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EFFECTIVENESS OF LOTUS PHYTOREMEDIATION (NYMPHAEA PUBESCENS) IN REDUCING AMMONIA AND PHOSPHATE CONCENTRATIONS IN COFFEE LIQUID WASTE

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Abstract

Based on laboratory tests of ammonia and phosphate concentrations in coffee wastewater from coffee factory in Semarang, the concentrations were found to be 8.55 and 1.01 mg/L, respectively. These high concentrations negatively impact fish farming production. Nymphaea pubescens has shown potential in remediating ammonia and phosphate concentrations in coffee wastewater. Therefore, the objective of this study is to determine the effectiveness of lotus phytoremediation (Nymphaea pubescens) in reducing ammonia and phosphate concentrations in coffee wastewater. The research method employed is true experiment with Completely Randomized Design, involving four treatments and three replications. According to ANOVA and Duncan's Multiple Range Test results, Nymphaea pubescens significantly reduced ammonia and phosphate concentrations in coffee wastewater. So far there have been no specific studies on the phytoremediation of Nymphaea pubescens for ammonia and phosphate concentrations in coffee wastewater, making this study a novel contribution. The findings suggest that this approach should be widely implemented, especially within educational settings and communities.

Keywords*: Ammonia; Coffee Waste; Lotus; Nymphaea pubescens; Phosphate; Phytoremediation*

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INTRODUCTION

Coffee agro-industry activities contribute to increasing the rate of economic growth of a country. However, unwise waste management has a negative impact on polluting the environment. The high concentration of organic matter in liquid coffee waste has an impact on water pollution in the form of organic pollutants (Bisekwa *et al*., 2020; Suryadi *et al*., 2016). The process of peeling and washing coffee beans produces organic liquid waste in the form of ammonia and phosphate (Arifin, 2016). Based on the results of ammonia and phosphate concentrations tests conducted by researchers on liquid coffee waste from coffee factory in Semarang City, the figures obtained were 8.55 and 1.01 mg/L respectively. Ammonia concentration testing uses Nesseler method with SNI 06.2479.1991. While phosphate concentration testing uses the Vanadate 4500 APHA P.C. analysis method. These concentrations are above the established quality standards.

Based on the results of interviews with pond cultivation owner, many fish died suddenly within two to three months. As a result, fish production in pond cultivation decreased. The decrease in pond cultivation production was due to coffee production pollutants that had polluted the ponds. Liquid coffee waste from the coffee factory was not treated first, so it flowed directly into Streambed and headed towards the ponds located near the coffee factory. According to Wahyuningsih & Arbi (2020), ammonia is toxic and reduces the oxygen supply by a large amount in the aquatic ecosystem. On the other hand, high phosphate concentrations disrupt the metabolic process, causing fish death (Lestari *et al*., 2015). Based on this, environmental pollution due to liquid coffee waste must be handled immediately.

One alternative waste treatment is through phytoremediation. Phytoremediation is a natural waste treatment method by utilizes plants as agents in changing contaminants to be less or non-hazardous (Manasika, 2015; Aslam, 2017). Coffee processing wastewater is biodegradable so processing with phytoremediation is one of the right choices (Manasika, 2015; Rattan *et al*., 2015). The advantages of using phytoremediation are not only environmentally friendly but also the maintenance and processing process is easy and more economical compared to conventional remediation technology (Ahmad, 2017; Melati 2020). Phytoremediation relies on the ability of plants to absorb, accumulate, and convert pollutants into safer or easier-to-process forms. In the phytoremediation process,

plants utilize the chemicals in the waste as nutrients to meet their nutritional needs (Padmaningsum *et al*., 2014; Hapsari *et al*., 2018).

