

COMPARISON OF PHYTOREMEDIATION *AZOLLA MICROPHYLLA* AND *PISTIA STRATIOTES* AGAINST CADMIUM (Cd) ABSORPTION IN NGIPIK LAKE

Perbandingan Fitoremediasi *Azolla microphylla* dan *Pistia stratiotes* terhadap Absorpsi Kadmium (Cd) di Danau Ngipik

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Abstract

Telaga Ngipik is water that is used by the community as a basic supply, and water supply to several industries. The content of heavy metal cadmium (Cd) in Ngipik lake can reduce water quality. The purpose of this study was to determine the phytoremediation ability of *Azolla microphylla* and *Pistia stratiotes* against the absorption of heavy metal Cd in Ngipik lake water samples. The method used in this study was a quasi-experimental method, with the independent variables being 5, 10, and 15 days of phytoremediation and types of aquatic plants. The research variables used were cadmium levels in the roots of aquatic plants. Sampling using random sampling. The sample used was 48 liters of Ngipik lake water for 4 replications. The results showed that *Azolla microphylla* was able to absorb the highest cadmium metal on day 15 with a level of 18.30 mg/kg, while *Pistia stratiotes* were able to absorb the highest cadmium metal on day 10 with a level of 11.28 mg/kg. Cadmium levels in water affect watercolor, water pH, and plant morphology. Following up on the findings in this study, it is suggested to use a wider variety of aquatic plants that have the potential to absorb Cd.

Keywords : Azolla microphylla; Ngipik Lake; heavy metal Cd; phytoremediation; Pistia stratiotes

INTRODUCTION

Water quality needs to be maintained for the preservation of ecosystems and habitats in the waters. Water quality can decrease if the water contains excessive chemicals. According to Aliyah (2019), in his research, he stated that the Ngipik lake contains a chemical substance in the form of the heavy metal cadmium (Cd) which can have a direct impact on organisms because Cd can accumulate in the body of living things. In order for the water quality to remain in its original state, it is necessary to manage it to reduce Cd levels in the waters.

Based on the data explanation the

researcher wants to know the ability to reduce pollutants by trying to use plants that have phytoremediation capabilities. Phytoremediation is the use of plants to remove pollutants from contaminated soil or waters (Ahmad & Adiningsih, 2019). Aquatic plants can absorb several types of pollutants effectively so that they can reduce pollutants in the waters. Aquatic plants have varied results in the ability to absorb metals in water (Irhami et al, 2018).

Pistia stratiotes and *Azolla microphylla* plants are aquatic plants that have a role to stabilize physical, chemical, and biological factors in water (Rahmi & Sajidah, 2017). According to the results of research conducted by Nugroho,

Wahyuningsih, & Ginandjar (2019), the *Eichornia crassipers* plant can be used to reduce levels of Cd waste in waters. In this study, it was shown that the accumulation of heavy metals was found in the roots. This is because the roots are the organs that first interact with the medium. Research by Oktavia, Budiyo, & Dewanti (2016), used the *Salvinia molesta* plant to reduce cadmium content by reducing cadmium metal. The higher the cadmium level in the water, the more Cd is contained in the plants. High levels of Cd in plant roots go hand in hand with decreasing levels of Cd in the water.

Apart from *Eichornia crassipers* and *Salvinia molesta*, *Azolla microphylla* and *Pistia stratiotes* have the ability to remediate heavy metals. This is evidenced by research by Naghipour (2018), which shows that there is a decrease in cadmium levels by using the *Azolla microphylla* plant. Then research Purnama (2018), shows that *Pistia stratiotes* can reduce cadmium content by reducing Cadmium metal. Phytoremediation treatment used *Pistia stratiotes*. *Azolla microphylla* plants have different remediation power from *Pistia stratiotes*. This research is needed to determine the ratio between the two plants in absorbing the heavy metal Cd.

