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Application of Biotic Ligand and Toxic Unit Modeling Approaches to Predict Improvements in Zooplankton Species Richness in Smelter-Damaged Lakes near Sudbury, Ontario

Farhan R. Khan,*[,†,‡] W. (Bill) Keller,[§] Norman D. Yan,[∥] Paul G. Welsh,[⊥] Chris M. Wood,[#] and James C. McGeer*[,‡]

[‡]Department of Biology, Wilfrid Laurier University, 75 University Avenue W, Waterloo, Ontario N2L 3C5, Canada

[§]Cooperative Freshwater Ecology Unit, Laurentian University, Sudbury, Ontario P3E 2C6, Canada

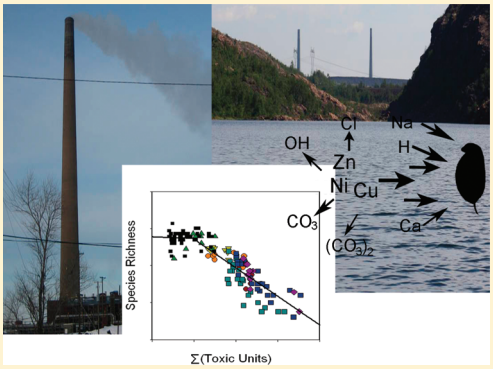
[∥]Department of Biology, York University, Toronto, Ontario M3J 1P3, Canada

[⊥]Ontario Ministry of the Environment, Toronto, Ontario M4 V 1M2, Canada

[#]Department of Biology, McMaster University, 1280 Main Street, Hamilton, Ontario, L8S 4K1, Canada

Supporting Information

ABSTRACT: Using a 30-year record of biological and water chemistry data collected from seven lakes near smelters in Sudbury (Ontario, Canada) we examined the link between reductions of Cu, Ni, and Zn concentrations and zooplankton species richness. The toxicity of the metal mixtures was assessed using an additive Toxic Unit (TU) approach. Four TU models were developed based on total metal concentrations (TM-TU); free ion concentrations (FI-TU); acute LC50s calculated from the Biotic Ligand Model (BLM-TU); and chronic LC50s (acute LC50s adjusted by metal-specific acute-to-chronic ratios, cBLM-TU). All models significantly correlated reductions in metal concentrations to increased zooplankton species richness over time ($p < 0.01$) with a rank based on r^2 values of cBLM-TU > BLM-TU = FI-TU > TM-TU. Lake-wise comparisons within each model showed that the BLM-TU and cBLM-TU models provided the best description of recovery across all seven lakes. These two models were used to calculate thresholds for chemical and biological recovery using data from reference lakes in the same region. A threshold value of TU = 1 derived from the cBLM-TU provided the most accurate description of recovery. Overall, BLM-based TU models that integrate site-specific water chemistry-derived estimates of toxicity offer a useful predictor of biological recovery.



1.0. INTRODUCTION

Over 7000 lakes around Sudbury (Ontario, Canada) were affected by acidification and increased metal concentrations from historic industrial emissions.[1] As a result, many became inhospitable for aquatic life; however, subsequent emission controls improved water quality and many plant, invertebrate, and fish species have returned. Zooplankton community changes in lakes with increasing pH and decreasing metal concentrations have been previously discussed.[2−5] Metals, particularly Cu^{2+} and Ni^{2+}, have been implicated as potential factors limiting the recovery of zooplankton diversity to levels typically found in reference lakes but consideration has been on an individual metal basis.[3,5] Although metal speciation (i.e., free ion concentrations) has been considered,[4] the combined toxicity of metal mixtures has yet to be investigated.

There are few studies linking the effects of metal mixtures to toxic impacts. Borgmann and colleagues[6,7] established a bioaccumulation modeling approach with *Hyalella azteca* but this requires knowledge of burden-to-effect relationships and has not been developed for metal concentrations in the exposure medium. One approach of combining metals is concentration or exposure additivity (as opposed to response addition) to produce a single linear variable, the "Toxic Unit" (TU[8]). The TU approach normalizes the exposure concentration for each contaminant by expressing it as a proportion of a toxicity end point and then these are summed to estimate toxicity on a proportional basis. Of the summation methods (additivity, antagonism, and synergism) additivity is generally used in the absence of data to demonstrate synergistic or antagonistic effects.[9] In this study we varied both the exposure concentration (numerator of each TU proportion) and the toxicity end point (TU denominator) to provide different estimates of metal mixture impacts for contaminated lakes over time.