Various studies have proven that phytoremediation is an effective method of reducing waste concentrations in the environment. However, not all plants have the potential to be bioremediation agents. In the study of Siswandari *et al* (2016), plants will be able to remediate pollutants if they have reached adulthood. Plants that can be used for phytoremediation include those that are hyperaccumulators, able to remediate more than one pollutant, and tolerant to the pollutants being remediated (Zahro & Nisa, 2020; Ghassani & Titah, 2022). One of the phytoremediation agents that is effective in reducing waste is the Lotus. In addition to being useful as an ornamental plant, the lotus also acts as a phytoremediation agent because it has hypertolerant and hyperaccumulator properties towards pollutants. In the study of Djibran *et al* (2015), the lotus plant can reduce phosphate to a concentration of 4.775 mg/l. In addition, Andiyanto's research (2019) stated that the lotus plant *Nymphaea pubescens* reduced 64% of ammonia concentration and 84% of phosphate concentration from domestic liquid waste.

There are no specific research examining the phytoremediation of *Nymphaea pubescens* on ammonia and phosphate concentrations of liquid coffee waste, thus creating a significant research gap. Although previous studies have shown the phytoremediation potential of lotus plants in reducing ammonia and phosphate concentrations of waste, so far there has been no study that specifically explores the effectiveness of lotus in dealing with liquid coffee waste. Pollution from untreated liquid coffee waste not only threatens environmental quality and ecosystem health but also has a direct impact on the income of local communities who depend on these natural resources. This is felt by fish farmers in Semarang City who are affected by liquid coffee waste pollution. Therefore, further research that measures the ability of *Nymphaea pubescens* to mitigate the impact of liquid coffee waste is very important to be developed. Thus, this study aims to determine the effectiveness of phytoremediation of *Nymphaea pubescens* in reducing ammonia and phosphate concentrations of liquid coffee waste.

RESEARCH METHODS

The liquid coffee waste used in this study was a mixture of residual water

from the coffee bean peeling and washing process obtained from one of the coffee processing factories in Semarang City. Meanwhile, the Nymphaea pubescens lotus was obtained from a swamp in Semarang City. Before the Nymphaea pubescens was used as a biofilter, acclimatization was carried out first. The aim is to adjust the condition of the lotus so that it has good pollutant absorption capacity. Acclimatization is carried out by placing the lotus in an acclimatization pond for 7 days before being used as a biofilter Rismawati *et al* (2020). This is strengthened by Muryani & Widiarti (2018) that the effective acclimatization time for lotus before phytoremediation treatment is 7 days. The acclimatization pond contains water from rainwater reservoirs and is left exposed to direct sunlight (Elizabeth *et al*., 2020; Hasibuan *et al*., 2020). If within 7 days the plants do not experience any morphological changes, the process of exposure to liquid coffee waste can be continued (Rismawati *et al*., 2020).

Research Subject

The subject of this study is *Nymphaea pubescens* which will be used for phytoremediation of coffee liquid waste, with research parameters are ammonia and phosphate. Phytoremediation process is carried out at the Biology Education Laboratory of PGRI Semarang University in December 2023. Meanwhile, the ammonia and phosphate consentrations of coffee liquid waste are tested at the Health Laboratory and Medical Device Testing Center in Semarang.

Research Design and Methods

This type of research is a true experiment with a Completely Randomized Design with 4 variations of lotus plant biomass *Nymphaea pubescens*. The variations of lotus biomass *Nymphaea pubescens* are 0 gram, 25 grams, 50 grams, and 75 grams with each biomass added as much as 20 liters of coffee waste. Each treatment was repeated 3 times, so that 12 experimental treatment units were obtained. The sampling technique in this study was purposive sampling which is carried out by taking coffee wastewater from a coffee processing factory that led to Tanggang Streambed. The experimental layout is arranged according to completely randomized design with a combination of treatments between concentration and duration of phytoremediation as shown in Figure 1.

