Selenate and selenite uptake, accumulation and toxicity in *Lemna minuta*

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**ABSTRACT**

The kinetics of Se uptake and toxicity to *Lemna* were studied over a period of 14 days of exposure to Se(IV) or Se(VI). The growth of *Lemna* stopped immediately after exposure to 5.0 mg/L of Se(IV) or Se(VI). The content of chlorophyll and phaeopigments of *Lemna* exposed to 5.0 mg/L of Se(IV) was two to three times less than in the control after 3 d exposure. *Lemna* took up Se rapidly within the first 3 d. The Se content in *Lemna* along with the exposure time fitted well the two-compartment and the hyperbolic model, which demonstrates that the mechanism of Se(IV) and Se(VI) uptake in *Lemna* is not only through passive diffusion, but also through other processes such as ion channel proteins or transporters. The kinetic bioconcentration factors (BCFs) were 231 and 42 for 0.5 mg/L Se(IV) and Se(VI) exposure, respectively. The uptake rate of *Lemna* reached 263 mg/kg/d and 28 mg/kg/d in the Se(IV) and Se(VI) treatments, respectively. This study showed that Se(IV) has a faster accumulation rate than Se(VI), but a higher toxicity, indicating *Lemna* could be a good candidate to remove Se(IV) from water, producing Se-enriched biomass which may eventually also be considered for use as Se-enriched feed supplement or fertilizer.

**Key words** | duckweed, selenium, Se uptake, toxicokinetic model

**HIGHLIGHTS**

- The kinetics of Se(IV) and Se(VI) accumulation and toxicity in *Lemna* were studied.
- Se(IV) had a faster Se accumulation rate but a higher toxicity than Se(VI) in *Lemna*.
- Se content in *Lemna* reached a steady state after 6 d of Se exposure.
- Both the two-compartment model and the hyperbolic kinetic model could simulate the accumulation of Se in *Lemna*.
- *Lemna* could potentially be used as a phytoextraction plant to remove Se(IV) from water.

**GRAPHICAL ABSTRACT**

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INTRODUCTION

Selenium (Se) is an essential micronutrient for humans and animals (Hatfield et al. 2014). However, the thresholds of Se in the environment that differentiate among deficiency, suitability and toxicity are very narrow (Fordyce 2013). Increasing anthropogenic activities such as mining, agriculture and industrial manufacturing produce wastewaters containing Se, for example those of coal (0.4–1,500 μg/L) and gold (1,700–33,000 μg/L) mining, flue gas desulfurization process water (1.0–10,000 μg/L) and agricultural drainage (140–1,400 μg/L) (Lemly 2004; Tan et al. 2016). These can result in elevated Se concentrations in the receiving water bodies that exceed the current chronic aquatic life criteria in lentic (1.5 μg/L) and lotic (3.1 μg/L) waters adopted by the United States Environmental Protection Agency (USEPA 2016). For instance, Se concentrations of 7–14 μg/L were found in Hyco Lake (North Carolina, USA) near the effluent source of a power plant and 9.6 μg/L Se was measured in Elk River (British Columbia, Canada) near coal mines (Young et al. 2010). Additionally, Flanders (Belgium) has adopted a surface water quality standard of a maximum of 5.0 μg/L of total Se (Vlarem II 1995), while the Canadian Council of Ministers of the Environment has established a limit of 20–50 μg/L of Se for irrigation water (Etteib et al. 2020).

Phytoextraction is an environmentally friendly method to remove excess contaminants and nutrients from water. Duckweed (Lemna sp.), as an aquatic floating plant, is a good candidate for the treatment of wastewaters because of its fast growth rates, easy harvest, and simple structure (Panfili et al. 2017). Additionally, duckweed containing high levels of protein (around 20%–40%) and starch can also be explored as a valuable protein and carbohydrate source for animal feed (Zhong & Cheng 2016). Particularly, Se taken up by plants is easily converted into organic Se compounds, such as the selenoamino acids Se-cystine (SeCys2) and selenomethionine (SeMet), which have a benefit for human and animal nutrient intake (Terry et al. 2000). Additionally, the use of the aquatic plant duckweed for the removal of Se from contaminated water and the production of Se-enriched biomass for use as an animal feed supplement or micronutrient fertilizer would contribute to the drive for the circular economy. However, there is limited information about Se accumulation in duckweed and the physiological and biochemical response of duckweed to Se oxianions.

