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The Effect of Phytoremediation Using Duckweed (*Lemna minor*) on Ammonia Levels in Catfish Farming Wastewater in Boyolali

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Abstract - *Farming activities, especially catfish Farming, as carried out by the community in Kampung Lele, Boyolali, Indonesia cannot be separated from producing waste. Water pollution caused by catfish farming waste can be identified through increased ammonia levels. This research aims to determine whether the density of duckweed (Lemna minor) can influence changes in ammonia levels in liquid waste from catfish Farming and to determine the ammonia levels in liquid waste from catfish Farming after treatment with duckweed. The research is phytoremediation research using duckweed. The research used a completely randomized design in data collection carried out with 6 variations (0 plants, 150 plants, 300 plants, 450 plants, 600 plants, 750 plants) for 15 days starting from the first day and continuing every 2 days. The repetition carried out in the research was 4 repetitions which were based on the Federer formula. The data obtained were analyzed using two way ANOVA analysis and further tested with Duncan's post hoc test using the SPSS application. The results obtained in the form of ammonia levels after treatment decreased, namely 0.6 ppm on A_{150}, 0.5 ppm on A_{300}, 0.5 ppm on A_{450}, 0.45 ppm on A_{600}, and 0.4 ppm on A_{750}. The density of duckweed can influence changes in ammonia levels in liquid waste from catfish Farming, through two way ANOVA analysis which obtained a sig value. equal to 0.00 or < 0.05, then there is a difference in the average ammonia based on density and time, so that in Duncan's post hoc follow-up test we found variations in A_{750} on the 11th day with 0.475 ppm ammonia and A_{600} on the 15th day with ammonia 0.45 ppm meets quality standards.*

Keywords *– Ammonia, Catfish Farming, Duckweed, Phytoremediation, Wastewater*
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1. Introduction

Increase in population results in increased human activity, especially activities that can provide income for humans. One of the increasing human activities is Farming activities which can be used to fulfill human needs and income. Intensive Farming results in a lot of waste being produced (Sianipar, et al., 2022). Farming activities, especially catfish Farming, as carried out by the community in Kampung Lele, Boyolali, Indonesia cannot be separated from producing solid and liquid waste. This can have an impact on the aquatic ecosystem, especially on local fish and plant life around the disposal of catfish farming waste. Apart from that, decreasing water quality also affects the physiological processes, behavior, growth and mortality of fish. Environmental pollution caused by aquaculture waste is triggered by the use of uncontrolled feed and the use of medicines that are less environmentally friendly (Sianipar, et al., 2022). Intensive accumulation and deposition of feed residues and fish feces is also a source of organic waste from fish farming (Maharani, et al., 2022).

Water pollution caused by catfish farming waste can be identified through increased ammonia levels. Catfish farming wastewater also contains a lot of N2 and NH3 (ammonia) as a result of the breakdown of protein and amino acids from leftover feed and feces (Septiani, et al., 2014). Increasing ammonia levels that do not comply with quality standards can of course result in environmental damage.

The problem resulting from catfish Farming waste has become one of the problems that needs attention, so various ways are needed to overcome this problem. One way to deal with catfish Farming waste which can pollute water is by using plants or what is called Phytoremediation. Aquatic plants act as aquatic aerators through the process of photosynthesis, regulating water flow, cleaning polluted streams through sedimentation processes and absorbing particles and minerals (Ahmad & Adiningsih, 2019). Compared to other waste processing methods, phytoremediation is quite economical because it does not require high operational costs. In addition, phytoremediation can reduce ammonia levels through aeration processes and plant metabolism.

There are several plants that can be used in the phytoremediation method, one of which is duckweed. Duckweed have effective phytoremediation capabilities in improving the quality of water contaminated by waste (Fatikasari & Purnomo, 2022). Duckweed has several advantages, such as a high rate of absorption of nutrients and water, a fast growth rate, easy to find, and high adaptability to climate. *Lemna minor* also has many benefits, namely as a biofertilizer to increase plankton growth, animal feed and fish feed (Nopriani, et al., 2014).

