligands. Such tendencies are notionally linked to metal binding of biomolecules and consequent toxic effects. For example, the log of the solubility product for the metal hydroxide (log $-K_{SO}$ MOH), which reflects metal ion affinity to intermediate ligands (e.g., those with oxygen donor atoms) was correlated with metal inhibition of algal growth [2]. The solubility of the metal sulfide, thought to reflect a metal ions tendency to bind to sulfhydryl groups of biomolecules, has been correlated with growth, reproduction, and survival of *Daphnia magna* exposed to different metals [1]. Kaiser [4] found correlations between sublethal and toxic effects and log $AN/\Delta IP$ or ΔE_0. $AN/\Delta IP$ and ΔE_0 (AN = atomic number, ΔIP = difference in ionization potentials between ion oxidation number OX and $OX - 1$, ΔE_0 = the absolute difference in electrochemical potential between the ion and its first stable reduced state) also reflect qualities affecting interactions with ligands. The atomic number reflects the size of the ion, and ΔIP and ΔE_0 reflect the effects of atomic ionization potential and the ability of the ion to change its electronic state, respectively. Based on hard and soft acid and base theory, the toxic effects of metals to mice were correlated with the softness index, σ_p, i.e., (coordinate bond energy of the metal fluoride − coordinate bond energy of the metal iodide)/(coordinate bond energy of the metal fluoride) [3,5].

Other ion characteristics which reflect metal affinities to ligands may also prove useful for predicting the relative toxicity of metal ions. The absolute value of the log of the first hydrolysis constant, $|\log K_{OH}|$ (K_{OH} for $M^{n+} + H_2O \rightarrow MOH^{n-1} + H^+$), was correlated with metal ion affinity to intermediate ligands, like those with oxygen donor atoms [6]. Ion characteristics with bivariate characterization can also reflect metal–ligand binding tendencies (e.g., Z^2/r, $\Delta\beta$) [7]. Z^2/r (Z = ion charge, r = ionic radius) is a surrogate measure of ionic bond stabilities for metal–ligand complexes, and $\Delta\beta$ (log of the stability constant for the metal fluoride − log of the stability constant for the metal chloride) reflects the tendency to form covalent bonds with soft ligands, such as those with sulfur donor atoms. Another set of bivariate characteristics which reflect metal–ligand binding tendencies are $\chi_m^2 r$ and Z^2/r [8]. The $\chi_m^2 r$ (χ_m = electronegativity, r = Pauling ionic radius) reflects the importance of covalent interactions with ligands relative to ionic interactions, and Z^2/r reflects the energy of an ion when interacting electrostatically with a ligand.

In our previous study, the relative toxicity of nine divalent metal ions (added as chloride salts) to bacteria (Microtox®) in a NaCl medium, which reflects marine speciation conditions, was predictable using ion characteristics [9]. The present study was expanded to 20 metals, including mono-, di-, and trivalent

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Table 1. Mean 15-min median effect concentration (EC50) values (μM/L, $n = 3$) for 20 metals using the Microtox® bacterial assay, pH of EC50 solution in 3.02% $NaNO_3$ (pH of 3.02% $NaNO_3$ solution alone was 5.33) used in speciation calculation, proportion free ion determined from speciation program and free metal ion EC50 (total metal ion unspeciated EC50 × proportion free ion)

Metal	Total metal ion EC50 (±SD)	pH	Proportion free ion[a]	Free metal ion EC50 (±SD)
Li^+	294,135 ± 19,201	5.93	0.97	285,311 ± 18,625
Na^+	401,000 ± 8,582	5.54	0.92	368,920 ± 7,895
Mg^{2+}	87,242 ± 6,162	5.48	0.62	54,090 ± 3,820
K^+	625,241 ± 34,863	5.59	0.75	468,931 ± 26,147
Ca^{2+}	94,702 ± 16,247	5.93	0.60	56,821 ± 9,748
Cr^{3+}	184 ± 11	4.23	0.91	168 ± 10
Mn^{2+}	1,571 ± 49	5.33	0.94	1,476 ± 46
Fe^{3+}	102 ± 5	3.45	0.22	22 ± 1
Co^{2+}	874 ± 84	5.81	0.87	761 ± 73
Ni^{2+}	566 ± 51	5.59	0.77	436 ± 39
Cu^{2+}	1.62 ± 0.13	5.35	0.76	1.23 ± 0.10
Zn^{2+}	35 ± 7	5.52	0.80	28 ± 6
Sr^{2+}	235,527 ± 4,885	5.29	0.39	91,856 ± 1,905
Ag^+	0.926 ± 0.013	5.39	0.98	0.907 ± 0.012
Cd^{2+}	27 ± 5	5.43	0.71	19 ± 4
Cs^+	403,890 ± 32,793	5.78	0.75	302,917 ± 24,595
Ba^{2+}	95,455 ± 15,349	5.65	0.40	38,182 ± 6,140
La^{3+}	1,693 ± 162	5.50	0.19	322 ± 31
Hg^{2+}	0.919 ± 0.023	5.38	0.000155	0.000142 ± 0.000004
Pb^{2+}	1.15 ± 0.12	5.56	0.40	0.46 ± 0.05

[a] Estimated using PC MINTEQA2, Version 3.10 [11].

metals. The 20 metals were selected to reflect wider differences in ligand binding affinities and tendencies compared to our previous study [9]. Therefore, the purpose of this study was to determine the 15-min median effect concentration (EC50) values for 20 metals to bacteria (Microtox) in a $NaNO_3$ medium reflecting freshwater speciation conditions and model these EC50 values with several ion characteristics. This information was used to determine (1) whether the test medium (NaCl vs. $NaNO_3$) had a significant effect on the effectiveness of EC50 modeling and (2) which ion characteristics best predicted the relative toxicity of metal ions using a simple bacterial assay (Microtox).