D_1	B_1	A_1	A_2
C_1	D_2	C_2	B_3
D_3	A_3	C_3	B_2

Figure 1. Experimental Layout

Information:

A: P0 (0 gr *Nymphaea pubescens* + 20 liters of coffee liquid waste)

B: P1 (25 gr *Nymphaea pubescens* + 20 liters of coffee liquid waste)

C: P2 (50 gr *Nymphaea pubescens* + 20 liters of coffee liquid waste)

D: P3 (75 gr *Nymphaea pubescens* + 20 liters of coffee liquid waste)

1,2,3: Repeat-

Phytoremediation Procedure

The phytoremediation process was carried out according to the experimental design for 6 days starting from when the lotus was placed in the experimental tank. Sampling for measuring ammonia and phosphate concentrations was carried out three times, on day-0 or before phytoremediation, day-3 of phytoremediation, and day-6 of phytoremediation. On day-0 or before the lotus was placed in the phytoremediation tank, initial measurements were carried out which aimed to identify the initial conditions of the wastewater before biological treatment was carried out to determine the efficiency of the treatment method (Elystia *et al*., 2021). Ammonia concentration testing uses the Nesseler method which adjusted to SNI 06.2479.1991. Phosphate concentration testing uses the Vanadate analysis method 4500 APHA P.C.

Data Analysis

The research data obtained was then tested using a two-way Analysis of Variance (ANOVA) to determine the effect of treatment on the observed parameters. If the ANOVA test results show a significant effect of the treatment, the next stage is to conduct a Duncan Multiple Range Test to compare the average between treatments. The Duncan Multiple Range Test aims to determine which treatment is significantly different from other treatments, thus providing a deeper understanding of the effect of each treatment in the study.

RESULTS AND DISCUSSION

Effectiveness of Phytoremediation Using *Nymphaea pubescens* on Ammonia Concentration

Coffee liquid waste is one of the industrial wastes that has the potential to cause water pollution. The largest potential for liquid waste is produced from the coffee bean washing process carried out after the fermentation process (Azizah *et al*., 2019). The main components of coffee liquid waste are organic materials such as protein, glucose, and mucus. The majority of organic material in coffee liquid waste contains high concentration of ammonia. Ammonia is the main product of the decomposition of organic nitrogen waste

so it can be used as a chemical parameter for water pollution (Hamonangan & Yuniarto, 2022). The ammonia content of coffee liquid waste before treatment was 2.15 mg/L. This concentration is above the quality standard set in the Decree of the Minister of Environment No. 28 of 2001, which is 0.5 mg/L. The high concentration of ammonia have an impact on the sudden mass death of fish in the ponds. The pond ecosystem is contaminated with coffee waste because the waste outlet from the coffee factory leads to the pond located close to the coffee production factory and flows through the Tenggang River.

Table 1. Average of Ammonia Concentrations on Day-3 After Treatment

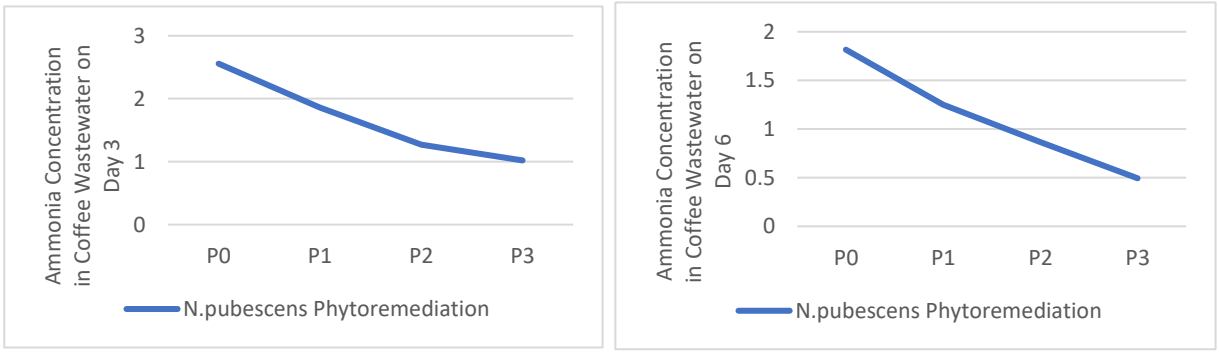
Treatment	Repeat - (mg/L)			Quality standards *) (mg/L)	Reduction (%)
	1	2	3		
P0	1.11	0.758	0.689	0.5	0
P1	0.797	0.529	0.534	0.5	27
P2	0.587	0.401	0.284	0.5	50
P3	0.426	0.347	0.247	0.5	60

