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Article

The effect of floating plant on water purification: Comparison of the water purification capability of Water Hyacinth, Duckweed, and Azolla

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SUMMARY

Water pollution is a critical issue for human health, aquatic plant and animal biodiversity. While there are several different approaches to resolve this issue, our research investigates one possible solution of using aquatic plants as a natural treatment system. To identify the optimal plant for treating polluted water in India, we selected water from the Mithi river and three types of floating plants: water hyacinth (*Eichhornia crassipes*), duckweed (*Lemna perpusilla*), and azolla (*Azolla pinnata*). We used a total of six measurements (dissolved oxygen, conductivity, turbidity, pH, color of water, and the number of colonies grown on the culture media) taken for seven days to compare the effect of each floating plant on the change in water quality. We hypothesized that duckweed would be the best plant to purify water in India since its effect on reducing turbidity or biochemical oxygen demand (BOD) was found in past research.

Our results show that all three floating plants are effective at purifying the water. Further, duckweed significantly reduced the turbidity and number of bacteria in the water, suggesting it may be the most optimal water purifier among those three. Based on the results from this experiment, we recommend floating plants as one alternative to resolving water pollution in India, which would effectively purify water as well as require less cost for construction and maintenance.

INTRODUCTION

Water, one of the fundamental constituents of the Earth covering three-quarters of its surface area, exists in the form of wetland. According to National Oceanic and Atmospheric Administration (NOAA), a wetland is classified as a land saturated with water and divided into five general types: estuarine (estuary), palustrine (marsh), riverine (river), marine (ocean), and lacustrine (lake). Wetland has significance in providing habitat for birds and fish, preventing erosion of soil, and maintaining water quality and rate of water flow (1, 2). However, studies suggest that these wetlands are becoming polluted due to the rapid industrialization, improper waste disposal, and the growing population (3). Many wetlands have a high concentration of nitrogen, phosphorus and organic pollutants, which exceeds wetland's self-purification capability, resulting in severe eutrophication–an excessive growth of algae or plants caused by a surfeit of nutrients in water (4,5). Some of the wetlands have algal blooms due to the overgrowth of blue-green algae, and those areas show a depletion or lack of dissolved oxygen (DO). Thus, countermeasures are required to resolve eutrophication, which threatens the ecosystem.

In India, clean water access is vital not only for economic purposes such as industrial use and irrigation, but also for various cultural and religious ceremonies, and sometimes for community bathing and washing (6). However, because contaminants from domestic sewage, large industries, or agricultural run-off flow into the water without filtration, water quality often does not meet the criteria for biochemical oxygen demand (BOD), total coliform numbers (TC), fecal coliform numbers (FC), dissolved oxygen (DO), and ions (7).

To solve the water pollution issue, scientists have conducted research on physical (8) and chemical treatments (9). However, due to the economic and technical constraints of these treatments, a new method for water purification is being explored: natural treatment (10). Particularly, the effect of aquatic plants on water purification has been consistently researched since 1980 (11), and recent studies investigate the application of aquatic plants such as creating an artificial wetland or island to purify water (12). Researchers have shown that aquatic plants significantly reduce nitrate, phosphate, and toxic metals (13). Unlike other physical and chemical treatments, the use of aquatic plants does not cause additional damage, such as destruction of the ecosystem, and involves less cost for construction and maintenance (14). However, it has limitations because it is a seasonal treatment, and not much research has been done for different types of water. Although some researchers investigate the effect of aquatic plants on purifying water, they use similar types of plants that have been researched previously.

