

Applications of Australian Native Aquatic Plants on Purifying Wastewater Sources

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ABSTRACT: Wastewater treatment plants play an important role in maintaining the health of ecosystems and ensuring the economic, social and political soundness of communities. However, current wastewater treatment methods are economically and environmentally unsustainable. Aquatic plant restoration has been receiving attention because of its high efficiency and eco-friendliness compared to previous methods. Three types of aquatic plants: duckweed (*Lemna disperma*), azolla (*Azolla pinnata*) and hornworts (*Ceratophyllum demersum*) were tested for their effects in removing constituents (pH, total alkalinity, hardness, sulfite, copper, nitrite, nitrate, total N) and reducing the number of bacteria colonies in a wastewater source, over the course of 7 days.

The results show that all three aquatic plants were capable of recovering and removing bacteria in water. Duckweed, however, was the most effective of all three plants. A 3D digital model of a duckweed based wastewater treatment plant was devised to showcase how duckweed could be incorporated into full-scale water treatment systems. The uptake of aquatic-plant based wastewater treatment systems has been slow. The conducted research adds to the advantages and the feasibility of full-scale aquatic-plant based wastewater systems.

KEYWORDS: Earth and Environmental Sciences; Water Science; Wastewater; Aquatic Plants; Phytoremediation.

■ Introduction

Literature Review:

Wastewater is the used water supply of a community which has been modified physically, chemically, or biologically as a result of the introduction of certain substances such as body wastes, hair, food scraps, fat, chemicals, dirt, and microorganisms.¹ Wastewater is a common source of water pollution which can affect human health and natural ecosystems.² Excess nutrients such as nitrogen and phosphorus in a wastewater source, when discharged to rivers and streams, can cause eutrophication. An abundance of nutrients leads to a large 'bloom' in growth of algae and phytoplankton, causing an increase in chemical oxygen demand (COD) in the water.³ As the algae and phytoplankton die, the bacteria consuming them have the potential to remove most of the available oxygen, hampering aquatic biota.⁴ The improvement of water quality requires reductions of excess nutrients, total dissolved solids, COD, total coliform numbers, and adjusting the pH, dissolved oxygen, and temperature.⁵ This ensures that the water that is disposed or recycled does not present harmful effects.⁶

In Australia, wastewater treatment systems are vital in maintaining the health of the diverse 'flora and fauna', and ensures economic, social and political soundness.⁷ Moreover, efficient wastewater management addresses the growing issue of water scarcity and allows efficient water recycling in regions with high water stress.⁸ Nevertheless, the conventional wastewater management system is economically and environmentally unsustainable.^{9, 10} It does not align with basic principles of water conservation, generates toxic sludge and chemicals as by-products, and does not enable effective

reclamation and reuse of water and nutrients.¹¹ Chemical remediation, for instance, may increase the toxicity of the water body, and chemical agents may cause secondary pollution.¹² In recent years, aquatic plant restoration (phytoremediation) has been receiving attention because of its high efficiency and eco-friendliness compared to conventional remediation techniques.^{13,14} Some aquatic plants, such as duckweed and water hyacinth, can store large amounts of inorganic nutrient salts such as nitrogen and phosphorus compounds.^{15,16} Furthermore, Dhir¹⁷ found that aquatic plants can remove a high level (90%) of pathogenic microbes such as *Enterococci*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Clostridium perfringens*, *Staphylococcus aureus*, and *Salmonella*.

The three aquatic plants tested in this research were duckweed (*Lemna disperma*), ferny azolla (*Azolla pinnata*) and hornworts (*Ceratophyllum demersum*). Duckweed is a floating hydrophyte with one solid root that is usually 1-4 cm long. The plant is often elliptic and does not have a stalk except in the bud stage.¹⁸ Duckweed is native to Western Australia, New South Wales, Victoria and Tasmania. Azolla is a native aquatic plant found in the southern states of Australia in still or slow-moving waterways. The plant is reddish-green with several branches arranged on each side and is usually triangular in shape.¹⁹ Hornwort is a submerged and rootless aquatic plant. It has stems that are free branching, reaching a length of up to 60 cm. The leaves are dark green with lengths of 1-4 cm long and two to three times palmately divided.²⁰ Hornworts are native to all Australian states except for Tasmania.