Therefore, the researchers wanted to use *Azolla microphylla* and *Pistia stratiotes* as remediators in phytoremediation of environmental pollution in Ngipik lake with chemical parameters in the form of cadmium levels in roots and pH, physical parameters in the form of watercolor, and biological parameters in the form of changes in the growth of the tested plants. Researchers chose *Azolla microphylla* and *Pistia stratiotes* because they are more effective than *Salvinia molesta* and *Eichornia crassipers* in absorbing heavy metals 116 mg/g (Rahmi & Sajidah, 2017). This research is important to do with the hope of knowing the phytoremediation ability of *Azolla microphylla* and *Pistia stratiotes* so that cadmium levels can be reduced so that aquatic ecosystems are

maintained and people who use lake water do not consume excess cadmium.

RESEARCH METHODS

Collection of Samples

Random sampling, a population that has the opportunity to be a research sample. *Azolla microphylla* plants were taken from the Integrated Laboratory of the University of Muhammadiyah Malang and *Pistia stratiotes* were obtained from the rice fields of Rusunawa 1 University of Muhammadiyah Malang. *Azolla microphylla* and *Pistia stratiotes* plants were selected with complete morphological characteristics of the plants and the same size. Determination of Cd levels can be tested by the Atomic Absorption Spectrophotometer (AAS) method. The measured Cd level was taken from the roots of the plant. The color of the water is determined based on the observations of the researcher. The pH value of water is measured using a pH meter.

Research procedure

Which will be used in this study to have the characteristics of a complete plant part, then the washing process is carried out until it is clean. After the washing process, several plants were weighed to a total of 100 g using analytical scales and then put into the wastewater. Plant weights were obtained based on research conducted by Purnama (2018).

Sample mapping is needed to facilitate observation activities. Mapping of samples starts with the preparation of tools and materials in advance by labeling the phytoremediation bath (plastic bucket).

The experimental design in this study used a completely randomized design (CRD) following the research that has been done by Munawwaroh & Pangestuti (2018). The research design used was factorial design with 6 treatments that were repeated 4 times, so the number of samples

used was 24 samples. This experiment consisted of 2 factors, factor I was the type of plant, and factor II was the long planting of phytoremediation.

Data Collection and Data Analysis

The data collection technique used for data collection in this study was observed through the stages of research procedures. There are two stages in this research, namely the preparation stage in the form of preparing the tools and materials to be used in the research and implementation of the research in the form of taking plants, lake water, and mapping samples. Then the phytoremediation process of the Azolla microphylla and Pistia stratiotes plants that have been carried out is washed clean, then transferred to a plastic bucket that has filled Ngipik lake water without diluting ± 2L. Azolla microphylla and Pistia stratiotes were tested according to the predetermined length of phytoremediation. The data retrieved was done by measuring watercolor, Cd levels in plant roots, and observations of water morphological damage, plant pH caused by Cd metal and documenting the results of observations to support existing data. Plant root preparation and measurement of Cd levels were carried out directly by laboratory analysts of University of Brawijaya.

The data analysis technique used in this study was the Statistical Package for the Social Sciences (SPSS). The methods used are descriptive and Two-Way Analysis of Variance (ANOVA) methods (Rohmah, Iw, & Hilal, 2018). Descriptive statistical analysis was carried out using the Kolmogorov-Smirnov normality test to determine whether the data variants were normal, after normal data the Levene test homogeneity was carried out to determine whether the data variants were homogeneous (Munawwaroh & Pangestuti, 2018). If the data is normal, then the test is continued with the two-way ANOVA test to determine whether there is an effect of the independent variable on the dependent

variable.

RESULT AND DISCUSSION

Cadmium (Cd) Levels in Plant Roots, WaterColor, Degree of Acidity (pH), and Plant Morphology

Based on testing the roots of plants that have received treatment, the results of cadmium levels can be seen in Table 1.