Selenium compounds exist in nature in five redox states: Se(-II), Se(0), Se(II), Se(IV) and Se(VI) (Terry et al. 2000). Different chemical forms of Se have different properties regarding bioavailability, mobility, and toxicity. Selenium enters freshwater mainly as selenite (Se(IV)) and selenate (Se(VI)) oxianions due to their high solubility. These are the two major forms of toxic Se in the ecosystem, because the two Se species are readily taken up by aquatic plants or animals and metabolized to organic Se compounds, resulting in Se bioaccumulation and toxicity to organisms (Mechora et al. 2015). High Se concentrations in plants can cause symptoms of toxicity such as growth inhibition, leaf chlorosis and premature death. For instance, Zhong et al. (2016) reported negative impacts of Se(IV) on the chlorophyll fluorescence and starch content of the duckweed Landoltia punctata after exposure to Se(IV) concentration higher than 3.2 mg/L. Carvalho & Martin (2001) recorded that the fresh weight of the duckweed Lemna obscura Aust. decreased from 50 to 20 mg when the Se(IV) dose was increased from 1.0 to 20 mg/L in the medium. Ohlbaum et al. (2018) found that the chlorophyll a content decreased from 0.36 to 0.13 mg/g fresh weight for Lemna when increasing the Se(VI) concentration from 0.05 to 0.5 mg/L in the leachate of a seleniferous soil. Mechora et al. (2015) studied the response of duckweed to various Se(IV) concentrations and found that Lemna minor L. was dying at the highest Se(IV) concentration (10 mg/L). However, which of the inorganic Se forms, Se(IV) or Se(VI), has a higher aquatic toxicity for specific organisms is not clear: some studies indicated that Se(IV) is more toxic (Li et al. 2020), while the opposite was found in other studies (Krofič et al. 2016). The difference may depend on the exposure time, plant species or ambient conditions. Therefore, it is necessary to characterize the toxicity of Se to duckweed before implementing it in phytoextraction.

Metal accumulation affects the cycling of pollutants and micronutrients in aquatic systems and impacts the ecosystem’s health. Because of the complexity of metal uptake, detoxification and excretion processes in plants, it is difficult to directly predict all mechanisms and routes of bioaccumulation. This has led to the development of theoretical kinetic models to predict metal uptake and retention in both terrestrial and aquatic species (Clason et al. 2005). First-order kinetics are characteristic of a compartment model where the uptake rate of a chemical is linear with exposure concentration. When passive transport processes like diffusion are the main uptake mechanism, first-order kinetic models make a reasonable prediction over a wide range of exposure concentrations. However, if metal uptake is facilitated not
only by diffusion, but also by ion channel proteins or carriers, the relationship between metal uptake and exposure concentrations will follow a saturation curve, indicating that first-order kinetic models are not suitable at the higher exposure concentrations. In this situation, the non-linear two-compartment and the hyperbolic model might be applied to metal bioaccumulation over a wide exposure range. For instance, Templeman & Kingsford (2015) evaluated the accumulation of Cu and Zn in the jellyfish Cassiopea maremetens through a two-compartment model. Clason et al. (2003) showed that the two-compartment and hyperbolic toxicokinetic models could simulate the bioaccumulation of the heavy metals Cd, Pb, Cu and Zn in the Antarctic amphipod Paramoera walker. Selenium, as a metalloid, to some extent has the same characteristics as metals, but there has been no research evaluating the theoretical kinetic models of Se accumulation in plants and quantifying Se uptake rates of duckweed.

This study aimed to (1) assess the dynamic effect of Se on the physiology of duckweed during a 14-d incubation period under Se(IV) or Se(VI) exposure, (2) quantify the capacity of duckweed to accumulate the two forms of Se from aqueous medium, and (3) evaluate if the toxicokinetic models can predict bioconcentration patterns of aqueous Se(IV) and Se(VI) in duckweed.