Aquatic plants are divided into two categories: hydrophytes attached to the substrate and free-floating hydrophytes, depending on how they grow and live (15). Hydrophyte refers to plants living in water. It can prevent erosion, stabilize the soil of wetland, absorb nutritive salts or harmful substances, and prevent excess growth of phytoplankton (16). Hydrophyte can be subdivided into three types: emergent hydrophytes, submerged hydrophytes and floating hydrophytes. Unlike emergent plants such as lotus, or submerged plants such as coontail, that need soil to fix their roots in the bottom of the water, floating plants require no soil and are easier to grow in a controlled environment.Therefore, our study compared the water purification effectiveness of three types of floating plants: water hyacinth (*Eichhornia crassipes*), duckweed (*Lemna perpusilla*), and azolla (*Azolla pinnata*). We

Use of water	Class	Criteria
Propagation of wildlife, fisheries	D	– pH: 6.5 to 8.5 – DO ≧ 4 mg/L – Free ammonia ≦ 1.2 mg/L

Table 1a. Water quality criteria for various uses of water suggested by the Central Pollution Control Board.

Measurement	Dissolved Oxygen	Conductivity	Turbidity	pH	Number of colonies
Low	Less preferred	NA	Preferred	NA	Preferred
High	Preferred	NA	Less preferred	NA	Less preferred
Permissible limit	>2 mg/L	150-500 µS/cm	Not specifically determined	6.5-8.5	< 2000 cfu/100mL

Table 1b. The criteria to evaluate water quality in this experiment.

determined their ability to purify water by taking several types of measurements to evaluate water quality. These floating plants were chosen because they are common floating plants inhabiting India and research suggests water hyacinth, azolla, and duckweed have an effect on quality (17,18,19). While that research provided deep analysis of each of the plants, there are no comparisons of those plants in India. Therefore, the comparison between the three different types of floating plant will be the key to this research.

In this research, we measured the pH, turbidity, conductivity, dissolved oxygen, color, and bacteria growth in the culture medium of the water samples in order to identify an optimal floating plant to solve the water pollution problem in India.

The conductivity of water is affected by the amount of ionic nutrients such as sodium, chloride, or sulfate dissolved in water. High conductivity, which indicates more solute salts and ions, can lead to poor plant growth and toxicity, whereas low conductivity results in slow-growing plants (20). Fresh streams ideally have a conductivity between 150 to 500 µS/cm. The pH determines the solubility and biological availability of chemical constituents such as metals and nutrients (21). Majority of aquatic creatures have a narrow pH tolerance range of 6.5-9, and excessively high or low pH is harmful to the animals and plants living in the water. The level of DO is the number of oxygen (O_2) molecules per million total molecules in a sample. It is affected by a physical condition such as temperature and pollutants and higher DO level is preferred for fish growth and activity. Turbidity is the amount of light scattered by particles in water. It is used to determine the water clarity and to estimate the amount of dissolved colored material and suspended solids in water. High turbidity levels may disrupt the natural movements, cause illness, or expose fish to potential pathogens or toxins, as well as reduce the aquatic plant's accessibility of light. Lastly, the number of bacteria living in water also determines water quality since the water with an excessive number of bacteria is considered impaired. Among two ways of culturing bacteria, culturing them in liquid or in a medium, we chose to use culture medium since it is easy to observe the group of fungi or bacteria in a form of colony despite their slow growth. Some research suggests that for water to be considered clean, the number of fecal coliforms should be less than 2,000 cfu and that of *E. coli* be less than 1,260 cfu in 100mL water sample (22). This is because fecal coliform bacteria may present in water as a result of disposal of human waste or overflow of domestic sewage, indicating the contamination of water. The presence of fecal coliform bacteria further indicates that there may be a harmful pathogen that can cause waterborne pathogenic diseases such as typhoid fever (23).

Indian governmental report suggests different criteria for evaluating water quality based on the usage of water: drinking, outdoor bathing, propagation of wildlife, and irrigation/industrial cooling. In this research, data was compared to the criteria for class D as the goal of the research was to have water that propagates wildlife and fisheries (24) (**Table 1a**). Based on the criteria used by Indian government, this research has set up new permissible limits to compare water purification capability of plants (**Table 1b**).

Prior to the experiment, we hypothesized that duckweed would be the best plant to purify water in India since past research suggested that duckweed was effective in decreasing turbidity by removing total suspended solids (TSS) and in reducing BOD (25).

