

**BIOTIC LIGAND MODEL WINDOWS[®] INTERFACE,
RESEARCH VERSION 3.41.2.45:
USER'S GUIDE AND REFERENCE MANUAL**

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Prepared by:  WindWard^{environmental} LLC

200 West Mercer Street, Suite 401 ♦ Seattle, Washington ♦ 98119
224 Harrison Street, Suite 705 ♦ Syracuse, New York ♦ 13202

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Acronyms

Acronym	Definition
Ag	silver
AWQC	ambient water quality criteria
BLM	biotic ligand model
Ca	calcium
Ca²⁺	calcium ion
CaCO₃	calcium carbonate
CCC	criterion continuous concentration
CEC	Commission for Environmental Cooperation
CHESS	chemical Equilibria in Soils and Solutions
Cl	chloride
CMC	criterion maximum concentration
Cu	copper
DIC	dissolved inorganic carbon
DOC	dissolved organic carbon
EA20	accumulation of metal on the biotic ligand that causes a defined effect in 20% of an exposed population
EPA	US Environmental Protection Agency
FAV	final acute value
FMB	fixed monitoring benchmarks
H₂CO₃	carbonic acid
HA	Humic acid
HC5	concentration corresponding to the 5 th percentile of the species sensitivity distribution (SSD)
IWQC	instantaneous water quality criteria
K	potassium
LA50	accumulation of metal on the biotic ligand that is lethal to 50% of an exposed population
LC50	concentration that is lethal to 50% of an exposed population
Mg	magnesium
Na	sodium
NO₃	nitrate
NOM	natural organic matter

Pb	lead
PO₄	phosphate
ppt	parts per thousand
QA/QC	quality assurance/quality control
SO₄	sulfate
SSD	species sensitivity distribution (showing the range in organism sensitivity to a toxicant ordered from most sensitive to least sensitive)
TOC	total organic carbon
TU	toxic unit
WHAM	Windermere Humic Aqueous Model
WQC	water quality criteria

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ADDITIONAL INFORMATION

Additional information can be found in the User's Guide, or online at:

<http://www.windwardenv.com/biotic-ligand-model>.

Contact Information

For questions or problems, including bug reports, relating to the use and the application of the biotic ligand model (BLM) or the BLM Windows® Interface, please contact either:

Robert Santore
Windward Environmental LLC
Phone: 206-812-5450
E-mail: roberts@windwardenv.com

or

Kelly Croteau
Windward Environmental LLC
Phone: 206-812-5452
E-mail: kellyc@windwardenv.com

Additional information can be found online at:

<http://www.windwardenv.com/biotic-ligand-model>.

1 Introduction to the Biotic Ligand Model

1.1 INTRODUCTION

Metal bioavailability and toxicity have long been recognized to be a function of water chemistry (Sunda and Guillard 1976; Sunda and Hansen 1979). For example, the formation of inorganic and organic metal complexes and their sorption to particle surfaces can reduce metal toxicity. As a result, metal toxicity can be highly variable and dependent on ambient water chemistry when expressed as total or dissolved metal concentrations. In contrast, the effects of water chemistry on metal toxicity can often be reduced or eliminated when metal toxicity is related to free metal ion concentrations (Sunda and Guillard 1976). Allen and Hansen (1996) have shown the relationship between metal speciation and toxicity and have used this relationship to predict the range of effects that site-specific water quality characteristics can have on copper (Cu) toxicity.

1.2 BLM FRAMEWORK AND CONCEPTUAL MODEL

The biotic ligand model (BLM) was developed to incorporate metal speciation and the protective effects of competing cations into predictions of metal bioavailability and toxicity (Di Toro et al. 2001; Paquin et al. 1999; Santore et al. 2001; Santore et al. 2002). The BLM is based on a conceptual model similar to the gill site interaction model proposed by Pagenkopf (1983) and is conceptually similar to the free ion activity model (Morel and Hering 1993). The BLM incorporates a version of Chemical Equilibria in Soils and Solutions (CHESS) (Santore and Driscoll 1995) that was modified to include the chemical and electrostatic interactions described in the Windermere Humic Aqueous Model (WHAM) (Tipping 1994). Metal toxicity is simulated as the accumulation of metal at a biologically sensitive receptor, the "biotic ligand," which represents the site of action of acute metal toxicity. Inorganic and organic ligands can also bind metal, however, thereby reducing accumulation at the biotic ligand. By incorporating the biotic ligand into a chemical equilibrium framework that includes aqueous metal complexation, the relationship between free metal ion concentrations and toxicity is an inherent feature of the model.

The BLM framework also incorporates the competitive effects of other cations that interact with the biotic ligand to mitigate toxicity. For example, at a fixed free metal concentration, as hardness increases, the increased calcium ion (Ca^{2+}) competes with the free metal for binding sites at the biotic ligand. A higher free metal concentration is therefore required to achieve the same toxic effect in the presence of the elevated Ca^{2+} concentration. The BLM uses this competitive mechanism to simulate the reduction in metal toxicity due to elevated hardness concentrations. Thus, the BLM can effectively account for a reduction in metal toxicity caused by elevated levels of hardness cations (Meyer et al. 1999).

The BLM has been developed using published information on metal toxicity and biotic ligand accumulation as a function of water chemistry. The most comprehensive data compiled to date for use with the BLM are for Cu toxicity to fathead minnows (*Pimephales promelas*). The biotic ligand, or site of acute Cu toxicity for fathead minnow, has been identified as sodium (Na) ion uptake channels in the gill membrane (Playle et al. 1993a). Cu accumulation on the gill has been associated with respiratory distress and decreased blood plasma Na concentrations caused by interference with these sites (Playle et al. 1992). The adsorption of Cu to gill surfaces in the BLM has been calibrated to measurements of Cu accumulation on the gill over a wide range of water quality conditions (Playle et al. 1992; Playle et al. 1993b). Additionally, MacRae (1999) has established a dose response relationship necessary to determine the biotic ligand LC50 (concentration that is lethal to 50% of an exposed population) in rainbow trout. In the BLM, metal toxicity is defined as the amount of metal necessary to result in accumulation at the biotic ligand equal to the biotic ligand LC50. While others have developed models capable of predicting metal bioaccumulation on the gill under short-term exposure conditions (Playle et al. 1993a, b), the BLM is the first that includes a scheme for predicting toxicity. The BLM for other metals and organisms is based on a similar approach.

1.3 BLM APPLICATIONS

The BLM can be used to calculate the chemical speciation of a dissolved metal, including complexation with inorganic and organic ligands and the biotic ligand. The biotic ligand represents a discrete receptor or site of action on an organism where accumulation of metal leads to acute toxicity. The BLM, therefore, can be used to predict the amount of metal accumulation at this site for a variety of chemical conditions and metal concentrations (i.e., the inorganic, organic, and biotic speciation of metals in aquatic settings).

According to the conceptual framework of the BLM, the accumulation of metal at the biotic ligand at or above a critical threshold concentration leads to acute toxicity. This critical accumulation on the biotic ligand is also termed the LA50 (accumulation of metal on the biotic ligand that is lethal to 50% of an exposed population) or, for example, the EA20 for chronic toxicity (accumulation of metal on the biotic ligand that causes a defined effect in 20% of an exposed population). The LA50 is expressed in units of nmol/g wet weight of the biotic ligand. Since the BLM includes inorganic and organic metal speciation and competitive complexation with the biotic ligand, the amount of dissolved metal required to reach this threshold varies, depending on the water chemistry. Therefore, in addition to calculating chemical speciation, the BLM can be used to predict the concentration of metal that would result in toxicity within a given aquatic system.

1.1.1 Fixed monitoring benchmark

The fixed monitoring benchmark (FMB) is a single value that can be used to monitor compliance with the time-variable water quality criteria (WQC). The FMB uses a BLM toxicity calculation in combination with concurrent metal concentrations to calculate the likelihood of exceeding the WQC, and determines a safe value based on their continuous distribution functions. The theory and background of the FMB is not described in this user guide, only the use of the BLM in calculating the FMB. For more information on the FMB development and method of calculation, refer to Ryan et al. (2018).

2 Overview and Help File Layout

2.1 WHAT'S NEW IN THIS DISTRIBUTION?

In Version 3.41.2.45, several features have been added, various improvements to the software have been made, and several bugs have been fixed. Changes include:

- ◆ A bug that prevented silver (Ag) from being run successfully with simple chemistry has been fixed.
- ◆ A bug that prevented metals other than copper from being run successfully in toxicity mode has been fixed.
- ◆ Units labeling has been fixed; unit conversion functionality in the simple chemistry tabs has been improved.
- ◆ The Options menu is no longer a sub-menu of the Edit menu; the menu was becoming so large as to be unwieldy.
- ◆ The BLM has been translated into French! This is thanks to support from Environment and Climate Change Canada. Translations are limited to the user interface, so labels in output files are only in English for the time being.
- ◆ A new option for running large datasets has been added: “Run Batches...” has been added to the File menu so that datasets larger than the maximum number of observations can be run sequentially with minimal supervision.
- ◆ Output files can now be written as CSV files.
- ◆ The user can now look up median ion ratios by US state, Canadian province, Canadian ecozone, or Commission for Environmental Cooperation (CEC) ecoregion.
- ◆ The FMB can now be calculated using WQC and HC5 (concentration corresponding to the 5th percentile of the species sensitivity distribution [SSD¹]) parameter files.

2.2 HELP FILE LAYOUT

The remainder of this document describes the hardware and software requirements for installing and running the BLM Windows® Interface, details the data requirements of the BLM, and provides a step-by-step guide to using the various functionalities of the BLM Windows® Interface and a walk-through of the application using an example BLM data file.

¹ Showing the range in organism sensitivity to a toxicant ordered from most sensitive to least sensitive.

3 Setup and Installation

3.1 SYSTEM REQUIREMENTS

The BLM Windows® Interface is designed for use on the PC family of computers running Microsoft Windows®. The memory requirements of the BLM Windows® Interface are modest and should not interfere with other resident programs.

3.2 INSTALLING THE BLM WINDOWS® INTERFACE

Installing from a setup file – To install the BLM Windows® Interface from the setup file “BLM_3.41.2.45_setup.exe,” simply double-click on the file and follow the prompts. The setup program (Figure 3-1) will guide the user through a fairly straightforward installation process, querying the user for information on where to install the necessary files. During the installation, a shortcut to the BLM Windows® Interface application will be added to the “Programs” sub-menu within the “Start” menu on the Microsoft Windows® desktop. In addition, the BLM Windows® Interface application will be registered in the system registry so that the BLM data files created by the user can be accessed directly by double-clicking on the file name.

