

# Application and Validation of a Biotic Ligand Model for Calculating Acute Toxicity of Lead to *Moina Dubia* in Lakes of Hanoi, Vietnam

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## Research Article

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# Abstract

It is increasingly being recognized that biotic ligand models (BLMs) are valuable in the risk assessment of metals in aquatic systems. The authors investigated the effect of pH, Ca, Mg, K, Na on the acute toxicity of Pb to *Moina dubia*, native zooplankton in lakes of Hanoi, Vietnam. Calcium, Magnesium and pH strongly influenced acute Pb toxicity to *Moina dubia*. Based on this data set, a acute Pb-BLM for *Moina dubia* was developed according to condition of Hanoi lakes. The developed BLM was shown, in an independent validation with data on acute toxicity test on natural water sets, to be capable of predicting chronic Pb toxicity with 81.3% accuracy. The results proved that BLM can be useful tool for calculating the acute toxicity based on water-quality criteria in lake of Hanoi.

# Introduction

Lead is one of the non-essential most toxic metals to the organism (Pareja-Carrera et al. 2020). The lead is used widely in battery manufacturing, aluminum industry, coating technology, printing and mining, and metallurgy. Lead emission in traffic and burning fossil fuels in industrial zones also cause lead pollution after it deposits to water or soil environment (Borase et al. 2021). In many areas, lead pollution happened on the water surface. The concentration of lead in the water surface of Bangladeshi rivers ranges 17-10180  $\mu\text{g/L}$  (Uddin and Jeong 2021). In the Honghu, Guchenghu, and Taihu rivers in China, lead deposited as high as 37.5-48.75 mg/kg (Yao et al. 2009). In Vietnam, lead concentrations in surface water of Hanoi such as in To Lich River and Kim Nguu Rivers were in the range of 100-220  $\mu\text{g/L}$  due to untreated wastewater (Nguyen, 2007). Another study studied lead concentrations in the northern rivers near the mining areas, Thai Nguyen the lead concentration in water used for agricultural irrigation was from 93.4 to 111.5  $\mu\text{g/L}$  (Nguyen, 2007). Lead is considered a dangerous element and may directly affect the growth and reproduction of organisms. The International Agency for Research on Cancer (IARC) has listed lead and its compounds as potentially carcinogenic substances in humans. The question is how to assess the risk of metal to aquatic ecosystems. The biotic ligand model (BLM) was built to predict the ecotoxicology and do a risk assessment. The BLM has been developed for more than twenty years and had been officially applied in many countries (British Columbia 2019, Tobiason et al. 2018). In Asia, the BLM was developed for aquatic species in China in 2012 (Wang et al. 2011) and recently in 2017 (Wang et al. 2017, Zhou et al 2011). In Japan, studies on BLM have been carried out since 2013 but mainly based on toxicological data in other countries (Hayashi 2013, Naito et al, 2010). Most studies on the BLM in tropical regions used the toxicological data collected from the temperate zone (Shoji anh Taniguchi 2016).

The BLMs had been developed for Cu (De Schamphelaere and Janssen 2002), Ni (Deleebeeck et al, 2008, 2009) and Cd (Clifford and McGeerand 2010). Lead is one of the metals studied by USEPA in the BLM development center (DeForest et al. 2017). The BLM has been evolving for a long time but mostly on endemic species in Europe and America such as *Baetis tricaudatus* (mayfly), *Ceriodaphnia dubia* (cladoceran) (Nys et al. 2014), *Daphnia magna* (DeForest et al 2017), and *Pimephales promelas* (Mageret al. 2011). Due to the differences in the body size, metabolism rate, and adsorption ability of surface ligan,

the application of those model constants of Europe and American species for those living in the tropical region. The characteristic of surface water in the Tropical area would affect the ability to predict the acute toxicology of metal. However, there are still limited studies on BLM in eutrophic lakes.

*Moina dubia* is a widely distributed zooplankton in the aquatic bodies in Viet Nam (Le et al., 1999), Thailand, Philippines, Indonesia (Korovchinsky 2013), and Sri Lanka (Fernando, 1979). The eutrophic lake plays controlling fertility and toxic algae in the lake. *Moina dubia* is important zooplankton in the food chain to maintain the diversity of species in the food chain of lakes (Adeyemov 1994). *Moina* in general and *M. dubia* are very sensitive to pesticides and metals (von der Ohe P.C., Liess M. 2004). Many studies have selected *Moina* to study metal toxicity (Gama-Flores 2008, Borase et al, 2021, Zou and Bu 1994, Sheedy et al. 1991). This paper discusses a BLM model development for urban eutrophic lakes on *Moina dubia*, native zooplankton in Vietnam (Borase et al, 2021).

## Materials And Methods

### 2.1 Study species and EC50 determination

*Moina dubia* were collected from Truc Bach Lake (+21°02' 40.8"N; +105°5'22,1'E) located in Hanoi. They are abundant in this small urban and highly eutrophic lake (Hoang et al 2017, Pham et al. 2018). In the laboratory, *M. dubia* were isolated using a Pasteur pipette. They were cultured in a common basal medium (Conklin and Provasoli 1977) at a temperature of ~24°C, pH = ~7.5, and under the ambient light and a photoperiod of 12h light: 12h dark cycle. The acclimation period lasted one month (ca. 5e7 generations) to minimize the lake environmental background on the toxicity test results (Sorin and Choi, 2012). *M. dubia* were fed ad libitum with *Chlorella vulgaris*. *C. vulgaris* was centrifuged and washed to remove culture nutrients before feeding *M. dubia* at a density of 1 10<sup>6</sup> cells/mL. The newborn *M. dubia* (<24 h old) were elected randomly from the culture for toxicity tests.

### 2.2 Water characteristic of eutrophic urban lake

Water samples were collected from 12 lakes surround Hanoi, including two natural suburban lakes and ten closed urban lakes. Collective sampling in five places in the lake was applied. Samples were transferred to the laboratory and stored at 4°C. The physical and chemical parameters (pH, DOC, chlorophyll-a, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>). Monitoring for water quality was conducted from January 2018 to May 2019.

