Ecotoxicology and Environmental Safety 148 (2018) 336–345

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Validation of Cu toxicity to barley root elongation in soil with a Terrestrial Biotic Ligand Model developed from sand culture☆

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ARTICLE INFO

Keywords:
Sand culture
TBLM
Osmotic effect
Soil toxicity

ABSTRACT

Constants for a Terrestrial Biotic Ligand Model (TBLM) to predict the Cu toxicity to barley root elongation (RE) were developed from controlled sand culture experiments. These constants were used to predict RE in soil culture. The competition of H^+, Ca^{2+}, and Mg^{2+} to Cu^{2+} toxicity were studied individually and independently, and linear relationships between EC50 free Cu^{2+} and H^+, Ca^{2+}, and Mg^{2+} activities were found, meaning that the cations H^+, Ca^{2+}, and Mg^{2+} will alleviate the toxicity of Cu^{2+} in solutions. Toxicity accompanying increasing concentration of solution ions other than Cu^{2+} was observed and modeled as an osmotic effect which improved soil culture toxicity prediction. The Root Mean Square Error (RMSE) of %RE and EC50 (50% effective concentration) for soil toxicity prediction using TBLM parameters developed from sand culture are 13.0 and 0.23 respectively, which are as good as that of 14.0 and 0.24 using parameters that developed from soil culture itself. A model including the activity at the root plasma membrane surface was tested and found not to provide improvement over the use of bulk solution activity to predict metal toxicity. TBLM parameters obtained from water solution culture were unable to accurately predict the EC50s in soils whereas the parameters obtained from sand culture were able to predict the toxicity in soils. Including the toxicity of $CuOH^+$ was found to improve the toxicity prediction slightly.

1. Introduction

The wide use of copper and its association with soil make it necessary to assess Cu toxicity in soil systems. The total Cu concentration or free Cu activity does not represent the metal bioavailability and it's toxicity in soil (Allen, 2002), so the Terrestrial Biotic Ligand Model (TBLM) based on metal speciation in soil and soil solution, and the biological interaction of metals with the biotic sites was proposed and developed to address Cu toxicity (Steenbergen et al., 2005; Thakali et al., 2006). It was assumed that it is the binding between Cu^{2+} and biotic sites that causes the toxicity, and competition for the same sites from the other cations and protons alleviates the toxicity. The toxicity and alleviation effects need to be studied to evaluate metal toxicity in soil systems.

Several Cu toxicity TBLMs have been developed– for earthworm survival (Steenbergen et al., 2005), for barley root elongation (Antunes et al., 2007; Lock et al., 2007; Wang et al., 2009, 2012), and for wheat root elongation (Luo et al., 2008) – mostly developed based on water solution bioassays. Water solution is used to differentiate hydroponic systems without solid substrate present from sand solution in which there is sand in addition to the solution. Among those TBLM studies only Steenbergen et al. (2005) performed direct comparison between measured pCu and predicted EC50 values for 6 field soils.

Instead of using a water solution system, Thakali et al. (2006) developed a TBLM directly from $CuCl_2$ amended soils for Cu toxicity to barley root elongation. However, adding the $CuCl_2$ salt will not only alter the Cu^{2+} activity in the soil solution, but also will cause the release of cations and protons from the soil organic matter (SOM) (Ponizovsky et al., 2006), so it is not possible to control cation concentrations in the soil solution to study each cation's effect on toxicity individually and independently. The release of cations also results in toxicity from an osmotic effect (Ben-Gal et al., 2009; Lin et al., 2015; Owojori et al., 2009), making it difficult to study Cu toxicity independently in the soil system.

Kinraide (2006) and Wang et al. (2010) reported that metal activity at the plasma membrane (PM) is better than the activity in bulk solution in predicting the metal toxicity for the plant. The alleviation of toxicity from the other cations was explained by the sequential processes that increase of cations concentration in the solution decrease the negativity of the plasma surface potential (ψ_{PM}), and then decrease the toxic metal

☆ Terrestrial Biotic Ligand Model constants developed from sand culture predict Cu toxicity for plant grown in soil culture.

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http://dx.doi.org/10.1016/j.ecoenv.2017.10.031
Received 24 July 2017; Received in revised form 10 October 2017; Accepted 12 October 2017
Available online 06 November 2017

activity at the plasma membrane and thereby the toxicity (Kinraide, 1998; Kinraide et al., 2004). Wang et al. (2011) also suggested a dual effects of membrane potential on ion uptake and toxicity.

The purpose of this study was to develop a TBLM for Cu toxicity to barley root elongation for the soil system, using a sand culture system to develop the required constants. The cations and pH were varied individually and independently and the competition from H^+, Ca^{2+} and Mg^{2+} was studied, and the co-toxicity from $CuOH^+$ and other Cu complexes (De Schamphelaere and Janssen, 2002; Wang et al., 2009) were analyzed. The TBLM developed from sand culture was validated by the soil culture bioassays that have been previously reported (Rooney et al., 2006; Thakali et al., 2006), and was compared with the prediction by the TBLM parameters that were developed from soil itself (Thakali et al., 2006) to evaluate the method of using sand culture to develop the toxicity model for soil culture. Analysis by the approaches with incorporation of plasma membrane surface potential effect (Kinraide, 2006; Wang et al., 2010), and prediction using TBLM parameters developed from water solution systems (Wang et al., 2009, 2012) were also included for comparing the ability to predicting the toxicity in soil culture.