RESULTS

Floating plants moderately change the conductivity, pH, and DO, but drastically reduce the turbidity of the water

To determine how three different types of floating plant play a role in improving water quality, four different groups, including a control group, were used. Here, each of three experiment groups—water hyacinth, duckweed, and azolla—had one type of floating plant in the water. The control group, on the other hand, had nothing inside the water to observe any possible change in water quality without any involvement of plants. In order to reduce the error, there were four set-ups

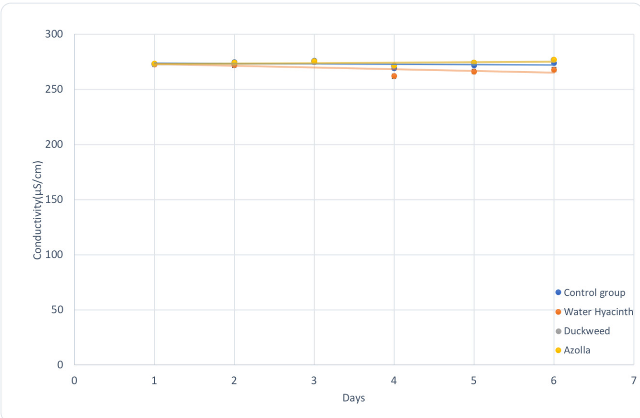

Figure 1. Effect of floating plants on conductivity. The change in conductivity of control group, water hyacinth, duckweed and azolla for 6 days; linear trendline; data represent the average; error bars represent standard deviation

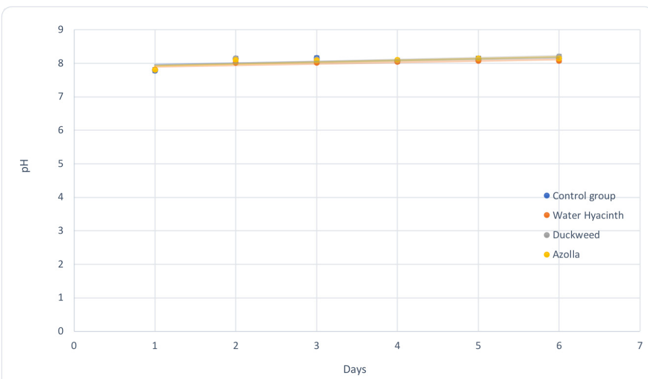

Figure 2. Effect of floating plants on pH. The change in pH of control group, water hyacinth, duckweed and azolla for 6 days; linear trendline; data represent the average; error bars represent standard deviation

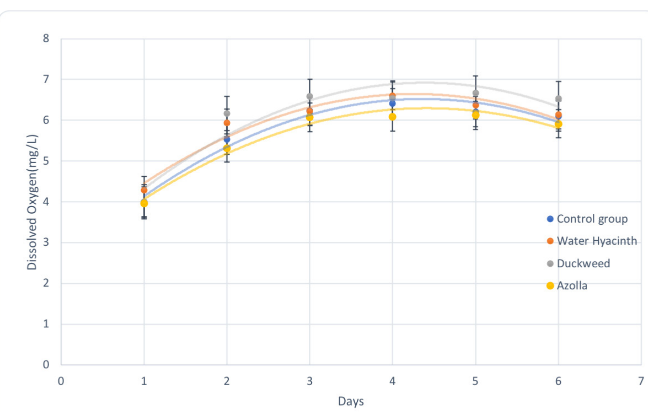

Figure 3. Effect of floating plants on increasing dissolved oxygen level. The change in dissolved oxygen of control group, water hyacinth, duckweed and azolla for 6 days; polynomial trendline; data represent the average; error bars represent standard deviation.

per group, and every measurement was taken five times per set-up. Then, the results were averaged within its group and plotted graphically.

First, the conductivity of all the groups showed a constant increase, except from day 3 to day 4 (**Figure 1**). The trend of both the control and experimental groups were similar, indicating that floating plants did not have a significant impact on conductivity. The increase in conductivity was not sharp and the conductivity of all groups was in the permissible level of 150-500 µS/cm from day 1 to day 6.

