# Phytoremediation of TSS, NH₃-N and COD from Sewage Wastewater by *Lemna minor* L., *Salvinia minima, Ipomea aquatica* and *Centella asiatica*

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**Abstract:** The rapid growth of industries has resulted in wastewater generation containing different organic and chemical substances channeled into the water body. This causes the arising of water pollution issues in many regions. The phytoremediation method was introduced in the process of treating water pollution as it is low cost and environmentally friendly. *Lemna minor, Salvinia minima, Ipomoea aquatica* and *Centella asiatica* were chosen in this study because they have tolerance to various pollution conditions and are able to remove organic pollutants and heavy metals. The objectives of this study were to determine the water quality before and after treatment, to determine the rate of reduction in total suspended solids (TSS), ammoniacal nitrogen (NH₃-N), and chemical oxygen demand (COD) in sewage water through the phytoremediation method and to assess the effectiveness of the plants in the phytoremediation of sewage wastewater. It was found that, *Lemna minor, Salvinia minima, Ipomoea aquatica* and *Centella asiatica* were able to reduce TSS by 50.8%, 77.6%, 85.6% and 67.6%, respectively; NH₃-N by 80.4%, 89.9, 97.3% and 79.1%, respectively; and COD by 75%, 82%, 44.8% and 36.46%, respectively. In this study, it was found that sewage wastewater treatment using *Ipomoea aquatica* was more efficient in reducing NH₃-N and *Salvinia minima* was more efficient in reducing TSS and COD values.

**Keywords:** phytoremediation; *Lemna minor* L.; *Salvinia minima; Ipomea aquatics; Centella asiatica*; sewage wastewater treatment

## 1. Introduction

Wastewater is generated from the industrial, domestic, commercial and agricultural sectors [1,2]. Untreated wastewater contains various types of pollutants consisting of organic and inorganic materials with different concentrations [3], depending on the type of human activity that produces the wastewater. The water body can be contaminated when exposed to a high volume of wastewater and high concentration of contaminants [4]. The untreated wastewater should not be released into the environment because it contains harmful pollutants that can affect human health and ecosystem quality. Three-quarters of the organic carbon in wastewater presents as carbohydrates, fats, proteins, amino acids and volatile acids and inorganic pollutants including sodium, calcium, potassium, magnesium, chlorine, sulfur, phosphate, bicarbonate, ammonium salts and heavy metals [5].

Domestic wastewater generated from the household includes water from the sink, bathroom, toilet, washing machine and dishwasher. Industry and business centers also produced wastewater that must be treated before being released to the environment. Hazardous materials such as heavy metals and hazardous chemicals from the road surface, parking lot, roof and building can penetrate and pollute the river, lake and water body. Accordingly, treatment plants were built in every industry, factory and even residential area to treat wastewater. A treatment plant can help in reducing the pollutants in wastewater to the extent that the environment can be sustained and not harmed. However, most of the wastewater treatment technologies require high capital investment and also contribute to the problem of sludge disposal [6]. Thus, an environmentally friendly method of phytoremediation has been introduced as one of the wastewater treatment technologies. Phytoremediation uses green plants which make it an attractive biological treatment method [7].

Phytoremediation is a technology or method that used natural or genetically modified plants to remove, transfer, stabilize or reduce various pollutants contained in soil or water media [8]. The phytoremediation agents can remove organic and inorganic contaminants and interact with microorganisms [9,10]. Wastewater treatment using a phytoremediation method offers several advantages, such as a cost-effective approach, minimum energy requirement, minimal environmental disturbance, conservation of soil biological activity, etc. [11]. Phytoremediation can also occur naturally in soil and water ecosystems [12]. This technology has been accepted in the last two decades as an environmentally friendly and low cost approach in wastewater treatment. According to Mojiri [13], the use of plants for the recovery of soil and water which was contaminated with heavy metals have been proved to be effective. Kasim and Rahman [1] found that lake water treatment using aquatic plants was beneficial to improving water quality, with high efficiency in reducing chemical oxygen demand (COD).