Table 1. Cadmium Metal Content in the Treatment of Aquatic Plants and Planting Time

Treatment	Cd Content in the Root (mg/kg)	
	Amount of 4 Replicates	Average of 4 Replicates
AL1	21,691	5,42
AL2	41,782	10,44
AL3	73,232	18,30
PL1	42,901	10,72
PL2	45,120	11,28
PL3	44,930	11,23

Keterangan:

A : Azolla microphylla

P : Pistia stratiotes

L1 : 5 days long planting

L2 :10 days long planting

L3 :15 days long planting

Sumber: Nisa Tahun 2020

Based on Table 1, the results of the absorption of Azolla microphylla on cadmium metal can be seen that the highest cadmium levels are shown at 15 days of planting with an average value of 18.30 mg/kg. The lowest average was shown at 5 days of planting with a value of 5.42 mg/kg. The results of the absorption of Pistia stratiotes on cadmium metal can be seen that the highest cadmium levels are shown in the 10-day planting period with an average value of 11.28 mg/kg. The lowest average was shown at 5 days of planting with a value of 10.72 mg/kg. The value of Cd levels shows the number of Cd levels that have been absorbed by plants in the water.

Based on the results of research on the color of water contained in the

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phytoremediation treatment using aquatic plants *Azolla microphylla* and *Pistia stratiotes*, data is obtained as in Table 2.







Table 2. Water Color in Phytoremediation Treatment

Treatment	Repeat			
	1	2	3	4
AL1	Green, quite cloudy	Green, quite cloudy	Green, quite cloudy	Green, quite cloudy
AL2	Green, quite cloudy	brown, quite cloudy	Green, quite cloudy	Green, quite cloudy
AL3	Brown, clear.	Brown, clear.	Brown, clear.	Brown, clear.
PL1	Green, quite cloudy	Green, quite cloudy	Green, quite cloudy	Green, quite cloudy
PL2	brown, quite cloudy	Brown, clear.	Brown, clear.	brown, quite cloudy
PL3	Brown, clear.	Brown, clear.	Brown, clear.	Brown, clear.

Sumber: Nisa Tahun 2020

Based on Table 2, the observation results show that in the treatment of *Azolla microphylla* and *Pistia stratiotes* plants, it can be seen that the color of the water on day 5 is green and quite cloudy, while on the 15th day the color of the water changes to brown and clear.

Table 4. Morphology of Plant Damage

Long Plating	Picture	<i>Azolla microphylla</i>	Picture	<i>Pistia stratiotes</i>
5 Days		Leaves: dark green. Roots: some light, bushy.		Leaves: green and fresh. Roots: light, bushy.
10 Days		Leaves: some of the leaves turn yellow. Roots: dark in color, partially fallen off		Leaves: some brown yellow and dry leaves. Roots: dark in color, mostly fall off.
15 Days		Leaves: brown, loose and dry. Roots: dark in color, mostly fall off.		Leaves: mostly brown yellow, shed and dry leaves. Roots: dark in color, mostly fall off.

Sumber: Nisa Tahun 2020

The degree of acidity (pH) obtained after phytoremediation treatment by aquatic plants is as in Table 3.

Table 3. Degree of Acidity (pH) of Water in the Treatment of Aquatic Plants

Treatment	Amount of 4 Replicates	Average of 4 Replicates
AL1	33,9	8,4
AL2	33,9	8,4
AL3	35,1	8,7
PL1	33,6	8,4
PL2	34,5	8,6
PL3	35,0	8,7

Sumber: Nisa Tahun 2020

Based on Table 3, shows that the lowest average degree of acidity (pH) in the *Azolla microphylla* treatment was on the 5th and 10th days of 8.4, while the highest average was on the 15th day, which was 8.7. In the *Pistia stratiotes* treatment, the lowest average was on the 5th day of 8.4, while the highest average was on the 15th day, which was 8.7.

The morphology of plant damage due to heavy metal cadmium contamination of aquatic plants *Azolla microphylla* and *Pistia stratiotes* were obtained as shown in Table 4.

Based on Table 4, the observation results show that the treatment of *Azolla microphylla* and *Pistia stratiotes* plants has chlorosis in the leaves and roots (Taufikurrahman, Juanda, & Suryati, 2020).