MATERIALS AND METHODS

Microtox toxicity assay

The Microtox bacterial assay was used to determine 15-min EC50 values for 20 metals (added as nitrate salts) using standard methods [10], except that a 3.02% w/v $NaNO_3$ solution was used as the diluant in the Microtox Basic Test in place of the standard Microtox diluant (2% w/v NaCl). The 20 metals tested included mono-, di-, and trivalent metal ions (Table 1). A reconstituted marine bacterium (*Vibrio fischeri* Beijerinck 1889, formerly *Photobacterium phosphoreum*) was exposed at 15°C to osmotically adjusted (3.02% w/v $NaNO_3$) solutions of metals. The osmotic adjusting solution was prepared by dissolving 30.2 g of reagent-grade $NaNO_3$ in 1 L of deionized water. The solution was then filtered through a 0.45-μm filter to remove insoluble particles. Using the Microtox Model 500 toxicity analyzer (Microbics Corp., Carlsbad, CA, USA), bioluminescence of the bacteria was quantified over a range of metal concentrations, and the metal concentration which resulted in a 50% decrease in light output after 15 min of exposure (15-min EC50) was calculated using the Microbics data collection and reduction software (Version 7.10).

The EC50 values were expressed as total unspeciated metal or calculated free ion concentration. The free ion concentration was used because this more accurately reflects the bioreactive concentration of the metal. Concentrations of the free ion species were predicted with PC MINTEQA2, Version 3.10 [11]. Metal hydrolysis and nitrate complexation were considered. The ionic strength of the medium was fixed at 355 mM/L Na and 355 mM/L NO_3. Median effect concentrations of dissolved metal, NO_3 from the added metal salt, and the pH of the metal solution at the EC50 concentration in 3.02% $NaNO_3$ were also used in speciation estimations (Table 1). Assumptions of a fixed pH, closed system, and no precipitation of solid phases were made during computations. The exception was Fe^{3+}, for which precipitation of solids was allowed. Equilibrium constants for nitrate complexation, hydrolysis, and precipitation (where appropriate) were taken from Smith and Martell [12] with the following exceptions: constants for nitrate complexation with La^{3+} were estimated from available data for Eu^{3+}, and complexation constants for Li^+ in nitrate were extrapolated from available data for Group IA metals.

Ion characteristics

Ion characteristics used for modeling (Table 2) were obtained from a variety of sources. Ionic radii (r) were obtained from Shannon and Prewitt [13,14] using ''IR'' values and the *CRC Handbook of Chemistry and Physics* [15]. The ΔIP values were calculated from ionization potentials obtained from the *CRC Handbook of Chemistry and Physics* [15]. The ΔE_0 values were obtained from Kaiser [4], and average electronegativity values (χ_m) were taken directly from Allred [16]. The first hydrolysis constants ($|\log K_{OH}|$) were obtained from Baes and Mesmer [6] and Brown and Allison [11]. The softness index values (σ_p) were calculated from coordinate bond energies obtained from Pearson and Mawby [17]. Other ion characteristics ($\chi_m^2 r$, Z^2/r, and $AN/\Delta IP$) were calculated from values obtained from previous references where necessary.

Model development

Regression models of 15-min EC50 values with several ion characteristics were performed using PROC GLM of the SAS package [18]. Models were developed using the total metal ion EC50s (unspeciated) and the free metal ion EC50s (speciated). Models were also developed using the EC50 values from mono-, di-, and trivalent metal ions separately. Models involving one independent variable included ΔE_0, $\chi_m^2 r$, $|\log K_{OH}|$, and σ_p, and models involving two independent variables included ($AN/\Delta IP$, ΔE_0), ($\log AN/\Delta IP$, ΔE_0), ($\chi_m^2 r$, Z^2/r), and ($\chi_m^2 r$, $|\log K_{OH}|$). Both ($AN/\Delta IP$, ΔE_0) and ($\log AN/\Delta IP$, ΔE_0) were used because Kaiser [4] found that log-transforming $AN/\Delta IP$ resulted in a better model, yet Newman and McCloskey [9] found no advantage to log-transforming $AN/\Delta IP$. Other ion characteristics and other combinations of two-variable models were also used to model log EC50 but were not included because of inferior results. Akaike's Information Criterion (AIC) was calculated for each model using the estimated log likelihood to determine which variable(s) gave the best fit. The formulas used to calculate log likelihood and AIC were obtained from Neter et al. [19] and Newman [20], respectively. The model with the smallest AIC was judged to be the most informative, regardless of the number of variables used in the model.