Table 1 shows several previous studies that were conducted in Malaysia on the removal of TSS, $NH_3$-N and COD by phytoremediation treatment. In Malaysia, the use of plants to treat polluted water or soil has gained popularity. Many studies have been conducted to study the capability of the plant in reducing different types of contaminants in soil or water, and the research outputs can be practiced in various sectors [14–16]. To date, phytoremediation is not only important for universities and research centers, but also creates new business for contractors and consulting firms. The objectives of this study were to determine the water quality before and after treatment, to determine the rate of reduction in total suspended solids (TSS), ammoniacal nitrogen ($NH_3$-N), and COD in sewage wastewater through the phytoremediation method by *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica*, and also to evaluate the effectiveness of these plants in the phytoremediation of sewage wastewater. These plants were selected as the phytoremediation agents in this study because of their rapid growth rate and tolerance to various environmental conditions.

**Table 1.** The removal of total suspended solids (TSS), ammoniacal nitrogen ($NH_3$-N) and chemical oxygen demand (COD) by phytoremediation treatment based on previous studies in Malaysia.

| Reference | Pollutant | Type of Plant | Removal Rate (%) | Duration |
|---|---|---|---|---|
| Safauldeen et al. [4] | COD, TSS | *Eichhornia crassipes* | 83, 92 | 28 days |
| Hanafiah et al. [17] | $NH_3$-N, TSS | *Salvinia molesta* <br> *Pistia stratiotes* | 96, 88 <br> 83, 95 | 7 days |
| Hazmi and Hanafiah [18] | COD, $NH_3$-N | *Lemna minor* <br> *Azolla filiculoides* | 93.7, 66.4 <br> 94.2, 52.7 | 6 days |
| Nizam et al. [19] | $NH_3$-N, TSS | *Centella asiatica* <br> *Ipomea aquatica* <br> *Salvinia molesta* <br> *Eichhornia crassipes* <br> *Pistia stratiotes* | 98, 90 <br> 73, 73 <br> 63.9, 89.3 <br> 74, 96 <br> 78, 98 | 14 days |
| Akinbile et al. [20] | $NH_3$-N | *Eichhornia crassipes* | 86 | 49 days |

**Table 1.** *Cont.*

| Reference | Pollutant | Type of Plant | Removal Rate (%) | Duration |
|---|---|---|---|---|
| Akinbile and Yusoff [21] | COD, $NH_3$-N | *Eichhornia crassipes*<br>*Pistia stratiotes* | 59, 85<br>54, 82 | 28 days |
| Akinbile et al. [22] | COD, $NH_3$-N, TSS | *Azolla pinnata* | 71, 62, 80 | 28 days |
| Darejah et al. [23] | COD | *Chrysopogon zizanioides* L. | 94 | 14 days |
| Ng and Chan [24] | COD | *Salvinia molesta* | 39 | 16 days |
| Rezania et al. [25] | COD, $NH_3$-N, TSS | *Eichhornia crassipes* | 41, 64, 34 | 21 days |

## 2. Materials and Methods

### 2.1. Sampling and Laboratory Analysis

The sewage wastewater samples were collected at the sewage treatment plant at a residential area located in Melaka. Initial data parameter analysis involved in-situ measurement (temperature, pH, conductivity and dissolved oxygen (DO)), while the ex-situ measurement parameters such as TSS, $NH_3$-N, COD and biochemical oxygen demand (BOD) were performed in the laboratory. The readings were taken in triplicate for each parameter. All recorded values were in the form of standard deviation and mean values. The reading of temperature, pH, conductivity and DO was recorded using the YSI 5000 multiparameter instrument (YSI Inc., Yellow Springs, OH, USA). BOD was determined by using the following formula (Equation (1)). After the first reading was taken ($DO_i$), the samples were kept in the incubator for five days at 20 °C ($DO_5$).

$$BOD_5 = DO_i - DO_5 \tag{1}$$

COD content was measured using HACH DR2500. The $NH_3$-N reading was determined using the Nessler method and the instrument used was a HACH DR/2010 spectrophotometer (HACH, Loveland, CO, USA). TSS was determined using the gravimetric method. Prior the analysis, distilled water was filtered through 0.45 μm filter paper to remove foreign substances. The filter paper was then dried in the oven at 105 °C and weighed. The sewage wastewater sample was filtered and the filter paper was dried again at 80 °C. The dried filter paper with residual substances was weighed. The calculation of TSS content was obtained using Equation (2):

$$TSS \text{ (mg/L)} = (A - B)/V \times 1000 \text{ mL} \tag{2}$$

where,

A = Weight of filter paper after filtration
B = Weight of filter paper before filtration
V = Volume of filtered water sample.