Effect of Length of Planting Time on Cadmium Absorption

Cadmium metal accumulation in the roots occurs from the absorption, translocation, and localization stages. This is by the research conducted by Mardikaningtyas, Ibrohim, & Suarsini (2016) that heavy metals accumulate more in the roots than in the leaves.. It is evident from the results of research that have been analyzed in Table 1, both plants can phytoremediation the absorption of cadmium levels. The roots of the *Azolla microphylla* plant experienced an increase in each variation of planting time for 5 days, 10 days, and 15 days with an average of 5.42 mg/kg, 10.44 mg/kg, and 18.30 mg/kg. The roots of the *Pistia stratiotes* plant experienced an increase in the planting time of 5 days and 10 days, while the planting time of 15 days decreased. The average amount of cadmium levels in the 5-day planting period was 10.72 mg/kg and the 10-day planting period was 11.28 mg/kg, while the 15-day planting period was 11.23 mg/kg. Based on data analysis statistics are presented in Table 5, it is proven that planting time affects even though the plant has an accumulation limit in the form of a saturation point (Ni'mah, Anshari, & Saputra, 2019).

Table 5. Two-Way ANOVA Test Results of Water Plant Absorption on Cadmium Metal Absorption (Cd)

Source	Sum of Squares	df	Mean Square	F	Sig.
Type	0,586	1	0,586	0,003	0,956
Long Planting	181,027	2	90,513	0,492	0,619
Interaction of types and Long Planting	157,153	2	78,576	0,427	0,659

Sumber: Nisa Tahun 2020

In the process of absorption of heavy metals, plants have a saturation point. The saturation point is the maximum tolerable limit for plants to absorb heavy metals/contaminants. The ability of plants to absorb metals will decrease after passing the saturation point. Metal concentrations in water can increase again because plants can release absorbed metals again by shedding their leaves and roots (Arasy, Elystia, & Andrio, 2016). *Azolla microphylla* and *Pistia stratiotes* have a maximum limit in absorbing metals. This is supported by Arifin & Goang (2018), which states that the *Azolla microphylla* plant has a metal absorption limit on the 20th day with the condition that most of the leaves are blackish-brown and the roots fall out. *Pistia stratiotes* plants have the maximum absorption of Cd until the 10th day with the condition of the plants partly yellow and dry leaves and loose roots.

Different types of plants can influence the phytoremediation process. *Azolla microphylla* plants which have a small root volume, lateral and tapered root types can absorb Cd longer than *Pistia stratiotes* which have long root systems and large root volumes. This is because the *Azolla microphylla* plants have a longer saturation point than *Pistia stratiotes* (Raras, Yusuf, & Alimuddin, 2015). So that Cd levels in the roots of *Azolla microphylla* plants are higher than in *Pistia stratiotes*.

Factors of different types of plants and planting time factors can influence the phytoremediation of the heavy metal Cd. The long planting time can cause the accumulation of absorption to be higher. This is because the longer the planting time, the more contaminant absorption in the water will increase until the saturation point for plants to absorb (Nugroho et al., 2019). According to Ni'mah (2019), planting time is a determination that results in maximum absorption of heavy metals. In *Azolla microphylla* plants with a planting time of 15 days can still absorb the metal content of Cd, while in *Pistia stratiotes* plants with a planting time of 15 days can no longer

absorb cadmium metal. This is because on the 10th day of *Pistia stratiotes* it is at its saturation point so that on the results of absorption on the 15th day the Cd levels in plant roots decrease (Purnama *et al.*, 2018).

Interaction of Plant Types and Planting Time

The difference in ability between the three variations in planting time is influenced by differences in plant morphology. Changes in morphological damage in plants depend on planting time in remediating cadmium metal, so that the higher levels of cadmium that are absorbed can increase the damage to plant morphology (Taufikurrahman *et al.*, 2020). Symptoms caused by heavy metal contamination are chlorosis on the tips and sides of the leaves (Kandowangko & Lamondo, 2015). Changes in plant morphology are plant reactions to adaptation due to direct contact with cadmium metal. Physical changes in *Azolla microphylla* plants after 5 days of planting were seen in dark green leaves, in addition to some light and bushy roots. After 10 days of planting, it was seen that some of the sides of the leaves had turned yellow and the roots were dark and some of them fell off, then at 15 days of planting, many sides of the leaves were brown, dry and many of the roots had fallen off. This occurs due to a decrease in metabolism caused by the absorption of excess cadmium metal ions (Oktavia *et al.*, 2016).

