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Doctoral Dissertation

Selection of duckweed species for efficient
phytoremediation of selenium-contaminated aquatic
environments

(セレン汚染水環境の効率的なファイトレメディエ
ーションに適用するウキクサ種の選定)

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Chapter 1: Introduction

1.1. Selenium (Se)

Se is an element often found in trace concentrations in natural aquatic environments (Conde and Sanz Alaejos, 1997). Se plays an important role particularly in aquatic ecosystems aiding at metabolic and physiological functions of fish, animals, and plants. Specifically, low concentrations of Se in the form of selenoproteins serve as a micronutrient and antioxidant required in immune functions, redox homeostasis, reproduction, and thyroid hormone production in humans and animals (Au et al., 2023; Tinggi and Perkins, 2022; Xia et al., 2021). In plants, Se has been suggested to promote photosynthetic activity, growth, and protein content (Saleem and Fariduddin, 2022). In addition, Se possesses broad applicability in various agricultural and industrial processes in the medical, electronic, and manufacturing sectors (Tan et al., 2016). Its nutritional and industrial significance has led to a notable increase in the global demand for Se from 2300 tons per year in 1999 to 3600 tons in 2023 (Haug et al., 2007; U.S. Geological Survey, 2024).

The beneficial role of Se in animals and humans, however, is dependent on its concentration. In humans, the recommended limit is below a threshold of 400 $\mu\text{g/day}$ (Fordyce, 2013), whereas the provisional guideline value for Se in drinking water sources set by the World Health Organization (WHO) is 40 $\mu\text{g/L}$ (World Health Organization, 2022). Above these levels, Se reportedly acts as an oxidant that promotes the release of reactive oxygen species affecting the normal metabolism of flora, fauna, and humans. For example, in humans, Se toxicity manifests as selenosis, heart disease, skin discoloration, hair loss, vomiting, diarrhea, and muscle spasms (Bai et al., 2025; Hira et al., 2004;

Lombeck et al., 1987), whereas in animals, Se toxicity has been shown to produce behavioral alterations and impaired growth and reproduction, which has been connected to the disappearance of fish species (Lemly, 2004; Uddin et al., 2024). Furthermore, in terrestrial and aquatic plants, exposure to Se levels > 2 mg-Se/L can cause oxidative stress producing chlorosis and cell damage (Mechora et al., 2015; Saleem and Fariduddin, 2022).

1.2. Pollution of aquatic environments by Se

Se exists in natural environments in four different oxidation states: selenide (Se(-II)), elemental selenium (Se(0)), selenite [Se(IV); SeO_3^{2-}], and selenate [Se(VI); SeO_4^{2-}]. Among them, Se(IV) and Se(VI) are the forms of primary concern due to their high mobility and solubility, exhibiting potential for bioaccumulation, biomagnification, and toxicity to aquatic life and humans (Khamkhash et al., 2017; Li et al., 2024; Stewart et al., 2010).

Aquatic Se pollution arises from the mobilization of naturally occurring Se from rocks, soils, and minerals through both natural and anthropogenic processes, as well as from poorly-treated gaseous and wastewater emissions. Among anthropogenic sources, coal and metal mining, industrial activities, and agriculture have been reported as predominant contributors in different aquatic environments (El-Ramady et al., 2014; Saha et al., 2017; Werkneh et al., 2023). As aquatic Se pollution is a recognized global environmental problem (Lemly, 2004), the implementation of effective remediation technologies is urgently needed.

1.3. Current technologies for soluble Se removal

Although various remediation or mitigation technologies for Se contamination have been extensively explored, ensuring their applicability to various water/wastewater compositions remains challenging due to limitations in economic feasibility and removal efficiency (He et al., 2018; Li et al., 2022; Lichtfouse et al., 2021, 2022). Furthermore, the majority of reported studies remain at the laboratory or pilot scale, focusing mainly on wastewater treatment rather than the remediation of contaminated aquatic environments.

Chemical reduction is a technique where soluble Se(VI) and Se(IV) are reduced to Se(0) and Se(-II) typically using a reductant like zero-valent iron, which is considered economically favorable due to the ready availability and ease to handling of elemental iron. However, several challenges arise during the reduction process, including quick cementation of the iron filings, pH and temperature dependence, long hydraulic retention time, and high costs associated with the sludge disposal (Guan et al., 2015).

The electrocoagulation process involves the electrochemical oxidation of a sacrificial anode to release metal ions into the water. These ions hydrolyze to form highly reactive species and metal hydroxides, which act as coagulants like flocs that destabilize contaminants including Se facilitating their removal (Aryanti et al., 2024). Despite showing high Se removal efficiencies when coupled with microfiltration in treating wastewater (Mavrov et al., 2006), economic feasibility, driven mainly by the consumption of energy and replacement of materials, limits its utilization (Aryanti et al., 2024).

Adsorption is a fast and reversible process used for contaminant removal by binding of pollutants to material surface commonly known as the adsorbent (Ike et al., 2017). Common adsorbents include organic synthetic resins, oxides and minerals, carbon-based

adsorbents, waste materials, and biosorbents. Despite their considerable efficiencies for Se removal (Gezer et al., 2011; You et al., 2001), conventional adsorbents such as synthetic resins are limited by high production costs, poor selectivity in complex matrices, and issues with degradation, and reusability (Satyam and Patra, 2024). In contrast, biosorbents seemingly offer a sustainable alternative for Se removal. Although known biosorbents have certain limitations, such as poor mechanical strength and low rigidity (Ramrakhiani et al., 2016), they provide several advantages, including cost-effectiveness, high removal efficiency, ease of implementation, and potential reuse in fuel or energy production (Dhabhai et al., 2018).

Biological treatment and remediation involve the use of microorganisms (e.g. bacteria) and plants to reduce, stabilize or uptake soluble Se species. Although microbial reduction of soluble Se species to less soluble Se(0) has been widely explored in wastewater treatment, its efficiency is limited, particularly due to the need for optimization of parameters such as pH and salinity, as well as the addition of a carbon source (Soda et al., 2011). In contrast, phytoremediation (e.g. in constructed wetlands (CWs)) emerges as a promising nature-based process for Se remediation. For instance, Zhao et al. (2020) evaluated the potential of a CW system operated with a 2-day wet/dry cycle, finding that Se removal efficiency exceeded 98%. Despite some limitations such as the need of long treatment time and influence of environmental conditions (Kuppan et al., 2024), phytoremediation is considered a cost-efficient and eco-friendly alternative for Se cleanup that attracts significant scientific interest (Kafle et al., 2022).

Although several technologies have been developed for Se removal as discussed above, with the exception of phytoremediation, their performance and applicability in the remediation of contaminated aquatic environments remain largely uncertain.

1.4. Duckweed as a remedial agent for Se pollution

The Lemnaceae family, commonly known as duckweeds, consists of 36 species of free-floating aquatic macrophytes distributed among five genera: *Spirodela*, *Landoltia*, *Lemna*, *Wolffiella*, and *Wolffia*. Duckweeds are characterized by high growth rates (Said et al., 1979), making them promising phytoremediation agents. Their utility is further enhanced by their global distribution (Wang et al., 2010), which ensures a local and stable supply, and their ease of cropping (Gupta and Prakash, 2013). In addition, their high-value biomass, rich in protein and/or starch content, suggests its potential for reuse after phytoremediation as animal feed, fertilizer, and feedstock for the production of biogas and bioethanol (Baek et al., 2021; Xu et al., 2023). In fact, duckweeds are recognized for their ability to remediate aquatic environments contaminated with excess nutrients, organics, and heavy metals (Baek et al., 2021; Mkandawire and Dudel, 2007; Zhao et al., 2017). Further, their live biomass has been utilized as biosorbents for removing microplastics and industrial dyes (Can-Terzi et al., 2021; Dovidat et al., 2020), which can be also regarded as a form of phytoremediation.

For Se, some studies on the phytoremediation using duckweeds have confirmed their potential to remove soluble Se, Se(VI), and Se(IV), though there has been no report on the use of live duckweeds as the biosorbent for Se removal. For instance, *Lemna minor* (Lm) used in a CW removed Se(VI) with removal efficiencies > 75% (Nattrass et al., 2022), and accumulated up to 5.8 mg-Se/g when exposed to Se(IV) in a synthetic medium (Mechora et al., 2015). Similarly, *Landoltia punctata* (LAp) demonstrated a superior uptake capacity of soluble Se (approximately 30%) in mining wastewater compared to other aquatic plants (Miranda et al., 2014).

1.5. Objectives of this study

There is a necessity to develop effective and sustainable remediation technologies for Se pollution in aquatic environments, especially those that can provide low-cost operation, environmental-friendliness, and the potential for Se resource recovery. While phytoremediation using duckweeds seems a promising alternative for Se removal, there is still limited knowledge on this approach, including their use as biosorbent, and its optimization is essential. The objective of this study is to select and evaluate effective duckweed species for the removal of soluble Se at typical environmentally relevant concentrations as the most fundamental study. Investigations were conducted to assess their performance both as a biosorbent in short-term experiments and as a sustainable phytoremediation agent in long-term experiments.

This introductory chapter (Chapter 1) provided the context for this study. It detailed the dual beneficial and toxic properties of Se, examined its potential pollution sources in aquatic systems, and reviewed conventional technologies for soluble Se removal. In addition, based on the review of the potential of duckweed as a Se phytoremediation agent, the objectives of this study were established as mentioned in the previous paragraph.

Chapter 2 presents a systematic review detailing the current status of Se contamination. This comprehensive review identified typical pollution sources and current concentration levels of Se in global aquatic environments. These findings were important to define the environmentally relevant testing ranges for the removal experiments conducted in subsequent chapters.

Chapter 3 focused on the short-term removal of soluble Se using six live duckweed species as biosorbents in batch experiments. The duckweed *Lemna gibba* (Lg) was selected as the most efficient Se biosorbent, and its adsorption capacity for Se(VI) and

Se(IV) was characterized for comparison with other typical Se adsorbents. In addition, the potential for valorization through the reuse of waste biomass and the adsorbed Se was discussed.

In Chapter 4, duckweeds used in Chapter 3 were assessed in long-term tests for their growth capacity and healthy status under various Se(IV) or Se(VI) concentrations to identify the most tolerant duckweed species. LAp and *Lemna aequinoctialis* (La) were selected as promising duckweeds for sustainable Se removal, thus their removal capacities were evaluated. Their Se uptake was also quantified to determine their potential for valorization as Se-enriched biomass.

Chapter 5 synthesizes the major findings and conclusions of this study, assessing the viability of duckweed as a short-term and long-term phytoremediation agent for soluble Se removal. Furthermore, the chapter outlines future research directions, emphasizing the necessity for testing duckweed performance under real-world water matrices and complex environmental conditions for its practical implementation as a suitable water remediation technology.

Chapter 2: Review of current status of Se pollution in aquatic environments

2.1. Introduction

This chapter presents a structured literature review of literatures published between 2015 and 2024 to identify global Se sources and contamination levels in the aquatic environment, namely surface water and groundwater. The review will provide essential insights for addressing and mitigating Se pollution, as well as to determine environmentally relevant concentration ranges for the removal studies presented in subsequent chapters.

2.2. Screening and analysis of literatures

The review in this chapter applies a methodology that comprises systematic review principles, analytical guidelines, and technical tools to analyze the existing literatures on Se contamination in aquatic environments. It follows some of the guidelines suggested by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (Page et al., 2021), which were divided into three stages (Figure 2-1).

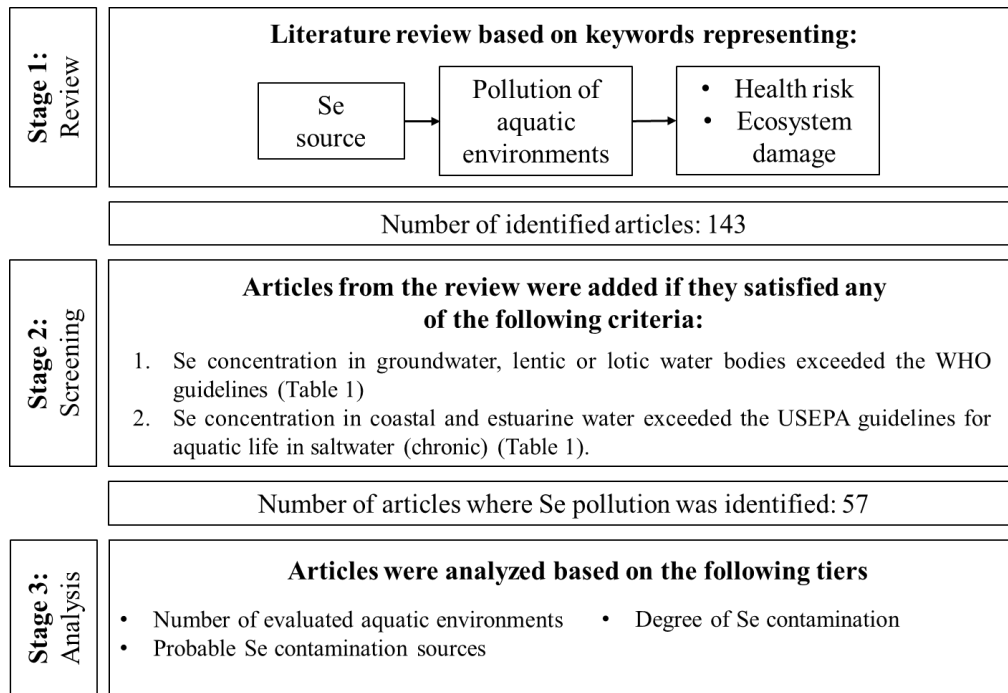


Figure 2-1 Design of the systematic review conducted in this chapter (adapted from Javan et al. (2025)).

In stage 1, the Scopus and Google Scholar databases covering the years 2015 – 2024 were explored between 2023 and 2024, to identify recent Se contamination cases. The keywords used in the search included “selenium”, “water”, “pollution”, “contamination”, heavy metals”, “surface water”, “groundwater”, “estuarine water”, and “health.” The obtained literatures included scientific articles and reports, which were reviewed for the occurrence of Se in aquatic environments. In total, 143 articles containing Se concentration data were considered for the screening stage.

In the screening stage, the 143 articles were evaluated to determine whether they describe significant Se contamination occurrence in the area of study. Specifically, an area was classified as contaminated if the maximum reported Se concentration exceeded the guideline values established by the WHO for freshwater ecosystems or the United States Environmental Protection Agency (USEPA) for coastal and estuarine waters. The

guideline concentrations used for reference screening are listed in Table 2-1. The screening stage yielded 57 articles where significant Se contamination was detected in aquatic environments.

Table 2-1 Se environmental standards for drinking water and aquatic life used for screening during the literature review in this study.

	Se guideline value (µg/L)	Target water bodies	Reference of the Se guideline values
WHO provisional guideline value of Se in drinking water	40	Groundwater Lotic Lentic	World Health Organization (2022)
USEPA guidelines for aquatic life in saltwater (chronic)	71	Coastal and estuarine	Environmental Protection Agency (1999)

The analysis stage involved a detailed examination of the 57 articles to identify the number of investigated water bodies, Se contamination level, and possible pollution sources. The evaluation of contamination occurrence only considered the maximum concentration of Se. Studies with spatial or temporal variability within the same water body were classified as polluted based on the maximum reported concentration or the most recent data, respectively. The concentration metrics for Se varied across the studies reviewed in this chapter. Some reports exhibited total concentrations, while others quantified only the dissolved fraction, thus, care should be taken during comparison and data aggregation.

2.3. Se contamination sources

Possible Se contamination sources were identified in most literatures as shown in Table 2-2. In Table 2-2, the pollution sources were categorized as “natural”, “anthropogenic”, “natural and anthropogenic”, and “unspecified” based on the description of the area of study. Consistent with Section 1.2, among anthropogenic sources, three subcategories were established as “mining activities”, “industrial activities”, and “agricultural activities”. For water bodies with multiple anthropogenic pollution sources, each source was counted individually.

Table 2-2 Summary of sources of Se contamination in groundwater and surface water.

Source	Groundwater	Surface water			Total cases per source
		Lentic	Lotic	Estuarine and coastal	
Natural	9 (19.6%)	1 (6.7%)	N.R.	N.R.	10 (10.5%)
Anthropogenic	Mining	20 (43.5%)	6 (40%)	9 (30%)	35 (36.8%)
	Industry	8 (17.4%)	4 (26.7%)	10 (33.3%)	22 (23.2%)
	Agriculture	4 (8.7%)	2 (13.3%)	10 (33.3%)	16 (16.8%)
Natural and anthropogenic	3 (6.5%)	1 (6.7%)	1 (3.3%)	N.R.	5 (5.3%)
Unspecified	2 (4.3%)	1 (6.7%)	N.R.	4	7 (7.4%)
Total cases per water body	46	15	30	4	95

Percentages indicate the ratio of reported cases in each water body or in the total count.

N.R., not reported

2.3.1. Natural sources

Se pollution originating from natural sources were identified in approximately 10% of the reported contamination cases, primarily in groundwater, while 5% were linked to

both natural and anthropogenic origins (Table 2-2). Although not consistently reported, several significant sources of natural Se pollution were identified as geological formations and seawater intrusion.

2.3.1.1. Geological formations

Se naturally occurs in various geological formations, including sedimentary rocks such as black shales, coals, and phosphatic deposits associated with sulfide minerals. Upon weathering, Se is primarily mobilized as the water-soluble species: Se(IV) and Se(VI) (Fordyce, 2013; Mehdi et al., 2013), which have high potential for migration through infiltration and runoff into aquatic environments. For instance, the oxidation of sulfides and weathering of sulfate-evaporites such as gypsum and anhydrite have been reported as the main cause of mobilization of Se causing the contamination of groundwater in Mexico (Mora et al., 2021), whereas Se pollution originating from Mancos Shale weathering, efflorescent salt dissolution, and redox processes has been reported in the United States of America (USA) (Linard et al., 2017). In addition, Se-containing sediments and rocks have been attributed as the main causes of groundwater pollution in India (Kumar et al., 2020). Similarly, natural pollution in surface water was reported to originate from weathering of carbonate rocks in China (Luo et al., 2024).

Weathering of parent rocks is also a primary factor in the formation of seleniferous soils (Saha et al., 2017). Seleniferous soils are abundant in the USA, Russia, India, and China where soils have reportedly exhibited elevated Se levels (Kieliszek, 2019; Saha et al., 2017). In India, mobilization from such soils has been reported to elevate Se concentration in groundwater (Lapworth et al., 2017). Although not clearly reported in the reviewed literature, it should be noted that depositions from eruptions and volcanic

plumes typically enrich Se in volcanic soils (Fordyce, 2013; Saha et al., 2017), which may be another considerable source of Se contamination in the aquatic environment.