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The recent development of physiologically based toxicity prediction models such as the biotic ligand model (BLM) may allow for new approaches to understand the recovery of metal-impacted lakes. The BLM is a geochemical equilibrium based model that builds on the free ion activity model (FIAM[10]) by quantifying the bioaccumulation of bioavailable forms of metal at the site of toxicity (the biotic ligand[11,12]). The acute toxicity of metals generally results from the free metal ion[10] but other forms (species) in solution, for example $Cu(OH)_2$, can also be associated with impacts and are accounted for in the BLM approach.[12] Complexation of metal cations by negatively charged ligands in the exposure medium, competition for uptake to the biotic ligand between metal ions and other cations (e.g., Na^+, H^+, Ca^{2+}, and Mg^{2+}), and estimates of metal uptake itself are provided by conditional equilibrium constants.[13] Thresholds for accumulation on the biotic ligand are associated with acute lethality (LC50 or EC50) and as a result the BLM provides water chemistry specific estimates of metal toxicity. The BLM approach has been demonstrated as a robust method for understanding the acute impact of metals in aquatic systems and models for Cu, Ni, Ag, Zn, and Cd have recently been published (see review 14).

In the present study four different TU models were used to estimate metal-mixture impacts on zooplankton species richness in the Sudbury lakes after the pH was no longer a limiting factor (i.e., pH > 6, see Section 2.2). These models were based on total metal concentrations (TM-TU); free ion concentrations (FI-TU); BLM predicted acute LC50s (BLM-TU); which were in turn adjusted by metal-specific acute-to-chronic ratios to produce a chronic BLM model (cBLM-TU). Each of the models represents an increased level of complexity with respect to estimating the potentially toxic fraction of metals. While the total metal concentrations in the exposure medium (TM-TU) are often used to gauge toxicity, it does not account for the bioavailable portion of metal that is associated with toxicity, the free ion.[15,16] A FI-TU model should provide improved estimates of toxic metal concentrations because it accounts for complexation reactions, and the BLM-based TU models may further improve estimates by integrating the toxicity mitigation influences of complexation and competition.

BLM modeling outputs are generally coupled with stressor variables such acute lethality[17] or whole body metal burdens.[18] Ecological effects are often expressed as community-level variables, such as species richness, diversity, or abundance, which better represent the overall health of an ecosystem.[19] In this study we develop TU modeling approaches to estimate the recovery of crustacean zooplankton species richness following water quality improvements in smelter-impacted lakes. Species richness was selected over total community abundance as the more sensitive metric for zooplankton community damage and recovery.[3,20,21]

2.0. METHODS

2.1. Background to Contamination in Sudbury Lakes. Lakes near metal smelters in Sudbury, Ontario, Canada, were impacted by both acidification and increased trace metal concentrations.[1] Six of our study lakes are located within 30 km of smelters and were severely affected by acidity (pH near 4) and exhibited very high concentrations of trace metals, in particular Cu and Ni[1] (Table S1). In this study we focused on Cu, Ni, and Zn. Although other trace metals had been measured in the Sudbury lakes they were either of negligible concentration after pH had risen above 6 (Cd and Pb, Section 2.2) or do not have a BLM currently available (Al). Between 1960 and the mid 1990s atmospheric SO_2 and metal emissions from the smelters were reduced by over 90% resulting in reduced acidity and metal inputs in impacted lakes.[1,22] Additionally, small-scale experimental,[23,24] and then large-scale liming programs[25] led to improved water quality in some lakes. These improved conditions permitted the return of many plant, invertebrate, and fish species to the previously inhospitable waters. Detailed descriptions of the impacts and subsequent partial recovery can be found in Keller et al.[2,26,27] and Yan et al.[3−5]

2.2. Data Set Handling. A 30-year monitoring program of lakes affected by the Sudbury smelters provided water chemistry and biotic data for this study. From this extensive record seven of the most studied lakes were selected and only those years where lake pH > 6, which has been described as a barrier to biological recovery[28,29] (see Supporting Information for full account of data selection), were used. The final data set consisted of 92 individual data-points from the Sudbury Lakes as follows: Clearwater (n = 9 years of data, 1998−2006), Joe (n = 7, 1985−2006), Hannah (n = 23, 1976−2003), Lohi (n = 11, 1975−76 and then 1995−2003), Middle (n = 24, 1975−2003), Nelson (n = 8, 1985−2006), and Whitepine (n = 10, 1997−2006). Hannah Lake and Middle Lake, and Clearwater Lake and Lohi Lake are connected, but none of the other lakes share a common watershed. The data set offered gradients of water chemistry variables both over time and across lakes (Table S1). A data set of water chemistry and zooplankton species richness from 23 unimpacted reference lakes from northeastern Ontario was used to generate recovery targets for the contaminated lakes.[3]

2.3. Toxic Unit Modeling. In applying the TU approach to derive an annual estimate of the combined impact of Ni, Cu, and Zn in each lake, the ratios of the waterborne exposure concentrations to the effect threshold concentration for each metal were summed.[7,8] Four different combinations of exposure and effect ratios were calculated according to eq 1, i.e., based on total metal concentrations, free ion concentrations, acute BLM-derived LC50s, and chronic BLM-derived LC50s (see detailed explanations of the four models in the Supporting Information). Exposure-to-effect ratios were summed to calculate the overall TU assuming additivity (i.e., individual metals do not interact toxicologically nor influence bioavailability and uptake). Thus additivity assumes different binding sites on the biological membrane for each metal.[9] This assumption is reasonable given our understanding of the differences in uptake pathways for Cu (via Na^+ channels[30]), Zn^{2+} (as an analog of Ca^{2+}[31]) and Ni^{2+} (Mg transport mechanism[32]).