A decentralised wastewater management system is an approach where each community manages and processes

wastewater in their locality. Wastewater in a decentralised system would not be sent to a large processing facility or landfill. This allows small-scale and low-cost wastewater treatment facilities to be integrated with urban agriculture. Water flow would remain small, implying less environmental damage. Moreover, the system enables effective and quick water recycling due to being in a neighbourhood level. As a result, this system targets the high water stress issue in rural and small communities. Aquatic plant purification technologies could be incorporated into decentralised wastewater management across a variety of communities.

Research Objectives:

While studies have analysed the functions of individual aquatic plants, there has yet been a comparison between those plants in terms of their effectiveness in purifying wastewater samples. Moreover, full scale applications of aquatic-plants-based wastewater treatment plants are still lacking in Australia and in many countries in the world. Therefore, this research aims to: (1) test the ability of three native Australian aquatic plants including: duckweed, azolla, and hornworts for their effectiveness in adjusting pH, total alkalinity, hardness, and removing chemicals in a sample of contaminated water; (2) test the ability of these aquatic plants in reducing the number of bacterial colonies in the sample of water; (3) propose an application of these aquatic plants in wastewater treatment systems by constructing a 3D digital model.

Research Questions and Hypotheses:

The research paper addresses the research question: What are the applications of Australian native aquatic plants such as duckweed, azolla, and hornworts on purifying wastewater sources?

This study hypothesized that all three aquatic plants would be able to remove nutrients and reduce the number of bacterial colonies to a high degree. Nevertheless, duckweed would be the most effective in recovering the amount of chemicals and reducing the amount of total coliform numbers in the contaminated water samples. Duckweed could be incorporated as secondary treatment in full-scale wastewater treatment systems.

■ Methods

Water samples were collected from the Bulimba Creek in Brisbane, Queensland. This water is speculated to have undergone only primary and secondary wastewater treatment. Leaves and rocks submerged under a rapid-flowing current were observed, indicating a high nutrient and oxygen level in the water body. The depth of the creek was measured at 30 ± 0.1 cm, and the temperature of the water was 28.0 ± 0.05 °C. Ten 2.5 L plastic containers with dimensions of 21.4 cm (L) x 12.5 cm (H) x 14.5 cm (W) were utilized to grow the aquatic plants. Six cups or approximately 1.42 L of water were collected from the middle of the creek in every container. The containers were rinsed out three times before the final collection to reduce existing contamination.

Each type of plant had 3 set-ups to reduce random error. The experiment also included one control group, which did not contain an aquatic plant. 5.00 ± 0.005 g of *Lemna*

disperma and 30.00 ± 0.005 g of *Azolla pinnata* were measured using a 0.01 g increment digital platform scale. When placed in the containers, the *Lemna disperma* and *Azolla pinnata* covered around 90% of the surface water. The *Ceratophyllum demersum* was measured using a ruler and cut to a length of 10 ± 0.1 cm using a scissor. Each container received two strands of hornworts. The ten containers were placed under a shelter in an outdoor and open environment. The temperature ranged from 25 ± 0.5 °C to 30 ± 0.5 °C over 7 days. This environment was ideal for aquatic plant growth since the optimum temperatures for temperate submerged aquatics plants are usually between 25 ± 0.5 °C and 32 ± 0.5 °C. Moreover, the experimental set up closely replicated the atmosphere in a constructed wetland while it reduced the exposure to rain and dust, which could affect the reliability of the results.