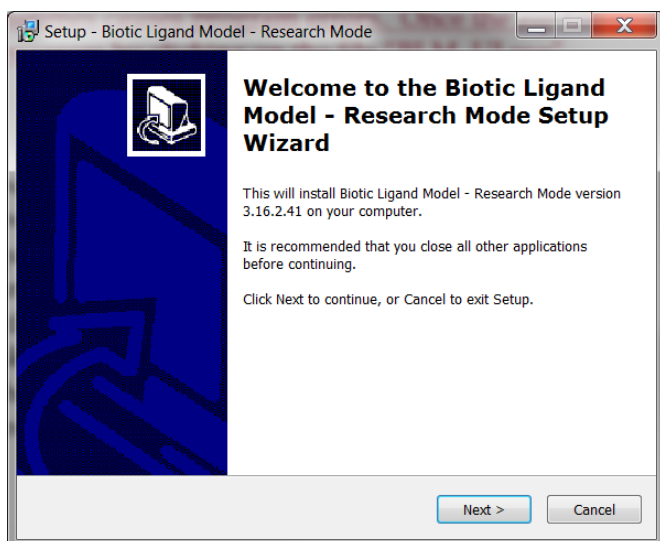


Figure 3-1. Opening screen for the BLM setup program

Installing from a compressed (.zip) file – To install the BLM Windows® Interface from the compressed file, simply unzip the entire package to a folder in the Documents directory on your hard disk. If your zip utility asks if you would like to preserve paths, say “yes.” Do not try to run it from the desktop or a server folder, since that sometimes causes program errors. Once the zip file is unpacked, you can start the program by double-clicking on the file “BLM_UI.exe,” which will have a BLM icon.

4 Data Requirements

4.1 FULL SITE CHEMISTRY

The BLM predicts metal toxicity and speciation for a particular site based on ambient water quality. Therefore, the user will be expected to provide data describing the physical and chemical properties of the site water. The data requirements of the BLM are conventional physical and chemical parameters that are easily measurable in the laboratory; certain parameters may be measured in the field or laboratory depending on the purpose of the BLM simulation (e.g., pH and temperature, as discussed below). This section describes the general physical and chemical data requirements for an application of the BLM to predict metal speciation, toxicity, or WQC in aquatic systems.

The ambient water quality information required to run the BLM is:

- ◆ Temperature
- ◆ pH
- ◆ Metal concentration
- ◆ Dissolved organic carbon (DOC)
- ◆ Major cations (calcium [Ca], magnesium [Mg], Na, and potassium [K])
- ◆ Major anions (sulfate [SO₄] and chloride [Cl])
- ◆ Alkalinity
- ◆ Sulfide
- ◆ Possible other components (e.g. nitrate [NO₃] for Ag runs)

For a given metal, some of these chemical inputs have an important effect on metal speciation, while other chemical inputs have only minor effects on BLM predictions. The user should be aware of the relative importance of each of the chemical inputs to decide whether adequate information is available for a meaningful application of the BLM. The guidelines described in the subsequent subsections may be helpful in that assessment.

Each water sample must be fully described in terms of the above-listed water quality inputs before the BLM can be used. However, if some of the parameters are known to be absent in a water sample, a nominal, negligible concentration should be input (a value on the order of 1E-10 mg/L is typically sufficient) rather than a zero concentration.

The BLM also allows the estimation of major ions from hardness and ion ratios (see Section 0) or salinity and base marine chemistry (see Section 0), thereby allowing the model to run with only temperature, pH, DOC, and hardness or salinity.

4.1.1 Temperature

Temperature measurements are typically the most common and basic of all water quality measurements and therefore available in most laboratory characterizations of site water chemistry. Because the BLM is based on a thermodynamic chemical equilibrium modeling framework, temperature measurements are important in determining the relevant thermodynamic reaction rates. Note that laboratory temperature conditions are relevant when the BLM is being used to explain or predict toxicity tests conducted in the laboratory. Field temperatures, on the other hand, are more appropriate when using the model to extrapolate to field conditions.

4.1.2 pH

Accurate pH values are important to BLM results for most metals. The chemical speciation of many metals, such as Cu, is directly affected by pH. Sample pH is also important in determining the metal complexation capacity of dissolved organic matter, and in determining the speciation of inorganic carbon, which relates to the formation of metal carbonate complexes. For these reasons, pH is considered a required chemical input to the BLM. If BLM results are to be compared with laboratory measurements of metal toxicity, then it is preferable that the pH be measured within the test chamber during the exposure. Otherwise, use the field pH to extrapolate to field conditions.

4.1.3 Dissolved organic carbon

Dissolved organic matter can play a critical role in determining metal speciation and bioavailability. In the BLM, the presence of dissolved organic matter is specified as a DOC concentration expressed in mg/L and is considered a required input for the BLM. For water with low DOC, it is important to make sure that analytical detection limits are sufficiently low. In toxicity studies, the test organisms themselves may be a significant source of organic matter, depending on the number of organisms and the volume of the test chamber.

Natural organic matter (NOM) is any organic component of the water; for example, leaf litter and detritus are common sources of organic matter. NOM is a complex mixture of organic acids, proteins, and many more complex organic molecules. Total organic matter can be broken down into particulate organic matter and dissolved organic matter. The accepted standard, although the exact cutoff is somewhat arbitrary, is that dissolved organic matter can pass through a 0.45-micron filter, whereas particulate organic matter gets caught in a filter that size. DOC is a way of quantifying dissolved organic matter and is simply the mass of carbon present in the

mixture of organic compounds. Sometimes total organic carbon (TOC) is reported instead of DOC. DOC is preferred for use in the BLM, but TOC can be used in lieu of DOC with the understanding that the actual amount of DOC will be less than the amount of TOC.

It should not be expected that DOC from artificial sources (e.g. acetate, sugar, MOPS or other buffers, etc.) will behave as NOM. The organic matter binding model that the BLM uses is based on natural sources of organic matter, and inputs should therefore not include known quantities of artificial organic matter. If there is a significant amount of artificial organic carbon in the solution, then a speciation model that includes that chemical component may be necessary to accurately model it.

4.1.4 Humic acid fraction of DOC

The BLM uses a description of organic matter chemistry developed for WHAM, Version 1.0 (Tipping 1994), that characterizes metal complexation with both humic and fulvic organic matter sources.² To be able to make use of this capability, it is necessary to specify the distribution of humic and fulvic acids in the organic matter present in a given water. Unfortunately, NOM composition is not routinely characterized, and information on humic and fulvic acid content is not likely to be available. In the absence of chemical characterization, a value of 10% humic acid (HA) content is recommended for most natural waters. The variability of the dissolved organic matter content in diverse water sources has not been found to be an especially critical parameter, and little benefit is achieved by characterizing NOM beyond DOC concentrations.

4.1.5 Metal concentrations

Metal concentrations are needed to run the model in speciation mode. It is not necessary to input metal concentrations for the model to calculate toxicity or instantaneous WQC/HC5; however, metal concentrations are required when calculating an FMB (see Sections 0 and 0). If the metal concentrations are censored (i.e., below the detection limit), then this can be indicated by making the number negative or using the "<" symbol before the number (e.g., if the detection limit is 0.2 µg/L, then enter either "-0.2" or "<0.2" in the metal column for observations that are below the detection limit). The censored data will display with the "<" symbol but will be stored as a negative number in the background (for computing purposes), so do not be confused if a censored value suddenly turns negative when you select a cell to edit it.

² Note that this version of WHAM is sometimes called WHAM Model V, due to a change in the version numbering around that time.

4.1.6 Major cations

The cations Ca, Mg, Na, and K are all necessary inputs to the BLM. For Cu and Ag, Ca and Na can compete directly with the metal at biotic ligand sites; these cations will, therefore, have a direct effect on predictions of metal toxicity. For some organisms, Mg may play a critical role as well. These cations, therefore, should be considered required inputs to the BLM. On the other hand, K currently has no direct effect on metal toxicity in the BLM and can be estimated if measurements do not exist. Although K does not have direct effects on metal toxicity, it is still often present in large enough quantities that it can affect the charge balance and ionic strength of the water. This would indirectly affect the metal toxicity, typically – but not always – to a small extent.

4.1.7 Major anions

The anions SO_4 and Cl are necessary inputs to the BLM (although bicarbonate is also an important anion, as discussed in Section 0). In fresh waters, SO_4 may be the dominant anion and is, therefore, important for determining charge balance and ionic strength. The chemistry of metals and NOM is dependent, to varying degrees, on ionic strength, so SO_4 has some importance as a BLM input. If measurements of SO_4 are not available, however, the concentrations can be estimated. For Cu and most other metal simulations, Cl is only important as a contribution to ionic strength, but for Ag simulations, Cl can have additional importance due to the formation of Ag-Cl complexes. Therefore, it is preferable that only measured Cl concentrations be used for BLM applications involving Ag, while estimates can be used for applications involving other metals.

4.1.8 Alkalinity

Inorganic carbon species in the BLM include carbonate (CO_3^{2-}), bicarbonate (HCO_3^-), and carbonic acid (H_2CO_3). The sum of these species is referred to as dissolved inorganic carbon (DIC). Bicarbonate is usually the most important DIC species in natural waters, because it is the dominant species between pH 6.35 and 10.33.

Inorganic carbon is a critical input to the BLM because many metals form carbonate complexes. The one exception is Ag, which does not form carbonate complexes; DIC is not a critical input to BLM applications for Ag. Unfortunately, measurements of DIC are not often taken in natural water samples, but DIC can be estimated from alkalinity and pH measurements, as in Equation 4-1.

$$DIC = Alk \cdot \frac{\frac{[H]}{K_1} + 1 + \frac{K_2}{[H]}}{1 + \frac{2 \cdot K_2}{[H]}} \quad \text{Equation 4-1}$$

where:

$$\begin{aligned} Alk &= \text{alkalinity in equivalents/L} \\ &= 2 \times 10^{-5} \times \text{alkalinity (as mg calcium carbonate [CaCO}_3\text{]/L)} \\ [H] &= 10^{-pH} \\ K_1 &= 10^{-6.352} \\ K_2 &= 10^{-10.329} \end{aligned}$$

The BLM Windows® Interface uses this equation to calculate the DIC internally, so only the alkalinity and pH need to be specified. Alkalinity should be measured on filtered samples to eliminate potential contribution from suspended calcium carbonate (CaCO₃) and expressed in units of mg/L CaCO₃. If there are chemical additions that affect alkalinity but not DIC, those quantities should be excluded from the input alkalinity concentrations. However, depending on the inorganic carbon option selected, the user may also opt to specify DIC concentrations directly, or choose to have the BLM calculate the DIC that would result from atmospheric CO₂ being in equilibrium with site chemistry. This last option assumes a pCO₂ of 10^{-3.2} by default (or whatever value is entered into the pCO₂ input box on the Simplified Site Chemistry tab; see Sections 0 and 0), and calculations are done within the BLM engine. It differs from the atmospheric alkalinity calculation used when converting between simple and full site chemistry (see Section 0) in that all of the chemical equilibria equations are considered, not just carbonate equilibria equations.

4.1.9 Sulfide

Although it has traditionally been assumed that sulfide concentrations are negligible in aerated waters, recent evidence suggests that appreciable sulfide concentrations persist in both marine and fresh waters. Waters affected by wastewater treatment plant effluents in particular can have elevated sulfide concentrations. Sulfide has a strong affinity for many metals, so it is an important consideration in determining metal speciation and bioavailability. Therefore, if sulfide is present, measured sulfide should be considered a required input to the BLM, especially when sulfide concentrations are similar to the predicted effect levels for a given metal and organism.