2.3.1.2. Seawater intrusion

Seawater intrusion is a natural process caused by the density difference between seawater and freshwater, which leads to the natural inland movement of seawater until equilibrium is achieved. This can be enhanced by anthropogenic activities such as groundwater overpumping and climate change (Tiwari et al., 2024). Seawater intrusion has been suggested to be an important mechanism of Se contamination in coastal aquifers in Djibouti, India, and Saudi Arabia (Ahmed et al., 2017; Alshehri et al., 2021; Kumar et al., 2024). Specifically, Se contamination during seawater intrusion into groundwater results from increased ionic competitions, which leads to ion exchange processes that enhance the desorption of Se(VI) and Se(IV) from carbonate and sodium-magnesium-sulfate minerals (Chen et al., 2019; Mora et al., 2020).

2.3.2. Anthropogenic sources

As shown in Table 2-2, most reported Se pollution in surface and groundwater can be attributed to anthropogenic activities. Nearly 77% of the total excess Se were linked to anthropogenic sources, although the contribution of each source subcategory as follows varied across different water bodies.

2.3.2.1. Mining activities

Mining brings Se-enriched deposits to the surface (USEPA, 2018) and account for

approximately 37% of reported pollution cases, representing the highest occurrence among the anthropogenic sources evaluated in this review (Table 2-2). This observation highlights their predominant role in Se pollution of both surface water and groundwater.

Se is mainly recovered as a by-product of copper (Cu) mining, which, consequently, can lead to elevated Se concentrations in mine waste, as observed in the Iberian Pyrite Belt, posing a potential risk of Se pollution to aquatic environments (Figueiredo et al., 2014; Haug et al., 2007). In addition, Cu mining is a primary contamination source of surface water and groundwater in India and Iran (Kazemi et al., 2022; Prasad et al., 2020). Other Se-containing mine waste such as dzharkenite and Se-substituted pyrite in a phosphate patch in the USA have been reported to generate leachate from backfilled pits and overburden storage piles causing Se to leach into groundwater (Hay et al., 2016; Ryser et al., 2005). Other metal mining sources of Se pollution included uranium mining in Mongolia (Ariunbileg et al., 2016), gold mining in Cameroon (Mambou-Ngueyep et al., 2021), and salt and lead-zinc mining in Nigeria, where environmental pollution is worse due to illegal mining (Egbueri and Enyigwe, 2020; Obasi et al., 2024; Obasi and Akudinobi, 2020). Notably, abandoned mines represent important Se pollution sources as demonstrated in surface waters in China (Jiao et al., 2023), although active mines present a higher risk (Obasi and Akudinobi, 2020).

Coal mining was identified as another significant source of Se pollution. Elevated Se content in coal has increased the hazardous risk to the aquatic environment in several regions in China and Canada (Lemly, 2009; Wang et al., 2010). Particularly, in Elk Valley, Canada, Se is present in coal waste dumps with various metals, including pyrite, sphalerite, and chalcopyrite, which could be responsible for surface water pollution at concentrations exceeding ecological safety thresholds (Hendry et al., 2015; Teck Coal

Limited, 2023). Similarly, a closed coal mine in Canada was shown to be a continuous Se pollution source in a lake below the mine spoil pile (Cooke et al., 2024). In India, Se contamination of groundwater was attributed to open-cast coal mining (Prathap and Chakraborty, 2019).

2.3.2.2. Industrial activities

Although Se pollution from industrial activities is widely mentioned in previous studies and is considered the second major anthropogenic source of Se pollution (Table 2-2), specific sources were scarcely or not clearly identified. However, some potential sources of Se pollution such as metal smelting, the semiconductor industry, and oil-refining and coal-fired plants (Lichtfouse et al., 2021) are considered contamination sources via emission of gaseous Se into atmosphere and Se-containing wastewater.

Metallurgical activities, specifically metal smelters, are recognized as major Se pollution sources (Maret and Skinner, 2000), as Se is a byproduct of metal smelting. The smelting processes mobilize Se into the atmosphere, which can settle on surface waters (Dudka and Adriano, 1997; USEPA, 2018). In addition, during the smelting process, a significant proportion of Se is lost in the slag, which, when discarded, can increase Se concentrations in the waste effluent (Desai et al., 2016; Kenzhaliyev et al., 2018).

Se is considered a useful raw material for semiconductor production in several industrial applications such as solar cells and X-ray flat-panel detectors (Liu et al., 2024; Qi et al., 2022). The semiconductor industry has generated significant amounts of Se-containing wastewater, likely increasing the risk to the environment (Gilles and Loehr, 1994).

Se pollution from oil refining occurs primarily through the discharge of Se-laden

wastewater, a byproduct of the refining process (Wang et al., 2024). Throughout the refining process, Se is introduced into wastewater in various chemical forms that could cause environmental concerns (Staicu et al., 2017). This issue is particularly prominent in San Francisco Bay, where Se pollution from oil refining has been confirmed (Hansen et al., 1998).

The high Se emissions from coal-fired power plants could also pose a threat to aquatic ecosystems (Brandt et al., 2017; Tian et al., 2011). Although volatilized Se generated in the coal-fired plants is captured in flue gas treatment systems, significant amounts of Se could enter wastewater streams (Ma et al., 2022).

2.3.2.3. Agricultural activities

Seleniferous agricultural drainage wastewater is a major source of Se pollution worldwide (Kharaka et al., 1996), whereas the number of pollution reports in aquatic environments from agricultural activities were less than those of mining and industrial activities in the literatures (Table 2-2).

Since Se is considered an essential nutrient for plants and helps mitigate heavy metal toxicity and against pests, it is often applied in fertilizer, which could lead to Se contamination in aquatic environments (Feng et al., 2021; Mechora, 2019; Saleem and Fariduddin, 2022). In Sri Lanka, fertilizers heavily applied for tea and vegetable cultivation are the main source of Se contamination in tributaries of the Mahaweli River (Diyabalanage et al., 2016). Other mechanisms involve the mobilization of Se from seleniferous soils through agricultural irrigation for crop production (Linard et al., 2017) and the exposure of Se-bearing sedimentary deposits caused by the construction of open drainage channels in agricultural areas, which have been implicated in surface water

contamination in the USA (USEPA, 2018).

2.4. Se contamination levels

It is crucial to understand the extent and origin of Se pollution in aquatic environments for effective remediation, particularly through selecting the appropriate cleanup technique and implementing appropriate prevention and mitigation strategies. The single-factor pollution index (P_i) was used to categorize Se environmental contamination levels reported in the literatures (Dey et al., 2021). P_i was calculated using the following Eq (2.1)

$$P_i = \frac{C_{max}}{C_{standard}} \quad \text{Eq (2.1)}$$

where C_{max} is the measured maximum Se concentration and $C_{standard}$ is the Se environmental standard criterion (Table 2-1). Herein, pollution levels in freshwater sources were categorized based on P_i and corresponding C_{max} values as: $1 \leq P_i \leq 2.5$ ($40 \mu\text{g/L} \leq C_{max} \leq 100 \mu\text{g/L}$), which indicated low pollution level; $2.5 < P_i \leq 25$ ($100 \mu\text{g/L} < C_{max} \leq 1000 \mu\text{g/L}$), which indicated moderate pollution level; and $P_i > 25$ ($C_{max} > 1000 \mu\text{g/L}$), which indicated high pollution level. For estuarine and coastal waters, the same P_i categories were applied, but with different corresponding C_{max} values based on their specific standard criterion (Table 2-1): $71 \leq C_{max} \leq 177.5 \mu\text{g/L}$ for low pollution level; $177.5 < C_{max} \leq 1775 \mu\text{g/L}$ for moderate pollution level; and $C_{max} > 1775 \mu\text{g/L}$ for high pollution level.

Se pollution levels varied among source types, water bodies, and geographical regions (Figures 2-2 – 2-4). Analysis of the entire dataset revealed that moderate Se pollution levels ($100 - 1000 \mu\text{g/L}$) were more frequently found than either high or low Se pollution

levels in all water bodies except estuarine and coastal waters. This suggests that global cleanup and remediation effort, particularly in drinking water sources, should prioritize Se concentrations within this range. Furthermore, anthropogenic sources reportedly generated all levels of Se pollution (from low to high), while natural sources mainly caused moderate and low pollution levels.

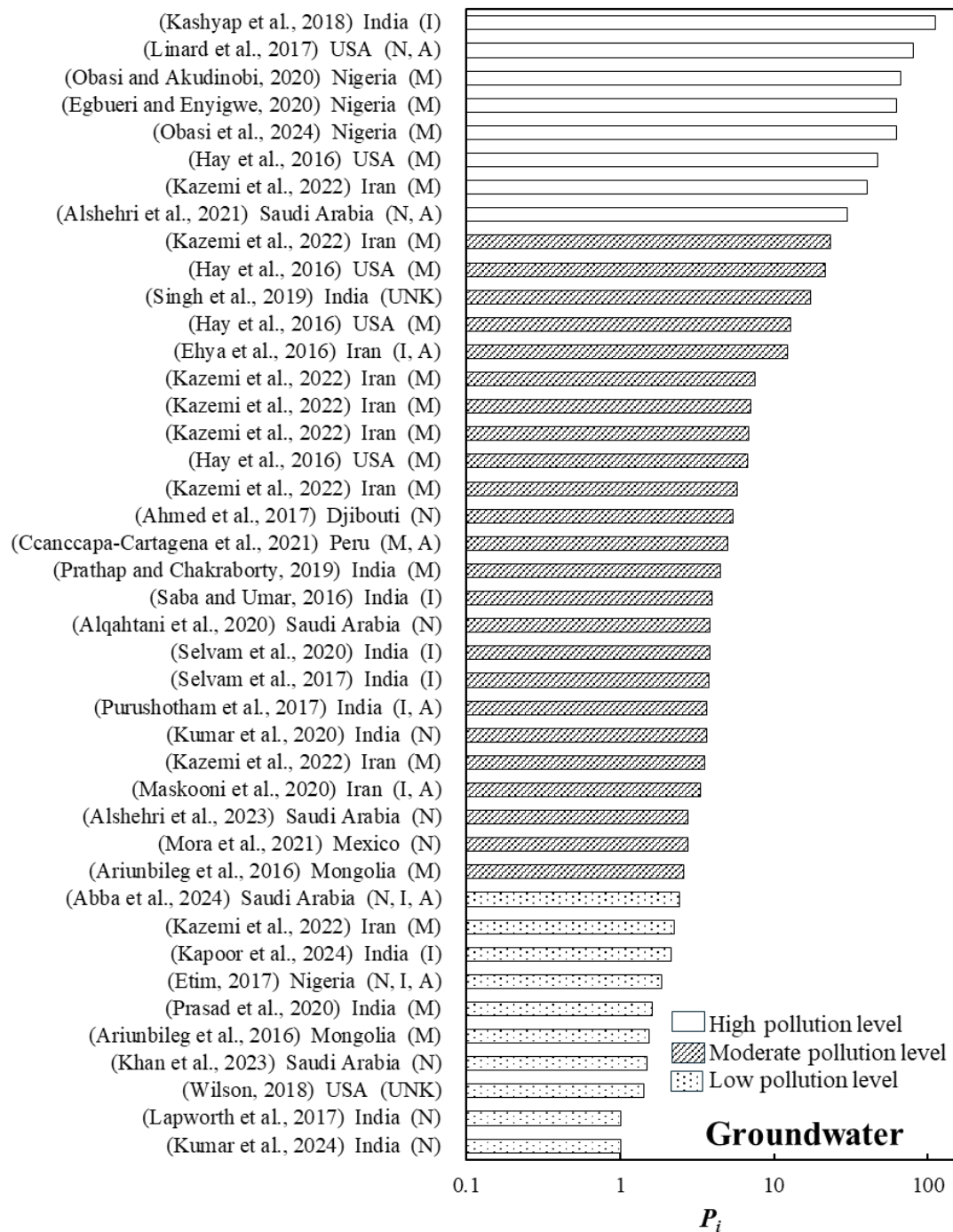


Figure 2-2 Se pollution indices calculated for groundwater worldwide. Letters in parentheses indicate the possible source of origin. N, natural; A, agriculture; M, mining; I, industrial activities; UNK, unknown. The data is shown in order of highest to lowest contamination levels.

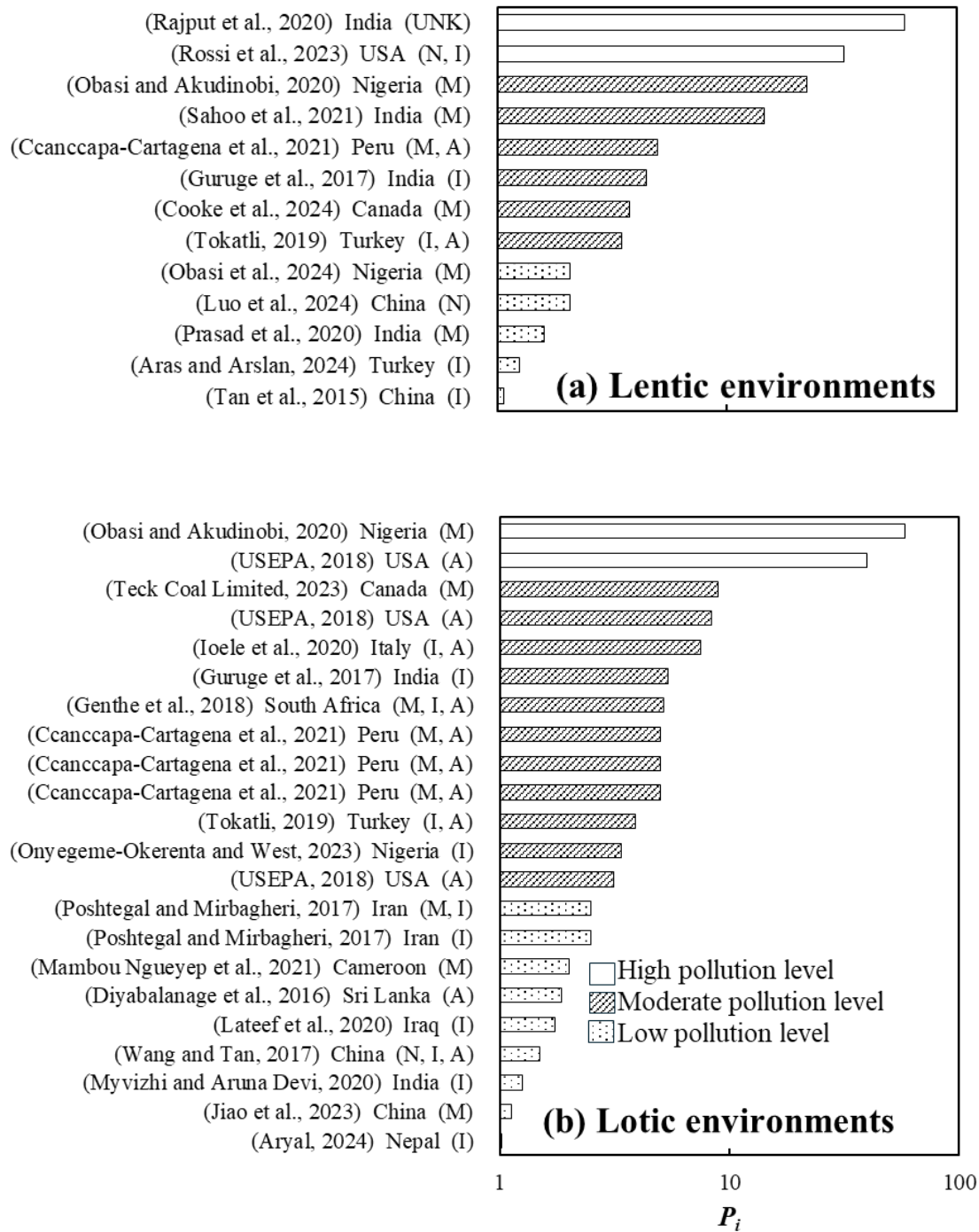


Figure 2-3 Se pollution indices calculated for freshwater sources worldwide. (a) Lentic environments and (b) lotic environments. Letters in parentheses indicate the possible source of origin. N, natural; A, agriculture; M, mining; I, industrial activities; UNK, unknown. The data is shown in order of highest to lowest contamination levels.

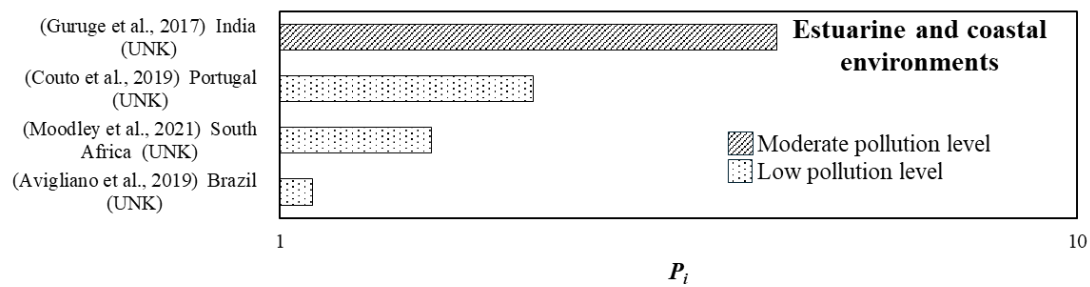


Figure 2-4 Se pollution indices calculated for estuarine and coastal water worldwide. Letters in parentheses indicate the source of origin. UNK, unknown. The data is shown in order of highest to lowest contamination levels.

2.4.1. Groundwater

The Se contamination cases in groundwater were illustrated in Figure 2-2 to compare the contamination levels with their respective source. Among 42 pollution cases observed in groundwater, eight cases (19%) exhibited high pollution levels ($>1000 \mu\text{g/L}$), and were predominantly attributed to mining activities (Egbueri and Enyigwe, 2020; Hay et al., 2016; Kazemi et al., 2022; Obasi et al., 2024; Obasi and Akudinobi, 2020). Other, less frequently reported sources of high pollution levels include industrial discharges, which produced Se concentrations up to $4475 \mu\text{g/L}$ (Kashyap et al., 2018), and a combination of geogenic and agricultural processes, which generated concentrations as high as $3220 \mu\text{g/L}$ (Alshehri et al., 2021; Linard et al., 2017). Overall, these findings suggest mining as the predominant source of high Se pollution in groundwater.