Levels of total alkalinity, hardness, sulfite, copper, nitrite, nitrate, and total N (nitrate, nitrite and ammonia) in ten water samples were measured using Allora 14-in-1 reagent water test strips. The pH level was measured using a digital pH meter. Measurements were taken every day for seven consecutive days.

The effectiveness of aquatic plants in removing bacteria such as *E. coli* were quantified by growing bacterial cultures in non-selective, nutrient agar plates at 37°. 1 ± 0.05 mL of a given water sample were collected using a 3.5 ± 0.05 mL sterilized transfer pipette. This solution was then transferred into the petri dish with a lit candle nearby. The lit candle could reduce the number of bacteria getting into the petri dish while performing the experiment. The solution was spread out evenly using a sterilized inoculation spreader. Plastic tapes were used to seal tightly along the side of the petri dish to reduce contamination from the environment. Petri dishes were placed in a dark and warm place to optimize bacteria growth. They were observed every 12 hours, and the final observation was made after 48 hours as bacteria was not likely to grow further after 48 hours. The number of different types of colonies were counted in order to measure the quality of water. Ten different water samples after the experiment and the water sample before the experiment were tested. Since the original and the control water sample had too many colonies of bacteria to count, serial dilution was performed to measure the number of bacterial colonies present. $1 \text{ mL} \pm 0.05 \text{ mL}$ of the water sample was diluted with $9 \pm 0.05 \text{ mL}$ of fresh water. Then $1 \pm 0.05 \text{ mL}$ of this sample was transferred into the petri dish, making the solution 100 times diluted. New pipettes were used in every step to avoid cross contamination. Three different colonies with distinct colours – white, orange, and red – were characterised as type 1, type 2 and type 3 respectively.

The 3D model of the proposed wastewater treatment plant incorporating aquatic plants was designed using SketchUp. Tools such as 2 Point Arc (A), rotated rectangle, Rectangle (R), Circle (C), Push/Pull (P), Freehand, Eraser (E), Paint Bucket (B) were used to create the model. The house model was designed by Paul Palamra under 3D Architectural Digest.

■ Results

Removal of Chemicals:

Three aquatic plants showed an ability to recover water constituents to a high degree. The pH level, for instance, indicated a reduction from 7.6 to 7.0 in duckweed, 7.2 in hornworts and 7.3 in azolla (Table 1). The total alkalinity level showed a great decrease in all three aquatic plant species. The initial concentration of 180 mg/L was reduced to 120 mg/L in duckweed, 130 mg/L in azolla and hornworts (Table 1). The level of hardness in the water sample also demonstrated a 60% recovery rate across three aquatic plants. The initial level of 125 mg/L was reduced to 50 mg/L hardness concentration (Table 1). Similarly, the nitrogen level showed a range of around 40 to 60% recovery rate in three aquatic plants, within which the duckweed group had the greatest reduction from 50 mg/L to 20mg/L. Levels of nitrite and nitrate dropped significantly in correlation with the total nitrogen concentration. Notably, aquatic plants such as duckweed and hornworts showed a complete removal of nitrite and nitrate from the initial water sample. Furthermore, the three aquatic plants removed all 10 mg/L of sulfite and 50% of the 2 mg/L of copper from the initial water sample.

