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PHYTOREMEDIATION OF LIVESTOCK WASTEWATER USING *Azolla filiculoides* AND *Lemna minor*

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ABSTRACT

The increment in livestock production leads to high risk of environmental problems such as water pollution due to the improper management of livestock operations. Phytoremediation is an alternative approach in treating wastewater from livestock from further contaminate the water bodies. For this study, *Azolla filiculoides* and *Lemna minor* were used as the phytoremediation plants to remove ammoniacal nitrogen and chemical oxygen demand in the wastewater from livestock. Livestock wastewater was treated using *Azolla filiculoides* and *Lemna minor* for six days. Using the reactor digestion and Nessler methods, the initial values before treatment for ammoniacal nitrogen and chemical oxygen demand were 109.83 mg/L and 11,742 mg/L, respectively. After six days of treatment, ammoniacal nitrogen was reduced to 51.93 mg/L and 43.93 mg/L by using *Azolla filiculoides* and *Lemna minor*, respectively. The chemical oxygen demand was decreased to 680 mg/L and 820 mg/L by using *Azolla filiculoides* and *Lemna minor*, respectively. The results showed that both *Azolla filiculoides* and *Lemna minor* were efficient in removing the nutrients in the livestock wastewater.

KEYWORDS

Azolla filiculoides, Lemna minor, phytoremediation, livestock wastewater.

1. INTRODUCTION

Water is one of the most important resources for all living organisms on earth. According to a research, water resources are the most fragile resources in the environment [1]. Due to its delicacy, water resources may have high probability of facing various environmental problems such as water pollution. In Malaysia, the rising in the productivity of livestock farming sectors is important for the economy [2]. World Bank stated that the global meat production was tripled from 45 million tonnes to 134 million tons [3]. These numbers are expected to further increase by 2050. Nevertheless, the increment in livestock production leads to high risk of environmental problems such as water pollution. This problem happens due to the improper management of livestock operations.

A study stated that the excessive amount of nutrients such as phosphorus and nitrogen in water will lead to vigorous weed growth and causing eutrophication [4]. Eutrophication is an event corresponding to the changes in the water environment where the sudden enrichment of nutrients in the water and causing the abundance growth of algae and aquatic macrophytes [5]. Phytoremediation is a treatment that can be applied to overcome these problems [6]. Phytoremediation is a nature-based approach using aquatic plants to breakdown and remove various environmental pollutants such as heavy metals, pesticides and organic compounds away from the environment [7-8]. This cost-effective approach is now widely used throughout the world.

Several aquatic plants such as water lettuce (*Salvinia stratiotes*), water hyacinth (*Eichhornia crassipes*) and water lilies (*Nymphaea spontanea*) are well-known as effective phytoremediation agents. *Azolla filiculoides* is a small water heterosporous fern and belongs to the family of *Azollaceae.* This particular species is commonly found at ponds, ditches, water reservoirs, wetlands, channels as well as at slow running rivers. Moreover, *A. filiculoides* has a worldwide distribution and native from the American subtropics [9]. A group researcher, reported that *A. filiculoides* is widely distributed in aquatic ecosystem and considered as an invasive species in some areas [10].

Lemna minor or also known as duckweed is a free floating aquatic plant and belongs to family of *Lemnaceae*. *L. minor* can be found in a rich nutrient content area such as ponds, ditches and even in swamp areas [11]. This species is widely distributed throughout the world expect for in the regions where the temperature pass 0°C in some time in a year [12]. Both of these prolific plant species are known to be utilized in the removal of organic and non-organic materials from the water bodies. Therefore, the objective of this study was to measure the effectiveness of *Azolla filicuoides* and *Lemna minor* in reducing nutrients in livestock wastewater sample.

2. METHODOLOGY

2.1 Experimental Design

The livestock wastewater samples were collected at the cattle farm located in Melaka, Malaysia. Prior to the experiments, the wastewater samples were filtered in order to remove the foreign substances that might affect the experiments. Then, wastewater samples were divided equally into 3 tanks filled with 6 liters of the livestock wastewater.

Azolla filiculoides and *Lemna minor* were selected as phytoremediation plants for treating the livestock wastewater. These aquatic plants were weighed at 30 g for each species. Prior to the experiment, both aquatic plants were rinsed thoroughly by using distilled water especially at the root zones in order to remove the foreign substances which may affect the experiment. Both *Azolla filiculoides* and *Lemna minor* were allocated into 4 tanks where 2 tanks were filled with wastewater samples and the other 2 tanks were filled with distilled water. The livestock wastewater was exposed to both plants species for a duration of 6 days where the analysis was measured on every alternate day. Figure 1 shows the illustration of the preparation of the plant and wastewater samples.