Based on these things, it is necessary to conduct research on catfish Farming waste using the phytoremediation method through plant metabolic processes, one of which is the duckweed. This research aims to determine whether the density of duckweed can influence changes in ammonia levels in liquid waste from catfish Farming and to determine the ammonia levels in liquid waste from catfish Farming after treatment with duckweed.

2. Research Methods

2.1. Study Area and Materials

This research is located in Kampung Lele, Boyolali, Indonesia with coordinates -7.539157, 110.620261 and lasts from February 2024 – March 2024. The research uses tools including containers or buckets, jerry cans and ammonia test kits. The materials used in this research include duckweed, catfish Farming wastewater, and ammonia test kit reagent.

2.2. Experimental Procedures

This research is quantitative research that is experimental. This research uses two factors, namely the length of time of the plant and the number of plants, so that the research design is obtained at Table 1.

Table 1. Research Design of Phytoremediation

A	Time (Day)							
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8
A_1	N_{1-1}	N_{1-2}	N_{1-3}	N_{1-4}	N_{1-5}	N_{1-6}	N_{1-7}	N_{1-8}
A_2	N_{2-1}	N_{2-2}	N_{2-3}	N_{2-4}	N_{2-5}	N_{2-6}	N_{2-7}	N_{2-8}
A_3	N_{3-1}	N_{3-2}	N_{3-3}	N_{3-4}	N_{3-5}	N_{3-6}	N_{3-7}	N_{3-8}
A_4	N_{4-1}	N_{4-2}	N_{4-3}	N_{4-4}	N_{4-5}	N_{4-6}	N_{4-7}	N_{4-8}
A_5	N_{5-1}	N_{5-2}	N_{5-3}	N_{5-4}	N_{5-5}	N_{5-6}	N_{5-7}	N_{5-8}
A_6	N_{6-1}	N_{6-2}	N_{6-3}	N_{6-4}	N_{6-5}	N_{6-6}	N_{6-7}	N_{6-8}

Note: A: Number of Duckweed; P: Time (day); N: Dependent Variable

In this research, the first factor is planting time (P) and the second factor is number of plants (A). In this study, 6 variations were observed and 7 observations of ammonia resulted in 42 observations. The variations used were 2 liters of catfish Farming waste each in a container filled with duckweed with variations of 0 plants, 150 plants, 300 plants, 450 plants, 600 plants, and 750 plants with ammonia testing treatment every 2 days for 15 days starting on day 1.

This research was carried out repeatedly during the data collection process to avoid as few errors as possible in the research process. The repetitions carried out in the research were based on Federer's formula. Federer Formula: (Irmawartini & Nurhaedah, 2017)

$$(k-1)(r-1) \geq 15 \quad (1)$$

Note: k: number of variations; r: number of repetitions

Based on Federer's formula, the repetitions that need to be carried out in this research are 4 repetitions. This repetition is due to the number of variations in the research, namely 6 variations. So 24 treatments were carried out with 6 variations which were repeated 4 times. A completely randomized factorial pattern design was also used in the research for data collection. The placement design was carried out by randomizing the placement of sample treatments using a lottery.

2.3. Data analysis

Data obtained from the results of data collection in this research is plant density by measuring the number of plants and ammonia levels, then analysis is carried out. Analysis was carried out using two way ANOVA analysis and further tested using Duncan's post hoc test with the SPSS application. Two way ANOVA analysis was carried out with density and time as independent variables and ammonia levels as the dependent variable. This analysis was used to determine the effect of plant number as plant density on ammonia levels in catfish Farming wastewater over a period of 2 days for 15 days starting from day 1. Through this analysis, the significant value will be known which is used to determine the average difference between the independent variable and the dependent variable, based on: Significant value (sig.) > 0.05, so there is no difference and Significant value (sig.) < 0.05, then there is a difference (Sufren & Natanael, 2013). After the two way ANOVA test, a further test was carried out, namely the Duncan post hoc test. Duncan's post hoc test was used to determine the average difference in the interaction of density and time on ammonia levels. The average difference is used to determine the density and efficient time for ammonia levels in accordance with water quality standards.