*Quality standards of Indonesia Government Regulation No. 82 of 2001

Table 2. Average of Ammonia Concentrations on Day-6 After Treatment

Treatment	Repeat - (mg/L)			Quality standards *) (mg/L)	Reduction (%)
	1	2	3		
P0	0.628	0.527	0.661	0.5	0
P1	0.622	0.415	0.215	0.5	31
P2	0.440	0.336	0.189	0.5	52
P3	0.113	0.305	0.176	0.5	72

*Quality standards of Indonesia Government Regulation No. 82 of 2001



(a) (b)

Figure 2. Graph of Decreasing Ammonia Concentrations (a) Day-3 (b) Day-6

Based on the research results, it was found that phytoremediation of *Nymphaea pubescens* was effective in reducing ammonia concentration in coffee liquid waste. This can be proven by the results of the analysis using the ANOVA test and Duncan's multiple range test which showed that the treatment of *Nymphaea pubescens* phytoremediation had a significant effect on reducing ammonia concentration in coffee liquid waste. Lotus can absorb toxins and

survive in polluted environments because it has long and dense rhizomes so it is very good at absorbing and expanding the surface area of toxin absorption (Dordio *et al*, 2011). On the third day, all P3 repetitions had experienced a decrease in ammonia levels to below the quality standard. The same thing happened to P2 although there was still one treatment that had not reached the quality standard. However, both P3 and P2 had decreased on average to below the quality standard. Meanwhile, on the third day, the ammonia levels of P0 and P1 were still above the quality standard. The ammonia levels of P0 on the sixth day were still high. However, P1 had experienced a decrease in ammonia levels to below the quality standard on the sixth day. Meanwhile, P2 and P3 continued to experience a decrease in ammonia levels. Of the four treatments, the most effective in remediating ammonia was P3 with a fairly large percentage decrease. The more Nymphaea pubescens biomass, the more ammonia from coffee liquid waste is reduced. This is as stated in Khaer & Nursyafitri (2017) and Sholehah *et al* (2022) which state that the more phytoremediator plants, the more organic matter will be absorbed so that pollutant levels will be lower.

Phytoremediation of *Nymphaea pubescens* on ammonia concentration begins with the rhizofiltration process. Ammonia is a compound that is soluble in water so that ammonia is absorbed by the long and dense lotus roots along with water. After the ammonia reaches the roots, the next stage of phytoremediation is phytodegradation. Although lotus can absorb ammonia, nitrate has the highest level of Nitrogen absorption efficiency compared to ammonia. According to Ismuhajaroh and Nisa (2020), the optimum pH for the growth of *Nymphaea pubescens* lotus is pH 5.7 (acidic atmosphere) so it requires more anions as nutrients. Thus, the ammonia stage will be degraded into nitrate, and with the help of the enzyme oxygenase ammonia will be broken down into nitrate compounds. The decomposition of ammonia (NH_3) into Nitrate (NO_{3-}) is also carried out with the help of nitrifying bacteria. This is as in the research of Rahmawan *et al* (2023) that the process of reducing pollutant levels is a collaboration between aquatic plants and microbes, then degraded by microorganisms into simpler compounds.

After ammonia is successfully degraded into nitrate, it is then forwarded to the upper part of the lotus through the transport network to other parts of the plant. Nitrate will be utilized by the lotus for the assimilation process in forming new cells, helping the photosynthesis

process, and the formation of amino acids. According to Mengel & Kirkby (2017), the range of Nitrogen concentrations needed in lotus tissue is 1.0-3.5% of the dry weight of the lotus or around 0.1-0.35 ppm. If the N content absorbed by the lotus is more than 3.5 ppm, the lotus tries to prevent pollutant poisoning of its cells by storing pollutants in the root vacuoles so as not to inhibit its metabolic process.