### 2.2 Experimental Design

To investigate the effects independently of main cations including Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup> and H<sup>+</sup> on lead toxicity, we change one cation concentration while keeping all other cation concentrations as low as possible. The Ca<sup>2+</sup> concentration changed from 4 - 56 mg/l, the Mg<sup>2+</sup> concentration changed from 2.99 – 71.2 mg/l, Na<sup>+</sup> concentration changed from 3.5 – 60.7 mg/l, K<sup>+</sup> changed from 1.85 – 2.29 mg/l, while pH was kept at 7.5. The pH changed from 7 to 8.5 since *M. dubia* adapt in the basic condition, so pH < 7

would cause uncomfortable for the metabolism of *M.dubia*. The composition of the experimental sets was described in Table 1. The range of the main cation was classified based on the characteristics of natural water in Hanoi lake. Each cation set was .ed pH and put into the room temperature until it met the stability condition before using for acute tests. Each set was repeated four times and comprised of at least five different cation concentrations. The selected cation concentrations represented a range of water surface characteristics of the urban Lake of Hanoi, Viet Nam.

### *Preparation of the Test Solutions*

CaCl<sub>2</sub>, MgSO<sub>4</sub>, NaCl, KCl and HCl, NaOH (purity > 98%, Merck, Germany) were used to make stock solutions. For each essay, Pb (NO<sub>3</sub>)<sub>2</sub> was added into the control mediums to make up the concentration series of lead. Except for the pH set, the medium was .ed at pH 7.5 in the range of eutrophic lakes. The buffer MOSP was used for the stability of the solution. All the medium was put into a 25oC room one day before being used for acute test to reach the near-equilibrium condition.

### *Acute Toxicity Tests.*

The 24-h immobilization assay was conducted with neonate *Moina dubia* (< 24h-old age) following the guideline 202 of OECD (24). The organisms were acclimated in the laboratory 5-6 generations before being used for toxicology tests. For each test, six treatments (control and five different lead concentrations) were performed from the lowest to the highest lead concentration with four replications. The medium was filled 50ml in each cup. After 24h exposure in the 25°C and 12h light: 12h dark, the number of immobilized neonates in each cup was checked and counted.

### *Estimate the equilibrium condition of ion and biotic ligand*

Prepare a Pb<sup>2+</sup> adsorbent solution with five different concentrations of 50 µg/L, 100 µg/L, 200µg/l and 300µg /l prepared with standard solution Pb(NO<sub>3</sub>)<sub>2</sub> 1g/l (Merk, Germany) in a soft water environment (hardness in Ca was 50 µg/L, with minimum nutritional ingredients). The pH was .ed by NaOH or HCl, to three levels of 6.5, 6.8, and 7. The reconstituted solution is allowed to stabilize for 30 minutes before placing 10 M. dubia (<24h old) neonates in beakers with different lead concentrations and the lead-free solution as the control medium. The volume of the adsorbent solution was 250ml. Samples of 10 ml were collected every 15 minutes at the initial adsorption stage until 1-hour and then every 30 minutes. Samples taken were then measured Pb<sup>2+</sup> concentration to find the adsorption equilibrium coefficient.

Metal adsorption on the contact surface of living organisms is a form of complex bonds between metals and functional groups on the surface of biological cells. The adsorption equilibrium coefficient was calculated by the Langmuir adsorption equilibrium equation (Farvus et al. 1989).

### *2.3 Model validation*

The acute toxicity tests of lead to *M.dubia* were conducted with the natural water collected from 12 lakes as the control medium. The acute tests were performed by six lead treatments (one control and five

different lead concentrations) and replicated four times (See 2.2). Values of EC50-24h received the test were then used for validating the developed BLM by .calibrating the lead accumulation coefficient.

#### 2.4. Mathematical Description of the BLM.

The equation of calculation BLM was described below:

Where  $EC_{50} (Pb^{2+})$  is the free lead ion activities resulting in 50% of 24h-*M.dubia* immobilized after 24 h of exposure.

$$EC_{50} = \frac{f_{PbBL}^{50}}{(1 - f_{PbBL}^{50}) \cdot K_{PbBL}} \cdot \{1 + K_{CaBL} (Ca^{2+}) + K_{MgBL} (Mg^{2+}) + K_{NaBL} (Na^{+}) + K_{KBL} (K^{+}) + K_{HBL} (H^{+})\} \quad (1)$$

( $Ca^{2+}$ ), ( $Mg^{2+}$ ), ( $Na^{+}$ ), ( $K^{+}$ ) and ( $H^{+}$ ) are concentration of described ions in the water bodies. The stability constants  $K_{HBL}$ ,  $K_{MgBL}$ ,  $K_{CaBL}$ ,  $K_{NaBL}$ ,  $K_{KBL}$  were calculated from the relation between pH,  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Na^{+}$ ,  $K^{+}$  and  $EC_{50_{Pb^{2+}}}$ . Assumption of the BLM concept is the linear relationships observed between  $EC_{50_{(Pb^{2+})}}$  and the mentioned ion.

#### Data treatment and Statistics

Speciation calculations were conducted using Windermere humic aqueous model (WHAM) Ver 7.02 (<http://www.ceh.ac.uk/products/software/wham/>). Speciation calculations were performed for all experimental treatments.  $EC_{50}$ -24h expressed as dissolved lead were calculated from observed immobilities at each lead concentration. Twenty –four- hours expressed as free lead ion activity were calculated from observed immobilities at each calculated free lead ion activity.  $EC_{50}$  were calculated using the trimmed Spearman Karber method (Hamilton, 1977). All linear regressions (see further) were calculated using SPSS 20. The stability constants were received by solving the system of equation coding in Matlab environment.

## Results And Discussion

### 3.1 Geochemistry of water in the trophic lakes

The water temperature in eutrophic lakes varied widely from 16 to 35.4°C. The temperature dropped from 15.5 -16.5°C in the winter but increased to 35.4°C on the summer day.

