



## Review

## Using biotic ligand models to predict metal toxicity in mineralized systems

Kathleen S. Smith<sup>a,\*</sup>, Laurie S. Balistrieri<sup>b,1</sup>, Andrew S. Todd<sup>a,2</sup><sup>a</sup> U.S. Geological Survey, Box 25046, Denver Federal Center M.S. 964D, Denver, CO 80225-0046, United States<sup>b</sup> U.S. Geological Survey, University of Washington, Box 355351, Seattle, WA 98195, United States

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## ABSTRACT

The biotic ligand model (BLM) is a numerical approach that couples chemical speciation calculations with toxicological information to predict the toxicity of aquatic metals. This approach was proposed as an alternative to expensive toxicological testing, and the U.S. Environmental Protection Agency incorporated the BLM into the 2007 revised aquatic life ambient freshwater quality criteria for Cu. Research BLMs for Ag, Ni, Pb, and Zn are also available, and many other BLMs are under development. Current BLMs are limited to ‘one metal, one organism’ considerations. Although the BLM generally is an improvement over previous approaches to determining water quality criteria, there are several challenges in implementing the BLM, particularly at mined and mineralized sites. These challenges include: (1) historically incomplete datasets for BLM input parameters, especially dissolved organic carbon (DOC), (2) several concerns about DOC, such as DOC fractionation in Fe- and Al-rich systems and differences in DOC quality that result in variations in metal-binding affinities, (3) water-quality parameters and resulting metal-toxicity predictions that are temporally and spatially dependent, (4) additional influences on metal bioavailability, such as multiple metal toxicity, dietary metal toxicity, and competition among organisms or metals, (5) potential importance of metal interactions with solid or gas phases and/or kinetically controlled reactions, and (6) tolerance to metal toxicity observed for aquatic organisms living in areas with elevated metal concentrations.

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\* Corresponding author. Tel.: +1 303 236 5788.

E-mail addresses: [ksmith@usgs.gov](mailto:ksmith@usgs.gov) (K.S. Smith), [balistri@usgs.gov](mailto:balistri@usgs.gov) (L.S. Balistrieri), [atodd@usgs.gov](mailto:atodd@usgs.gov) (A.S. Todd).<sup>1</sup> Tel.: +1 206 543 8966.<sup>2</sup> Tel.: +1 303 236 1426.

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## 1. Introduction

Biotic ligand models (BLMs) are computational models that determine metal speciation and predict metal toxicity to biota in aqueous systems (Niyogi and Wood, 2004; Erickson, 2013). BLMs combine an equilibrium geochemical speciation model, a metal–organic binding model, and a toxicological model. Specific BLMs vary in regard to specific model construction, metals and organisms addressed. Niyogi and Wood (2004) described 15 different BLMs that had been developed and more have been developed since their publication. In the United States, the most mature BLM is probably that for the acute toxicity of copper (Di Toro et al., 2001; Santore et al., 2001), and when we refer to the BLM here, we refer specifically to that model. The U.S. Environmental Protection Agency (USEPA) incorporated the BLM into its recently revised aquatic life ambient freshwater quality criteria for Cu (USEPA, 2007), thus updating the previous hardness-based criteria for Cu (USEPA, 2002b).

In this review we discuss some of the scientific concepts behind BLMs, evolution of Water Quality Criteria (WQC) to arrive at incorporation of BLMs, features of BLMs, case studies using BLMs, and some caveats of using BLMs to predict metal toxicity in mined and mineralized systems. We concentrate on the research version of the BLM available from HydroQual, Inc., at [http://www.hydroqual.com/wr\\_blm.html](http://www.hydroqual.com/wr_blm.html). This version of the BLM predicts acute toxicity and speciation of Cu, Ag, Cd, and Zn for a variety of aquatic organisms including fathead minnow (*Pimephales promelas*), rainbow trout (*Oncorhynchus mykiss*), and water fleas (*Daphnia magna*, *Daphnia pulex*, and *Ceriodaphnia dubia*). We emphasize Cu and Zn in our discussions of metal speciation, bioavailability, and toxicity. Table 1 provides a list of selected acronyms that we use in this report.

## 2. Metal toxicity to aquatic biota—the basics

### 2.1. Metals in the aquatic environment

Metals are generally ubiquitous in trace amounts within the aquatic environment, originating from diverse natural and anthropogenic sources. Human sources of metals to the aquatic environment can include both point sources (e.g., municipal wastewater discharges, mine drainage) and non-point sources (e.g., agricultural and urban runoff, atmospheric deposition). Importantly, many trace metals (e.g., Zn, Ni, Cu) play an essential role in the normal metabolic processes of aquatic organisms; however, they can become increasingly toxic outside of this “window of essentiality” (Hopkin, 1989; Wood, 2011). Other “non-essential” metals (e.g., Ag, Cd, Pb) play no known role in normal metabolic function of aquatic organisms, and as such are toxic once the “no-effect” concentrations are exceeded (Kapustka et al., 2004). The realized

toxicity of individual metals within the aquatic environment is dependent on a suite of factors, including the identity and chemical form of the metal, duration and magnitude of metal exposure, the pathways of metal exposure, and the relative sensitivities of resident aquatic organisms to the metal of concern. While many potential pathways exist for metals to lead to toxic effects in aquatic organisms (e.g., aqueous exposure, dietary exposure, sediment, maternal transfer), the following discussion will primarily focus on the aqueous metal exposure and toxicity pathway because it forms the basis for the revised USEPA aquatic life ambient freshwater quality criteria for Cu (USEPA, 2007).

### 2.2. Types of toxic effects

The dose–response relationship is a fundamental underlying principle to understanding and interpreting the toxicity of contaminants to living organisms. Simply defined, dose–response relationships relate the quantity of toxicants to which an organism is exposed to the degree of resultant harmful effects (Rand et al., 1995). Typically, to precisely estimate the toxic effect of a given contaminant on a given organism, these dose–response relationships are individually quantified in a laboratory environment according to highly standardized protocols, featuring highly prescribed exposure conditions, durations, and measured toxicity endpoints. The upside of a stripped-down, standardized approach

**Table 1**  
List of acronyms used in this report.

ALC	Aquatic life criteria
BL	Biotic ligand
BLM	Biotic ligand model
CCC	Criterion Continuous Concentration (chronic)
CMC	Criterion Maximum Concentration (acute)
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EC50	Median effect concentration
FA	Fulvic acid
FAV	Final Acute Value
FIAM	Free-ion activity model
GMAV	Genus mean acute value
HA	Humic acid
HS	Humic substances
LA50	Lethal accumulation; 50% mortality
LC50	Median lethal concentration
NOM	Natural organic matter
SMAV	Species mean acute value
TMDL	Total maximum daily load
TOC	Total organic carbon
TU	Toxic unit
WER	Water effect ratio
WHAM	Windermere Humic Aqueous Model
WQC	Water Quality Criteria

is that it facilitates the evaluation of the contaminant-specific effects on organisms of interest in a manner that can be easily replicated. The downside is that real contaminant exposures and resultant ecological impacts in the environment usually are significantly more complicated, involving mixtures of exacerbating and mitigating chemical influences, as well as complex ecological communities and structures. The extrapolation of laboratory-derived results to the field is a significant source of uncertainty in predicting the realized ecological effects of metal contamination (Buchwalter et al., 2007).

Within this standardized framework, the terms “acute” and “chronic” are commonly used to describe the toxic effects of a contaminant on an organism. These terms function as descriptors of the duration of exposure, the magnitude of the exposure and/or the type of toxic effect elicited. Acute toxicity tests are typically designed to quantify easily detectable, severe effects (e.g., death, immobilization) that manifest over short durations of exposure (typically less than 96 h) across a range of increasing concentrations of the contaminant of interest. The most commonly expressed metric defining acute toxicity is the median lethal/effect concentration (LC50/EC50), which is the time-dependent concentration of contaminant that is estimated to elicit a severe effect in 50% of the test organisms (e.g., Rand et al., 1995). In contrast, chronic toxicity tests are typically designed to characterize sublethal toxic effects (e.g., reduced growth, diminished reproductive function, changes in behavior) through the use of longer term exposures and the assessment of multiple biological endpoints. As such, chronic toxicity testing typically yields effects at lower exposure concentrations for a given metal. Historically, chronic toxicity testing employed very long-duration exposures (i.e., life-cycle toxicity tests) such that potential toxicant effects could manifest at any point “from embryo to embryo” (Cooney, 1995). In practice, the high cost and time-consuming nature of these life-cycle tests have rendered them largely impractical, and as such, new tests designed to estimate chronic effects to critical life stages (e.g., early life stages) within a shorter test time-frame (e.g., 30 days) have emerged as standard practice. Acute-to-chronic toxicity ratios are sometimes used to estimate chronic criteria (Mebane et al., 2008).

### 2.3. Mechanisms of uptake

In general, toxicity requires the transfer of a contaminant from the exposure environment to the site within the organism where it elicits a toxic effect. As noted above, there are many routes by which an aquatic organism can be exposed to a given contaminant (e.g., diet, interaction with sediment), with the route of exposure often determining the site of toxic action and toxicity mechanism for the organism (e.g., Clearwater et al., 2002; Meyer et al., 2005; Wood, 2011). The site of acute toxic action during aqueous metal exposures in fish is typically the gill, where metals induce

ionoregulatory impairments. The physiological mechanism of toxicity at the gill site typically varies by metal, with disruption of Na/K exchange (by Cu; e.g., Taylor et al., 2003) and Ca uptake (by Zn; e.g., Hogstrand et al., 1996) as known examples. Through the detailed characterization and comprehension of these routes of exposure and mechanisms of toxicity for a given toxicant, the role(s) that external biotic and abiotic factors play in modulating toxicity can be better understood and modeled (e.g., BLMs).

### 2.4. Differences in organismal susceptibility

Susceptibility of a given organism to toxicity from a given metal is a function of both factors related to the type of exposure (as detailed above) and factors inherent to the organism. By compiling and normalizing results from studies conducted using the standardized toxicity-testing methodologies detailed above, it is possible to calculate generalized toxicity values for individual species for individual metals. The routine use of a standardized suite of test organisms and subsequent calculation of species mean acute values (SMAV) and genus mean acute values (GMAV) allows for the comparison of relative sensitivities of potentially resident aquatic organisms to a given metal (Table 2). Most metals yield a wide range of sensitivities among organisms potentially resident in aquatic ecosystems. For example, for Zn, the GMAV for the most sensitive genus in the USEPA's 1995 Zn database (*Ceriodaphnia*; 93.95 µg/L) is many orders of magnitude lower than the GMAV for the least sensitive genus (*Argia*; 88,960 µg/L) (USEPA, 1995; DeForest and Van Genderen, 2012). Importantly, relative susceptibility of a given organism is not necessarily consistent across the variety of metals of concern. For example, while the genus (*Pimephales*) containing the fathead minnow (*P. promelas*) is listed as the seventh most sensitive genus for Cd, it is the seventeenth most sensitive genus for Zn and Cu (USEPA, 1995, 2001, 2007). *Oncorhynchus*, a salmonid, is the most sensitive to Cd but only moderately sensitive to Cu or Zn (Mebane, 2006; DeForest and Van Genderen, 2012). Further, different life stages of a given organism often are differentially susceptible to metal toxicity, with immature or younger life-stages frequently being more sensitive than their adult counterparts. In short, in evaluating the potential toxicity of a metal to an aquatic ecosystem, the wide range of sensitivities and life stages of potentially resident organisms should be considered. Illustrating this point, the derivation of metal-specific WQC typically relies on the development of species-sensitivity distributions for a given metal, with the criteria ultimately designed to be protective of all but a small percentage of organisms with toxicology data in the database.