Similarly, moderate Se pollution levels in groundwater ($100 - 1000 \mu\text{g/L}$) were predominantly linked to mining activities (Ccanccapa-Cartagena et al., 2021; Hay et al., 2016; Kazemi et al., 2022; Prathap and Chakraborty, 2019), accounting for 42% of the reported moderate cases. Industrial and agricultural sources contributed to moderate

pollution levels to a lower degree (Ccanccapa-Cartagena et al., 2021; Ehya and Marbouti, 2016; Maskooni et al., 2020; Purushotham et al., 2017; Saba and Umar, 2016; Selvam et al., 2017, 2020). In contrast, moderate pollution from solely natural sources was observed in only five sites (Ahmed et al., 2017; Alqahtani et al., 2020; Alshehri and Zaidi, 2023; Kumar et al., 2020; Mora et al., 2021), whereas one pollution case was generated from an unknown source (Singh et al., 2019).

The number of low-level pollution cases ($<100\text{ }\mu\text{g/L}$) were comparable to that of high-level cases in terms of the contribution of anthropogenic and natural sources. As observed in high and moderate pollution cases, mining activities were the predominant cause of low-level groundwater pollution (Ariunbileg et al., 2016; Kazemi et al., 2022; Prasad et al., 2020). In addition, geogenic, agricultural, and industrial processes, either individually or in combination, generated low-level Se pollution (Abba et al., 2024; Etim, 2017; Kapoor et al., 2024; Khan et al., 2023). One case had Se-contamination of $57\text{ }\mu\text{g/L}$ from an unknown source (Wilson, 2018), whereas in other two cases, Se was enriched in groundwater up to $40\text{ }\mu\text{g/L}$ from natural sources, reaching the environmental standard limits (Kumar et al., 2024; Lapworth et al., 2017).

2.4.2. Surface water

Se contamination in surface water was analyzed across freshwater (lentic and lotic) (Figure 2-3) and estuarine and coastal waters (Figure 2-4). Reports of high Se pollution ($>1000\text{ }\mu\text{g/L}$) in surface water were less frequent than those in groundwater. For example, high pollution cases in lentic waters generated from an unknown source, and natural and industrial activities, reached Se concentrations of $2383\text{ }\mu\text{g/L}$ (Rajput et al., 2020) and $1300\text{ }\mu\text{g/L}$ (Rossi et al., 2023), respectively (Figure 2-3a). For lotic waters, mining and

agricultural processes generated Se concentrations of 2330 µg/L (Obasi and Akudinobi, 2020) and 1591 µg/L (USEPA, 2018), respectively (Figure 2-3b).

Primary sources of moderate pollution (100 – 1000 µg/L) varied among lentic and lotic environments. Mining activities alone (Cooke et al., 2024; Obasi and Akudinobi, 2020; Sahoo et al., 2021), and together with agriculture (Ccanccapa-Cartagena et al., 2021) were predominant sources of moderate Se pollution in lentic environments, while in one case, industrial activity (Guruge et al., 2017) also generated moderate Se pollution. Conversely, in lotic environments, agriculture was the major source of moderate Se pollution either alone (USEPA, 2018), or combined with other sources (Ccanccapa-Cartagena et al., 2021; Genthe et al., 2018; Ioele et al., 2020; Tokatli, 2019). Mining (Teck Coal Limited, 2023) and industrial discharges (Guruge et al., 2017; Onyegeme-Okerenta and West, 2023) also reportedly yielded moderate pollution levels.

Low Se pollution cases (<100 µg/L) were slightly less frequent than those of moderate levels. Mining and industrial activities were the dominant sources of low Se pollution in both lentic and lotic environments (Aras and Arslan, 2024; Aryal, 2024; Jiao et al., 2023; Lateef et al., 2020; Mambou-Ngueyep et al., 2021; Myvizhi and Aruna Devi, 2020; Obasi et al., 2024; Poshtegal and Mirbagheri, 2017; Prasad et al., 2020; Tan et al., 2015). Natural sources alone, or together with anthropogenic sources, also produced low pollution levels both in lentic and lotic environments, but at lower frequency (Luo et al., 2024; Wang and Tan, 2017).

Reports of Se pollution in coastal and estuarine waters were much less frequent than those in surface water or groundwater, with reports only exhibiting low to moderate pollution levels (Avigliano et al., 2019; Guruge et al., 2017; Couto et al., 2019; Moodley et al., 2021). The pollution sources were unspecified in all cases, and the highest Se

concentration was 298 µg/L (Guruge et al., 2017).

2.5. Summary

In this chapter, a systematic reference review detailing the current status of Se contamination was performed, and confirmed that Se pollution ubiquitously exists in global aquatic environments.

Natural geogenic processes predominantly impact groundwater through oxidation and weathering of minerals and seawater intrusion, while anthropogenic processes exhibited a significantly higher impact on both surface water and groundwater. Metal and coal mining were the dominant sources of anthropogenic Se pollution, whereas industrial discharges, and agricultural activities involving Se fertilizer application or mobilization through irrigation of seleniferous soils posed a relatively lower impact.

Se contamination levels varied depending on the classification of the aquatic environments. High pollution levels >1000 µg/L, primarily generated by anthropogenic sources, were observed in groundwater, and with less frequency in surface water, reaching as high as 4475 µg/L. Notably, moderate pollution levels, ranging from 100 – 1000 µg/L, are the most prevalent across all freshwater environments caused via natural and anthropogenic sources. These indicate that remediation and cleanup efforts should prioritize these sources and higher concentration ranges.

Chapter 3: Screening of duckweeds as the biosorbent for soluble Se removal

3.1. Introduction

As noted in Section 1.4, duckweeds seem to possess significant potential for Se phytoremediation, and a possible application includes their use as biosorbents. While live duckweed biomass has successfully removed microplastics and industrial dyes (Canterzi et al., 2021; Dovidat et al., 2020), there has been no previous report on metal-adsorption. On the other hand, a few studies have primarily focused on metal adsorption using dried or powdered duckweed (Chen et al., 2015; Tang et al., 2017); however, this approach is a less cost-effective and environmentally-friendly compared with the use of live duckweeds as the biosorbent. Since the potential of live unprocessed duckweeds for Se biosorption remains largely unexplored, this chapter aims to identify a duckweed species that can serve as an effective biosorbent for Se removal. A comparative evaluation of six live duckweed species for their Se(VI) and Se(IV) adsorption capacity was first performed in the screening tests over a range of environmentally relevant Se concentrations. The most efficient duckweed species selected in this initial step was further characterized, assessing its Se(VI) and Se(IV) removal capacity and determining the best fit isotherm model. Its adsorption capacity was also compared to other typical Se adsorbents to determine its feasibility as a biosorbent. In addition, the potential for valorization, via the reuse of waste biomass and the adsorbed Se across the tested concentration ranges, was discussed.

3.2. Materials and methods

3.2.1. Duckweeds

Six duckweed species from three genera were used in the present study: *Spirodela polyrhiza* (Sp, clone UY0001), LAp (clone RDSC9387), La (clone OYA0001), Lg (clone RDSC7741), *Lemna japonica* (Lj, clone RDSC8695), and Lm (clone RDSC5512). Their origins and gene accession numbers have been reported by Ishizawa et al. (2021). All duckweeds were sterilized using 0.5 %-Cl sodium hypochlorite for 7 min, washed twice with sterile ion-exchange water for 7 min, and cultivated with sterile modified Arnon and Hoagland hydroponic medium (A&H medium) (pH 7.0 adjusted with KOH or HCl). The A&H medium had the following composition (in mg/L): KNO₃ (36.1), KCl (250.7), MgCl₂·6H₂O (76.47), MgSO₄·7H₂O (10.3), CaCl₂·2H₂O (147), FeSO₄·7H₂O (3.33), H₃BO₃ (0.95), MnCl₂·4H₂O (0.39), CuSO₄·5H₂O (0.03), ZnSO₄·7H₂O (0.08), NaMoO₄·2H₂O (0.34), NaH₂PO₄ (3.87), and EDTA·Na (5.0). Each duckweed culture was maintained under standard laboratory conditions (28 °C, photon flux of 80 µmol/m²/s, and a 16/8-h light/dark cycle) in a growth chamber (LH-220S; NK Systems, Osaka, Japan) for routine maintenance (Ishizawa et al., 2021).

3.2.2. Test media and cultivation of duckweeds

The tests conducted in this study were performed using A&H medium as basal medium. Se stocks were prepared using sodium selenate (Na₂SeO₄) (Mitsuwa Chemicals Co. Ltd., Osaka, Japan) or sodium selenite (Na₂SeO₃) (Sigma-Aldrich Japan G.K., Tokyo, Japan) and filter-sterilized using a 0.20-µm cellulose-acetate filter (Toyo Roshi Kaisha Ltd., Tokyo, Japan).

For use in the tests, duckweeds were pre-cultivated for 7 days in 500 mL Erlenmeyer flasks containing 200 mL of A&H medium under standard laboratory conditions as specified in Section 3.2.1. These controlled laboratory conditions were chosen to standardize the experimental environment, ensuring consistent results and allowing for the accurate measurement of Se effects without confounding variables such as fluctuating water chemistry or temperature.

3.2.3. Screening tests

Screening tests were carried out first to determine effective duckweed species for Se removal via sorption. Sterile duckweed fronds, corresponding to approximately 0.1 g fresh weight (30 – 50 fronds for each duckweed, excepting 12 fronds for Sp) were carefully blotted dry on sterile tissue paper and transferred to 100 mL Erlenmeyer flasks containing 60 mL sterile A&H medium supplemented with 0.05 – 2.0 mg-Se/L as Se(IV) or Se(VI). Flasks were agitated at 40 rpm using a horizontal orbital shaker inside a constant-temperature dark chamber for Se adsorption tests in triplicate. This agitation rate was chosen to mimic natural movement of water in aquatic environments and to ensure uniform distribution of elements in the solution. Each flask was destructively sampled for residual Se at each time point, and the duckweed dry weight (DW) was measured at the end of the tests. Se adsorption capacity was evaluated as in Eq (3.1):

$$Adsorption\ capacity = \frac{(C_0 - C_f)V}{m} \quad Eq\ (3.2)$$

where C_0 and C_f (mg/L) are the Se concentrations at time zero and at the end of the tests (or at the selected contact time), respectively, V (= 0.06 L) is the solution volume, and m (g) is the DW of the duckweeds.

3.2.4. Se sorption tests

Based on the screening test results, the most effective duckweed species for Se adsorption (Lg), was selected for the Se removal tests. Lg was pre-cultivated under the same conditions as in the screening tests. One hundred twenty (120) fronds (approximately 9.39 mg DW) of Lg were introduced into a 200 mL Erlenmeyer flask containing 100 mL sterile A&H medium. The removal tests were performed under the same environmental conditions as the screening tests, but either Se(VI) or Se(IV) was added up to 1.25 mg-Se/L to the test media, considering duckweed morphology tolerance limits. Se removal was evaluated by measuring the Se concentration in the media at the start and equilibrium time. The Se adsorption capacity at equilibrium (q_e) was calculated using Eq (3.2):

$$q_e = \frac{(C_0 - C_e)V}{m} \quad \text{Eq (3.2)}$$

where C_0 and C_e (mg/L) are the Se concentrations at time zero and at equilibrium, respectively, V (= 0.1 L) is the solution volume, and m (g) is the DW of Lg.

3.2.5. Isotherm studies

Experimental data of q_e obtained from the removal tests were fitted to various adsorption isotherms. Most popular two-parameter Langmuir and Freundlich models (Vargas et al., 2011), and the three-parameter Sips model (de Vargas Brião et al., 2023) were used to determine the adsorption process characteristics (Table 3-1).

Table 3-1 Non-linear forms of the fitting two-parameter (Langmuir, Freundlich) and three-parameter (Sips) isotherm models.

Isotherm	Equation	Parameters
Langmuir	$q_e = \frac{q_{max}K_L C_e}{1 + K_L C_e}$	q_{max} (mg/g) = maximum adsorption capacity; K_L (L/mg) = Langmuir constant
Freundlich	$q_e = K_F C_e^{1/n}$	K_F, n = Freundlich constants
Sips	$q_e = \frac{q_{max}(K_S C_e)^n}{1 + (K_S C_e)^n}$	q_{max} (mg/g) = maximum adsorption capacity; K_S (L/mg), n = Sips constants

3.2.6. Analytical methods

Aliquots taken in the screening and Se removal tests were filtered through a 0.20- μ m cellulose-acetate filter (Toyo Roshi Kaisha Ltd., Tokyo, Japan), diluted with ultra-pure water, and stored in a refrigerator at 4 °C before soluble Se analysis. Se(VI) and Se(IV) concentrations were determined using ion chromatography (HIC-Sp system; Shimadzu, Kyoto, Japan) with an IonPac AS4A-SC column (Dionex, CA, USA) at 45 °C and a 3-mM Na₂CO₃ solution as the eluent. Duckweed DW was determined after harvesting and drying whole plant biomass in an oven at 60 °C for 2 – 4 days to a constant weight. Duckweed roots were measured at the beginning of the screening tests for the longest one in each plant of 2 – 4 fronds, using a gridded glass slide (2 × 5 cm).

3.2.7. Statistical analysis

The data in this study are presented as mean $\pm$ standard deviation. One-way ANOVA followed by Tukey's test, or pairwise *t*-tests were used to examine whether differences were statistically significant. Differences at $p < 0.05$ were considered significant. All

statistical analyses and nonlinear regression analysis to fit the experimental data and estimate model parameters were performed using R software v.4.3.1 (<https://www.R-project.org/>; R Foundation for Statistical Computing, Vienna, Austria).

3.3. Results and discussion

3.3.1. Time course of Se sorption

To determine the time period necessary for achieving equilibrium for Se sorption in the screening test systems, Se sorption was periodically monitored at 25 °C. Sorption was measured at set intervals (5, 10, 20, 30, 60, 90, and 120 min) for the six duckweeds on 0.5 mg-Se/L Se(VI) or Se(IV) (Figure 3-1). While most duckweeds exhibited fairly reproducible Se sorption time courses in the triplicate tests here, large experimental errors were observed in the sorption tests for Se(IV) by Sp and Se(VI) by Lj. Se sorption tests were standardized by using approximately the same wet weight of healthy duckweed fronds to minimize the experimental errors. However, since it has been pointed out that metal sorption occurs mainly in root tissues of aquatic plants (Yasui et al., 1984), heterogeneous root distribution particularly in Sp and Lj (Table 3-2) likely caused the observed adsorption variability, though number of roots of each duckweed should also be considered.

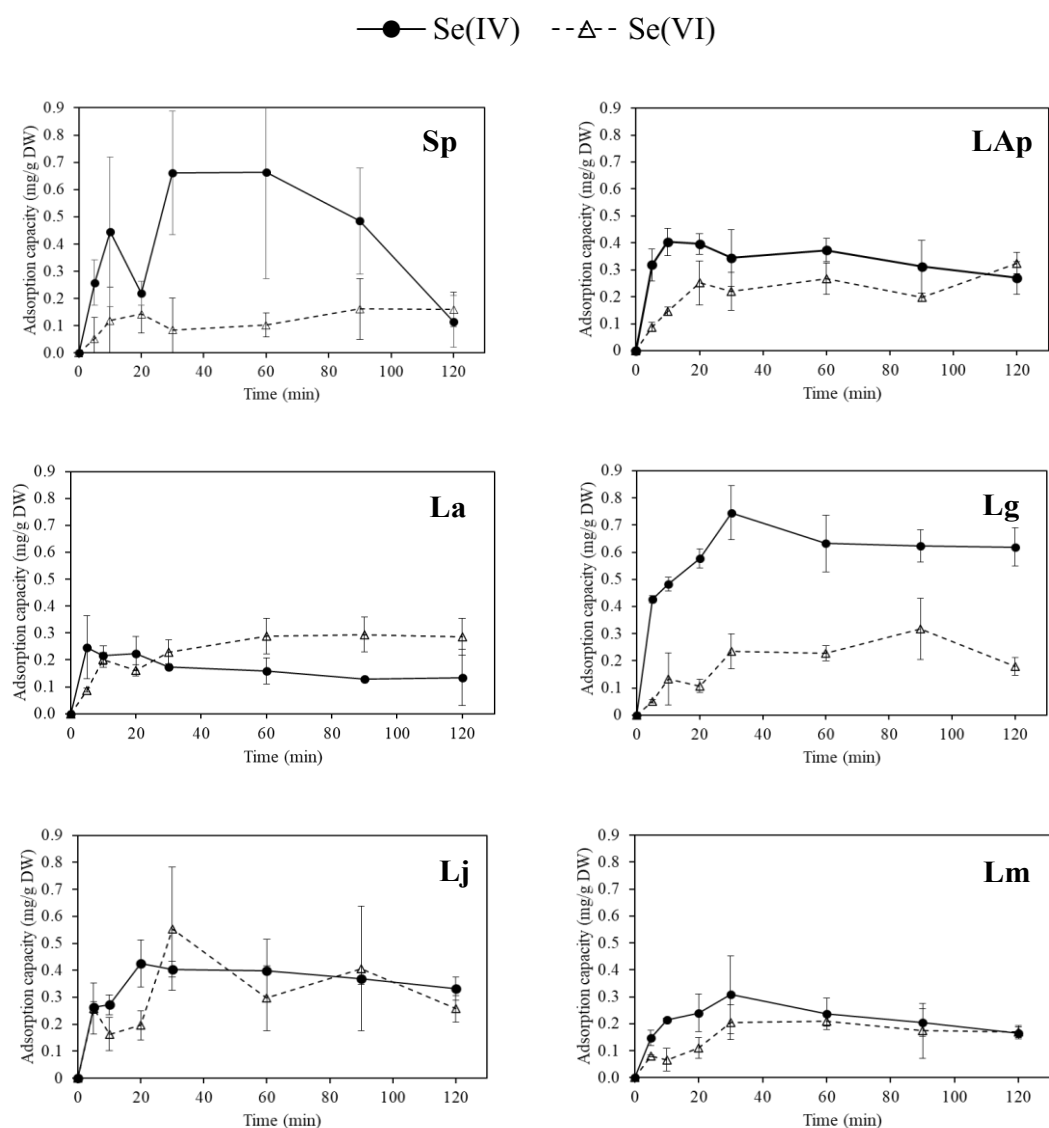


Figure 3-1 Time course of Se(IV) and Se(VI) sorption by six duckweed species in the screening test systems. Values are mean $\pm$ standard deviation ($n = 3$). Sp, *Spirodela polyrhiza*; LAp, *Landoltia punctata*; La, *Lemna aequinoctialis*; Lg, *Lemna gibba*; Lj, *Lemna japonica*; Lm, *Lemna minor*.

