# Comparison of Lemna minor and Spirodela polyrhiza duckweed's ability to remove cadmium and lead from heavy metals polluted water

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**ABSTRACT**

Excluding heavy metals in water using aquatic plants is a recent trend that has attracted a lot of attention due to its effectiveness and environmental friendliness. In this study, the adaptability and accumulation of cadmium and lead in polluted water environments by *Lemna minor* (L) and *Spirodela polyrhiza* (S) collected in Dalat (Lam Dong) – $L_{DL}$, $S_{DL}$ and Phan Thiet (Binh Thuan) – $L_{PT}$, $S_{PT}$, Vietnam were investigated. The results showed that the adaptability and growth of Lemna minor followed the order: $L_{PT} > L_{DL} > S_{DL} > S_{PT}$. The study also examined the optimal environmental conditions for the growth of the plant, which were a pH of 8.43, a temperature of 23.57°C, and a growth period of 13.79 days. Furthermore, in a cadmium and lead-polluted environment, both species of plants showed a higher adaptability to lead pollution compared to cadmium pollution. While *Lemna minor* had a higher ability to accumulate Cd in biomass than Pb, the opposite was observed in *Spirodela polyrhiza*.

**Keywords:** duckweeds, *Lemna minor, Spirodela polyrhiza*, accumulation, cadmium, lead.

## 1. Introduction

The pollution rate is increasing rapidly with increasingly serious levels that have had a big impact on the global ecosystem. Among them, heavy metal pollution, especially in water sources, has caused many repercussions, adversely affecting the life and survival of organisms, and is one of the urgent issues that need effective solutions [1]. When water sources are contaminated with heavy metals, it can endanger the ecosystem as well as human health. In the environment, cadmium is one of the most toxic metals that can be observed [2, 3]. It is important to note that cadmium and its compounds pose significant health hazards. They are known to cause cancer in multiple systems, including the cardiovascular, renal, digestive, nervous, reproductive, respiratory, and other organs. Cadmium, due to its low excretion capacity from the body, can cause combined toxicity when combined with other metals, making it particularly hazardous [4]. This toxicity directly affects the kidneys, increases blood pressure, and can lead to kidney and cardiovascular diseases, posing a significant risk to human life [5]. Lead, another heavy metal found in the environment as a consequence of industrial activities, lead smelting and refining, mining, and so on [4], is known to contribute to high blood pressure, reproductive abnormalities, as well as nervous and metabolic disorders in humans [6, 7]. Inhalation of lead can result in poisoning and damage to various organs such as the liver and kidneys, leading to heart problems and brain damage, weakened bones, and overall damage to the nervous system. Acute lead poisoning can cause symptoms such as memory loss, headaches, and nausea [8].

The reduction of heavy metal content in water sources is of great significance in protecting the environment and human health. Various methods have been proposed as potential solutions for treating heavy metals in water. These include membrane filtration, ultrafiltration, solvent extraction electrolysis, ion exchange, reverse osmosis, chemical and electrochemical precipitation, as well as coagulation [9], ... However, most of these methods are expensive and may only be suitable when the concentration of heavy metal ions is relatively high. Recent studies have indicated that certain plant species can thrive in water environments contaminated with heavy metals and have the ability to accumulate these metals in their biomass [10]. Utilizing plants to absorb heavy metals from water is considered an environmentally friendly and cost-effective approach that is also easy to implement [11]. Furthermore, it can be combined with other methods to enhance treatment efficiency. Duckweed, which is a collective term encompassing a group of floating aquatic plants in the *Lemnaceae* family, is found on every continent except Antarctica. Among the plant species capable of acting as bioaccumulation agents, the *Lemnaceae* duckweed is of particular interest because they possess many advantages that are rarely found in aquatic and terrestrial plants, such as small size, the ability to grow the fastest among flowering plant groups, adaptability to various ecological conditions, ability to absorb many heavy metals, easy-to-harvest biomass for the production of biofuels [12], and more.

Therefore, *Lemnaceae* duckweed is one of the very promising subjects for application in treating water sources contaminated with heavy metals. In this study, the ability to absorb cadmium and lead metals in water environments by *Lemna minor* and *Spirodela polyrhiza* duckweeds in the laboratory range was investigated to evaluate the efficiency of Lemna minor duckweed in treating heavy metal-contaminated water. The impact of factors such as water pH, heavy metal concentration, and growth time on the level of metal ion accumulation on duckweed biomass was also studied to determine the optimal conditions for these duckweed species to adapt, grow, and develop well. Comparing the adaptability, growth, and development in heavy metal-polluted environments, as well as the ability to accumulate heavy metals in biomass, among different species of aquatic plants is the basis for selecting a species of plant with effective water environment remediation capabilities.