The median pH value of eutrophic lakes in Hanoi in the database was deviated from neutral to slightly basic (7.3 - 8.5). Aquatic organizations would get troubles when living in permanently alkaline environments (Sakamoto et al. 2018). Many factors would affect the change in the pH of water bodies. The bloom of algae, which uptake bicarbonate during the photosynthesis process, can break the H<sup>+</sup> balance in the water, causing the pH variation day and night (Acuña-Alonso 2020). Second, anthropogenic activities, including discharging untreated domestic wastewater, would also affect the pH

of urban lakes. The data on nutrients (N total =  $30,4 \pm 6,6$  mg/L; P total =  $0,97 \pm 0,2$  mg/L; and chlorophyll-a ( $201,1 \pm 0,44$  mg/m<sup>3</sup>) in the eutrophic lakes which was in agreement with a previous study (von der Ohe and Liess 2004) (Table 1). The blooming of algae would significantly change the pH value and the daily dissolved oxygen (DO) in the surface water. The DO varied widely from 4.06 to 11.24 mg/L depending on the metabolism of the algae (Cook and Gale 2005). The calcium and magnesium concentration was approximately varied 20.5 mg/L – 56.3, and 4.8 mg/L – 33 mg/L respectively.

### *3.1 Effect of major cation on acute Pb toxicity*

An increase in the concentration of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ , and  $\text{K}^+$  resulted in an elevated 24-h  $\text{EC}_{50}$ -  $\text{Pb}^{2+}$ . In these bioassay sets, observed  $\text{EC}_{50}$ s ranged from 283 to 518 nM for free lead ion activity. The result show a positive linear relations ( $p < 0.05$ ) between activities of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ , and  $\text{K}^+$  (Figure 1a-d).

A positive linear regression between calcium concentration and value of  $\text{EC}_{50}$ s indicated that calcium could reduce the toxic effect of lead on *M.dubia*. The impact of calcium on metal toxicity has been observed on many different aquatic species (Markich and Jeffree, 1994, Chun Sang 2016). According to Riethmuller, N., (2000), hardness does not directly affect the types speciation of lead in the medium but is influenced by strong changes in the acid-base balance of the solution, thereby indirectly changing the existence of the lead in the solution (Riethmuller et al. 2000). The mobility lead concentration decreased when increasing the concentration of calcium in the medium. The second reason, when  $\text{Ca}^{2+}$  and  $\text{Pb}^{2+}$  are highly concentrated in the ligand surface, *M.dubia* will prioritize absorption of calcium over the lead during metabolism and cell charge balance. High calcium concentration on the cell membranes of organisms can decrease the transport rate of lead across the cell membrane. thereby reducing the penetration of lead into the body (Favus et al. 1989, Markich and Jeffree 1994).

The  $\text{Mg}^{2+}$  was strongly bound to function groups (-COOH, CHO-..) on the surface of ligand and compete with II valence metals on ligand surfaces (Shen et al. 2016). The  $\text{Mg}^{2+}$  concentration on the surface of the ligand created a positive charge potential on the surface of the organism which can limit lead absorption into the body. Experimental results show that when the magnesium concentration in the environment increases, the amount of lead entering the organism decreases, thereby reduced the toxicity of lead to *M.dubia*.

$\text{Na}^+$  and  $\text{K}^+$  were two metal competed strongly with  $\text{Pb}^{2+}$  on the surface of the organism (Zhao et al. 2017, Renaudin et al. 2018). The density of  $\text{Na}^+$  and  $\text{K}^+$  would create a positive electric potential on the biological absorption surface, which reduces the possibility of  $\text{Pb}^{2+}$  transported inside the organism. During the transportation of a  $\text{Pb}^{2+}$  ion through the cell membrane, two  $\text{Na}^+$  or  $\text{K}^+$  ions would be pumped out to maintain the charge balance on the membrane. Thus,  $\text{Na}^+$  and  $\text{K}^+$  also play a crucial role in regulating the absorption of lead into the body. An increase of  $\text{Na}^+$  or  $\text{K}^+$  concentration makes it difficult to absorb  $\text{Pb}^{2+}$ , thus reduces the toxicity of lead.

### *Effect of pH on the lead toxicity*

The pH test was conducted in the range of 7-8.5 to give observed EC<sub>50</sub>-24h range from 241 to 518 nM for dissolved lead. The linear relationship between (H<sup>+</sup>) and 24-h EC<sub>50</sub>(Pb<sup>2+</sup>) would indicate the possibility of proton competition at the ligand surface (Figure 1e). Two possible mechanisms can be proposed. First, pH can affect the speciation of lead in the medium. When pH > 7.5, lead exists in the flexible forms Pb<sup>2+</sup> and Pb(OH)<sub>2</sub> or PbCO<sub>3</sub> of these forms together have a toxic effect on *M.dubia* (Farrell 2012). When the pH is low, there was mainly Pb<sup>2+</sup> which highly interacted with functional groups on the ligand surface. Second, the pH in the medium would affect the micro-environment of the biotic ligand such as for fish (Playle 1992) or for invertebrates (Gensemer and Playle 1999) which can affect the interaction between the specification of lead at the organism-water interface (Playle 1992). There may be a limitation in using pH to predict EC<sub>50</sub>(Pb<sup>2+</sup>) in the pH range from 7 to 8.6 (R<sup>2</sup> = 0.65). For pH in the range from 7 to 8.3, the BLM concept succeeds to calculate EC50s from pH. The reason for that could be, at pH => 8.3 the lead in the medium mostly in PbCO<sub>3</sub> and Pb(OH)<sub>2</sub> form. In that case, not only Pb<sup>2+</sup> but other speciation of lead were bound to the ligand and transport to act toxin. Other studies reported that the forms as Pb (OH)<sub>2</sub> or PbCO<sub>3</sub> had low toxicity than the flexible form (Antunes and Kreager 2014). For this reason, we used the formula that expressed the relationship between pH in the range from 7 to 8.3 and EC50 to calculate the constants K<sub>HBL</sub>, K<sub>MgBL</sub>, K<sub>CaBL</sub>, K<sub>NaBL</sub>, K<sub>KBL</sub>.