MATERIALS AND METHODS

**Lemna source and cultivation**

Duckweed (Lemna minuta) was randomly collected from a natural freshwater canal in Delft (The Netherlands), and cultivated in modified Hoagland solution (Ohlbaum et al. 2018) at pH 6 to acclimatize for 7 days in a greenhouse. The modified Hoagland solution contained: 118 mg/L Ca(NO3)2·4H2O, 5.0 mg/L KNO3, 5.0 mg/L MgSO4·7H2O, 0.3 mg/L FeSO4·7H2O, 0.15 mg/L MnSO4·7H2O, 8.0 μg/L CuSO4·5H2O, 300 μg/L H3BO3, 1.28 μg/L (NH4)6Mo7O24·4H2O, 1.79 μg/L Na2WO4·2H2O, 22 μg/L ZnSO4, 5.0 μg/L NiSO4·7H2O and 4.0 μg/L CoCl2·6H2O. The temperature in the greenhouse varied between 25 and 30 °C and light was provided with a minimum intensity of 100 μmol photons/m²/s.

**Kinetics of Se accumulation and tolerance**

After 7 days of incubation, 1.0 g (wet weight) of Lemna was transplanted to 100 mL of Hoagland solution supplemented with 0.5 or 5.0 mg/L of Se(IV) or Se(VI) added as sodium selenite (Na2SeO3) or sodium selenate (Na2SeO4), respectively. Medium without Se served as control. Three replicate pots were prepared for each treatment and incubation period (0, 1, 3, 6, 8, 10, and 14 d). The total production of Lemna in each pot was harvested at every time-point for analysis. The harvested biomass was washed with deionized water (DI) and analyzed for growth indicators (fresh weight), tolerance index of roots (root length), total Se content, and photosynthetic pigment concentrations.

**Analytical methods**

For total Se determination, the plants were oven-dried at 60 °C until constant weight and then digested with 10 mL concentrated HNO3 in a microwave (CEM Mars 5, Matthews, NC, USA). The digested solution was diluted with DI water and analyzed for the total Se content using an atomic absorption spectrophotometer coupled to a graphite furnace (GF-AAS, Thermo Elemental Solaris MQQ, GF95, Thermo Fisher Scientific, Waltham, MA, USA). The multi-element standard solution as quality control was always analyzed along with each batch sample to evaluate the accuracy of total Se determination.

Chlorophyll α and β and their decomposition product phaeopigments α and β were determined following the procedure of Wintermans & De Mots (1965). Fresh weight of 0.1 g of Lemna was ground in a mortar with 5 mL ethanol (96%), transferred to a centrifuge tube and left overnight in dark conditions for extraction. The samples were centrifuged at 3,000 rpm for ten minutes. The absorbance of the supernatant was measured at 750, 665 and 649 nm in a UV-Vis spectrophotometer (Lambda 365 UV/Vis, PerkinElmer, Waltham, MA, USA) for the measurement of chlorophyll α and β. Afterwards, 0.5 mL of 0.06 M HCl was added to 3 mL of supernatant for acidification. The absorbance of the acidified supernatant was determined at 750, 666 and 655 nm for the measurement of phaeopigments α and β.

**Data analysis**

Statistical differences of the data were analyzed with analysis of variance (ANOVA) and Duncan’s multiple comparison tests in SPSS 20.0.

The tolerance index of the Lemna roots was evaluated by measuring the average length of ten roots of each
sample and calculated by the following Equation (1):

\[
Tolerance \ index = \frac{Average \ root \ length \ of \ sample}{Average \ root \ length \ of \ control}
\]  
(1)

The content of chlorophyll \( \alpha \) and \( \beta \) and phaeopigments \( \alpha \) and \( \beta \) were calculated according to the following Equations (2)–(5):

Chlorophyll \( \alpha = \)

\[
\frac{[13.70 \times (A665 - A750) - 5.76 \times (A649 - A750)] \times V \times D}{FW \times 1000}
\]
(2)

\[
Chlorophyll \ \beta = \frac{[25.80 \times (A649 - A750) - 7.60 \times (A665 - A750)] \times V \times D}{FW \times 1000}
\]
(3)