## 2. EXPERIMENTAL

### 2.1. Material and Methods

#### 2.1.1. Sample collection

The survey was conducted on individuals of *Lemna minor* and *Spirodela polyrhiza* duckweed collected from two different regions, including: Phong Nam (Phan Thiet, Binh Thuan): *Lemna minor* is designated as $L_{PT}$, *Spirodela polyrhiza* is designated as $S_{PT}$; Cam Ly (Da Lat, Lam Dong): *Lemna minor* is designated as $L_{DL}$, *Spirodela polyrhiza* is designated as $S_{DL}$.

#### 2.1.2. Cultivation of duckweed

Before arranging the experiment, sterilization was carried out to eliminate the presence of microorganisms in the samples. The samples were rinsed under running water, then placed in an aseptic cabinet to be washed with 70% ethanol solution for 30 seconds and washed again with sterilized water at least 3 times. Continue to place the samples in a 0.9% bleach solution for 30 seconds, gently shake, and wash again 3 times with sterilized water. Select the duckweed samples that are less damaged and cultivate them in the nutrient medium to use as a sample source for the experiments. The sterilized samples are grown in a sterilized nutrient medium to obtain a sufficient amount of sample for the experiment.

The nutrient medium used to cultivate all samples in the experimental model was the nutrient medium published by Appenroth et al. (1996) [13]. Depending on each experiment, heavy metal ions such as cadmium and lead were used from $CdCl_2$ and $Pb(NO_3)_2$ salt solutions with different concentrations. After preparing the medium, it was poured into 1.5L flasks, tightly closed with nylon membrane, and sterilized at 121ºC for 20 minutes. After that, it was taken out of the sterilizer, allowed to cool, and then the experiment was carried out. The experiments were conducted at an appropriate temperature and were illuminated with white LED lights for 16 hours per day.

The optimal growth conditions for duckweed species were determined using the response surface methodology and the Design Expert 12.0.3.0 software [14]. To investigate the possible interactions between the studied parameters and their effects on duckweed development, the Box-Behnken design was employed. pH of water (5–8.5), temperature (18–25◦C) and the grow time (2–16 day) are variable input parameters. The factor levels were coded as −1 (low), 0 (central point or middle) and 1 (high) [15]. The survey model consists of 17 experiments, of which 5 were conducted at the center of the experimental area. In Table 1, the experimental parameters and corresponding levels of the Box-Behnken design are provided.

**Table 1. The levels of the selected variables in the experimental design**

| **Variables** | **Unit** | **Level in Box-Behnken design** | | |
|---|---|---|---|---|
| | | **Low (-1)** | **Middle (0)** | **High (+1)** |
| pH of water | | 5 | 6.75 | 8.5 |
| Temperature | ºC | 18 | 21.5 | 25 |
| Time | day | 2 | 9 | 16 |

### 2.2. General Procedure

The formulae were contaminated with cadmium at concentrations of 0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, and 0.40 mg/L and lead at concentrations of 0, 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, 1.40, and 1.60 mg/L. The growth and development of the duckweed were monitored for 25 days and 50 days. After 25 days and 50 days, the amount of accumulated cadmium and lead in the biomass of the duckweed was analyzed in order to assess the level of accumulation of each species of duckweed. Besides, control area was prepared by growing these duckweeds species under the same conditions as the models mentioned above in water uncontaminated.

### 2.3. Detection Method

For sample preparation and quantification, heat drying, microwave-assisted acid digestion, and graphite furnace atomic absorption spectrophotometry techniques were employed. These methods were adopted based on the procedures outlined in Romero-Estévez et al. [16].

Upon completion of the investigation period, the plants were carefully extracted from the stock solution and thoroughly rinsed. Subsequently, they were subjected to drying at a temperature of

60°C in a Memmert UM 500 stove (Schwabach, Germany) until reaching a constant weight. The dried plant samples were individually homogenized using a porcelain mortar.