Comparing speciation media

In our previous study, 15-min EC50 values for nine metals (Ca, Mg, Mn, Ni, Cd, Zn, Cu, Pb, and Hg) were modeled with

Table 2. Metal ion characteristics used in regression models

Metal ion	Ion electron configuration	Outer shell electrons	*AN*	*r* (Å)	*ΔIP* (eV)	ΔE_0 (V)	χ_m	$\lvert\log K_{OH}\rvert$	$\chi_m^2 r$	Z^2/r	*AN*/*ΔIP*	σ_p
Li^+	{He}	8[a]	3	0.74	5.39	3.05	0.98	13.6	0.71	1.35	0.56	0.247
Na^+	{Ne}	8[a]	11	1.02	5.14	2.71	0.93	14.2	0.88	0.98	2.14	0.211
Mg^{2+}	{Ne}	8[a]	12	0.72	7.39	2.38	1.31	11.6	1.24	5.56	1.62	0.167
K^+	{Ar}	8[a]	19	1.38	4.34	2.92	0.82	14.5	0.93	0.72	4.38	0.232
Ca^{2+}	{Ar}	8[a]	20	1.00	5.76	2.76	1.00	12.7	1.00	4.00	3.47	0.181
Cr^{3+}	{Ar}$3d^3$	3	24	0.62	14.46	0.41	1.66	4.0	1.71	14.52	1.66	0.107
Mn^{2+}	{Ar}$3d^5$	5	25	0.83	8.21	1.03	1.55	10.6	1.99	4.82	3.04	0.125
Fe^{3+}	{Ar}$3d^5$	5	26	0.65	14.46	0.77	1.83	2.2	2.18	13.85	1.80	0.103
Co^{2+}	{Ar}$3d^7$	7	27	0.75	9.19	0.28	1.88	9.7	2.65	5.33	2.94	0.130
Ni^{2+}	{Ar}$3d^8$	8	28	0.69	10.52	0.23	1.91	9.9	2.52	5.80	2.66	0.126
Cu^{2+}	{Ar}$3d^9$	9	29	0.73	12.57	0.16	1.90	8.0	2.64	5.48	2.31	0.104
Zn^{2+}	{Ar}$3d^{10}$	10	30	0.75	8.57	0.76	1.65	9.0	2.04	5.33	3.50	0.115
Sr^{2+}	{Kr}	8[a]	38	1.13	5.34	2.89	0.95	13.2	1.02	3.54	7.12	0.174
Ag^+	{Kr}$4d^{10}$	10	47	1.15	7.57	0.80	1.93	12.0	4.28	0.87	6.21	0.074
Cd^{2+}	{Kr}$4d^{10}$	10	48	0.95	7.91	0.40	1.69	10.1	2.71	4.21	6.07	0.081
Cs^+	{Xe}	8[a]	55	1.70	3.89	2.92	0.79	14.9	1.06	0.59	14.14	0.218
Ba^{2+}	{Xe}	8[a]	56	1.36	4.79	2.90	0.89	13.4	1.08	2.94	11.69	0.183
La^{3+}	{Xe}	8[a]	57	1.05	7.74	2.37	1.10	8.5	1.27	8.57	7.36	0.171
Hg^{2+}	{Xe}$4f^{14}5d^{10}$	10	80	1.02	8.32	0.91	2.00	3.4	4.08	3.92	9.62	0.065
Pb^{2+}	{Xe}$6s^24f^{14}5d^{10}$	10	82	1.18	7.61	0.13	2.33	7.7	6.41	3.39	10.78	0.131

[a] Noble gas configuration.

several ion characteristics [9]. These EC50 values were determined using a NaCl medium. The model which provided the best fit using these nine metals was the log of the first hydrolysis constant, $\lvert\log K_{OH}\rvert$. The EC50 values for the same nine metals tested in a $NaNO_3$ medium from the present study were modeled with $\lvert\log K_{OH}\rvert$, and the plots were compared to the previous study [9].

RESULTS

EC50 values

The 15-min EC50 values (± standard deviation) of the total metal ion (unspeciated) EC50 values and the free metal ion (speciated) EC50 values are provided in Table 1. The total metal ion EC50 values ranged from 0.919 μM/L for Hg to 625,241 μM/L for K, and the free metal ion EC50 values ranged from 0.000142 μM/L for Hg to 468,931 μM/L for K (Table 1). The proportion free ion values determined from PC MINTEQA2 are also provided in Table 1.

Comparing test media

Whether the medium was NaCl or $NaNO_3$, EC50s were similar (Fig. 1). This was true for both the total metal ion (Fig. 1A) and free metal ion EC50s (Fig. 1B). The regression lines for the NaCl and $NaNO_3$ media were also similar if total EC50s (Fig. 1A) or free ion EC50s (Fig. 1B) were used in the model. Modeling the free metal ion EC50 for Hg in the NaCl medium required considering the neutral $HgCl_2^0$ species, which has extremely high membrane permeability relative to the free ion [21–26]. If the neutral $HgCl_2^0$ species were not included in the model, the regression lines for the NaCl and $NaNO_3$ media were very different because the log of the EC50 for Hg using the free ion species was −12.31 versus −1.31 if the neutral $HgCl_2^0$ species were included.

Models for all metals

If models were fit using total dissolved metal concentrations, all variables except those using *AN*/Δ*IP* were statistically sig-

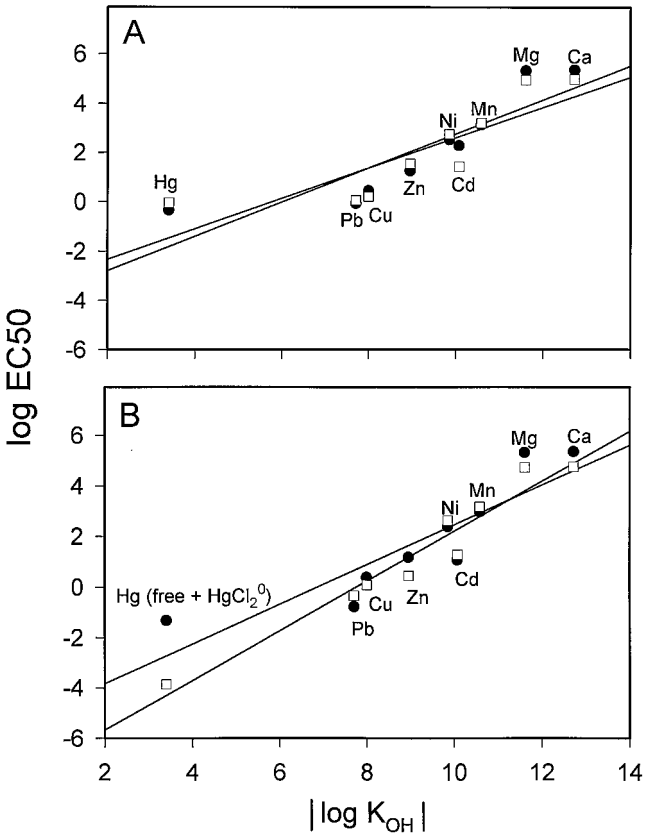

Fig. 1. The model for log median effect concentration (EC50) and $\lvert\log K_{OH}\rvert$ in a NaCl or $NaNO_3$ medium using the total metal ion (unspeciated) EC50 (**A**) and the free metal ion (speciated) EC50 (**B**) for nine divalent metals. ● = EC50 determined in a NaCl medium, and □ = EC50 determined in a $NaNO_3$ medium.

Table 3. Regression models of log median effect concentration (EC50) (unspeciated) and several ion characteristics. Models were generated for all ions together (n = 20), monovalent ions alone (n = 5), divalent ions alone (n = 12), and trivalent ions alone (n = 3). The models with the smallest Akaike's Information Criterion (AIC) within a valence group (i.e., total, monovalent, divalent, and trivalent) were judged to have the most information regardless of the number of independent variables

Log EC50 = $f(x)$	r^2	Model (log EC50 =)	MSE[a]	AIC
ΔE_0				
Total	0.76	$0.69 + 1.58(\Delta E_0)$*	1.063	61.12
Monovalent	0.98	$-2.07 + 2.64(\Delta E_0)$*	0.433	10.30
Divalent	0.72	$0.83 + 1.51(\Delta E_0)$*	1.150	39.24
Trivalent	0.87	$1.82 + 0.57(\Delta E_0)$	0.332	6.56
$\chi^2_m r$				
Total	0.71	$5.75 - 1.24(\chi^2_m r)$*	1.172	65.06
Monovalent	0.99	$7.09 - 1.66(\chi^2_m r)$*	0.334	8.70
Divalent	0.66	$5.33 - 1.08(\chi^2_m r)$*	1.261	37.52
Trivalent	0.89	$4.79 - 1.33(\chi^2_m r)$	0.305	6.26
$\lvert\text{Log } K_{OH}\rvert$				
Total	0.51	$-1.02 + 0.41(\lvert\log K_{OH}\rvert)$*	1.527	76.72
Monovalent	0.84	$-23.92 + 2.05(\lvert\log K_{OH}\rvert)$*	1.153	17.36
Divalent	0.80	$3.83 + 0.66(\lvert\log K_{OH}\rvert)$*	0.973	35.46
Trivalent	0.99	$1.53 + 0.19(\lvert\log K_{OH}\rvert)$	0.073	1.72
Softness index (σ_p)				
Total	0.81	$-2.11 + 35.50(\sigma_p)$*	0.958	57.14
Monovalent	0.95	$-2.47 + 35.41(\sigma_p)$*	0.626	12.72
Divalent	0.75	$-3.43 + 46.46(\sigma_p)$*	1.068	37.52
Trivalent	0.98	$0.38 + 16.68(\sigma_p)$	0.134	3.64
$AN/\Delta IP$, ΔE_0				
Total	0.81	$1.20 - 0.12(AN/\Delta IP) + 1.65(\Delta E_0)$*	0.982	59.12
Monovalent	0.98	$-2.08 + 0.003(AN/\Delta IP) + 2.64(\Delta E_0)$*	0.530	13.06
Divalent	0.81	$1.68 - 0.18(AN/\Delta IP) + 1.61(\Delta E_0)$*	0.987	36.78
Trivalent	>0.99	$1.84 + 0.48(AN/\Delta IP) - 0.90(\Delta E_0)$	0.000	–
Log $AN/\Delta IP$, ΔE_0				
Total	0.80	$1.39 - 1.24(\log AN/\Delta IP) + 1.59(\Delta E_0)$*	0.991	59.42
Monovalent	0.98	$-2.33 + 0.27(\log AN/\Delta IP) + 2.69(\Delta E_0)$*	0.494	12.64
Divalent	0.80	$2.13 - 2.16(\log AN/\Delta IP) + 1.59(\Delta E_0)$*	1.006	37.20
Trivalent	>0.99	$1.63 + 5.17(\log AN/\Delta IP) - 1.21(\Delta E_0)$	0.000	–
$\chi^2_m r$, Z^2/r				
Total	0.80	$6.54 - 1.24(\chi^2_m r)$* $- 0.16(Z^2/r)$*	1.006	59.96
Monovalent	0.99	$8.03 - 1.68(\chi^2_m r)$* $- 0.99(Z^2/r)$*	0.057	0.98
Divalent	0.72	$7.96 - 1.13(\chi^2_m r)$* $- 0.55(Z^2/r)$	1.192	40.88
Trivalent	>0.99	$5.06 - 0.69(\chi^2_m r) + 0.11(Z^2/r)$	0.000	–
$\chi^2_m r$, $\lvert\log K_{OH}\rvert$				
Total	0.84	$2.82 - 0.96(\chi^2_m r)$* $+ 0.23(\lvert\log K_{OH}\rvert)$*	0.910	56.36
Monovalent	0.99	$-0.31 - 1.33(\chi^2_m r)$* $+ 0.50(\lvert\log K_{OH}\rvert)$*	0.115	4.58
Divalent	0.85	$-0.95 - 0.44(\chi^2_m r) + 0.48(\lvert\log K_{OH}\rvert)$*	0.891	34.70
Trivalent	>0.99	$0.48 + 0.44(\chi^2_m r) + 0.26(\lvert\log K_{OH}\rvert)$	0.000	–