Phaeopigments \( \alpha = \)

\[
\frac{[24.50 \times (A666a - A750a) - 9.32 \times (A655a - A750a)] \times V \times D}{FW \times 1000}
\]
(4)

\[
Phaeopigments \ \beta = \frac{[36.97 \times (A655a - A750a) - 18.48 \times (A666a - A750a)] \times V \times D}{FW \times 1000}
\]
(5)

where: \( V \) = extraction volume (mL), \( D \) = sample dilution factor, \( FW \) = fresh weight of sample (g), and \( A665, A666a, A655a, A750a \) = absorbance at 655, 666 and 750 nm after acidiﬁcation, respectively.

The time course of Se uptake was evaluated by the two-compartment model (Clason et al. 2004a, 2004b), in which the Se exposure and the \( \text{Lemna} \) plants were considered as the first and second compartments, respectively. The model parameters for uptake and clearance were estimated by Equation (6), taking into account only data from the uptake phase and using non-linear iterative least square methods:

\[
C_A = C_0 + C_w \frac{K_a}{K_b} (1 - e^{-K_b t})
\]
(6)

where: \( C_A \) is the Se concentration in \( \text{Lemna} \) (mg/kg), \( C_0 \) is the background Se concentration in \( \text{Lemna} \) from the control (mg/kg), \( C_w \) is the Se exposure concentration (mg/L), \( K_a \) is the rate constant of Se uptake (mg/kg/d) and \( K_b \) (mg/kg/d) is the rate constant for clearance, which occurs in parallel with the uptake.

For the two-compartment model, the bioconcentration factor (BCF) at the theoretical equilibrium was calculated with the following Equation (7):

\[
BCF = \frac{K_a}{K_b}
\]
(7)

where \( K_a \) and \( K_b \) are derived from the two-compartment model (Equation (6)).

Alternatively, the time course of Se uptake was analyzed with a hyperbolic model (Clason et al. 2005) estimated with the following Equation (8):

\[
C_A = C_0 + \frac{C_{max} t}{t_{max/2} + t}
\]
(8)

where: \( C_A \) is the Se concentration in the \( \text{Lemna} \) (mg/kg), \( C_0 \) is the background Se concentration in \( \text{Lemna} \) from the control (mg/kg), \( C_{max} \) is the maximum Se concentration in the plants at theoretical equilibrium (mg/kg) and \( t_{max/2} \) is the time to reach half of the \( C_{max} \) (d).

### RESULTS AND DISCUSSION

**Effect of Se(IV) and Se(VI) on the growth rate of \( \text{Lemna} \)**

The biomass production of \( \text{Lemna} \) significantly decreased upon Se application compared with the control (Figure 1). The fresh biomass of \( \text{Lemna} \) significantly increased with incubation time, but at a slower growth rate under Se exposure when compared with the control. After 14 d of incubation, the biomass of \( \text{Lemna} \) increased up to 1.7 and 1.5 g in the control and the 0.5 mg/L Se treatments (both Se(IV) and Se(VI)), respectively. The fresh biomass in the 5.0 mg/L Se amendments remained unchanged during the whole incubation period. This indicated that Se toxicity (both Se(IV) and Se(VI)) stunts \( \text{Lemna} \) biomass growth, especially at 5.0 mg/L Se application. Similarly, Li et al. (2020) found that the dry weight of \( \text{Azolla cristata} \) decreased signiﬁcantly (from 100 mg to 80 mg) \((P < 0.01)\) when the Se(IV) exposure increased to 0.5 mg/L in the medium. The biomass of stem and leaves formed during Se exposure were markedly reduced in alfalfa (\( \text{Medicago sativa} \) cv.) exposed to 100 and 900 \( \mu \)M Se (\( \sim 7.9 \) mg/L and 71 mg/L) compared with the non-exposed controls (Dai & Jia 2017). Ohlbaum et al. (2018) found that 0.5 mg/L of
Se(VI) in Hoagland solution caused the death of approximately 5% of the exposed *Lemna minor* and *Egeria densa*.