2. Materials and methods

2.1. Toxicity bioassays

Barley, *Hordeum vulgare* (Doyce), as suggested by ISO 11269-1 (ISO, 1993) was used as toxicity test organism in the experiments. After germination, seeds with radical about 3–5 mm were planted in acid washed quartz sand containing defined composition solution. After four days of growth the root length was determined and % root elongation (%RE, root elongation = root length after harvest – root length before planting) relative to the exposure control which had no added Cu^{2+} (% RE = 100) was calculated for each plant harvested (see Supplementary data for experimental procedures).

2.2. Experimental design

A total of 17 experiments were conducted with concentrations of Ca^{2+} and Mg^{2+} and pH of the sand solution varied independently and individually, including 9 H^+ experiments (pH 4.5–8.0), 5 Ca^{2+} experiments (concentration 2.93–100 mM), and 5 Mg^{2+} experiments (concentration 0.998 – 30 mM). In each set of experiments, only the concentration of single cation or the proton activity was changed, while the others were kept constant at standard pH or concentration of Ca^{2+} and Mg^{2+}. The influence of K^+ on barley root elongation was not considered because Lock et al. (2007) showed that K^+ concentration did not affect Cu toxicity to barley. Experiment 1 was the experiment with standard pH and concentration of Ca^{2+} and Mg^{2+} (pH = 6, Ca^{2+} = 2.93 mM, Mg^{2+} = 0.998 mM). This generally follows the approach used by De Schamphelaere and Janssen (2002) to determine BLM parameters. Details of the experimental design are presented in the Supplemental data (Table SD1).

2.3. Soils for validation

The European soils that were previously reported in the studies of Rooney et al. (2006) and Thakali et al. (2006) and were selected to validate the TBLM parameters that were developed from the sand culture experiments. The same as in the study of Thakali et al. (2006), not all 18 soils were selected, but only 11 non-calcareous were used for validation due to the unavailability of a model to accurately predict the metal speciation in calcareous soils (Ponizovsky et al., 2006). For the soils to which Cu salt was added, the dissolved Cu in soil solution can be generally predicted within a half log unit (Ponizovsky et al., 2006). The soils have a wide range of properties, with pH from 3.7 to 7.4, and organic carbon content from 0.41% to 23.32%.

Additionally it was reported that increased soil pH reduced the metal toxicity to plants (Lexmond, 1980; Smith, 1994), as at high pH most of the metal was bound to the soil organic matter rather than to the plant's biotic ligand (Plette et al., 1999; Weng et al., 2003, 2004), thus making the metal less bioavailable and resulting in reduced toxicity when pH increased. So it is less important to include the calcareous soils that have high pH for the toxicity study.

2.4. Physical and chemical measurements

The sand solution was replaced daily due to the water loss by evaporation, and sand solutions before and after planting were collected for measurement. The pH was determined with an Orion 370 pH meter with anaccupHast combination pH electrode. Cu, Ca, and Mg were measured using a PerkinElmer Optima 5300 ICP – OES. Measured pH was within 0.2 units and measured metal concentrations were within 15% of nominal values. Total inorganic carbon was measured for the sand solution of pH larger and equal to 7, by a Tekmar-Dohrmann Apollo 9000 TOC analyzer, and this value and pH were used to calculate the activities of $CuOH^+$, $CuHCO_3^+$ and $CuCO_3^\circ$ in the sand solution.

2.5. TBLM equations

It was assumed that the complexation between free Cu^{2+} and biotic ligand (BL) sites results in toxicity, and competition of H^+, Ca^{2+}, and Mg^{2+} will alleviate toxicity. The toxicity is determined by the fraction (f) of total BL bound by Cu^{2+} (Di Toro et al., 2001; Thakali et al., 2006):

$$f = \frac{[CuBL^+]}{[TBL]} = \frac{K_{CuBL}\{Cu^{2+}\}}{1 + K_{CuBL}\{Cu^{2+}\} + K_{HBL}\{H^+\} + K_{CaBL}\{Ca^{2+}\} + K_{MgBL}\{Mg^{2+}\}} \quad (1)$$

where $[CuBL^+]$ is the concentration (moles g^{-1}) of BL sites bound by free Cu^{2+} and [TBL] is the total BL sites (moles g^{-1}), K_{XiBL} is the conditional binding constant for interaction between cation X_i and BL (L mol^{-1}), and {} represents the cation activities.

The biological toxic response is presented by the fraction (f) with the log-logistic dose response equation:

$$R = \frac{100}{1 + \left(\frac{f}{f_{50}}\right)^{\beta}} = \frac{100}{1 + \left(\frac{K_{CuBL}\{Cu^{2+}\}}{f_{50}(1 + K_{CuBL}\{Cu^{2+}\} + K_{HBL}\{H^+\} + K_{CaBL}\{Ca^{2+}\} + K_{MgBL}\{Mg^{2+}\})}\right)^{\beta}} \quad (2)$$

where R = biological response as % of the control, f_{50} is the fraction of the total BL sites occupied by Cu^{2+} at which a 50% response is observed and β is the shape parameter.

The EC50 is derived from Eq. (2) with R = 50%:

$$EC50\{Cu^{2+}\} = \frac{f_{50}}{(1-f_{50})K_{CuBL}}(1 + K_{HBL}\{H^+\}_{50} + K_{CaBL}\{Ca^{2+}\}_{50} + K_{MgBL}\{Mg^{2+}\}_{50}) \quad (3)$$

where the subscript "50" represents the activities of cations at the 50% effect level. Eq. (2) can be used to calculate the toxicity response as percent of control, and Eq. (3) shows the relationship between EC50 and cations' activities.

If toxicity of $CuOH^+$ is included, Eq. (2) will be: