Korean Journal of Environmental Biology

Original article

https://doi.org/10.11626/KJEB.2021.39.3.298

Korean J. Environ. Biol.
39(3) : 298-310 (2021)
ISSN 1226-9999 (print)
ISSN 2287-7851 (online)

Mathematical modeling to simulate the adsorption and internalization of copper in two freshwater algae species, *Pseudokirchneriella subcapitata* and *Chlorella vulgaris*

Yongeun Kim, Minyoung Lee[1], Jinsol Hong[1] and Kijong Cho[1,*]

Ojeong Resilience Institute, Korea University, Seoul 02841, Republic of Korea
[1]Department of Environmental Science and Ecological Engineering, Korea University, Seoul 02841, Republic of Korea

***Corresponding author**
Kijong Cho
Tel. 02-3290-3064
E-mail. kjcho@korea.ac.kr

Received: 24 August 2021
Revised: 4 September 2021
Revision accepted: 6 September 2021

Abstract: Prediction of the behavior of heavy metals over time is important to evaluate the heavy metal toxicity in algae species. Various modeling studies have been well established, but there is a need for an improved model for predicting the chronic effects of metals on algae species to combine the metal kinetics and biological response of algal cells. In this study, a kinetic dynamics model was developed to predict the copper behavior (5 μg L^{-1}, 10 μg L^{-1}, and 15 μg L^{-1}) for two freshwater algae (*Pseudokirchneriella subcapitata* and *Chlorella vulgaris*) in the chronic exposure experiments (8 d and 21 d). In the experimental observations, the rapid change in copper mass between the solutions, extracellular and intracellular sites occurred within initial exposure periods, and then it was slower although the algal density changed with time. Our model showed a good agreement with the measured copper mass in each part for all tested conditions with an elapsed time (R^2 for *P. subcapitata*: 0.928, R^2 for *C. vulgaris*: 0.943). This study provides a novel kinetic dynamics model that is compromised between practical simplicity and realistic complexity, and it can be used to investigate the chronic effects of heavy metals on the algal population.

Keywords: intracellular, extracellular, adsorption isotherms, kinetic dynamics

INTRODUCTION

The effects of heavy metal contamination on ecosystems have received intensive attention over the past 30 years (Cooper *et al.* 2010). To evaluate the effects of heavy metals on aquatic ecosystems, many studies with various species have been reported. Among the most studied biota, freshwater algae are important species for assessments of heavy metals due to not only their ecological role as primary producers in aquatic ecosystems (Janssen and Heijerick 2003) and the basis of many food chains (Stoiber *et al.* 2012), but also they have the advantages such as easy culturing, low maintenance cost, and high sensitivity to pollutants.

To increase our mechanistic understanding of the relationship between heavy metals and algae species, modeling approaches have been widely used to describe the kinetics of metal binding and uptake into algal cells (Levy *et al.* 2008). Two modeling approaches, the free ion activity model (FIAM) and the biotic ligand model (BLM), have been developed to predict the toxicity and metal bioavailability in aquatic ecosystems (Zeng *et al.* 2009). The FIAM and BLM predict the toxicity on aquatic organisms based on the assumptions that toxic effects are determined by the free metal ion activity in the solutions and accumulation of

 ©2021. Korean Society of Environmental Biology.

metal to the biotic ligand, respectively. These models have been proven to predict the acute toxicity fairly well in algae that generally expressed as 48 or 72 h EC_{50} (half-maximal effective concentration) value. However, predicting metal accumulation and toxicity under chronic exposure is still challenging issues in the BLM approach because it focused on short term uptake of cationic metals and their toxicity with assuming that biotic ligand does not change upon exposure to a toxicant (Levy *et al.* 2008; Paquet *et al.* 2015). Furthermore, the BLM is an equilibrium model that assumes the target organisms are at equilibrium with their surroundings although physicochemical conditions are rarely at equilibrium (Slaveykova and Wilkinson 2005). Other limitations and exceptions of the BLM approach have been deeply discussed by Slaveykova and Wilkinson (2005) and Miao and Wang (2007).

Other modeling approaches, adsorption isotherms and kinetic model, have been used widely to describe the distribution of introduced metal in organisms and interaction between the sorbate and sorbent from a physiochemical perspective (Xiong *et al.* 2013) in many algae species (Chen *et al.* 2008; Xiong *et al.* 2013; Jeyakumar and Chandrasekaran 2014). However, these models are overlooked or ignored possible biological responses from heavy metals, and developed with data from experiments conducted in only a few hours, and represent the interaction between solutions and algae surface without uptake process. Even though these approaches are useful to represent the metal behavior in algae systems through a relatively simple equation, some modifications are still needed to utilize them to investigate the chronic effects of metal on algae species. Therefore, a new kinetic modeling approach that can combine the biological response of algae and metal kinetics for long-term exposure is critically needed to better understand chronic toxicity with metal binding and uptake process in algae. Very few studies have been attempted which investigate the interaction between metal and algal cells through their own modified model (Fortin *et al.* 2007), but the long-term observation and prediction were not conducted.

When heavy metals are introduced into algae systems, metal mass flows between the culturing solutions, extracellular and intracellular binding sites occur continuously. The effects of heavy metals on algae can be changed dynamically with time because the metal mass in each part may be determined by the mass flow rate between them. De Schamphelaere *et al.* (2005), Miao and Wang (2007), and Levy *et al.* (2008) suggested that the extracellular or intracellular metal mass has a strong association with the toxicity in algae. It is also suggested by Franklin *et al.* (2002) who reported that initial algal density determined the extracellular and intracellular metal mass, and it led to an increase or decrease of toxicity. Thus, the prediction for dynamics of extracellular and intracellular metal mass with algal density is also required to better understand the chronic metal effects on algae for a cause-and-effect relationship.

In this study, copper was selected as a test heavy metal since it is observed in aquatic ecosystems in a wide range of concentrations and has high toxicity above nutritional requirements on aquatic organisms (Levy *et al.* 2008; Dang *et al.* 2009; Stoiber *et al.* 2012). It is also increasingly used to date, as antifouling biocides since tributyltin was banned in 2003 (Gatidou and Thomaidis 2007). Two freshwater algae species, *Pseudokirchneriella subcapitata* (formerly known as *Rhaphidocelis subcapitata* and *Selenastrum capricornutum*) and *Chlorella vulgaris* were chosen since these species are one of the most widely used test organisms in ecotoxicology due to its availability, ecological relevance, and sensitivity to toxicants (Machado *et al.* 2015; Kim *et al.* 2018, 2020).

Taking into consideration the limitations in the existing approaches, this study aimed at the development of the long-term kinetic model to predict the copper distribution in solution, extracellular, and intracellular binding sites in *P. subcapitata* and *C. vulgaris* for long-term exposure. In particular, we focused on representing the copper mass changes in each part separately and describing them with time for long-term exposure through the modifying existing model. We conducted in increasing concentration of copper with two algae species: (a) verification for the applicability of three kinetic models for 8-d exposure, (b) development of long-term kinetic model and comparison with observed and simulated copper kinetics for 8-d exposure, and (c) validation of the developed model for 21 d.