### 2.5. Role of metal speciation

The chemical speciation of metals in water is of great importance in determining their reactivity, transport, fate, bioavailability, and potential toxicity. Templeton et al. (2000, p. 1456) define chemical species of chemical elements as the “specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.” The chemical parameters that define various metal species are the identity of the metal, oxidation state, chemical formula, molecular structure, and physical state. Metal speciation constrains potential exposure pathways of metals to biota and controls metal bioavailability and toxicity. Some processes and geochemical conditions can redistribute cationic dissolved metals in the environment (Fig. 1). Because BLMs considered in this review deal exclusively with dissolved systems, the remaining discussion focuses on aqueous speciation. There are many water-quality parameters that can substantially alter metal speciation, bioavailability, and toxicity including pH, DOC, temperature, alkalinity ( $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ), hardness ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ),

**Table 2**

Species mean acute values (SMAV; as µg/L) for five commonly tested aquatic laboratory organisms. Superscript numbers indicate the relative sensitivity ranking of each organism (among these five species) for each given metal.

Species	Zn <sup>a</sup>	Cu <sup>b</sup>	Cd <sup>c</sup>
<i>C. dubia</i>	174 <sup>(1)</sup>	5.8 <sup>(3)</sup>	31.4 <sup>(4)</sup>
<i>D. pulex</i>	253 <sup>(2)</sup>	2.5 <sup>(1)</sup>	46.4 <sup>(5)</sup>
<i>D. magna</i>	356 <sup>(3)</sup>	5.0 <sup>(2)</sup>	13.4 <sup>(2)</sup>
<i>P. promelas</i>	3830 <sup>(5)</sup>	72.0 <sup>(5)</sup>	29.2 <sup>(3)</sup>
<i>O. mykiss</i>	689 <sup>(4)</sup>	21.6 <sup>(4)</sup>	2.1 <sup>(1)</sup>

<sup>a</sup> Zn data from USEPA (1995).

<sup>b</sup> Cu data from USEPA (2007).

<sup>c</sup> Cd data from USEPA (2001).

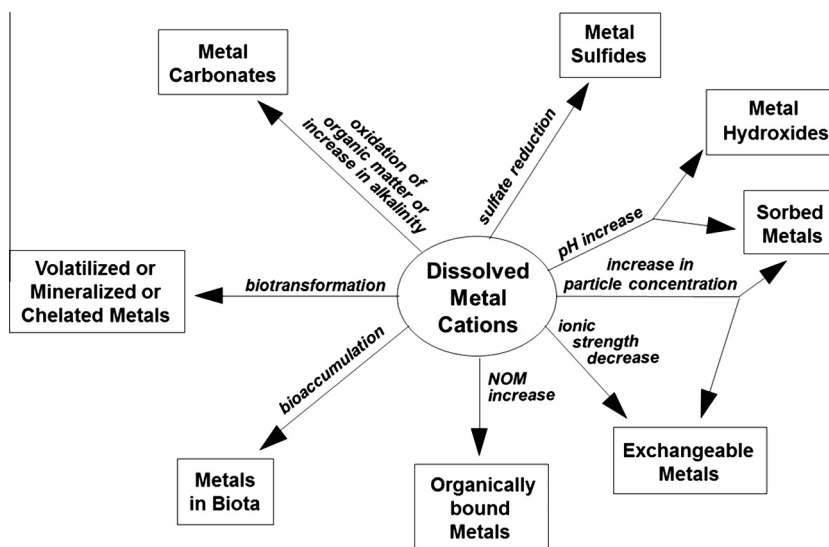


Fig. 1. Some processes and geochemical conditions that can redistribute cationic dissolved metals (from Smith and Huyck, 1999).

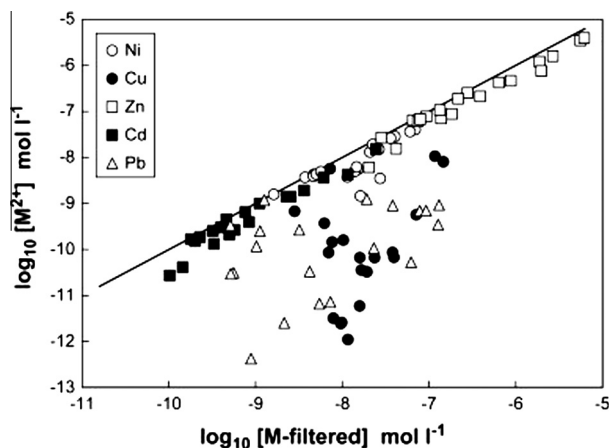


Fig. 2. Free ion concentrations of Ni, Cu, Zn, Cd, and Pb compared with total filtered concentrations (with permission from Tipping et al., 2008, Fig. 1).

salinity ( $\text{Na}^+$ ,  $\text{Cl}^-$ ), inorganic ligands, and interactions with sediments (e.g., Pagenkopf et al., 1974; Wang, 1987; Janes and Playle, 1995; Wood et al., 1999).

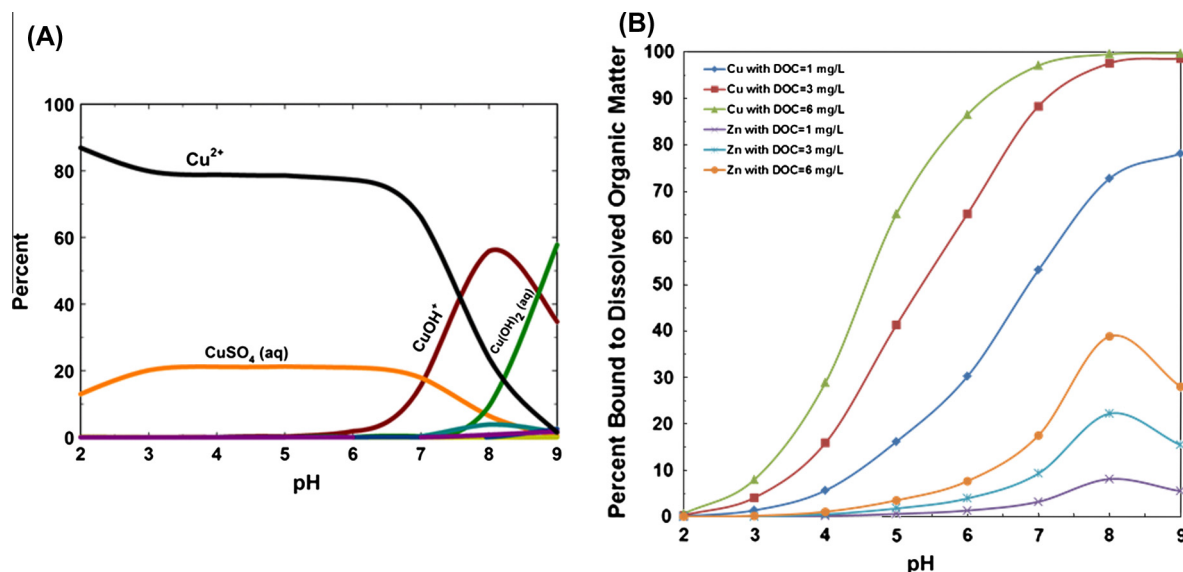
#### 2.5.1. Free-ion activity model

Generally, free-ion ( $\text{M}^{2+}$ ) concentrations of dissolved metals are the best indicator of bioavailability and toxic effects to aquatic organisms (O'Donnel et al., 1985). Morel (1983) and Morel and Hering (1993) formulated the free-ion activity model (FIAM), which demonstrates that the free-metal ion activity reflects the overall reactivity of the metal. This reactivity is a major control on metal bioavailability and toxicity. The FIAM does not assume that the free-metal ion is the only bioavailable (or toxic) metal species. Instead, the FIAM states that biological response is proportional to the free-metal ion activity in solution. The BLM approach is based on the FIAM and uses geochemical equilibrium models to estimate the amount of metal bound to a biotic ligand (BL; e.g., fish gill) for a particular water composition. In aqueous systems metals are present as various inorganic and organic complexes. Using Cu as an example, the free ionic form ( $\text{Cu}^{2+}$ ) and possibly some small inorganic complexes (e.g.,  $\text{CuOH}^+$ ) can pass through biological membranes (e.g., Sunda and Lewis, 1978;

De Schamphelaere et al., 2002), and toxicological studies have demonstrated a direct relationship between free  $\text{Cu}^{2+}$  ions and toxicological effects (e.g., Sunda and Guillard, 1976; Winner and Gauss, 1986).

Campbell (1995) provided a critique of the FIAM and highlights some possible exceptions to the FIAM when applied to natural systems. In particular, he points out potential problems in systems containing natural dissolved organic matter (DOM), both due to difficulties in determining metal speciation in these systems and due to observed direct interactions between DOM and aquatic organisms that are not taken into account in the FIAM. He also commented on the need to determine conditional constants for DOM–metal complexes at biologically relevant ratios, as outlined by Buffle et al. (1990).

Determining aqueous metal species is somewhat problematic because analytical laboratories generally report aqueous metal concentrations as total or total filtered ('dissolved'; operationally defined by filtration procedures). To determine metal speciation, it is necessary to either compute or directly measure the various species. Although direct measurement techniques are being developed, there is no 'cookbook' method (Batley et al., 2004; Sigg et al., 2006; Pesavento et al., 2009; Sturgeon and Francesconi, 2009; Hamilton-Taylor et al., 2011). There are several thermodynamically based chemical-equilibrium models that can compute metal speciation. For example, MINTEQA2 (Allison et al., 1991) and PHREEQC (Parkhurst and Appelo, 1999) are commonly used computer programs, but they either do not include metal binding with organic matter or have a limited ability to consider metal binding with organic matter. In contrast, Visual MINTEQ (Gustafsson, 2012) includes several choices for metal binding with organic matter in addition to the original choices from MINTEQA2. Several models have been developed that include multiple metal-binding sites for organic matter. For example, the Windermere Humic Aqueous Model (WHAM) incorporates the humic ion-binding model (Tipping, 1994, 1998). The WHAM version V model is included in the BLM. A plot of computed free-ion concentrations versus measured total filtered concentrations for Ni, Cu, Zn, Cd, and Pb using WHAM version VI (Tipping et al., 2008) illustrates that different metals exhibit different amounts and types of aqueous speciation, and that total 'dissolved' metal concentrations often do not reflect the potentially bioavailable fraction of metals (Fig. 2). When using programs to compute speciation, it is important to be aware of the



**Fig. 3.** Copper speciation with inorganic ligands as a function of pH (A) and binding of Cu and Zn to dissolved organic carbon (DOC) as a function of pH (B) in the Capulin leachate from waste rock at the Questa mine site, New Mexico (composition given in Table 3).

**Table 3**

Chemical analysis for Capulin mine-waste leachate (18-h test) from waste rock at the Questa mine site, New Mexico. Data from Smith et al. (2007, Table 4).

Constituent	Value
pH	4.1
Al (mg/L)	1.7
Ca (mg/L)	100
Cd (μg/L)	1.4
Cu (μg/L)	100
Fe (μg/L)	<50
K (mg/L)	1.4
Mg (mg/L)	4.1
Na (mg/L)	1.2
Zn (μg/L)	326
Chloride (Cl) (mg/L)	<0.6
Sulfate ( $\text{SO}_4$ ) (mg/L)	310

possible limitations. Failure to include all of the important chemical elements and species, consider all of the important reactions, and verify the validity of any inherent assumptions (i.e., thermodynamically versus kinetically controlled reactions) may lead to erroneous conclusions. Turner (1995) discussed problems in modeling trace metal speciation. Hering (2009) provided an overview of metal speciation and bioavailability assessment and cautions that BLM data need to be scrutinized for confounding factors.

### 2.5.2. Metal concentration and pH

As previously noted, total metal concentration often has little to do with metal bioavailability and toxicity (see Fig. 2). Instead, it is the bioavailable forms (primarily the free metal ion) that correspond to metal bioavailability and toxicity. The bioavailable forms are a function of the water composition, presence of inorganic and organic ligands that bind with the metals, the presence of competing ions, and the properties of the metals themselves (see Smith and Huyck, 1999; Smith, 2007). In natural fresh waters, sulfate ( $\text{SO}_4^{2-}$ ), phosphate ( $\text{PO}_4^{3-}$ ), and fluoride ( $\text{F}^-$ ) tend to be important ligands under acidic conditions, and carbonate ( $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ) and hydroxide ( $\text{OH}^-$ ) complexes become increasingly important with increasing pH values (Nordstrom and Munoz, 1994; Stumm and Morgan, 1996; Langmuir, 1997). Fig. 3A illustrates Cu speciation with inorganic ligands as a function of pH for a mine-waste leachate; Table 3 provides the composition of this leachate. Cu

complexation with sulfate is important under low-pH conditions, and hydroxide complexes become important with increasing pH values (Fig. 3A). Metal binding with DOM (Fig. 3B) can also be very important and will be discussed in later sections.

Many waters in mined and mineralized areas are impacted by acidification, and low-pH conditions can be detrimental to biota. Hirst et al. (2002) noted that it is difficult to distinguish between the effects of dissolved metals and the effects of acidification. Campbell and Stokes (1985) discussed two types of behavior in response to acidification: (1) Type I (Cd, Cu, Zn) results in  $\text{H}^+$  competition for binding sites or  $\text{H}^+$  effects on membrane potential, which occurs when the proportion of the free-metal ion is low and metal binding at biological surfaces is weak, and (2) Type II (Pb) results in an increase in metal bioavailability, which occurs when pH decrease strongly affects metal speciation and metal binding at biological surfaces is strong. O'Halloran et al. (2008) studied the effect of different pH values and acid-mine drainage (AMD) on the survival of a type of mayfly (*Deleatidium* spp.) in 96-h laboratory trials. Their results showed that the primary driver of toxicity was pH, and that the chemistry of the natal stream strongly influenced the sensitivity of the mayflies to AMD. Turnpenny et al. (1987) reported that the absence or scarcity of salmonids correlated with high levels of metal toxicity due to low-pH conditions. Reports on the effect of low pH on metal bioavailability and toxicity in the literature differ between metals and between biota (e.g., Campbell and Stokes, 1985; Gerhardt et al., 2005). Several researchers have attempted to partition the effects of metals and pH (e.g., Dsa et al., 2008; O'Halloran et al., 2008), but the details warrant further study.

The pH (and  $\text{H}^+$  ion) plays a dual role in metal toxicity. First, it has a role in metal speciation, both from a metal hydrolysis standpoint and from its competitive role in metal binding to various ligands, especially organic matter. Second, the  $\text{H}^+$  ion can compete with metals for binding at the BL. Seemingly contradictory results reported in the literature may be at least partially due to this dual role. Other factors that may be important involve interactions between dissolved and particulate phases; these interactions commonly are not considered in toxicological studies and are not included in BLMs. For example, decreasing pH may release metals sorbed to particulate phases and make the metals bioavailable in the dissolved phase. This mechanism has been demonstrated by

Stillings et al. (2008). Alternately, increasing pH may cause metal sorption onto particulate phases (see Smith, 1999). Metal-solubility reactions also are pH-dependent and can release or remove metals to or from the dissolved phase. Hence, in order to adequately understand (and model) metal toxicity in natural systems, especially dynamic systems such as waters influenced by mining or mineralization, it is necessary to consider the whole system, not merely the dissolved phases.

## 2.6. Metal complexation by natural organic matter

Natural organic matter (NOM) is a diverse mixture of organic molecules that is ubiquitous in natural water (Thurman, 1985; Świątlik et al., 2004). NOM can form complexes with dissolved metals, thereby reducing their bioavailability and/or toxicity to aquatic organisms. For example, Richards et al. (2001), De Schampelaere et al. (2004), and Luiders et al. (2004) demonstrated that NOM reduces metal bioavailability in proportion to its concentration in water. Consequently, an accurate depiction of metal binding with NOM is critical to predictive modeling of metal bioavailability and toxicity. The importance of NOM complexation with metals varies with the metal. This concept is illustrated in Fig. 3B for Cu and Zn. Note the stronger binding for Cu compared with Zn.

NOM molecules are derived from terrigenous sources (also known as allochthonous; contain many aromatic groups, have larger molecules, generally dark in color) and/or are produced within a water body (also known as autochthonous; contain few aromatic groups, have smaller molecules, generally light in color) (McKnight et al., 2001). There is a correlation between various optical parameters of DOM and its degree of protection against metal toxicity such that optically dark DOM appears to be more protective against metal toxicity (e.g., Al-Reasi et al., 2011, 2012, 2013). Because it is so complicated, NOM has traditionally been classified according to various physical properties, such as dissolved versus particulate, allochthonous versus autochthonous, hydrophobic versus hydrophilic, and degree of acidity. The polarity and acidity properties are determined by retention on resins. An arbitrary filter-size cutoff of 0.45  $\mu\text{m}$  has been used to define DOM. Perdue and Ritchie (2003) provided an overview of DOM in freshwater systems.

The concentration of NOM in water is measured by complete oxidation, using either high-temperature combustion or UV photo-oxidation in the presence of a strong oxidizing agent, which converts all the organic material to carbon dioxide. Consequently, the concentration of NOM in water is expressed in terms of carbon; unfiltered samples are reported as total organic carbon (TOC) and filtered samples are reported as DOC. DOC generally accounts for about 50% of DOM, and DOC concentrations generally range between 1 and 15 mg C/L in natural water (Thurman, 1985; Mulholland, 1997). Aiken et al. (2002) reported some difficulties in organic carbon analyses based on results from an interlaboratory comparison. They discussed the importance of complete removal of inorganic carbon prior to DOC analysis, and they report that the skill of the analyst is crucial in obtaining accurate data. Therefore, the choice of the laboratory and analyst is an important consideration for DOC data. In addition, proper sampling, preservation, and storage of DOC samples are essential to minimize errors (Kaplan, 1994). Contamination from sampling equipment, such as filter membranes, is an important consideration (Karanfil et al., 2003). Accepted sampling, preservation, storage, and analytical procedures need to be adopted to obtain accurate DOC determinations.

Geochemists have historically focused on humic substances (HS; organic material extractable onto resins), which were fractionated into two classes based on solubility at low pH:

1. Humic acid (HA), which precipitates at  $\text{pH} \leq 2$  and contains larger molecules, and

2. Fulvic acid (FA), which is soluble at low pH and contains smaller molecules.

These two fractions exhibit different binding affinities for different metals.

Modeling of metal complexation by NOM has progressed from one or two binding-site fits to empirical data to representations of electrostatic interactions and binding-site heterogeneity that can depict metal binding to NOM over a range of geochemical conditions. There are two main approaches to modeling metal binding to NOM: (1) a discrete distribution approach (WHAM; Tipping, 1994, 1998), and (2) a continuous distribution approach (NICA-Donnan; Kinniburgh et al., 1996). The BLM incorporates WHAM to characterize metal binding to NOM, which treats HA and FA differently. The BLM has an adjustable parameter for percent HA; the default recommendation is 10% HA and 90% FA. Ryan et al. (2004) found a strong correlation between toxic effects and measured HA. However, they found that varying the %HA from 1% to 100% in the BLM input had almost no influence on the relationship between predicted and observed LC50 values.

A topic of current research is the relative importance of DOC concentration (i.e., DOC quantity) versus DOC composition (i.e., DOC quality, which relates to the source of the DOC) for metal complexation. For example, Luiders et al. (2004) examined Cu toxicity to rainbow trout in the presence of NOM from different sources and concluded that both the NOM metal-binding quality and the NOM quantity are important in assessing Cu bioavailability. Baken et al. (2011) compared Cd, Cu, Ni, and Zn affinity for several different DOC samples from various sources and found that anthropogenically influenced samples had a significantly larger metal-binding affinity than did DOC samples from natural sources. Hence, DOCs from different sources can have very different abilities to reduce metal bioavailability and toxicity. De Schampelaere et al. (2004) suggested that BLMs be corrected for DOC aromaticity through a UV-absorbance based correction, and other researchers have suggested a modifying factor that is dependent on optical properties of the DOC (e.g., Schwartz et al., 2004). There has been some debate as to whether 100% of DOC should be assumed to be metal reactive. For example, Welsh et al. (2008) found that assumptions regarding the nature and reactivity of the DOM in their flow-through toxicity tests were important in Cu toxicity predictions. Several researchers have noted that less than 100% of the DOM in natural systems is metal-reactive, with the metal-reactive percentage ranging between 40% and 80% (e.g., McKnight et al., 1983; Dwane and Tipping, 1998; Bryan et al., 2002).

Another topic of current research is the direct interaction of DOM with biological surfaces. Sorption of DOM onto biological surfaces/membranes has been reported to be pH-dependent and more prevalent under acidic conditions (e.g., Campbell et al., 1997). Galvez et al. (2008) reported that DOM has direct actions on the ionic transport and/or permeability properties of fish gills. Elayan et al. (2008) conducted a molecular-level study of the sorption of HA to model membranes and found that the HA-membrane interactions appear to be a combination of hydrogen bridging and hydrophobic interactions that involve a minimum of two steps. Gheorghiu et al. (2010) concluded that their observed differences in Cu toxicity to *O. mykiss* in the presence of different types of DOM likely are due to DOM-quality-dependent direct actions of DOM on the gills. Hence, the direct interaction of DOM with biological surfaces can result in a number of physiological effects that are not taken into account in speciation approaches such as the BLM. These direct effects of DOM can be of critical importance in ecological understanding and risk assessment (Galvez et al., 2008), and at least some of the observed protective effects of DOM may be due to direct beneficial effects on organism physiology (Wood et al., 2011). However, DOM is not always beneficial to organisms (e.g., Steinberg et al., 2006).

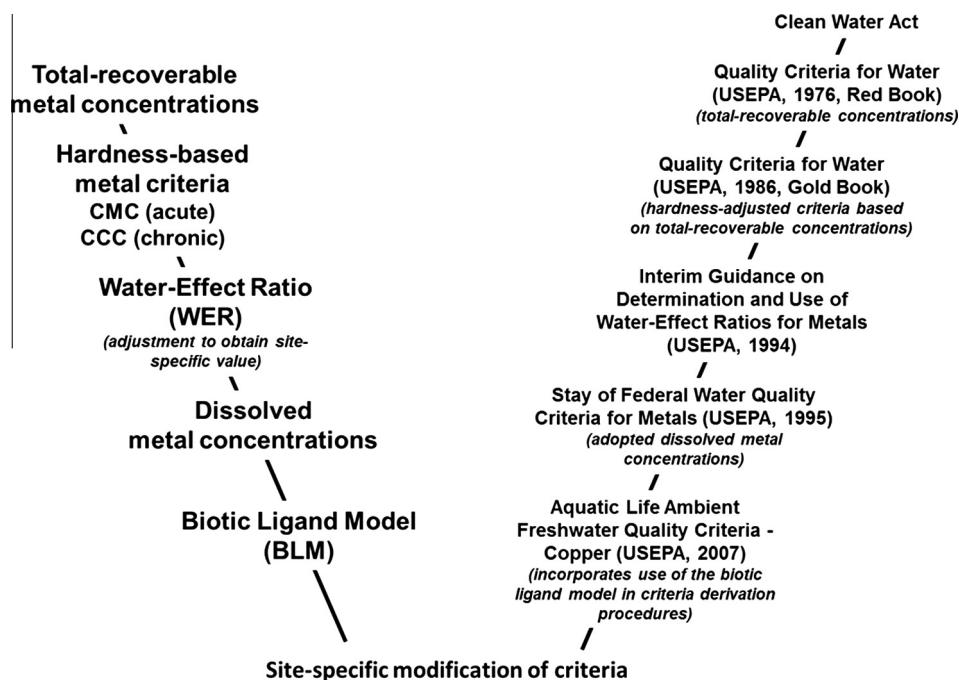


Fig. 4. Evolution of USEPA's numeric aquatic life criteria (ALC) for metals (prepared with information from Reiley, 2007).

Table 4

Comparison of hardness-based Cu Criterion Maximum Concentration (CMC) values with biotic ligand model (BLM) based Cu CMC values for varying concentrations of dissolved organic carbon (DOC) at pH 7.0 and hardness of 80 mg/L CaCO<sub>3</sub>. Data excerpted from USEPA (2007, Appendix G).

DOC (mg/L)	Hypothetical hardness-based CMC (μg/L)	BLM-based CMC (μg/L)
2	11.3	4.4
4	11.3	8.8
8	11.3	18.0
16	11.3	37.0

### 3. Evolution of metal water quality criteria

Over the past 35 (plus) years USEPA's WQC have evolved from 'one size fits all' national criteria to allowances for site-specific conditions. Some of the main steps of the evolution of USEPA's WQC (Fig. 4) and the following discussion highlight several of those steps in greater detail.

#### 3.1. Hardness-based criteria

Since very early in their development, aquatic life WQC for most metals have recognized and accounted for the influence that water hardness has on metal toxicity. Specifically, by compiling metal concentration and other water quality data (including water hardness) from metal toxicity studies conducted across wide ranges of hardness (e.g., Bradley and Sprague, 1985), comparison of toxic metals concentrations versus hardness have yielded regressions that typically illustrate an ameliorative relationship between increasing hardness and resultant metal toxicity (Stephan et al., 1985). Indeed, most current metals criteria are not single numbers, but instead are hardness-dependent equations (e.g., USEPA, 2013) designed to be protective across a range of water hardness. From a toxicology standpoint, the ameliorative effect of increasing hardness reflects increasing concentrations of physiologically essential cations (e.g., Ca<sup>2+</sup> and Mg<sup>2+</sup>) relative to concentrations of competing metal ions. Importantly, however, in many of these same

source studies where hardness varied, other factors co-varied with hardness (e.g., alkalinity, pH), and so the resultant correlations reflect the cumulative relationship of metal toxicity with overall exposure water chemistry (e.g., Bradley and Sprague, 1985). Further, many of these early studies did not measure or report the levels of constituents now known to influence the toxicity of specific metals (e.g., DOC and Cu toxicity) (USEPA, 2007), constituents that are frequently present in natural and sites waters. Table 4 provides a comparison of hardness-based WQC versus BLM-based WQC for Cu over a range of DOC concentrations. Note that the hardness-based WQC remain constant because they do not consider DOC concentration whereas the BLM-based WQC range from values far less than the hardness-based WQC to values significantly greater than the hardness-based WQC.

#### 3.2. Water-effect ratio

Acknowledging that many of the physical and chemical characteristics known to influence the toxicity of metals in surface waters were not taken into account with hardness-based aquatic criteria, the USEPA developed guidance in the early 1980s that could be used to resolve observed differences in toxicity between laboratory dilution waters and site waters (USEPA, 1994). The water-effect ratio (WER) approach calculated a ratio by dividing toxicity (acute LC50) measured in site water with that measured in laboratory water adjusted to a similar hardness, with the hardness-based acute metal criteria multiplied by this resultant ratio to yield a site-specific metal criterion. In theory, this lab-based approach factored in the important influences of water quality constituents present in the site water but not recognized by the hardness-based criterion. In practice, however, the approach was vulnerable to producing artificially high multiplicative ratios, depending on whether the laboratory waters were a good match to laboratory waters used in the USEPA criterion to be modified, among other factors (Welsh et al., 2000).

#### 3.3. Biotic ligand models

Limitations of the hardness-based criteria and WER necessitated the development of a new approach, a BLM, that (1) explicitly

links chemical speciation, bioavailability of metals, and WQC, and (2) is cost effective and easily incorporated into regulatory frameworks. Early research on the FIAM and gill site interaction model provided the conceptual and quantitative framework for BLMs (Sunda et al., 1978; Pagenkopf, 1983; Morel and Hering, 1993; Paquin et al., 2002). These early models recognized the important role of chemical speciation, particularly the activity of free metal ions, in determining metal bioavailability and toxicity, and that toxicity to fish could be viewed as the interaction of metals with anionic surface sites of the gill. The BLM couples chemical speciation calculations with toxicological information to produce WQC (Di Toro et al., 2001), and was proposed as an alternative to expensive WER toxicological testing. In 2007, the USEPA issued new aquatic life ambient freshwater quality criteria for Cu that includes a BLM and new information on the toxicity of Cu (USEPA, 2007). Currently, Cu is the only metal that has a BLM incorporated into its aquatic life criteria (ALC), although aquatic BLMs for other metals (Ag, Cd, Pb, Ni, and Zn) are in development (Niyogi and Wood, 2004).

BLMs have three primary components: (1) thermodynamic speciation calculations that determine the distribution of dissolved chemical species among their free and complexed forms, (2) relationships between the physiology of organisms, uptake of metal by biological receptors, and metal toxicity, and (3) in one case to date (USEPA, 2007), prediction of site-specific WQC based on defined ecological or toxicological effects. The following discussion addresses each of these components; first from a conceptual perspective, then from an operational perspective.

### 3.3.1. The BLM—A conceptual view (Fig. 5)

Water contains dissolved cations, such as  $H^+$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ , and metal ( $Me^{2+}$ ) ions, as well as dissolved inorganic and organic ligands, such as hydroxyl ( $OH^-$ ), bicarbonate ( $HCO_3^-$ ), carbonate ( $CO_3^{2-}$ ), sulfate ( $SO_4^{2-}$ ), chloride ( $Cl^-$ ), and sulfide ( $HS^-$ ) ions, and DOM. In BLMs, there are additional ligands that represent biological receptors, i.e., biotic ligands ( $BL^-$ ). These BLs are considered to be on the surface of an organism, such as a fish gill, so they can interact with dissolved ions. The BLs are not necessarily sites of toxic action for the organism; however, accumulation of metal on the BLs is related to toxicological effects. Dissolved cations

interact with inorganic, organic, and BLs forming inorganic complexes (e.g.,  $NaHCO_3$ ,  $CuOH^+$ ,  $CaCO_3$ ), organic complexes (e.g.,  $Ca-DOM$ ,  $Cu-DOM$ ), and cation-BL complexes (e.g.,  $CuBL^+$ ,  $MgBL^+$ ). The distribution of a dissolved chemical species (e.g., Cu) among its free ( $Cu^{2+}$ ) and complexed (e.g.,  $CuOH^+$ ,  $CuCO_3$ ,  $Cu-DOM$ ,  $CuBL^+$ ) forms depends on the chemical conditions (temperature, pH, concentrations of cations and ligands) of the solution and the binding strength or relative affinity between given cations and ligands.

Of interest, from a toxicological perspective, is what factors control the chemical speciation of the BL. This speciation is affected by solution composition (pH, types and concentrations of cations), competition among cations for a BL, and complexation of cations with the BL and with other inorganic and organic ligands in solution. Major cations (e.g.,  $Ca^{2+}$ ), which have concentrations that are generally greater than metal concentrations, can out-compete trace metals for BLs. The DOM typically binds Cu and some other metals more strongly than the BL and, thereby, prevents the metal from binding with the BL or reaching the site of toxic action. Depending on the relative affinity of the metal for inorganic and biotic ligands, the formation of dissolved metal-inorganic complexes also may inhibit binding between metals and the BL.

In addition to chemical speciation, another component of BLMs is the relationship between chemical speciation and toxicity. Within BLMs, specific ecological and toxicological effects (e.g., mortality) are related to the chemical speciation of the BL or the amount of metal bound by the BL. For example, LA50 values are the lethal accumulation of metal on the BL at which 50% of organisms die after a fixed exposure time. A key operational component within BLMs is that the accumulation of metal on the BL that is related to a specific effect (e.g., LA50) is independent of solution composition. In USEPA's development of the BLM for Cu, this cause-effect relationship between a threshold level of metal accumulation and mortality was documented for at least two organisms with which it was possible to directly measure accumulation at the BL (e.g., fathead minnow and rainbow trout gills) (USEPA, 2003). However, due to the absence of accumulation studies for the majority of species within databases used to calculate WQC, it is assumed the accumulation/toxicity relationship (and resultant BLM input parameters) observed with these select organisms are valid across a wide range of other, unmeasured

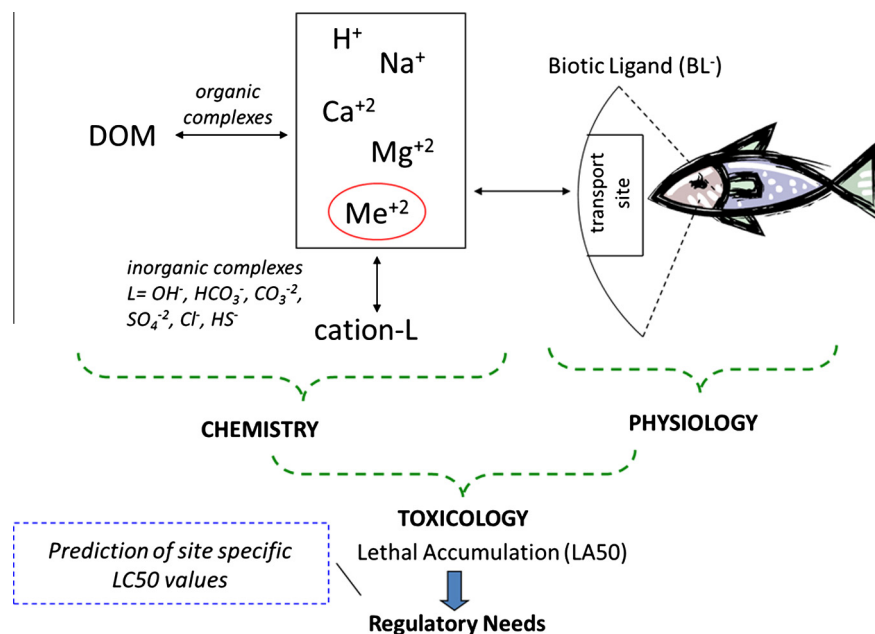


Fig. 5. Illustration of the three components of biotic ligand models (BLMs; see text for discussion; modified from Paquin et al., 2002).

organisms. The LA50s for these other organisms are typically calculated by relating experimental water chemistry and observed mortality (LC50s) through the speciation mode of the BLM. This assumption is critical for the last component of BLMs, i.e., prediction of site-specific WQC. It follows that these criteria (e.g., LC50 values) are predicted by determining the concentration of dissolved metal at a given field site that results in the lethal accumulation of metal on the BL (i.e., LA50).