$$TU_{ij} = \frac{[Cu_{Ex}]_{ij}}{[Cu_{Eff}]_{ij}} + \frac{[Ni_{Ex}]_{ij}}{[Ni_{Eff}]_{ij}} + \frac{[Zn_{Ex}]_{ij}}{[Zn_{Eff}]_{ij}} \quad (1)$$

where TU_{ij} is the summed toxic unit for metals Cu, Ni, and Zn for each lake i (i = 1 to 7) for each year j. For the TU model based on total metal, free ion, Biotic Ligand Model, and chronic BLM (cBLM-TU), numerators (exposure concentrations (Ex)) were total metal concentrations for TM-TU, free ion concentrations for FI-TU, and dissolved metal concentrations for both BLM-TU and cBLM-TU models. Denominators (effect concentrations or toxicity end points (Eff)) for each model were as follows: water hardness-based U.S. EPA Water Quality Criteria (WQC, criterion continuous concentration) concentrations (for TM-TU), free ion environmental no effect

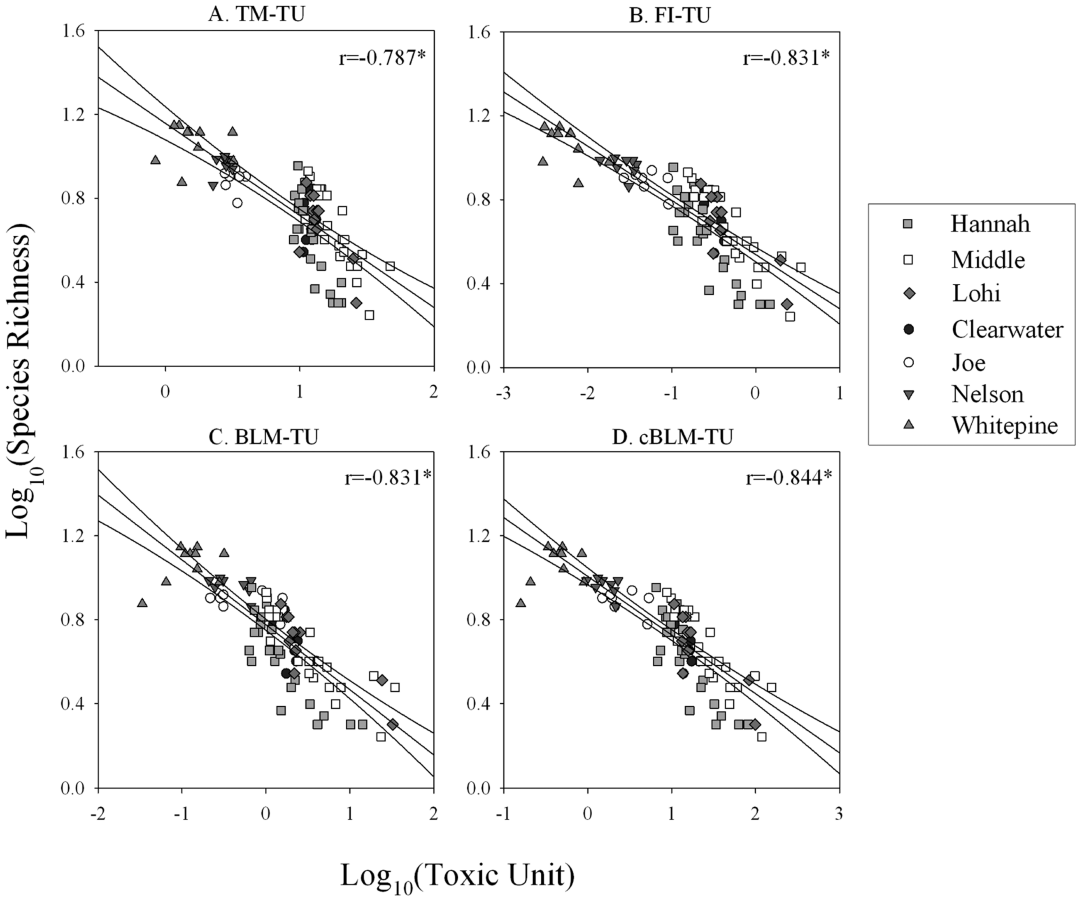

Figure 1. Comparisons of scattergrams of zooplankton species richness vs Toxic Unit scores for the four compared models: (A) Total Metal TU (TM-TU), (B) Free Ion TU (FI-TU); (C) BLM-TU, and (D) cBLM-TU. Data on both axes are Log_{10} transformed, and the displayed Pearson's r coefficients, all of which are significant at $P = 0.01$ ($n = 92$), were calculated on logged data. Lines show the best fit linear regression with 95% confidence interval.

values (ENEVs for FI-TU[33]), BLM estimates of acute toxicity (LC50s for BLM-TU[12,34]), and BLM estimates of chronic toxicity (acute LC50s to which acute to chronic ratios were applied for cBLM-TU[35−38]). It is worth noting that ENEVs and WQC are chronic criteria and the latter incorporates ACRs (for details see Supporting Information).