MATERIAL AND METHODS

1. Test algae and culture conditions

The freshwater microalgae *Pseudokirchneriella subcapitata* (strains CCAP 278/4) and *Chlorella vulgaris* (strains AG40003) were obtained from the Culture Collection of Algae and Protozoa (CCAP, Scottish Marine Institute, UK) and the Korean Collection for Type Cultures (KCTC, Korea Research Institute of Bioscience and Biotechnology, Korea), respectively. All cultures were maintained in the

synthetic freshwater media (EPA 2002) in 200 mL Erlenmeyer flasks and placed in the environmental chamber controlled at 20 ± 1°C with a 16 : 8 h light/dark cycle (70 μmol photons m^{-2} s^{-1}, Philips TLD 30 W cool white fluorescent lighting). For the copper exposure experiments, the test medium excluded copper and ethylenediaminetetracetic acid (EDTA). The test medium consisted of the macronutrients (N, Mg, Ca, S, P, Na, K, and C) and micronutrients (B, Mn, Zn, Co, Mo, Fe, and Se). All glass-wares used for algae culture and exposure experiments were soaked with 10% HNO_3 for 1 d and rinsed 5 times with distilled water. The glass-wares were dried in a heating oven at 80°C.

2. Exposure experiments

The two algae species were exposed to copper for two exposure duration conditions, 8- and 21-d exposure. The exposure conditions were the same as the algae culture conditions described above. Copper stock solutions were prepared by dissolving reagent-grade copper sulfate ($CuSO_4 \cdot 5H_2O$, ≥ 99% purity, Sigma-Aldrich, USA) in the algal medium, and the aqueous aliquots were added to a 100 mL glass beaker filled with 70 mL algal medium to make the final exposure concentrations of copper as 5, 10, and 15 μg L^{-1}. These exposure concentrations were selected based on the finding by Franklin *et al.* (2002) reported that EC_{50} values for *P. subcapitata* and *Chlorella* sp. from the 72-h copper exposure test were 17 and 16 μg L^{-1}, respectively.

The initial algal density for both algae species was 2.0×10^6 cells mL^{-1} for all treatments. The test beakers were shaken twice daily by hand. For the 8-d exposure experiments, changes of algal density and copper masses (dissolved, extracellular, and intracellular algae) were monitored 0.25, 0.5, 1, 2, 4, 6, and 8 d after treatments. Five replicates (5 glass beakers) were prepared for each copper concentration (0, 5, 10, and 15 μg L^{-1}) and observation date and placed in the environmental chamber. Each observation date, five beakers in total 240 beakers were randomly selected and determined the algal density and copper masses by destructive sampling. Before the destructive sampling operation, the algal density was measured using a haemacytometer (Marienfeld, Germany) under an optical microscope (E2200; Nikon, Japan). Procedures in copper mass determinations were described in section 2.3 below.

The same procedures used in the 8-d exposure experiment were used for the 21-d exposure experiments, but the observation date was 0.25, 1, 2, 4, 6, 8, 10, 13, 16, and 21 d after treatments.

3. Dissolved, extracellular, and intracellular Cu determination

To investigate the behavior of copper in the exposure experiment over time, copper masses in dissolved, extracellular, intracellular algae were determined with the procedure of Franklin *et al.* (2002) and Ayed *et al.* (2015). Briefly, a 40 mL algae medium from each test beaker was moved into a 50 mL sterilized polypropylene conical tube (SPL, Korea), and centrifuged for 30 min at 3,500 rpm in a large capacity centrifuge (Combi 514R; Hanil, Korea). After centrifugation, 20 mL supernatant solutions were pipetted into a 50 mL disposable conical tube and acidified with 40 μL of HNO_3 (trace analysis grade, Sigma-Aldrich, USA) to analyze dissolved copper. The remaining supernatant solutions were gently discarded, and the remaining algal pellet dissolved in 20 mL of 0.02 M EDTA. This sample was shaken for 30 s and then centrifuged for 20 min at 3,500 rpm to separate the extracellular and intracellular copper. The supernatant solutions from these processes were referred to as extracellular (surface-bounded) copper. The remaining algae pellets were air-dried until moisture contents were removed and then 2 mL of concentrated HNO_3 were added. Algal cells were acid digested in a sonicator water bath (Sonic420; Hwashin Tech., Korea) for 20 min. After cooling, the samples were made up to 10 mL distilled water to analyze intracellular copper. Leached copper from the test beakers with 50 mL of 1 M HNO_3 was determined to calculate the mass balance. In all experiments, a proportion of absorbed copper mass on the surface of glass beakers was remained constant ranged from 5.41 to 10.63% regardless of elapsed exposure times. Thus, the mass balance of copper in the exposure systems was estimated as total copper input - copper on the glass surface - copper in the media - copper adsorbed and internalized to algal cells.

Copper mass in all fractions of samples was measured using an inductively coupled plasma mass spectrometer (ICP-MS 7700x; Agilent, USA). Copper concentration in the samples was calculated using six-point calibration curves using a serial dilution of copper standard (Sigma-Aldrich, USA). The detection limit by ICP-MS for copper was 0.5 μg L^{-1}.

4. General kinetic models

Adequacy of three kinetic models such as the pseudo-first-order, pseudo-second-order, and intra-particle diffusion models to describe sorption behaviors of copper on the

 ©2021. Korean Society of Environmental Biology.

two algae species was evaluated using 8-d exposure data. These kinetic models have been successfully employed to describe the interactions between metals and algal cells under short-term exposure conditions, usually less than 1 d duration (Chen *et al.* 2008; Areco and dos Santos Afonso 2010). Units of these kinetic model parameters were modified to represent the algal density instead of algae mass because the present study was focused on the behavior of copper with an algal density over time. The kinetic models are as follow (Ho and Mckay 1999; Ma *et al.* 2007):

Pseudo-first-order model:

$$q_t = q_e - q_e \exp(-k_1 t), \quad \text{(Eq. 1)}$$

Pseudo-second-order model:

$$q_t = \frac{t k_2 {q_e}^2}{1 + t k_2 q_e}, \quad \text{(Eq. 2)}$$

Intra-particle diffusion model:

$$q_t = k_d t^{1/2} + C, \quad \text{(Eq. 3)}$$

where q_t is the adsorbed metal mass by the algae at time t (μg mL^{-1} 10^{-8} cells), q_e is the adsorbed metal mass by algae at equilibrium (μg mL^{-1} 10^{-8} cells), k_1 is the pseudo-first-order sorption rate constant (1 d^{-1}), k_2 is the pseudo-second-order reaction rate constant (10^8 cells μg^{-1} mL^{-1} d^{-1}), k_d is the intra-particle diffusion rate constant, and C is the adsorption rate-intercept.

5. Long-term kinetic model development

To predict the adsorption and internalization of copper in the two green algae species under long-term exposure conditions (8-d and 21-d), a long-term kinetic model (LKM) was developed using a concept of the pseudo-second order kinetic model. Biological responses of algae to copper were incorporated into the LKM: algal cell density changes, a ratio of occupied sites to maximum adsorption sites on the surface algal cell, and internalization of adsorbed metal into cells. General assumptions of the LKM were followed as (a) algae count data represent well the algal population density, (b) algal density includes all effects of biological response such as exudates, (c) uniform mixing is attained that copper and algal cells are evenly distributed in the experimental conditions, and (d) chemical properties of the test medium are not drastically affected by the growth of algae (observed pH: 7.5–8.0) during the experiments.

A combination of three ordinary differential equations comprising three state variables is used to represent the copper behavior in solutions (Cu_d), extracellular (Cu_e), and intracellular (Cu_i) with elapsed time. The mass flows of copper were represented J_{de} (from dissolved to extracellular), J_{ei} (from extracellular to intracellular), and J_{di} (from dissolved to intracellular). The model parameter descriptions and units are presented in Table 1.