### 3.2. Estimation of BLM Parameters.

The intercept and slope were obtained by conducting a Linear regression analysis of the relationship between EC<sub>50</sub>(Pb<sup>2+</sup>) and cation activity Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>, H<sup>+</sup>). Combination the effect of the main cation, the matrix was expressed in equation (2)

$$\begin{aligned}
 -1K_{CaBL} + 0,025K_{MgBL} + 0,013K_{NaBL} + 0,06K_{KBL} + 0,085K_{HBL} &= -0,23 \\
 0,037K_{CaBL} - K_{MgBL} + 0,267K_{NaBL} + 0,012K_{KBL} + 0,175K_{HBL} &= -0,45 \\
 0,014K_{CaBL} + 0,017K_{MgBL} - 1K_{NaBL} + 0,008K_{KBL} + 0,049K_{HBL} &= -0,146 \\
 0,0164K_{CaBL} + 0,022K_{MgBL} + 0,117K_{NaBL} - 1K_{KBL} + 0,06K_{HBL} &= -0,196 \\
 0,056K_{CaBL} + 0,022K_{MgBL} + 0,107K_{NaBL} + 0,011K_{KBL} - 1K_{HBL} &= -0,18
 \end{aligned} \quad (2)$$

The solution of this matrix after speciation calculations and linear regression analyses (Figure 1) resulted in the estimation of the stability constants K<sub>CaBL</sub> = 0,282; K<sub>MgBL</sub> = 0,549; K<sub>NaBL</sub> = 0,173; K<sub>KBL</sub> = 0,247; K<sub>HBL</sub> = 0,229

The constants for Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Na<sup>+</sup> were similar to those obtained for fish gills (Clifford and McGeerand 2010) and used in the BLM (Mager et al. 2011, Nys et al. 2014). The constant binding of lead to biotic ligand surface was (K<sub>PbBL</sub> = 7.301) conducted in the low pH toxicity test based on the Langmuir equation (pH = 6.7) (Zhou et al. 2011). The binding between lead and biotic ligand was similar as the K<sub>PbBL</sub> = 7 reported from Niyogi and Wood (2003). The biding competition between Pb<sup>2+</sup> and (H<sup>+</sup>) on the

biotic ligand was (3.72) which was less one unit than the  $\log K_{HBL} \sim 5.4$  for fish and much lower than  $K_{HBL} = 7.6$  for *Ceriodaphnia dubia* when they were exposed in chronic test (Nys et al. 2014).

A large difference between  $K_{PbBL}$  and  $K_{HBL}$  showed  $H^+$  had significant contribution in proton competition to lead on the ligand surface. In this study, the results in calculations of  $\log K_{CaBL} = 4.39$ ,  $\log K_{MgBL} = 4.47$ , and  $\log K_{NaBL} = 3.92$ ,  $K_{KBL} = 4.34$  (Table 2). These  $\log K$ 's were in the same range as the constants reported by Playle et al (1993), except for the  $K$ -constant, which has not been included in current BLMs so far (McGeer et al. 2000).

In comparison with another  $\log K_{CaBL}$  and  $\log K_{MgBL}$ , the  $\log K_{NaBL}$ , and  $K_{KBL}$  seem to quite high. The reason for that may be because they are binding to the typical biogenic chelating ligands such as aspartate, citrate, etc (Stumm and Morgan). This result suggested that  $Na^+$  and  $K^+$  reduce the lead toxicity not only by competition on the ligand surface but also by preventing the loss of plasma electrolytes (De Schampelaere and Janssen 2002).

### 3.2. BLM development and validation

To validate the BLM, the natural water samples were collected from 10 urban lakes in Ha Noi, Viet Nam. 28 synthetic tests were used to improve the model predictions. A single overall intrinsic density to Pb toxicity was used for all the data sets.

The predicted EC50s and observed EC50s from synthetic test waters were demonstrated in Fig 2 and Fig. 3 with  $R^2=80.5\%$ . It was recognized that the BLM-parameters estimated EC50 fit with the observed EC50s data.

$$EC_{50} = \frac{f_{PbEL}^{50}}{(1 - f_{PbEL}^{50}) \cdot K_{PbEL}} \cdot \{1 + K_{CaBL}(Ca^{2+}) + K_{MgBL}(Mg^{2+}) + K_{NaBL}(Na^+) + K_{KBL}(K^+) + K_{HBL}(H^+)\}$$

$f^{50}$  was calibrated from 0.35 đến 0.47 and received the best performance at 0.42

Although the developed *M.dubia* BLM shows promise, there was still a need to further validate the data from experiments with a broader range of exposure conditions in the field with the variations of DOC concentrations. For the validation with nature water, ANOVA analysis results show that the experimental EC50 value has a significant correlation with the EC50s value calculated from the model ( $P\text{-value} = 0.0001$ ). Predictive ability of the coefficient determination model coefficient  $R^2 = 75.3\%$ . (Fig. 4, 5)

In general, the  $EC_{50}\text{-Pb}^{2+}$  values predicted by the model in the suburban lakes were greater than  $EC_{50}\text{-Pb}^{2+}$  for the urban lakes. The results of the experiment also show the same trend. In urban, in addition to the highly toxic metals such as lead, there are also other metals such as arsenic and cadmium present in natural water, which increase lead toxicity.

The results showed a significant dispersion between EC50 obtained in the lab experiments with natural water and the calculated EC50s from the model. However, the differences are acceptable. The model-calculated EC<sub>50</sub>-Pb<sup>2+</sup> values tend to be greater than EC<sub>50</sub>-Pb<sup>2+</sup> values tested in inner urban lakes but was less than those tested with nature water from lakes in a suburban city (Quan Son and Tuy Lai lake). The reason is that in natural water conditions, organic matter ranges widely, from trophic lakes and eutrophic. Therefore, the coefficient of a model can be calibrated within the range of 0.38-0.9. Table 3. shows the calculated results of the model before and after the calibration, with the prediction accuracy (coefficient determination) of 81.3%.

## Conclusion

Biotic ligand models have proven their usefulness in predicting acute metal toxicity in natural waters based on a more complete knowledge of bioavailability-influencing water characteristics including pH, Ca, Mg, Na, K. The BLM concept can be used to support development of discharge regulation into the aquatic environment. This illustrates that the incorporation of bioavailability of metals in current waterquality guidelines and risk assessments is indispensable and that further research is needed to develop Pb BLMs with aquatic zooplankton and also with other organisms and to investigate how these can be implemented in the risk-assessment process. Based on the present research with *Moina dubia*, BLM can provide appropriate criteria to address the risk of Pb in surface waters.