### 3.3.2. The BLM—The operational view

One primary component of the BLM is thermodynamic calculations that partition dissolved elements among their free and complexed forms. Two chemical equilibrium programs—CHESS (Santore and Driscoll, 1995) and WHAM V (Tipping, 1994)—as well as metal-organism parameter files that include reactions and equilibrium constants for cation interactions with BLs are used in the BLM along with dissolved concentrations of organic carbon, cations, anions, and BLs to calculate chemical speciation. The reactions and equilibrium constants necessary for the calculations and the BL concentration already have been summarized and are included in the model. The required input information includes pH, temperature, dissolved concentrations of the metal of interest, major cations (Na, K, Ca, Mg), major anions (Cl, SO<sub>4</sub>), DOC, an estimate of the percent humic (versus fulvic) acid fraction of organic matter, alkalinity, and sulfide. Although metal–sulfide complexes may be important in certain oxic environments, there are limited measurements of sulfide concentrations in such systems, and, presently, sulfide only is a placeholder for future versions of the model.

The second component of the BLM is the relationship between the amount of metal that binds with the BL and a specific endpoint for toxicity. In the BLM, LA50 values are included in the metal-organism parameter files and are based on previously conducted experiments (Paquin et al., 2002).

The final component in the BLM is the prediction of site-specific LC50 values. The BLM calculates the concentration of dissolved metal that results in the lethal accumulation of metal on the BL (i.e., LA50 values).

The research version of the BLM has three modes of operation. The speciation mode calculates the chemical speciation of elements among their various chemical forms (free ions, inorganic complexes, organic complexes, and BL complexes) for a site-specific solution composition. The toxicity mode predicts acute toxicity as a LC50 value. Once again, the LC50 value is for a site-specific solution composition. The third operational mode is prediction of WQC for Cu, and only Cu (i.e., no other metals, at present). This mode gives Final Acute Value (FAV), Criterion Maximum Concentration (CMC = FAV/2), Criterion Continuous Concentration (CCC = FAV/ACR, where ACR is the Acute to Chronic Ratio and equal to 3.22 for Cu), and Acute Toxic Unit (TU = observed Cu concentration/CMC) for the site-specific solution composition (HydroQual, Inc., 2007).

Thus, in order to run the research version of the model, the user must input the complete solution composition, choose the metal and organism of interest (i.e., metal-organism parameter file), and choose the mode of operation. The program checks that all input concentrations fall within the range for which the BLM has been calibrated (Table 5). Input concentrations are highlighted in red if they fall outside of the calibrated range. It should be noted that input concentrations for many mined and mineralized sites exceed the concentration ranges that were used to develop and calibrate the BLM for Cu. For example, the lower pH limit of calibration for the BLM is 4.9 and many mine-drainage sites have pH values below pH 4.9. Furthermore, acid rain in the northeastern United States commonly is lower than this pH value. Another common exceedance is that sulfate concentrations at mine-drainage sites are commonly greater than the calibration limit of

278.4 mg/L. Likewise, the upper limit for DOC calibration is 29.65 mg/L and surface waters in high-latitude wetland settings, such as those found in northern Minnesota, Maine, and Alaska, can locally exceed this concentration.

Once run, the model output includes two files. One is a detailed summary of the element concentrations and speciation of the solution, whereas the other is a subset of the information that is relevant to most users. If the model is run in the WQC mode for Cu, a third file containing FAV, CMC, CCC and TU is generated. More detailed information is available in the user manual for the BLM (HydroQual, Inc., 2007). Although not explicitly detailed in the user manual, numerous other user modifications can be made within the “Research Mode” of the HydroQual software by changing the parameter files. These include parameters such as modifying the fraction of DOC that is active to bind with metals, which biotic ligand binding constants to include, binding affinity values, binding site density, and critical accumulation values. Many of the numerous publications with new BLMs or new critical accumulation values for new species or sublethal endpoints have done so by modifying the parameter files for the HydroQual, Inc. (2007) software, and its previous versions (e.g., De Schampelaere et al., 2002, 2005; Paquin et al., 2011; Wang et al., 2011).

### 3.3.3. Assumptions within the BLM

There are several important assumptions inherent in the use of the readily available versions of the BLM.

1. The model only considers dissolved equilibria. It does not consider mineral precipitation or other interactions between dissolved and particulate phases (e.g., adsorption).
2. The calculations assume that the system is at chemical equilibrium, i.e., kinetic reactions are not important.
3. The model only considers one metal and one organism at a time. No metal mixtures or multiple aquatic organisms can be modeled at the same time.
4. The equilibrium constants among a given metal, major cations, hydrogen ions, and BLs are the same for all organisms. The LA50 values, which are included in the metal-organism parameter files and do not change with solution composition, account for variations in the sensitivity of organisms to a given metal. For example, the binding constants for Cu interactions with BLs of water fleas and fish are the same, but the LA50 values for water fleas (*C. dubia* and *D. magna*) are lower than those for rainbow trout (*O. mykiss*) and fathead minnows (*P. promelas*) because water fleas are more sensitive to Cu concentrations than are fish.
5. The BLM is calibrated for specific ranges of element concentrations (Table 5). Input chemical concentrations that fall outside of the calibrated range are highlighted in red in the input file of the BLM.

### 3.3.4. The BLM and supporting documentation

Two versions of the HydroQual BLM software are available. The research version is obtained from the HydroQual website ([http://www.hydroqual.com/wr\\_blm.html](http://www.hydroqual.com/wr_blm.html)) and has the capability to output solution speciation data, LC50 values for a variety of organisms and metals, and WQC calculations (e.g., Final Acute Values (FAV), Criterion Continuous Concentrations (CCC), Criterion Maximum Concentrations (CMC), Acute Toxic Units (TU) for Cu). The version available from the USEPA only calculates the WQC for Cu.

## 3.4. Issues for use of the biotic ligand model in mineralized systems

### 3.4.1. Metal mixtures

Mixtures of aqueous metals are common in mined and mineralized areas, but the BLM allows only one metal and one organism to

**Table 5**

Calibration ranges for element concentrations used in the biotic ligand model (BLM) for speciation and toxicological computations.

Parameter	Lower boundary	Upper boundary
Temperature (°C)	10	25
pH	4.9	9.2
Dissolved organic carbon (DOC) (mg/L)	0.05	29.65
Humic acid content (%)	10	60
Ca (mg/L)	0.204	120.24
Mg (mg/L)	0.024	51.9
Na (mg/L)	0.16	236.9
K (mg/L)	0.039	156
Sulfate (SO <sub>4</sub> ) (mg/L)	0.096	278.4
Chloride (Cl) (mg/L)	0.32	279.72
Alkalinity (mg CaCO <sub>3</sub> /L)	1.99	360
Dissolved Inorganic Carbon (DIC) (mmole/L)	0.056	44.92
Sulfide (mg/L)	0	0

be considered at a time. It is likely that the dissolved metals compete for inorganic and organic ligands, and may or may not bind to the same sites on the ligands. Consequently, the presence of multiple metals can influence the overall toxicity of the water to aquatic organisms; some interactions may be additive and other interactions may be synergistic or antagonistic. Norwood et al. (2003) reviewed a number of studies dealing with the effects of metal mixtures on aquatic biota and found a wide range of results. Vijver et al. (2011) found that toxicological responses of organisms to metal mixtures were difficult to predict and were often slightly less than or slightly more than additive. However, they concluded that the assumption of additivity serves as a conservative prediction of multi-metal toxicity for the purposes of environmental risk assessment.

One approach to metal mixtures is the toxic unit (TU) modeling approach, which predicts additive toxicity of metal mixtures to aquatic organisms (Playle, 2004). Applications of this approach are discussed in the case studies below, and in Balistrieri et al. (2007) and Balistrieri and Blank (2008). Khan et al. (2012) successfully applied a TU approach to recovery of aquatic biota in lakes near Sudbury, Ontario. Schmidt et al. (2010) developed a TU model of additive metal toxicity derived from BLM outputs. Their approach allows for a broader ecological assessment of metal toxicity than single metal simulations.

Another approach for assessing toxicity of metal mixtures is to consider humic acid as an analogue for biological receptors (Stockdale et al., 2010, 2014). This approach uses the extensive database on metal interactions with natural organic matter and, thereby, considers multiple metal binding to an array of different binding sites as well as competitive interactions among toxicants (H and metal ions) for those sites. A toxicity function that incorporates the accumulation of multiple toxicants on humic matter and weighting coefficients for the toxicants is then related to biological health effects. Balistrieri and Mebane (2014) use a similar approach to account for the toxicity of metal mixtures to trout but use a two site-multiple metal BLM to evaluate toxicant accumulation on the biotic ligand. It is clear that developing models that apply to metal mixtures can be an important extension of the BLM (Paquin et al., 2002).

#### 3.4.2. Metal tolerance

Organisms living in areas with elevated metal concentrations can become tolerant to metals with prolonged exposure. This metal tolerance has been observed for bacteria (e.g., Bruins et al., 2000), plants (e.g., Monni et al., 2000), and aquatic biota (e.g., Klerks and Weis, 1987). Current research addresses whether this tolerance reflects genetic adaptation or acclimation to reduce metal sensitivity (e.g., Laurén and McDonald, 1987; Bossuyt and

Janssen, 2003; Lopes et al., 2004; Muysen and Janssen, 2005; Martins et al., 2009).

#### 3.4.3. Influence of dissolved Al and Fe on BLM predictions in mineralized systems

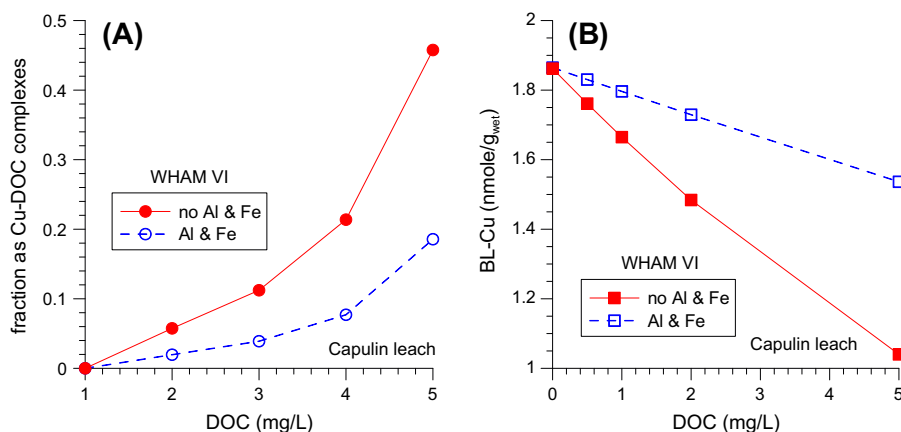
Available BLMs currently do not consider dissolved Al or Fe in the chemical speciation of natural waters. However, dissolved Al and Fe frequently are found in waters from mineralized systems, and these metals could compete with other cations (H<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>) and other metals (e.g., Cd<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>) for inorganic and organic ligands. Because the formation of metal–DOC complexes, particularly Cu–DOC complexes, is an important process that minimizes the amount of metal that binds with BLs, we now examine the influence of dissolved Al and Fe on chemical speciation in mineralized waters.