Copper mass flows into extracellular from solutions (J_{de}) is controlled by the copper concentration difference and ratio of occupied sites to maximum adsorption sites. Pseudo-second order model (Ho and Mckay 1999), one of the widely used kinetic sorption models, is applied to represent the copper mass flow by rate of occupation of adsorption site. We used this concept with some modification to formulate the equation of J_{de} as $(Cu_d - Cu_e)^2 (1 - Cu_{de}/Cu_{me})$. Leby *et al.* (2008) found that changes in algal cell ultrastructure and increase of extracellular copper concentration occurred within the initial exposure period, suggesting that the flow of copper from solutions to extracellular sites is decreased by algae response to copper toxicity with time (k_{de}). The scaled time variable (t) is added for the response of algal cells to represent the decrease of adsorption rate with time. The kinetic equation for the above interactions between dissolved copper and extracellular sites is as follows:

Table 1. A list of the model parameter descriptions and units

Parameter	Description	Unit
Cu_d, Cu_e, Cu_i	Copper mass of dissolved, extracellular, and intracellular, respectively.	μg
k_{de}, k_{ei}, k_{di}	Variable related to copper transfer at dissolved-extracellular, extracellular-intracellular, and dissolved-intracellular, respectively.	k_{de}, k_{di}: μg^{-2} d^{-1}, k_{ei}: mL cells^{-1} d^{-1}
Cu_{me}, Cu_{de}	Maximum and present extracellular copper densities, respectively.	μg mL cells^{-1}
Cu_{mi}, Cu_{di}	Maximum and present intracellular copper densities, respectively.	μg mL cells^{-1}
A_{dt}, A_{d0}	Initial and present algae densities, respectively.	cells mL^{-1}
C_{de}, C_{ei}, C_{di}	Specific constant related to k_{de}, k_{ei}, and k_{di}, respectively.	C_{de}, C_{di}: cells mL^{-1} μg^{-2} d^{-1}, C_{ei}: mL cells^{-1} d^{-1}
t	Scaled-elapsed day	–

$$J_{de} = (Cu_d - Cu_e)^2 k_{de}\left(1 - \frac{Cu_{de}}{Cu_{me}}\right)$$

$$\text{where} \quad k_{de} = \frac{C_{de}}{ln(A_{d0})(t+1)}, \qquad \text{(Eq. 4)}$$

Copper mass flow from solutions to intracellular sites (J_{di}) can be described by the concentration difference between solutions and intracellular sites. Copper has to pass through the extracellular layer to move into the intracellular sites. However, these processes can occur so quickly during the initial exposure period caused by plenty of unoccupied extracellular and intracellular sites. Therefore, intracellular copper mass in the early period of exposure is expressed as follows:

$$J_{di} = (Cu_d - Cu_i)^2 k_{di}$$

$$\text{where} \quad k_{di} = \frac{C_{di}}{ln(A_{d0})}, \qquad \text{(Eq. 5)}$$

In the conceptual framework of the biotic ligand model (BLM) and the free ion activity model (FIAM), internalization of adsorbed metal into cells is characterized by the rate constant and a reaction with an intracellular ligand (Hassler *et al.* 2004) as $J_{int} = k_{int}\{M\text{-}R_{cell}\}$, where J_{int} is the metal internalization flux, k_{int} is the internalization rate constant, and $\{M\text{-}R_{cell}\}$ is the metal-bound to the sensitive sites. Based on these concepts, biological response with time (k_{ei}) and the ratio of occupied to maximum intracellular sites is added to the equation. Thus, the kinetic equation describing the internalization flux of copper is as follows:

$$J_{ei} = k_{ei} A_{dt}\left(1 - \frac{Cu_{di}}{Cu_{mi}}\right)$$

$$\text{where} \quad k_{ei} = \frac{C_{ei}}{(t+1)}, \qquad \text{(Eq. 6)}$$

Mass balance of copper mass of dissolved (Cu_d), extracellular (Cu_e), and intracellular (Cu_i) is simply expressed as $Cu_d = initial\ copper\ input - J_{di} - J_{de}$, $Cu_e = J_{de} - J_{di}$, and $Cu_i = J_{di} + J_{ei}$, respectively. Therefore, based on Eq. (4), (5), and (6), the ordinary differential equations for the three state variables were written as:

$$\frac{dCu_d}{dt} = -(Cu_d - Cu_e)^2 \frac{C_{de}}{ln(A_{d0})(t+1)}\left(1 - \frac{Cu_{de}}{Cu_{me}}\right) - (Cu_d - Cu_i)^2 \frac{C_{di}}{ln(A_{d0})}, \qquad \text{(Eq. 7)}$$

$$\frac{dCu_e}{dt} = (Cu_d - Cu_e)^2 \frac{C_{de}}{ln(A_{d0})(t+1)}\left(1 - \frac{Cu_{de}}{Cu_{me}}\right) - \frac{C_{ei}}{(t+1)} A_{dt}\left(1 - \frac{Cu_{di}}{Cu_{mi}}\right), \qquad \text{(Eq. 8)}$$

$$\frac{dCu_i}{dt} = (Cu_d - Cu_i)^2 \frac{C_{di}}{ln(A_{d0})} + \frac{C_{ei}}{(t+1)} A_{dt}\left(1 - \frac{Cu_{di}}{Cu_{mi}}\right). \qquad \text{(Eq. 9)}$$

The equations presented above for the copper mass balance over time were represented in a dynamic model using Powersim studio (Powersim software As, Norway), and then simulated by the Euler integration method with 0.01 d time-step. To calibrate the parameters in the presented model, parameter values that minimized the weighted sum of squares deviation between observed data and model predictions were estimated by Powersim solver analysis tools.

The LKM developed in this study was developed with 8-d exposure data and validated with 21-d exposure data to

Table 2. Estimated parameter values for pseudo-first-order, pseudo-second-order, and intra-particle diffusion models

Model	Parameter	Copper concentration (μg L^{-1})					
		P. subcapitata			*C. vulgaris*		
		5	10	15	5	10	15
Pseudo-first-order	q_e (μg mL 10^8 cells^{-1})	7.82	16.77	36.60	17.35	29.57	38.94
	k_1 (d^{-1})	5.90	7.16	3.76	1.31	1.01	1.55
	R^2	0.91	0.78	0.691	0.96	0.95	0.98
Pseudo-second-order	q_e (μg mL 10^8 cells^{-1})	8.06	16.54	36.92	18.91	32.59	42.25
	k_2 (10^8 cells μg^{-1} mL^{-1} d^{-1})	1.52	4.04	0.27	0.11	0.05	0.06
	R^2	0.92	0.76	0.611	0.98	0.97	0.98
Intra-particle diffusion	k_d (μg mL 10^8 cells^{-1} d^{-1})	1.98	2.69	6.29	5.96	10.63	12.95
	C (μg mL 10^8 cells^{-1})	3.56	10.06	19.86	3.77	5.17	9.58
	R^2	0.73	0.55	0.40	0.91	0.93	0.86

 ©2021. Korean Society of Environmental Biology.