**Effect of Se(IV) and Se(VI) on the *Lemna* root length**

Both low and high Se(IV) concentrations caused a significant inhibition in the root growth of *Lemna*, while the low Se(VI) exposure concentration did not show any notable effect, compared with the control (Figure 2). The root length of *Lemna* significantly increased along with the incubation time in the control and low Se exposure. During the 14 d cultivation period, the root length of *Lemna* exposed to 0.5 mg/L Se(IV) and Se(VI) increased by 1.2 cm and 2.1 cm, respectively, which indicated a slower root growth in the Se(IV) medium than in the Se(VI) medium. The growth of the *Lemna* roots stopped immediately when exposed to 5.0 mg/L of Se(IV), and after 3 d of incubation in the 5.0 mg/L Se(VI) medium. The slower growth rate of the roots under Se(IV) exposure confirms the higher Se(IV) toxicity to *Lemna*.

The root tolerance-index is the ratio of the root length of a treatment to that of the control (Equation (1)) and reflects the tolerance of plants to contaminants. The roots of plants are directly in contact with the contaminant, so the root tolerance-index is a sensitive indicator. *Lemna* tolerated higher concentrations of Se(VI) than those of Se(IV), which was also evidenced by the higher tolerance index in the Se(VI) treatments (Figure 3). Generally, the root tolerance-index declined with increasing exposure time to both Se(IV) and Se(VI) medium, which indicated that root growth under Se exposure becomes slower along with the incubation time.

The higher Se(IV) toxicity can be explained by the different transformation pathways of Se in plants. Se(IV) taken up by plants is easily converted into organic Se (e.g., SeMet and SeCys) and then misincorporated into proteins by replacing cysteine and methionine, resulting in the malformation of proteins and inactivation of enzymes (Sabbagh & Van...
Specifically, the amino acid cysteine is often found at the active site of enzymes and thus is involved in catalytic reactions. In addition, cysteine is essential for the formation of disulfide bridges, which have an important role in maintaining protein function and structure. Given cysteine's role in proteins, replacing cysteine with SeCys by misincorporating it into plant proteins could impair or misfold proteins, resulting in Se toxicity in plants (Sabbagh et al. 2015; Van Hoewyk 2020). This is thought to be the cause of Se toxicity in Lemna. Se(VI) transformation in plants is a slow and energy-consuming process (Van Hoewyk 2013), therefore Se(VI) taken up by Lemna most likely exists as an inorganic Se(VI) form (Li et al. 2020). Additionally, oxidative stress is another mechanism of Se toxicity (Van Hoewyk 2013). Several studies have shown the ability of some cell types to catalyze the bioconversion of SeMet to alternative forms capable of producing superoxides (Ponce et al. 2018). Thus, organic Se forms, such as SeMet, possibly present in Lemna exposed to Se(IV) in this study, may be metabolized to superoxides, resulting in a higher toxicity of Se(IV) than Se(VI).

Effect of Se(IV) and Se(VI) on the pigment content

The pigment content in plants gives an indication of the physiological changes after Se exposure. The content of pigments (chlorophyll α, chlorophyll β, phaeopigments α, and phaeopigments β) in Lemna calculated by Equations (2)–(5) decreased significantly with increasing time of exposure to 5.0 mg/L Se(IV) (Figure 4(a) and 4(b)). After 3 d of incubation, the pigment content in Lemna exposed to 5.0 mg/L of Se(IV) was two to three times less than that of the control, while no significant influence was observed in the first 3 d of growth at 0.5 mg/L Se(IV) exposure (Figure 4(a)). This indicated that the exposure to high Se(IV) concentration (5.0 mg/L) inhibited the synthesis of pigments. On the other hand, the Se(VI) amendment did not cause any notable inhibition of the pigment content of Lemna compared with the control, although the pigment content of all treatments, including the control, decreased slightly upon prolonging the incubation time (Figure 4(c) and 4(d)). These results further confirmed that Lemna tolerates Se(VI) better than Se(IV).