For pH testing, all groups showed an increase between day 1 and day 2, then remained constant from day 2 to day 6 (**Figure 2**). All the groups were in the permissible limit of pH 6.5 to 8.5. The slopes of duckweed, azolla and water hyacinth were similar to the control group, which indicated that these plants did not affect the pH of water.

For dissolved oxygen level, the overall trend of the graph indicated that dissolved oxygen of water increased until day 4, then started to decrease (**Figure 3**). Although dissolved oxygen started to decrease, all the measurements were in the permissible range, exceeding 2 mg/L. By comparing the polynomial trendline for each group in the graph, it can be said that the most effective plants to increase dissolved oxygen was Duckweed > Control group > Azolla > Water hyacinth. In this sense, the experiment suggested that plants were not effective in terms of increasing DO since only duckweed ranked higher than the control group. However, a drastic increase of DO for all four groups from day 1 to day 2 indicated that between day 1 and day 2, water might have been in the stage of going towards equilibrium. Thus, when considering the trend from day 2 to day 6, the control group and water hyacinth group showed steeper decrease in DO from day 4 to day 6 than the duckweed group and azolla group. This indicated that duckweed and azolla were more effective than water hyacinth in impeding the decrease in DO. Thus, the order of the groups which showed effectiveness in increasing or maintaining dissolved oxygen level was Duckweed > Azolla > Control group > Water Hyacinth.

Lastly, for turbidity, all groups showed a decrease in turbidity over time (**Figure 4**). This might also be due to the precipitation of floating matter as time went on since the turbidity of the control group also decreased as the time proceeded. Comparing the trendlines of the experimental groups to that of the control group, all three plants—water hyacinth, duckweed, and azolla—were effective at decreasing the turbidity level since they had a steeper slope, which indicated that turbidity was decreasing faster. The measurement of turbidity for each group from day 1 to day 6 showed that the order of the most effective group for reducing turbidity was Water Hyacinth > Duckweed > Azolla > Control group.

Floating plants led to an improvement in the transparency of water

In addition to quantitative measurement from an apparatus, qualitative data were also taken when evaluating the water quality of experiment groups. To determine the effectiveness of floating plants, the clearness and the color of the water were taken into account since if the water had a green color,

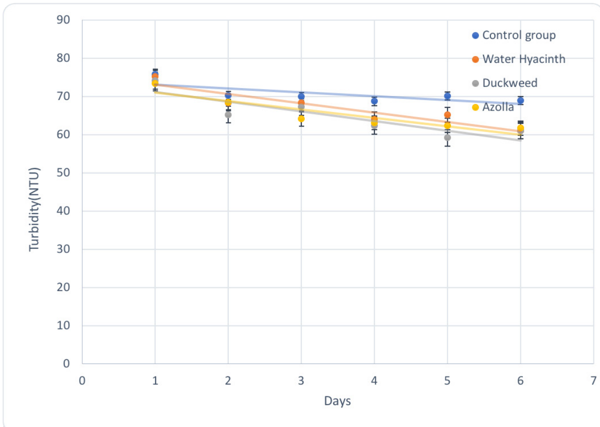

Figure 4. Effect of floating plants on decreasing turbidity. The change in turbidity of control group, water hyacinth, duckweed and azolla for 6 days; linear trendline; data represent the average; error bars represent standard deviation.