Researchers at several universities continue to look into the nature of sulfide-metal complexes in aqueous systems. The persistence of sulfide in aerated waters may be linked to the formation of stable metal-sulfide clusters, and these clusters may not be

detected by traditional sulfide measurements. Alternatively, strong metal complexes believed to be due to sulfide compounds may actually be due to other forms of reduced sulfur that are also missed by traditional sulfide measurements. Suitable analytical methods that measure the target form of sulfide and do not measure other non-reduced forms of sulfur are under development. Also, sulfide levels in some locations may be known to be low and well below the effect levels of interest for a given metal. Therefore, sulfide measurements may not be critical in all instances.

Since these research questions are still being addressed, metal-sulfide reactions have not yet been incorporated into the BLM. The sulfide column in the input file is a reminder that these interactions are likely to be added to subsequent versions of the model. Sulfide concentrations added in that column will not affect the BLM calculation. It is recommended that typical users enter a near-zero concentration as a default value (i.e., 1.0E-10 mg/L). When sulfide reactions are omitted from model simulations, the BLM will always predict a lower (i.e., more protective) estimate of a concentration associated with toxicity.

4.1.10 Other components

The current marine chemistry file also includes phosphate (PO_4) as a component. PO_4 does have reactions associated with it, and measured concentrations should be entered if available. PO_4 can bind very strongly with some metals and may affect solubility. If no PO_4 measurements are available, it is suggested that the user estimate them with salinity and base marine chemistry (see Section 0).

Parameter files for silver include a NO_3 component as well, and measured concentrations should be included if available. It is also possible, through the use of a user-generated parameter file, to have other non-standard components (recommended for advanced users only). Typically, when a non-standard component is included in a parameter file, it is because it will have a significant effect; measured concentrations should be entered whenever possible.

4.2 SIMPLIFIED SITE CHEMISTRY

In addition to the full site chemistry, the model can be run with simplified site chemistry inputs. “Simplified Site Chemistry” differs from (full) “Site Chemistry” in that there are fewer inputs required for each site, making data collection easier. The ionic composition of the water is estimated based on some aggregate measure of ionic strength and assumed values of the relative compositions of the ions. The ambient water quality requirements to run the BLM with simplified site chemistry are:

- ◆ Temperature
- ◆ pH
- ◆ Metal concentration (not required in toxicity mode)

- ◆ DOC
- ◆ Freshwater only:
 - ◆ Hardness
 - ◆ Ion ratios (default values may be used if unknown)
 - ◆ pCO₂ (defaults to 3.2)
- ◆ Marine only:
 - ◆ Salinity
 - ◆ Base marine chemistry

The requirements for temperature, pH, metal concentration, and DOC are the same as for full site chemistry (see Section 4.1). The ion ratio and pCO₂ or base marine chemistry values can be changed to match the particular conditions of the site or test.

4.2.1 Freshwater simple chemistry

While in freshwater mode, the ionic composition of the water will be estimated based on measured hardness values and median, assumed, or regional ion ratios. The alkalinity will be estimated based on atmospheric alkalinity.

4.2.1.1 Hardness

The hardness measurement is used, with pH and ion ratios, to estimate the major cations and anions. Hardness is typically the total Ca and Mg ion concentrations, although if hardness is measured directly, other ions can sometimes affect it also. For this calculation, hardness is assumed to be equivalent to Ca + Mg, expressed in units of mg/L CaCO₃.

4.2.1.2 Ion ratios

The ion ratios determine the proportion of each ion that makes up hardness and the charge balance of the water, so Ca:Mg is the ratio of Ca ions to Mg ions (in mol/L). These ratios will vary among water samples depending on watershed geology and various inputs. The default values are median values based on water quality monitoring data from across the United States and Canada. The data used to calculate these values are publicly available. The user may also select values specific to a Canadian ecozone (Ecological Stratification Working Group 1995) or province, or US state or ecoregion based on the delineations of the CEC (Commission for Environmental Cooperation 1997). Ecozones and ecoregions are ecologically and geologically similar regions that are groups at various spatial scales. The CEC has three spatial scales, Level I being the broadest and Level III being the finest. Canadian ecozones generally have borders that are the same as or very similar to CEC Level I ecological regions, with a few ecozones corresponding to Level II ecological regions

instead. The data for these smaller delineations are the same as for the default North American values.

4.2.1.3 pCO_2

Alkalinity is estimated from pH assuming an open system in equilibrium with CO_2 in the atmosphere. The relatively small differences between indoor and outdoor CO_2 concentrations may significantly affect the calculated alkalinity. For this reason, the user is allowed to adjust the pCO_2 value (i.e., ambient CO_2 concentration in atmospheres). In most natural settings, it is a reasonable assumption that the CO_2 concentration will be $10^{-3.5}$ atmospheres, but for indoor settings (where many toxicity tests are performed) this might not be the case. CO_2 concentrations are typically higher indoors, resulting in a lower pCO_2 value. Most indoor air quality standards suggest CO_2 to be at concentrations of less than 10^{-3} atmospheres, so the pCO_2 should generally be greater than $10^{-3.5}$ and less than $10^{-3.0}$ atmospheres. The indoor CO_2 concentration depends on the amount of ventilation and respiration occurring in the building, and there would not be any physical effects from CO_2 until it reached much higher concentrations than 10^{-3} atmospheres. Hence, pCO_2 values greater than 10^{-3} atmospheres are possible but not likely.

4.2.1.4 Simple to full chemistry conversion process

When the user switches from the “Simplified Site Chemistry” tab to the full “Site Chemistry” tab, the software copies the labels, temperature, pH, DOC, and copper concentrations to the full chemistry input grid. Then the pH and pCO_2 are used to estimate alkalinity, and the hardness and ion ratios are used to estimate the major ion concentrations.

Alkalinity is estimated with the assumption that carbonate species are in equilibrium with atmospheric CO_2 , following Henry’s Law. The following equations perform this operation:

$$[H_2CO_3^*] = K_H * P_{CO_2} \quad \text{Equation 4-2a}$$

$$[HCO_3^-] = 10^{-6.352} * \frac{[H_2CO_3^*]}{[H^+]} \quad \text{Equation 4-2b}$$

$$[CO_3^{2-}] = 10^{-10.329} * \frac{[HCO_3^-]}{[H^+]} \quad \text{Equation 4-2c}$$

$$\left[Alk \left(\frac{mg}{L} \text{ as } CaCO_3 \right) \right] = ([HCO_3^-] + 2 * [CO_3^{2-}] + [OH^-] - [H^+]) * \left(\frac{100086}{2} \right) \quad \text{Equation 4-2d}$$

where $[H_2CO_3^*]$ is the sum of dissolved CO_2 gas and the carbonic acid (H_2CO_3) species, K_H , is Henry’s Law constant (equal to $10^{-1.5}$ M/atm), so that with the default pCO_2 value of $10^{-3.2}$, the concentration of $[H_2CO_3^*] = 10^{-4.8}$ M. The alkalinity is then pH dependent (e.g., the alkalinity is 4.44 mg/L $CaCO_3$ when the pH is 7). At low pH, this

calculation will often yield a negative alkalinity, which is replaced with an alkalinity of 1 mg/L CaCO₃ to avoid errors.

The hardness is converted to ion concentrations using the ion ratios and a charge balance. Calcium concentrations are calculated first, as:

$$[Ca^{2+}] = \left(\frac{\text{Hardness as } \frac{mg}{L} \text{ CaCO}_3}{100086 \frac{mg}{mol}} \right) * \left(\frac{Ca:Mg}{Ca:Mg+1} \right) \quad \text{Equation 4-3}$$

The remaining cations are calculated as:

$$[X] = \frac{[Ca^{2+}]}{Ca:X} \quad \text{Equation 4-4}$$

where X is Mg, Na, or K. The anions, SO₄ and Cl, are calculated by first calculating the anion deficit:

$$\begin{aligned} \text{Deficit} \left(\frac{eq}{L} \right) &= 2 * [SO_4^{2-}] + [Cl^-] && \text{Equation 4-5} \\ &= \Sigma(\text{charge} * \text{Cations}) - \Sigma(\text{charge} * \text{Anions}) \\ &= (2 * [Ca^{2+}] + 2 * [Mg^{2+}] + [Na^+] + [K^+] + [H^+]) \\ &\quad - ([OH^-] + [HCO_3^-] + 2 * [CO_3^{2-}]) \end{aligned}$$

Then, the concentrations of SO₄ and Cl are calculated as follows:

$$[SO_4^{2-}] = \frac{\text{Deficit}}{2 + \frac{1}{SO_4:Cl}} \quad \text{Equation 4-6a}$$

$$[Cl^-] = \frac{[SO_4^{2-}]}{SO_4:Cl} \quad \text{Equation 4-6b}$$

If there is a negative deficit, the concentration of SO₄ would be calculated as a negative number: an impossible condition. In such cases, [SO₄²⁻] is set at an arbitrary low number (10⁻⁷ mol/L) before calculating [Cl⁻] in order to avoid errors.

4.2.2 Marine simple chemistry

While in Marine mode, the ionic composition of the water will be estimated based on measured salinity values and base marine chemistry. Inorganic carbon is also based on the base marine chemistry, not an atmospheric estimate like in the hardness-based simple chemistry.

4.2.2.1 Salinity

Salinity should be input using units of parts per thousand (ppt). Full chemistry is calculated from salinity and base marine chemistry. It should be noted that the BLM interface estimates full ion chemistry from salinity under the assumption that estuarine water is essentially diluted sea water with negligible ionic inputs from the freshwater source. This assumption is not valid in some cases, namely when the estuarine water's salinity approaches that of freshwater, or when the estuary's freshwater source has a high hardness. For example, when the freshwater input to an estuary has a hardness of 100 mg/L CaCO₃ and the estuarine water has a salinity of 10 ppt, the estimated ion concentrations will differ from actual by about 14% for Ca, 2% for Mg, 0.3% for Na, 1% for K, 6% for SO₄, and 1% for Cl. However, a similar magnitude of departure from actual concentrations is seen at only about 25 ppt when the freshwater input has a hardness of 500 mg/L CaCO₃.

4.2.2.2 Base marine chemistry

The base marine chemistry is the chemistry that would be expected from full-strength sea water. This defaults to sea water with a salinity of 35 ppt, Ca of 10.02 mmol/L, Mg of 51.48 mmol/L, Na of 457.12 mmol/L, K of 9.950 mmol/L, SO₄ of 27.52 mmol/L, Cl of 532 mmol/L, DIC of 1.998 mmol/L, and PO₄ of 2.33 mmol/L.

4.2.2.3 Simple to full chemistry conversion process (salinity)

The Ca, Mg, Na, K, SO₄, Cl, and PO₄ ions and DIC are all calculated from salinity and base marine chemistry. The calculation assumes that the contribution from fresh water is negligible, and therefore the concentrations of ions and DIC are proportional to the base chemistry as per the following equation:

$$[X] = \frac{(\text{Salinity}) * [X]_{\text{base}}}{(\text{Salinity})_{\text{base}}} \quad \text{Equations 4-7}$$

where [X] is the concentration of one of the ions or DIC and "base" denotes the values from the base marine chemistry. As noted, this relationship breaks down when the freshwater source has high hardness, or when the site is near the freshwater source (i.e., has a low salinity). In those cases, it is best to measure the full chemistry of each sample, or to manually estimate the full chemistry while taking the freshwater source's ions into account.