Table 3-2 Root length (in cm) of the duckweeds utilized in the time screening tests. Values are the mean $\pm$ standard deviation (n = 42).

Duckweed	Sp	LAp	La	Lg	Lj	Lm
Root length (cm)	4.24 $\pm$ 0.29	1.81 $\pm$ 0.28	1.52 $\pm$ 0.11	7.26 $\pm$ 0.66	5.20 $\pm$ 0.92	6.24 $\pm$ 0.30

Sp, *Spirodela polyrhiza*; LAp, *Landoltia punctata*; La, *Lemna aequinoctialis*; Lg, *Lemna gibba*; Lj, *Lemna japonica*; Lm, *Lemna minor*

Se sorption rate varied depending on the Se and duckweed species. However, there was a general trend observed that Se(IV) sorption proceeded faster than that of Se(VI) for all duckweed species, though the trend was not so clear for Lj. The maximum Se adsorption capacity, also varied among the duckweed species: about 0.2 – 0.7 mg/g DW for Se(IV) and 0.15 – 0.4 mg/g DW for Se(VI). Considerable difference in maximum sorption among duckweeds highlights the importance of selecting appropriate species for efficient Se(VI)/Se(IV) removal from aquatic environments.

3.3.1.1. Se(VI) sorption time course

Se(VI) sorption reached equilibrium within 60 min. Specifically, maximum Se(VI) sorption was achieved after 20 min for Sp and LAp, 30 min for Lg, Lj, and Lm, and 60 min for La.

Se(VI) sorption remained at stable levels after equilibrium was reached. For La and Lm, Se(VI) sorbed by the equilibrium time remained stable over extended exposure in the tests. For the other duckweeds, there were certain temporal fluctuations of Se(VI) sorption, including increase even after the equilibrium, during the test period, as typically shown for Lj. The observed fluctuations suggest that sorption of Se(VI) is dynamic, exhibiting continuous sorption/desorption even after apparent equilibrium. Se(VI) has

been shown to have weak outer-sphere complexation with a sorbent (Wei et al., 2018), indicating its sorption is reversible by competition with coexisting ions. This mechanism likely explains the fluctuations of Se(VI) adsorption observed in this study; i.e. Se(VI) sorption by duckweeds may be achieved by other-sphere complexation. Based on these results, sorption of Se(VI) by duckweeds is considered to be completed within 60 min.

3.3.1.2. Se(IV) sorption time course

In contrast to Se(VI) sorption, Se(IV) sorption by the tested duckweeds proceeded in a relatively fast process, reaching equilibrium within 30 min. Se(IV) removal exceeded 0.2 mg/g DW already at 10 min for all duckweeds, which was consistently higher than for Se(VI), with the exception of La. Specifically, maximum Se(IV) removal was reached after 20 – 30 min for Sp, Lg, Lj, and Lm, while it was reached only after 5 and 10 min for La and LAp, respectively, indicating very rapid Se(IV) sorption can be achieved by these two duckweeds. Although the reason for the very quick sorption of Se(IV) by LAp and La is unknown, their comparatively short root length among the tested duckweeds (Table 3-2) seems to be related to this phenomenon, while the number of roots could also be a contributing factor. This is because the root zone is known to strongly affect metal sorption by plants (Yermiyahu and Kinraide, 2005).

While Se(VI) sorption remained stable after reaching equilibrium, a common trend of gradual decrease in Se(IV) sorption was observed for all the duckweeds as the tests prolonged. The decrease mostly remained at non-significant levels ($p > 0.05$) for all duckweeds, but for Sp the decrease became significant from 90 to 120 min ($p < 0.05$). These indicated that extended exposure may negatively affect Se(IV) sorption or cause its desorption by duckweeds. Although desorption of adsorbed metals in aquatic plants

has not been clearly documented so far, stress conditions such as continuous shaking in darkness could induce root exudation of organic acids (e.g. citric acid) in aquatic plants, which can mobilize and desorb metals (Hou et al., 2015). This phenomenon underscores the necessity to select appropriate sorption time for the Se(IV) removal using duckweeds. In the range of the test results, it can be said that Se(IV) sorption by duckweeds is completed within about 30 min and is maintained relatively stable up to 90 min.

3.3.2. Screening of duckweeds for efficient Se removal

It is important to select duckweed species to be used as biosorbents for efficient Se removal. As the duckweed screening tests, the six species were examined for their Se sorption capacity as shown in Figure 3-2. The sorption tests were conducted at different Se(VI) and Se(IV) concentrations at 28 °C for 90 min for achieving the equilibria.

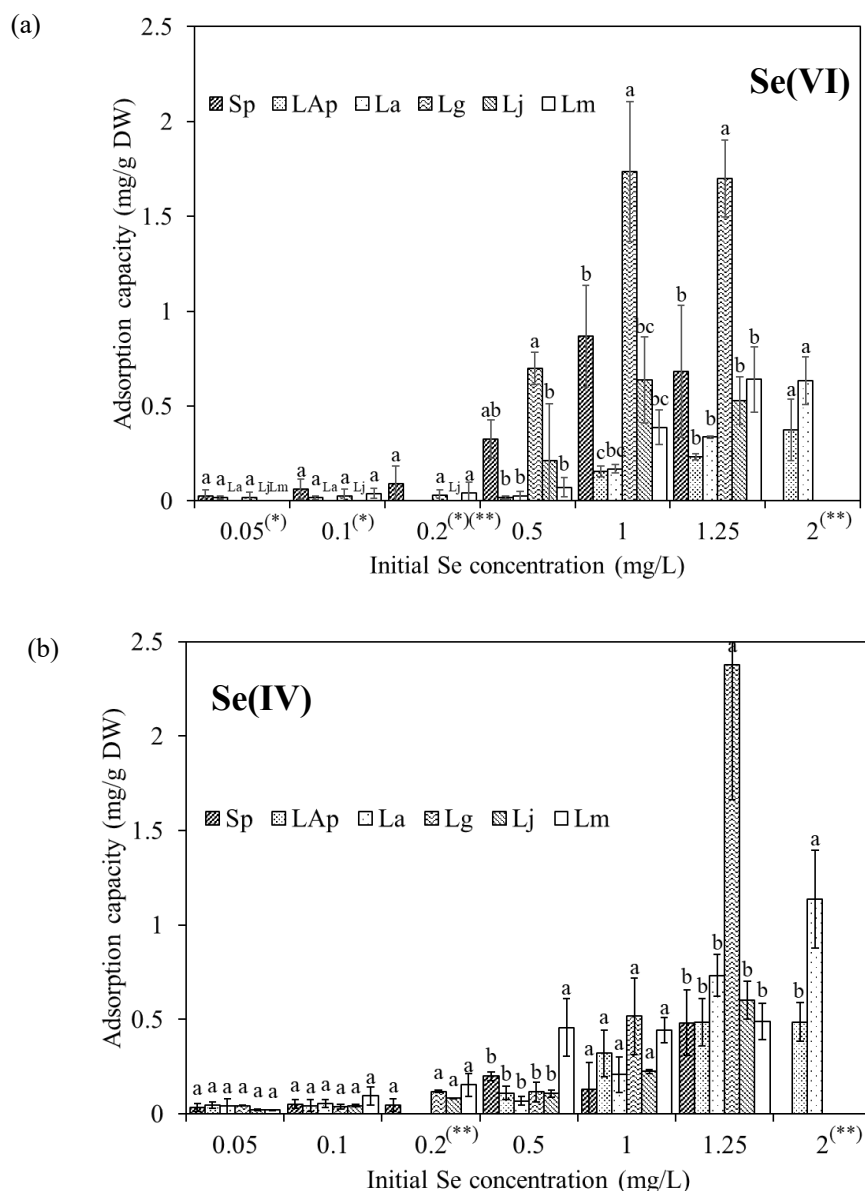


Figure 3-2 Adsorption capacity of Se by six duckweed species. (a) Se(VI) sorption and (b) Se(IV) sorption. Values are mean $\pm$ standard deviation ($n = 3$). Different letters indicate statistically significant differences among species at the same Se concentration, according to *Tukey's* test ($p < 0.05$). (*) For Se(VI) sorption, zero adsorption capacity was observed for La, Lj, and Lm at low Se(VI) concentrations, which is represented by the species names in the graph. (**) No evaluations were conducted for LAp and La at 0.2 mg-Se/L, or for Sp, Lg, Lj, and Lm at 2.0 mg-Se/L. Sp, *Spirodela polyrhiza*; LAp, *Landoltia punctata*; La, *Lemna aequinoctialis*; Lg, *Lemna gibba*; Lj, *Lemna japonica*; Lm, *Lemna minor*.

3.3.2.1. Se evaluation ranges for six duckweeds

Adsorption capacity of Se(VI) or Se(IV) was first evaluated for all duckweed at initial Se concentrations of 0.05, 0.1, 0.5, 1.0, 1.25, and 2.0 mg-Se/L. However, the maximum concentration tested (2.0 mg-Se/L) visibly altered the morphology in some duckweeds even within a short test period of 90 min (Table 3-3). Specifically, Sp, Lg, Lj, and Lm evidenced root loss when exposed to this Se concentration. Although not previously reported in aquatic macrophytes, the root loss mechanism seems like a survival response that prevents the introduction of hazardous elements into plant structure, since root plays an essential role in the adsorption of elements into aquatic plants (Yasui et al., 1984). Loss of root could hinder the use of duckweeds as biosorbents owing to the difficulty recovering fragmented roots that adsorbed Se. Thus, the adsorption capacity of the four duckweeds (Sp, Lg, Lj, and Lm) was evaluated at a maximum Se concentration of 1.25 mg-Se/L which did not cause the root loss, instead a concentration of 0.2 mg-Se/L was added to standardize the number of concentration points.

Table 3-3 Observation of root loss in duckweeds exposed to 2.0 mg-Se/L Se(VI)/Se(IV) in the screening tests.

Duckweed	Sp	LAp	La	Lg	Lj	Lm
Root loss	+	-	-	+	+	+

+: root loss occurred, -: root loss did not occur. Sp, *Spirodela polyrhiza*; LAp, *Landoltia punctata*; La, *Lemna aequinoctialis*; Lg, *Lemna gibba*; Lj, *Lemna japonica*; Lm, *Lemna minor*

3.3.2.2. Se(VI) sorption

Figure 3-2(a) shows Se(VI) sorption capacity of six duckweeds at different initial

Se(VI) concentrations. All duckweeds show very low or negligible Se(VI) adsorption at Se(VI) concentrations below 0.2 mg-Se/L in the screening tests. Significant adsorption levels were observed at Se(VI) concentrations higher than 0.5 mg-Se/L, while adsorption capacity by Sp and Lg was significantly higher compared to the other duckweeds at from 0.5 to 1.0 mg-Se/L ($p < 0.05$). At initial Se(VI) concentrations of 1.0 and 1.25 mg-Se/L, especially Lg demonstrated effective Se(VI) sorption, showing much higher adsorption than the other duckweeds. Se(VI) adsorption capacity of the other duckweeds including LAp and La which did not lose roots even at 2.0 mg-Se/L showed comparable levels at concentrations from 1.0 to 2.0 mg-Se/L ($p > 0.05$).

3.3.2.3. Se(IV) sorption

Se(IV) sorption capacity of six duckweeds at different initial Se(IV) concentrations are compared in Figure 3-2(b). Similar to Se(VI) sorption, most duckweeds exhibited almost negligible Se(IV) adsorption capacity at initial concentrations below 0.2 mg-Se/L, though Se(IV) sorption was slightly higher than that of Se(VI), as a general tendency. At Se(IV) concentrations from 0.5 to 1.25 mg-Se/L, all the duckweeds showed significant Se(IV) adsorption capacity, which was increased with increasing initial Se(IV) concentration. Lm showed considerably higher adsorption capacity than the other duckweeds at 0.5 mg-Se/L ($p < 0.05$). Similarly, Lg remarkably outperformed the other duckweeds in Se(IV) sorption at 1.25 mg-Se/L ($p < 0.05$). As for the other duckweeds which can be applied to higher Se(IV) concentrations (up to 2.0 mg-Se/L) due to the lack of root loss, La demonstrated a higher adsorption capacity than LAp ($p < 0.05$).

3.3.2.4. Candidates of Se sorbents

The results of the screening tests indicated that Lg could serve as a most promising biosorbent for Se removal, since Lg showed the highest adsorption capacity for both Se(VI) and Se(IV), though it is available for Se concentrations up to 1.25 mg-Se/L. High Se(VI) and Se(IV) adsorption capacity by Lg may result from its relatively long root length compared with the other duckweeds (Table 3-2), which provides an increased surface area, and thus a high density of available adsorption sites (Goldberg, 1991). For applying to higher Se concentrations, La seems to be a candidate sorbent, because it showed a higher Se(IV) sorption capability than LAp; however, its Se sorption capability is considered inferior to that of Lg. As a result, Lg was selected here as the candidate duckweed for further characterization as the sorbent for Se removal.

3.3.3. Characterization of Lg as biosorbent for Se removal

The Se removal potential of the selected duckweed, Lg, was evaluated at similar concentrations as the screening tests at 0.05, 0.1, 0.2, 0.5, 1.0, and 1.25 mg-Se/L for 90 min at 28 °C in the Se removal test system using larger numbers of the fronds. Figure 3-3 shows the Se adsorption and removal performance of Lg at different initial Se(VI) or Se(IV) concentrations.

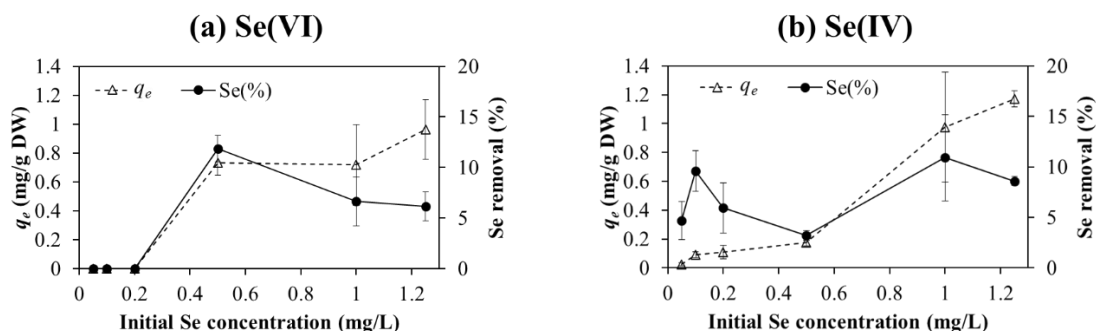


Figure 3-3 Se adsorption performance of Lg at different initial Se(VI)/Se(IV) concentrations in the Se removal tests. (a) Se(VI) removal efficiency (%) and q_e (mg/g DW). (b) Se(IV) removal efficiency (%) and q_e (mg/g DW). Values are mean $\pm$ standard deviation (n = 3). q_e , adsorption capacity at equilibrium; Lg, *Lemna gibba*.

3.3.3.1. Se(VI) removal

Lg exhibited negligible Se(VI) sorption (q_e) in the Se removal tests at initial Se concentrations below 0.2 mg-Se/L ($p < 0.05$); however, it showed an approximately constant Se(VI) adsorption capacity of 0.73–0.96 mg/g DW at higher initial Se concentrations (0.5–1.25 mg-Se/L) (Figure 3-3a). Although no significant Se(VI) removal was observed at Se concentrations below 0.2 mg/L, substantial fractions of Se(VI) were removed at higher initial Se concentrations: the removal efficiency peaked at 11.5% at 0.5 mg-Se/L and decreased at higher Se concentrations (6.2% at 1.25 mg-Se/L).

3.3.3.2. Se(IV) removal

Se(IV) removal by Lg followed a different trend from that of Se(VI). Lg showed low but statistically significant Se(IV) sorption (q_e) even at concentrations below 0.2 mg-Se/L (0.02–0.11 mg/g DW) ($p < 0.05$). Furthermore, q_e values increased stepwise with

increased initial Se(IV) concentrations from 0.5 to 1.25 mg-Se/L, reaching a maximum of 1.17 mg/g at 1.25 mg-Se/L DW (Figure 3-3b). Resultantly, Lg showed substantial and relatively consistent Se(IV) removal efficiencies across all tested Se concentrations: the lowest and highest removal efficiencies were 3.2% and 10.9% at initial Se(IV) concentrations of 0.05 mg-Se/L and 1.0 mg-Se/L, respectively.

3.3.3.3. Adsorption isotherms

The data for Se(VI)/Se(IV) adsorption by Lg (q_e) in the Se removal tests were fitted to the Langmuir, Freundlich, and Sips models to determine the appropriate adsorption isotherm, which is useful for designing and operating Se removal processes using Lg as a biosorbent. The results and their corresponding best-fit parameters and correlation coefficients (R^2) are shown in Figure 3-4.

Among the examined models, the Sips model best described the sorption of Se(VI) and Se(IV), indicating adsorption onto a heterogeneous surface (de Vargas Brião et al., 2023). This was consistent with the characteristics of the observed adsorption process. Specifically, the sinusoidal behavior of the adsorption of Se(VI) and Se(IV) (Figure 3-4) suggests a Type V isotherm according to the Brunauer classification (Brunauer et al., 1940), which is characteristic of a relatively weak and non-spontaneous process well represented by the Sips model (de Vargas Brião et al., 2023; Inglezakis et al., 2018).

The inflection point in the Sips isotherm is a key indicator of when the adsorption begins to overcome competing factors, causing the curve to transition from concave upward to concave downward (de Vargas Brião et al., 2023; Inglezakis et al., 2018). The q_e values at the inflection points were derived from Eq (3.3) using the n and q_{max} values obtained from the Sips fitting (de Vargas Brião et al., 2023). The corresponding C_e values

were 0.372 and 0.597 mg-Se/L for Se(VI) and Se(IV), respectively. The earlier inflection point for Se(VI) suggests less competitive adsorption than that of Se(IV).

$$\frac{q_e}{q_{max}} = \frac{n-1}{2n} \quad \text{Eq (3.3)}$$

The weaker adsorption of Se(VI) may be due to outer-sphere complexation, which involves weaker electrostatic forces and is sensitive to the ionic strength of the solution (Wang et al., 2018). This finding is consistent with the observation that the Se(VI) sorption levels were not significant at low Se concentrations. In contrast, stronger Se(IV) adsorption likely involves inner-sphere complexation, attributed to the high density of high-affinity surface binding sites (Mishra et al., 2010), resulting in significant adsorption levels even at low Se concentrations.