In this study, the lower pigment content in Lemna upon exposure to 5.0 mg/L of Se(IV) may be due to lipid peroxidation of the chloroplast membranes, resulting in cell damage and photosynthesis disruption. Studies have demonstrated the formation of reactive oxygen species (ROS) in plants under Se exposure, which is reflected in a higher malondialdehyde (MDA) and superoxide radical (O₂⁻) content in plants exposed to Se (Dai & Jia 2017). The produced ROS, including hydroxyl radicals (OH), superoxide radicals (O₂⁻) and hydrogen peroxide (H₂O₂), can cause oxidative damage to plant cell structures, cell membranes and photosynthetic pigments (Uruç Parlak & Demirezen Yilmaz 2020), which could lead to decreased pigment content. Similarly to the present study, Zhong & Cheng (2016) found that 40–80 μmol/L of Se(IV) (equivalent to 3.2–6.4 mg/L Se) decreases the carotenoid and chlorophyll content of the duckweed Landoltia punctata. Similarly, exposure of the duckweed Lemna minor to concentrations of Se(IV) exceeding 2 mg/L negatively affected photochemical efficiency as well as electron transport system activity (Mechora et al. 2015).

Effect of Se(IV) and Se(VI) on Se uptake

The Se content in Lemna increased as a function of the Se concentration in the medium, both for Se(IV) and Se(VI) exposure (Figure 5). Lemna accumulated between four and nine times more Se(IV) than Se(VI). For instance, after 1 d of incubation, the Se content in Lemna was 431 and 96 mg/kg at 5.0 mg/L Se(IV) and Se(VI) exposure, and 77 and 9 mg/kg at 0.5 mg/L Se(IV) and Se(VI) application, respectively. Lemna showed a rapid accumulation of Se during the first 3 d, followed by a slower accumulation phase. Specifically, under 5.0 mg/L Se(IV) and Se(VI) exposure, the Se content of Lemna increased, respectively, by 99.7% and 98.8% in the first 3 d of incubation compared with the beginning of the experiment, and by only 5.9% and 7.8% in the remaining 11 d of incubation, compared with the Se content at 3 d.
The higher and faster accumulation of Se(IV) than Se(VI) has also been observed in other plants. For instance, soybean (Glycine max) took up four times more Se(IV) than Se(VI) after 50 h of exposure to 5.0 μmol/L Se (equivalent to 0.4 mg/L Se) (Zhang et al. 2003) and tomato (Solanum lycopersicum L.) accumulated ten-fold more Se(IV) than Se(VI) in roots and shoots when exposed to concentrations of Se higher than 0.5 mg/L (Wang et al. 2013). The higher uptake of Se(IV) than Se(VI) can be explained by their different uptake mechanisms and metabolisms. Se(IV) is mostly taken up in a faster passive diffusive way and quickly converted into organic Se forms by plants (e.g., SeMet, SeCys, Me-SeCys and Se-methyl-selenocysteine (SeMet-SeCys)) (Arvy 1993; de Oliveira et al. 2017; Li et al. 2020). In contrast, Se(VI) is taken up in an active way through the facilitation of an S transporter and easily redistributed from roots to shoots (Arvy 1993; Li et al. 2008). Afterwards, Se(VI) is reduced to Se(IV) and then converted into organic Se compounds. Se(VI) reduction occurs via substitution of sulfate in the ATP sulfurylase reductase system, which is an ATP-consuming process and the rate-limiting step in the Se(VI) transformation (Van Hoewyk 2013). The Se(VI) reduction rate is thus much slower than its uptake rate, resulting in Se(VI) saturation and lower accumulation in the plant tissues (Li et al. 2020).