During the growth of the various aquatic plant species over 7 days, the water quality parameters were measured at 8 time points. Data in Figure 1 indicates that duckweed showed the steepest downward trajectory in alkalinity level, pH, total alkalinity, hardness, copper, nitrite, nitrate and total nitrogen. The pH level of the water sample with the presence of duckweed follows a simple linear decrease and reached a plateau after two days of the experiment (Figure 1A). In comparison, data points for azolla and hornworts demonstrate a regressed logarithmic trend in terms of their alkalinity level over time (Figure 1A). This is evident by the high R² value of 0.9391 for azolla and 0.9417 for hornworts in log-linear regression. Figure 1B shows that the pH level in the duckweed group followed a steep downward trajectory, reducing from 7.6 to 7 and reached a plateau on day 4. The pH level in the azolla and hornworts experimental groups, in contrast, declined gradually over the experimental period, and only reached a plateau on the two final days. Changes in the level of hardness amongst three plants were similar. As shown in Figure 1C, duckweed, azolla and hornwort groups decreased 62% by the end of the experiment, from 130 to 50 mg/L. The duckweed group reached 50 mg/L on day 3, whereas the hornwort group reached the same level on day 4 and the azolla on day 5. The changes in sulfate level is similar in the duckweed and hornwort groups. They removed all 10mg/L of sulfate after 2 days whereas it took azolla 4 days to recover the same level of sulfate. The duckweed, hornwort and azolla recovered 50% of copper and reached a plateau after 3, 4 and 5 days respectively (Figure 1E). This trend is similar to the changes over time in nitrate level where duckweed recovered 100% of 1mg/L of nitrate, the fastest amongst three aquatic plants (Figure 1F). On the other hand, azolla was only able to recover 80% of the nitrate in the initial water sample whereas the duckweed and hornwort groups

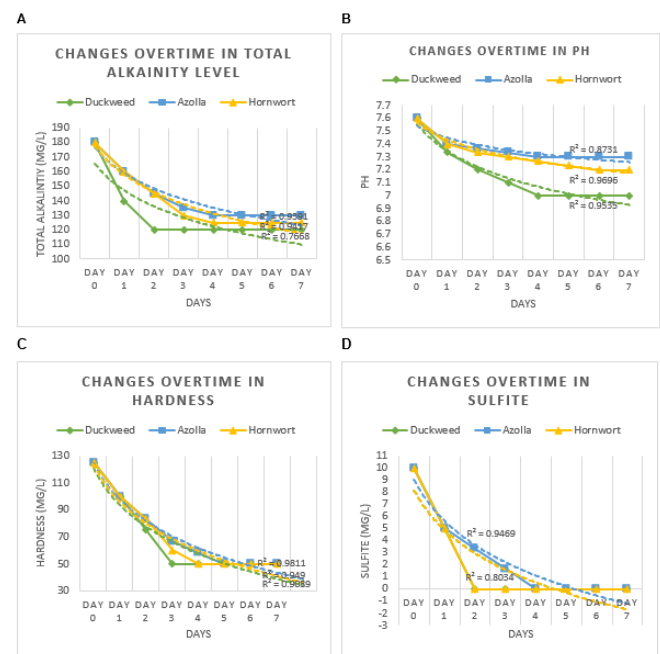
significantly across three experimental groups. Nevertheless, the duckweed group showed the steepest linear slope in all three groups, significantly across three experimental groups. Nevertheless, the duckweed group showed the steepest linear slope in all three groups.

Removal of Pathogens:

Three aquatic plants groups indicated a decrease in the number of colonies grown on the culture media. The duckweed group indicated a significant reduction on all three colony types. Figure 2 shows an 87% reduction in colony type 1, 100% in type 2 and 63% in type 3. The water sample containing azolla showed a 77% reduction in colony type 1, 71% in type 2 and 64% in type 3. Finally, the water sample containing hornworts showed a 63% reduction in colony type 1, 77% in type 2 and 58% in type 3. The control experimental group had a similar number of bacterial colonies compared to the initial number of bacteria.

Table 1: Chemical analysis of the water sample before and after the experiment.