Effectiveness of Phytoremediation Using *Nymphaea pubescens* on Phosphate Concentration

Phosphate is one of the compounds whose presence can be used as a chemical parameter of environmental pollution. In water, phosphate is found in inorganic form, and its presence affects the balance of the ecosystem in it. The phosphate content in liquid coffee waste before treatment was 0.756 mg/L. This concentration is above the standard quality standards set in the Decree of the Minister of Environment No. 28 of 2001, which is 0.2 mg/L. The high phosphate concentration are also one of the factors in the decline in the production of pond cultivation located close to the coffee factory. This is reinforced by Sutamihardja *et al*., (2018) that high phosphate concentration in waters cause phytoplankton blooming so that the amount of dissolved oxygen decreases and causes mass fish deaths.

Table 3. Average of Phosphate concentration on Day-3 After Treatment

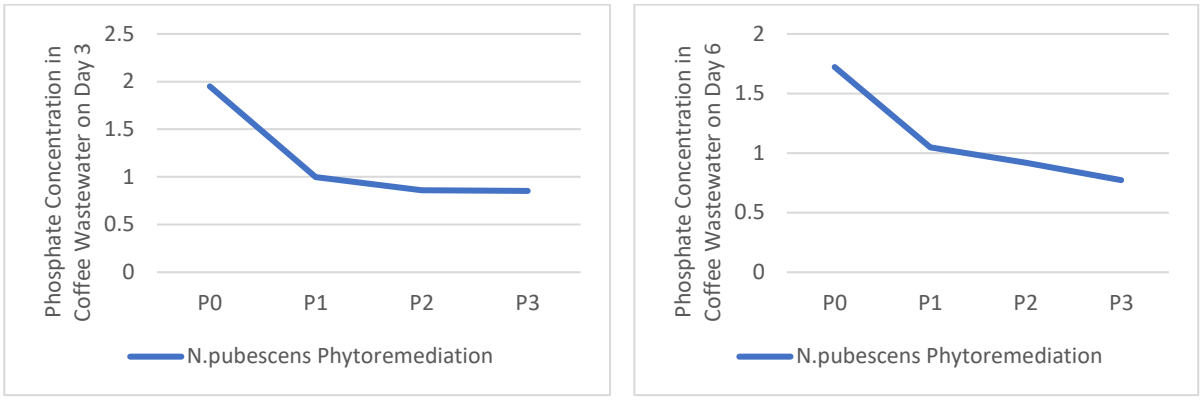
Treatment	Repeat - (mg/L)			Quality standards *) (mg/L)	Reduction (%)
	1	2	3		
P0	0.378	0.353	0.319	0.2	0
P1	0.333	0.264	0.402	0.2	48
P2	0.293	0.288	0.278	0.2	55
P3	0.266	0.336	0.251	0.2	56

*Quality standards of Indonesia Government Regulation No. 82 of 2001

Table 4. Average of Phosphate concentration on Day-6 After Treatment

Treatment	Repeat - (mg/L)			Quality standards *) (mg/L)	Reduction (%)
	1	2	3		
P0	0.333	0.358	0.331	0.2	0
P1	0.333	0.259	0.455	0.2	39
P2	0.306	0.322	0.291	0.2	47
P3	0.285	0.273	0.215	0.2	55

*Quality standards of Indonesia Government Regulation No. 82 of 2001



(a) (b)

Figure 3. Graph of Decreasing Phosphate concentration (a) Day-3 (b) Day-6

High phosphate concentration in coffee wastewater can be reduced through the phytoremediation process *using Nymphaea pubescens* plants. Based on the results of research that has been carried out, *Nymphaea pubescens* phytoremediation is effective in reducing phosphate concentration in coffee wastewater. This can be proven by the results of the analysis using the Anova test and Duncan's Multiple Range Test, which show that the *Nymphaea pubescens* phytoremediation treatment has a significant effect on reducing phosphate concentration in coffee wastewater. From the four treatments, P1, P2, and P3 decrease in phosphate concentration. However, from the third to the sixth day, phosphate concentration of all treatments had not reached the quality standard. In the research of Afasyimi (2022) and Hastutik (2023), it was stated that the longer the absorption time, the greater the pollutants absorbed by plants. Considering that, phosphate concentration before treatment is very high, 0.756 mg/L, additional time and concentration needed to remediate the phosphate concentration to meet the established quality standards. However, the average of phosphate concentration in P3 was not much different from the quality standards.