## Declarations

### Ethics approval and consent to participate

The submitted work is original and have not been published elsewhere in any form or language (partially or in full).

### Consent for publication

We co authors of this article give our consent for the publication of identifiable details, which can include photograph(s) and/or videos and/or case history and/or details within the text (“Material”) to be published in the Journal and Article. Therefore, anyone can read material published in the Journal.

### Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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## Authors' contributions

- **HP:** Conceptualization, Methodology, Investigation, Data curation, Investigation, Visualization, Writing- Original draft preparation.
- **LV:** Methodology, Investigation, Coding, Visualization,
- **NL:** Conceptualization, Methodology, Investigation, Data curation, Investigation,
- **T-HH:** Supervision, Project Administration, Funding acquisition, Conceptualization, Writing- Reviewing and Editing
- All authors read and approved the final manuscript

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## Tables

**Table 1. Characteristics of water quality in Lake of Hanoi and values of experiment EC<sub>50</sub> (Pb<sup>2+</sup>) with *M.dubia***

Lakes	pH	DOC, mg/l	Ca <sup>2+</sup> , mg/l	Mg <sup>2+</sup> , mg/l	Na <sup>+</sup> , mg/l	K <sup>+</sup> , mg/l	SO <sub>4</sub> <sup>2-</sup> , mg/l	Cl <sup>-</sup> , mg/l	EC50 T <sub>Pb</sub> , µg/l
Lang	7,5	2.3	37,8	12,98	1,39	1,85	7,98	65,34	510
Hai Ba Trung	8,1	5	45,23	7,86	9,13	2,5	15,98	124,23	980
Thanh Nhan	8,3	7.3	54,7	8,75	18,5	2,43	9,54	345,41	950
Dong Da	7,2	12	45,78	7,98	1,63	2,23	12,54	45,67	300
Bay Mau	7,85	11,8	64	4,8	54,2	8,23	15,65	111,825	600
West Lake	8,2	2,78	38,23	5,73	21,6	14,2	23,65	167,56	320
Nam Dong	8,3	5	40,54	6,95	16,3	8,34	7,87	115,33	960
Van Chuong	8,1	5.2	32,56	3,24	2,98	2,32	20,56	168,54	890
Kim Lien	7,85	4.5	38,96	8,64	2,63	3,23	23,94	345	680
Linh Dam	7,3	18	35,9	14,4	7,16	10,34	31,45	79	430
Truc Bach	8,3	4,8	47,82	8,64	60,1	16,2	23,94	275	980
Quan Son	8,35	6,98	46,82	18,4	1,48	19,6	7,12	45,23	1200
Tuy Lai	8,37	8,74	87,68	20,4	1,004	20,43	7,34	23,45	1450

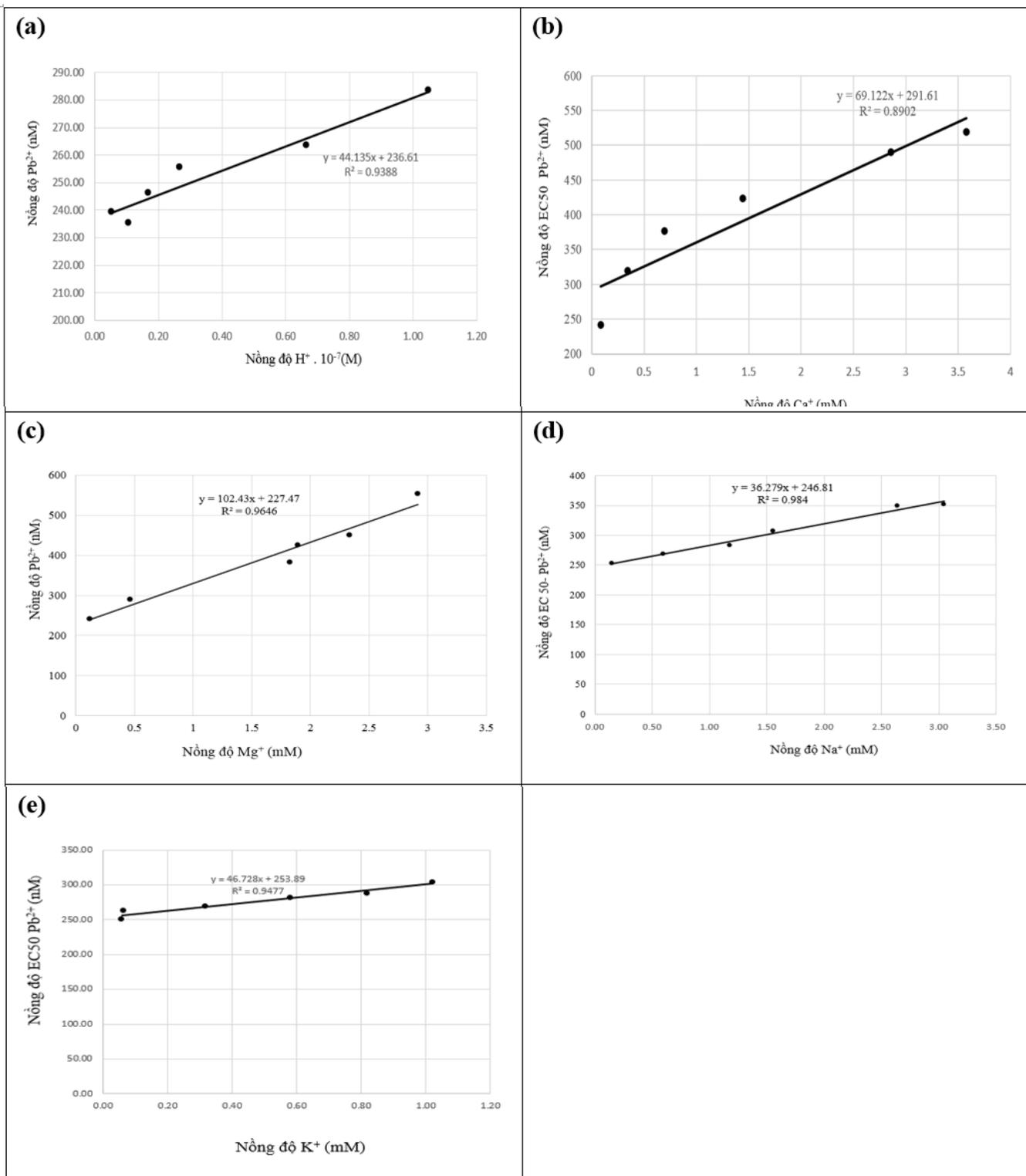
**Table 2.** Comparison of Biotic Ligand Model Constants ((95% Confidence) for Binding of Cations to the Biotic Ligand of *Moina dubia* (This Study) and Stability Constants for Inorganic Copper Complexes *Daphnia magna* (Naito et al 2010) và *Pimephales promelas* (Shoji et al . 2016)