Physical changes in *Pistia stratiotes* after 5 days of planting can be seen in the leaf color is still fresh green, besides that the plant roots are light and dense. After 10 days of planting, can see that some of the edges of the leaves have a yellowish color and the roots have started to turn dark and some have fallen out. Furthermore, at 15 days of planting, most of the sides of the leaves were dry and yellow and the roots were dark and most of them fell off. According to Yosephine, Tistama, Adinugroho, & Dalimunthe (2020), the

morphology of plant damage due to heavy metal contamination makes plants begin to show symptoms of chlorosis, namely the leaves change from green to yellow with brownish brown. Chlorosis occurs because cadmium metal inhibits the action of the phytochelatin synthase enzyme, which catalyzes the synthesis of chlorophyll, besides that plants are also lacking in nutrients. During chlorosis, plants also shed their leaves or roots. This happens because cadmium metal that has entered the plant will be excreted by shedding old leaves so that later it can reduce metal levels (Sholikah & Rachmadiarti, 2019). Apart from affecting the damage to plant morphology, the interaction of plant types and length of time in the garden can be seen from the degree of acidity (pH) and watercolor.

The data from the measurement of environmental physical and chemical parameters prove that the phytoremediation of cadmium metal using *Azolla microphylla* and *Pistia stratiotes* can increase the pH in each treatment. The highest increase in pH occurred during the planting period of 15 days with an average of 8.7, the lowest increase in pH was at the planting time of 5 days with an average pH of 8.4. The degree of acidity can affect the solubility of heavy metals in water and the conditions of plants that interact directly with the growing media (Purnama *et al.*, 2018). The increase in pH is caused by photosynthesis, denitrification, organic nitrogen breakdown, and sulfate reduction (Sugiyanto, Yona, & Kasitowati, 2016). Increasing the pH will decrease the solubility of water oxygen and increase the toxicity of heavy metal Cd. The decrease in pH value is due to the metal cadmium being absorbed or bound by plant roots (Purnama *et al.*, 2018).

The results showed that the lowest average degree of acidity (pH) in the *Azolla microphylla* treatment was on the 5th and 10th days of 8.4, while the highest average was on the 15th day, which was 8.7. In the *Pistia stratiotes* treatment, the lowest mean

were on day 5 of 8,4, while the highest average was on day 15, which was 8,7. The pH value in water is influenced by the content of the heavy metal cadmium. Water that has high cadmium levels will have an acidic pH. Meanwhile, the lower the cadmium level in the water, the pH becomes alkaline. An increase in pH can reduce the solubility of cadmium metal in water because it will change the metal from carbonate to hydroxy form. In addition to the pH of the water, changes due to the phytoremediation process can be seen based on physical parameters in the form of watercolor.

The observations show that in the treatment of *Azolla microphylla* and *Pistia stratiotes*, it can be seen that the color of the water on day 5 is green and quite cloudy, while on day 15 the color of the water changes to brown and clear. Watercolor is a physical factor that is influenced during the phytoremediation process. The watercolor at the beginning of the treatment was green and quite cloudy, but after the treatment for a long time of planting 5,10 and 15 days, the color of the water changed to brown and became clearer than before. This indicates that the presence of high levels of cadmium makes the water green and quite cloudy (Poernomo, Razif, & Mansur, 2020).

CONCLUSION

The *Azolla microphylla* plant was able to absorb the highest heavy metal cadmium at 15 days of planting with an average of 18.30 mg/kg. *Pistia stratiotes* were able to absorb the highest heavy metal cadmium in 10 days with an average of 12.28 mg/kg. There is an interaction between plant types and planting time on the absorption of cadmium metal in the form of damage to plant morphology, the higher the pH value and the clearer the watercolor.

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