Constitutes	Initial (mg/L)	<i>Lemna dispema</i>		<i>Azolla pinnata</i>		<i>Ceratophyllum demersum</i>		Control	
		After (mg/L)	Recovery (%)	After (mg/L)	Recovery (%)	After (mg/L)	Recovery (%)	After (mg/L)	Recovery (%)
pH	7.6	7.0	5.2	7.3	2.6	7.2	3.9	7.6	-
Total alkalinity	180	120	33	130	28	130	28	180	-
Hardness	125	50	60	50	80	50	80	125	-
Sulfite	10	0	100	0	100	0	100	10	-
Copper	2	1	50	1	50	1	50	2	-
Nitrite	1	0	100	1	-	0	100	1	-
Nitrate	25	0	100	5	80	0	100	25	-
Total N	50	20	60	30	40	28	44	48	4



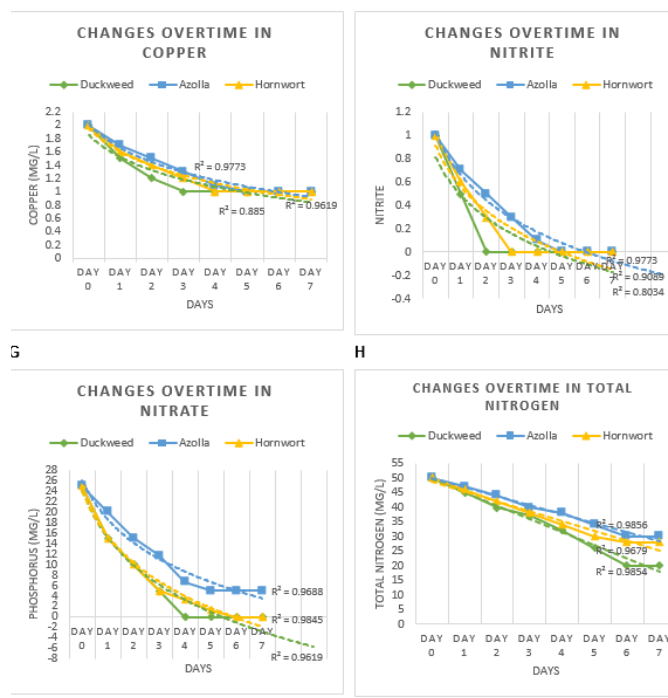


Figure 1: Changes overtime in levels of constituents in the water sample.

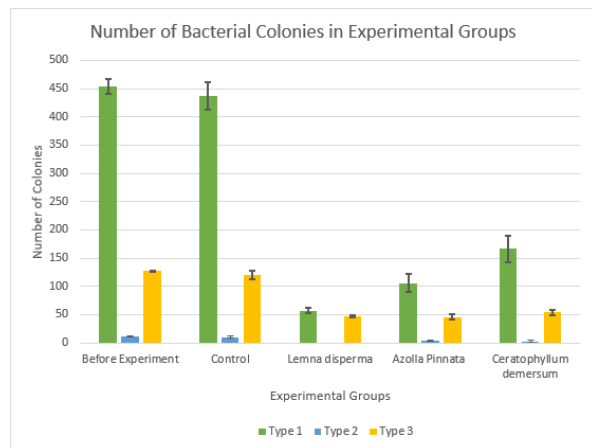


Figure 2: Levels of bacterial colonies in experimental groups.

Discussion

Interpretation of Results:

Assimilation of nutrients from water by aquatic plants is an eco-friendly, efficient and cost-effective wastewater treatment approach. The results align with the hypothesis that all three aquatic plants were capable of reducing the amount of nutrients and bacteria in water, but duckweed was the most effective of all. Duckweed was capable of absorbing, on average, the highest number of constituents from the wastewater sample in the shortest amount of time. Moreover, duckweed also removed the greatest number of bacterial colonies amongst all three aquatic plants. Similar observations have been made in a study by Badr El-Din and Abdel-Aziz¹³ where they found that duckweed had much higher pollutant removal efficiencies, especially, nitrogen, phosphorus, and potassium than water hyacinth and green algae. They also found that COD and biological oxygen demand (BOD) in a wastewater sample