### 2.2. Phytoremediation Treatment

*Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica* were selected as phytoremediation agents in this study. These plants were chosen because they have a rapid growth rate and were tolerant to various conditions. Prior to the experiment, the plant samples were first rinsed thoroughly, especially at the root area, to remove foreign substances, and were left in a tank filled with distilled water for a week to neutralize the plants. The sewage wastewater samples were filtered to remove foreign substances before being used for the experiment. Four containers with dimensions of 38 cm × 25 cm × 15 cm were filled with plants and sewage wastewater for treatment. Each plant was weighed 30 g and were placed in a container containing 6 L of sewage wastewater. All containers were placed under lighting in the laboratory to maintain the optimum surrounding conditions for

the aquatic plants. The reading of COD, TSS and $NH_3$-N values of sewage wastewater samples were recorded alternately every two days for a duration of 8 days. Figure 1 shows the experimental set up in this study.
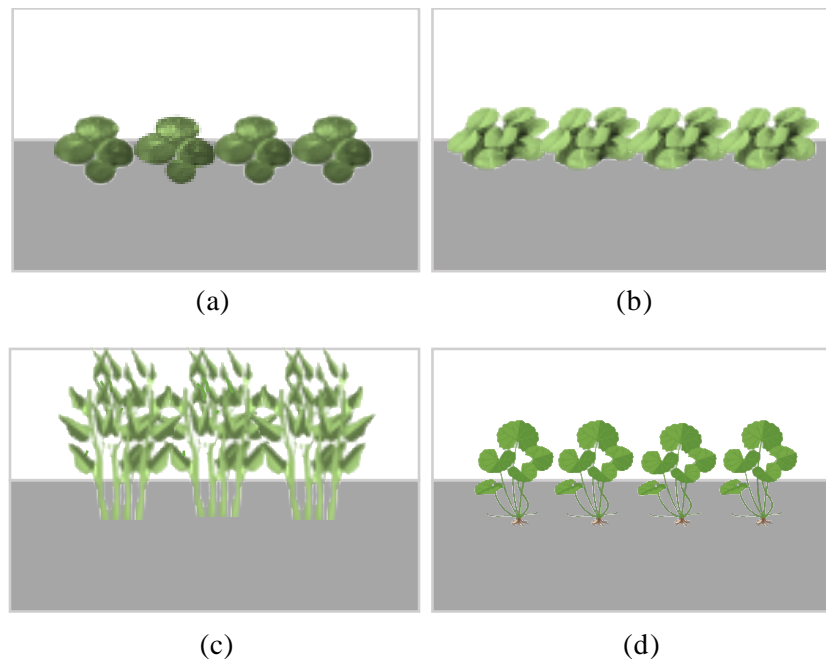


**Figure 1.** The phytoremediation treatment performed in the laboratory using (**a**) *Lemna minor*, (**b**) *Salvinia minima*, (**c**) *Ipomea aquatica* and (**d**) *Centella asiatica*.

### 2.3. Statistical Analysis

One-way ANOVA test using the IBM SPSS Statistics software (Version 23, IBM, New York, NY, USA, 2019) was performed in this study to determine the significant differences between the parameters of sewage wastewater quality before and after the 8 days of phytoremediation treatment by *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica*.

## 3. Results and Discussion

### 3.1. Initial Parameters for Water Quality Prior Treatment

The results of preliminary data on water quality in the study area found that the average temperature reading for the sewage wastewater samples was 26.77 °C and the average reading of pH was 7.33. The pH of sewage wastewater showed an alkaline value because the samples were collected at a sewage treatment plant at a residential area which may contain wastes such as soap, urine and detergent generated from the household activities. The average reading of DO content was 3.19 mg/L. The low value of DO in the study area was due to the high amounts of bacteria and excessive amount of BOD from the untreated sewage which used DO. DO refers to the level of free oxygen, which does not accumulate in water or other fluids. It is an important parameter in assessing water quality because of its influence on living organisms in water body. Too high or too low a level of DO can endanger the aquatic organisms and affect the water quality [17].