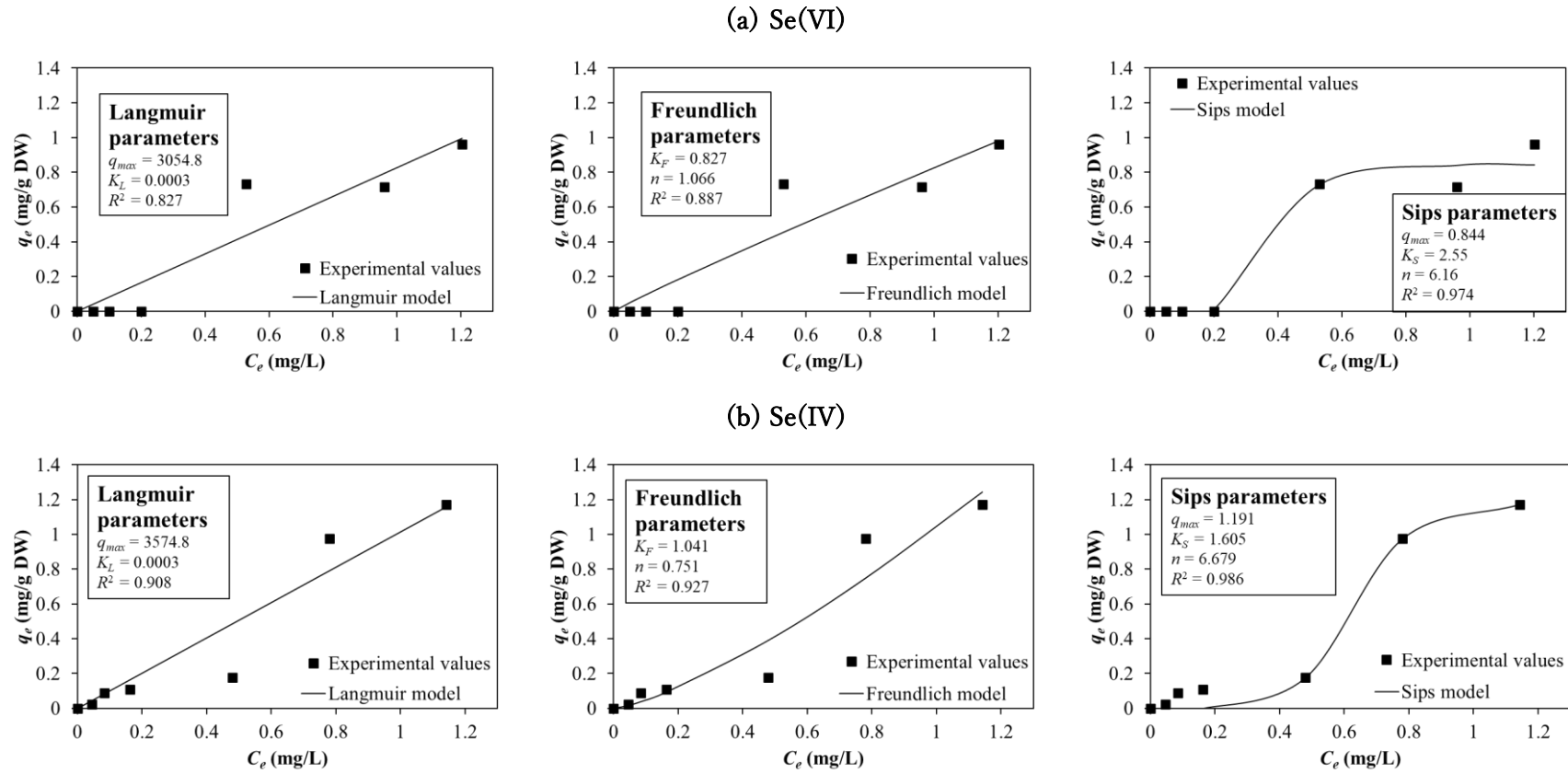


Figure 3-4 Fits of Se(VI)/Se(IV) adsorption by Lg to the Langmuir, Freundlich, and Sips isotherms with its corresponding fitting parameters and R^2 values. (a) Adsorption of Se(VI) and (b) adsorption of Se(IV). q_e , adsorption capacity at equilibrium; C_e , equilibrium concentration; Lg, *Lemna gibba*.

3.3.4. Se sorption capability of Lg in comparison with reported sorbents

To evaluate the potential of live Lg as a Se sorbent, its adsorption capacity for Se(VI) and Se(IV) was compared with that of other reported Se sorbents, whose adsorption isotherms were determined (Table 3-4). For standardized comparison, the values of reported q_{max} and q values at adsorption equilibrium concentrations of 0.5 and 1.0 mg-Se/L (q (0.5) and q (1.0), respectively) calculated using respective isotherms were compared for each material.

3.3.4.1. Se(VI) sorption capability

Although mineral sorbents such as magnetite and magnetic Fe/Mn oxide have been tested at higher initial Se(VI) concentrations, their q_{max} values of 0.245 and 0.77 mg/g, respectively, are significantly lower than that of Lg (0.844 mg/g). In addition, the q (0.5) and q (1.0) values of Lg were greater than those reported for magnetite. The synthesized oxidized carbon nanotubes exhibited q (0.5) of 0.733 mg/g and a slightly higher q (1.0) of 0.919 mg/g, indicating a comparable Se(VI) removal capacity to that of Lg. In contrast, biosorbents such as dried wheat bran had a markedly inferior capacity, with q_{max} and q (0.5) values of 0.062 and 0.036 mg/g, respectively. Overall, these comparisons highlight that Lg possesses a superior Se(VI) removal capacity compared to several other common materials.

Table 3-4 Comparison of the adsorption capacities of Se(VI) and Se(IV) on various sorbents.

Sorbent	Magnetite at pH 4	Magnetic Fe/Mn oxide	Oxidized carbon nanotubes at 30 °C	Wheat bran at 30 °C	Algae <i>Cladophora sericea</i> at 20 °C	Lg	
Reference	Martínez et al. (2006)	Gonzalez et al. (2010)	Kamaraj and Vasudevan (2015)	Hasan and Ranjan (2010)	Filote et al. (2017)	This study	
Se(VI)	Concentration range	0.24–39.5	0.25–10	0.5–2.0	0.1–1.0	N.D.	0.05–1.25
	q_{max} (mg/g)	0.245	0.77	1.231	0.062	N.D.	0.844
	q (0.5) (mg/g)	0.161	N.D. ^a	0.733	0.036	N.D.	0.705
	q (1.0) (mg/g)	0.195	N.D. ^a	0.919	N.D.	N.D.	0.841
Se(IV)	Concentration range	0.24–39.5	0.25–10	N.D.	0.1–1.0	2.0–50	0.05–1.25
	q_{max} (mg/g)	0.22	6.57	N.D.	0.060	0.74 ^b	1.17 ^b
	q (0.5) (mg/g)	0.194	N.D. ^a	N.D.	0.042	0.016	0.223
	q (1.0) (mg/g)	0.207	N.D. ^a	N.D.	N.D.	0.03	1.143

N.D., not determined

^a Could not be calculated due to the lack of parameter information in the isotherm^b Measured value

3.3.4.2. Se(IV) sorption capability

The Se(IV) sorption capacity varied significantly among the sorbents. In contrast to its performance with Se(VI), the magnetic Fe/Mn oxide exhibited a higher q_{max} of 6.57 mg/g for Se(IV) than that of Lg (1.17 mg/g). However, this superior performance could be attributed to the magnetic Fe/Mn oxide being tested at a significantly higher initial concentration (10 mg-Se/L). Conversely, magnetite showed a significantly lower performance, with q_{max} and q (1.0) being less than that of Lg, whereas q (0.5) was slightly lower. Among the biosorbents, dried wheat bran exhibited markedly inferior q_{max} and q (0.5) values compared to Lg. Similarly, the dried algae *Cladophora sericea* demonstrated a lower capacity, with q_{max} , q (0.5), and q (1.0) values lower than those of Lg, despite being tested at much higher initial concentrations (up to 50 mg/L). Overall, Lg is one of the most effective sorbents for Se(IV) removal among the compared materials.

In addition to its high performance as a Se(VI) and Se(IV) biosorbent, Lg offers several practical advantages over common sorbents. Its simple preparation and easy recovery highlight its potential as an efficient and sustainable Se sorbent, although its capacity is affected by root loss.

3.3.5. Possibility of duckweed biomass and recovered Se utilization after Se sorption

Duckweed biomass can be utilized as animal feed, fertilizer, and feedstock for various fuels and chemicals. This versatility implies that when Lg is used as a biosorbent to remove Se, the waste biomass and recovered Se can be repurposed as valuable resources.

For instance, Se-enriched duckweed, with a Se content of approximately 0.21 mg-Se/g

DW, has been successfully utilized as a slow-release Se biofertilizer, because the duckweed biomass contains important macronutrients such as nitrogen and phosphorous (Li et al., 2021). This suggests that Lg used for Se removal could be similarly repurposed, particularly when it is used for Se removal at concentrations of over 0.5 mg-Se/L for Se(VI) and 1.0 mg-Se/L for Se(IV). However, because the adsorbed Se is likely in a soluble form, plant absorption may be limited.

Trace elements such as Se enhance biogas production during anaerobic digestion (AD), as Se acts as a cofactor for essential enzymes such as hydrogenase (Ariunbaatar et al., 2016). Duckweed biomass is a good source of carbohydrates that can be converted into organic acids such as lactic acid after deproteinization, and this process can be accelerated in the presence of Se (Kotamraju et al., 2024). Enhancement of these valorization methods have been successfully demonstrated with duckweed containing 0.17–0.23 mg-Se/g DW. This suggests that Se-enriched Lg with comparable Se contents can also be repurposed for these applications (Kotamraju et al., 2023, 2024).

Biomasses enriched with low and high Se concentrations can be directly utilized for animal and human nutrition. Studies on biomass other than duckweed, such as *Chlorella* and various plant foods, have shown promising results. For example, *Chlorella* enriched with 0.13 mg-Se/g significantly improved the reproduction capability of breeding boars (Doucha et al., 2009). Furthermore, Tangjaidee et al. (2023) highlighted the benefits of Se-enriched plant foods in treating conditions such as diabetes and liver injury in mice. Similarly, daily doses of < 300 µg of Se-enriched plant foods have been reported to improve several functional properties in humans (Tangjaidee et al., 2023). These observations suggest that Se-enriched Lg can be prepared (e.g., pulverized) for use in nutritional trials. However, special care must be taken before such applications, including

appropriate sterilization and consideration of potentially hazardous elements in the water matrix.

3.4. Summary

In this chapter, six live duckweed species were examined for their Se adsorption capability to identify effective biosorbents. Lg demonstrated the highest Se adsorption capacity among the tested duckweeds, making it a suitable candidate as a Se biosorbent, while consideration of Se concentration ranges is necessary, since elevated levels of Se can alter the morphology of the duckweeds (i.e. causing root loss). The experimental results revealed that Lg can remove significant amounts of Se(VI) and Se(IV) within 90 min. However, while Se(IV) removal remained significant across all tested concentrations Se(VI) adsorption was negligible at concentrations < 0.2 mg-Se/L. The adsorption pattern for both Se(VI) and Se(IV) followed sinusoidal behavior best described by the Sips isotherm, with maximum equilibrium adsorption capacities of 0.96 and 1.17 mg/g DW, respectively. The best fitting to the Sips isotherm suggested heterogeneity of the sorption sites. The adsorption capacities of Lg for Se(VI) and Se(IV) are higher than those of most reported sorbents at similar equilibrium concentrations, demonstrating their practical feasibility as Se(VI) and Se(IV) sorbents. It was suggested that Lg biomass after Se adsorption can be utilized as a Se-enriched biofertilizer, substrate for the AD processes for effective production of methane and organic acids, or as animal or human feed.

Chapter 4: Screening of Se-tolerant duckweeds for sustainable Se removal

4.1. Introduction

While the remarkable capacities of duckweed as the biosorbent for fast Se removal were demonstrated in Chapter 3, development of long-term remediation of Se pollution using growing duckweeds is also necessary for vast area contamination. However, previous studies predominantly utilized only locally available duckweed species (Mechora et al., 2015; Miranda et al., 2014; Nattrass et al., 2022), hindering the potential for optimization. To ensure a sustainable remediation process, it is essential for duckweeds to maintain healthy growth and exhibit continuous Se removal capacity over time. Consequently, selecting duckweed species that exhibit both high Se-tolerance and removal efficiency is critical for constructing effective and sustainable phytoremediation systems.

The studies in this chapter aim to identify Se-tolerant duckweeds for the removal of soluble Se during their growth. Six duckweed species were comparatively evaluated for their tolerance against Se(VI) and Se(IV) based on their growth and healthy status under exposure to various Se concentrations. The selected Se-tolerant duckweed species were assessed for their Se(VI) and Se(IV) removal efficiency over a range of environmentally relevant Se concentrations. Both tolerance and removal tests were conducted for seven days in the presence of high and low sulfate concentrations, as sulfate reportedly affects Se bioaccumulation and poses toxicity in a duckweed (Lo et al., 2015; Severi, 2001) and occurs naturally and anthropogenically with Se (Kharaka et al., 1996; Morrison et al.,

2012). Furthermore, the Se content in duckweed biomass after the removal tests was quantified for potential valorization as Se-enriched biomass.

4.2. Materials and methods

4.2.1. Duckweeds and their pre-cultivation

In this chapter, six duckweed species; Sp, LAp, La, Lg, Lj, and Lm were comparatively evaluated for their Se tolerance. The sterilization step and growth conditions of the duckweeds were described in Section 3.2.1. Prior to experimental tests, duckweeds were pre-cultivated for seven days in 300 mL Erlenmeyer flasks containing 100 mL of either low-sulfate A&H medium (LS-A&H), which is the modified A&H medium specified in Section 3.2.1 ($[\text{SO}_4^{2-}] = 5.2 \text{ mg/L}$), or high-sulfate A&H medium (HS-A&H). The HS-A&H medium was previously described by Toyama et al. (2006) and contains a higher sulfate concentration ($[\text{SO}_4^{2-}] = 203 \text{ mg/L}$), while maintaining the same K^+ and Mg^{2+} concentrations as the LS-A&H medium. Its composition was as follows (in mg/L): KNO_3 (36.1), K_2SO_4 (293), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (103), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (147), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (3.33), H_3BO_3 (0.95), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.39), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.03), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.08), $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ (0.34), NaH_2PO_4 (3.87), and $\text{EDTA} \cdot \text{Na}$ (5.0). Se stocks were prepared as specified in Section 3.2.2.

4.2.2. Se tolerance tests

Se tolerance tests were conducted for the six duckweed species (Table 4-1). Twenty fronds of each pre-cultivated duckweed were placed in 100 mL of test medium contained

in 300-mL Erlenmeyer flasks and cultivated for seven days under standard laboratory conditions. The test medium contained Se(IV), added to both LS-A&H and HS-A&H media at 1, 2, and 4 mg-Se/L, or Se(VI), added at 1, 2, and 4 mg-Se/L for LS-A&H medium and 10, 20, 40, 60, and 100 mg-Se/L for HS-A&H medium. The control tests were performed without the addition of Se to the media, and three replicates were prepared for each test. Duckweed growth was evaluated by counting the frond number at the beginning and end of the experiments, and the average relative growth rate (RGR) of duckweeds was calculated based on the increase in frond number during the 7-day experiment. In addition, frond appearance was visually observed during the test period.

Table 4-1 Experimental design for all tests conducted in the studies in this chapter.

Tests	Tested duckweeds	Initial frond number	Growth media	Se(IV) (mg-Se/L)	Se(VI) (mg-Se/L)
Se tolerance tests	Sp, LAp, La, Lg, Lj, Lm	20 fronds	HS-A&H	0, 1, 2, 4	0, 10, 20, 40, 60,
			LS-A&H		100 0, 1, 2, 4
Se removal tests	LAp, La	50 fronds	HS-A&H	0, 0.2, 0.5, 1.0	0, 0.2, 0.5, 1.0
			LS-A&H		

LAp, *Landoltia punctata*; La, *Lemna aequinoctialis*; Sp, *Spirodela polyrhiza*; Lg, *Lemna gibba*; Lj, *Lemna japonica*; Lm, *Lemna minor*

4.2.3. Se removal tests

Based on the Se tolerance test results, two Se-tolerant duckweeds (LAp and La) were selected for the Se removal tests (Table 4-1). Fifty fronds of each duckweed species, corresponding to 5.7 and 4.5 mg dry weight (DW) for LAp and La, respectively, were

introduced into the test media. The Se removal tests were conducted independently for Se(IV) or Se(VI) in both LS-A&H and HS-A&H media in the same manner as those of the Se tolerance tests for seven days; however, Se(IV) or Se(VI) was added at 0.2, 0.5, and 1.0 mg-Se/L to the test media. Control tests using test media without Se addition were also conducted. Tests with a Se concentration of 0.5 mg-Se/L for Se(IV)/Se(VI) without duckweed were also performed as another control. Se removal was evaluated by measuring the Se concentration in the medium at the start and the end of the experiments. To estimate the evaporation of the medium, the volume of the remaining solution in each flask was measured after the experiments. At the end of the experiments, the DW of the duckweeds was measured.

4.2.4. Analytical methods

Soluble Se analysis was performed as detailed in Section 3.2.6. In the Se removal tests, all the duckweed biomass in the flasks was harvested at the end of the tests, washed with ion-exchange water, and dried in an oven at 60 °C for 3–5 days to a constant weight. The biomass was further digested with an HNO₃ (68.5 %): HClO₄ (70 %) mixture (1 mL, 10:1 v/v) (Singh et al., 2008) in a dry heating block at 100 °C for 6 min until no plant material was observed. After cooling to temperatures between 17 and 20 °C, the samples were filtered through a 0.45-μm PTFE filter (Toyo Roshi Kaisha Ltd.), diluted with ultra-pure water, stored in a refrigerator at 4 °C and analyzed for total Se to evaluate the Se content of the biomass. The Se concentration in the digestate was measured using inductively coupled plasma-atomic emission spectrometry (ICPS-7510; Shimadzu).