Log K	Model coefficient		
	<i>Moina dubia</i> (Pb)	<i>Daphnia Magna</i> (Cu) (Naito et al 2010)	<i>P.promelas</i> (Cu ) (Shoji et al 2016)
Log $K_{CaBL}$	4,39	3,47	3,6
Log $K_{MgBL}$	4,47	3,58	3,6
Log $K_{NaBL}$	3,92	3,19	3,19
Log $K_{KBL}$	4.34	-	-
Log $K_{HBL}$	3,72	5,4	5,4

**Table 3.** Comparing EC50 values of lead to *M.dubia* from experiments with natural lake water and results from pre- and post-calibrated models

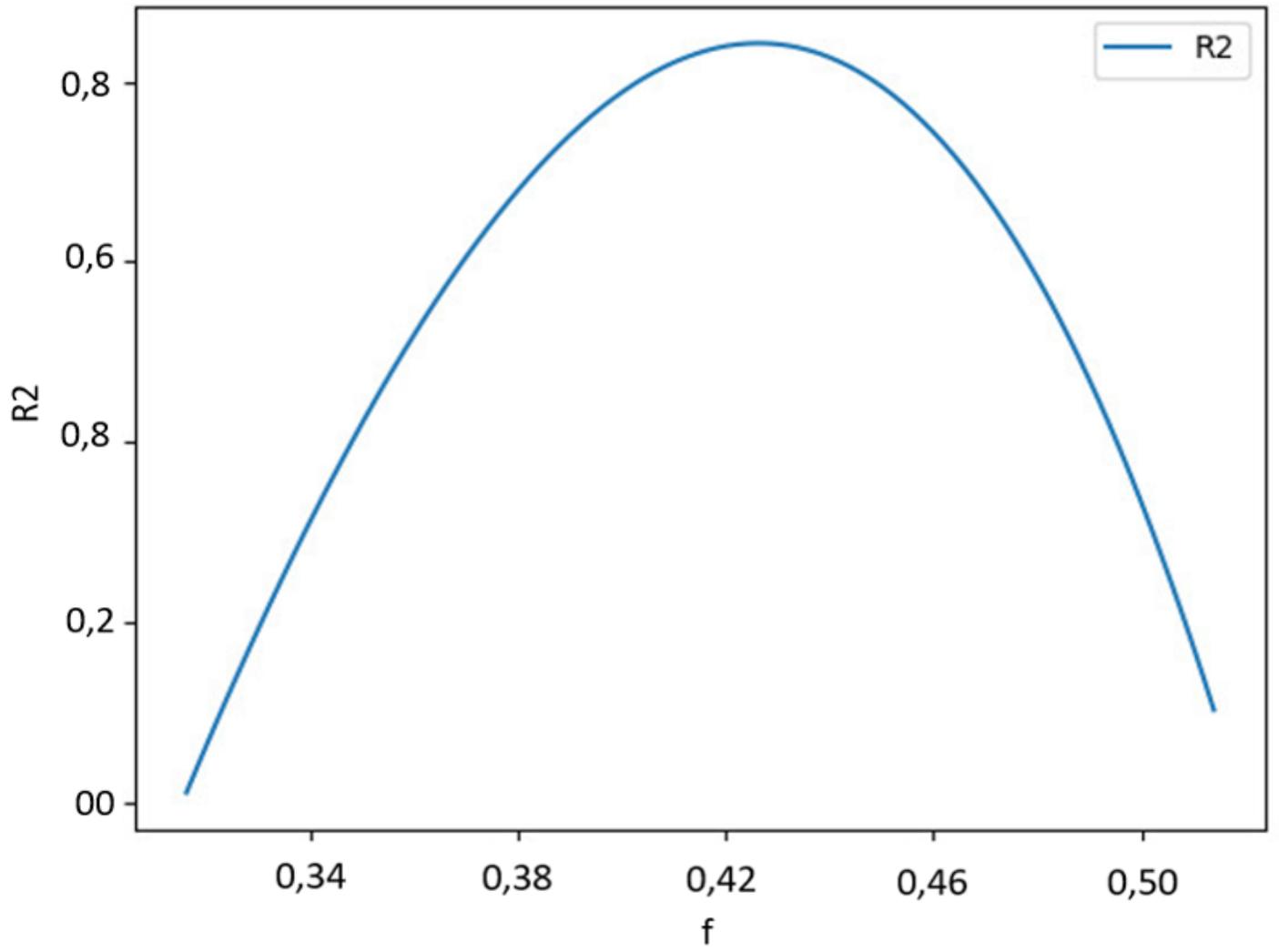
Lakes	Experimental $EC_{50-Pb^{2+}}$	Calculated $EC_{50Pb^{2+}}$ before calibration	Calculated $EC_{50Pb^{2+}}$ after calibration
Lang	2.54	3.24	2.51
Hai Ba Trung	2.46	3.12	2.42
Thanh Nhan	2.65	3.43	2.66
Dong Da	2.55	3.28	2.55
Bay Mau	3.04	3.94	3.06
Ba Mau	3.07	3.97	3.08
West Lake	2.54	3.25	2.53
Nam Dong	2.52	3.18	2.47
Van Chuong	2.05	2.65	2.06
Kim Lien	2.32	3.01	2.34
Linh Dam	3.76	3.55	2.76
Truc Bach	3.25	4.10	3.18
Truc Bach	2.30	3.39	2.63
Quan Son	3.05	3.68	2.86
Tuy Lai	3.35	4.33	3.36
Quan Son	3.00	3.85	2.99
Tuy Lai	3.20	4.20	3.26