For the acid digestion process, 0.5000 grams of each sample were precisely weighed and placed into Teflon vials. Gradually, 5 mL of 70% nitric acid (Merge, Germany) and 3 mL of 30% hydrogen peroxide (Merge, Germany) were added. Acid digestion was accomplished using a MARS 6 microwave (CEM, Matthews, NC, USA).

To quantify the concentrations, the filtered digestions were subjected to analysis. An absorption spectrophotometer coupled with a graphite furnace (AA7000, Shimadzu, Japan) was employed to determine the levels of Cd and Pb.

### 2.4. Data analysis

Data for evaluation were obtained in triplicate (n = 3) and are presented as the average ± standard deviation (SD). Excel 2013 software was utilized to create the database and generate diagrams.

## 3. Results and discussion

### 3.1. Optimal growth conditions for duckweed species

The results of the survey on the optimal growth conditions of duckweed species are presented in Table 4. Under these conditions, the rate of increase in surface area of duckweed species is shown in Table 5.

The results show that *Lemna minor* has a better ability to adapt, grow, and develop than *Spirodela polyrhiza*. Among them, the *Lemna minor* collected in Phan Thiet (Binh Thuan) has a better growth and development ability than the species collected in Da Lat (Lam Dong). The opposite is true for *Spirodela polyrhiza* species.

The response variable, the rate of increase in surface area (%), was assessed based on the design matrix, and the obtained results are presented in Table 2. Figures 1, 2, 3, and 4 portray the response surface plots, illustrating the relationship between pH, temperature, and growth duration on the rate of increase in surface area for the examined species of duckweed.

The analysis of variance (ANOVA) reveals a linear relationship between the main effects of pH, temperature, and time, as well as a quadratic relationship involving these factors. The Design-expert software determined the final mathematical equation in terms of natural factors, with a confidence level above 95%. The equation is presented below:

$$S_{L(PT)} = 213.82 + 98X_1 + 200.73X_2 – 8.40X_3 + 131.47X_1X_2 – 5.88X_1X_3 – 2.22X_2X_3$$

**Table 2. Experimental and empirical values of Y for four examined duckweed species**

| Std | Run | Factor 1 A: pH | Factor 2 B: time (day) | Factor 3 C: temp (ºC) | Response 1 S of L(PT) (%) | Response 2 S of L(DL) (%) | Response 3 S of S(PT) (%) | Response 4 S of S(PT) (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 5 | 2 | 21.5 | 15.4 | 57.7 | 6.3 | 22.1 |
| 11 | 10 | 6.75 | 2 | 25 | 30.5 | 33.5 | 14.7 | 13.2 |
| 9 | 7 | 6.75 | 2 | 18 | 37.7 | 37.3 | 15.5 | 15.1 |
| 2 | 11 | 8.5 | 2 | 21.5 | 43.5 | 29.5 | 8.7 | 16.1 |
| 7 | 1 | 5 | 9 | 25 | 109.6 | 397.1 | 45.6 | 129.7 |
| 5 | 14 | 5 | 9 | 18 | 119.8 | 417.2 | 40.7 | 130.1 |
| 8 | 8 | 8.5 | 9 | 25 | 198.8 | 173.4 | 31.9 | 53.2 |
| 3 | 15 | 5 | 16 | 21.5 | 214.7 | 1041.3 | 86.2 | 266 |
| 14 | 3 | 6.75 | 9 | 21.5 | 214.8 | 203.7 | 66.7 | 162.3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 15 | 5 | 6.75 | 9 | 21.5 | 219.7 | 198.2 | 68.2 | 165.1 |
| 17 | 13 | 6.75 | 9 | 21.5 | 221.5 | 207.2 | 69.1 | 163.7 |
| 13 | 6 | 6.75 | 9 | 21.5 | 225.8 | 215.3 | 64.3 | 164.9 |
| 16 | 12 | 6.75 | 9 | 21.5 | 232.4 | 195.7 | 65.8 | 159.2 |
| 6 | 17 | 8.5 | 9 | 18 | 232.5 | 192.1 | 38.2 | 56.8 |
| 12 | 9 | 6.75 | 16 | 25 | 366.7 | 468.5 | 118.6 | 251.9 |
| 10 | 16 | 6.75 | 16 | 18 | 382.8 | 492.1 | 35.7 | 270.3 |