4.2.5. Calculations

RGR was calculated using Eq (4.1) (Naumann et al., 2007) from the values of the frond numbers at the start (x_0) and at the end of the 7-day experiments (x_7) as the mean values.

$$RGR(d^{-1}) = \frac{\ln(x_7) - \ln(x_0)}{\text{Number of days}} \quad \text{Eq (4.1)}$$

Se removal (μg) and removal efficiency (%) were calculated using Eqs (4.2) and (4.3), respectively:

$$\text{Se removal } (\mu\text{g}) = Se_0 \times V_0 - Se_7 \times V_7 \quad \text{Eq (4.2)}$$

$$\text{Se removal efficiency } (\%) = \left(\frac{Se_0 \times V_0 - Se_7 \times V_7}{Se_0 \times V_0} \right) \times 100\% \quad \text{Eq (4.3)}$$

where Se_0 and Se_7 are the Se(Se(VI)/Se(IV)) concentrations (in mg/L) in the medium, and V_0 and V_7 represent the volume of the solution (in L) at the start and end of the experiments, respectively.

The Se recovery (%) was calculated based on Eq (4.4):

$$\begin{aligned} \text{Se recovery } (\%) \\ = \frac{\text{Se mass in the duckweed at the end of the experiments } (\mu\text{g})}{\text{Se mass removal } (\mu\text{g})} \times 100\% \end{aligned} \quad \text{Eq (4.4)}$$

4.2.6. Statistical analysis

The statistical methods used in this chapter were the same as those described in Section 3.2.7, including one-way ANOVA followed by Tukey's test or pairwise t -tests. Similar to the referred section, data are presented as mean $\pm$ standard deviation, and a p value < 0.05 was considered statistically significant.

Data standardization was conducted using Z-scores to allow comparison between parameters with different measurement scales. Specifically, the Z-score for the parameters was calculated using Eq (4.5):

$$Z = \frac{x - \mu}{\sigma} \quad \text{Eq (4.5)}$$

where x is the raw value, μ is the mean of the dataset for a given parameter, and σ is the standard deviation of the dataset for the given parameter.

4.3. Results and discussion

4.3.1. Screening of Se-tolerant duckweeds

Se tolerance tests were first conducted for the six duckweeds to select species that can sustain healthy growth under the exposure to Se. Figure 4-1 shows the RGR of the duckweeds grown at different Se(IV)/Se(VI) concentrations under high- and low-sulfate conditions. Appearance observations (Tables 4-2 – 4-5) were used to evaluate the health status of the grown duckweeds at the end of the tests (Figure 4-2). The presence of healthy fronds indicates duckweed tolerance to Se (Mechora et al., 2015). Mean Z scores were calculated to rank the tolerance of the evaluated duckweeds (Table 4-6).

In the control tests without Se, the observed RGR of the six duckweeds ranged from 0.2 to 0.33 (d⁻¹), and the sulfate concentration in the test media did not significantly affect the RGR of all duckweeds (Figure 4-1a). Among the six duckweeds tested, LAP and La showed relatively higher RGR, where Sp showed relatively lower RGR compared with that of the other duckweeds ($p < 0.05$).

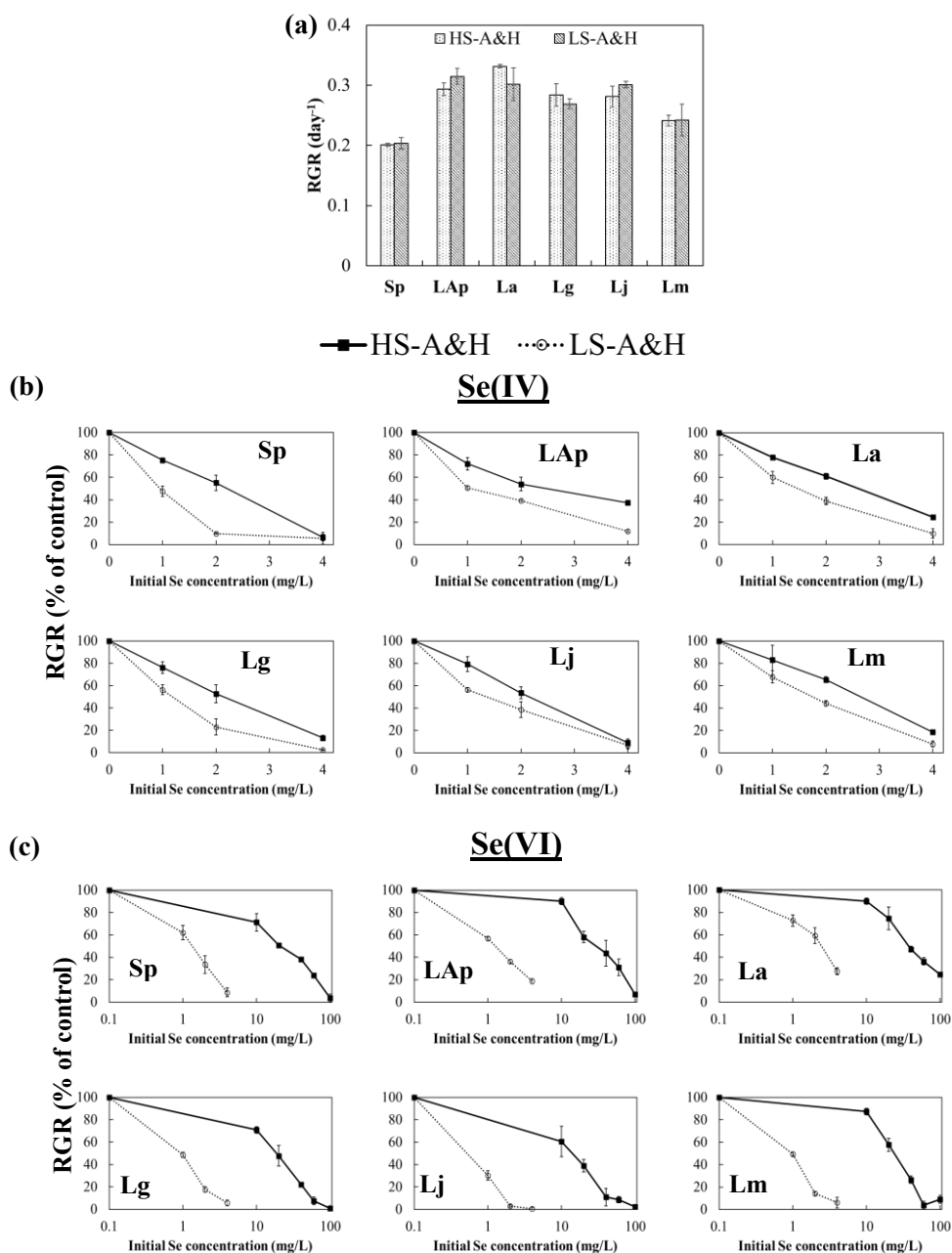


Figure 4-1 Relative growth rate (RGR) of the six duckweeds grown with or without Se exposure in LS-A&H and HS-A&H media: (a) Duckweeds grown without exposure to Se, (b) duckweeds grown on Se(IV), and (c) duckweeds grown on Se(VI). Values are mean $\pm$ standard deviation ($n = 3$). To allow log transformation, a value (0.1) was added to the zero Se concentration in the Se(VI) tests. Sp, *Spirodela polyrhiza*; LAp, *Landoltia punctata*; La, *Lemna aequinoctialis*; Lg, *Lemna gibba*; Lj, *Lemna japonica*; Lm, *Lemna minor*.

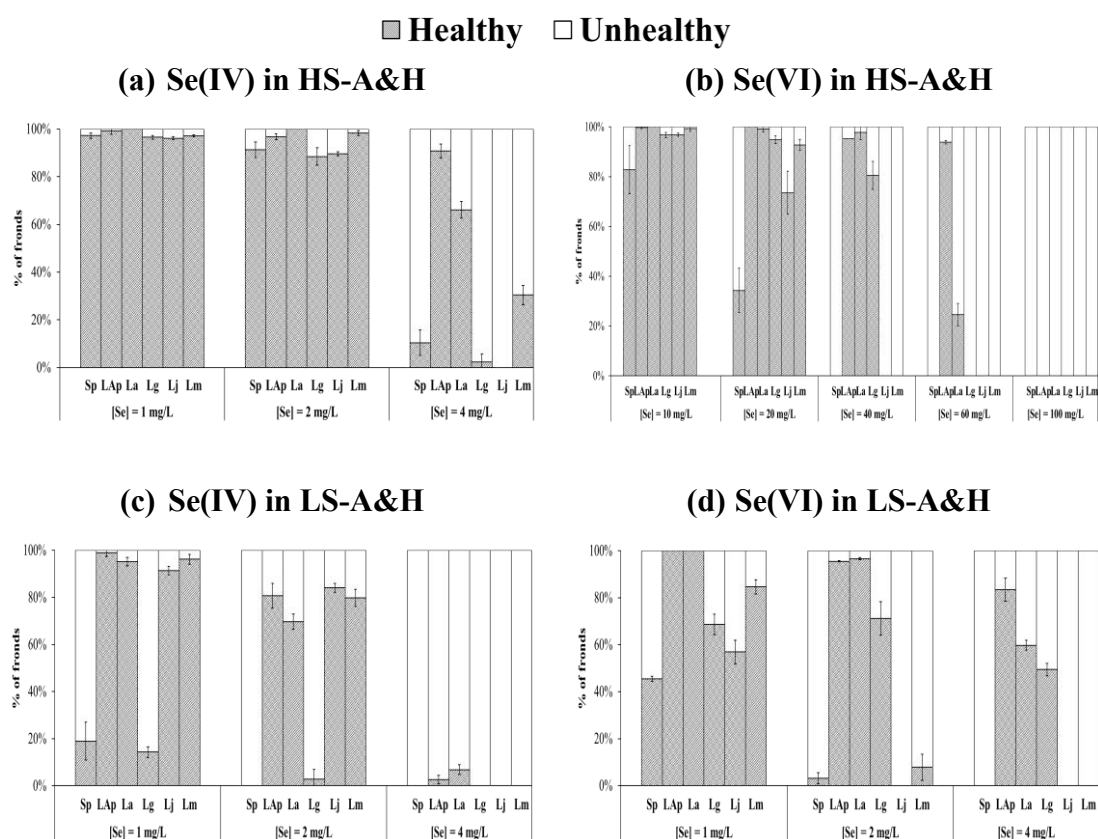


Figure 4-2 Ratio of healthy and unhealthy fronds after 7-day treatment with different concentrations of Se. Frond with green and yellowish or whitish colors were judged healthy and unhealthy, respectively. (a) Duckweeds exposed to Se(IV) in HS-A&H medium, (b) duckweeds exposed to Se(VI) in HS-A&H medium, (c) duckweeds exposed to Se(IV) in LS-A&H medium, and (d) duckweeds exposed to Se(VI) in LS-A&H medium. Values are mean $\pm$ standard deviation ($n = 3$). *Sp*, *Spirodela polyrhiza*; *LAp*, *Landoltia punctata*; *La*, *Lemna aequinoctialis*; *Lg*, *Lemna gibba*; *Lj*, *Lemna japonica*; *Lm*, *Lemna minor*.

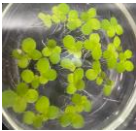
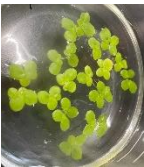
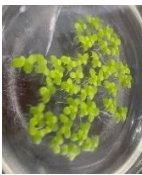
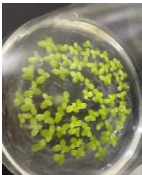
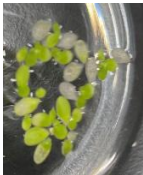
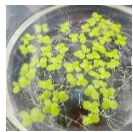
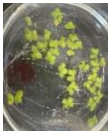
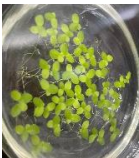
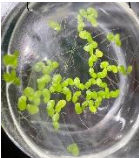
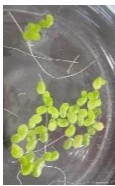
4.3.1.1. Tolerance against Se(IV)

In HS-A&H medium with high-sulfate concentration, all duckweeds showed a similar pattern of growth inhibition by Se(IV). Specifically, a linear reduction in RGR associated

with an increase in Se(IV) concentration from 1 to 4 mg-Se/L was observed (Figure 4-1b). Overall, the degree of RGR reduction was significantly less for LAp and La compared with that in the other duckweeds ($p < 0.05$). Notably, despite the considerable reduction in RGR at 2 mg-Se/L Se(IV), most duckweed species exhibited healthy fronds at this concentration (Figure 4-2a). However, 4 mg-Se/L Se(IV) caused not only the drastic decrease in RGR but also the discoloration and even necrosis of duckweed fronds (Table 4-2), except in LAp and La, which maintained high ratios of healthy fronds (more than 70%) (Figure 4-2a). These results indicated a higher tolerance of these two duckweeds to Se(IV) under high-sulfate conditions.

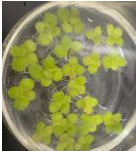
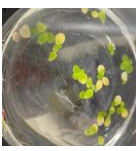
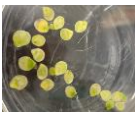
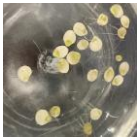
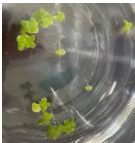
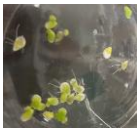
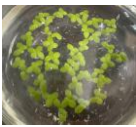
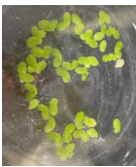
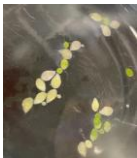
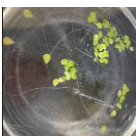
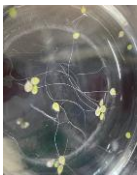
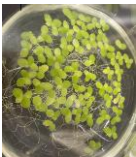
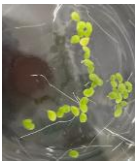
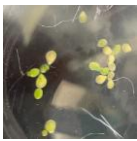
Conversely, in LS-A&H medium with low-sulfate concentration, Se(IV) exhibited significantly higher inhibitory effects on the growth of all duckweeds than those in HS-A&H medium (Figure 4-1b). Higher toxic Se(IV) effects under low sulfate concentration have been previously reported for algae by Ponton et al. (2020). Among the tested duckweeds, Sp and Lg showed a much greater reduction in RGR at 2 mg-Se/L, and severe frond necrosis and discoloration were observed even at 1 mg-Se/L Se(IV) (Table 4-3) ($p < 0.05$). All duckweed species showed little to no growth at 4 mg-Se/L Se(IV), and healthy fronds were scarcely observed at the end of the tests (Figure 4-2c). However, it should be noted that LAp and La maintained healthy fronds, albeit in a very small fraction, even at this concentration, suggesting their relatively high tolerance to Se(IV) under low-sulfate conditions.

Table 4-2 Appearance of duckweed species at the end of the Se(IV) tests in HS-A&H medium.

Duckweed	Initial Se(IV) concentration (mg-Se/L)			
	0 (Control)	1	2	4
Sp				
LAp				
La				
Lg				
Lj				
Lm				

Sp, *Spirodela polyrhiza*; LAp, *Landoltia punctata*; La, *Lemna aequinotialis*; Lg, *Lemna gibba*; Lj, *Lemna japonica*; Lm, *Lemna minor*

Table 4-3 Appearance of duckweed species at the end of the Se(IV) tests in LS-A&H medium.

Duckweed	Initial Se(IV) concentration (mg-Se/L)			
	0 (Control)	1	2	4
Sp				
LAp				
La				
Lg				
Lj				
Lm				

Sp, *Spirodela polyrhiza*; LAp, *Landoltia punctata*; La, *Lemna aequinoctialis*; Lg, *Lemna gibba*; Lj, *Lemna japonica*; Lm, *Lemna minor*

4.3.1.2. Tolerance against Se(VI)

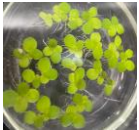
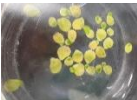
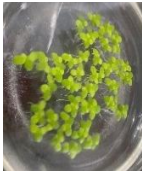
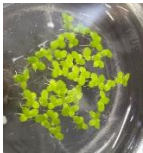
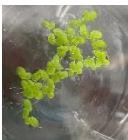
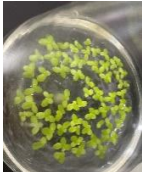
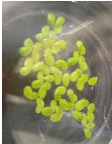
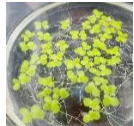
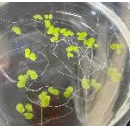
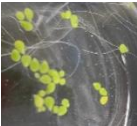
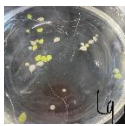
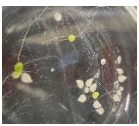
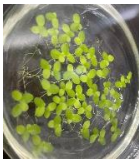
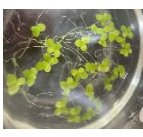
In HS-A&H medium, all duckweed species exhibited a similar growth inhibition

pattern due to Se(VI), which was characterized by a linear decrease in RGR as Se(VI) concentration increased from 10 to 100 mg-Se/L (Figure 4-1c). It should be noted that the growth inhibition of duckweed species by Se(VI) under high-sulfate concentration conditions was much less severe than that by Se(IV) which caused severe growth inhibition even at 2–4 mg-Se/L. Generally, RGR was significantly higher for LAp and La compared with that for the other duckweeds ($p < 0.05$). Notably, these two duckweeds maintained more than 90% of healthy fronds at up to 40 mg/L of Se(VI). In addition, despite the considerable reduction in RGR at 40 mg-Se/L Se(VI), Lg also exhibited healthy fronds (approximately 80 %) at this concentration (Figure 4-2b). However, 60 mg-Se/L Se(VI) caused not only a decrease in RGR but also the discoloration and necrosis of most duckweed fronds. In contrast, LAp and La maintained 90 and 20% of healthy fronds, respectively, whereas, at 100 mg-Se/L Se(VI), no healthy fronds were observed in any duckweed (Table 4-4). The observed healthy fronds, together with the higher RGR at all Se(VI) concentrations ($p < 0.05$), indicated a higher tolerance of LAp and La to Se(VI) under high-sulfate conditions.