declined by 43% and 42% respectively with the presence of duckweed. On the other hand, hornworts show a greater capacity to remove constituents than azolla (Figure 2). This builds on existing evidence by Yang *et al.*²² which state that emergent aquatic plants such as hornworts, have strong roots, allowing them to effectively absorb and store nutrients compared to other types of aquatic plants. Constituent levels in the azolla experimental group demonstrated a more gradual decreasing trend, which usually resulted in a plateau at a later time compared to duckweed and hornworts (Figure 1). Azolla, however, removed a larger number of bacteria than hornworts. Notably, standard deviation error bars for the initial and control experimental group overlap for all three colony types (Figure 2). Error bars for type 3 colonies in azolla and duckweed also overlap. These results indicate that the difference between experimental groups is not statistically significant. Nevertheless, similar data for the initial and control groups implies that no significant random errors occurred, boosting the reliability of the research.

3D Model:

A 3D model was devised to propose a duckweed based sewage treatment system to treat household wastewater for surface water discharge. As shown in Figure 3, household wastewater is first passed through an anaerobic baffled reactor (ABR) for advanced primary filtration. It contains a number of upflow sludge blanket reactors, which the wastewater passes over and under. This allows efficient treatment of almost all soluble organic wastewater.²³ Moreover, it has a simple structure and operation with no special gas or sludge separation equipment.²³⁻²⁵ As a result, it could be easily incorporated in decentralized communities or in tropical and subtropical areas of developing countries.²⁶ There will be a control point at the effluent of the ABR to monitor the quality of the water. The water is then discharged into a four-chamber duckweed pond. The depth of the pond is suggested to be between 0.6 meters and 1.5 meters since it is suggested as the most suitable for large-scale duckweed wastewater treatment systems.^{27, 28} The hydraulic retention time would be a minimum of 20 days to ensure that the effluent meets acceptable discharge standard.²⁷ The floating duckweed mat works in collaboration with aerobic and anaerobic bacteria to purify wastewater sources. Heterotrophic bacteria decomposes organic waste into mineral components (ammonia nitrogen and orthophosphates) that are readily recovered by the duckweed plants.²⁹ As a result, the nutrients in the water are reduced for safe discharge to surface water. Duckweed needs to be harvested every 20 to 30 days to prevent the release of nutrients back into the water when the duckweed dies and to allow the fastest rate of nutrient recovery since overcrowding inhibits duckweed reproduction and growth. As duckweed floats on the water surface, harvesting involves netting or scooping duckweed. In large and broader ponds, duckweed can be harvested using a mechanical harvester. Harvested duckweed can become a food source for cultured animals such as fish, ducks, chickens, pigs and ruminants.^{27, 30-34}

Implications:

Nutrients and pathogens removal mechanisms of aquatic plants can help construct a more eco-friendly and efficient wastewater treatment system, which, ultimately, enables effective water recycling for communities experiencing high water

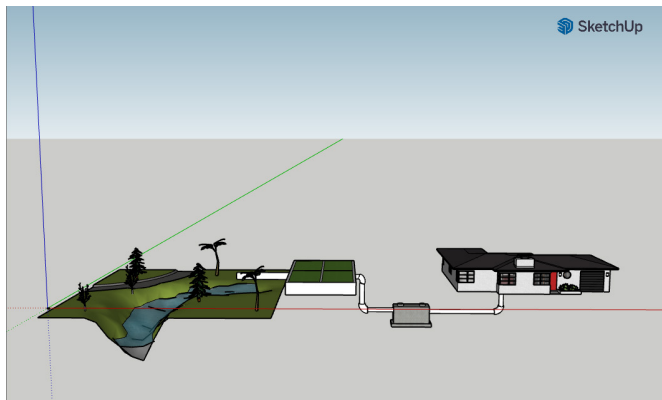


Figure 3: Duckweed based wastewater treatment plant.