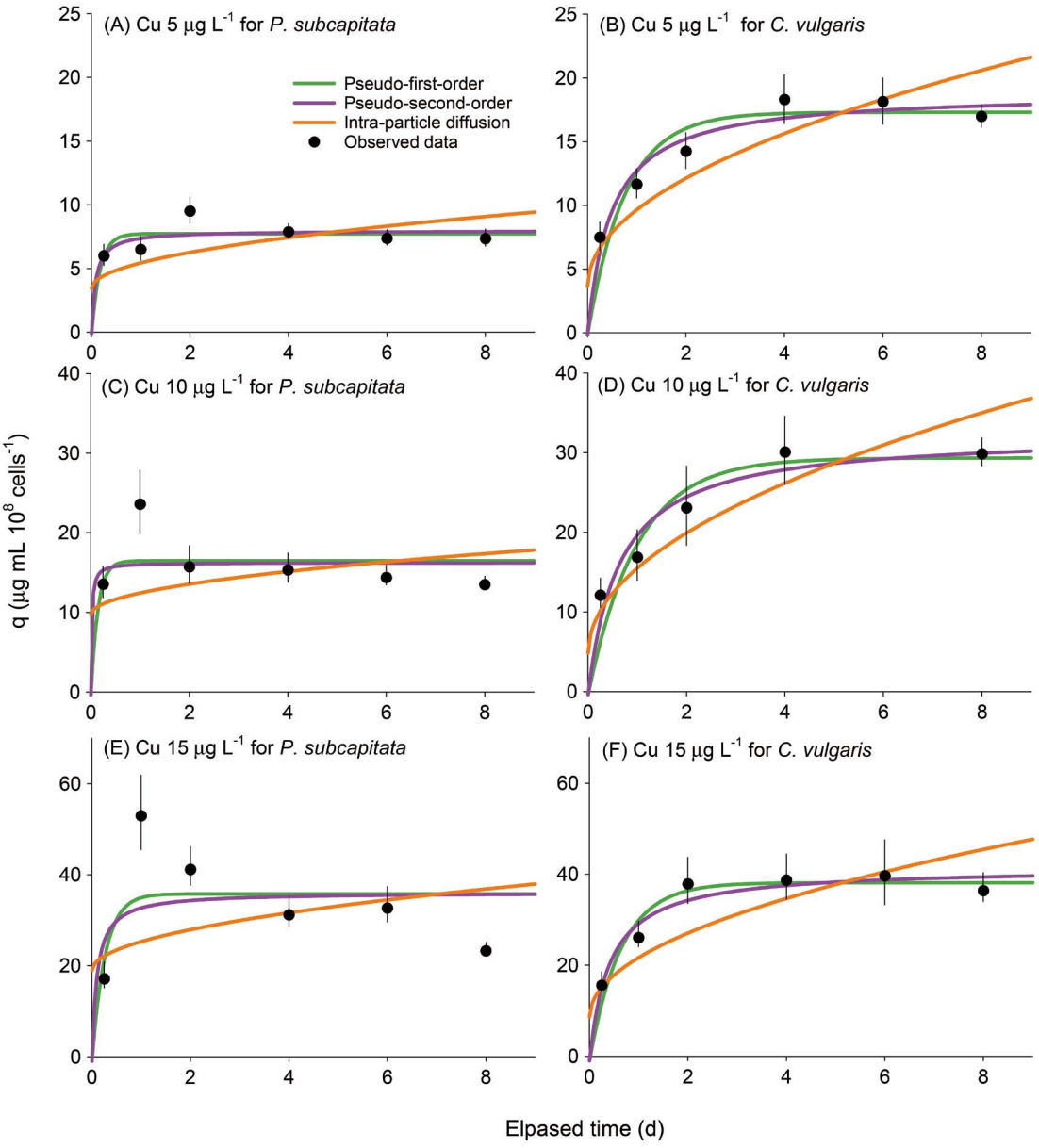

Fig. 1. A general kinetic model for exposed copper concentrations: (A) 5 μg L^{-1} for *Pseudokirchneriella subcapitata*, (B) 5 μg L^{-1} for *Chlorella vulgaris*, (C) 10 μg L^{-1} for *P. subcapitata*, (D) 10 μg L^{-1} for *C. vulgaris*, (E) 15 μg L^{-1} for *P. subcapitata*, and (F) 15 μg L^{-1} for *C. vulgaris*.

find the applicability of this model under long-term exposure conditions.

RESULTS

1. Evaluations of three general kinetic models

The observed data for adsorbed copper mass were fitted to the pseudo-first-order, pseudo-second-order, and intra-particle diffusion models for *P. subcapitata* and *C. vulgaris* over exposure times (Fig. 1). The adsorbed copper mass on *P. subcapitata* began to increase during early exposure periods (< 2 d), and then slightly decreased with time, while the copper mass on *C. vulgaris* was continuously increased and enter the equilibrium.

For both algae species, the pseudo-first-order and pseudo-second-order models were better fits than the intra-particle

diffusion models according to the values of correlation coefficients (R^2) (Table 2). The intra-particle diffusion model underestimated the copper masses before exposure days of 4 but overestimated after that. The adsorption kinetics of *C. vulgaris* was well represented by three kinetics models compared with those for *P. subcapitata*. The R^2 values for *C. vulgaris* remained constant regardless of the kinetic models and treated copper concentrations, but the values for *P. subcapitata* decreased as the concentrations increased from 5 to 15 μg L^{-1} (Table 2). These results suggested that copper adsorption kinetics with algal cells, especially for *P. subcapitata* under copper exposed conditions were affected by some factors which were not considered in these general kinetic models.

2. Development of the long-term kinetic model

The parameter estimates used for the LKM were listed in Table 3. Maximum copper densities of intracellular (Cu_{mi}) and extracellular (Cu_{me}) were higher at *P. subcapitata* and *C. vulgaris*, respectively. These parameter values do not mean that tested algal cells can hold a specific amount of copper, but higher values of the maximum copper loading density indicate that the effect of present copper concentration on copper mass flux between each part is lower in the observed data. In our model, Cu_{mi} and Cu_{me} are parameters related to copper mass flow from extracellular to intracellular (J_{di}) and from solution to extracellular (J_{de}), respectively. Thus, higher values of Cu_{mi} and Cu_{me} represent that J_{di} and J_{de} may have a stronger influence on the overall copper kinetics in the experimental sets, respectively. The parameters, C_{de}, C_{di}, and C_{ei}, showed the same trend in exposure tests of both *P. subcapitata* and *C. vulgaris* with increasing the copper concentration. Increased C_{ei} with increasing the copper concentration indicated that copper mass flow from extracellular to intracellular occurred rapidly. Whereas decreased C_{de} and C_{di} with increasing the copper concentration represented that the squares of the difference between the dissolved and algal cell-related copper mass were a major contributory factor to copper kinetics in increasing input copper mass.

Table 3. Estimated long-term kinetic model parameters for *Pseudokirchneriella subcapitata* and *Chlorella vulgaris* for the exposed copper concentration

Parameter	Copper concentration (μg L^{-1})			Unit
	5	10	15	
P. subcapitata				
Cu_{me}	8.37	8.37	8.37	μg mL 10^8 cells^{-1}
Cu_{mi}	3.50	3.50	3.50	μg mL 10^5 cells^{-1}
C_{de}	87.29	47.24	44.19	cells mL^{-1} μg^{-2} d^{-1}
C_{di}	146.25	126.12	53.99	cells mL^{-1} μg^{-2} d^{-1}
C_{ei}	1.80	1.91	8.42	mL d^{-1} 10^8 cells^{-1}
C. vulgaris				
Cu_{me}	5.34	5.34	5.34	μg mL 10^6 cells^{-1}
Cu_{mi}	1.19	1.19	1.19	μg mL 10^8 cells^{-1}
C_{de}	81.19	47.34	30.72	cells mL^{-1} μg^{-2} d^{-1}
C_{di}	327.28	95.72	49.77	cells mL^{-1} μg^{-2} d^{-1}
C_{ei}	1.19	5.70	6.19	mL d^{-1} 10^8 cells^{-1}

As shown in Fig. 2, the copper mass dynamics during 8-d exposure duration in three fractions (dissolved in medium, extracellular, and in intracellular algae) were well described by LKM (mean of R^2 for dissolved Cu mass: 0.962, extracellular Cu mass: 0.789, and intracellular Cu mass: 0.951), and the distribution patterns between the algae species were similar. In all the concentrations tested, drastic changes in copper mass in the three fractions occurred first 1−2 d after exposure. The dissolved copper mass in the medium decreased greatly and continued to decrease with exposure durations after 3 d, while the opposites were observed for intracellular copper masses. The extracellular copper mass increased slightly and decreased 3 d after exposures. At the final exposure day, the predicted copper mass in the intracellular was highest, followed by the dissolved. The extracellular copper mass was the smallest proportion of the total copper during the exposure tests, ranged from 5.64 to 21.22%. For low (5 μg L^{-1}) and medium (10 μg L^{-1}) copper treatments, the distribution amounts were similar between the algae species, but for high (15 μg L^{-1}) copper concentration, the intracellular copper mass in *P. subcapitata* was 60% higher than that in *C. vulgaris*.