**Table 3. Optimal growth conditions of duckweed were collected**

**Factors**

| Factor | Name | Level | Low Level | High Level | Std.Dev. | Coding |
|---|---|---|---|---|---|---|
| A | pH | 8.43 | 5.00 | 8.50 | 0.0000 | Actual |
| B | Time | 13.79 | 2.00 | 16.00 | 0.0000 | Actual |
| C | Temp | 23.57 | 18.00 | 25.00 | 0.0000 | Actual |

$S_{L(DL)} = 204.02 - 155X_1 + 265.27X_2 - 8.28X_3 - 183.7X_1X_2 + 0.35X_1X_3 - 4.95X_2X_3 + 92.39X_1^2 + 55.29\,X_2^2 - 1.46\,X_3^2$

$S_{S(PT)} = 66.82 - 5.70X_1 + 31.21X_2 + 10.09X_3 - 8.55X_1X_2 - 2.80X_1X_3 + 20.92X_2X_3 - 17.47X_1^2 - 10.45X_2^2 - 10.25\,X_3^2$

$S_{S(PT)} = 66.82 - 5.70X_1 + 31.21X_2 + 10.09X_3 - 8.55X_1X_2 - 2.80X_1X_3 + 20.92X_2X_3 - 17.47X_1^2 - 10.45X_2^2 - 10.25\,X_3^2$

$S_{S(DL)} = 163.04 - 30.31X_1 + 112.63X_2 - 3.04X_3 - 20.18X_1X_2 - 6.80X_1X_3 - 4.12X_2X_3 - 43.67X_1^2 + 1.51X_2^2 - 26.92\,X_3^2$

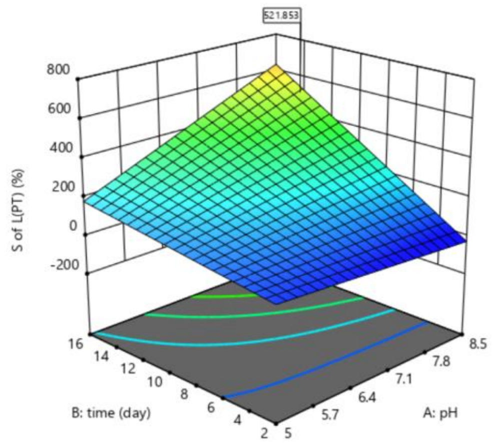



**Figure 1. 3D response surface graph for the rate of increase in surface area of *Lemnar minor PT* vs. pH and time**

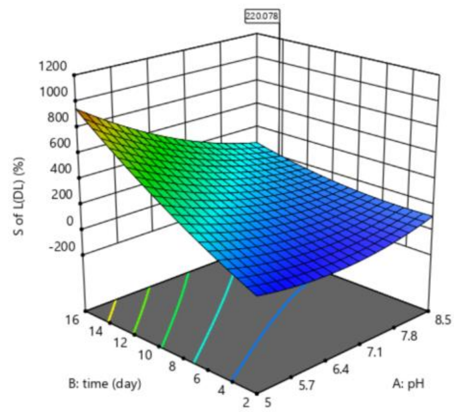



**Figure 2. 3D response surface graph for the rate of increase in surface area of *Lemnar minor DL* vs. pH and time**

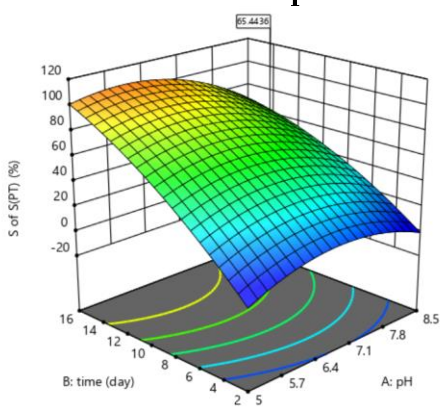



**Figure 3. 3D response surface graph for the rate of increase in surface area of *Spirodela polyrhiza* PT vs. pH and time**

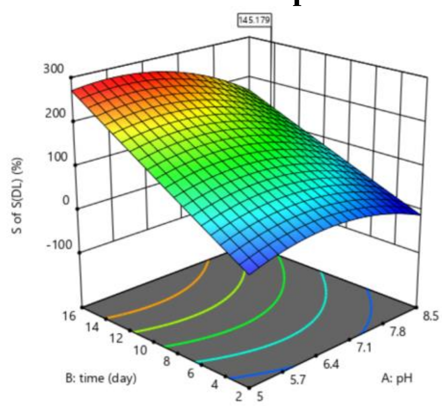



**Figure 4. 3D response surface graph for the rate of increase in surface area of *Spirodela polyrhiza* DL vs. pH and time**

### 3.2. Impact of cadmium and lead on the morphological changes of duckweed

For *Lemna DL* species, under the influence of Cd, after 50 days, samples showed poor growth and development compared to those after 25 days. The samples showed signs of yellowing and weakening leaves at concentrations ranging from 0.25 to 0.4 mg/L.