The mean value of the TSS of the sewage water was 83.33 mg/L. The TSS value was influenced by the presence of algae and also the presence of soil in the water body. When it rains, soil from the surrounding area will enter the water body and cause an increase in suspended solids volume. TSS is a particle larger than two microns in a water body. The suspended solids were made up of inorganic materials, bacteria and algae. Organic particles from the decomposition materials can also contributed to the increase in suspended solids volume. When algae, plants and animals decompose,

the decomposition process allows small organic particles to enter the water body as suspended solids. TSS is an important factor in determining the purity of water. The higher the amounts of suspended solids present in the water, the cloudier the water [26]. The average reading for COD was 40 mg/L. COD value was related to the DO parameter because a higher COD value means more organic matter was oxidized in the water which will reduce the DO level. COD is a measure of the oxygen required to oxidize the dissolved materials and organic matter in water [27]. It is an important water quality parameter as it provides an index to assess whether the discharged sewage will have an impact on the environment.

The average value of BOD recorded was about 26 mg/L. BOD represents the amount of dissolved oxygen needed by microorganisms in the aerobic process of the decomposition of organic matter. BOD was used as an indicator to determine the amount of organic pollutants in most aquatic ecosystems [28]. The permitted limit for BOD issued by the Department of Environment (DOE) in Malaysia is 50 mg/L. A low BOD value indicated a good quality, whereas a high BOD value indicated a contaminated water quality. Finally, the average content of $NH_3$-N in the sewage sample was 10.20 mg/L. $NH_3$-N is a common pollutant in a domestic wastewater which results from the degradation of nitrogenous organic matter. The degradation of organic matter in the biological treatment stage also produced a large amount of ammonia compounds [29]. The Environmental Quality (Sewage) Regulations 2009 has been revised by the DOE Malaysia, which significantly reduced the limit for the released of $NH_3$-N from 50 mg/L to 5 mg/L. Sewage wastewater which contains high ammonia nitrogen will prevent the natural nitrification and reduce the water purification capacity and consequently endanger the water environment. Excess nitrogen released into the water body can contribute to eutrophication and rapid growth of algae, which results in a reduction of oxygen in water. Figure 2 shows the preliminary data on the water quality of sewage wastewater samples in this study.
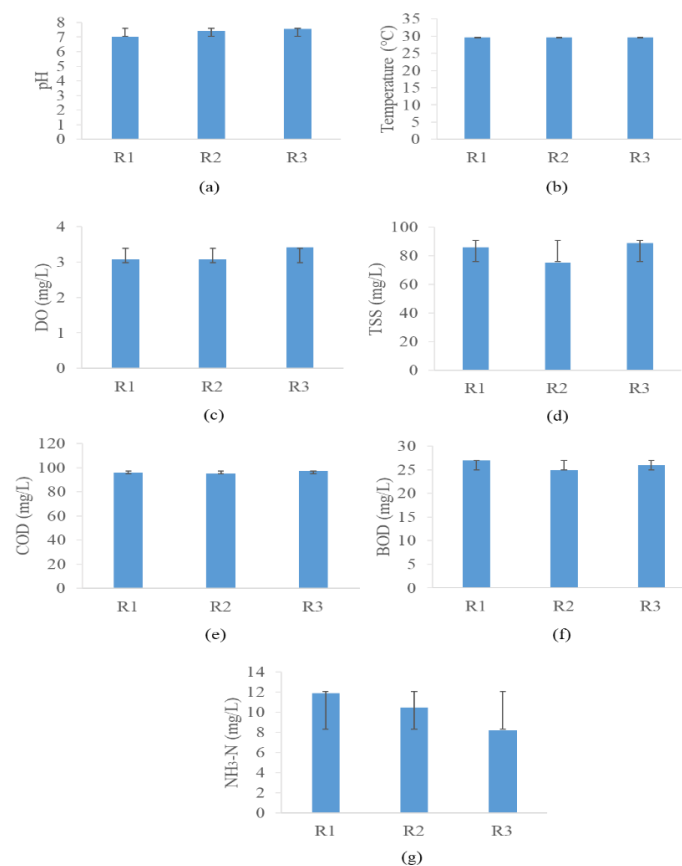


**Figure 2.** The initial parameters reading of sewage wastewater samples for (**a**) pH, (**b**) temperature, (**c**) DO, (**d**) TSS, (**e**) COD, (**f**) BOD and (**g**) $NH_3$-N.