Additionally, the plant exposure to high Se concentrations resulted in a transmembrane potential gradient between the inside and outside of the cells. Thus, Se is transported across the cell membrane through ion channels and rapidly enters into the plant cells at the beginning of the exposure (Reid & Hayes 2003). This could partially explain the fast Se accumulation during the first 3 d of incubation. Likewise, studies have demonstrated that Se(VI) is taken up by plants via sulfate transporters through expression of SULTR1:2, whereas Se(IV) is transported by phosphate transporters present in the root plasma membranes (Li et al. 2008; Gupta & Gupta 2017). In this study, the fast Se accumulation during the first 3 d of incubation could be
partially attributed to a higher amount of transporters in the root plasma membrane at the beginning of the exposure phase, which could support the rapid transportation of Se from the surrounding medium into the plants. Accordingly, the depletion of specific transporters through the incubation time could result in a slower accumulation after 3 d. In addition, Se absorption by the plant could also partially contribute to the fast Se accumulation during the first 3 d of growth.

Table 1 | Kinetic parameters of bioaccumulation of Se in *Lemna* at different Se concentrations according to the fitting to the two-compartment and hyperbolic models

<table>
<thead>
<tr>
<th>Se dosage (mg/L)</th>
<th>Two compartment</th>
<th>Hyperbolic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>$K_a$</td>
</tr>
<tr>
<td>Se(IV) 0.5</td>
<td>0.93</td>
<td>263</td>
</tr>
<tr>
<td>Se(IV) 5.0</td>
<td>0.97</td>
<td>142</td>
</tr>
<tr>
<td>Se(VI) 0.5</td>
<td>0.83</td>
<td>8.0</td>
</tr>
<tr>
<td>Se(VI) 5.0</td>
<td>0.99</td>
<td>28</td>
</tr>
</tbody>
</table>

**Kinetic model of Se uptake by *Lemna minuta***

The time course of Se uptake and clearance in toxicokinetic studies were analyzed using the two-compartment model (Equation (6)) (Clason et al. 2004b). Selenium exposure was considered as the first compartment and *Lemna* as the second compartment. Likewise, a hyperbolic model (Equation (8)) was applied to analyze the Se uptake with time by *Lemna*. The estimated parameters of the two-compartment and hyperbolic models are shown in Table 1. Both models provided a good fit to the experimental Se uptake data at low and high Se concentrations. The coefficients of determination ($R^2$) for the fitting ranged from 0.85 to 0.97 ($P < 0.0001$) in both models, indicating that the two-compartment model and hyperbolic model can be used to estimate the Se uptake by *Lemna*.

According to the two-compartment model, both the uptake rate ($K_a$) and the $BCF$ (obtained by Equations (6) and (7)) of Se(IV) in *Lemna* were much higher compared with those of Se(VI) (Table 1). Additionally, the increase in Se concentration affected the uptake rate differently depending on the Se species. The uptake rate decreased from 263 to 142 mg/kg/d upon increasing the Se(IV) dosage from 0.5 to 5.0 mg/L, while it increased from 8 to 28 mg/kg/d with increasing Se(VI) concentration. In contrast, the $BCF$ decreased with increasing Se dosage both in Se(IV) and Se(VI) treatments. The $BCFs$ at the theoretical equilibrium were 125 and 251 for Se(IV), and 35 and 42 for Se(VI) at 5.0 and 0.5 mg/L Se dosage, respectively. Ohlbaum et al. (2018) also found that increasing the ambient Se concentration from 0.1 mg/L to 0.5 mg/L decreased the $BCF$ values in both *Lemna minor* and *Egeria densa*.

The higher values of the $BCF$ and $K_a$ in the Se(IV) treatment confirmed the ability of *Lemna* to accumulate Se(IV) in larger amounts and faster than Se(VI). This partially supports a faster passive uptake of Se(IV), but a slower active
uptake of Se(VI) by plants. The BCF is generally used to measure the capability of aquatic organisms to bioconcentrate pollutants. The kinetic Se BCFs in *Lemna* obtained in the present study are higher than in other plants (Table 1). For example, Dai & Jia (2017) studied the effect of Se on the growth, tolerance and antioxidative system of three alfalfa cultivars and evidenced that the maximum BCF of alfalfa is 15.4 at 900 μmol/L Se(VI) (equivalent to 63 mg/L Se). It should be noted that there are two different approaches to calculating the BCF: (1) using the ratio of $K_a$ and $K_b$ from kinetic data not assuming that an equilibrium has been reached during the experiment; or (2) using the ratio of the element concentration in the plants and the exposure concentration assuming that an equilibrium has been reached (Clason et al. 2003). The second method is mostly applied to quantify the BCF value, resulting in an inaccurate estimation of the accumulation ability of organisms, as the equilibrium state is difficult to reach or takes a long time (Dai & Jia 2017; Ohlbaum et al. 2018). Although some studies have applied the toxicokinetic model to evaluate the kinetic accumulation and BCF of heavy metals, such as Cd, Pb, Cu and Zn, there has been no investigation of Se kinetic accumulation and BCF evaluation by *Lemna* yet.