4.3 MINIMUM DATA REQUIREMENTS

The BLM can be run with observations numbering from 1 to 1,000. In toxicity mode, a corresponding number of instantaneous WQC values will be calculated. Any observations with missing chemistry will be skipped in the calculation process. Missing metal concentrations will not prevent an observation from being run in

toxicity mode, since the metal concentration is the component being calculated. However, calculation of the FMB will require metal concentrations and at least 10 complete observations (including metal). See Section 0 for a full description of the required chemistry inputs.

4.4 DATA TEMPLATE

To assist users in collecting and organizing the information required to run the BLM, a BLM Data Template is now installed in the data directory of all new installations, and is available online (www.windwardenv.com/biotic-ligand-model/). The template is in Microsoft Excel®.xlsx format and contains two data template sheets in the workbook, one for toxicological data ("Toxicity Data Template") and one for site chemistry ("Site Chars Data Template"). The word "METAL" in some of the column headers should be replaced with the metal of concern when entering data. Although it is expected that the data in the Site Chars Data Template sheet will mostly be used in toxicity mode, metal concentration columns are still included to make it easier to compare to the predicted HC5/WQC, and also to make the template usable to set up inputs for FMB calculations.

The user should fill in this template to the greatest extent possible when preparing data for use in the BLM. If water quality parameters need to be estimated, it is suggested that the green columns be copied to the right of the table and filled in with the estimated values there, making it clear which ones are the estimated values and which are the measured/reported.

5 Running the Application

The following subsections describe how to use the software, the various functions and features available in the BLM Windows® Interface, and the use of the BLM with its various predictive capabilities.

5.1 STARTING THE APPLICATION

To start using the BLM Windows® Interface, the user should select the application using “Start -----> Programs -----> Biotic Ligand Model -----> Biotic Ligand Model, Version 3.16.2.41” on the Microsoft Windows® desktop. The user will be presented with the screen shown in Figure 5-1, which contains the user input areas and the various functions included in this version of the BLM Windows® Interface.

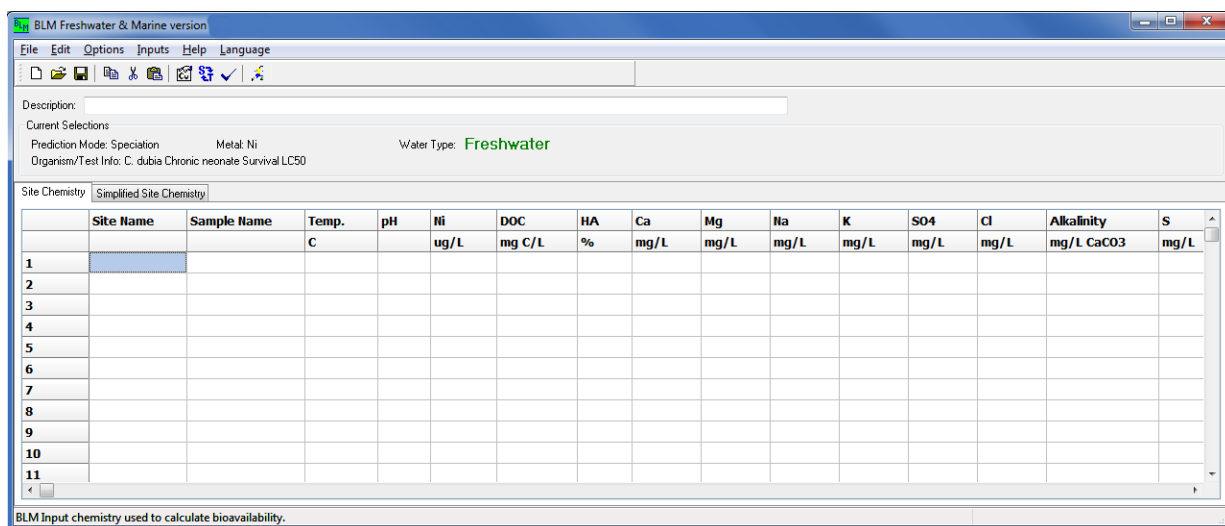


Figure 5-1. Opening screen for the BLM Windows® Interface application

If the user already has a BLM data file created using the BLM Windows® Interface, the file can be opened directly by double-clicking on the file name through a file system manager, such as Microsoft Windows® Explorer.

5.2 DESCRIPTION OF INTERFACE

Figure 0-1 shows a snapshot of the BLM Windows® Interface application. The main purposes of this section of the interface application are:

- ◆ To provide an easy-to-use editor to develop input files containing water chemistry information for the BLM
- ◆ To facilitate checks and validate user inputs for the various parameters

- ◆ To perform checks on whether the values entered for any given parameter are within the range for which the BLM has been calibrated
- ◆ To run the BLM for predictions of aquatic speciation or toxicity for a variety of metals and organisms

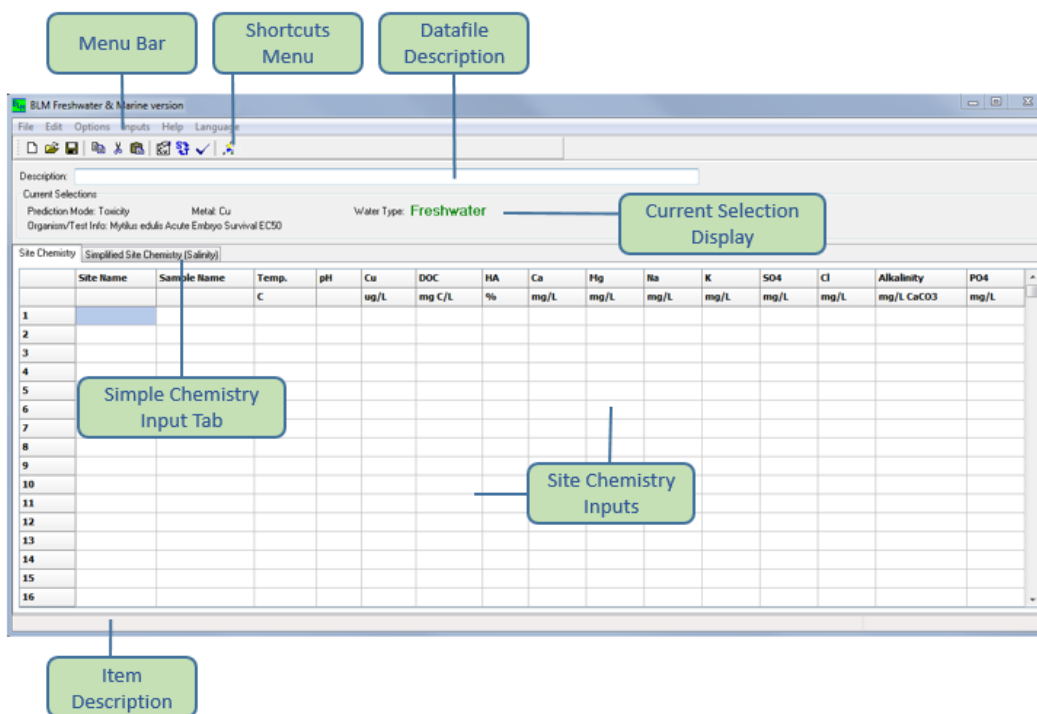


Figure 0-1. Snapshot of the BLM Windows® Interface

As shown in Figure 0-1, the interface window is divided into seven areas broadly based on their functionality. Each of these is described in the subsections that follow.

5.3 SITE CHEMISTRY INPUTS TAB

This region of the interface window contains a spreadsheet-based editor, which organizes the various BLM input parameters in a columnar format such that the chemistry for each discrete water sample can be specified in a separate row. Apart from the water chemistry information, two additional columns are also provided for labeling the sites and samples described in a given BLM data file. Figure 0-2 shows the various columns typically available for user input. See Section 0 for a full description of each of the required chemistry inputs and their importance in the model.

BLM Freshwater & Marine version

File Edit Options Inputs Help Language

Description:

Current Selections
 Prediction Mode: Toxicity Metal: Cu Water Type: Freshwater
 Organism/Test Info: Mytilus edulis Acute Embryo Survival EC50

Site Chemistry Simplified Site Chemistry (Salinity)

	Site Name	Sample Name	Temp.	pH	Cu	DOC	HA	Ca	Mg	Na	K	SO4	Cl	Alkalinity	PO4
			C		ug/L	mg C/L	%	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L CaCO3	mg/L
1															
2															
3															
4															

Figure 0-2. Columns for data input in the BLM Windows® Interface

The first column, “Site Name,” is meant to contain information about the site under consideration. For example, it could be the name of the river or site ID if the same file contains water chemistry data for more than one location along a particular river. The “Sample Name” field can be used to distinguish among the various water chemistry samples available for a particular site. For instance, at a given site, this field could represent the date and time at which the site water samples were collected. For both the site and the sample descriptor fields, the maximum number of characters allowed in each field is 20.

5.4 SIMPLE CHEMISTRY INPUTS TAB

By clicking on the “Simplified Site Chemistry” tab, a grid similar to the full site chemistry data inputs will be displayed, as shown in Figure 0-3 for freshwater mode and Figure 5-5 for marine mode. The contents of the tab will be slightly different depending on whether the software is in freshwater or marine mode. The “Simplified Site Chemistry” view is an alternate place where the user can save chemical characterization of the water chemistry. See Section 0 for a description of the required chemistry inputs for this tab.

BLM Freshwater & Marine version

File Edit Options Inputs Help Language

Description:

Current Selections
 Prediction Mode: Toxicity Metal: Cu Water Type: **Freshwater**
 Organism/Test Info: Ceriodaphnia dubia Acute Neonate (<24 hr) EC50; LC50

Site Chemistry Simplified Site Chemistry

	Site Name	Sample Name	Temp.	pH	Cu	DOC	Hardness
			C		ug/L	mg C/L	mg/L CaCO3
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							

Ion Ratios
 Ion ratios are custom values.

Ca : Mg 2.04
 Ca : Na 1.33
 Ca : K 12.61
 SO4 : Cl 0.79
 pCO2 = 10⁻ 3.5

Restore Default Values
 Select Ion Ratios...

Figure 0-3. Snapshot of Simplified Site Chemistry inputs and ion ratios

BLM Freshwater & Marine version

File Edit Options Inputs Help Language

Description:

Current Selections
 Prediction Mode: Toxicity Metal: Cu Water Type: **Marine**
 Organism/Test Info: Mytilus edulis Acute Embryo Survival EC50

Site Chemistry Simplified Site Chemistry (Salinity)

	Site Name	Sample Name	Temp.	pH	Cu	DOC	Salinity
			C		ug/L	mg C/L	ppt
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							

Base Marine Chemistry

Salinity (ppt) 35
 Ca (mmol/L) 10.02
 Mg (mmol/L) 51.48
 Na (mmol/L) 457.12
 K (mmol/L) 9.950
 SO4 (mmol/L) 27.52
 Cl (mmol/L) 532
 DIC (mmol/L) 1.998
 PO4 (mmol/L) 2.33

Restore Default Values

Figure 0-4. Snapshot of Simplified Site Chemistry (Salinity) inputs and base marine chemistry

The ion ratios and pCO₂ or base marine chemistry can be viewed and edited in a side bar with editable boxes, and can be returned to the starting values at any time by clicking the “Restore Default Values” button. If ion ratios or base marine chemistry are unknown for the water source being considered, use the default values in the boxes or (for freshwater mode) select the ecozone, ecoregion, state, or province the water source is in from the “Select Ion Ratios...” dialog. The “Select Ion Ratios...” button will open a dialog so that the user may select values specific to a Canadian ecozone (Ecological Stratification Working Group 1995) or province, or US state or ecoregion based on the delineations of the CEC (1997). The pCO₂ is inserted in a box to the right of the grid, below the ion ratios in freshwater mode. The value to be entered is the negative logarithm of the actual pCO₂ value.