[a] MSE = mean square error of the model.
* Variable had a significant effect in model (α = 0.05).

nificant (α = 0.05) (Table 3). The best one-variable model used the softness index (σ_p) (r^2 = 0.81, AIC = 57.14), although ΔE_0 and $\chi^2_m r$ also provided adequate fits (Table 3). The best two-variable model was a combination of $\chi^2_m r$ and $\lvert\log K_{OH}\rvert$ (r^2 = 0.84, AIC = 56.36) (Table 3), although all other two-variable models, ($AN/\Delta IP$, ΔE_0), (log $AN/\Delta IP$, ΔE_0), and ($\chi^2_m r$, Z^2/r) also provided adequate fits. The two-variable model using $\chi^2_m r$ and $\lvert\log K_{OH}\rvert$ also provided the best overall model among the total ion EC50 values as judged by the lowest AIC value.

If models were fit using free metal ion concentrations, all variables except those using $AN/\Delta IP$ and Z^2/r were statistically significant (α = 0.05) (Table 4). The best one-variable model used the softness index (σ_p) (r^2 = 0.73, AIC = 71.04) (Table 4). As the softness index (σ_p) increased, the log of EC50 also increased (Fig. 2). If metals are grouped according to their softness (e.g., soft ions, borderline ions, and hard ions), the soft ions were more toxic than the hard ions (Fig. 2). The best two-variable model was a combination of $\chi^2_m r$ and $\lvert\log K_{OH}\rvert$ (r^2 = 0.81, AIC = 65.48) (Table 4). Contour plots of the log of free metal ion EC50 versus ($\chi^2_m r$ and $\lvert\log K_{OH}\rvert$) and ($\chi^2_m r$, Z^2/r) are shown in Figure 3. The metal ions were grouped according to metal type (e.g., alkaline earth/rare earth metals and transition metals), with the highest EC50 values being from the alkaline earth metals (Fig. 3). Toxicity generally increased as $\lvert\log K_{OH}\rvert$ decreased and $\chi^2_m r$ increased (Fig. 3A); toxicity increased as $\chi^2_m r$ increased and Z^2/r increased (Fig. 3B). Overall, the modeling of free metal ion (speciated) EC50 did not significantly improve models compared to those based on total metal ion (unspeciated) EC50 values. Generally, two-variable models provided better fits than one-variable models.

Table 4. Regression models of free metal ion log median effect concentration (EC50) and several ion characteristics. Models were generated for all ions together ($n = 20$), monovalent ions alone ($n = 5$), divalent ions alone ($n = 12$), and trivalent ions alone ($n = 3$). The models with the smallest Akaike's Information Criterion (AIC) within a valence group (i.e., total, monovalent, divalent, and trivalent) were judged to have the most information regardless of the number of independent variables