For both species, under the influence of Cd, after 50 days, samples showed poor growth and development compared to those after 25 days. The samples showed signs of yellowing and weakening leaves at concentrations ranging from 0.25 to 0.4 mg/L. With regards to *Lemna minor* PT species, under the influence of Pb ions, compared to the 25th day, the leaf size on the 50th day was larger, more uniform but more yellowed, and the number of leaves increased rapidly compared to the first day of plant cultivation. As the Pb concentration increased, the leaf size gradually

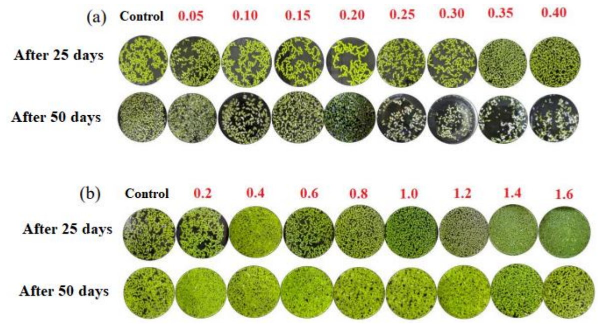



**Figure. Morphological changes of *Lemna minor PT* duckweed in environments supplemented with Cd (a) and Pb (b) after 25 and 50 days**

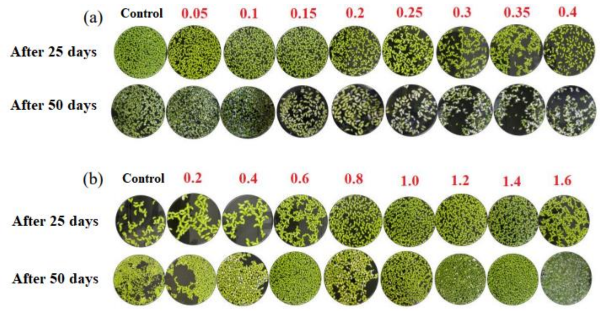
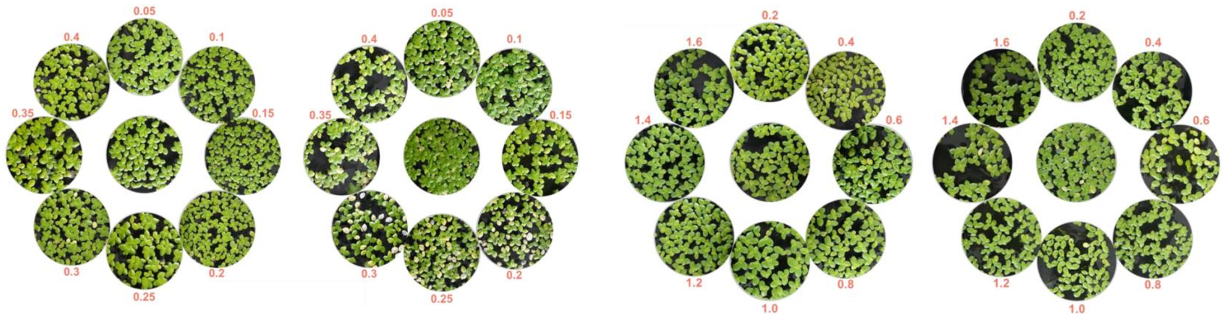
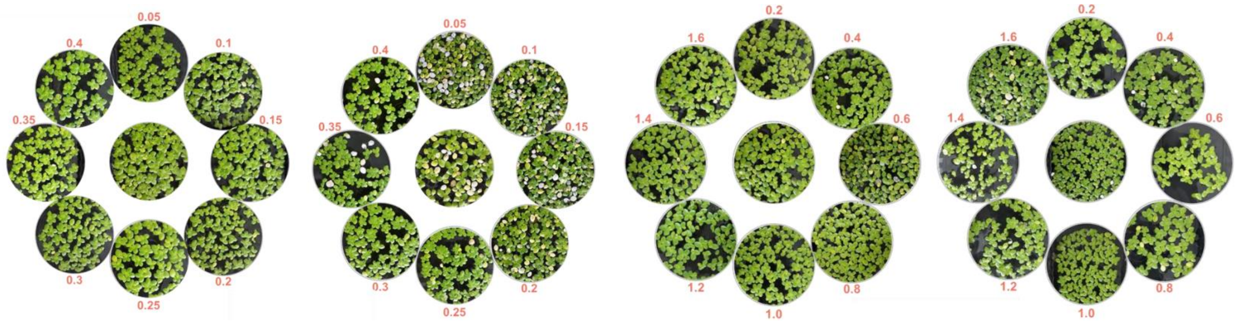