While in the “Simplified Site Chemistry” tab, clicking the “Open” option will filter for files with the extension “.blme” rather than “.blm” in freshwater mode, or with “.swblme” rather than “.swblm” in marine mode. The “.blme” and “.swblme” file types save the ion ratios and base marine chemistry, respectively. An example of each of these files is included with the software download.

After the simplified site chemistry data has been inserted into this grid, simply run the BLM as normal. Alternatively, the user can select the “Site Chemistry” tab again to have the full site chemistry estimated. This is helpful if only some of the chemistry needs to be estimated, since the user can use any measured values to replace the estimated values in the “Site Chemistry” tab, ultimately resulting in a better prediction of toxicity. Switching from the simplified tab to the full chemistry tab is a one-way conversion – *the user cannot go back to the simple chemistry inputs after converting.*

5.5 MENU BAR

Located at the very top of the interface window, the menu bar provides the user with a range of functions and features, including:

- ◆ Mechanism for managing the BLM data files
- ◆ Text editing functions
- ◆ Model and output options
- ◆ Options relating to data inputs
- ◆ A help function
- ◆ Language selection

These features are described in the sections that follow.

5.5.1 File

Figure 0-5 shows the functions available under this menu item. Basic file management utilities to create a new BLM data file, open an existing BLM data file, and save a BLM

data file are provided. The user can also open a dialog to run batches from this menu (see Section 5.12) or open a file that was previously used (only the last five files are accessible via this dialog).

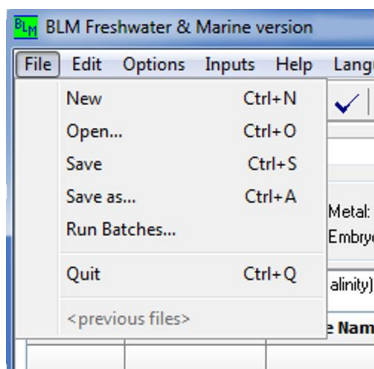


Figure 0-5. Snapshot of file menu item

Shortcut keys (shown to the right of each item) are also available for all of the different functions in the “File” menu.

For ease of access, BLM data files can also be opened directly by double-clicking on the BLM data file in a file system manager, such as Microsoft Windows® Explorer. This avoids having to first start the application and then locate the BLM data file of interest.

Note that the BLM data files created by the interface application are given a “.blm” extension in freshwater mode and a “.swblm” extension in marine mode by default. Even though the BLM data file created by the interface application basically is an ASCII text file, it is recommended that the user not modify this file using a program other than the BLM Windows® Interface application, as doing so may result in the BLM data file becoming corrupted. If this happens, the next time the user tries to edit that BLM data file using the BLM Windows® Interface, the file may not be read correctly by the BLM interface application.

5.5.2 Edit

Figure 0-6 shows the editing functions available in the BLM Windows® Interface. Basic editing functions such as “Cut,” “Copy,” and “Paste” are available in the interface application. The editing functions can be performed on a single or multiple cells by highlighting and dragging the cell(s), or by using the Shift and Arrow functions on the keyboard. These editing functions can also be accessed by using the shortcut keys shown to the right of each. Note that it is also possible to copy and paste data from external programs, such as a spreadsheet application, into the BLM Windows® Interface.

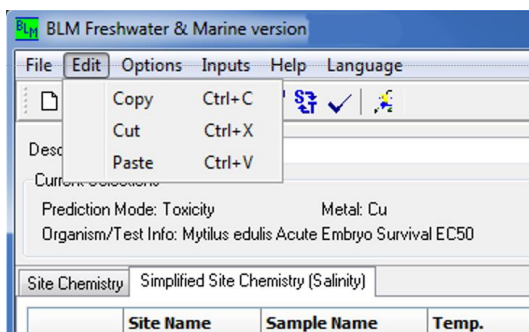


Figure 0-6. Snapshot of edit menu item

5.5.3 Options

Figure 0-7 shows the “Options” menu. There are the options to save output as Microsoft® Excel (*.xls), space-delimited (*.txt), or comma-delimited (*.csv), and to show the BLM “engine” window while the model is running. The latter option has been added so that the user can see when the BLM run gets stuck on an observation, as the window shows the progress through the observations. The BLM engine occasionally encounters a problem that it cannot overcome, and it will not proceed as normal. This is more likely with a toxicity mode run than a speciation run, since the optimization may fail to converge. The problem can usually be fixed by changing the order of the observations so that there is a different starting point for the optimization.

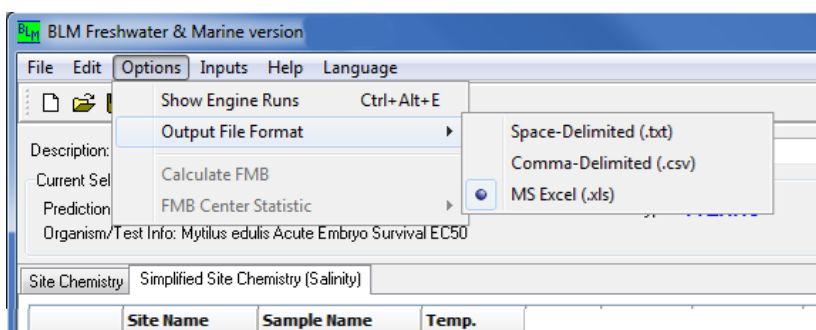


Figure 0-7. Snapshot of options menu item

The second set of options is for determining the behavior of the program in relation to calculating the FMB. One menu item can be un-checked to have the program not calculate the FMB. The second item gives the user the option of the center statistic to use when the FMB is calculated. The center statistic can be calculated as the median or the geometric mean. With a perfectly lognormal distribution, both options would give the same answer, but if the distribution’s shape is odd, then the geometric mean tends to give a better answer.

5.5.4 Inputs

Figure 0-8 shows the options available under the “Inputs” menu item. The units for all parameters can be changed by selecting the “Set Units” menu item. When changing a unit for a given parameter, data already input for that parameter can be converted to the new unit to prevent any loss of data. The “Set Inorganic Carbon” menu item can be selected to change how the user inputs inorganic carbon in the full “Site Chemistry” tab. There are two options to input inorganic carbon in closed systems: DIC or alkalinity. The third option is for an open system, in which the inorganic carbon is in equilibrium with the atmosphere. See Section 0 for further discussion of the inorganic carbon options. The “Set Exceedance Frequency” menu item is for setting the exceedance or return period for the FMB calculation. It can be specified as either days or years, and the Z_{EF} (inverse normal) value will be displayed below the input boxes.

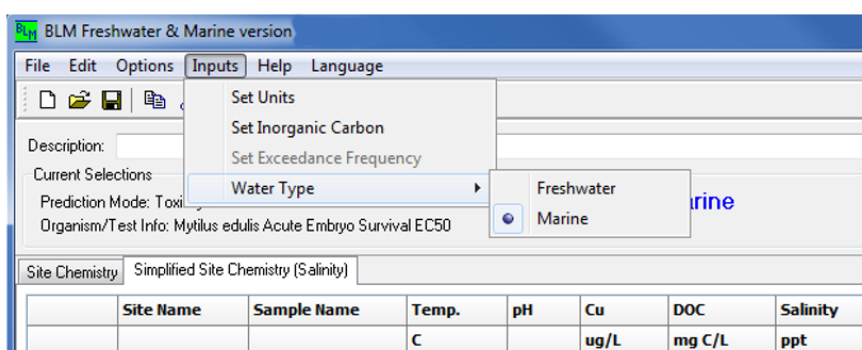


Figure 0-8. Snapshot of inputs menu item

The “Water Type” submenu item lets the user toggle between freshwater and marine environments for the simulations. This will change some of the inputs that are needed and the format of the “Simplified Site Chemistry” tab (hardness- or salinity-based, respectively), and will bring up the metal and organism selection screen for the user to choose an appropriate parameter file for their simulation. The user should select the water type before entering data into the input grid, since the input grid will be cleared when switching to a new water type.

5.5.5 Help

Figure 0-9 shows the various features available under the “Help” menu item.

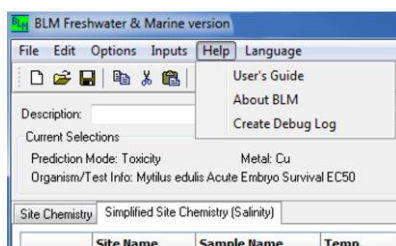


Figure 0-9. Snapshot of help menu item

The User's Guide for the BLM Windows® Interface can be accessed via this menu item. In addition, under the "About BLM" sub-item, there is information on contacts for technical support. The "Create Debug Log" option will direct the program to make a detailed log of everything that happens in it, which is useful for sending to the developers if a problem is occurring.

5.6 SHORTCUTS MENU

Icons located on the toolbar provide shortcuts to some of the menu bar items, as well as additional functions that are not available on the menu bar. Figure 0-10 shows the various icons and their functions; brief descriptions are provided in the subsections that follow.

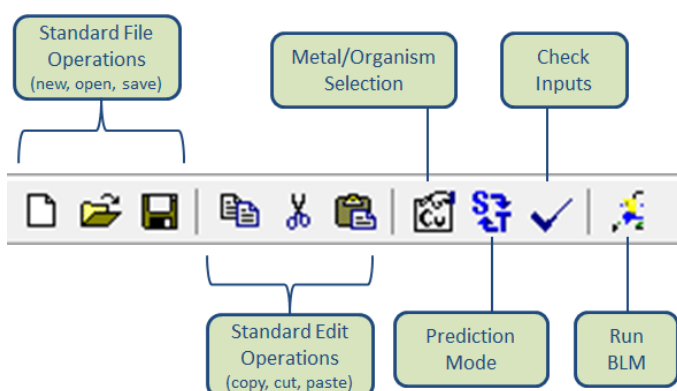


Figure 0-10. Shortcut menu icons

5.6.1 Open file

This is a shortcut to the menu bar item under "File -----> Open" and serves as a quick way to access the BLM data files. If the BLM data file being edited has changed since the last time it was saved and the user tries to open another file, the user will be queried as to whether the current data file should be saved prior to opening another data file.

5.6.2 Save file

This is a shortcut to the menu bar item under "File -----> Save" and serves as a quick way to save the BLM data file. The data file will be saved with the same, existing name. If the user wishes to save the file under a different file name, the menu bar item "File -----> Save As" should be chosen.

5.6.3 Metal/organism selection

As mentioned, the BLM can be used to study the toxicity and speciation of a variety of metals and organisms. This action button is provided to allow the user to select the metal and the organism for which toxicity or speciation needs to be predicted. Clicking

on this icon will present the user with the window shown in Figure 0-11. The user can then choose the desired metal and organism for the BLM predictions. The current metal and organism selections are displayed in the “Current Selection” display area (see Figure 0-1).