The decrease in phosphate concentration in liquid coffee waste occurs due to the absorption of phosphate by lotus roots. Phosphate is the main source of phosphorus needed in large quantities for plant growth and development. Phosphate is a compound that plays a vital role for plants, such as energy transfer, root growth, assimilation, and respiration, and helps flowering and fruit ripening. In several samples that were given treatment, there were some that experienced an increase in phosphate concentration, this was because the phosphate that settled dissolved again, so the phosphate concentration in the wastewater

increased. However, overall there was a decrease in phosphate concentration.

Phosphorus is absorbed by plants in the form of inorganic ions orthophosphate. Orthophosphate with compounds such as H_2PO_4-, HPO, and PO can be utilized directly by the lotus without having to break it down first. Phytoremediation of lotus *Nymphaea pubescens* begins with the rhizofiltration process. Phosphate is a compound that is soluble in water, so phosphate is absorbed by the long and dense lotus roots along with water. The process of phosphate absorption in *Nymphaea pubescens* is carried out by the tips of the root surface by diffusion with the meristem tissue so that there is a pulling movement by the water molecules in the lotus (Rusyani, 2014). Through the phosphate transport network, it is translocated to the upper part of the lotus following the transpiration flow. According to Mengel & Kirkby (2017), the range of phosphorus concentrations needed in lotus tissue is 1-1.5% of the dry weight of the lotus or around 0.1-0.15 ppm. If the phosphate content in the environment is more than 0.15 ppm, the phytoextraction process will occur. The lotus tries to prevent pollutant poisoning of its cells by accumulating phosphate in the root cell vacuoles so as not to inhibit its metabolic processes.

Figure 3. Chlorosis and Necrosis of Nymphaea pubescens

Based on the results of observations during the study, it was found that the condition of *Nymphaea pubescens* leaves in sample P1 turned yellow and the tips of the leaves became wrinkled. This is because *Nymphaea pubescens* experienced poisoning caused by the large volume of liquid coffee wastewater which was not balanced with the large amount of phytoremediator plant biomass. As a result, there was damage to the morphology of *Nymphaea pubescens* with a biomass of 25 grams, which was marked by yellowing of the leaves and disruption of the physiological process of the plant which resulted in the plant not

being able to absorb large amounts of pollutants optimally. This is reinforced by Rukmi (2013) who stated that phytoremediator plants can experience poisoning which is marked by symptoms of chlorosis and necrosis. Chlorosis is poisoning in plants which is marked by symptoms of yellowing of the leaves of the plant due to loss of green leaf substance while necrosis is a symptom of plant cell death which is marked by rolling and wrinkling of the leaves so that the physiological process of the plant is disrupted and causes the inability of the plant to absorb large amounts of waste. Thus, the lotus that is effective in absorbing liquid coffee waste is P3 with a biomass of 75 grams *Nymphaea pubescens*.

CONCLUSION

Phytoremediation of *Nymphaea pubescens* can effectively reduce ammonia and phosphate concentrations in liquid coffee waste. During the phytoremediation process, both remediation time and plant biomass significantly influence the amount of pollutants absorbed. The more plant biomass used and the longer remediation process, more pollutants are absorbed. This study not only contributes to environmentally friendly and sustainable waste management, but also introduces new insights into phytoremediation with a focus on *Nymphaea pubescens* as a means to reduce ammonia and phosphate concentrations in liquid coffee waste. Implementing the research findings on a large scale, particularly within the coffee industry, local communities, or educational institutions, is highly recommended to validate the practical benefits of this phytoremediation technology. Simultaneously, this will help raise awareness about the importance of maintaining and preserving the environment in a sustainable manner.

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