2.4. Model Comparison and Analysis. Toxic Unit model outputs and zooplankton species richness were Log_{10} transformed to allow for the analysis of linear relationships. A two-level analysis was undertaken to determine, first, how well each model described the relationship between mixture toxicity and zooplankton species richness in the Sudbury lakes, and second, whether this relationship was representative of all 7 lakes. In the first step, the TU output from each model was correlated to zooplankton species richness (Pearson's correlation). This analysis provided an r^2 value and a slope (the overall slope) that described each model's fit to the zooplankton species richness irrespective of lake or time (i.e., all 92 data-points). However, there is potential for an overestimation of the fit of a model when a data set is composed of nested subsets,[39] as is the case of individual lakes nested within Sudbury lakes data set. Thus the second step of analysis for each model involved regressing zooplankton species richness to TUs on an individual lake basis, producing individual lake slopes, and comparing these to the overall slope. Assuming that the overall fit of the model was reflective of the fit of each individual lake, the slope of each individual lake would be expected to align to the overall slope. We adopted a descriptive approach to express the individual lake slopes as deviations from the overall slope (see ref 40). The variance (S^2) of the individual lake to the overall slope was calculated as the sum of squared error per lake divided by the number of observations in the individual lake data subset.

2.5. Estimating Chemical and Biological Recovery in Metal-Impacted Lakes. The two TU models that offered the best fit to the Sudbury lakes data (the BLM-TU and cBLM-TU) were used to model the reference lake data (described in Section 2.3 and Supporting Information). BLM-TU and cBLM-TU outputs were calculated for these reference lakes to estimate potential recovery limits from metal toxicity. Recovery limits were used to deduce whether zooplankton species richness in the Sudbury lakes, following water improvements, was comparable to the number of zooplankton species in the reference lakes. Under the assumption that species richness in reference lakes was typical of unimpacted sites, a zooplankton recovery threshold was calculated at two standard deviations below the mean species richness. This accounts for natural variability that occurs in reference conditions, and lakes with higher species richness values are considered as recovered. The recovery threshold was applied to each of the Sudbury lakes to determine the state of biological recovery in relation to predicted TU values. Yan et al.[3] used a similar approach for zooplankton metrics from nonacidified reference lakes to establish whether the zooplankton community in acidified experimental lakes had recovered. The ability of the TU model

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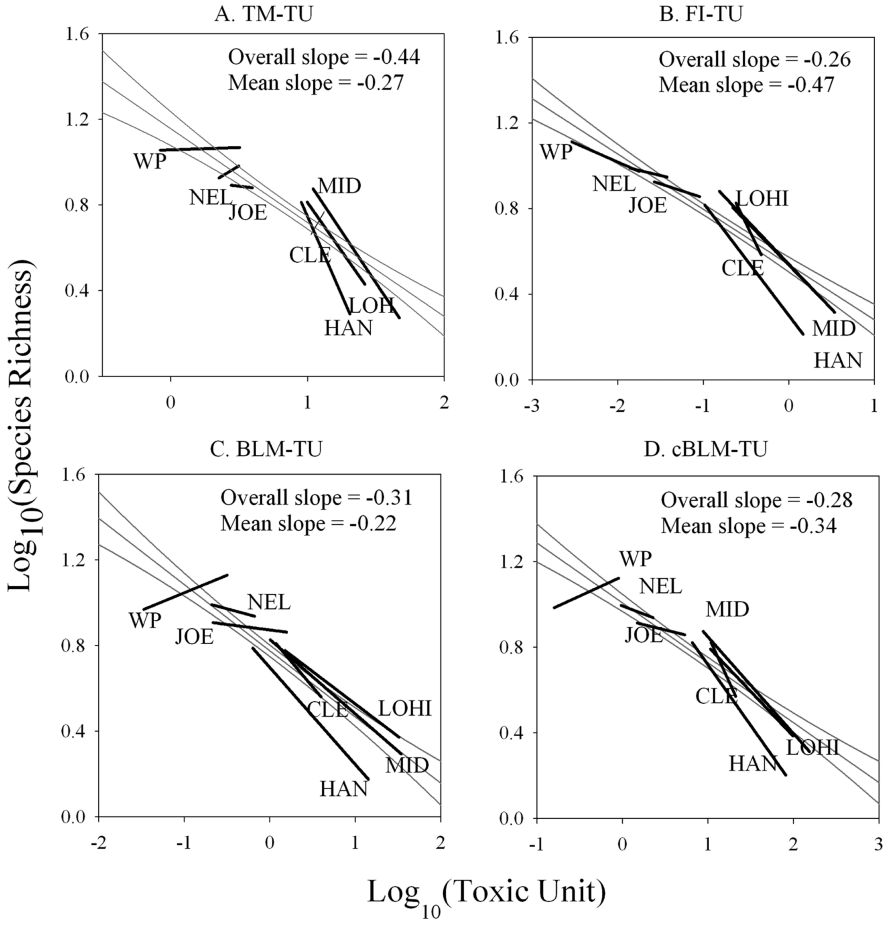

