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Research Article

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Ability of greater duckweed (*Spirodela polyrrhiza*) to absorb cadmium and its potency as phytoremediator

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ABSTRACT

Phytoremediation is a method that uses the ability of plants to absorb pollutants in the environment both on land and in water. This study aims to explore duckweed which is a candidate for phytoremediator. Duckweed is a small aquatic plant that grows very fast. In this study, duckweed used is *Spirodela polyrrhiza* and cadmium was chosen as a model pollutant with concentrations of 0.05 and 0.1 ppm. Cultivation was done for 7 days. The specific growth rates in the 0.05 and 0.1 ppm treatments were decreased, 0.118 ± 0.001 and 0.11 ± 0.002 days^{-1}, respectively, when compared to the control, 0.143 ± 0.002 days^{-1}. *S. polyrrhiza* was able to absorb cadmium with absorption capacity of 66.4 and 67.2 % at 0.05 and 0.1 ppm, respectively. These showed the potential of duckweed to be further developed as a phytoremediator for the environment.

Keywords: cadmium; duckweed; phytoremediation; phytoremediator; *Spirodela polyrrhiza*

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INTRODUCTION

The increase in population was in line with the increase in industrial activities to meet market demands and needs (Sembada, 2022). The metal coating industry, plastics, batteries, and other human activities such as agriculture, mining, and pharmaceuticals would produce wastes that were harmful to the environment if they were not treated immediately and disposed directly to the environment. One of the elements contained in the waste was cadmium (Cd). Unlike other pollutant compounds, heavy metals could not be degraded, therefore some methods needed to reduce the metal content in polluted lands or water bodies (Zhang et al., 2020). Many of factory sewage drains end up in the rivers and other waterways. These would cause cadmium levels to exceed government regulatory limits. Some of the water polluted streams were entered the stream used for irrigation, so the heavy metals could accumulate on agricultural crops in the long-term exposure (Ismail et al., 2014). If these contaminated crops were consumed by humans, they could cause various diseases. Excessive consumption of cadmium could cause kidney and bone damage (Rahimzadeh et al., 2017). In Indonesia, the cadmium levels in waters should not exceed 0.01 mg/L (Rachmaningrum et al., 2015).

One way to reduce the cadmium levels in water bodies was the use of phytoremediation. Phytoremediation defined as pollutants clean-up methods using plants as the main agents (Yan et al., 2020). *Spirodela polyrhiza* is a plant that belongs to the duckweed family and floated in water surface (Faizal et al., 2021). *S. polyrhiza* had short doubling time and was easy to adapt with various environmental conditions (Sembada & Faizal, 2019) made this plant was suitable for phytoremediation. Besides cadmium, this plant could also be used as a phytoremediator of arsenic, lead, and other heavy metals. Rhizofiltration was one of the routes used to reduce pollution in waters by absorbing, concentrating, and depositing the pollutants in the plant roots (Kanwar et al., 2020). Rhizofiltration was also a suitable method to remediate Pb, Cd, Cu, Ni, Zn, and Cr metals which generally could be bound with plant roots (Yan et al., 2020). These kinds of binding could be also accompanied by a process of transforming heavy metals into less toxic forms (Dixit et al., 2015). Phytoremediation had been carried out so far using many plants such as *Eichhornia crassipes* (Ting et al., 2018), *Typha domingensis* (Gomes et al., 2014), *Scirpus grossus* (Al-Baldawi et al., 2014), and Phragmites australis (Hechmi et al., 2014), but after the phytoremediation process was complete, the residue biomass would accumulate and not be utilized. By using duckweed, the residual biomass could be converted into several other bioproducts such as bioethanol (Sembada & Faizal, 2022). This study would be investigated the absorption capacity of cadmium in *S. polyrrhiza* and the growth characteristics affected by the presence of heavy metal.

RESEARCH METHODS

This study used an experimental type of research that begins with duckweed cultivation followed by exposure to heavy metals to evaluate the phytoremediation ability. Then also measured the levels of heavy metals in plant biomass by using atomic absorption spectrometry. Experiment was conducted at the Laboratory of Biomass Production, School of Life Sciences and Technology, Institut Teknologi Bandung. *S. polyrrhiza* was weighed for 20 g and grown in 1L plastic container for 7 days. Each container was filled with 750 mL half-strength Hoagland medium (Sembada & Faizal, 2019). Hoagland medium was prepared from a mixture of macronutrients, micronutrients, and distilled water as shown in Table 1 (Yadav et al., 2015).