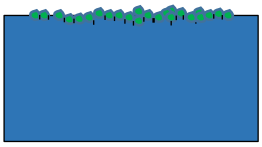
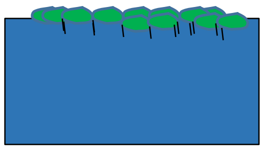
Weight of plant	Type of plant with wastewater sample/control	
	Azolla filiculoides	*Lemna minor*
30 g	a) 	b) 

Figure 1: a) Azolla filiculoides; b) Lemna minor

2.2 In-Situ Analysis

In-situ analysis was done to determine the physico-chemical characteristics of the wastewater samples. The multiparameter YSI 556 was used in this study for the measurement of pH, temperature, turbidity, conductivity and dissolved oxygen (DO). Prior to in-situ analysis, the multiparameter YSI 556 was calibrated according to the specific parameter measurement procedure.

2.3 Ex-situ Analysis

Ex-situ analysis was done for chemical oxygen demand and ammoniacal nitrogen using Spectrophotometer UV/VIST60. Closed pump and Nessler methods were used to measure the chemical oxygen demand and ammoniacal nitrogen, respectively.

3. RESULTS AND DISCUSSION

3.1 Physico-chemical Parameters

Table 1 shows the results for the physico-chemical parameters of livestock wastewater before treatment. According to a study, pH is an indicator of the acidity of water solution [13]. In this study, the pH level of livestock wastewater is 8.16 and it is considered as alkaline. This condition is maybe due to eating habit of the livestock. Turbidity is a result of the existence of the organic and inorganic constituents in the water. Organic constituent promotes the microorganism growth thus may lead to waterborne disease, whereas inorganic constituent did not cause any harm. The value for turbidity in livestock wastewater was 117.67 NTU. This condition is due to the cattle's dung upon the cleaning of the barn. Apart from that, cattle's dung consists a lot of organic constituents which promotes the microorganism growth. Conductivity indicates the presence of cations and anions in the water bodies. In this study, the value for conductivity in livestock wastewater was 3456.67 µS/cm. This situation is due to amount of salt concentration in the livestock wastewater resulted from the feeding pattern of the livestock where they were fed with acid salts in a pellet form in order to reduce the ammonia emission.

Dissolved oxygen is the dissolved form of oxygen and essential for aquatic organisms. According to a study, dissolved oxygen enters the water through diffusion from the atmosphere as well as the by-product of photosynthesis process by the aquatic plants and algae [13]. A low number of dissolved oxygen with value less than 2 mg/L indicates poor water quality, thus affect the sensitivity of aquatic organisms. In this study, the value of livestock wastewater is 3.23 mg/L. Total dissolved solid is a measure of all constituents dissolved in water. The total dissolved solid in water comprises inorganic salts and dissolved materials. Salts comprise the anions such as carbonates, chlorides, sulfates, nitrates and cations such as potassium, magnesium, calcium and sodium [13]. For livestock wastewater, the value for total dissolved solid was 2312.67 mg/L and high total dissolved solid affects the odor and taste of the water.

Table 1: Physico-chemical parameters of livestock wastewater

Parameter	Unit	S1	S2	S3	Mean
pH	-	8.17	8.16	8.15	8.16
Turbidity	NTU	92	123	138	117.67
Conductivity	µS/cm	3340	3530	3500	3456.67
Dissolved oxygen	mg/L	3.2	3.4	3.1	3.23
Total dissolved solid	mg/L	2271	2350	2317	2312.67
Ammoniacal nitrogen	mg/L	109	110	110.5	109.83
Chemical oxygen demand	mg/L	109	110	110.5	109.83

3.2 Phytoremediation of ammoniacal nitrogen and chemical oxygen demand

3.2.1 Phytoremediation of ammonical nitrogen using Azolla filiculoides and Lemna minor

Figure 2 shows the rate of reduction for ammoniacal nitrogen in livestock wastewater by using *Azolla filiculoides* and *Lemna minor*. The value of ammoniacal nitrogen before treatment was 109.83 mg/L. Based on the Figure 2, the plot graph for livestock wastewater with *Azolla filiculoides* shows a constant rate of reduction of ammoniacal nitrogen from day zero to day six with 52.7% of reduction. Whereas for the plot graph for wastewater with *Lemna minor*, it shows 12% rate of reduction of ammoniacal nitrogen from day zero to day two. Then, the pattern for the plot graph continues declining from day two until day six with the final value of 43.93 mg/L (54.4% rate of reduction) for ammoniacal nitrogen.