Figure 2. Slope variation in each Toxic Unit model: (A) Total Metal TU (TM-TU); (B) Free Ion TU (FI-TU); (C) BLM-TU, and (D) cBLM-TU\. For each model the TU is correlated against the species richness (on a Log10 scale). The overall slope is shown with 95% confidence limits of all data-points (n = 92, gray lines) and is compared to the mean of the individual lake slopes (WP = Whitepine, JOE = Joe, NEL = Nelson, HAN = Hannah, CLE = Clearwater, MID = Middle, LOHI = Lohi, black lines).

to estimate recovery was evaluated by setting the toxicity threshold at TU = 1 (corresponding to acute toxicity for BLM-TU and chronic end points for cBLM-TU). For comparative purposes we also derived an upper TU limit for reference data, calculated at two standard deviations above the mean TU value for reference lakes.

3.0. RESULTS

3.1. Comparison of TU Models with Data Pooled Across Lakes. The four models significantly correlated decreasing TU to increasing zooplankton species richness (p < 0.01 Pearson's correlation, Figure 1). However, the strength of the correlations differed, ranging from the TM-TU model (r^2 = 0.619) to the cBLM-TU (r^2 = 0.712), with intermediate values of 0.69 for both the FI-TU and BLM-TU models. Using overall correlation as a measure of how well each model described the increase in zooplankton species richness, the models ranked as follows: cBLM-TU > BLM-TU = FI-TU > TM-TU. While the sum of TUs was correlated with species richness for all four models, the relative contributions of Cu, Ni, and Zn varied considerably (Table S2). The BLM-TU model tended to predict that Cu was most likely the main contaminant while the FI-TU model generally assigned a much lower proportion of the TUs to Cu, with its proportion replaced mainly by Zn (data not shown).

3.2. Comparison of TU Models between Lakes. The TU models based on the BLMs (BLM-TU and cBLM-TU, Figure 2C and D) were more consistent across lakes than the TM-TU and FI-TU models (Figure 2A and B). Both TM-TU and FI-TU models produced predictions with larger variation between individual slopes of each lake and the overall slope across lakes (Figure 2 and Figure S1) and this is supported by the calculated S^2 values (Table 1). In comparison, both the BLM-TU and the cBLM-TU models exhibited lower variability between individual lakes and across all lakes (Table 1). In all

Table 1. Comparison of the Model Diagnostics for the Toxic Unit Models Used to Predict Zooplankton Species Richness[a]

	overall model fit			lake-wise model fit		
model	R^2	slope	p-value	mean slope	SD	S^2
TM-TU	0.619	−0.44	<0.01	−0.27	0.89	0.83
FI-TU	0.690	−0.26	<0.01	−0.47	0.37	0.19
BLM-TU	0.690	−0.31	<0.01	−0.22	0.23	0.06
cBLM-TU	0.712	−0.28	<0.01	−0.34	0.35	0.12

[a]For each model the overall model parameters (n = 92) are given as r^2 value, slope, and p-value (Pearson's correlation). The mean slope calculated from the slope of each individual lake (n = 7) with standard deviation is shown for each model. The individual lake slope to overall slope deviation is described as variance (S^2).