The chemical speciation of metals (Cd, Cu, and Zn) and loading of the BL by metal as a function of the concentration of DOC in the presence and absence of dissolved Al and Fe are evaluated using PHREEQC (Parkhurst and Appelo, 1999), WHAM VI (Tipping, 1998), and the composition of a mine-waste leachate (Smith et al., 2007). PHREEQC and WHAM VI, like the BLM, perform thermodynamic speciation calculations, and, unlike the currently available BLM, include reactions and equilibrium constants to determine the speciation of dissolved Al and Fe. PHREEQC is used to determine the partitioning of dissolved Fe between oxidized and reduced species for oxic conditions, which is then put into WHAM VI. Like WHAM V, which is incorporated into the BLM, WHAM VI is capable of partitioning cations among inorganic and organic species. The database of WHAM VI was modified to include a BL and reactions that describe the interactions between BLs and cations, which is similar to information in the metal-organism parameter files of the BLM. The model results only are shown for Cu because the fraction of Cd and Zn as metal–organic complexes was <0.014 for the given solution conditions.

The fraction of dissolved Cu as Cu–organic complexes increases as the concentration of DOC increases (Fig. 6A). At a given DOC concentration, the fraction of total dissolved Cu as Cu–organic complexes is greater in the absence of dissolved Al and Fe than in their presence because Al and Fe compete with Cu for organic ligand sites. Because a greater amount of Cu is bound by DOC in the absence of dissolved Fe and Al at a given DOC concentration, the amount of Cu binding to the BL is less than in the presence of dissolved Al and Fe (Fig. 6B). Thus, if dissolved Al and Fe concentrations are considered in the speciation calculations, the loading of the BL at a given DOC concentration is greater. This result implies that the predicted LC50 value would be lower in such a system, and that the presence of dissolved Al and Fe should be included in BLM calculations in mineralized waters.

#### 3.4.4. Temporal variability

Dissolved metal concentrations can show large temporal variations in natural waters (e.g., Nimick et al., 2011). One emerging challenge in the widespread implementation of the BLM in a regulatory setting is the influence of time-variability input on the BLM output. Although this problem is not unique to the BLM, as hardness-based WQC can also fluctuate with diel and seasonal variability of water hardness, the issue is somewhat more complicated with the BLM as the model output is influenced by multiple time-variable water quality parameters. An example of time-variable output is presented in Fig. 7, which shows the WQC Cu FAVs generated by the BLM using the Kansas River data file that is provided with the model as an example input file. Significant variability is exhibited in the amount of Cu that is predicted to be toxic at this site, ranging from a low of 50 µg/L to a high of more than 250 µg/L. This five-times difference in predicted toxicity threshold makes the development of a defensible fixed site-specific criterion



**Fig. 6.** The fraction of total dissolved Cu as Cu-organic complexes (A) and the loading of the biotic ligand by Cu (BL-Cu) (B) as a function of dissolved organic carbon (DOC) in the absence and presence of dissolved Al and Fe in the Capulin leachate from waste rock at the Questa mine site, New Mexico (composition given in Table 3).

very challenging, as that resultant number often has many implications, including the actual protectiveness of the final criterion for resident aquatic life, permit limits for entities discharging to that stream, and for the establishment of wasteload allocations in a total maximum daily load (TMDL) framework. As the BLM begins to be applied in the regulatory arena, approaches to address this time variability have been developed. For example, in response to Colorado's challenges with site-specific implementation of the Cu BLM, the USEPA commissioned the developers of the BLM to build a tool called the Fixed-Monitoring Benchmarks (FMB) approach (HydroQual, Inc., 2008). Briefly, the tool utilizes a probability-based approach to generate a "benchmark" value from the time-variable output to evaluate compliance with WQC. To date, application of this FMB approach has been limited.

#### 4. Studies related to mined and mineralized systems

##### 4.1. Application of the BLM in watersheds impacted by historical mining activities

Predicted toxicity of Cd, Cu, and Zn to several organisms [*C. dubia* and *D. magna* (water fleas), *P. promelas* (fathead minnow), and *O. mykiss* (rainbow trout)] is examined at two field areas that are affected by historical mining activities using the BLM, metal-organism parameter files supplied with the program, and site-specific water composition. It should be noted that the following results are dependent on the magnitude of binding constants for cations with biotic ligands and LA50 values, both of which are not always referenced for the organisms in the parameter files supplied with the BLM program. With this awareness, the objectives of the case studies are to illustrate (1) differences in dissolved chemical speciation of Cd, Cu, and Zn, and (2) differences in predicted toxicity for various metals and organisms at the sites. In addition, predicted toxicity using the BLM will be compared with field studies of organism survival. Previous publications present additional details about the studies conducted at the two sites (Balistrieri et al., 2007; Balistrieri and Blank, 2008).

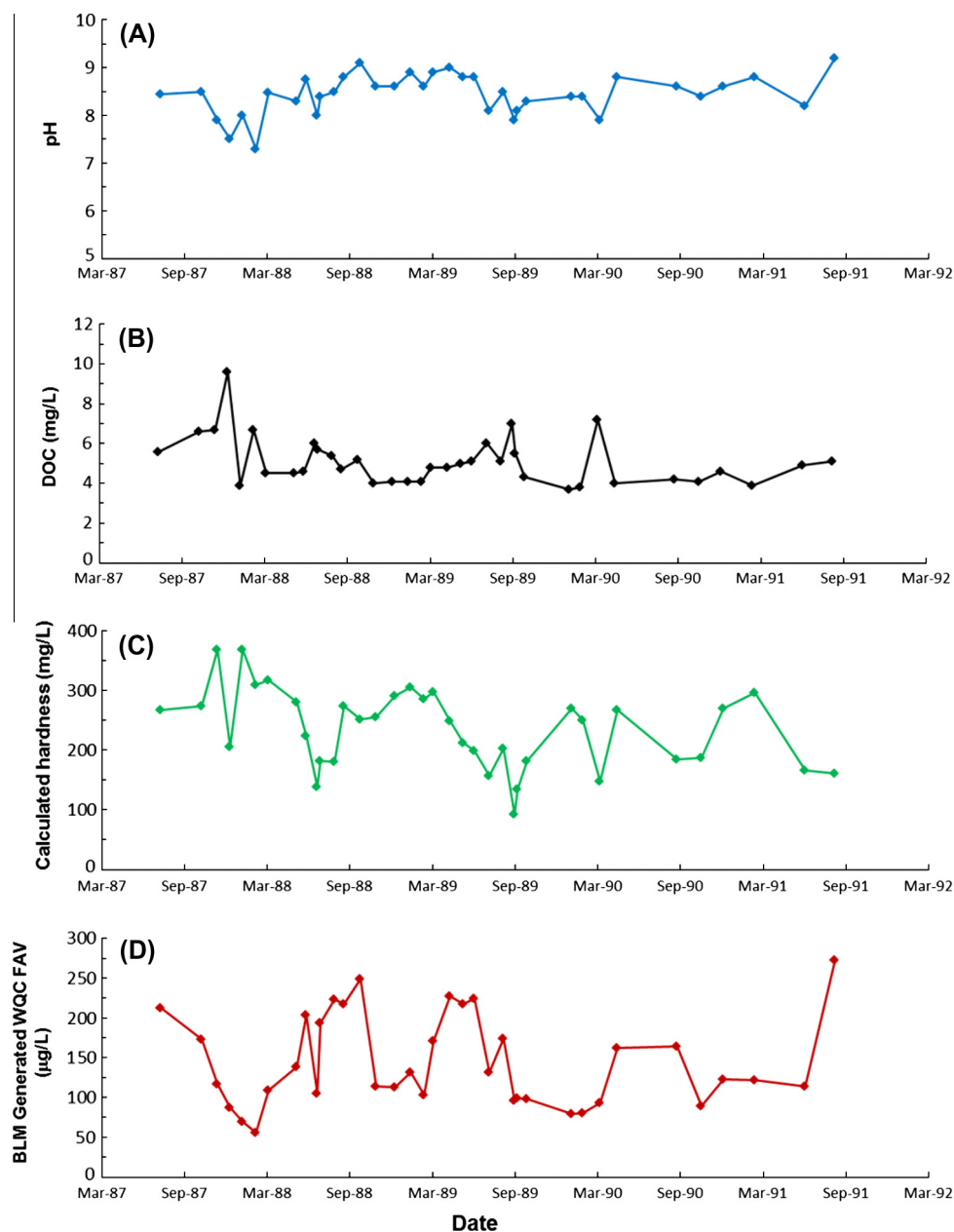
The first study area is the Coeur d'Alene River basin in northern Idaho (Fig. S1, Supplementary Material). The river flows through the Coeur d'Alene mining district where Pb–Zn–Ag-rich ores were mined, milled, and smelted. The legacy of this historical mining is metal-enriched sediment and water throughout the Coeur d'Alene River basin and detrimental impacts on the health of humans, wildlife, and fish (National Research Council, 2005). Due to high metal concentrations, fish are absent in some sections of Canyon and Ninemile Creeks and fish abundance is lower in the South Fork

Coeur d'Alene River compared to sites upstream of the mining district (Maret and MacCoy, 2002; National Research Council, 2005). Water samples were collected from six sites within the South Fork and main stem of the Coeur d'Alene River (1 above, 3 within, and 2 below the mining district) and from three tributaries within the mining district, including Canyon and Ninemile Creeks, and analyzed for the constituents needed by the BLM (Balistrieri and Blank, 2008).

The second study area is the West Branch of the Ompompanoosuc River, which is downstream from the Elizabeth Copper Mine Superfund site in east-central Vermont (Fig. S2, Supplementary Material). The primary ore minerals were pyrrhotite, chalcocite, and sphalerite. Acidic, metal-enriched water emerges from mine-waste rock and tailings piles forming Copperas Brook, which then flows into the Ompompanoosuc River. Water samples were collected in the river about 1 km upstream of the confluence with Copperas Brook, in Copperas Brook, and at four sites within the mixing and reaction zone just below the confluence of Copperas Brook and the Ompompanoosuc River. These samples also were analyzed for the constituents needed by the BLM (Balistrieri et al., 2007). Surface water toxicity studies indicated that 80–100% of fathead minnows and water fleas survived at a site upstream of the confluence, whereas there was little to no survival of these organisms in Copperas Brook or in the mixing and reaction zone 16 m downstream of the confluence (Fig. 8) (Hathaway et al., 2001).

Values of pH, water hardness, and concentrations of DOC and metals influence the amount of metal that binds to BLs. The Coeur d'Alene River basin sites have near neutral to slightly basic pH, low DOC concentrations (<0.7 mg/L), and water hardness that is soft to moderately hard (Fig. S3, Supplementary Material). The upstream site in the Ompompanoosuc River is slightly basic whereas Copperas Brook is very acidic and sites downstream of the confluence are acidic (Fig. S4, Supplementary Material). Concentrations of DOC in this system are low (1–1.4 mg/L; Fig. S4, Supplementary Material), but higher than observed in the Coeur d'Alene River basin. Water around the Elizabeth Copper Mine site is moderately hard to very hard. Samples collected above the mine sites in both study areas have the lowest concentrations of dissolved Cd, Cu, and Zn (Figs. S3 and S4, Supplementary Material). Dissolved Cd and Zn concentrations are comparable between the study areas, whereas Copperas Brook and the Ompompanoosuc River downstream of the mine site have much greater concentrations of dissolved Cu than the rivers within the Coeur d'Alene River basin (Figs. S3 and S4, Supplementary Material).

The BLM was run in the speciation mode to provide information about the distribution of metals among their free ions, inorganic



**Fig. 7.** Calculated pH (A), dissolved organic carbon (DOC) (B), hardness (C), and output of the biotic ligand model (BLM v. 2.2.3) for Cu run in Water Quality Criteria (WQC) mode (D) using the program's default water chemistry input file (Kansas River). Model results demonstrate significant variability in predicted toxicity resulting from significant variability in model-sensitive input parameters, such as those shown.

complexes, and organic complexes at the two sites (Figs. S3 and S4, Supplementary Material). The calculations indicate that, in general, free ions and metal–inorganic complexes dominate the speciation of Cd and Zn at the sites, whereas Cu speciation is dominated by metal–organic complexes with some notable exceptions. When pH is low, like in Copperas Brook and at sites closest to the confluence in the Ompompanoosuc River, Cu exists as free ions and metal–inorganic complexes. Note that the fraction of metal associated with BLs is not included in these plots because the concentration of BLs in the system is very low and their presence virtually has no influence on the speciation of the dissolved metal.

Using the speciation mode, the concentration of metal associated with the BL ([BL–metal]) for in-situ conditions is calculated. These values are compared to the LA50 values of different organisms to evaluate whether the in-situ water composition is predicted to be toxic to the organisms. If the loading of the BL by

metal is larger than the LA50 values for the organism, then the in-situ chemical conditions are considered to be toxic to the organism. Comparisons between the predicted concentrations of BL–metal (Cd, Cu, and Zn) complexes determined from the BLM and LA50 values for water fleas (*C. dubia* and/or *D. magna*) and rainbow trout (*O. mykiss*) in the Coeur d'Alene River basin indicate that in-situ chemical conditions upstream of the mining district (site 1) are good for all modeled organisms (Fig. 9). Concentrations of Cu do not pose a threat to the health of the modeled organisms within or downstream of the mining district, whereas the predicted concentration of BL–Cd complexes is greater than the LA50 value for rainbow trout, but less than the LA50 value for water fleas, at all sites except site 7 (Pine Creek) and the farthest downstream site (site 9). The predicted concentration of BL–Zn complexes is greater than the LA50 value for rainbow trout at all sites within and downstream of the mining district, whereas the concentrations of BL–Zn

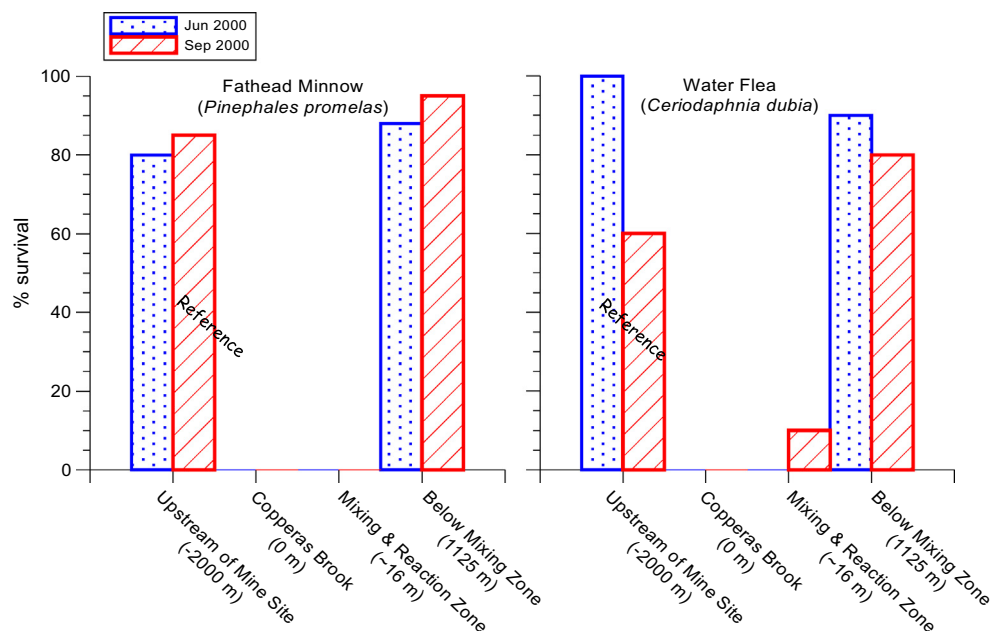


Fig. 8. Results of surface water toxicity tests at the Elizabeth Copper Mine Superfund site in Vermont (prepared with data from Hathaway et al., 2001).

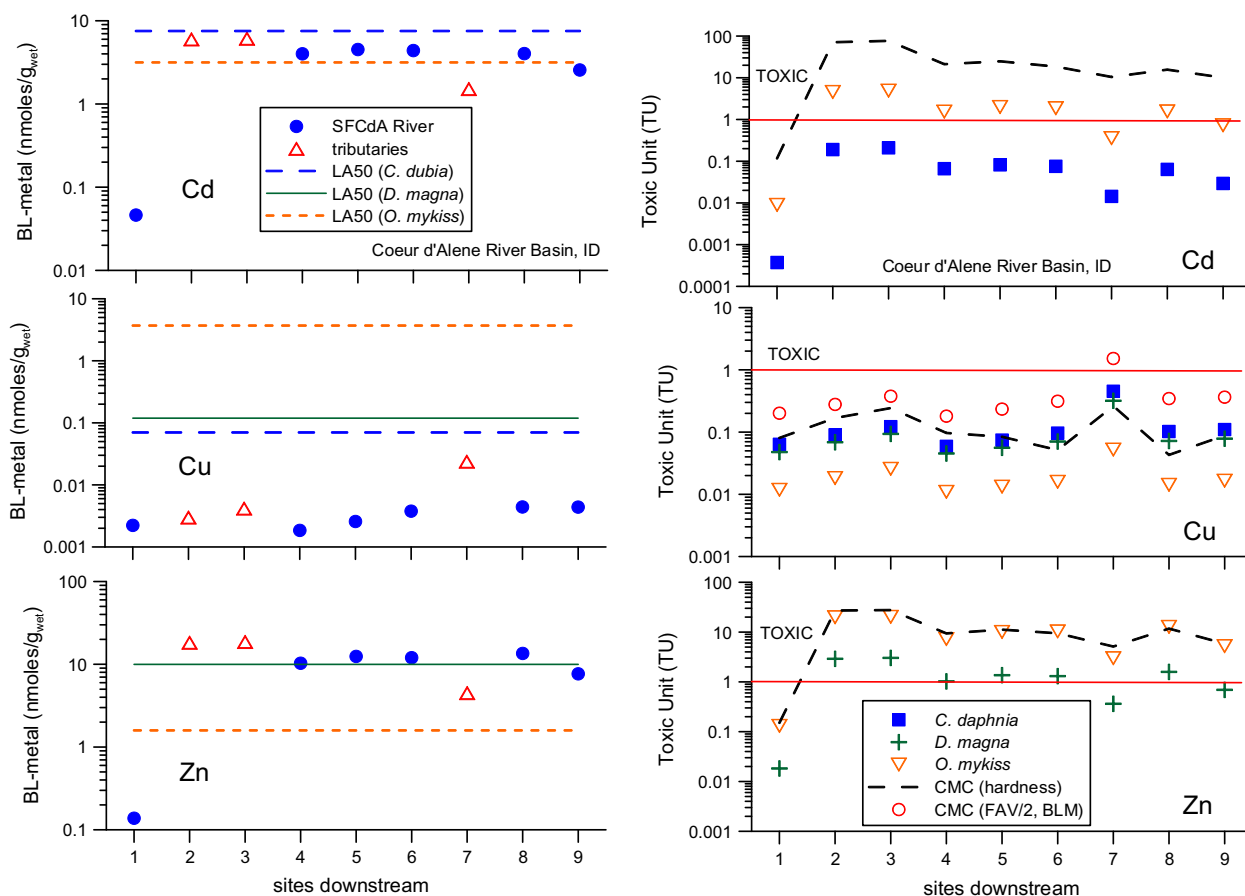
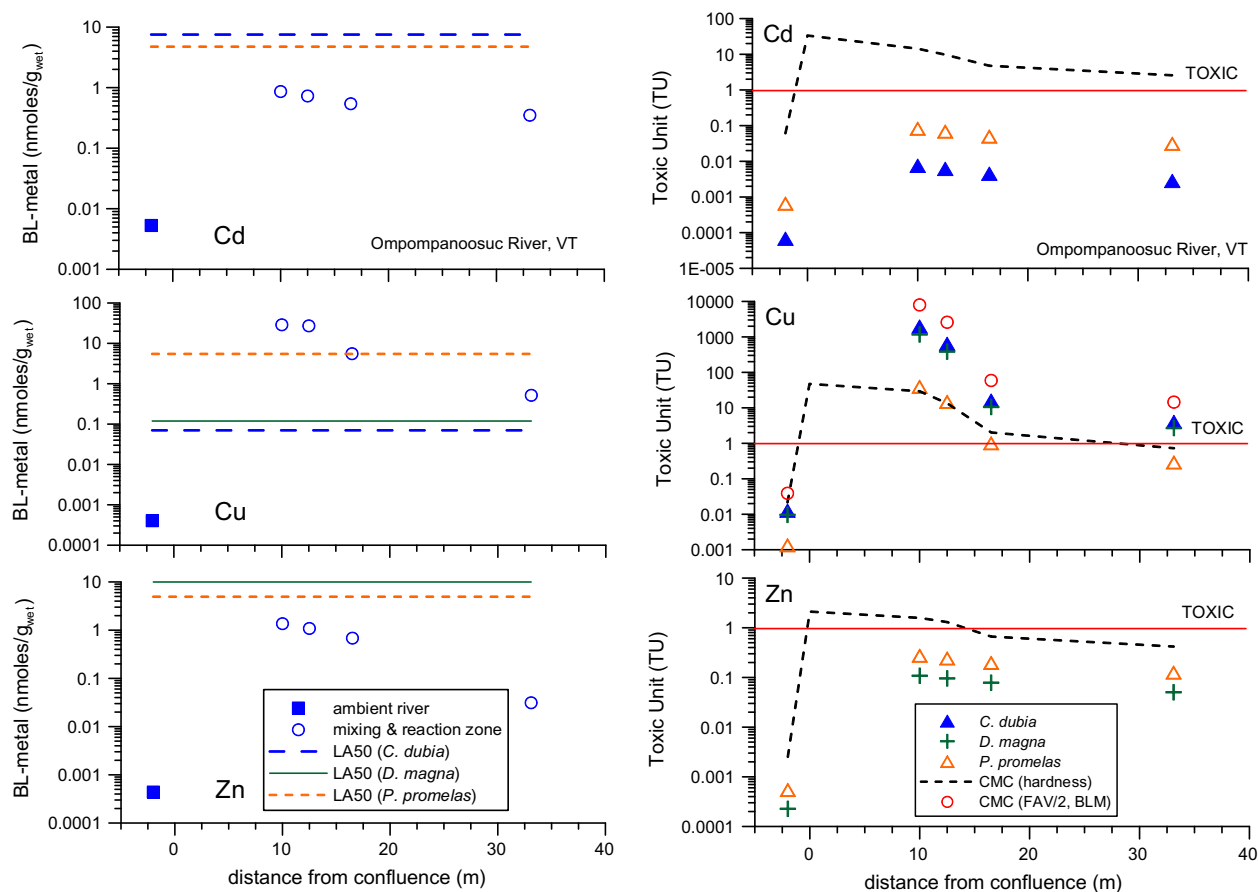


Fig. 9. The loading of Cd, Cu, or Zn on the biotic ligand and LA50 values for water fleas (*C. dubia* or *D. magna*) and rainbow trout (*O. mykiss*) (left panel) and toxicity units (TU) (right panel) at sites within the Coeur d'Alene River basin. Toxicity units are defined as the observed metal concentration/Water Quality Criteria (WQC), where WQC are defined as LC50 for water fleas and rainbow trout from the biotic ligand model (BLM), Criterion Maximum Concentrations (CMC) predicted from the BLM, or hardness-based CMC.

are greater than or equal to the LA50 value for water fleas at all sites in the district except for site 7 (Pine Creek) and the farthest sampling site (site 9) downstream in the South Fork Coeur d'Alene

River. At the Vermont study area, the predicted loading of the BL by Cd and Zn is lower than the LA50 values for water fleas and fathead minnows (Fig. 10). Except for the site upstream of the confluence,



**Fig. 10.** The loading of Cd, Cu, or Zn on the biotic ligand and LA 50 values for water fleas (*C. dubia* or *D. magna*) and fathead minnow (*P. promelas*) (left panel) and toxicity units (TU) (right panel) at the Elizabeth Copper Mine Superfund site. Toxicity units are defined as the observed metal concentration/Water Quality Criteria (WQC), where WQC are defined as LC50 for water fleas and fathead minnow from the BLM, Criterion Maximum Concentrations (CMC) predicted from the BLM, or hardness-based CMC.

Cu loading of the BL is predicted to exceed the LA50 for water fleas at all sites downstream of the confluence and is greater than or equal to the LA50 for fathead minnows at 3 of the 4 sites in the mixing and reaction zone. Note that loading of the ligand by metal was not predicted for the Copperas Brook site because the chemical conditions at this site exceed the concentration ranges that were used to develop and calibrate the BLM for Cu.

Values of LC50 for the metals at each sampling site are predicted by running the BLM in the toxicity mode. These values can be compared to the observed metal concentrations at the sampling sites in the form of TUs, where  $TU = [\text{observed metal concentration}] / LC50$ . For comparison, the observed concentrations also are compared to the hardness-based CMC for Cd, Cu, and Zn and to the CMC predicted by the BLM for Cu ( $TU = [\text{observed metal}] / CMC$ ). If TU is greater than 1, then the chemical composition at the site is considered to be potentially toxic.

Chemical conditions at the site upstream of the Coeur d'Alene mining district (site 1) are not predicted to be toxic for water fleas and rainbow trout (Fig. 9). However, the chemical concentrations of Cd and Zn, but not Cu, at most sites within or downstream of the mining district are predicted to be toxic to fish. Concentrations of Cd and Cu are not toxic ( $TU < 1$ ) to water fleas, whereas Zn concentrations are toxic to these organisms at most sites within or downstream of the district. The hardness-based criteria indicate equal or higher toxicity for Cd, Cu, and Zn for fish than the BLM criteria. The exceptions are the BLM predictions for Cu and water fleas at the most downstream sites and the CMC predicted by the BLM, which is more sensitive for Cu than the hardness-based

CMC. The likely culprit for toxicity to water fleas and fathead minnows in the mixing and reaction zone of the Ompompanoosuc River is predicted to be Cu, as  $TU < 1$  in the mixing and reaction zone for Cd and Zn for the organisms (Fig. 10). The hardness-based criteria predict equal or greater toxicity for Cd and Zn at all sites compared to the BLM predictions. In contrast, the BLM predictions in the mixing and reaction zone indicate equal or higher toxicity for water fleas than the hardness-based criteria.

As expected, conclusions drawn from the TU approach are very similar to those that were drawn by comparing predictions of metal loading to LA50 values. Concentrations of Cd and Zn are likely responsible for the observed lack of fish or their low abundance in stream waters within the Coeur d'Alene mining district. For the mixing and reaction zone of the Ompompanoosuc River, high Cu concentrations likely account for the low survivability of water fleas and fathead minnows in the mixing and reaction zone downstream of the river's confluence with acid mine drainage.

This case study has illustrated variability in the chemical speciation and toxicity of metals at two sites impacted by mining activities. Chemical speciation of Cd, Cu, and Zn, as predicted by the BLM, is sensitive to pH and total concentrations of dissolved metal and DOC. In general, Cd and Zn primarily exist as free metal ions ( $Cd^{2+}$ ,  $Zn^{2+}$ ) and complexes with inorganic ligands. In contrast, Cu speciation can vary from free metal ions ( $Cu^{2+}$ ) and inorganic complexes at low pH to primarily Cu-organic ligand complexes at higher pH. Predicted toxicity is variable depending on site-specific chemical conditions, the organism of interest, and metal concentrations and their chemical speciation. Rainbow trout and fathead

minnows tend to be more sensitive to Cd and Zn, whereas water fleas are more sensitive to Cu. The toxicity predictions of the BLM are consistent with observed toxicity at the field sites, and provide a site-specific means to identify the metals of most concern.

#### 4.2. Influence of dissolved organic-matter fractionation on Cu toxicity in mined and mineralized areas

The primary objective of this study was to determine whether chemical fractionation of DOM resulting from sorption of DOM by Fe- and Al-rich suspended sediment in stream water is sufficient to produce observable differences in the protective ability of the remaining DOM against acute Cu toxicity to aquatic organisms. This study builds on previous studies that observed that DOM fractionates in the presence of precipitating Fe and Al oxides (McKnight et al., 1992, 2002), and that the presence of Fe decreased the protective ability of DOM on Cu toxicity to aquatic biota (Smith et al., 2006, 2009).

Streams in mined and mineralized areas commonly are unusual geochemical systems in that the chemical composition of water originating from mineralized areas can be quite different from that of water originating from non-mineralized areas (Smith, 2005; Nordstrom, 2011). For example, water originating from areas underlain by mineralized rock commonly has lower pH and alkalinity values, higher sulfate concentrations, and higher dissolved Fe and Al concentrations than water originating from non-mineralized areas. The Fe and Al can precipitate, form suspended sediment, and coat the bottom of streams, especially downstream of confluences between impacted and relatively pristine streams.

As previously discussed, the presence of DOC tends to reduce metal toxicity to aquatic biota (see Fig. 3B and associated discussion), and the added presence of dissolved Fe and Al tends to decrease the amount of Cu–DOC binding, thus reducing the protective effect of DOC (see Fig. 6 and associated discussion). McKnight et al. (1992, 2002) demonstrated that DOM undergoes chemical fractionation when some of the DOM sorbs onto precipitating Fe and Al oxides. Fractionation occurs because DOM molecules that have the greatest metal-binding capacity are preferentially sorbed onto the precipitating Fe and Al oxides. As a result, the DOM remaining in the stream water is depleted in constituents that were preferentially sorbed onto the sediment, and hence the remaining DOM has reduced metal-binding capacity.

Cu toxicity studies were conducted in synthetically prepared water (USEPA formulation for moderately hard reconstituted water) amended with freeze-dried FA samples collected and isolated by McKnight et al. (1992) from below the confluence of a relatively pristine stream (Deer Creek, Colorado) with an acidic metal-enriched stream (Snake River, Colorado). The samples included (1) FA isolated from stream water that was collected below the confluence (Snake River Dissolved), (2) FA extracted from suspended sediment that was collected below the confluence (Snake River Sediment), and (3) Suwannee River Fulvic Acid (SRFA; a standard reference material). Acute 48-h toxicity tests were performed using standard USEPA methods with a water flea (*C. dubia*) as the test organism (USEPA, 2002a). The FA–Cu test waters were allowed to equilibrate for at least 24 h before test organisms were introduced to allow time for Cu to bind to the organic matter. Multiple tests were performed with variable Cu concentrations in order to bracket the LC50.

Toxicity test results are summarized in Fig. S5 (Supplementary Material). Although the test waters had nearly identical DOC values (3 mg C/L), pH values (pH 8), and chemical composition, there are distinct differences in the estimated LC50 values for the different sources of FA. The estimated LC50 value for test waters with no added FA was low (4.3  $\mu\text{g Cu/L}$ ), which means that a concentration of 4.3  $\mu\text{g Cu/L}$  was detrimental to 50% of the test organisms.

Estimated LC50 values for test waters with added FA were higher (13 to 60  $\mu\text{g Cu/L}$ ), which demonstrates the expected protective effect of DOC on acute Cu toxicity. Estimated LC50 values for the two different sources of FA from the Snake River are distinctly different; the dissolved FA LC50 is 13  $\mu\text{g Cu/L}$  whereas the sediment-derived FA LC50 is 60  $\mu\text{g Cu/L}$ . The LC50 of the SRFA standard reference material is intermediate between the two Snake River results (41  $\mu\text{g Cu/L}$ ). Hence, there is more than three times greater Cu toxicity measured in the presence of fractionated DOM than for unfractionated DOM. These results suggest that chemical fractionation of NOM in streams where Fe and Al minerals precipitate can be an important factor in determining Cu toxicity to stream-water biota. These findings highlight the importance of considering the potential effects of Fe and Al, and that stream ecosystems downstream of Fe- and Al-rich streams may be more vulnerable to adverse effects from metal toxicity.

The LC50 values predicted by the BLM were also computed for these test waters (Fig. S5, Supplementary Material). Default values for FA complexation constants and binding site densities were used in BLM computations. The BLM-predicted values were similar (65–68  $\mu\text{g Cu/L}$ ) for the different toxicity test-water solutions. Therefore, the effects of DOC fractionation on Cu binding and toxicity are not reflected in the BLM predictions. The aquatic life ambient freshwater quality criteria document for Cu (USEPA, 2007) discusses this issue, but does not require users to include Fe and Al in BLM simulations. It is likely that a modified version of the BLM may be necessary to refine site-specific water-quality criteria and to predict potential metal toxicity in Fe- and Al-rich mined and mineralized sites.

## 5. Challenges in implementation of BLMs—A summary

BLMs represent significant steps forward in determining site-specific WQC. Despite this advancement, several caveats should be considered in the implementation of BLMs, especially in mined and mineralized areas. The following list summarizes some of the main challenges discussed in this report.

1. Incomplete sets of BLM input parameters, which historically have not been measured at most sites, and poor estimation techniques for missing historical data (e.g., DOC).
2. Differences in DOC quality, particularly at field sites receiving anthropogenic DOC, and fractionation of DOC in Fe- and Al-rich systems. Both DOC quality and fractionation affect the strength of interactions between DOC and metals, especially for Cu.
3. Spatial and temporal variations in water-quality parameters and metal toxicity predictions.
4. Influence of multiple metals, competition among organisms and metals, and dietary metal uptake on metal toxicity predictions.
5. Metal interactions with solid or gas phases and kinetically controlled reactions.
6. Different metal sensitivities and acclimation to high metal concentrations for various organisms.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.apgeochem.2014.07.005>.

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