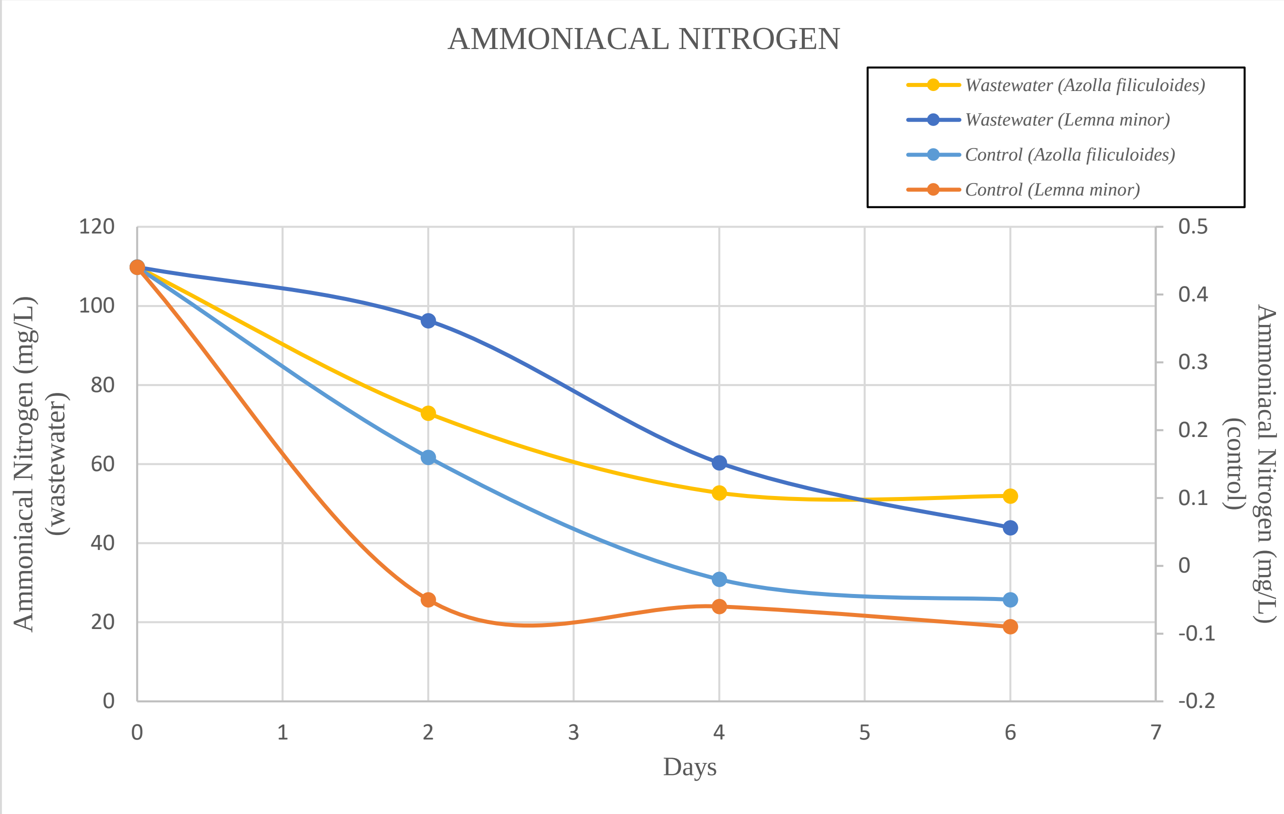


Figure 2: Rate of reduction for ammoniacal nitrogen in livestock wastewater by using Azolla filiculoides and Lemna minor

According to a study, the reduction of ammoniacal nitrogen is due to the absorption by the *Lemna minor* [14]. Apart from that, a group researcher mentioned that both roots and fronds of family *Lemnaceae* are capable to take up nutrients [15]. *Lemna minor* is said to be drawn more into NH_4^+ as a nitrogen source, however can lead to the retardation in their growth if in high concentration [16, 17]. According to Forni et al., lower reduction of ammoniacal nitrogen by *Azolla filiculoides* compared to *Lemna minor* can be endorsed to its capability in fixing nitrogen in the atmosphere, therefore leads to little demand of nitrogen from water [18]. Apart from that, this situation is maybe driven by the phosphorus deficiency of the *Azolla filiculoides*.