**Figure 6. Morphological changes of *Lemna minor DL* duckweed in environments supplemented with Cd (a) and Pb (b) after 25 and 50 days**



(a) (b) (c) (d)

**Figure 7. Morphology of *Spirodela* PN duckweed when cultured in an environment supplemented with Cd after 25 days (a), 50 days (b); Pb after 25 days (c) and 50 days (d)**



(a) (b) (c) (d)

**Figure 8. Morphology of *Spirodela* DL duckweed when cultured in an environment supplemented with Cd after 25 days (a), 50 days (b); Pb after 25 days (c) and 50 days (d)**

increased and became more widely distributed. With the *Spirodela polyrhiza* species, after 50 days, the duckweed leaves were still green, but there were a lot of algae in the duckweed culture environment, and yellow leaves appeared. The adaptability of *Lemna minor* and *Spirodela polyrhiza* to the lead-polluted environment was much better than to cadmium.

### 3.3. Cadmium accumulation ability of Lemna minor and Spirodela polyrhiza duckweeds

The analysis results show that each duckweed species has different ability to absorb and accumulate cadmium. Figure 8 show in detail the level of cadmium accumulation in the biomass of the surveyed duckweed species. *Lemna minor* PT duckweed has the best ability to accumulate cadmium (with an average of 72.06%). Meanwhile, *Lemna minor* DL with an average ability to accumulate 64.51% of cadmium in the water environment. After 50 days, the level of cadmium absorption and accumulation in the biomass of *Lemna minor* duckweed collected in Phan Thiet and Da Lat decreased compared to after 25 days. *Spirodela polyrhiza* duckweed has a lower ability to accumulate cadmium than Lemna minor, with the lowest being *Spirodela polyrhiza* duckweed collected in Phan Thiet (eliminating an average of 33.88% of cadmium in the water). In contrast to *Lemna minor*, after 50 days, the ability to accumulate cadmium in the biomass did not decrease, but increased slightly (about 5% with S*pirodela* PT and about 7% with *Spirodela* DL duckweed). This may indicate the good resistance ability of *Spirodela polyrhiza* duckweed in the cadmium-polluted water environment.

### 3.4. Lead accumulation ability of Lemna minor and Spirodela polyrhiza duckweeds

The results in Figure 9 showed differences in the ability to accumulate lead among the surveyed duckweed species compared to cadmium. *Spirodela* duckweed has a higher ability to accumulate lead than *Lemna minor* duckweed. Specifically, after 25 days, *Spirodela* DL duckweed can eliminate up to 75.43% of lead in the water, while it can remove only 39.03% of cadmium. Similar results were also observed in *Spirodela* PT duckweed. However, *Lemna minor* duckweed has a lower ability to accumulate lead in its biomass compared to cadmium. The amount of accumulated lead in the biomass of *Lemna minor* duckweed collected from both different locations had a level of eliminating lead in the water decreased by nearly half compared to cadmium. In all four surveyed duckweed species, the level of accumulated lead in their biomass after 50 days was higher than after 25 days. This result is consistent with the survey results on the effect of lead on the growth and development of *Lemna* and *Spirodela* duckweed. These duckweed species all have good adaptability in lead-polluted water environments.