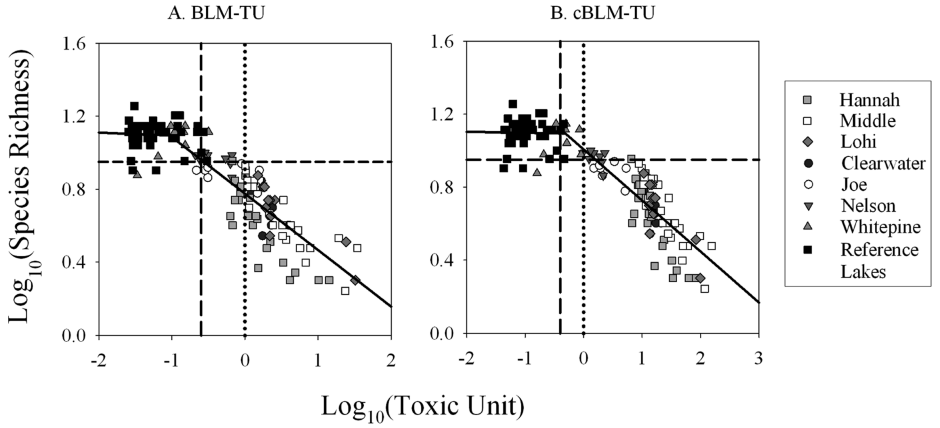

Figure 3. Zooplankton species richness plotted against (A) BLM-TU and (B) cBLM-TU for the 7 Sudbury lakes and reference lakes. On each figure, geochemical recovery end points are fixed at TU = 1 (0 on the Log10 scale, vertical dotted line) or derived from the from the reference data set (2 SD above the mean TU, vertical dashed line). In the latter scenario the upper TU limit for geochemical recovery occurs at TU = −0.6 and TU = −0.4 (on a Log10 scale) when derived using the BLM-TU and cBLM-TU models, respectively. A recovery threshold for species richness (2 S D below the mean species richness, horizontal dashed line) is positioned at 0.95 (equivalent to 9 species). Upper TU limits based on 2 SD from the mean of the reference lakes are most stringent, but using a fixed threshold (TU = 1) derived from the BLM-TU (A) classifies many more lakes (and years) as chemically recovered although species richness is lower compared to reference lakes. The most accurate indicator of recovery is TU = 1 derived from the cBLM-TU model as only Whitepine Lake, which has similar TU and richness values to the reference data, is considered to be recovered. The two solid lines show the mean species richness for reference lakes (horizontal) and the overall best fit line for the seven study lakes.

model estimates, Whitepine Lake stood out as contributing significantly to the variation in slopes among lakes (Figure 2). Based on their consistency among lakes in describing the relationship of species richness to TUs, the four models were ranked BLM-TU > cBLM-TU > FI-TU >TM-TU (from least to most variation).

Although the application of ACRs to the BLM-TU did increase the overall fit (cBLM-TU r^2 = 0.712 compared to BLM-TU r^2 = 0.69; Figure 1D and C), individual lake slope to overall slope variation increased (Figure 2, Figure S1). These differences between BLM-TU and cBLM-TU demonstrate the influence of the high ACR value for Ni (19.4) relative to that of Cu (2.07) and Zn (2.21). This increased the relative contribution of Ni to the cBLM-TU values while reducing that of both Cu and Zn (see shift in range of contributions to toxicity between BLM-TU and cBLM-TU in Table S2).

3.3. Estimates of Chemical and Biological Recovery. The BLM-TU and cBLM-TU models provided good correlations between mixture toxicity and zooplankton species richness in Sudbury lakes, and were subsequently used to model the reference lakes data set (Figure 3). Species richness in the reference lakes did not correlate to toxic units (e.g., BLM-TU r^2 = 0.02, p = 0.90) as metal concentrations were not expected to be toxic in these lakes. A zooplankton species richness of 9 (0.95 on a Log_{10} scale) was determined as a lower recovery threshold (i.e., 2 standard deviations below the mean species richness of reference lakes). This species richness value was applied to Sudbury Lakes, and only Whitepine Lake and Nelson Lake (for the majority of years) had 9 or above zooplankton species, therefore these two lakes could be considered as biologically recovered (with regards to species richness).

In terms of the ability of the BLM based toxicity prediction models to estimate species recovery, both a fixed TU value of 1 and the upper TU limit derived from reference lakes were examined. The upper TU limits were calculated as TU = 0.25 (−0.6 on Log10 scale) and 0.4 (−0.4) for BLM-TU and cBLM-TU models, respectively (Figure 3). When the threshold for chemical recovery was fixed at TU = 1 (0 on a Log_{10} scale, i.e., bioavailable metal concentrations equal the LC50) derived using the BLM-TU model, the water quality in Whitepine and Nelson lakes would be sufficiently recovered for species richness to be comparable to the reference lakes (Figure 3A). The later years of Joe, Hannah, and Middle lakes were also calculated as TU < 1 but actual species richness was below the species richness threshold for recovery (i.e., water chemistry-based TU models predicted chemical recovery, but measured species richness was not comparable to the reference lakes, Figure 3A). This fixed threshold of TU = 1 derived from the BLM-TU model appears to overpredict biological recovery. When the same TU = 1 value was applied to data in the cBLM-TU model, the fixed threshold was closer to the threshold based on the 2 SD from the mean of the reference data (Figure 3B). Using the cBLM-TU model and applying the fixed threshold (i.e., TU = 1) appears to be the most accurate estimate of geochemical recovery and agreed with measured biological recovery. Sudbury lakes data-points that are classed as having a water quality similar to the reference lakes also have a similar number of zooplankton species present. As with the upper limit based on 2 SD from the mean of the reference lakes data, the TU =1 threshold applied to the cBLM-TU model identified only Whitepine Lake as recovered and therefore is a conservative estimate of species recovery.

4.0. DISCUSSION

In modeling the effects of Cu, Ni, and Zn concentrations on the recovery of zooplankton in lakes surrounding Sudbury smelters we compared four additive TU models. Each model successfully correlated declining mixture toxicity to increasing species richness. This demonstrates the usefulness of these models in predicting environmental impact, as calculated metal mixture toxicity using a toxic unit approach reasonably predicted real world community responses. Of the four TU models tested, there was an increasing correlation to zooplankton species richness with increasing estimates of metal bioavailability: cBLM-TU > BLM-TU > FI-TU > TM-TU. The ranking of the

models demonstrates a progression in the mechanistic understanding of the toxicity of inorganic forms of dissolved metal. The TM-TU offers no consideration of the bioavailabilty of dissolved metal species as it is based on total metal thresholds for toxicity as influenced by ambient water hardness. BLM-based TU models provided the best correlations which is consistent with our expectation that free ion based models are more accurate than those that use total metal and, in turn, models that incorporate cation competition at the site of biological uptake in addition to complexation reactions are more accurate still. Although pH was specifically removed as a stressor from our analysis (i.e., selecting only years in which pH > 6), the influence of pH on Cu, Ni, and Zn availability would also have been incorporated into the models that accounted for speciation.