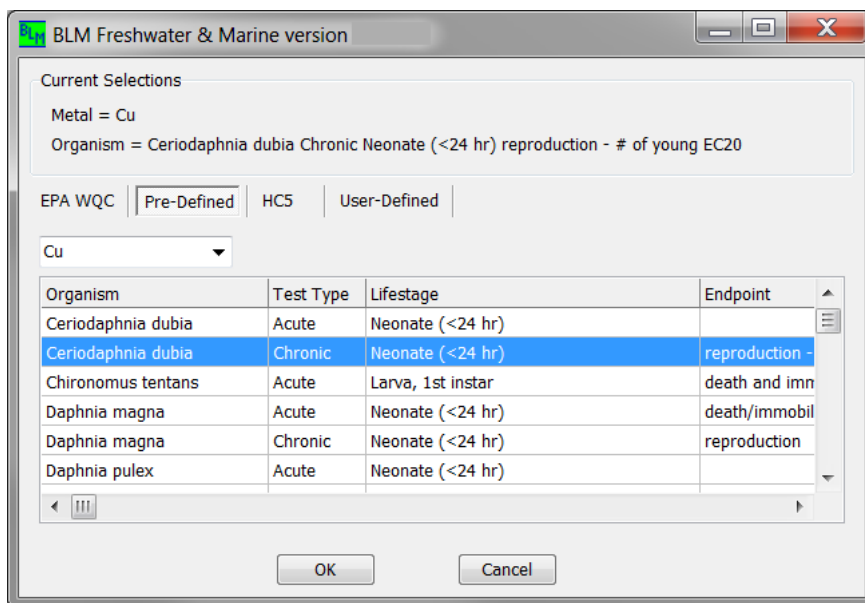


Figure 0-11. Metal and organism selection options

Acute and chronic parameter files have been developed for several metals in freshwater and marine environments, and they will be added to the package as they are finalized. Unless otherwise specified, the available parameter files are currently for acute effects.

5.6.3.1 Metal and organism options

The metal- and organism-specific parameter files that are included in the current distribution of the BLM Windows® Interface can be accessed by selecting the “Pre-defined” tab button, then selecting first the metal and then a row from the table, as shown in Figure 0-11. The user can resize the column widths by clicking and dragging between two column headers. The user can sort the table by any of the columns by clicking on the column header (sorting will cycle through original-, descending-, and ascending-order sorting). Note that the organism-, life stage-, and endpoint-specific options available in the table are subject to change as part of the ongoing refinement of the calibration and application of the BLM, and may therefore undergo revisions from time to time.

In order to provide enough information to accommodate chronic endpoints, a new parameter file format was created for starting in this distribution. Old parameter files only contained information about the organism; nothing about the life stage, endpoint, references, or methods was in any format that could be interpreted by the BLM

Windows® Interface program. In order to make the old parameter files available to users, many of the columns in the table will be blank for some metals and organisms, until we have converted everything to the new parameter file format.

It is recommended that the user develop separate BLM data files for separate metals, even though the application of the BLM may be for the same set of observations. The current distribution of the BLM can be applied to only one metal at a time. Because the input metal concentrations in the BLM Windows® Interface “Site Chemistry” tab grid are expressed in units of µg/L, the interface application internally converts these to mol/L using the molecular weight for the metal selected by the user when saving the input file. Changing the metal for the BLM application within an existing data file developed for a different metal may result in an erroneous conversion from µg/L to mol/L when the user saves and opens the data file the next time.

5.6.3.2 User-defined files

In addition to the metal- and organism-specific parameter files that are included with the current distribution, users may opt to develop and use their own versions of these files for BLM predictions. This is achieved by selecting the “User-defined” tab button shown in Figure 0-11 and clicking “Select Parameter File...” to browse for the desired parameter file. New parameter files can be developed by the user along the lines of the parameter files supplied with this distribution (i.e., files with the extension “.dat” located in the “Model” sub-directory within the BLM directory in Program Files). Note that the user should first select the appropriate parameter file when opening a BLM input file that was previously developed for a user-selected parameter file.

5.6.3.3 EPA WQC & HC5 Calculations

The US Environmental Protection Agency’s (EPA’s) nationally recommended ambient water quality criteria (AWQC) for Cu are based on the BLM (EPA 2007). The ability to calculate criteria has been included in previous publicly available BLM software versions (2.2.1, 2.2.3) and is included in this version of the BLM Windows® Interface. The freshwater Cu AWQC can be generated by selecting the “EPA WQC” tab button shown in Figure 0-11, and then selecting “Cu” in the “Select Metal” drop-down menu. When the BLM is run in the Cu WQC mode, it generates the EPA (2007) freshwater dissolved Cu WQC (acute and chronic) for each row of input and provides these results in the “WQC” report (see Section 5.10) (Figure 5-13). The report provides the BLM-based final acute value (FAV) and the acute instantaneous water quality criteria (IWQC) (which is calculated as the FAV divided by two; see EPA 2007). The acute and chronic criteria are also known as the criterion maximum concentration (CMC) and criterion continuous concentration (CCC), respectively. If dissolved Cu input data are provided by the user in the BLM input data file, the values are tabulated in the criteria output file and used to calculate acute and chronic toxic units (TUs). These TUs are computed as the ratio of the input dissolved Cu concentration to the IWQC output for each input row, with TU values >1 indicating a potential exceedance of the particular

Cu IWQC. Given the basis of EPA's aquatic life WQC derivations (Stephan et al. 1985), a TU >1 does not explicitly mean actual toxicity would be expected to occur. Rather, it simply indicates an exceedance of the particular magnitude of the criterion.

Site Label	Sample Label	Final Acute Value (FAV), ug/L	CMC (CMC=FAV/2), ug/L	CCC (CCC=FAV/ACR), ug/L	Cu	Acute Toxic Units (Acute TU=Cu/CMC)	Chronic Toxic Censored Flag (Chronic TU=0 = quantified, 1 = BDL)
"Kansas River 6892350" "7/22/1987	"	212.10	106.05	65.87	6.00	0.06	0.09
"Kansas River 6892350" "10/20/1987	"	173.43	86.71	53.86	2.50	0.03	0.05
"Kansas River 6892350" "11/23/1987	"	116.44	58.22	36.16	1.20	0.02	0.03

Figure 0-12. Instantaneous copper criteria report

The acute and chronic HC5 option is available for other metals for which BLM-normalized toxicity data have been used to construct a SSD, which was then used to calculate the 5th percentile of organism sensitivity. The BLM-predicted HC5 can be interpreted in a manner similar to EPA's derivation of aquatic life WQC, but HC5 calculations have not been reviewed and adopted by EPA.

For additional information on the terminology and the exact workings of the WQC calculation, the user is advised to refer to EPA (2007). The criteria report shown in Figure 5-13, being in Microsoft® Excel file format, can also be copied for pasting onto any external application or report.

5.6.4 Prediction mode

The BLM Windows® Interface application allows the user to run the BLM in either toxicity mode or speciation mode. When run in toxicity mode for a metal and organism specified by the user, the BLM will predict the amount of metal required to cause acute or chronic toxicity in each observation/line in the input file. However, when the BLM is run in speciation mode for a metal concentration specified by the user, the BLM will predict the organic and the inorganic chemical speciation in the water column and the metal accumulation on the biotic ligand that would result.

The "Prediction Mode" button allows the user to toggle between the speciation and toxicity prediction modes in the BLM. The current prediction mode is also displayed in the "Current Selection Display" area (see Section 5.7). The current prediction mode setting is saved when the application is closed, and the application will default to the last prediction mode used.

5.6.5 Check inputs

In order to provide users with an idea of the range of water chemistry to which the BLM can be applied, the range of parameter values to which the BLM has been developed and calibrated is defined in the BLM Windows® Interface application. After creating a BLM data file, the user may wish to check the water chemistry inputs to verify whether the parameter values are within the overall range for which the BLM has been calibrated, and to check whether all the parameters necessary for a BLM prediction have been specified. Clicking the “Check Inputs” icon generates an input check report that contains information on which parameters are out of range (i.e., too high or too low when compared with the range for which the BLM has been calibrated) and which parameters are missing for any given input row. The ranges prescribed for each of the BLM input parameters are shown in Table 0-1. A range represents the minimum and maximum of the parameter values observed in toxicity studies of several metals. They are sometimes just a range of values that would be believable for a parameter in a natural setting. These ranges are meant to help the user find erroneous data but are not true model validation/calibration ranges. The BLM will still run when input data are outside of these ranges, and it is up to the user to evaluate whether chemistry in these cases is relevant for the organism under consideration. Figure 5-14 shows an example of an input check report.

Table 0-1. Prescribed ranges for BLM input parameters

Parameter	Lower Bound	Upper Bound
Temperature (°C)	10	25
pH	4.9	9.2
DOC (mg/L)	0.05	29.65
Humic acid content (%)	10	60
Calcium (mg/L)	0.204	120.24
Magnesium (mg/L)	0.024	51.9
Sodium (mg/L)	0.16	236.9
Potassium (mg/L)	0.039	156
Sulfate (mg/L)	0.096	278.4
Chloride (mg/L)	0.32	279.72
Alkalinity (mg/L)	1.99	360
DIC (mmol/L) ^a	0.056	44.92
Sulfide (mg/L)	0.001	0.006
Hardness (mg/L)	7.9	525
Salinity (ppt)	5	38
All other parameters (mol/L)	0	1E+6

^a DIC is included because it is an alternate input to alkalinity. Since the conversion to DIC from alkalinity is pH dependent, a separate range was determined for alkalinity.

DIC – dissolved inorganic carbon
DOC – dissolved organic carbon
ppt – parts per thousand

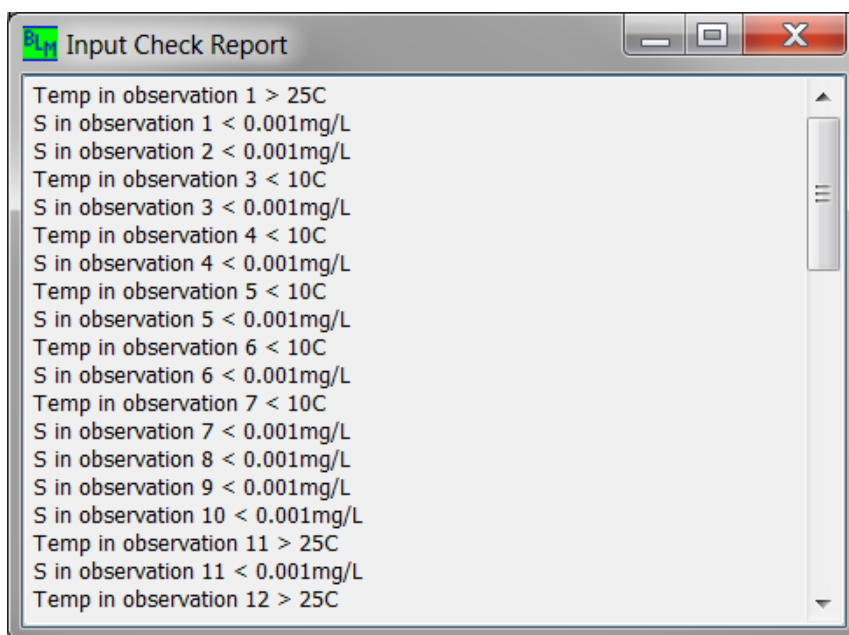


Figure 0-13. Example of an input check report generated by the check inputs function

5.6.6 Run BLM

The “Run BLM” icon is used to launch the BLM calculation engine to predict either metal toxicity or speciation for the user-specified selections for the site water chemistry described in the BLM data file currently open in the BLM Windows® Interface. If the BLM data file has changed since the last time it was saved and the user tries to run the BLM, the user will be queried as to whether the current file should be saved prior to the BLM run. If the option to calculate the FMB is selected but the user does not have enough valid data in the input file, then a warning will display when this button is clicked (the model will still complete its run, just without the FMB calculation).