The copper mass distributions per 10^8 algal cells over exposure times were presented in Fig. 3 and LKM was also well described these changes (mean of R^2 for intracellular Cu mass per algal cell: 0.878 and extracellular Cu mass per algal cell: 0.750). The distribution patterns were very similar among initial copper concentrations. For *C. vulgaris*, the copper fluxes into algal cells (intracellular) increased abruptly with time, while the extracellular concentrations increased slightly until 0.5 d and then remained constant, regardless of the initial copper concentrations. For the results of *P. subcapitata*, the copper fluxes increased until 1 d, and then remained constant level throughout the exposure times, but LKM described the fluxes poorly at 0.5 d showing over-

 ©2021. Korean Society of Environmental Biology.

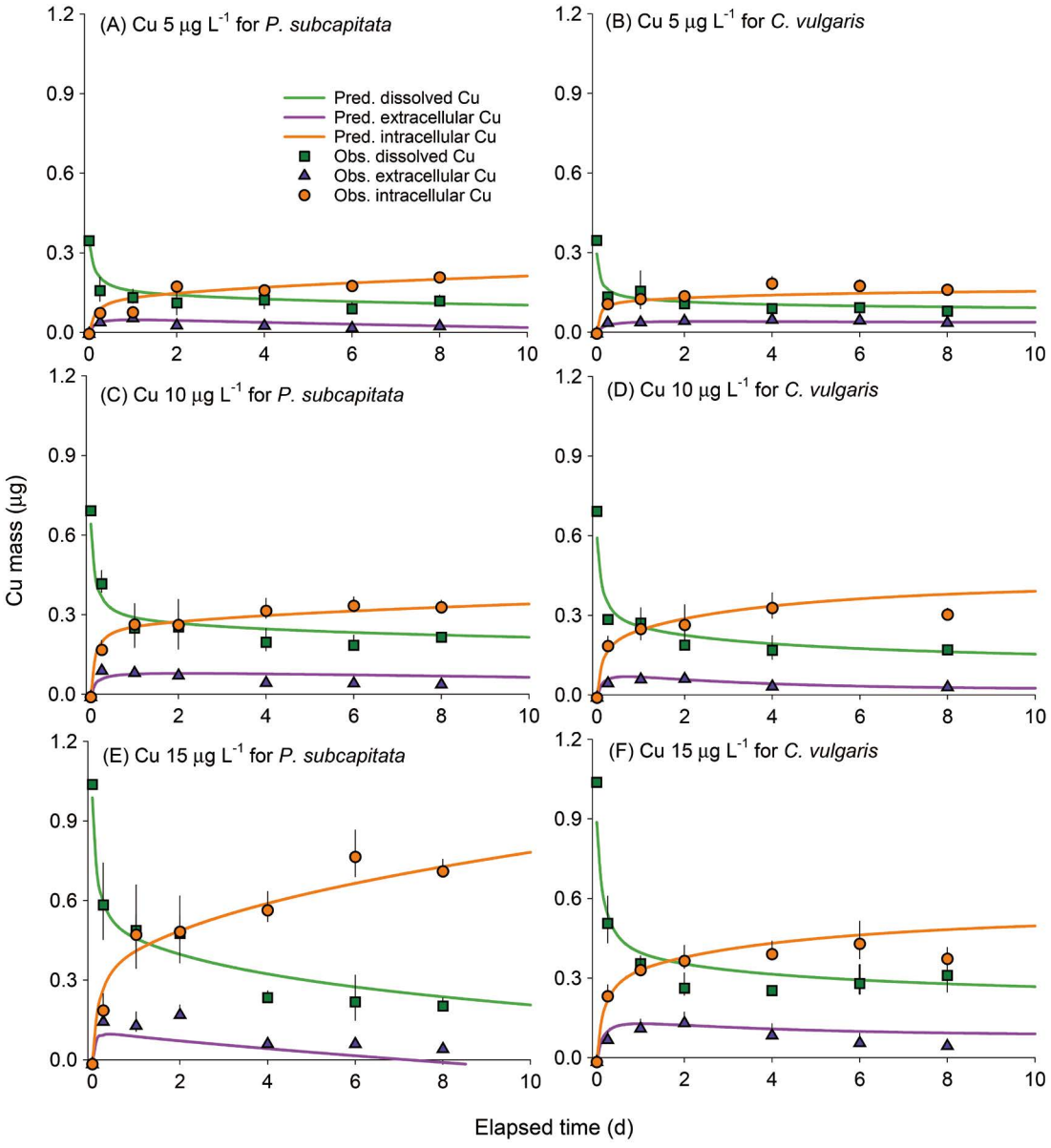

Fig. 2. Observed and predicted mass of dissolved, extracellular, and intracellular copper for exposed copper concentrations: (A) 5 μg L^{-1} for *Pseudokirchneriella subcapitata*, (B) 5 μg L^{-1} for *Chlorella vulgaris*, (C) 10 μg L^{-1} for *P. subcapitata*, (D) 10 μg L^{-1} for *C. vulgaris*, (E) 15 μg L^{-1} for *P. subcapitata*, and (F) 15 μg L^{-1} for *C. vulgaris*.

estimated at 5 μg L^{-1} but underestimated at 10 and 15 μg L^{-1}. The changes of extracellular concentrations in *P. subcapitata* were different from those in *C. vulgaris*; the concentrations increased slightly and then decreased after 1-d exposure. These results suggested that the constant binding capacity (numbers of binding sites) on the surface of *C. vulgaris* cells was maintained throughout the exposure times, but the capacity for *P. subcapitata* decreased with exposure times.

3. Model validation

LKM developed in this study was validated using an experimental data set obtained from 21-d exposures (Fig. 4). LKM described the dynamics of copper distributions for 21-d exposure conditions, and the patterns were similar with 8-d exposure. There was no large change in copper mass of dissolved, extracellular, and intracellular sites, although algae densities were increased or decreased over