In LS-A&H medium, Se(VI) showed a much more severe inhibitory effect on the growth of all duckweeds than that of HS-A&H medium (Figure 4-1c) ($p < 0.05$), which was almost comparable to the inhibitory effects of Se(IV). This was consistent with previous findings on Lm by Severi (2001), who argued that low-sulfate concentrations cannot compete for the same carrier on the cell membrane as Se(VI), leading to higher disruptive effects by Se(VI). Exposure to 4 mg-Se/L Se(VI) considerably reduced the RGR or completely inhibited the growth of all duckweeds; however, La, LAp, and Lg maintained > 50 % of healthy fronds even at this concentration (Table 4-5) (Figure 4-2d). Overall, a relatively higher RGR along with higher proportions of healthy fronds in LAp

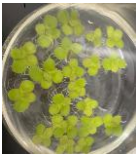
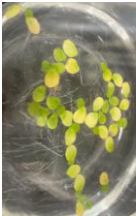
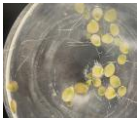
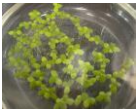
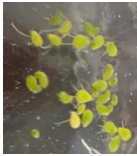
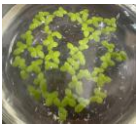
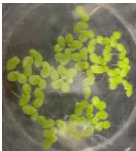
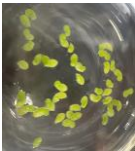
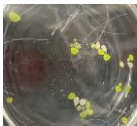
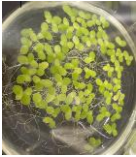
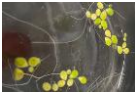
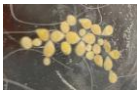
and La evidenced their relatively higher tolerance to Se(VI) under low-sulfate conditions among the tested duckweed species.

Table 4-4 Appearance of duckweed species at the end of the Se(VI) tests in HS-A&H medium.

Duckweed	Initial Se(VI) concentration (mg-Se/L)					
	0 (Control)	10	20	40	60	100
Sp						
LAp						
La						
Lg						
Lj						
Lm						

Sp, *Spirodela polyrhiza*; LAp, *Landoltia punctata*; La, *Lemna aequinoctialis*; Lg, *Lemna gibba*; Lj, *Lemna japonica*; Lm, *Lemna minor*

Table 4-5 Appearance of duckweed species at the end of the Se(VI) tests in LS-A&H medium.

Duckweed	Initial Se(VI) concentration (mg-Se/L)			
	0 (Control)	1	2	4
Sp				
LAp				
La				
Lg				
Lj				
Lm				

Sp, *Spirodela polyrhiza*; LAp, *Landoltia punctata*; La, *Lemna aequinocialis*; Lg, *Lemna gibba*; Lj, *Lemna japonica*; Lm, *Lemna minor*

The difference in the toxicity of Se(IV) and Se(VI) on duckweed growth could be

explained by the difference in their metabolism mechanisms. Li et al. (2020) demonstrated that Se(IV) conversion into organic Se species occurs more rapidly than that of Se(VI) in *Lemna minuta*, which may lead to the formation of malformed selenoproteins that disrupt cellular metabolism, inducing physiological stress in plants (Severi, 2001).

4.3.1.3. Selection of candidate duckweed species for Se removal

Mean Z-scores were calculated for each duckweed across all Se concentrations in the Se tolerance tests based on the RGR reduction and observations of frond health. Data for duckweed grown on 100 mg-Se/L Se(VI) in HS-A&H medium were excluded as no healthy fronds were observed (Table 4-6). Following normalization, the Z-scores for each duckweed across all Se(IV)/Se(VI) concentrations were aggregated to calculate an overall score in each growth medium (HS-A&H and LS-A&H). The six duckweeds were ranked, based on mean and individual Z-scores, for their tolerance against Se(IV) in the order of LAp, La, Lm > Sp, Lj ≥ Lg in HS-A&H medium, and LAp, La, Lm ≥ Lj > Sp, Lg in LS-A&H medium. As for tolerance against Se(VI), they were ranked in the order of LAp, La > Lm ≥ Sp, Lg, Lj in HS-A&H medium, and LAp, La > Sp, Lg ≥ Lm ≥ Lj in LS-A&H medium.

Table 4-6 Z-scores for the six duckweed species exposed to Se(IV)/Se(VI) based on the RGR reduction and % of healthy fronds under different Se concentrations in high sulfate (HS-A&H) and low sulfate (LS-A&H) conditions.

Duckweed species	HS-A&H medium							LS-A&H medium					
	Se(IV) (mg-Se/L)			Se(VI) (mg-Se/L)				Se(IV) (mg-Se/L)			Se(VI) (mg-Se/L)		
	1	2	4	10	20	40	60	1	2	4	1	2	4
Sp	-0.40	-0.45	-0.82	-1.28	-1.1	-0.21	-0.07	-1.24	-1.51	-0.58	-0.36	-0.29	-0.56
LAp	-0.25	0.02	1.61	0.76	0.49	0.93	1.44	-0.04	0.61	0.88	0.65	0.75	1.07
La	0.84	0.78	0.72	0.78	1.16	1.08	0.71	0.57	0.46	1.32	1.20	1.34	1.20
Lg	-0.62	-0.92	-0.64	-0.24	-0.04	0.008	-0.67	-0.68	-0.97	-1.01	-0.32	0.03	-0.04
Lj	-0.20	-0.75	-0.84	-0.64	-0.83	-1.19	-0.62	0.26	0.62	-0.40	-1.22	-1.09	-0.96
Lm	0.63	1.33	-0.02	0.62	0.33	-0.63	-0.80	1.13	0.79	-0.20	0.05	-0.73	-0.70

RGR, relative growth rate; Sp, *Spirodela polyrrhiza*; LAp, *Landoltia punctata*; La, *Lemna aequinoctialis*; Lg, *Lemna gibba*; Lj, *Lemna japonica*; Lm, *Lemna minor*

The higher tolerance observed in both LAp and La may be attributed to the rapid enhancement of antioxidant enzymatic activity, such as that of guaiacol peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD). This phenomenon reduces the oxidative stress and facilitates the attenuation of toxic inorganic Se(IV) and Se(VI). Previous findings demonstrated that Se exposure enhances GPX, CAT, and SOD activity in LAp and Lm, suggesting a potential link between increased enzyme production and Se detoxification (Severi, 2001; Zhong and Cheng, 2016).

Since both Se(IV) and Se(VI) contamination has been reported in aquatic environments (Conde and Sanz Alaejos, 1997), LAp and La were selected here as the candidate duckweeds for Se phytoremediation for further study.

4.3.2. Se removal of the selected duckweeds

The Se removal potential of the two Se-tolerant duckweed species, LAp and La, was

evaluated at 0.2–1.0 mg-Se/L of Se(IV)/Se(VI). Figures 4-3 and 4-4 show the Se removal amount (μg) in the test system, removal efficiency (%), and biomass increase at different Se(IV)/Se(VI) concentrations under high- and low-sulfate conditions. No significant removal was observed in the controls without duckweed.

4.3.2.1. Se(IV) removal by LAp and La

Healthy, green-colored fronds were observed in both duckweed species across all Se(IV) concentrations under both high and low- sulfate conditions, indicating their potential for sustained growth and prolonged Se(IV) removal. In fact, both duckweed species exhibited a stepwise increase in Se removal amount with increasing Se(IV) concentrations in growth media, regardless of sulfate concentrations after the 7-day test period.

In HS-A&H medium, LAp showed an almost consistent Se(IV) removal efficiency, ranging from 17.6% to 23.9% regardless of the initial Se(IV) concentration (Figure 4-3a). This is associated with a comparable increase in biomass observed in the control without Se and across all Se concentrations, with a slight decline at 1.0 mg-Se/L ($p > 0.05$) (Figure 4-3a). These observations indicated that the Se(IV) concentrations tested here did not affect the Se removal efficiency of LAp. Contrastingly, La showed a higher Se removal efficiency of 40.7% when exposed to 0.2 mg-Se/L of Se(IV), which decreased to 19.0% when grown on 1.0 mg-Se/L Se(IV) (Figure 4-3b). However, the substantial biomass growth of La indicated that growth capacity was not influenced by the Se concentrations tested here ($p > 0.05$) (Figure 4-3b).

In LS-A&H medium, the Se removal efficiency of LAp decreased linearly from 33.5%

to 17.8% with an increase in initial Se(IV) concentration from 0.2 to 1.0 mg-Se/L (Figure 4-3a). Moreover, biomass growth was significantly reduced after exposure to 1.0 mg-Se/L (Figure 4-3a). For La, Se(IV) removal efficiency decreased from 40.7% to 18.0% in a pattern similar to that under high-sulfate conditions, while biomass growth remained similar across all Se(IV) concentrations, with a slight decrease when grown on 1.0 mg-Se/L ($p > 0.05$) (Figure 4-3b). Overall, the higher ratio of Se removal by La compared with that by LAp, together with a consistent biomass increase, suggested that La could be a preferred Se(IV) removal agent than LAp under both high and low-sulfate conditions.

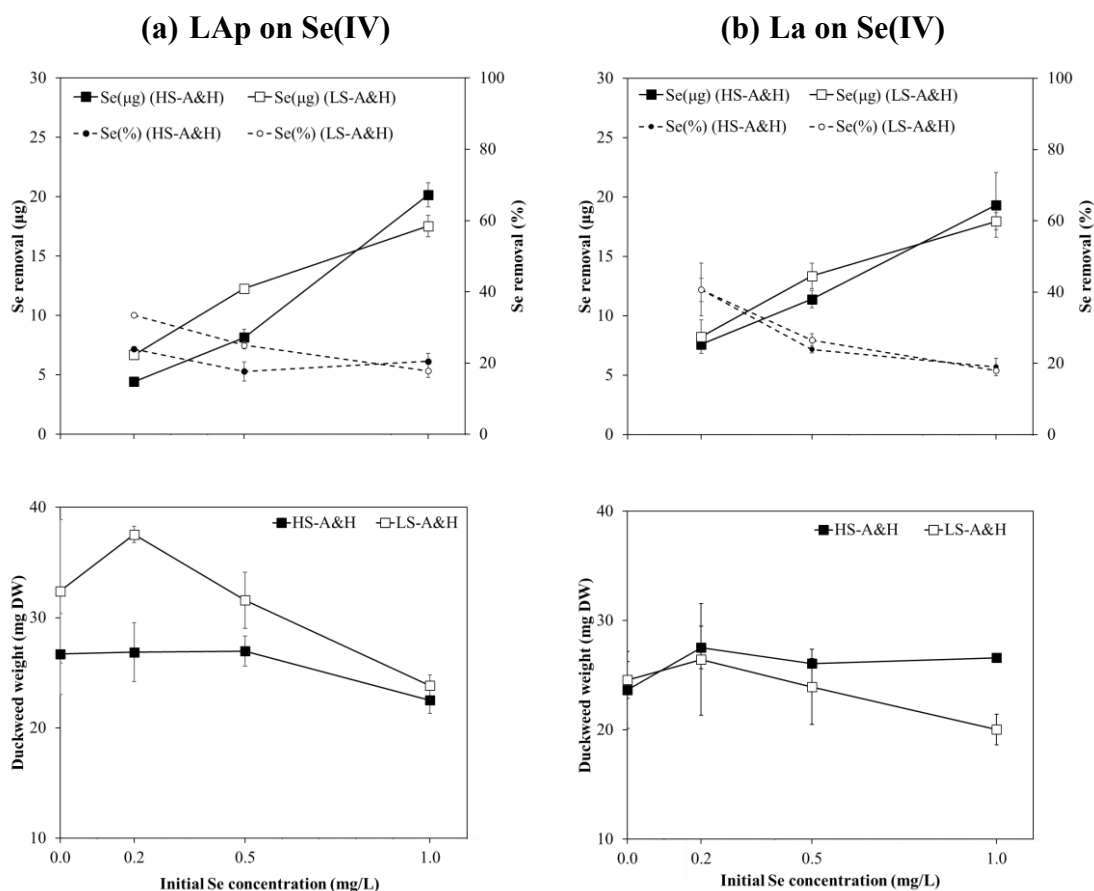


Figure 4-3 Se removal amount (μg), removal efficiency (%), and biomass growth (mg DW) of duckweeds grown at different Se(IV) concentrations and on different media. (a) Se removal and growth of LAp and (b) Se removal and growth of La. Values are mean $\pm$ standard deviation ($n = 3$). LAp, *Landoltia punctata*; La, *Lemna aequinoctialis*.

4.3.2.2. Se(VI) removal by LAp and La

In both HS-A&H and LS-A&H media, LAp and La exhibited healthy fronds across all Se(VI) concentrations, as was the case in the Se (IV) removal tests, thus indicating their ability to grow sustainably on Se(VI).

In HS-A&H medium, both LAp and La exhibited low Se(VI) removal efficiencies of less than 5% across all Se concentrations, indicating their limited capacity to remove

Se(VI) when grown in high-sulfate concentrations of 203 mg/L (Figure 4-4). At the used concentrations, Se(VI) had no significant effect on biomass growth in either species. This observation is in agreement with the findings of Gupta and Gupta (2017), regardless of their study focusing on other higher plants. They reported that Se(VI) transporters in plant roots show higher selectivity for sulfate over Se(VI) under high exogenous sulfate concentrations, thus reducing Se(VI) uptake by plants. This mechanism could explain the inability of the two duckweeds to remove Se(VI) under the high-sulfate concentration conditions in the present study.

In contrast, LAp and La exhibited a stepwise increase in Se(VI) removal amount associated with an increase in Se(VI) concentration from 0.2 to 1.0 mg-Se/L in LS-A&H medium. Se(VI) removal efficiency by LAp was comparable to that of Se(IV) at initial Se concentrations of 0.2 and 0.5 mg-Se/L, showing a linear decreasing pattern in Se removal efficiency across all Se concentrations ($p > 0.05$) (Figure 4-4a). Specifically, Se removal decreased from 32.6% at 0.2 mg-Se/L to 19.9% at 0.5 mg-Se/L and 14.9% at 1.0 mg-Se/L. Furthermore, the biomass yield of LAp decreased from 26.6 to 17.3 mg DW, which was associated with increased Se(VI) concentrations (Figure 4-4a). A maximum Se(VI) removal efficiency of 41.2% was detected when La was exposed to 0.5 mg-Se/L, with no significant differences across all Se(VI) concentrations ($p > 0.05$) (Figure 4-4b). However, La growth decreased linearly from 24.6mg DW at 0.2 mg- Se/L to 13.4mg DW at 1.0 mg-Se/L (Figure 4-4b), though it showed similar Se(VI) removal amounts when exposed to 0.5 and 1.0 mg-Se/L.

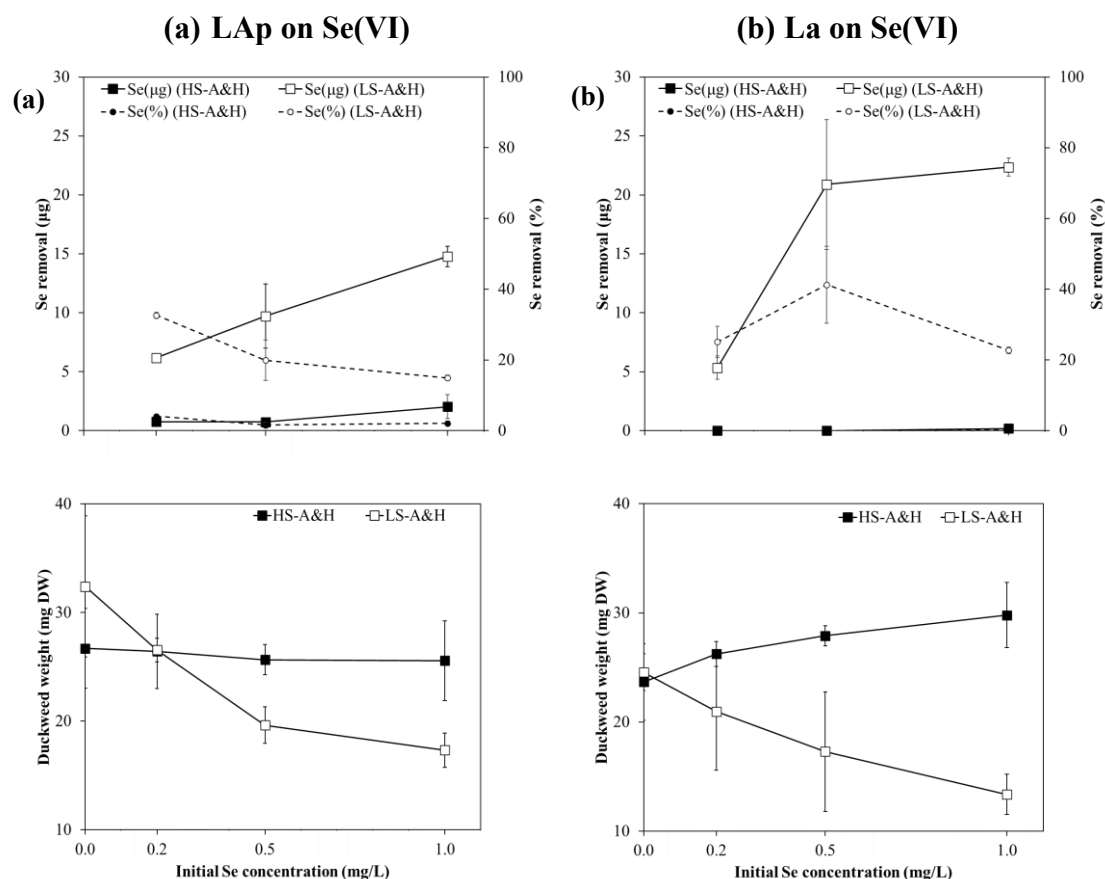


Figure 4-4 Se removal amount (μg), removal efficiency (%), and biomass growth (mg DW) of duckweeds grown at different Se(VI) concentrations and on different media. (a) Se removal and growth of LAp and (b) Se removal and growth of La. Values are mean $\pm$ standard deviation ($n = 3$). LAp, *Landoltia punctata*; La, *Lemna aequinoctialis*.

Based on the results across all removal tests, La was identified as a preferred Se phytoremedial agent under both high- and low-sulfate conditions. Specifically, La showed comparable and higher Se removal efficiencies than those of LAp at 0.2 mg-Se/L Se (IV) and 0.5–1.0 mg-Se/L Se(VI), respectively. This robust performance across a broad range of Se concentrations, commonly found in Se-impacted environments, highlights its suitability for practical application in diverse contaminated environments.

4.3.2.3. Accumulation of Se by LAp and La

Se accumulation by LAp and La during the Se(IV)/Se(VI) removal tests varied depending on the chemical form and initial Se concentration (Figure 4-5). For both duckweeds, the increase in Se accumulation was significantly correlated with the increase in the initial Se(IV)/Se(VI) concentration ($p < 0.01$).