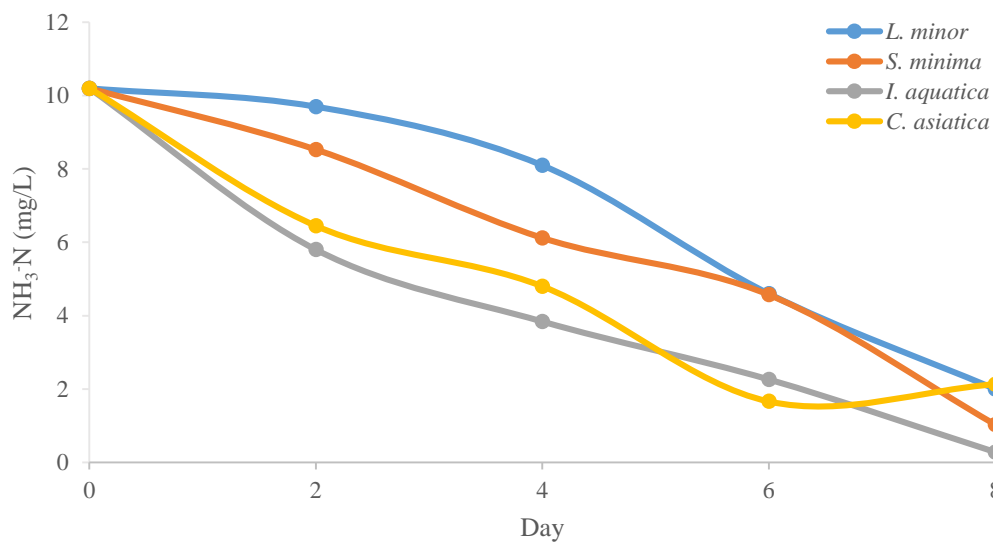*3.2. Removal of TSS, NH₃-N and COD*

3.2.1. Reduction Rate of TSS

Phytoremediation treatment of sewage wastewater using *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica* showed a decreasing in TSS reading (Figure 3). The average initial reading for TSS was 83.33 mg/L. On the second day of phytoremediation treatment, the TSS value was decreased to 67.33 mg/L, 61.67 mg/L, 68 mg/L and 70 mg/L for the treatment by *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica*, respectively. On the final day of treatment, the TSS value in the treatment sample for *Ipomoea aquatica* recorded the lowest average reading, which was 12 mg/L, followed by *Salvinia minima* (18.67 mg/L), *Centella asiatica* (27 mg/L) and *Lemna minor* (41 mg/L). The reduction in the average reading for TSS was primarily due to the plants' root systems. On that account, plants with fibrous roots were usually able to accumulate more TSS compared to those with taproot [30]. The root capacity increases with the growth of the plant, thus more suspended solids can be filtered [31]. Besides, a lower water flow also caused the increase in suspended solids and solids filtration by plant tissues.
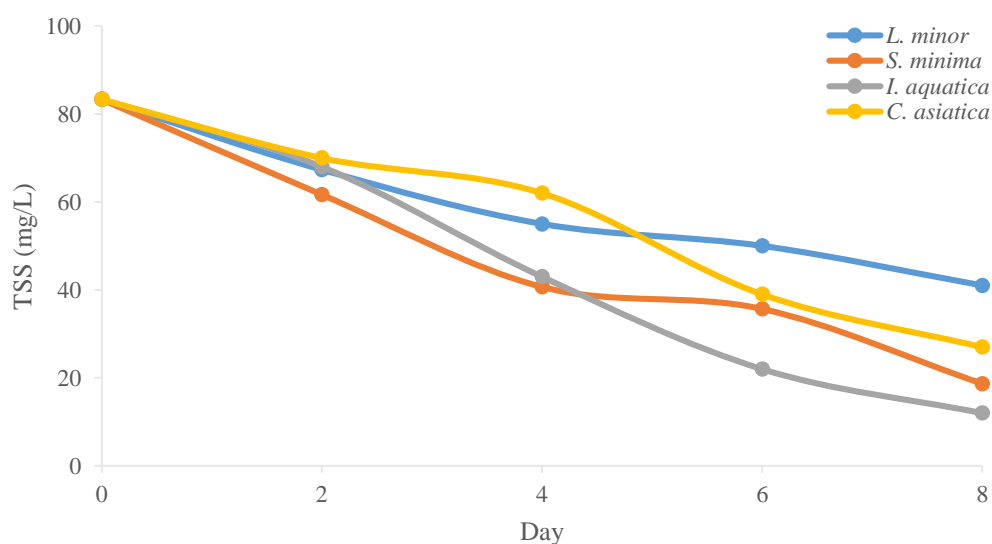


**Figure 3.** Graph of the average of TSS reading by the phytoremediation using *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica*.