3. Result and Discussion

3.1. Sampling Conditions

Sampling was carried out in Kampung Lele, Boyolali, Indonesia. The catfish Farming pond is located in an open area on the side of the village road. There are rivers and waterways on the outskirts of the catfish farming area. Catfish Farming water has a pungent odor and is green and brownish red in color. The strong odor from catfish farming water, especially during the draining process and after rain, can disturb the comfort of local people and road users near catfish farming sites.

Water for farming catfish will usually be drained and replaced after 2-3 weeks. During the process of draining a catfish Farming pond, catfish Farming waste water will usually be disposed of around the pond, where the water will flow into the ground or follow the water channel into the river. This is what can cause soil and river pollution where catfish Farming waste is disposed of. Apart from that, high levels of pollutants, especially ammonia, in catfish farming water can disrupt catfish farming activities. Therefore, it is necessary to manage waste to reduce pollutant levels in catfish farming wastewater so that it complies with water quality standard regulations.

3.2. Preliminary Measurement

In this study initial measurements were carried out in the field and before treatment. Initial measurements in the field were carried out before being taken to the treatment site in order to determine the condition of the quality of catfish Farming waste in the field, sampling and initial measurements before treatment to determine the initial ammonia levels before administering duckweed to each treatment sample.

Table 1. Preliminary Measurement of the Sample

Measurement	Ammonia (ppm)
Sampling	0.8
Before Treatment	0.8

Results of measurements in the field and measurements before treatment showed that there were no differences in the values of the measured ammonia levels, namely in field measurements and measurements before treatment the ammonia levels were 0.8 mg/L. This is because after the catfish Farming waste water is put into the container, there are no other objects added to the catfish Farming waste water such as fish food or catfish. Where, uneaten feed remains and fish feces are the origin of ammonia in waters (Salamah & Zulpikar, 2020). Therefore, there are no external factors that can change the ammonia levels in catfish farming wastewater, except for the role of fungi or microbes in the ammonification process in the wastewater. An increase in ammonia levels can be caused by the ammonification process of wastewater being left to sit or an increase in ammonia sources in wastewater due to the decomposition of organic matter in wastewater by fungi and microbes (Effendi, 2003).

3.3. Duckweed (*Lemna minor*) Density

In this study, density was calculated by counting the number of duckweed in each treatment and repeating phytoremediation of catfish Farming liquid waste. The calculation was carried out at the beginning of the treatment until the treatment lasted 15 days starting from day 1 and continuing every 2 days by counting one by one the number of duckweed. The results of calculating the number of individual duckweed can be seen in Table 3.

Table 2. Duckweed Density

A	Time (Day)							
	1	3	5	7	9	11	13	15
A_0	0	0	0	0	0	0	0	0
A_{150}	150	274.25	403.5	506	585.75	763	927.75	1189.25
A_{300}	300	372.5	450.5	548.25	663	824.75	1150.25	1256.75
A_{450}	450	550.5	670	816	1132.25	1252.75	1323.25	1351.5
A_{600}	600	778.75	979.75	1282.25	1314.5	1354	1381.75	1405.5
A_{750}	750	957.25	1285.25	1329.5	1362.5	1384.75	1418.25	1426.75

Note: A: Number of Duckweed

Table 3. Ammonia Levels

A	Time (Day)								Efficiency
	1	3	5	7	9	11	13	15	
A_0	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0%
A_{150}	0.8	0.8	0.8	0.8	0.8	0.6	0.6	0.6	25%
A_{300}	0.8	0.8	0.8	0.75	0.6	0.6	0.6	0.5	37.5%
A_{450}	0.8	0.8	0.8	0.6	0.6	0.6	0.525	0.5	37.5%
A_{600}	0.8	0.8	0.65	0.6	0.6	0.5	0.5	0.45	43.75%
A_{750}	0.8	0.8	0.6	0.6	0.5	0.475	0.4	0.4	50%