Table 1. Composition of Hoagland medium

Composition	Compounds
Macronutrient	$Ca(NO_3)_2.4H_2O$ 1 M KNO_3 1 M KH_2PO_4 1 M $MgSO_4.7H_2O$ 1 M
Micronutrient	H_3BO_3 2,86 g $MnCl_2.4H_2O$ 1,81 g $ZnSO_4.7H_2O$ 0,22 g $CuSO_4.5H_2O$ 0,08 g $NaMoO_4.2H_2O$ 0,025 g
Fe-EDTA	$FeSO_4.7H_2O$ Na_2EDTA

The metal used in this study was cadmium chloride ($CdCl_2$) with the following variation of concentration: 0 (control), 0.05, and 0.1 ppm. The metal was mixed with the Hoagland solutions according to the concentration that has been determined. In each treatment there were three replications. The specific growth rate and doubling time will be calculated during the cultivation. The calculation followed these following equations (Faizal et al., 2021).

$$\frac{dx}{dt} = \mu . x$$

$$\int_{t_0}^{t} \frac{dx}{x} = \int \mu \, dt$$

$$\ln x - \ln x_0 = \mu . t$$

$$\mu = \frac{\ln x_t - \ln x_0}{\Delta t}$$

$$dt = \frac{\ln 2}{\mu}$$

Where x is fresh biomass weight (g), t is number of cultivation days (days), μ is specific growth rate (day^{-1}), and dt is doubling time (days). The cadmium contents were evaluated in plant biomass on the initial, 2nd, 4th, and last day of cultivation. Plant biomass must be dissolved first by wet ashing method (Edgar et al., 2021). The plant samples including roots and fronds were dried, blended, and sieved to become uniform powder, then weighed for 1 g and added with 5 mL of nitric acid (HNO_3). The mixture was heated to boiling then cooled to room temperature and added with 2 mL of hydrogen peroxide (H_2O_2). The mixture then left until it looked clear and all organic matter had precipitated. The clear suspension separated from the precipitated matter then analysed with atomic absorption spectrometry (AAS). The results obtained will be used in calculating the percentage of absorption capacity (Cuadrado et al., 2019) according to the following formula.

$$\text{absorption capacity } (\%) = \frac{\text{cadmium content on biomass (ppm)}}{\text{cadmium content on medium (ppm)}} \times 100\%$$

Data obtained in this study will be evaluated by using descriptive statistics (mean and population standard deviation) in Microsoft Excel and using analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) in IBM SPSS Statistics.

FINDING AND DISCUSSION

The growth of *S. polyrrhiza* was analyzed based on the growth curves in various treatments as shown in Figure 1. The growth curve showed the increasing sloping lines which means that the duckweeds were still in the exponential growth stage and had not entered the stationary phase for 7 days of cultivation. From this growth curve, the value of the specific growth rate and doubling time would be obtained as shown in Table 2 (different letters indicate significant differences ($P < 0.05$) according to the Duncan test). The presence of cadmium in the growing media could have an effect on plant growth.

This was indicated by a decrease in the value of the specific growth rate and an increase in the value of the doubling time. This indicated that *S. polyrrhiza* need a longer time to double their biomass when compared to the control. The presence of heavy metals could affect plant growth and development (Carlos et al., 2016). It would also affect the physiological and metabolic processes that occur in plants. Even for very high levels of heavy metals would make plant cell death (Ashfaque et al., 2016). Duckweed could still grow even there were heavy metals in the growing media, this showed the potential use of duckweed as phytoremediator. The phytoremediation mechanism used by duckweed was rhizofiltration. This method used roots to absorb heavy metals in the environment (Fallahizadeh et al., 2019). Another mechanism used was phytoextraction which cleaned up the heavy metals in the environment and concentrated them on the plant tissues (Suman et al., 2018). The process that occured in the phytoremediation using duckweed was the absorption of heavy metals in the root system which would then be concentrated in plant tissues such as fronds.

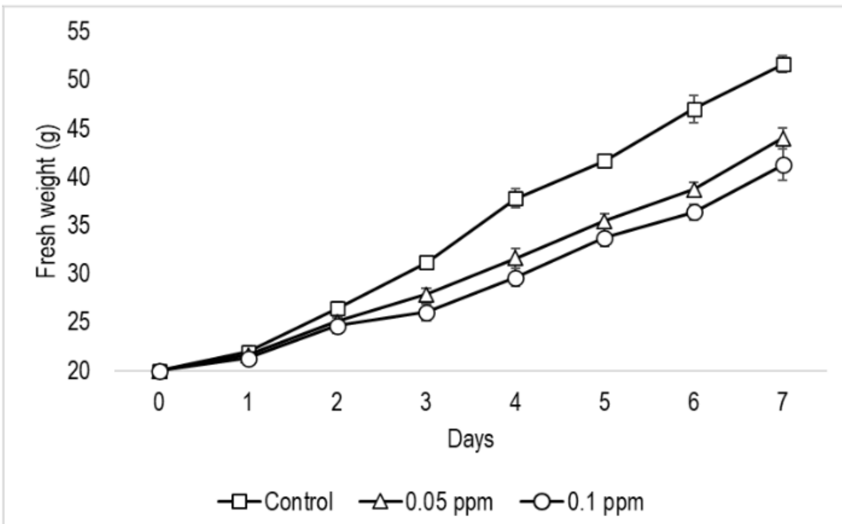

Figure 1. *S. polyrrhiza* growth curve in various treatments (Source: experimental data, 2022)