3.2.2. Reduction Rate of NH₃-N

Figure 4 shows the average reading value of NH₃-N in the treatment sample by the four plants. The average of initial reading for NH₃-N in the sewage sample was approximately 10.20 mg/L. On day 2 of treatment, the graph shows a slightly decreased NH₃-N reading when treated with *Lemna minor* (9.70 mg/L), *Salvinia minima* (8.53 mg/L) and *Centella asiatica* (6.45 mg/L). While the average reading of NH₃-N reduction by *Ipomea aquatica* was significant, with a value of approximately 5.8 mg/L. On day 8, the phytoremediation of sewage wastewater by *Ipomea aquatica* shows the highest reduction in NH₃-N content, which was about 0.28 mg/L, followed by *Salvinia minima*, *Lemna minor*, and *Centella asiatica*, with average values of 1.03 mg/L, 2 mg/L and 2.13 mg/L, respectively. Plants need nitrogen as a nutrient requirement for growth. The decreased NH₃-N value observed when the ammonium ions and nitrogen were absorbed by plants through the root system. The selection of plants to reduce the NH₃-N content in wastewater is important because the effectiveness of the treatment through phytoremediation can be enhanced by selecting aquatic macrophytes with a high tolerance towards ammonia level in wastewater [32]. The present of NH₃-N content in wastewater was due to the processes of organic waste matter decomposition and nitrogen fixation [33].

**Figure 4.** The average of NH$_3$-N reading by the phytoremediation using *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica*.

### 3.2.3. Reduction Rate of COD

The graph of COD content reduction in sewage wastewater sample by phytoremediation treatment by *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica* is shown in Figure 5. The average of initial reading for COD was about 96 mg/L. The reading of COD content was decreased on day 2 of treatment with the average value of 66.33 mg/L, 56.33 mg/L, 90 mg/L and 91 mg/L when treated with *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica*, respectively. The average of final COD value was approximately 24 mg/L, 17.33 mg/L, 53 mg/L and 61 mg/L for the phytoremediation by *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica*, respectively. The reduction in COD was obtained because the plants underwent the process of photosynthesis. The photosynthesis activity increases the dissolved oxygen in water, thus creating aerobic conditions in wastewater in favor of aerobic bacterial activity to reduce BOD and COD contents [34]. This can be an indication that wastewater would have a lesser impact on the environment after being treated using the phytoremediation method [35].
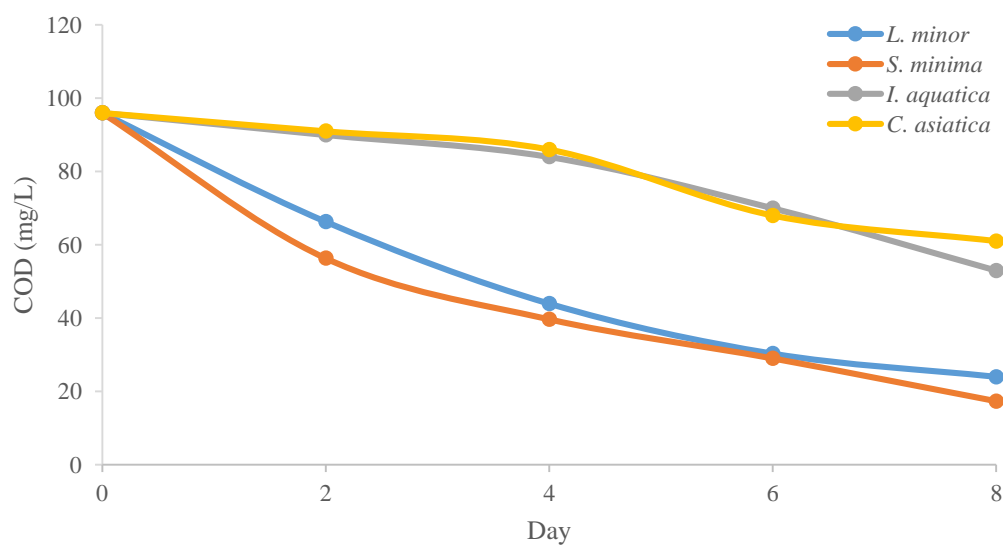


**Figure 5.** The average COD reading by the phytoremediation method using *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica*.