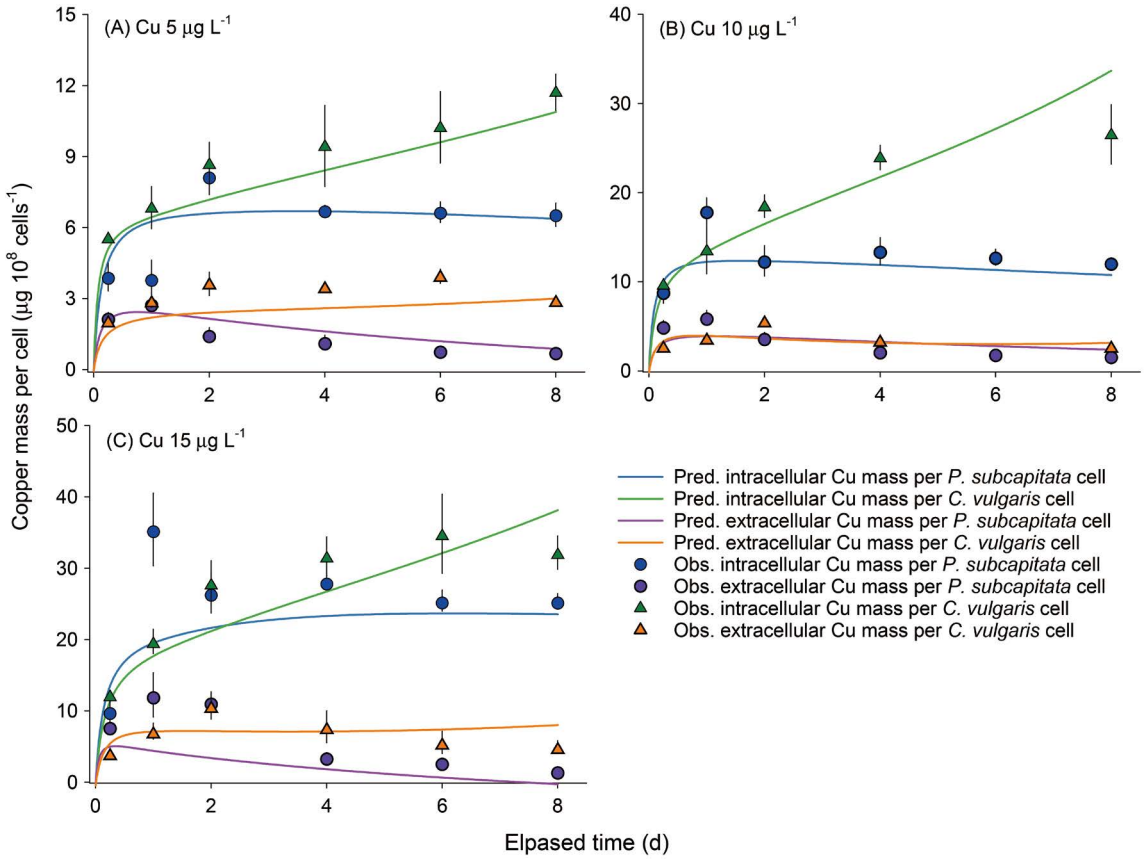

Fig. 3. Observed and predicted mass of extracellular and intracellular copper per 10^8 algal cells for exposed copper concentrations: (A) 5 μg L^{-1}, (B) 10 μg L^{-1}, and (C) 15 μg L^{-1}.

time. Especially, densities of *P. subcapitata* at 21-d were increased 2.4- and 1.9-fold when compared with densities at 4-d in 5 and 10 μg L^{-1} copper concentrations, respectively, but the copper mass of intracellular was almost no changed.

The correlations between predicted and observed copper masses in intracellular, extracellular, and dissolved with 1 : 1 lines for reference are shown in Fig. 5. In the results of *P. subcapitata*, a relatively large variance was also observed in a high copper mass of intracellular. For the *C. vulgaris*, estimated copper mass was overestimated in high copper mass by LKM. Overall, however, estimated copper mass by LKM is in good agreement with the measured value for both *P. subcapitata* ($R^2 = 0.928$) and *C. vulgaris* ($R^2 = 0.943$).

DISCUSSION

Our results showed that the total copper mass of dissolved, extracellular, and intracellular was slowly changed over time regardless of changes in algal density. Hence, uptake and internalization of copper mass per algal cell could be strongly influenced by the algal population over time. This suggests that the effects of copper on the algal population dynamically change over time, and toxicity will be increasing or decreasing by copper kinetics and the algal density dynamics in the long term.

Adsorption isotherms and sorption kinetic models have been widely used to investigate the mechanism of metal uptake and adsorption capacity by algae (Chen *et al.* 2008; Areco and dos Santos Afonso 2010; Jayakumar *et al.* 2014). Adsorption isotherms are apposite to describe the interaction between sorbate and adsorbent, and general kinetic models such as pseudo-first order, pseudo-second order, and intra-particle diffusion, are useful to investigate how rates are affected by sorbent character or sorption capacity (Jayakumar *et al.* 2014). In the results of the general kinetic model study, the fitness of the model was lower for *P. subcapitata* with increasing exposed copper concentration (Ta-

 ©2021. Korean Society of Environmental Biology.

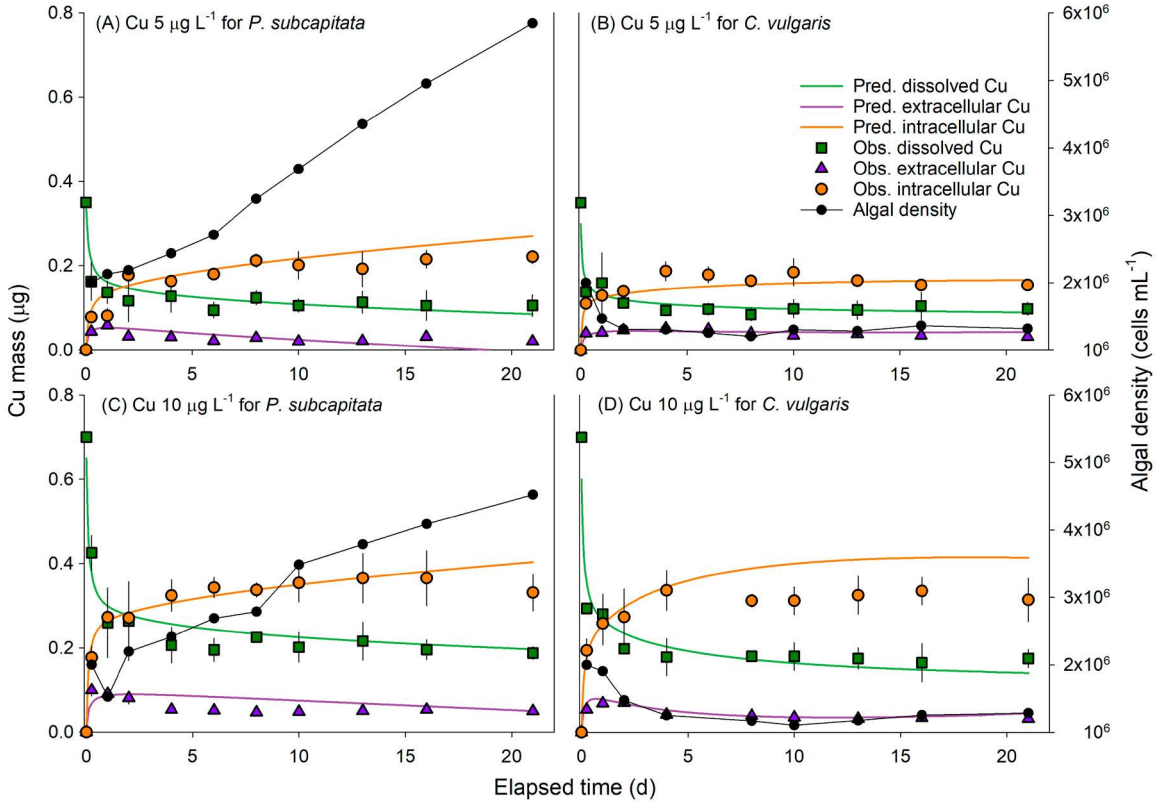

Fig. 4. Observed and predicted mass of dissolved, extracellular, and intracellular copper with algal density during a 21-d exposure for exposed copper concentrations: (A) 5 μg L^{-1} for *Pseudokirchneriella subcapitata*, (B) 5 μg L^{-1} for *Chlorella vulgaris*, (C) 10 μg L^{-1} for *P. subcapitata*, and (D) 10 μg L^{-1} for *C. vulgaris*.