Table 2. Growth kinetics parameter measured from *S. polyrrhiza*

Treatment	Specific growth rate (days^{-1})	Doubling time (days)
Control	0.143 ± 0.002[b]	4.86 ± 0.06[a]
0.05 ppm	0.118 ± 0.001[a]	5.85 ± 0.02[b]
0.1 ppm	0.11 ± 0.002[a]	6.29 ± 0.11[c]

Cadmium levels analyzed in plant biomass were shown in Table 3. From these results, it could be concluded that there was accumulation of cadmium in *S. polyrrhiza* biomass from the first day to the last day of cultivation. Cadmium transport into plant tissue in the roots could be through the symplast and also apoplast pathway. Apoplast is a transport pathway that involves the movement of solutes through the extracellular fluid in the cell wall. However, symplast is a transport pathway that occurs intracellularly between cells through plasmodesmata (Lux et al., 2011). There are also several transporters or proteins that help regulate the uptake of cadmium into plant cells such as NRAMP family, P-type ATPase, ABC transporter, CAX family, ZIP family, LCT transporter, and CE family (Song et al., 2017). This showed the potential of *S. polyrrhiza* as a phytoremediator of heavy metals. The absorption value of heavy metals was calculated based on the adsorption capacity of cadmium as shown in Figure 2. During 7 days of cultivation, *S. polyrhiza* was able to absorb 66.4 and 67.2 % of cadmium for 0.5 and 0.01 ppm treatment, respectively. A study conducted by Chaudhuri *et al*. (2014) showed the ability of duckweed (*Lemna minor*) to absorb 0.5 – 3 mg/L cadmium with absorption capacity of 42 – 78%. Other study conducted by Panfili et al. (2017) showed the ability of *L. minor* to absorb 2 mg/L copper with an absorption capacity of 54.2%. This showed that the *S. polyrrhiza* had good absorption capacity when compared to *L. minor*. Duckweed application as phytoremediator could be done in the constructed wetland setting (Adhikari et al., 2015; Li et al., 2017). With this constructed wetland system, the heavy metal cleaning process could take place in a sustainable manner. This process was expected to be a more environmentally friendly and cost-effective heavy metal treatment.

Table 3. Cadmium contents on *S. polyrrhiza* observed after 7 days of cultivation

Treatment	Day 0 (ppm)	Day 2 (ppm)	Day 4 (ppm)	Day 7 (ppm)
Control	0.005	0.005	0.005	0.005
0.05 ppm	0.005	0.0061	0.0178	0.0332
0.1 ppm	0.005	0.0128	0.0368	0.0672

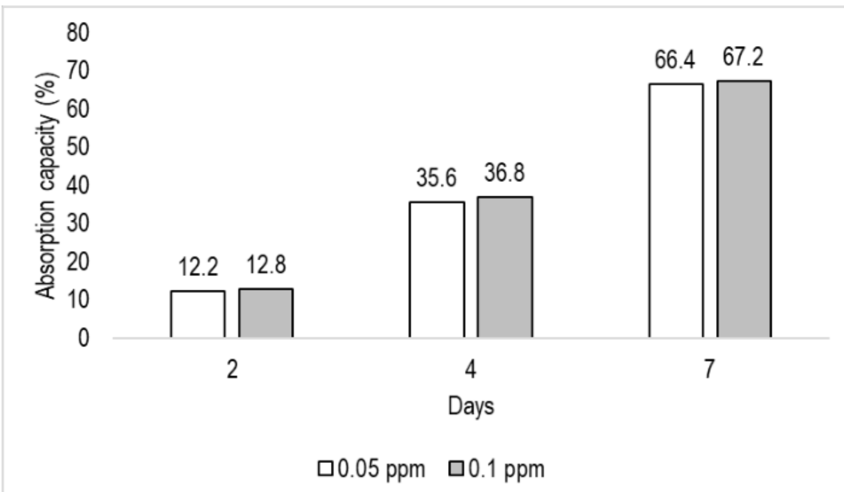

Figure 2. Cadmium absorption capacity of *S. polyrrhiza* (Source: experimental data, 2022)

CONCLUSION

Duckweed has the potential to be developed as a heavy metal phytoremediator. The results obtained from this study showed that *S. polyrrhiza* was able to absorb cadmium (Cd) with absorption capacity of 66.2 and 67.2% at 0.05 and 0.1 ppm, respectively. Further research is needed to conduct technoeconomic analysis on a pilot scale for heavy metal phytoremediation using duckweed.

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