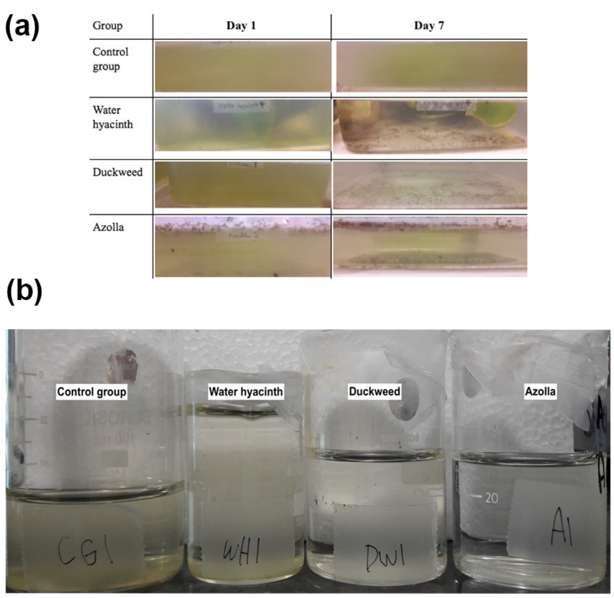

Figure 5. Change in color of water over 7 days. (**a**) Representative images of the color and transparency of water on day 1 and day 7; plants weren't removed from the water to identify possible contaminants attached to plant's roots or subsided on the bottom of containers. (**b**) Representative images of the color and transparency of water samples that were collected on day 7; water samples were taken from the setup to only observe contaminants dissolved in water.

it was assumed that the algae and other chemicals were included in the water and thus, water was contaminated.

The color of water was compared in two ways. First, the color was compared when there were plants in the water (**Figure 5a**). The color of water was observed when the plants were not displaced from water to observe density of color without any outside movement. After seven days from the start of the experiment, the water of all experimental groups turned almost transparent, whereas the water color of the control group did not change. The order of most purified water was therefore water with duckweed > water hyacinth > azolla > control group. This order corresponded to the order of turbidity, indicating that the results were consistent. The color of water was also compared through taking the water sample on the last day (**Figure 5b**). Although all samples were more transparent than the color observed when plants remained in water, the extent of its clarity varied. The observation showed that the order of the most purified water was the water with duckweed > water hyacinth > azolla > control group, which corresponded with the order with the color of the water with plants (**Figure 5a**).

Floating plants drastically reduced the number of colonies that grew on culture medium

On day 7, the sample of water was taken from each set up to measure the number of bacteria inhabiting in the water. We cultured water samples from day 1 and day 7 on culture media to compare the water quality before and after exposure to the floating plants. Then, we counted the number of colonies grown from each sample as a measurement of water quality. We considered that if there was a decrease in the number of colonies grown on culture medium, plants would have reduced the bacterial growth in water.

As expected, the number of colonies varied across the culture media of the water samples from different groups (**Figure 6a**). The control group had the highest density of colonies, while the duckweed and azolla groups had the lowest density. The samples grew five different types of colonies, which indicated the existence of different types of bacteria inhabiting the water. To count the number of colonies for each type of bacteria, the colonies were categorized into five different types depending on their color, size, and morphology (**Figure 6b**). Type 1 was the orange colony, type 2 was the white colony with a solid border line, type 3 was the small and white colony, type 4 was the white colony with the inside filled, and type 5 was the red colony. Then, the average number of colonies was counted. In summary, the water hyacinth had reduced the number of type 3 bacteria, duckweed reduced type 2 and 4, and azolla reduced type 3 and 4 (**Figure 6c**). All groups did not satisfy the permissible limit of fecal coliform numbers (FC) for drinking water: 20 cfu/mL. However, compared with the number of colonies that grew in the water before the experiment, all the experimental groups showed reduction in the number of bacteria. Thus, we concluded that the order of the most effective at reducing bacteria growth was azolla > duckweed > water hyacinth > control group.