Log EC50 = $f(x)$	r^2	Model (log EC50 =)	MSE[a]	AIC
ΔE_0				
Total	0.57	$0.14 + 1.62(\Delta E_0)$*	1.696	82.06
Monovalent	0.95	$-1.95 + 2.49(\Delta E_0)$*	0.601	12.44
Divalent	0.45	$0.18 + 1.56(\Delta E_0)$*	2.091	53.44
Trivalent	0.31	$1.65 + 0.32(\Delta E_0)$	0.709	9.19
$\chi^2_m r$				
Total	0.64	$5.59 - 1.40(\chi^2_m r)$*	1.560	77.74
Monovalent	0.97	$6.69 - 1.57(\chi^2_m r)$*	0.448	11.42
Divalent	0.56	$5.28 - 1.29(\chi^2_m r)$*	1.872	52.30
Trivalent	0.93	$4.22 - 1.28(\chi^2_m r)$	0.225	5.25
$\lvert\text{Log } K_{OH}\rvert$				
Total	0.56	$-2.51 + 0.51(\lvert\log K_{OH}\rvert)$*	1.716	82.44
Monovalent	0.78	$-21.84 + 1.88(\lvert\log K_{OH}\rvert)$*	1.303	18.42
Divalent	0.94	$-7.12 + 0.93(\lvert\log K_{OH}\rvert)$*	0.710	29.06
Trivalent	0.75	$1.24 + 0.16(\lvert\log K_{OH}\rvert)$	0.430	7.42
Softness index (σ_p)				
Total	0.73	$-3.25 + 39.95(\sigma_p)$*	1.349	71.04
Monovalent	0.92	$-2.29 + 33.20(\sigma_p)$*	0.777	14.24
Divalent	0.75	$-5.88 + 60.60(\sigma_p)$*	1.397	44.06
Trivalent	0.53	$0.57 + 11.47(\sigma_p)$	0.586	8.50
$AN/\Delta IP$, ΔE_0				
Total	0.65	$0.95 - 0.19(AN/\Delta IP) + 1.73(\Delta E_0)$*	1.571	78.50
Monovalent	0.95	$-1.97 + 0.004(AN/\Delta IP) + 2.49(\Delta E_0)$*	0.735	15.12
Divalent	0.61	$1.65 - 0.31(AN/\Delta IP) + 1.73(\Delta E_0)$*	1.841	51.96
Trivalent	>0.99	$1.69 + 1.02(AN/\Delta IP) - 2.82(\Delta E_0)$	0.000	–
Log $AN/\Delta IP$, ΔE_0				
Total	0.65	$1.30 - 2.03(\log AN/\Delta IP) + 1.64(\Delta E_0)$*	1.569	78.44
Monovalent	0.95	$-1.94 - 0.002(\log AN/\Delta IP) + 2.49(\Delta E_0)$*	0.736	15.12
Divalent	0.61	$2.46 - 3.79(\log AN/\Delta IP) + 1.69(\Delta E_0)$*	1.857	52.20
Trivalent	>0.99	$1.23 + 11.03(\log AN/\Delta IP) - 3.50(\Delta E_0)$	0.000	–
$\chi^2_m r$, Z^2/r				
Total	0.70	$6.36 - 1.39(\chi^2_m r)^* - 0.16(Z^2/r)$	1.464	75.28
Monovalent	0.97	$6.90 - 1.57(\chi^2_m r)^* - 0.22(Z^2/r)$	0.541	13.18
Divalent	0.57	$6.98 - 1.33(\chi^2_m r)^* - 0.36(Z^2/r)$	1.937	53.42
Trivalent	>0.99	$4.03 - 1.75(\chi^2_m r) + 0.08(Z^2/r)$	0.000	–
$\chi^2_m r$, $\lvert\log K_{OH}\rvert$				
Total	0.81	$1.48 - 1.00(\chi^2_m r)^* + 0.32(\lvert\log K_{OH}\rvert)$*	1.161	65.48
Monovalent	0.97	$3.47 - 1.43(\chi^2_m r) + 0.22(\lvert\log K_{OH}\rvert)$	0.521	12.96
Divalent	0.94	$-6.37 - 0.11(\chi^2_m r) + 0.88(\lvert\log K_{OH}\rvert)$*	0.737	31.04
Trivalent	>0.99	$7.40 - 2.58(\chi^2_m r) - 0.19(\lvert\log K_{OH}\rvert)$	0.000	–

[a] MSE = mean square error of the model.
* Variable had a significant effect in model ($\alpha = 0.05$).

Modeling metals by valence

Modeling metal ion EC50 values (unspeciated) by valence (mono-, di-, and trivalent) separately as opposed to together improved the model fits (Table 3), although the number of data points used for monovalent ($n = 5$) and trivalent ($n = 3$) metal ions was low. Modeling monovalent metal ions alone, $\chi^2_m r$ provided the best fit ($r^2 = 0.99$, AIC = 8.70) among one-variable models, and a combination of $\chi^2_m r$ and Z^2/r provided the best fit ($r^2 = 0.99$, AIC = 0.98) among two-variable models (Table 3). Modeling divalent metal ions alone ($n = 12$), $\lvert\log K_{OH}\rvert$ provided the best fit ($r^2 = 0.80$, AIC = 35.46) among one-variable models, and a combination of $\chi^2_m r$ and $\lvert\log K_{OH}\rvert$ provided the best fit ($r^2 = 0.85$, AIC = 34.70) among two-variable models (Table 3). Modeling trivalent metal ions alone, $\lvert\log K_{OH}\rvert$ provided the best fit ($r^2 = 0.99$, AIC = 1.72) among one-variable models, and all two-variable models provided equally good fits ($r^2 > 0.99$) (Table 3).