5.7 CURRENT SELECTION DISPLAY

This area of the interface window displays the current water type, metal, organism, and prediction mode selections made by the user. In the example shown in Figure 5-1, the user has opted to predict the toxicity of Cu for the EPA WQC calculation in fresh water. The options selected by the user are saved in the BLM data file and by the program when it closes. When the program is opened, it will default to the set of options selected when last closed. When a BLM input file is opened, the application

either switches to the selections made by the user when the input file was last saved, or it asks the user to select an input file.

5.8 DATA FILE DESCRIPTION

This area is provided for the user to insert comments describing the BLM data file; these comments will then be saved along with the water chemistry parameters input by the user. Although this function is not of critical importance to the use of the BLM, for record-keeping and possibly quality assurance/quality control (QA/QC) purposes, it is useful.

5.9 ITEM DESCRIPTION

Located at the very bottom of the interface window, this area is designed to display a brief description of the icon/image/area over which the cursor is currently positioned. In the example shown in Figure 5-1, the cursor is positioned over the “Data Inputs” area. Similar messages are displayed when the cursor is moved over other areas of the interface window.

5.10 DESCRIPTION OF OUTPUT FILES

The BLM creates two or three output files within the directory containing the BLM input file. The files have the same filename as the input file, with “.sim,” “.det,” or “.wqc” added before the file extension. For example, using the input file “test.blm” would create the following output files: “test.sim.txt,” “test.sim.csv,” or “test.sim.xls” (the simple version of the model output); “test.det.txt,” “test.det.csv,” or “test.det.xls” (the detailed version); and “test.wqc.xls” (the water quality report, created only when running the model for an EPA WQC or HC5). The format of the output files (i.e., text, csv, or Microsoft Excel®) is selected under the “Options” menu (see Section 0).

The “.det,” or detailed, output file contains all the chemical species accounted for in the BLM. Because the detailed file can be quite large, the “.sim,” or simple, output contains the following selected output results that may be of interest:

- ◆ Site and sample names
- ◆ BLM mode chosen (Speciation, Toxicity, LC50, etc.)
- ◆ pH
- ◆ Dissolved metal concentration in mol/L (the predicted FAV for Cu)
- ◆ Free metal concentration in mol/L
- ◆ Concentration of metal and metal hydroxide bound to DOC in mol/L
- ◆ Concentration of metal on the biotic ligand(s) in nmol/g_{wet}
- ◆ DOC in mg/L

- ◆ Percent humic acid
- ◆ Other input water chemistry in units of mol/L

The “.wqc” file contains a pared-down version of the results and is meant for those users interested in only the points relevant to compliance:

- ◆ Calculated FMB value(s) at the top (when that option is enabled)
- ◆ Site and sample labels
- ◆ Calculated WQC or HC5 value(s) in $\mu\text{g/L}$
- ◆ Measured metal concentration in $\mu\text{g/L}$
- ◆ Calculated toxic units (metal divided by WQC)
- ◆ Flags indicating censored metal concentrations

5.11 FMB CALCULATION

The user may have the option to calculate the FMB for WQC and HC5 parameter files. Follow these steps to run FMB calculations:

1. Load or enter data and save an input file as normal. There must be at least 10 observations in the input file for the FMB to be calculated. Note that censored metal data should be marked with a “<” or entered as a negative number, which will then be displayed with a “<.”
2. Select a WQC or HC5 parameter file from the “Metal/Organism Selection” dialog (see Section 0).
3. Under the “Options” menu, two options will now be enabled: “Calculate FMB” and “FMB Center Statistic.”
 - a. Make sure that “Calculate FMB” is checked.
 - b. If you would like to change the center statistic from the default, which is the geometric mean (geomean), select your option in the “FMB Center Statistic” menu.
4. Under the “Inputs” menu, the “Set Exceedance Frequency” option will now be enabled. Select it to change the target exceedance frequency for the FMB calculation. The default is once in three years.
5. Click the “Run” button.
6. Open the “*.wqc.xls” output file. The calculated FMB(s) will be located above the table in rows 5 through 8.

5.11.1 Using user-created HC5 files

The BLM software does not currently have user interface support for specifying that a user-defined file contains an HC5 that can be used to calculate an FMB. However, it is still possible to use a user-created file for this purpose, but it involves manually changing some of the installation files for the software. An advanced user may be able to make these changes without compromising the software integrity, but we suggest contacting us for support if you have an HC5 you would like to use for FMB calculations. We can send you a custom software update with the necessary changes in place.

5.12 BATCH MODE

The BLM's batch mode dialog can be accessed from the "File" menu by clicking the "Run Batches..." menu item. The BLM software can only accept 1,000 site chemistry observations at a time (because of memory issues), but monitoring datasets often contain many times that number. Therefore, a way to import large amounts of data and save and/or run them in (up to) 1,000-observation batches is useful. A screenshot of the batch mode dialog is shown in Figure 0-14, and the components shown therein are explained below.

Component	Header	Units
Site Name		
Sample Name		
Temp.		C
pH		
Cu		ug/L
DOC		mg C/L
HA		%
Ca		mg/L
Mg		mg/L

Figure 0-14. The batch run utility dialog

5.12.1 File type

In this section of the dialog, the user can choose between the two file types: “Spreadsheet with Data” or “Collection of Input Files.” The “Spreadsheet with Data” option allows the user to import data from a *.csv or *.xls file, have the program create BLM input files, and then optionally run the BLM with those files. The “Collection of Input Files” option allows the user to choose one or more BLM input files that have already been created and run each of them in sequence. The “Input Format,” “Component-Mapping Grid,” and “Batch Size” sections will disappear when the “Collection of Input Files” option is selected, since they are only relevant when importing data to create input files.

5.12.2 Data file(s)

The “Data File(s)” section has a box where the name of the single data file or the list of BLM input files that is/are selected will appear. Click the “Browse...” button next to this box to select which file(s) to use. Note that only one data file can be selected, but multiple BLM input files can be selected. A data file should have all relevant site chemistry data in columns (i.e., a column for temperature, a column for pH, etc.) and expressed as one of the accepted units used for that component (go to the “Inputs” menu and click “Set Units” to see these options). The columns should correspond to either full site chemistry components (temperature, pH, Cu, DOC, HA, Ca, Mg, Na, K, SO₄, Cl, and alkalinity) or simple chemistry components (temperature, pH, Cu, DOC, and hardness) and should have unique names indicated in the first row. Accepted file types for data files are *.csv (comma-separated values) and *.xls (1997–2003 Microsoft® Excel). *Note that the row limit for *.xls files is 65,536 rows, and larger datasets will require the use of a *.csv file.* Conversely, the “Collection of Input Files” option allows the user to select any number and combination of full BLM chemistry and simple BLM chemistry files that it will then open and run in sequence.

5.12.3 Input format

This section only appears when the “Spreadsheet with Data” file type is selected. Here the user can select which set of chemical components to use (i.e., full or simple chemistry). If simple chemistry is selected, all of the input files that are created will use the ion ratios that are currently selected in the simple chemistry tab. The “Batch Run” dialog does not need to be closed to change the ion ratio selection.

5.12.4 Component-mapping grid

This section only appears when the “Spreadsheet with Data” file type is selected. When a data file is selected, the program will read the first row of the file for the column headers. These headers will appear as drop-down box options in the column labeled “Header” of the component-mapping grid. The program makes guesses wherever possible to fill in these options (e.g., if a column is called “DOC [mg/L],” the

program will fill in that header as the one to use for the DOC component). Check these assignments (the computer is only *slightly* smart) and fill in any that are missing by selecting an appropriate column header from the drop-down box that appears when \ a cell is selected for editing. Also make sure to check that the units are correct; they should match the units of the data in the data file. All of the components need to have a header assignment in order to run batch mode. The same header can be used more than once (e.g., if there is only one “ID” column, it can be used as both “Site Name” and “Sample Name”).

5.12.5 Batch size

This section only appears when the “Spreadsheet with Data” file type is selected. The “Batch Size” section of the dialog has a small text box and a slider for selecting the size of the batches into which the program should divide the data. It defaults to the maximum number of observations possible in one input file (1,000), but it can be adjusted if a smaller number of observations per file is desired. Move the slider or type a number in the box to change the batch size. If the number of observations is not a multiple of the batch size, then the last batch will be smaller than the batch size number.

5.12.6 Output directory

The “Output Directory” section is for choosing and/or displaying where the files are going to be saved. The “Spreadsheet with Data” option lets the user choose a different directory for saving the newly created BLM input files and their associated output files, or keep the default directory (the same directory the data file is in). Clicking the “Browse...” button opens a separate dialog that the user can use to navigate to an existing directory. A new directory can be created by modifying the “Directory Name” field of the selection dialog. When the “Collection of Input Files” option is selected, the output directory cannot be changed; it is being displayed for clarity only and will be the same directory from which the input files come.

5.12.7 Execute

The “Execute” button tells the program to perform the action displayed in the drop-down box next to the “Execute” button. The “Create input files only” option is available only when the “Spreadsheet with Data” file type option is selected. With this option selected, the program will not normalize any of the data; it will just make the input files and stop. If a “Run...” option is selected instead, then the program will do the equivalent of reading an input file and clicking the “Run” button in the main interface. When all batches are finished running, a message will appear that tells the user when it finished and how long it took. It takes about one hour to run one 250-observation batch, but the exact amount of time depends on computing power and how difficult the chemistry is for the BLM engine to converge on. Be patient and remember that the BLM engine is doing around 80 chemical equilibria calculations

consisting of more than 100 chemical reactions and more than 25 mass balances, and solving a non-linear optimization algorithm for each site chemistry observation.

6 Example Application

The BLM Windows® Interface installation also features an example application for demonstration purposes. This file, named “LakeSuperior.blm,” is installed along with the application and is located in the BLM data directory asked for during installation (by default within the “My Documents” folder). The file can be opened directly, by double-clicking on the file name through a file system manager such as Microsoft Windows® Explorer, or by first starting the BLM Windows® Interface application and selecting the file through the “File -----> Open” action. This example data file contains the 2009 average water quality observations from Lake Superior, as reported by Tetra Tech (2013).

The data file “LakeSuperior.blm” can be used to predict metal speciation using the input metal concentration, or to predict the LC50 for a variety of metals and organisms. It is recommended that separate BLM data files always be maintained for each metal. In this case, the data file contains dissolved Cu concentrations; the BLM can be used to predict the inorganic, organic, and biotic speciations by setting the BLM prediction mode to “Speciation” using the shortcut Menu button “Prediction Mode.” Metal toxicity for the specified site water chemistry can also be predicted by setting the prediction mode to “Toxicity” and selecting the metal and organism for which toxicity is to be predicted using the shortcut Menu button “Metal/Organism Selection.”