Note: A: Number of Duckweed

Calculation results showed that there was an increase in the number of individual duckweed with each treatment and repetition. The greatest increase in density during the 15th day occurred on A_{450} with a difference of 1039.25 plants from the 1st day, while the least increase in density during the 15th day occurred on A_{750} with a difference of 676.75 plants from the 1st day 1.

Increase in density was slight because the size of the container used was not sufficient to support the growth of duckweed. Plant growth and development are influenced by the size of the media and the extension of the root boundary

(Onggo, et al., 2017). In this study, because the area of the container did not change during the treatment, the density of approximately 1250 plants did not provide much space for the growth of duckweed after covering the water surface. Therefore, the growth space becomes increasingly narrow, resulting in several duckweed overlapping each other, resulting in the plants drying out and dying.

Plant growth and development is influenced by factors, namely nutrients, water, carbon dioxide and a suitable climate. Plants need nutrients to support their growth and development (Astutik, et al., 2019). In this study, there were no additional nutrients and only used waste water media for cultivating catfish, therefore duckweed may not get enough nutrients for growth and development. However, the absence of adding nutrients in the research process was intended to not interfere with checking the amount of ammonia levels, so that during the research the amount of ammonia levels in catfish Farming wastewater was only influenced by the duckweed. Apart from that, the absence of the addition of other nutrients proves that duckweed can grow in polluted environments such as catfish farming wastewater.

3.4. Ammonia

Ammonia is the most widely produced product of nitrogen metabolism. Ammonia comes from the decomposition of dead organisms, apart from that, ammonia also comes from the metabolism of feed containing nitrogen and uneaten feed residue (Wahyuningsih & Gitarama, 2020). The overall results of measuring ammonia levels during treatment for each sample of catfish farming liquid waste can be seen in Appendix 2, while the average results of measuring ammonia levels can be seen in Table 4.

Ammonia levels decreased during the study period. In measurements before treatment, the ammonia level of liquid waste from catfish Farming in the field reached 0.8 ppm and measurements in the greenhouse reached 0.8 ppm. The measurement value before treatment shows that the ammonia level of liquid waste from catfish Farming exceeds the class 3 water quality standard limit based on PP Number 22 of 2021, which is the maximum limit for the class intended for cultivating freshwater fish with a maximum ammonia level of 0.5 ppm. However, after phytoremediation treatment using duckweed, the ammonia level of catfish Farming liquid waste was reduced to 0.4 ppm on the 15th day of treatment with 750 duckweed. The ammonia level is below the maximum limit for class 3 ammonia quality standards based on PP Number 22 of 2021.

Based on table 4.3, the average decrease in ammonia levels on the 15th day showed that the lowest efficiency of reducing ammonia levels was obtained in the variation with an initial number of plants of 150 plants, namely 25% with initial ammonia levels of 0.8 ppm to 0.6 ppm. Then, the highest efficiency in reducing ammonia levels was obtained in the variation with an initial number of plants of 750 plants, namely 50% with initial ammonia levels of 0.8 ppm to 0.4 ppm. Calculations of the efficiency of reducing ammonia levels with phytoremediation were also carried out in Ramadani, et al., (2022) research. This research showed that the apu wood plant (Pistia Stratiotes) could reduce ammonia by 26.5%. The reduction efficiency was greater in this study because in (Ramadani, et al., 2022) study the treatment was only carried out for 5 days, whereas in this study it was carried out for 15 days. Apart from the length of treatment, the plants used as phytoremediators were also different, namely in that study the apu wood plant was used, while in this study the duckweed was used.