3.2.2 Phytoremediation of chemical oxygen demand using Azolla filiculoides and Lemna minor

Figure 3 shows the rate of reduction for chemical oxygen demand in livestock wastewater by using *Azolla filiculoides* and *Lemna minor*. The value of chemical oxygen demand before treatment was 11,742 mg/L. Based on the Figure 3, the plot graph for livestock wastewater with *Lemna minor* shows a steep slope for the rate of reduction from day zero to day six with 93.7% of reduction. Whereas the plot graph for livestock wastewater with *Azolla filiculoides* shows a 94.2% of reduction from day zero to day six. Thus, the final values of chemical oxygen demand for livestock wastewater treated with *Lemna minor* and *Azolla filiculoides* were 740 and 680 mg/L, respectively.

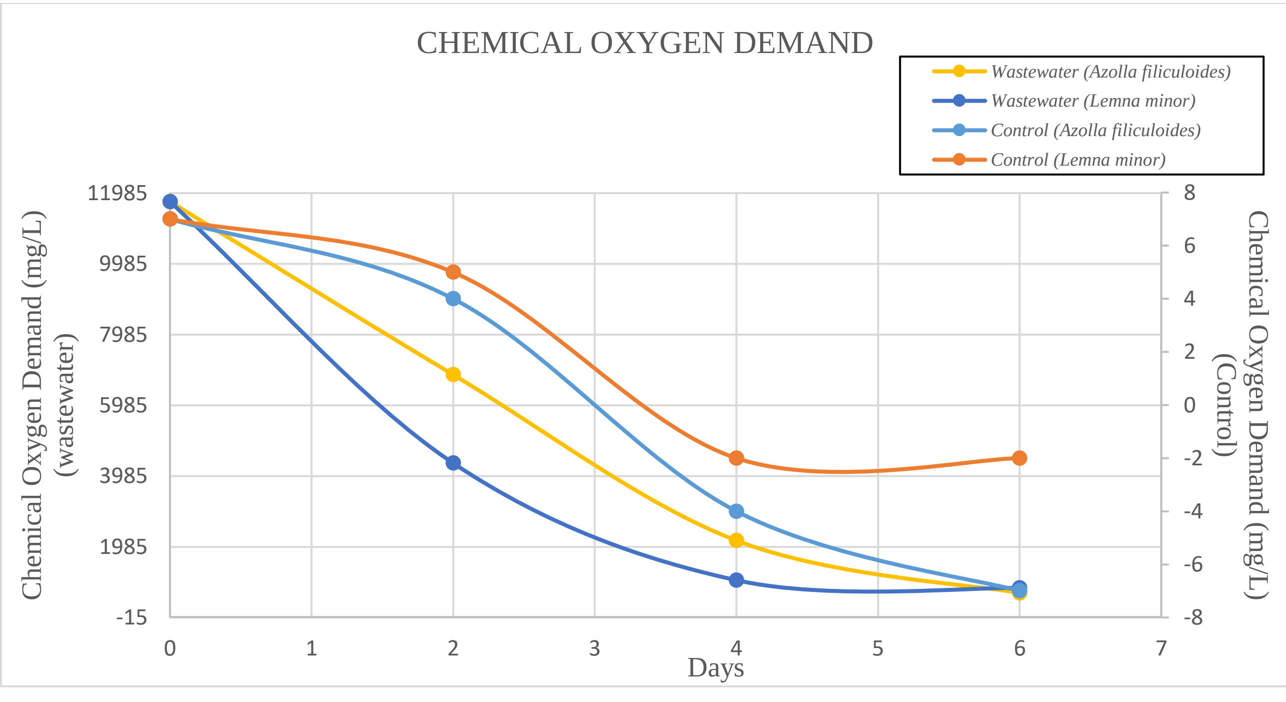


Figure 3: Rate of reduction for chemical oxygen demand in livestock wastewater by using Azolla filiculoides and Lemna minor

Based on a study done by a group researcher, *Azolla* species can remove chemical oxygen demand by 89.10% [19]. According to Singh et al., the reduction in chemical oxygen demand is the result from the oxidation process of organic compounds done by microorganisms' during phytoremediation treatment [20]. The effectiveness of chemical oxygen demand removal differed based on various species to different contaminants [21].

4. CONCLUSION

Lemna minor shows a great performance in reducing the ammoniacal nitrogen by reaching 66.4% reduction until the last day of experiment. As for chemical oxygen demand removal, *Lemna minor* able to reduce approximately 93.7%. *Azolla filiculoides* able to reach 52.7% of reduction in the ammoniacal nitrogen, whereas in chemical oxygen demand, *Azolla filiculoides* able to reduce to 94.2% until the last day of experiment. It can be concluded that phytoremediation is a great alternative way to remove the contaminants in the water bodies along with its cost effective and green technology-based. The selection of plant is a crucial part in phytoremediation process as it is the main tool in removing the contaminants.

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