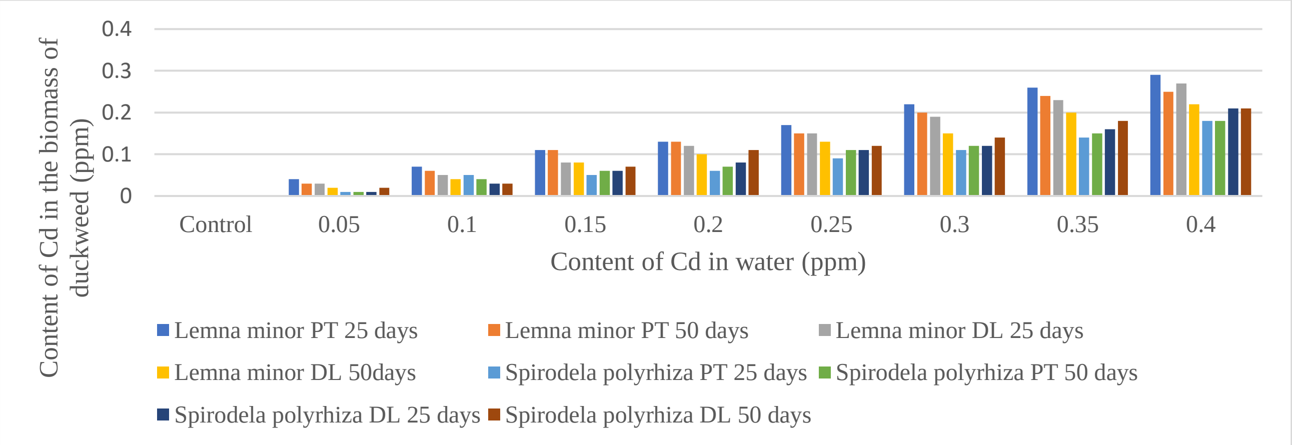



**Figure 8.** Cadmium accumulation ability of *Lemna minor* and *Spirodela polyrhiza* duckweeds

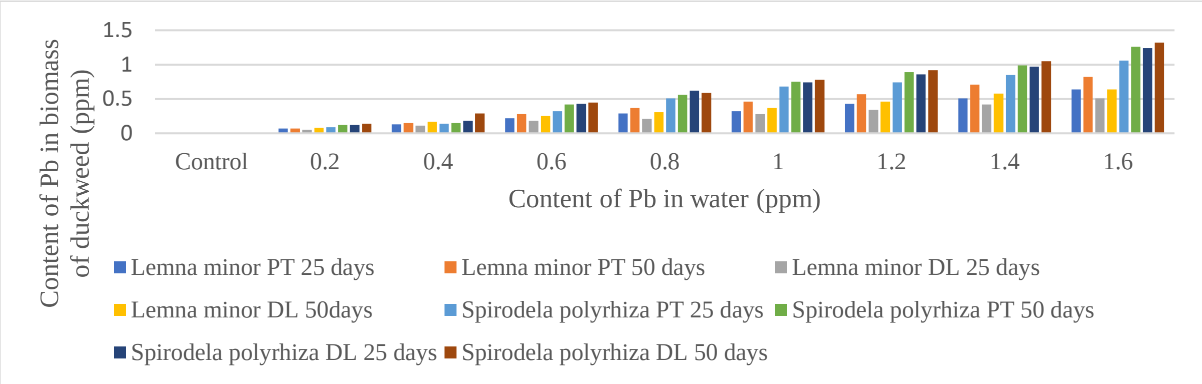



**Figure 9.** Lead accumulation ability of *Lemna minor* and *Spirodela polyrhiza* duckweeds

## 4. Conclusion

The research results have demonstrated the ability to use duckweed in treating water sources polluted with cadmium and lead. Depending on their ecological characteristics, each duckweed species has different abilities to adapt, grow, develop, absorb, and accumulate heavy metals. The study has identified the best growth conditions for the surveyed duckweed species. Among the collected duckweed species, *Lemna* and *Spirodela* in Phan Thiet showed good adaptability to the polluted environment, especially in lead-polluted water. Each duckweed species has different adaptability and ability to accumulate heavy metals. While *Spirodela* duckweed has a higher ability to accumulate lead than *Lemna minor* duckweed, *Lemna minor* has a higher ability to accumulate cadmium. *Lemna minor* duckweed can eliminate from 49.68% to 72.06% of cadmium and from 28.37% to 45.11% of lead in the water, while *Spirodela polyrhiza* duckweed can remove from 33.36% to 45.11% of cadmium and from 56.71% to 75.43% of lead in the water. This result shows that this characteristic of duckweed species can be exploited to effectively use them in treating water environments polluted with heavy metals. The potential of duckweed species in treating polluted water environments needs to be further exploited to bring about effective, environmentally friendly, easy-to-perform, and cost-effective solutions.

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