In the control samples there was no decrease in ammonia levels. This is because the control samples did not undergo phytoremediation treatment using the duckweed, so there were no factors that could help in the process of converting ammonia compounds into simpler compounds in catfish Farming wastewater. Meanwhile, a rapid decrease in ammonia levels compared to other samples occurred in samples with an initial density of 750 plants.

Reduction in ammonia levels by duckweed can occur due to the help of nitrifying bacteria, namely Nitrosomonas and Nitrobacter bacteria. Bacteria that can break down ammonia into nitrates or nitrites will make the roots of aquatic plants a place for bacteria to attach. Nitrosomonas bacteria will convert ammonia levels into nitrite through an oxidation process, while Nitrobacter bacteria will convert nitrite into nitrate through an oxidation process (Putri, et al., 2022). Nitrate will be absorbed by the roots of duckweed. The degradation results that have been absorbed by the roots will be reabsorbed by the internal parts of the plant such as stems and leaves (Ramadani, et al., 2022).

3.5. Analysis of the Effect of Duckweed Density on Ammonia

Results of the two way ANOVA analysis with SPSS can be seen in Appendix 3. Based on these results, it is known that the density has a sig value. is 0.00 or <0.05, then there is a difference in the average ammonia content of catfish Farming wastewater based on density. Time based results have a sig value. is 0.00 or <0.05, then there is a difference in the average ammonia content of catfish Farming liquid waste based on time. Then the interaction of density and time has a sig value. is 0.000 or <0.05, then there is a difference in the average ammonia content of catfish Farming liquid waste based on the interaction of duckweed density and treatment time.

Duncan's post hoc test show the lowest and highest levels of ammonia as well as similarities in density and time variations. Based on PP no. 22 of 2021, ammonia corresponding to group 3 has a maximum threshold of 0.5 ppm, so the appropriate variation is A750 on the 11th day. The A750 variation on day 11 had an ammonia level of 0.475 which is considered the same as an ammonia level of 0.45 ppm and 0.5 ppm. Then, based on the number of plants alone, A600, which has fewer plants than A750, can be used as an option to reduce ammonia levels, because the A600 variation can reduce ammonia levels to 0.45 ppm on the 15th day. This shows that the reduction in ammonia levels is influenced by plant density and treatment time. This also has similarities with the results research of (Nabila, 2021), that the density of duckweed also influences the reduction of parameters such as temperature, DO, BOD, COD, TSS and pH.

Ammonia levels decreased by duckweed because more duckweed experiences growth and development, the higher the density will be, which affects the number of plant roots which help in phytoremediation, such as a place for bacteria to attach and the plant's absorption capacity for contaminants. The longer the age of the plant, the more numerous and long the plant roots will grow. The more plant roots there are, the more ammonia the plant can absorb (Aka, et al., 2017). The main mechanism for preventing toxicity is primarily through the root system, where the absorption of contaminants by the plant occurs. The root system provides a very large surface area for the absorption and accumulation of contaminants along with water and nutrients essential for plant growth (Sukono, et al., 2020).

4. Conclusion

Ammonia levels in catfish Farming liquid waste after treatment with duckweed decreased from 0.6 ppm on A_{150}, 0.5 ppm on A_{300}, 0.5 ppm on A_{450}, 0.45 ppm respectively. on the A_{600}, and 0.4 ppm on the A_{750}. The highest ammonia level after treatment occurred in the A_{150} treatment, namely 0.6 ppm, and the lowest occurred in A_{750}, namely 0.4 ppm. Density of duckweed can influence changes in ammonia levels in liquid waste from catfish Farming, through two way ANOVA analysis which obtained a sig value. equal to 0.00 or <0.05, then there is a difference in the average ammonia based on density and time, so that in Duncan's post hoc follow-up test we found variations in A_{750} on the 11th day with 0.475 ppm ammonia and A_{600} on the 15th day with ammonia 0.45 ppm meets quality standards.

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