The maximum Se content in *Lemna* ($C_{max}$) at the theoretic equilibrium and the time to reach half of the $C_{max}$ ($t_{max/2}$) were obtained from the hyperbolic model. Specifically, the $C_{max}$ was 123 and 670 mg/kg for the 0.5 and 5.0 mg/L Se(IV) application, and 26 and 196 mg/kg for the Se(VI) exposure, respectively (Table 1).

The suitability of bioaccumulation models is influenced by the involvement of intracellular element-handling mechanisms in organisms (Clason et al. 2004b). In this study, Se accumulation with increasing exposure time followed the saturation curve for both Se species (Figure 5, Table 1), instead of the linear first-order kinetic model. This indicated that both Se(IV) and Se(VI) uptake by *Lemna* are not only through passive diffusion, but also through other processes such as ion channel proteins or transporters (Clason et al. 2004b). This is in agreement with other studies, which showed that the Se(VI) uptake mechanism is an active process, facilitated by energy and S transporters (Arvy 1995; de Oliveira et al. 2017; Wang et al. 2019), which has been verified by studying the effects of respiratory inhibitors, hydroxylamine and sulfur on Se uptake by plants (Arvy 1995; Li et al. 2008). However, the mechanism of Se(IV) uptake by plants remains unclear. Some researchers reported that Se(IV) uptake is mainly controlled by passive diffusion (Arvy 1995; de Oliveira et al. 2017). Wang & Dei (1999) studied the kinetics of metal accumulation (Cd, Cr, Se and Zn) in two macroalgae species and verified that metal uptake follows a linear pattern only over a 2 d exposure period, indicating that metal uptake proceeded by passive diffusion. Li et al. (2008) studied Se(IV) uptake in phosphorus-starved plants and concluded that Se(IV) uptake is an active process likely mediated, at least partly, by phosphate transporters. This partially supports the findings in the present study. Yet, many studies have been investigating the mechanism of Se uptake by organisms through biochemical or genetic methods (El Mehdawi et al. 2018), while only a few studies have validated the Se uptake mechanism by applying toxicokinetic models.

Additionally, together with the experimental data in Figure 5 and the $t_{max/2}$ from the hyperbolic model (Table 1), the Se concentration in *Lemna* apparently reached a relatively steady state after 6 d of exposure to both Se species, which could give a reference for other studies to choose the equilibrium time before implementing a hydroponic experiment in an Se-spiked medium or provide the information for some other similar systems to design the size, flow rate and retention time.

**CONCLUSIONS**

This study demonstrated that *Lemna* has a higher tolerance of Se(VI) compared with Se(IV), as shown by the lower root tolerance-index of *Lemna* upon Se(IV) exposure and the decrease of pigment content upon 5.0 mg/L Se(IV) exposure. The content of Se in *Lemna* increased with increasing Se exposure concentration for both types of Se oxyanions. The Se content in *Lemna* reached a steady state after 6 d of exposure. The accumulation of Se by *Lemna* could be modeled by both the two-compartment model and the hyperbolic kinetic model, indicating that the uptake of both Se species by *Lemna* is controlled by complex processes. The higher BCFs at theoretical equilibrium and faster uptake rates ($K_a$) obtained with the two-compartment model for the Se(IV) treatment evidenced that *Lemna* rapidly takes up and accumulates Se(IV). These results indicate that *Lemna* could potentially be used as a phytoextraction plant to remove Se(IV) from wastewater, which may eventually also lead to the production of Se-enriched crop fertilizers or protein-rich food/feed supplements.
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