stress. The SDG 6 Synthesis Report by the United Nations³⁵ on Water and Sanitation states that over 2 billion people live in countries experiencing high water stress. In Australia, some areas near the north and east coast experience high (40-80%) to extremely high (>80%) water stress.³⁶ This study proposes that local government agencies can capitalize on aquatic plants (phytoremediation technology) for treating and reusing wastewater in small and decentralized communities. Wastewater treatment technology utilizing aquatic plants is highly cost-effective and efficient compared to previous nutrients remediation technology. On the other hand, the incorporation of aquatic plants in wastewater treatment plants in local communities also poses benefits such as ground water recharge, climate enhancement, and agricultural or landscape irrigation.⁸ Furthermore, aquatic plants could be incorporated to treat wastewater from agricultural or industrial run-off with low cost and energy consumption.³⁷ Leng *et al.*³⁸ found that full scale applications of duckweed based wastewater treatment systems exist in USA, Bangladesh, and China. This boosts the feasibility of a duckweed wastewater treatment system in Australia and countries around the world. This study adds to the important contributions of phytoremediation technology to help enable availability and sustainable management of water and sanitation for all individuals in the future.

Limitations

The experimental design has not considered the combination of aquatic plants as an option to purify wastewater sources. A study by Zirschky and Reed³⁹ shows that a mixture of several aquatic plant species can be less susceptible to diseases than a monoculture in a pond. Furthermore, it is beyond the scope of this study to quantify the effect of aquatic plants on water quality indicators such as dissolved oxygen, turbidity, and phosphorus. Moreover, the study did not consider factors that may affect the ability of aquatic plants to purify wastewater sources such as the number of plants covering the water surface, temperature, and the wastewater medium. Failing to consider these factors may decrease the reliability of the results. Furthermore, the experiment was unable to imitate identical environments to

those of a pond used in full-scale wastewater treatment plants. The containers were not big and deep enough to showcase the effect of aquatic plants on purifying a large amount of wastewater influent. This may have led to aquatic plants recovering nutrients in a short amount of time, restricting the identification of clear trends. Figure 1 lacks control data points, reducing reliability. In the bacterial study, it is unknown if all microbes, especially pathogenic microbes, were removed from the phytoremediation method. The bacterial species in type 1, type 2, and type 3 colonies were also not identified. On the other hand, the 3D model did not consider a water recycling system for household sewage. Furthermore, the model did not separate grey and black household water to enable efficient wastewater treatment.

Recommendations

Future studies should take into account:

1. The combination of multiple types of aquatic plants as an option to purify wastewater sources.
2. The relationship between the number of aquatic plants covering the water surface and water purification level.
3. The relationship between temperature and water purification level of aquatic plants.
4. The effect of different water mediums on water purification level of aquatic plants.
5. The separation of grey and black household water in aquatic plant-based wastewater treatment plants.
6. Aquatic plant-based wastewater treatment plants for wastewater recycling.
7. The performance of phytoremediation in comparison with other biological wastewater treatment methods such as microalgae wastewater treatment.

Conclusion

This research aimed to identify the application of three Australian native aquatic plants (duckweed, azolla, and hornworts) on purifying wastewater sources. Based on analysis of the results, it can be concluded that all three aquatic plants were capable of effectively purifying wastewater sources. More specifically, a reduction in values of major water-quality indicators such as pH, total alkalinity, hardness, sulfite, copper, nitrite, nitrate, total N, and numbers of bacterial colonies were seen across three plants. Duckweed was identified as the most effective in recovering constituents and reducing pathogens. A 3D model of a duckweed based household sewage treatment system has been proposed. This could be valuable to future treatment plants designs. Conventional wastewater treatment methods are economically and environmentally unsustainable. Aquatic plants offer an environmentally friendly and cost-effective technology for treatment of wastewater sources. It is proposed that phytoremediation technology could be utilized in decentralized communities with high water stress to allow efficient water treatment and recycling. Overall, this research adds to the advantages and the feasibility of full-scale aquatic-plant based wastewater systems.

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