Modeling free metal ion EC50 values (speciated) by valence also significantly improved the model fits compared to modeling all metal ions together (Table 4), although the number of data points used for monovalent ($n = 5$) and trivalent ($n = 3$) metal ions were also low. Modeling monovalent metal ions alone, $\chi^2_m r$ provided the best fit ($r^2 = 0.97$, AIC = 11.42) among one-variable models, and a combination of $\chi^2_m r$ and $\lvert\log K_{OH}\rvert$ provided the best fit ($r^2 = 0.97$, AIC = 12.96) among two-variable models (Table 4). Modeling divalent metal ions alone ($n = 12$), $\lvert\log K_{OH}\rvert$ provided the best fit ($r^2 = 0.94$, AIC = 29.06) among one-variable models, and a combination of $\chi^2_m r$ and $\lvert\log K_{OH}\rvert$ provided the best fit ($r^2 = 0.94$, AIC = 31.04) among two-variable models (Table 4). Modeling trivalent metal ions alone, $\chi^2_m r$ provided the

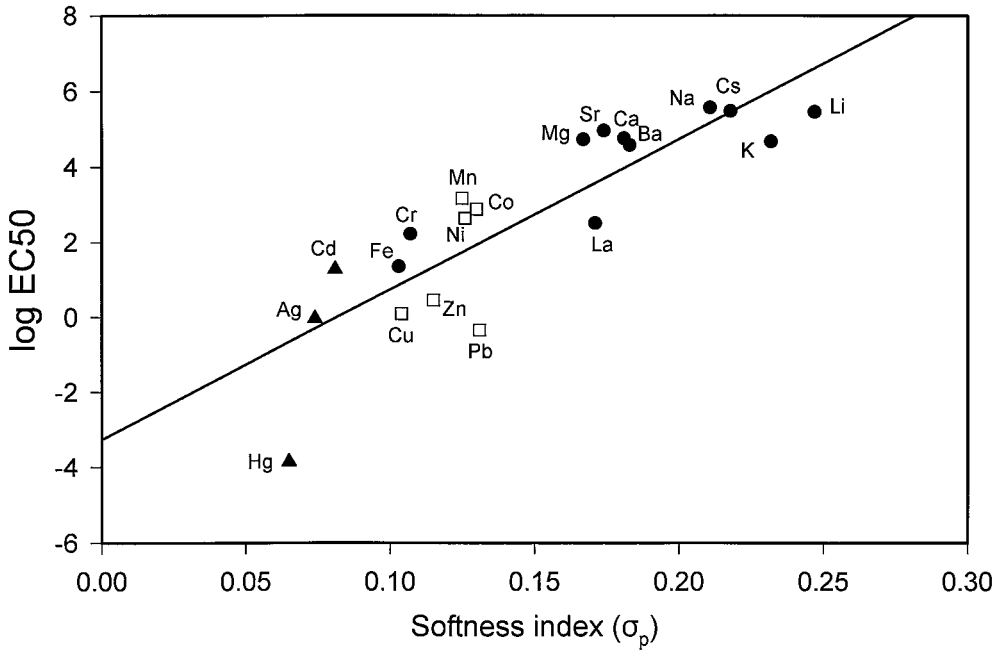

Fig. 2. The model for log free ion median effect concentration (EC50) (speciated) and the softness index (σ_p) for 20 metals. ● = hard ions, □ = borderline ions, and ▲ = soft ions.

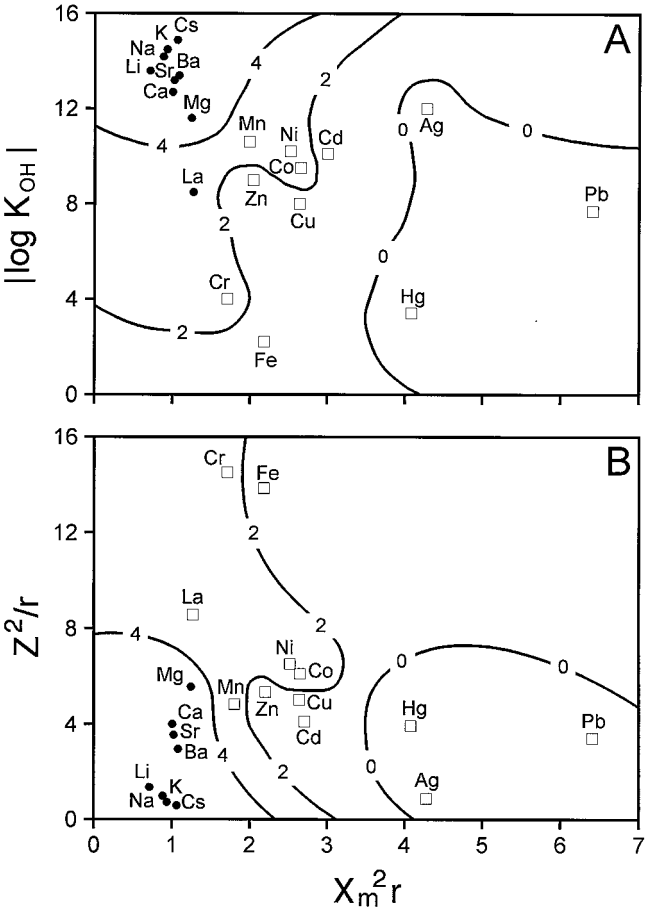

Fig. 3. Plots of $\chi_m^2 r$ and |log K_{OH}| (**A**) and $\chi_m^2 r$ and Z^2/r (**B**). Contour lines refer to the log free ion median effect concentration (EC50). ● = alkaline/rare earth metals, and □ = transition metals.

best fit (r^2 = 0.93, AIC = 5.25) among one-variable models, and all two-variable models provided equally good fits ($r^2 >$ 0.99) (Table 4).