3.2.4. Phytoremediation Efficiency by *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica*

The results indicated that all plants used as phytoremediation agents in this study were able to reduce the TSS, $NH_3$-N and COD contents in sewage wastewater samples with different rates of reduction. Figure 6 shows the percentage of reduction of the three parameters after the 8 days of phytoremediation treatment by *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica*. Based on the results, *Lemna minor* reduced about 50.8% of the TSS content. While the average reading of $NH_3$-N was reduced by up to 80.4%. For COD content in the sewage wastewater sample, *Lemna minor* able to absorbed approximately 75% of COD after 8 days of treatment. A one-way ANOVA test showed that there was a significant difference ($p > 0.05$) in the contaminants reduction. According to Hazmi and Hanafiah [18], *Lemna minor* was able to remove COD and $NH_3$-N by 93.7% and 66.4%, respectively. El-Khair et al. [36] found that *Lemna minor* reduced the TSS content by 96.3%. *Lemna minor* has been reported as a highly efficient floating macrophyte in organic pollutant removal [37,38].
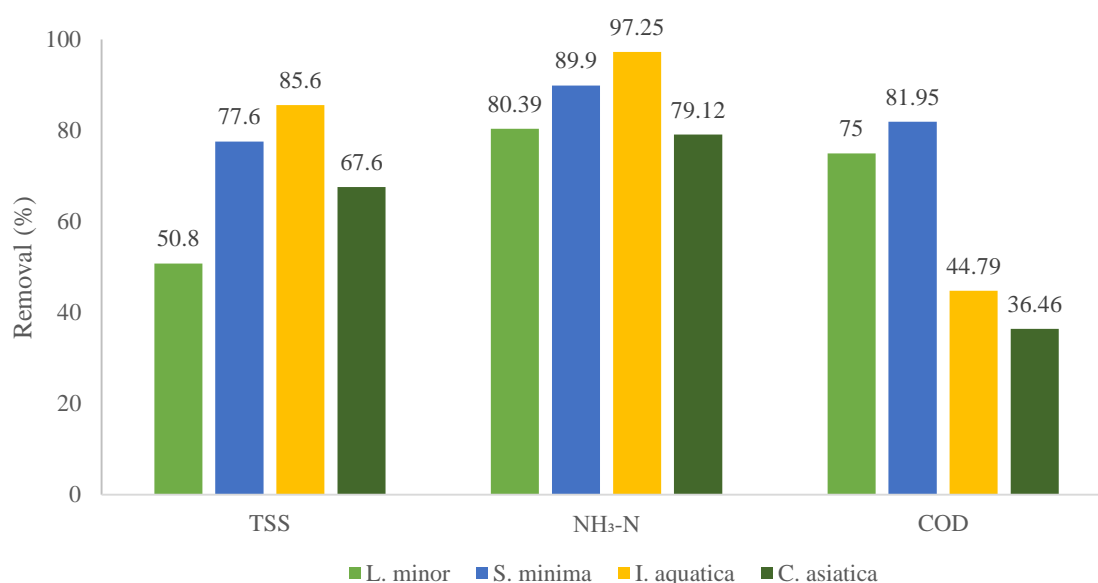


**Figure 6.** Percentage of reduction in TSS, $NH_3$-N and COD for *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica*.

As for *Salvinia minima*, the results showed that after 8 days of treatment, this plant had reduced the content of TSS, $NH_3$-N and COD by 77.6%, 89.9% and 82%, respectively. The ANOVA analysis conducted shows that there was a significant difference ($p > 0.05$) in the reduction reading of pollutants. *Salvinia minima* is a fast-growing aquatic fern which has high adaptability to various aquatic environments [39–41]. Salvinia minima was able to accumulate pollutants in different physiological conditions, as it consists of floating leaves and a submerged leaves structure [42,43]. According to Olguin et al. [44], their study indicated that *Salvinia minima* was more effective compared to *Spirodela polyrrhiza* in the phytoremediation of high-strength organic wastewater at a maximum initial $NH_3$-N concentration of 70 mg/L and at a pH of 6.