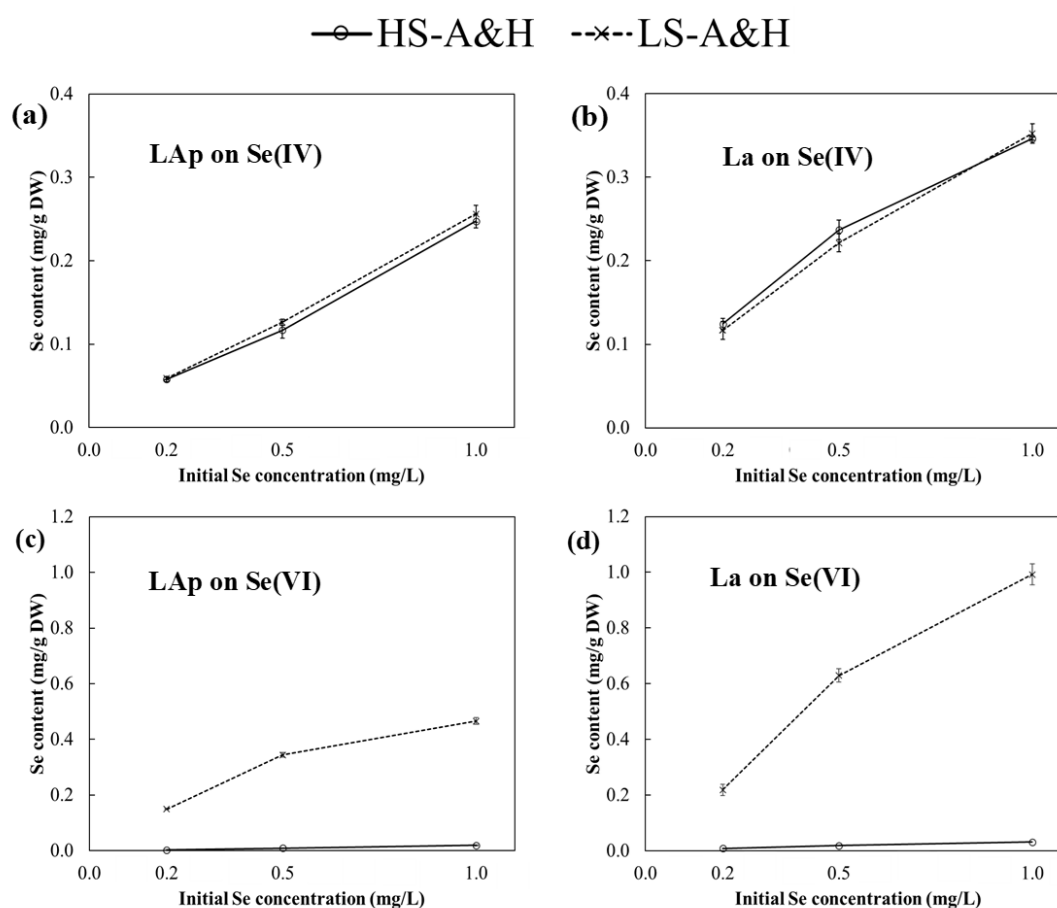


Figure 4-5 Se content (mg-Se/g DW) in duckweeds grown at different Se concentrations and on different media. (a) LAp grown on Se(IV), (b) La grown on Se(IV), (c) LAp grown on Se(VI), and (d) La grown on Se(VI). LAp, *Landoltia punctata*; La, *Lemna aequinoctialis*.

No significant difference was detected in the accumulation of Se by both LAp and La grown under high and low-sulfate conditions in the Se(IV) removal tests, indicating negligible sulfate effects on Se(IV) accumulation by both duckweed species (Figure 4-5a and b) ($p > 0.05$). Se content ranging from 0.11 mg/g at 0.2 mg-Se/L to 0.35 mg/g at 1.0 mg-Se/L and from 0.06 mg/g at 0.2 mg-Se/L to 0.26 mg/g at 1.0 mg-Se/L in La and LAp, respectively, indicated that La could accumulate more Se than did LAp.

Both LAp and La accumulated minimal amounts of Se in the Se(VI) removal tests using HS-A&H medium, which was in coherence with the negligible Se(VI) removal efficiency by both duckweed species in this series of tests. However, in Se(VI) removal tests under low-sulfate conditions, both LAp and La exhibited considerable Se accumulation ranging from 0.15 mg/g at 0.2 mg-Se/L to 0.47 mg/g at 1.0 mg-Se/L in LAp (Figure 4-5c) and from 0.22 mg/g at 0.2 mg-Se/L to 0.99 mg/g at 1.0 mg-Se/L in La (Figure 4-5d), which were notably higher than those shown in the Se(IV) removal tests using LS-A&H medium. La accumulated Se at high levels compared with those in LAp, irrespective of the Se chemical form and concentration.

The high Se content achieved by La, together with the high biomass production (Figure 4-3b and 4-4b), indicated its potential for valorization as a micronutrient source, as previously discussed in Section 3.3.5. Consequently, all La exposures, except for the 0.2 mg-Se/L Se(IV) treatment, are suitable for several applications, since their Se content in biomass is > 0.17 mg-Se/g DW. These applications include their use as a Se-enriched biofertilizer and as a substrate for AD for the production of methane and organic acids. Furthermore, La biomass, regardless of the Se concentration tested, could also be utilized as animal or human feed.

4.3.2.4. Se recovery by LAp and La

Se mass balance was performed to determine the recovery fraction of Se by the duckweeds. This was assessed by examining the Se content and removal in duckweed biomass and from the test system, respectively. This assessment aimed to quantify the fraction of Se potentially recovered as duckweed biomass for use as a resource. The recovery is summarized in Table 4-7.

Table 4-7 Recovered Se (%) by duckweed after the 7-day removal tests in HS-A&H and LS-A&H media.

Duckweed (medium)	Se(IV) (mg/L)			Se(VI) (mg/L)		
	0.2	0.5	1.0	0.2	0.5	1.0
LAp (HS-A&H)	43.6±8.5	47.8±8.7	34.9±3.2	N.A.	N.A.	N.A.
LAp (LS-A&H)	38.1±1.7	38.6±3.9	43.1±1.6	78.3±4.5	95.9±21.5	72.8±5.4
La (HS-A&H)	52.7±5.6	63.8±4.6	56.8±8.0	N.A.	N.A.	N.A.
La (LS-A&H)	44.1±6.2	46.9±1.1	48.1±0.4	106.5±23.5	68.6±16.6	79.1±6.0

Values are mean ± standard deviation ($n = 3$). N.A.: not applicable. LAp, *Landoltia punctata*; La, *Lemna aequinoctialis*

Overall, Se recovery as duckweed biomass ranged between 35% and 65% in the Se(IV) removal tests. A higher Se recovery of more than 70% was observed in the Se(VI) removal tests, while La exposed to 0.2 mg-Se/L and LAp exposed to 0.5 mg-Se/L achieved 100% Se recovery from the system. The observed loss of Se from the system could be attributed to the removal of Se adsorbed on the duckweeds during the washing step after biomass cropping for analyses. As demonstrated in Chapter 3, duckweeds are efficient Se biosorbents, implying that a fraction of the removed Se could be held on the plant/root

surface. This observation suggests that higher Se recovery could be achieved if the washing step at the end of the removal tests was avoided. Another potential pathway for Se loss from the system could be Se volatilization. Although Se volatilization has not been studied in duckweeds, it has been noted to occur in other aquatic plants at higher rates for Se(IV) than for Se(VI) (Pilon-Smits et al., 1999).

4.4. Summary

In this chapter, six duckweed species were examined for their Se tolerance to screen duckweeds which can be utilized in long-term phytoremediation of Se-contaminated aquatic environments. Two duckweed species, LAp and La, were identified to have a high Se tolerance among six duckweed species in the Se tolerance tests, making them suitable candidates for sustainable Se-phytoremedial agents. Their Se(IV)/Se(VI) removal capacity was evaluated under high and low-sulfate conditions. The results revealed that LAp and La could substantially remove a broad range of soluble Se(IV) and Se(VI) concentrations, with removal efficiencies higher than 40% while maintaining healthy and sustainable growth. However, Se(VI) removal and accumulation by both duckweeds were severely impaired under high-sulfate conditions due to the uptake preference of sulfate over Se(VI). A substantial portion of the removed Se was confirmed to accumulate in duckweed biomass and can be repurposed as a biofertilizer, AD stimulant, and animal or human feed. The experimental results strongly suggested that LAp and La are promising duckweed species that can be utilized for the phytoremediation of Se contamination, simultaneously achieving the recovery of Se as a resource. However, considering sulfate concentration as an inhibitor of Se(VI) removal is necessary. Based on the comparative

evaluation of the Se removal and accumulation capabilities, La appears to be a more suitable Se phytoremediation agent than LAp.

Chapter 5: Summary and conclusions

Despite the benefits of Se, being utilized as a micronutrient and a key element in various industries, its toxicity at elevated concentrations poses significant risks to natural aquatic ecosystems and human health. Phytoremediation by duckweed is considered a promising alternative technology for successfully removing Se from aquatic environments, possibly offering a cost-effective and eco-friendly solution. However, for practical applications, further studies for optimization of duckweed-based phytoremediation is necessary. Particularly the potential of live duckweed as a biosorbent for Se removal has not been examined at all, and also long-term remediation sustainability has not been fully investigated using various duckweed species. This study aimed to select effective live duckweeds for fast Se biosorption, and identify Se-tolerant species capable of sustainably removing Se during the treatment period.

Chapter 1 presented the general introduction, reviewing the necessity and the risks associated with Se toxicity, as well as the limitations and applicability of current Se removal technologies. Based on the remarkable potential of duckweed-based phytoremediation of Se contamination, the objective of this study was set as mentioned above.

Chapter 2 established the background for this study by systematically reviewing literatures on global aquatic Se pollution between 2015 and 2024. This review identified that most prevalent pollution levels range from 100 to 1000 $\mu\text{g/L}$; however, concentrations as high as 4475 $\mu\text{g/L}$ were generated from anthropogenic sources. These findings were essential to define the environmentally relevant concentrations applied in

the experimental designs in Chapters 3 and 4.

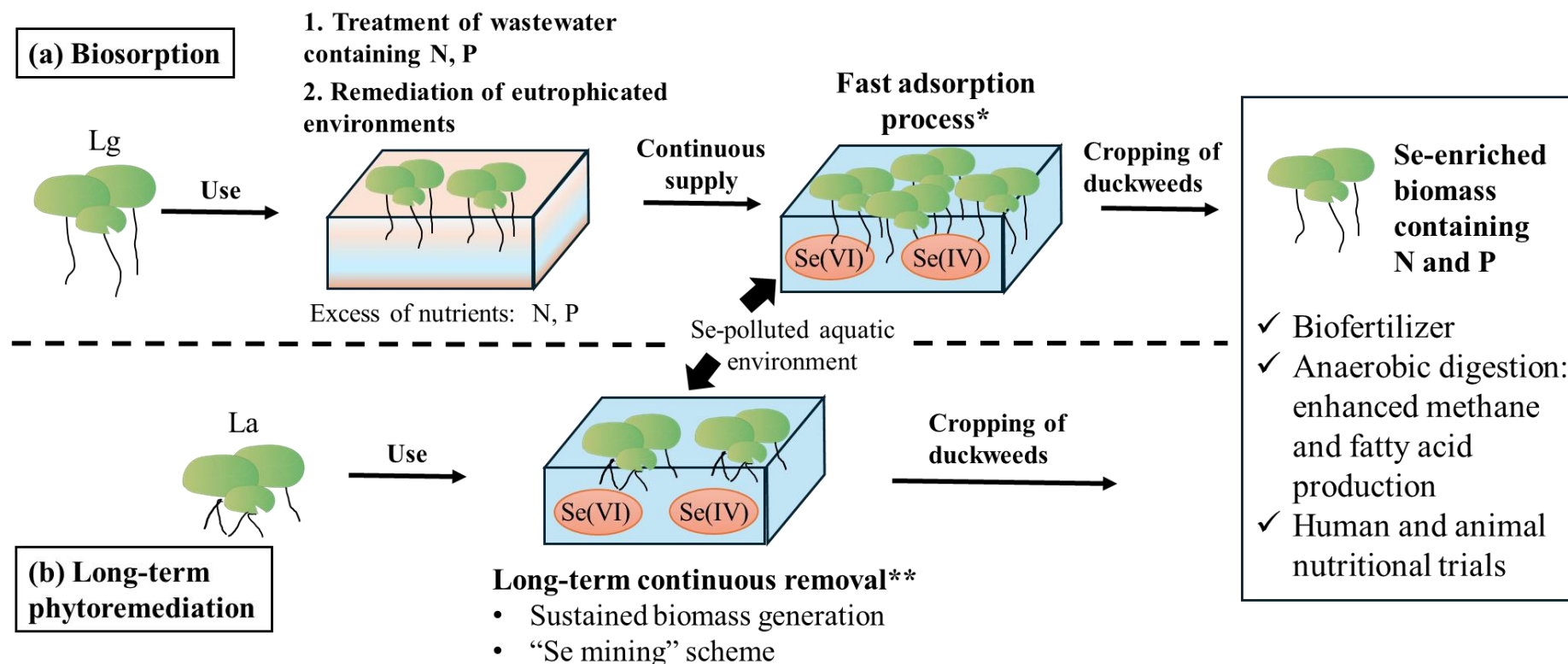
Chapter 3 introduced a novel approach utilizing live duckweed as a biosorbent for soluble Se removal. In this study, Lg was identified as a promising candidate and was further evaluated for its Se removal properties. Lg successfully removed significant amounts of Se(VI) and Se(IV) at their environmentally relevant concentrations within a short period of namely 90 min, with the adsorption pattern best described by the Sips isotherm model. Comparison of the Se adsorption capacity of Lg with those of typical reported sorbents confirmed its feasibility to remove soluble Se, while recovering it as a potential resource for various uses.

In Chapter 4, experimental studies were done for selecting Se-tolerant duckweeds suitable for long-term Se phytoremediation. Among the evaluated species, LAp and La were capable of sustaining high removal efficiencies of both Se(VI) and Se(IV) at relevant environmental concentrations while maintaining healthy growth. However, this study also noted the necessity of considering sulfate concentration as a potential inhibitor for Se(VI) removal. Notably, La demonstrated superior Se removal and accumulation capabilities compared to LAp, which suggested its potential for valorization.

This study established two distinctive approaches utilizing duckweed species for Se phytoremediation while recovering Se as a resource. Lg was confirmed as a superior biosorbent for rapid Se removal, outperforming typical sorbents, whereas La was identified as a promising agent for long-term phytoremediation, exhibiting sustained growth and high removal efficiency suitable for continuous water treatment systems.

Figure 5-1 synthesizes these approaches into a practical framework. The first process utilizes Lg as a biosorbent for rapid removal of Se (Figure 5-1a). This approach can be coupled with the phytoremediation of nutrient-rich water/wastewater. Excess biomass of

Lg used for the treatment of wastewater or cleanup of eutrophicated environments containing N and P, serves as a supply source of biosorbents for subsequent Se removal. On the other hand, for long-term remediation, the Se-tolerant duckweed, specifically La can be applied directly to Se contaminated water bodies for continuous removal of Se(VI)/Se(IV) during its sustainable growth (Figure 5-1b). The use of La ensures consistent Se removal efficiencies while generating high yields of Se-enriched biomass, representing a scheme of “sustainable Se mining”. Furthermore, the resulting biomass from both short-term and long-term processes is repurposed as fertilizer, anaerobic digestion stimulant, or for animal and human feed, contributing to a circular economy through resource recovery.



* Available for Se(VI) removal at concentrations > 0.2 mg-Se/L

** Available for Se(VI) removal when $[\text{SO}_4^{2-}] < 203$ mg/L

Figure 5-1 Strategies for the use of Lg (*Lemna gibba*) and La (*Lemna aequinoctialis*) in selenium (Se) removal from polluted aquatic environments, including the potential uses of Se-enriched biomass after phytoremediation. (a) Application as a fast biosorbent and (b) as a long-term phytoremedial agent.

To ensure the practical viability of the proposed duckweed-based remediation systems, further evaluations under conditions present in real-world Se-contaminated environments are required. These are outlined as follows.

(1) The impact of co-pollutants should be evaluated, as they could affect the Se removal efficiency of duckweed through various mechanisms. These include competition for sorption sites, alterations in plant morphology, or elevated toxicity that could disrupt sustainable growth.

(2) The influence of biotic interactions within the aquatic environment also warrants consideration. Future studies should elucidate the role of local microbial communities, which may enhance remediation (e.g. through plant-growth-promoting bacteria), while also evaluating competitive interactions (e.g. with algae) that could inhibit duckweed growth, thus affecting its remediation capacity.

(3) Validating the performance of duckweed-based phytoremediation systems under fluctuating environmental conditions (e.g. temperature and pH) is crucial for field implementation. Therefore, future research should implement pilot-scale tests for assessing system effectiveness and identifying necessary improvements. Within these evaluations, quantifying Se loss through phytovolatilization, especially under Se(IV) exposure, should be considered during long-term exposure to establish a complete mass balance.

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Achievements

Publications

1. **Ascencio-Damian J.E.**, Inoue D., Ike M. (2025) Screening of selenium-tolerant duckweeds and evaluation of their selenium removal capability. *Environmental Technology & Innovation*, 40, 104486. <https://doi.org/10.1016/j.eti.2025.104486>
2. **Ascencio-Damian J.E.**, Inoue D., Ike M. (2025) Selection of duckweed as a biosorbent for efficient soluble selenium removal. *Japanese Journal of Water Treatment Biology*, 61(4), 81-93. (In press).
3. **Ascencio-Damian J.E.**, Inoue D., Ike M. A systematic review of selenium pollution in global aquatic environments: Sources, levels, and co-pollutants. *Environmental Quality Management*. (Under review)

International and domestic conferences

1. **Ascencio-Damian J.E.**, Inoue D., Ike M. (2023) Evaluation of phytoremediation potential of six duckweed species for selenium contamination. The Water and Environment Technology Conference 2023 (WET2023), online.
2. **Ascencio-Damian J.E.**, Inoue D., Ike M. (2024) Removal and recovery of selenate and selenite using an aquatic plant. International Symposium on Resilient Planet, New Delhi, India.
3. **Ascencio-Damian J.E.**, Inoue D., Ike M. (2024) Recovery of critical elements using a duckweed system. Sustainable Remediation of Radionuclide Impacts on Land and Critical Materials Recovery (SURRI) Southampton Workshop, online.

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