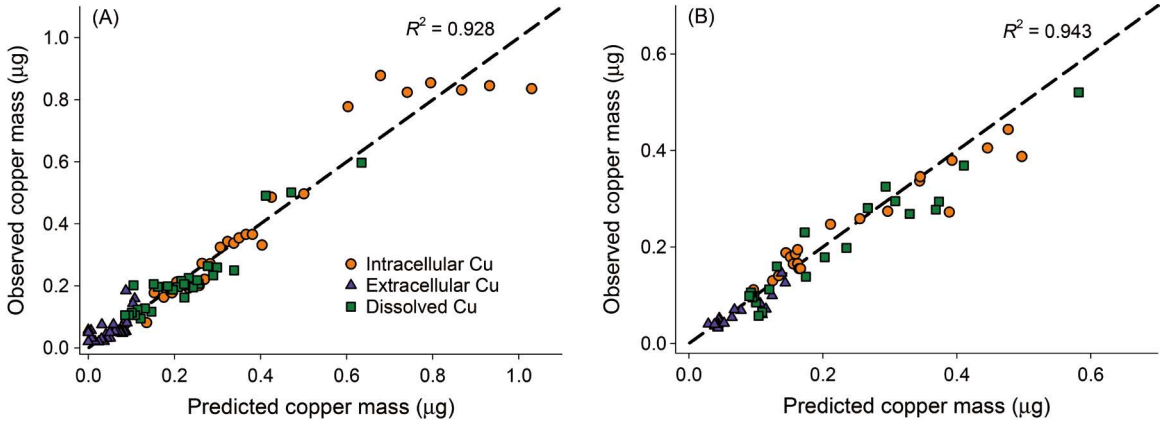

Fig. 5. Correlation between predicted and observed mass of dissolved, intracellular, and extracellular copper: (A) *Pseudokirchneriella subcapitata* and (B) *Chlorella vulgaris*. The dotted line represents the same value between predicted and observed copper mass. Each point represents the mean of five observed values.

ble 2). These kinetic model approaches were focused on the interaction between the dissolved metal and algal cell as sorbent in the equilibrium state. However, biological responses such as changes in cell ultrastructure and decreased uptake rate, are occurred during the initial exposure period (Levy *et al.* 2008). A remarkable difference between the

observed and predicted values by the general kinetic model in *P. subcapitata* may be induced by biological responses to copper exposure because they have less sensitivity to copper toxicity than *C. vulgaris* (Franklin *et al.* 2002). Another limitation of the general kinetic models was that extracellular and intracellular metal mass were not separately predicted. Extracellular and intracellular copper mass per algal cell is showed a different trend with an elapsed time (Levy *et al.* 2008). Thus, existing kinetic model approaches may not be suitable to investigate the interaction between metal and living algal cells or long-term kinetics although it is useful to describe the metal kinetics for the short-term.

Franklin *et al.* (2002) and Stoiber *et al.* (2012) reported that extracellular and intracellular metal are important factors to predict the heavy metal toxicity on algal cells. In this respect, our LKM was developed to describe the mass flow between the solutions, extracellular, and intracellular sites with time, and showed a good performance with experimental data (Figs. 2–5). FIAM and BLM have been also widely used in ecotoxicology with mechanistic descriptions for chemical speciation and bioavailability (Campbell *et al.* 2002). Especially, BLM with FIAM has been successfully utilized to understand how physiochemical water characteristics such as pH, hardness, dissolved organic carbon, and metal mixtures influence on toxicity of metals to freshwater biota under short-term exposure (De Schamphelaere *et al.* 2005; Chen *et al.* 2010). In this study, because the distribution of copper introduced water with time under long-term exposure was a major concern for describing the dynamic interaction between the metal and algae, a new kinetic model was developed using three state variables and three mass flow equations by adjusted existing approaches.

The ratio of copper in solution to total exposed copper decreased below 40% for all experimental sets for *P. subcapitata* and *C. vulgaris* (Fig. 2). Similarly, Levy *et al.* (2008) reported that the dissolved copper was decreased by 40–50% for *Tetraselmis* sp. (Prasinophyceae), and by below 11% for *Phaeodactylum tricornutum* (Bacillariophyceae) and *Dunaliella tertiolecta* (Chlorophyceae). Decrease ratio difference of dissolved copper between the algae species may occur by characteristics of the cell membrane and binding sites (Franklin *et al.* 2002; Levy *et al.* 2008). While dissolved copper in solution rapidly decreased, intracellular copper mass was increased up to 50% of total introduced copper. Meanwhile, extracellular copper mass was below 10% over time in all experimental sets. This result was not surprising as Franklin *et al.* (2002) and Wilde *et al.* (2006) showed that the intracellular copper mass was much higher than extracellular in low copper concentration (< 10 μg L^{-1}) for *Chlorella* sp. and *P. subcapitata*. They also reported that the ratio of extracellular to intracellular copper was increased in high copper concentrations, but it was not found in the range of concentrations tested in this study. The increasing ratio of extracellular to intracellular copper mass may be induced by the death of algal cells in high copper concentrations because dead cells have higher adsorption capacity (Kaduková and Virčíková 2005).

The two phases of the kinetics between algae and metals, the initial rapid adsorption and then a slower transport of metal, have been reported by several researchers (Mehta *et al.* 2002; Chen *et al.* 2008; Areco and dos Santos Afonso 2010). Franklin *et al.* (2002) suggested that metal toxicity was proportional to the amount of copper per cell. Thus, the effect of copper on *P. subcapitata* and *C. vulgaris* can be controlled by algal density. The variation of algal density during the initial exposure period can be a crucial factor in copper toxicity, since copper loading density determined by algal density led to accelerating the increase or decrease of copper mass per algal cell (Fig. 3).

Interestingly, dissolved copper, extracellular and intracellular copper masses were changed very slowly after initial adsorption, compared to changes in algal density (Fig. 4). According to Franklin *et al.* (2002) and Kim *et al.* (2020), the initial algal density is an important factor in the kinetics of copper introduced into the solution, as a high initial density leads to more adsorption and accumulation of copper on the algal cells. However, our results showed that change in algal density during the exposure period was not an influential factor for overall copper kinetics. Several factors such as the decrease in metal uptake rate, the increase in the number of vacuoles in cell ultrastructure, and the decrease in concentration difference between the solutions and algal cells (Tien *et al.* 2005; Levy *et al.* 2008; Paquet *et al.* 2015), might offset the effects of increased algal cell density on the total accumulated copper mass. To clearly understand the effect of copper on algal populations, it is necessary to specifically study the responses and changes of algal cells over time against copper toxicity.

A possible limitation of our modeling study is that description for the mechanism of slower copper transport between the solutions, extracellular, and intracellular regardless of algal density variation was not included. Changes in cell ultrastructure, exudate produced by algae, dynamics of copper-binding sites, and other detoxification mechanisms may occur during long-term exposure (Franklin *et al.* 2002; Tien *et al.* 2005; Levy *et al.* 2008). However, kinetic

 ©2021. Korean Society of Environmental Biology.

dynamics of copper with algal cell are influenced by these factors in a too complex manner, it may increase the model complexity and uncertainty. Thus, possible biological responses with time by algae were simplified by using model parameters and the scaled time parameter (t). Another limitation is that the elution of copper from algal cells was not considered. During the long-term exposure, copper mass flow from intracellular and extracellular to solutions may occur by decomposition of dead cells and disability of damaged cell surface (Levy *et al.* 2008). In our observation, an increase in dissolved copper after the initial rapid decrease was not founded within 21 d, but it can occur when the algal population enters into the decline phase. Despite these limitations, our kinetic model strikes a trade-off between practical simplicity and realistic complexity. The developed model can be used to clearly understand the dynamics of the algal population and the behavior of heavy metals by adding formulas expressing new mechanisms in the future.

ACKNOWLEDGMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2020R1I1A01074894, NRF-2021R1A6A1A10045235).

REFERENCES

Areco MM and M dos Santos Afonso. 2010. Copper, zinc, cadmium and lead biosorption by *Gymnogongrus torulosus*. Thermodynamics and kinetics studies. Colloid Surf. B-Biointerfaces 81:620–628.

Ayed HBA, B Taidi, H Ayadi, D Pareau and M Stambouli. 2015. Effect of magnesium ion concentration in autotrophic cultures of *Chlorella vulgaris*. Algal Res. 9:291–296.

Campbell PGC, O Errécalde, C Fortin, VP Hiriart-Baer and B Vigneault. 2002. Metal bioavailability to phytoplankton-applicability of the biotic ligand model. Comp. Biochem. Physiol. C-Toxicol. Pharmacol. 133:189–206.

Chen Z, W Ma and M Han. 2008. Biosorption of nickel and copper onto treated alga (*Undaria pinnatifida*): Application of isotherm and kinetic models. J. Hazard. Mater. 155:327–333.

Chen Z, L Zhu and KJ Wilkinson. 2010. Validation of the biotic ligand model in metal mixture: bioaccumulation of lead and copper. Environ. Sci. Technol. 44:3580–3586.

Cooper S, L Hare and PGC Campbell. 2010. Modeling cadmium uptake from water and food by the freshwater bivalve *Pyganodon grandis*. Can. J. Fish. Aquat. Sci. 67:1874–1888.

Dang F, H Zhong and WX Wang. 2009. Copper uptake kinetics and regulation in a marine fish after waterborne copper acclimation. Aquat. Toxicol. 94:238–244.

De Schamphelaere KAC, JL Stauber, KL Wilde, SJ Markich, PL Brown, NM Franklin, NM Creighton and CR Janssen. 2005. Toward a biotic ligand model for freshwater green algae: surface-bound and internal copper are better predictors of toxicity than free Cu^{2+}-ion activity when pH is varied. Environ. Sci. Technol. 39:2067–2072.

EPA. 2002. Method 1003.0: Green Alga *Selenastrum capricornutum*, Growth Test: Chronic Toxicity. Environmental Protection Agency. Washington, D.C.

Fortin C, FH Denison and J Garnier-Laplace. 2007. Metal-phytoplankton interactions: Modeling the effect of competing ions (H^+, Ca^{2+}, and Mg^{2+}) on uranium uptake. Environ. Toxicol. Chem. 26:242–248.

Franklin NM, JL Stauber, SC Apte and RP Lim. 2002. Effect of initial cell density on the bioavailability and toxicity of copper in microalgal bioassays. Environ. Toxicol. Chem. 21:742–751.

Gatidou G and NS Thomaidis. 2007. Evaluation of single and joint toxic effects of two antifouling biocides, their main metabolites and copper using phytoplankton bioassays. Aquat. Toxicol. 85:184–191.

Hassler CS, VI Slaveykova and KJ Wilkinson. 2004. Some fundamental (and often overlooked) considerations underlying the free ion activity and biotic ligand models. Environ. Toxicol. Chem. 23:283–291.

Ho YS and G Mckay. 1999. Pseudo-second order model for sorption processes. Process Biochem. 34:451–465.

Janssen CR and DG Heijerick. 2003. Algal toxicity testing for environmental risk assessments of metals: Physiological and ecological considerations. Rev. Environ. Contam. Toxicol. 178:23–52.

Jayakumar R, M Rajasimman and C Karthikeyan. 2014. Sorption of hexavalent chromium from aqueous solution using marine green algae *Halimeda gracilis*: Optimization, equilibrium, kinetic, thermodynamic and desorption studies. J. Environ. Chem. Eng. 2:1261–1274.

Jeyakumar RPS and V Chandrasekaran. 2014. Adsorption of lead (II) ions by activated carbons prepared from marine green algae: Equilibrium and kinetics studies. Int. J. Ind. Chem. 5:10.

Kaduková J and E Virčíková. 2005. Comparison of differences between copper bioaccumulation and biosorption. Environ. Int. 31:227–232.

Kim Y, J Son, HH Mo, YS Lee and K Cho. 2018. Modeling the influence of initial density and copper exposure on the interspecific competition of two algal species. Ecol. Model.

383:160–170.

Kim Y, J Son, YS Lee, J Wee, M Lee and K Cho. 2020. Temperature-dependent competitive advantages of an allelopathic alga over non-allelopathic alga are altered by pollutants and initial algal abundance levels. Sci. Rep. 10:4419.

Levy JL, BM Angel, JL Stauber, WL Poon, SL Simpson, SH Cheng and DF Jolley. 2008. Uptake and internalisation of copper by three marine microalgae: Comparison of copper-sensitive and copper-tolerant species. Aquat. Toxicol. 89:82–93.

Ma W, FQ Ya, M Han and R Wang. 2007. Characteristics of equilibrium, kinetics studies for adsorption of fluoride on magnetic-chitosan particle. J. Hazard. Mater. 143:296–302.

Machado MD, AR Lopes and EV Soares. 2015. Responses of the alga *Pseudokirchneriella subcapitata* to long-term exposure to metal stress. J. Hazard. Mater. 296:82–92.

Mehta SK, A Singh and JP Gaur. 2002. Kinetics of adsorption and uptake of Cu^{2+} by *Chlorella vulgaris*: influence of pH, temperature, culture age, and cations. J. Environ. Sci. Health Part A-Toxic/Hazard. Subst. Environ. Eng. 37:399–414.

Miao AJ and WX Wang. 2007. Predicting copper toxicity with its intracellular or subcellular concentration and the thiol synthesis in a marine diatom. Environ. Sci. Technol. 41:1777–1782.

Paquet N, M Lavoie, F Maloney, JFL Duval, PGC Campbell and C Fortin. 2015. Cadmium accumulation and toxicity in the unicellular alga *Pseudokirchneriella subcapitata*: Influence of metal-binding exudates and exposure time. Environ. Toxicol. Chem. 34:1524–1532.

Slaveykova VI and KJ Wilkinson. 2005. Predicting the bioavailability of metals and metal complexes: Critical review of the biotic ligand model. Environ. Chem. 2:9–24.

Stoiber TL, MM Shafer and DE Armstrong. 2012. Relationships between surface-bound and internalized copper and cadmium and toxicity in *Chlamydomonas reinhardtii*. Environ. Toxicol. Chem. 31:324–335.

Tien CJ, DC Sigee and KN White. 2005. Copper adsorption kinetics of cultured algal cells and freshwater phytoplankton with emphasis on cell surface characteristics. J. Appl. Phycol. 17:379–389.

Wilde KL, JL Stauber, SJ Markich, NM Franklin and PL Brown. 2006. The effect of pH on the uptake and toxicity of copper and zinc in a tropical freshwater alga (*Chlorella* sp.). Arch. Environ. Contam. Toxicol. 51:174–185.

Xiong Y, J Xu, W Shan, Z Lou, D Fang, S Zang and G Han. 2013. A new approach for rhenium (VII) recovery by using modified brown algae *Laminaria japonica* adsorbent. Bioresour. Technol. 127:464–472.

Zeng J, L Yang and WX Wang. 2009. Cadmium and zinc uptake and toxicity in two strains of *Microcystis aeruginosa* predicted by metal free ion activity and intracellular concentration. Aquat. Toxicol. 91:212–220.

 ©2021. Korean Society of Environmental Biology.