## Figures



**Figure 1**

Relation between concentration of  $H^+$  (a),  $Ca^{2+}$  (b),  $Mg^{2+}$  (c),  $Na^+$  (d),  $K^+$  (e) and  $EC_{50}-Pb^{2+}$



**Figure 2**

Validation fPbBL50 at lab experiment in control medium

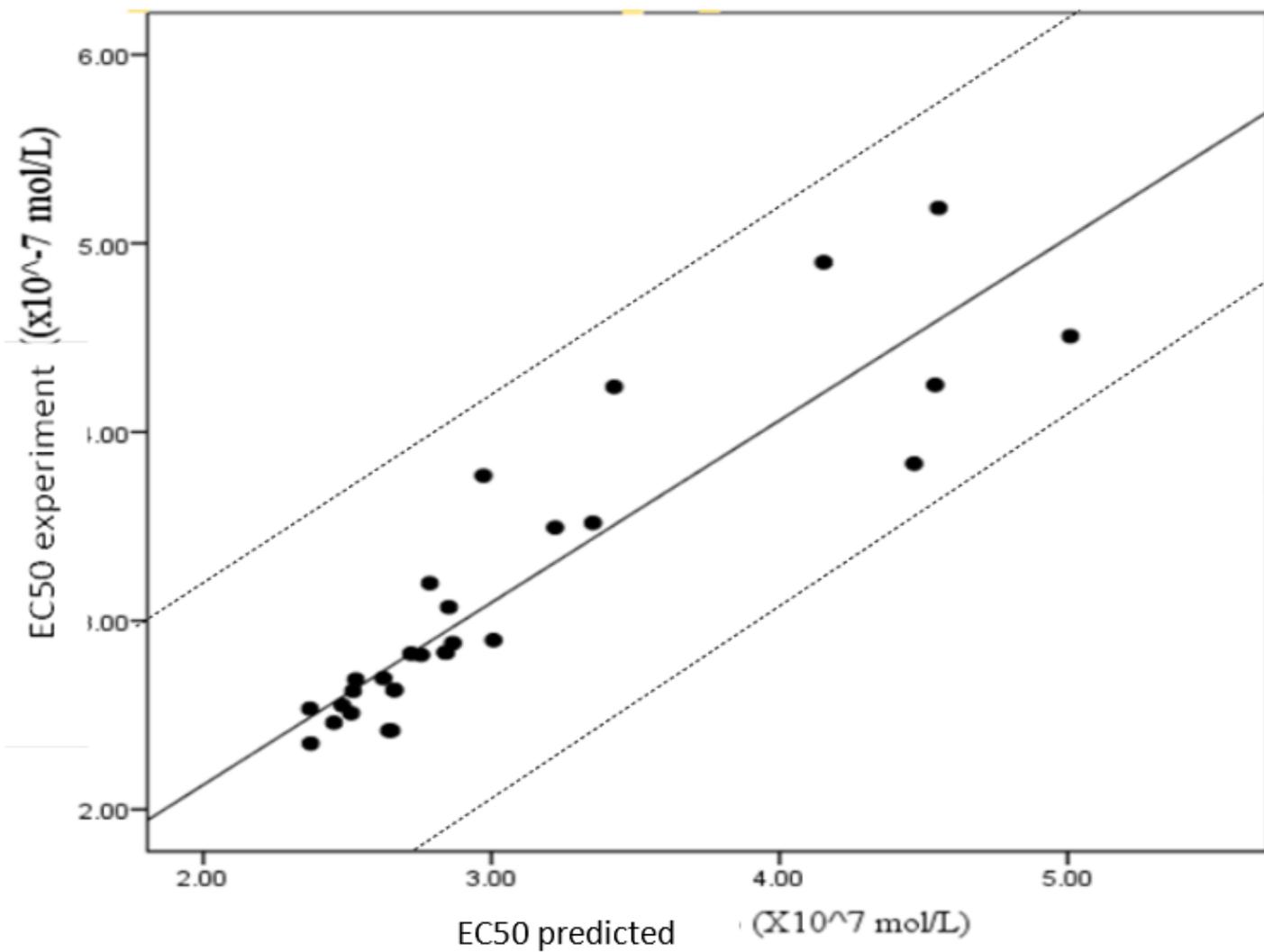
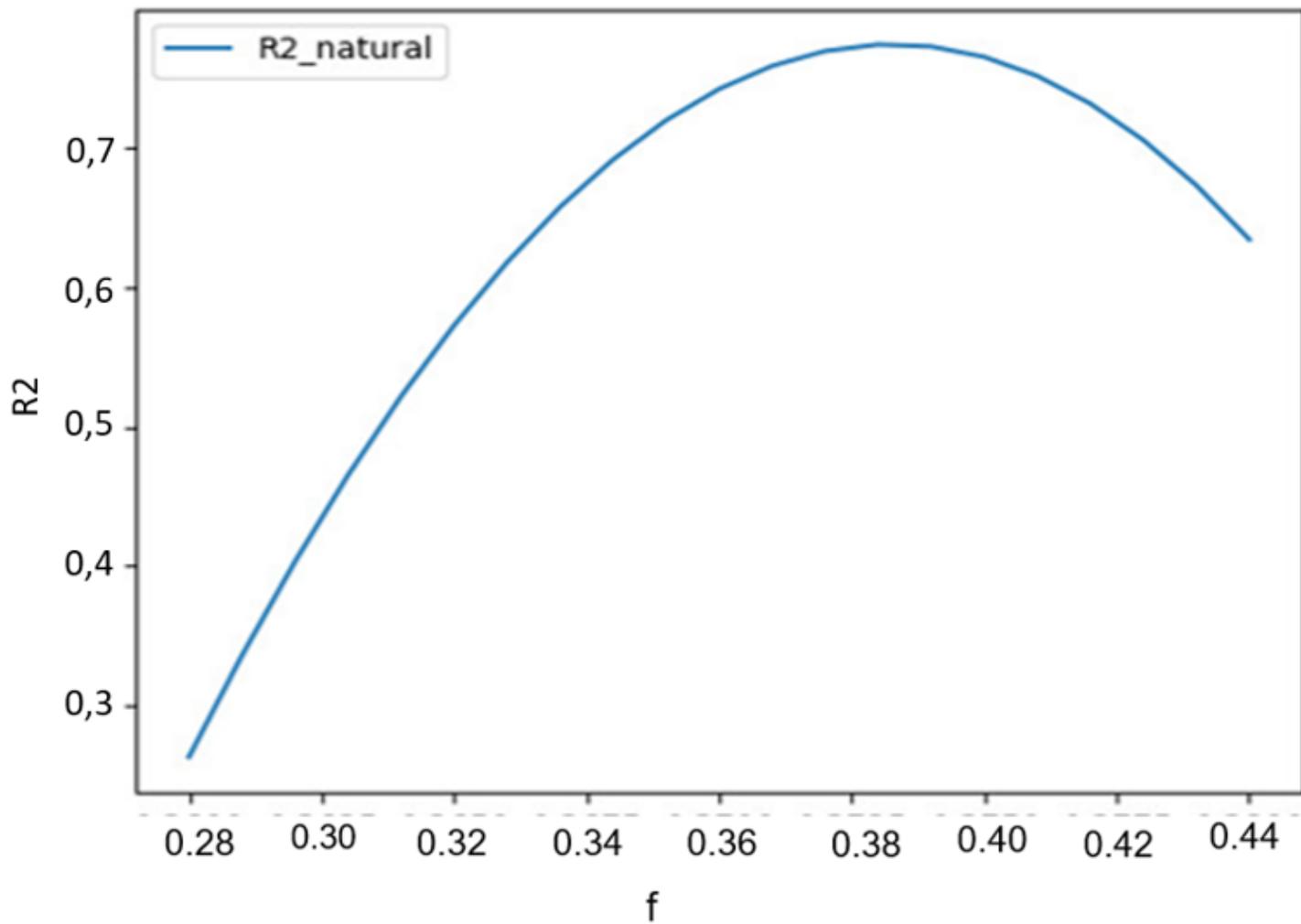