Once the user has defined the scope of the BLM predictions, the BLM can be run using the shortcut Menu button “Run BLM.” Depending on the number of lines of inputs in the BLM data file, the runtime for the BLM predictions can vary from a few seconds to several minutes. Upon completion of the BLM run, the user is informed of the names and locations of the output files and given the option to open the directory, as shown in Figure 0-1. The contents of the output files are described in Section 5.10.

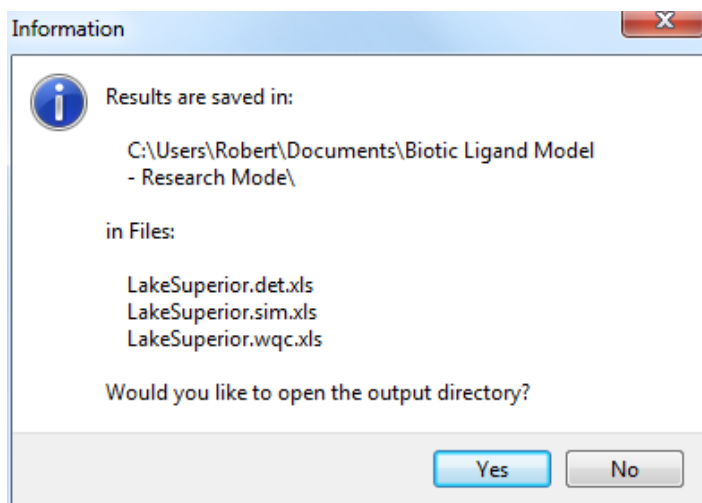


Figure 0-1. Example of notification window shown at completion of BLM run

7 Uninstalling the BLM

To uninstall the BLM Windows® Interface, select the uninstall utility using “Start -----> Programs -----> Biotic Ligand Model -----> Uninstall...” All files installed by the BLM during setup will be uninstalled. However, none of the files created by the user and saved in the BLM installation directory will be deleted during the uninstall. These files will have to be deleted manually by the user, if so desired.

8 Ion Ratio Tables

Monitoring data were gathered from across the United States and Canada (Table 8-1). Data from the United States were collected using the dataRetrieval package in R statistical software, totaling 21,685 observations from 47 states (Hirsch and De Cicco, 2015) (Table 8-2). The Canadian data were mostly collected from publicly available monitoring data, totaling 40,498 observations from 13 provinces and territories (Table 8-3).

Table 8-1. Median ion ratios in North America

Geographic Unit	Ca:Mg	Ca:Na	Ca:K	SO4:Cl
North America	2.04	1.33	12.61	0.79
Canada	2.14	1.47	13.94	1.70
United States	2.11	1.19	12.21	0.65

Table 8-2. Median ion ratios in Canadian provinces

Geographic Unit	Ca:Mg	Ca:Na	Ca:K	SO4:Cl
Alberta	1.82	1.96	29.27	4.70
British Columbia	3.33	3.30	23.51	3.86
Manitoba	1.28	1.29	8.69	1.52
New Brunswick	4.15	1.35	16.92	0.48
Newfoundland and Labrador	2.49	0.42	6.79	0.10
Northwest Territories	1.87	2.15	7.32	1.23
Nova Scotia	1.28	0.15	3.49	0.12
Nunavut	2.61	3.58	6.66	4.92
Ontario	2.27	1.34	13.19	1.41
Prince Edward Island	1.53	2.51	31.86	0.16
Quebec	2.48	1.41	16.26	0.56
Saskatchewan	0.83	0.48	4.61	3.29
Yukon	3.35	8.07	37.53	22.14

Table 8-3. Median ion ratios in US states

Geographic Unit	Ca:Mg	Ca:Na	Ca:K	SO4:Cl
Alabama	2.81	0.89	8.26	0.81
Alaska	2.87	5.26	25.22	5.87
Arizona	1.76	0.54	18.93	0.95
Arkansas	2.84	0.79	11.38	0.29
California	2.20	0.54	9.73	0.41

Geographic Unit	Ca:Mg	Ca:Na	Ca:K	SO4:Cl
Colorado	2.81	1.23	12.68	1.81
Connecticut	2.05	0.47	8.13	0.08
Delaware	1.70	0.67	6.26	0.19
Florida	4.83	1.68	5.75	0.48
Georgia	2.39	0.57	3.17	0.35
Hawaii	0.86	0.38	5.35	0.08
Idaho	1.67	1.04	11.56	0.76
Illinois	1.73	1.80	13.68	0.75
Indiana	1.99	1.95	16.93	0.64
Iowa	1.56	2.60	17.31	0.53
Kansas	3.04	0.71	9.11	0.36
Kentucky	3.40	2.59	11.55	0.58
Louisiana	1.97	1.13	10.85	0.63
Maryland	2.31	1.19	11.59	0.47
Massachusetts	3.70	0.23	6.22	0.07
Michigan	2.11	0.90	21.53	0.10
Minnesota	0.89	1.31	18.91	0.73
Mississippi	1.89	1.34	6.60	0.74
Missouri	1.76	1.19	10.65	1.62
Montana	2.01	1.02	13.58	5.09
Nebraska	1.88	0.75	10.04	2.23
Nevada	1.65	0.46	15.07	1.01
New Hampshire	2.39	0.12	5.39	0.05
New Jersey	1.76	0.60	10.64	0.21
New Mexico	3.64	1.19	12.76	2.51
New York	2.86	1.42	20.94	0.47
North Carolina	1.62	0.57	1.84	0.24
North Dakota	1.49	0.54	12.09	5.68
Ohio	2.02	1.52	11.41	0.51
Oklahoma	1.86	0.53	7.68	0.34
Oregon	1.90	1.32	11.45	0.38
Pennsylvania	2.10	1.48	13.61	0.53
South Carolina	1.38	0.45	2.12	0.23
South Dakota	1.55	0.48	10.90	7.59
Tennessee	2.18	2.06	11.08	1.04
Texas	2.67	0.46	14.99	0.49
Utah	1.46	0.51	19.04	1.12

Geographic Unit	Ca:Mg	Ca:Na	Ca:K	SO4:Cl
Virginia	1.39	0.70	4.13	0.18
Washington	1.83	1.06	10.09	1.28
West Virginia	1.12	1.20	11.46	4.29
Wisconsin	1.34	0.89	20.05	0.21
Wyoming	2.12	0.62	14.15	2.35

9 References

- Allen HE, Hansen DJ. 1996. The importance of trace metal speciation to water quality criteria. *Wat Environ Res* 68(1):42-54.
- Commission for Environmental Cooperation. 1997. Ecological regions of North America. Toward a common perspective. Commission for Environmental Cooperation, Montreal, Quebec, Canada.
- Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR, Santore RC. 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ Toxicol Chem* 20(10):2383-2396.
- Ecological Stratification Working Group. 1995. A national ecological framework for Canada. Agriculture and Agri-Food Canada, Research Branch, Centre for Land and Biological Resources Research and Environment Canada, State of the Environment Directorate, Ecozone Analysis Branch, Ottawa, Canada.
- EPA. 2007. Aquatic life ambient freshwater quality criteria - copper, 2007 revision. EPA-822-R-07-001. Office of Water, US Environmental Protection Agency Washington, DC.
- MacRae RK, Smith DE, Swoboda-Colberg N, Meyer JS, Bergman HL. 1999. Copper binding affinity of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) gills: implications for assessing bioavailable metal. *Environ Toxicol Chem* 18(6):1180-1189.
- Meyer JS, Santore RC, Bobbit JP, Debrey LD, Boese CJ, Paquin PR, Allen HE, Bergman HL, Ditoro DM. 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free-ion activity does not. *Environ Sci Tech* 33:913-916.
- Morel FMM, Hering JG. 1993. Principles and Applications of Aquatic Chemistry. John Wiley & Sons, Inc., New York, NY.
- Pagenkopf GK. 1983. Gill surface interaction model for trace-metal toxicity to fishes role of complexation, pH, and water hardness. *Environ Sci Technol* 17:342-347.
- Paquin PR, Di Toro DM, Santore RC, Trivedi D, Wu K-B. 1999. A biotic ligand model of the acute toxicity of metals: III. Application to fish and daphnia exposure to silver. In: Integrated Approach to Assessing the Bioavailability and Toxicity of Metals in Surface Waters and Sediments, EPA-822-E-99-001. US Environmental Protection Agency, Washington, DC, pp 3-59 - 3-102.
- Playle RC, Gensemer RW, Dixon DG. 1992. Copper accumulation on gills of fathead minnows: influence of water hardness, complexation and pH of the gill micro-environment. *Environ Toxicol Chem* 11:381-91.

- Playle RC, Dixon DG, Burnison K. 1993a. Copper and cadmium binding to fish gills: estimates of metal-gill stability constants and modelling of metal accumulation. *Can J Fish Aquat Sci* 50:2678-2687.
- Playle RC, Dixon DG, Burnison K. 1993b. Copper and cadmium binding to fish gills: modification by dissolved organic carbon and synthetic ligands. *Can J Fish Aquat Sci* 50:2667-2677.
- Ryan A, Santore R, Delos C. 2018. Application of a fixed monitoring benchmark approach to evaluate attainment of time-variable water quality criteria: copper BLM as a case study. *Integr Environ Assess Manag*.
- Santore RC, Driscoll CT. 1995. The CHESS model for calculating chemical equilibria in soils and solutions. In: Loeppert R, Schwab AP, Goldberg S, eds, *Chemical Equilibrium and Reaction Models*. American Society of Agronomy, Madison, WI, pp 357-375.
- Santore RC, Di Toro DM, Paquin PR, Allen HE, Meyer JS. 2001. A biotic ligand model of the acute toxicity of metals: II. Application to acute copper toxicity in freshwater fish and daphnia. *Environ Toxicol Chem* 20(10):2397-2402.
- Santore RC, Mathew R, Paquin PR, DiToro D. 2002. Application of the biotic ligand model to predicting zinc toxicity to rainbow trout, fathead minnow, and *Daphnia magna*. *Comp Biochem Physiol Part C* 133:271-285.
- Stephan CE, Mount DI, Hansen DJ, Gentile JH, Chapman GA, Brungs WA. 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. PB85-227049. Office of Research and Development, US Environmental Protection Agency, Washington, DC.
- Sunda WG, Guillard RRL. 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *J Mar Res* 34:511-529.
- Sunda WG, Hansen PJ. 1979. Chapter 8. Chemical speciation of copper in river water. Effect of total copper, pH, carbonate, and dissolved organic matter. In: Jenne EA, ed, *Chemical Modeling in Aqueous Systems*, ACS Symposium Series 93. ACS, Washington, DC, pp 147-180.
- Tetra Tech. 2013. Feasibility study for the remediation of sediments adjacent to Lockheed Martin Middle River Complex, Middle River, Maryland. Tetra Tech, Inc.
- Tipping E. 1994. WHAM - a chemical equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances. *Comput Geosci* 20:973-1023.