The strength of the BLM approach is that it is mechanistically based, accounting for threshold accumulations at the site of toxicity. It accounts for the geochemical species associated with toxicity which include Cu^{2+} and $Cu(OH)_2$,[12] Ni^{2+},[41] and Zn^{2+}.[12] Therefore both of the BLM-TU models account for speciation and complexation reactions in solution as well as cationic competition at the site of toxicity. BLM-based predictions for individual metals offer improvements over hardness-based methods for deriving acute water quality criteria and guidelines and are gaining acceptance within the regulatory community.[36,37,42] Here we provide evidence that BLM models can also be used to predict biological responses at the community level for mixtures of metals.

Despite the success of using the BLM approach to predict the recovery of zooplankton species richness, it is likely that further improvements to this approach might be realized by calibrating model parameters to meet site-specific needs. These might include soft water specific models, biotic ligand characteristics that are appropriate for zooplankton residing in these lakes (rather than the generalized *D. magna* model), and true chronic BLMs with experimentally derived stability constants. The waters of the Canadian Shield can be very soft (<3 mg/L Ca[43]) and Ca concentrations are subject to ongoing decline.[44] At very low Ca concentrations, the assumptions of the acute BLM (developed for moderately hard water) will likely not be accurate[13] as the bioavailability and toxicity of divalent metals ions are greater owing to reduced competition from cations.[41,45]

Our BLM-based TU models were based on the existing *D. magna* models, which used stability constants derived from the original fish gill BLM model,[13] adjusted to reflect the relative sensitivity of the new species.[17] *D. magna* BLMs based on fish gill stability constants have been shown to lack predictive accuracy in some cases.[46] Furthermore, as *D. magna* is not present the Sudbury lakes, the most accurate model would be derived from zooplankton that are, or at least one zooplankton species that represents zooplankton sensitivities in the Sudbury lakes. *D. magna* is more tolerant than smaller zooplankton species (e.g., *Daphnia mendotae* and *Chydorus sphaericus*) to pollutants as smaller species are susceptible to greater uptake as a function of greater surface area to size ratio.[47,48] A model based on *D. magna* metal sensitivity would be expected to underestimate toxicity when extrapolated to more sensitive zooplankton.

By applying ACRs to the acute BLM-derived LC50s, we reduced the LC50s which increased the overall correlation to zooplankton species richness, but appeared to weaken the within-lake relationships (Figures 2 and S1, Table 1). The application of ACRs increased the contribution of Ni relative to Cu by virtue of the high Ni ACR, but the performance of our cBLM-TU model may have been increased if the LC50 was derived from chronic BLMs rather than acute LC50s adjusted with ACRs. *D. magna* chronic BLMs are now available for Cu,[49] Zn,[50] and Ni.[51] Although using the chronic Zn BLM would have been feasible, the Cu and Ni models were unsuitable for our cBLM TU model. The chronic Zn BLM is mechanistically similar to the acute model, although stability constants have been altered to account for long-term exposure,[50] but the chronic Cu model requires the consideration of $CuCO_3$ uptake and toxicity, a species which is not incorporated in the acute Cu BLM. The Ni model[51] is even more problematic as the nonlinear effect of H^+ (pH) could only be expressed when superimposed onto the effects of Ca and Mg. Therefore, we chose to apply ACRs to acute BLM estimates of lethality which is consistent with current U.S. EPA water quality criteria guidelines. However, further refinement of the cBLM TU model by incorporating LC50s derived from chronic BLMs would certainly warrant more consideration.

By recalibrating the existing acute BLM-TU model as described we would expect greater accuracy in correlating mixture toxicity to community metrics. However, interpretation of the correlation is key to understanding the underlying processes within each lake. Our analysis suggested that the overall correlation (slope) between calculated TU and zooplankton species richness did not fully explain the descriptive (and predictive) powers of each model. Within the data set were seven lakes in which the processes of chemical and biological recovery differed, but lakes sharing similar characteristics can be grouped. Hannah, Middle, and Lohi are the closest lakes to the Sudbury smelters and as such were most severely affected. Artificial neutralization in the mid 1970s[24] reduced trace metal concentrations dramatically.[52] Within our data set (pH > 6), Hannah, Middle, and Lohi lakes show the greatest reduction of TU (*x*-axis) and increase in zooplankton richness (*y*-axis), i.e., steepest correlation slopes. Although, Nelson Lake was also limed, metal levels were lower (relative to Hannah, Middle, and Lohi) because it is further from the emission source, and the pH of the lake never fell below 5. Joe and Whitepine lakes also had relatively low trace metal concentrations and therefore TU values did not change as dramatically over time (Figure 2). Two groups of lakes provide the primary reason for the overall slope to individual lake slope variation observed among the TU models. The BLM-TU and cBLM-TU align more to the influence of Hannah, Middle, and Lohi lakes, and overpredict the rise in species richness in Whitepine, Nelson, and Joe lakes. Models that align well to Whitepine, Nelson, and Joe lakes (e.g., FI-TU model), underpredict the changes that happened over a greater range of TUs.

Taking Middle lake as an example of the neutralized lakes, following the addition of base, pH 6 was reached within a few years. Although phytoplankton community composition recovered fairly quickly,[24] zooplankton species richness did not increase. The only Daphnia species to re-establish a population was the acid-sensitive species *Daphnia mendotae*.[4,5] While other zooplankton species re-established (e.g., copepods), other Daphnid species failed to persist despite new colonists arriving. In contrast, Whitepine Lake recovered naturally, but started with the lowest metal concentrations and the most diverse zooplankton community, with at least eight species present in all sampling years. As acidity and metal levels decreased, new colonists arrived (e.g., *Eubosmina*

longispina) and/or relict populations increased in size (e.g., *Epischura lacustris*[2]). In effect, at the start of our data set, Whitepine Lake was more similar to the reference lakes than to the contaminated lakes, and it is not surprising that Whitepine Lake was predominantly classed as chemically and biologically recovered (Figure 3). It appears that individual lake recovery trajectories (correlations of TU to species richness) depend on the initial severity of damage and the timing since chemical recovery, as well as the current condition.[3,4] As Whitepine, Joe, and Nelson lakes were not as severely damaged as Hannah, Middle, and Lohi lakes, and correspondingly had a more species-rich starting point, they show less dramatic changes in richness. After all, they did not have as far to go.

BLM-based TU models best explained the relationship between species richness and mixture toxicity. In the final step in our modeling and analysis we included reference lake data to determine if the chemical recovery end points in the impacted Sudbury lakes, as predicted by the TU models, equated to actual biological recovery. The upper TU limits, derived from the reference data set, were determined for both BLM-TU and cBLM-TU models as 0.25 and 0.4 TU, respectively (Figure 3). These limits under-predicted the extent of biological recovery in the Sudbury lakes, in particular during the most recent years of our data set. When recovery thresholds were fixed at TU = 1, where the effect concentration is equal to the BLM-derived bioavailable metal concentration, lakes and years that were predicted to be geo-chemically recovered were also similar to the reference lakes in species richness. This was especially true in the case of the cBLM-TU model. Furthermore, in the case of the BLM-TU there is a degree of discrepancy between the reference data set-derived upper limit and the fixed TU = 1 threshold. However, with the cBLM-TU these two values are closer to unity, suggesting that through the application of ACRs, the acute BLM can be calibrated to in situ monitoring data, such as our univariate community metric, species richness.

Although the reduction of Cu, Ni, and Zn concentrations was correlated to increases in zooplankton species richness in all of our models (once pH had risen above 6), there are numerous other factors that influence the re-establishment of zooplankton communities in the Sudbury lakes. The process of biological recovery in contaminated lakes is far more complex than the simple removal of the metal stressors. Assuming there has been adequate time for recovery, factors such as propagule arrival rates and size, differing establishment probabilities of the propagules, predation, and/or inadequate habitat are all likely to affect the success of new colonists.[5,27,53,54] These factors can be divided into two categories: (a) chemical, physical, and biological factors related to habitat quality (local factors), and (b) factors relating to the dispersion of would-be colonists (regional factors).[2] As the lakes used in our analysis are geographically close and among numerous other lakes, propagule delivery rates were unlikely to limit recovery.

In this study we have demonstrated that dissolved metal concentrations can explain a large proportion of the trends in zooplankton community richness once the threat of acidity itself is removed. TU models that included the BLM provided better predictions of species richness most likely because they consider the integrated effects of water chemistry on bioavailability. Models could be further improved by accounting for site- and local species-specific parameters, as well as by moving from ACR to mechanistically based chronic models. Nonetheless summarizing physiological-relevant water chemistry into a single linear variable by combining the BLM and the Toxic Unit approaches holds real promise to predict both damage and recovery where the aquatic environment is affected by mixtures of metals, but not other stressors.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Methodologies relating to the selection of the final data set and derivation of the four TU models. Figure S1 describing the variance of individual lake slopes compared to the overall slope. Table S1 summarizing annual lake water chemistry parameters and zooplankton species richness for the seven Sudbury study lakes that were used in the TU modeling. Table S2 showing the percentage contributions of Cu, Ni, and Zn to each TU model. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 0044 0207-942-6688; fax: 0044 0207-942-5054; e-mail: f.khan@nhm.ac.uk (F.K.). Phone: 001 519-884-3537; fax: 001 519-746-0677; e-mail: jmcgeer@wlu.ca. (J.M.).

Present Address

[†]Department of Zoology, Natural History Museum, Cromwell Road, London SW7 5BD.

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