Figure 3

EC50 experiment and EC50 from model with  $f=0.42$



**Figure 4**

Validation fPbBL50 at lab experiment in natural medium

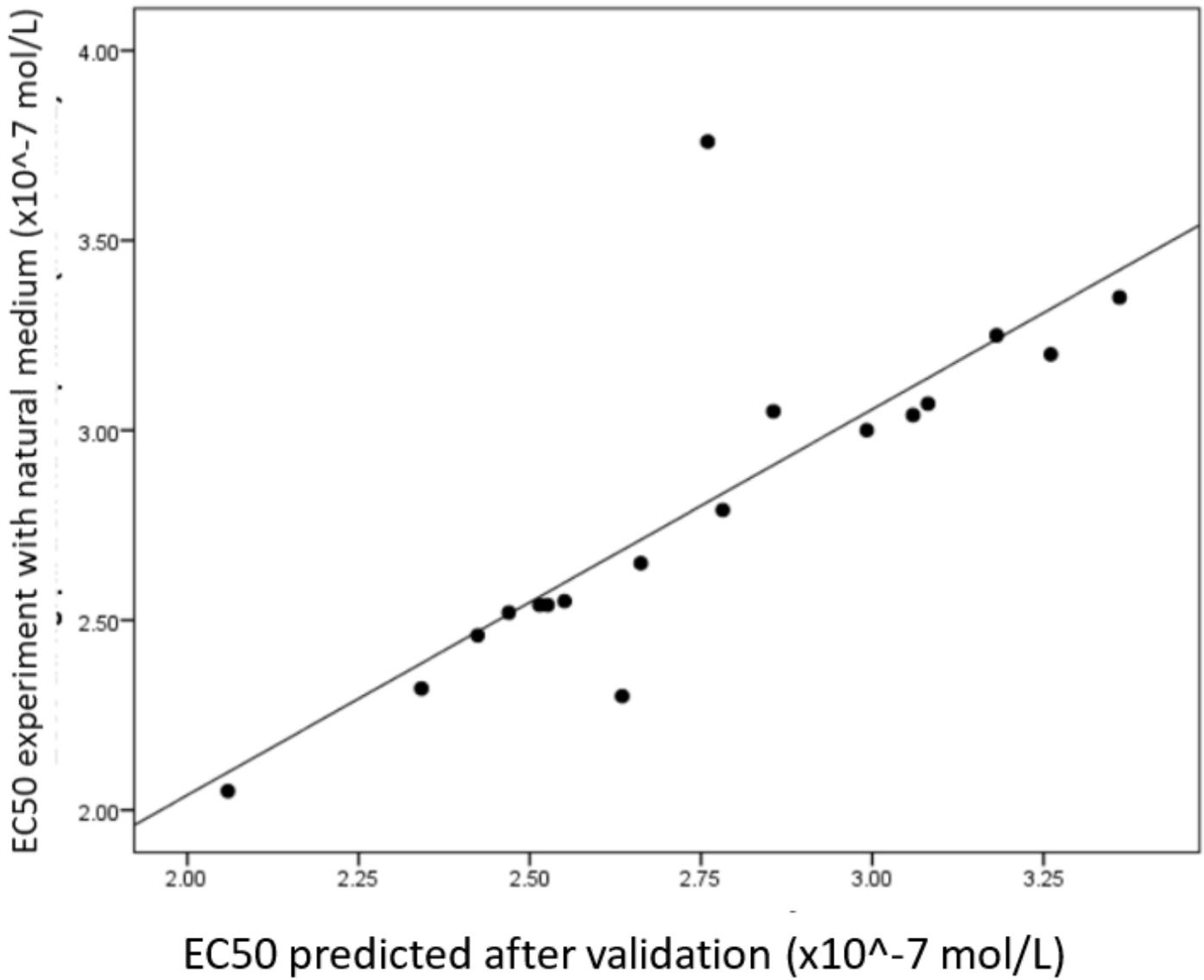


Figure 5

EC50 experiment with natural medium and EC50 from model after validation