Farraji et al. [45] reported that *Ipomoea aquatica* can accumulate up to 80% of TSS, 36.3% of COD and 30% of $NH_3$-N in palm oil mill wastewater. In this study, 85.6% of TSS was removed by *Ipomoea aquatica* after the phytoremediation treatment of sewage wastewater for 8 days. *Ipomoea aquatica* was able to trap TSS because of its larger and longer root structure with a lot of hair. Ammonia is one of the nutrients that macrophytes need to survive. It can be seen in this study which the average reading of $NH_3$-N was decreasing during the phytoremediation treatment. *Ipomoea aquatica* reduced approximately 97.3% of $NH_3$-N in the sewage wastewater sample. This plant was useful for removing nitrates from polluted water. By means of COD content, *Ipomoea aquatica* has absorbed about 44.8% of COD in the sewage

wastewater sample. The long and fibrous roots of *Ipomoea aquatica* make it able to absorb nutrients in wastewater efficiently. This species is a fast-growing plant and needs pollutants as food to grow [46]. The statistical analysis using a one-way ANOVA test showed a significant difference ($p < 0.05$) for the rate of reduction in the TSS, $NH_3$-N and COD contents during the 8 days of phytoremediation treatment of sewage wastewater.

With regards to *Centella asiatica*, the reduction percentage of TSS, $NH_3$-N and COD in the sewage wastewater sample was about 67.6%, 79.1% and 36.46%, respectively. According to Nizam et al. [19], *Centella asiatica* can remove 98% of $NH_3$-N from wastewater. A medicinal plant such as *Centella asiatica* can serve as phytoremediation agent as it has the ability to absorb different concentrations of contaminants in wastewater [47]. *Centella asiatica* can accumulate heavy metals through its roots, stems and leaves [48,49]. The roots of *Centella asiatica* play an important role in this study which the roots trapped the suspended particles in wastewater sample. At the beginning of the phytoremediation treatment, the average reading of $NH_3$-N was high. This is due to the decomposition of organic solid matter by the existing bacterial reaction in the sewage wastewater sample [50,51]. Ammonia is one of the important nutrients for *Centella asiatica* to grow. The reduction in pollutants such as TSS, $NH_3$-N and COD in this study showed that sewage wastewater can be treated using *Centella asiatica*. The one-way ANOVA analysis conducted showed that there was a significant difference ($p < 0.05$) in the reduction rate of the selected parameters.

Based on the percentage of reduction as shown in Figure 5, it can be observed that *Ipomoea aquatica* was more efficient in reducing the contents of TSS and $NH_3$-N, while *Salvinia minima* was more efficient in accumulating COD in the sewage wastewater sample. In a study conducted by Hanafiah et al. [52], *Ipomea aquatica* was found to be more efficient than *Centella asiatica* by its ability to accumulate higher concentration of aluminum (Al) and iron (Fe). Nizam et al. [19] found that *Centella asiatica* was the most efficient for $NH_3$-N removal compared to *Pistia stratiotes*, *Salvinia molesta*, *Ipomea aquatica* and *Eichhornia crassipes*. It was indicated that each plant that acts as phytoremediation agent has its own efficiency rate in removing different pollutants. However, there are several factors that may affect the reduction percentage of contaminants, such as the type of pollutant and wastewater, treatment duration and pollutant concentration [52–54].

## 4. Conclusions

This study was conducted to evaluate the potential of *Lemna minor*, *Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica* as phytoremediation agents in removing TSS, $NH_3$-N and COD from sewage wastewater samples. These plants were chosen because of their high tolerance to various pollutants and also because of their rapid growth rate. The results indicated that after the 8 days of treatment, the sewage wastewater treated by *Lemna minor Salvinia minima*, *Ipomoea aquatica* and *Centella asiatica* showed a reduction in TSS content at 50.8%, 77.6%, 85.6% and 67.6%, respectively. For the $NH_3$-N removal, the four treatment samples showed a reduction of approximately 80.4%, 89.9%, 97.3% and 79.1%, respectively. The value of COD in sewage wastewater treated by *Salvinia minima* reduced about 82% compared to *Lemna minor*, which reduced only 75% of COD content. *Ipomoea aquatica* able to remove up to 44.8% of COD and *Centella asiatica* removed 36.46% of COD. The one-way ANOVA test showed a significant different on sewage wastewater quality before and after the treatment by all plants. Based on the reduction percentage of TSS and $NH_3$-N, sewage wastewater treatment with *Ipomoea aquatica* showed a more efficient reduction, while *Salvinia minima* was more efficient in reducing the COD content. Hence, phytoremediation treatment method was proven to be able to remove pollutants and enhance the water quality with a low capital cost. This method is also safe to be implemented as it is environmentally friendly.

**Conflicts of Interest:** The authors declare no conflict of interest.

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