DISCUSSION

The relative toxicity of metal ions to bacteria (Microtox) was predictable with linear regression using ion characteristics. Modeling all metals together, the softness index (σ_p) provided the best one-variable model. The softness index separates metal ions into three groups based on their softness: (1) hard ions, which prefer to bind to oxygen or nitrogen (e.g., Ca, Li, Na, K, Cs, Mg, Sr, and Ba); (2) soft ions, which prefer to bind to sulfur (e.g., Cd, Hg, and Ag); and (3) borderline ions, which form complexes with oxygen, nitrogen, and sulfur to varying degrees (e.g., Co, Ni, Cu, Zn, and Pb) [3]. The present study showed that EC50 values increased as softness increased (Fig. 2). The soft ions (e.g., Hg, Cd, and Ag), which preferentially bind covalently to sulfur, were more toxic than the hard ions. This suggested the relative importance of metal binding to sulfur (e.g., sulfhydryl groups) on biomolecules. The best two-variable model was one that used a combination of $\chi_m^2 r$ and |log K_{OH}|. The correlation with $\chi_m^2 r$ also suggested the importance of metals covalently binding to soft ligands (e.g., those with sulfur donor atoms), and the correlation with |log K_{OH}| suggested the importance of metals binding covalently with intermediate ligands, e.g., those with oxygen donor atoms. Two-variable models generally fit EC50 values better than one-variable models.

The present study used a $NaNO_3$ medium, in contrast to a previous study that used a NaCl medium for the nine divalent metals [9]. If EC50 values for these nine metals were compared between the two media, EC50 values were similar. This suggests that similar predictive models can be developed between EC50s and ion characteristics regardless of whether the medium reflects freshwater ($NaNO_3$) or saltwater (NaCl) speciation conditions.

The results also suggest that these types of relationships can be developed for water with very different water quality characteristics.

When performing speciation on these nine metals, the free ion was used because this reflected the most bioreactive species, especially in the freshwater medium ($NaNO_3$). The exception was Hg species in the NaCl medium, which also included the neutral chloro-complex ($HgCl_2^0$) because of its extremely high membrane permeability [21–26]. Including $HgCl_2^0$ with free Hg increased the log of the speciated EC50 from −12.31 to −1.31, which resulted in regression lines of $|\log K_{OH}|$ and log EC50 with similar slopes (Fig. 1). This suggested the importance of including $HgCl_2^0$ when considering the bioreactive forms of inorganic mercury.

Because such a wide range of metals was used with respect to ion characteristics, separating the metal ions into smaller groups improved the correlations. Higher correlation coefficients were obtained by developing separate models for monovalent, divalent, and trivalent metals. For example, if modeling free metal ion EC50 values with the log of the first hydrolysis constant ($|\log K_{OH}|$), correlation coefficients increased from 0.56 for all ions modeled together to 0.78, 0.94, and 0.75 for mono-, di-, and trivalent metal ions, respectively (Table 4). (Akaike's Information Criterion values among total, mono-, di-, and trivalent metal ions cannot be directly compared due to differences in sample sizes.) This good correlation with $|\log K_{OH}|$ was consistent with our previous study [9], which found that $|\log K_{OH}|$ (designated $|\log K_H|$ in our previous study) was the ion characteristic which provided the best model fit when modeling nine divalent metal ions against log free metal ion EC50 values ($r^2 = 0.93$). These correlations with $|\log K_{OH}|$ suggested that metal binding to intermediate ligands (e.g., those containing oxygen donor atoms) on biomolecules can be important. Although $|\log K_{OH}|$ provided one of the lowest correlation coefficients if modeling all metal ions together, modeling metal ions by valence separately dramatically improved these correlations.

Kaiser [4] got much higher correlation coefficients when modeling *Daphnia magna* reproductive impairment (3-week EC16) with ΔE_0 and log $AN/\Delta IP$ by developing separate models for metals with noble gas electron configurations, those with partially or completely filled d orbitals, and those with filled s and d orbitals but incomplete p orbitals. Using the log transformation of $AN/\Delta IP$ instead of $AN/\Delta IP$ in models or separating metals by electron configuration did not improve the models in the present study. Modeling EC50 values for nine divalent metal ions with log $AN/\Delta IP$ also did not improve model fits in our previous study [9].

Relatively high correlation coefficients were obtained in the present study between ion characteristics and inhibition of bacterial bioluminescence. While these relationships were developed using a very simple bacterial system, additional studies suggest that these relationships may be applicable to more complex organisms. Biesinger and Christensen [1] found correlations between *Daphnia magna* reproductive impairment and the solubility of metal sulfides, while Jones and Vaughn [3] found correlations between mouse median lethal dose (LD50) values and the softness index (σ_p). Kaiser [4] took toxicity data from the literature and successfully modeled fish and macroinvertebrate median lethal concentration (LC50) values with ΔE_0 and log $AN/\Delta IP$. These predictive models, if developed, could prove very useful in areas where data on metal toxicity or sublethal effects are lacking or incomplete. Once a model has been developed with representative metals for a particular organism under certain environmental conditions, the relative effect of additional metals could be predicted. These types of information could prove extremely useful in ecological risk assessment, where it may not be feasible to collect data on all metals for all species and conditions. While further development of such models will be necessary to determine their range of applicability, the present study supports the continuing development of predictive models of metal ion toxicity using ion characteristics.

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