DISCUSSION

The six measurements in this experiment—conductivity, pH, turbidity, dissolved oxygen, color, and the number of bacteria colonies—showed that water hyacinth, azolla and duckweed did not always have an effect on water quality. For instance, there were subtle differences between experimental groups and the control group for conductivity, pH, and dissolved oxygen. On the other hand, turbidity and

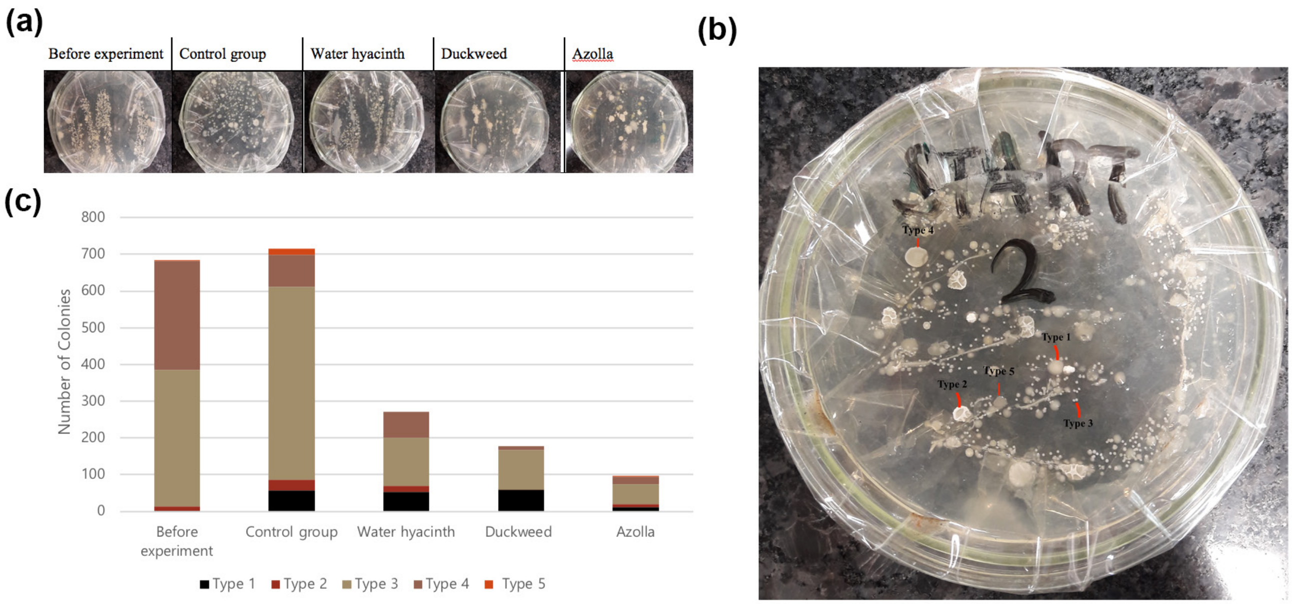

Figure 6. Colonies grown on culture media. (**a**) Representative images of bacteria grew on agar badges with water samples from day 7; The photo was taken 24 hours after the culture media were kept in 31^{o}C incubator; "Before experiment" refers to the water sample from day 1. (**b**) Representative image of different types of bacteria grew on agar plate with water sample taken on day 7; bacteria were differentiated based on its physical feature (**c**) The average number of different types of colonies found on agar badges with water samples from day 7; The number was counted 48 hours after the culture media were kept in 31^{o}C incubator; Before experiment refers to the water sample from day 1.

the number of bacteria in water changed drastically when the plants were added into the water; experimental groups were more effective at water purification compared to the control group since they had a larger decrease in turbidity and less bacteria growing on the culture media.

To determine the most effective group for purifying water, we ranked the groups based on their results from each measurement over the six-day experiment. The results from the experiment supported my hypothesis that the duckweed would be the most effective plants to purify water compared to water hyacinth or azolla. The water from the duckweed group showed the highest effectiveness of purification for most of the results; had the measurements of conductivity (276.9 μS/cm ± 0.95%) and pH (8.20 ± 3.13%) in the acceptable range set up before the experiment, had greatest increase in DO level (4.00 mg/L→ 6.53 mg/L ± 10.%) and the second greatest decrease in turbidity (74.16 NTU→61.06 NTU ± 0.36%) on average with almost colorless water on day 7, and had the second-lowest FC (178 cfu/mL) grown on culture media.

The results from each of the measurements showed correlation, which added to the reliability of the data. For example, turbidity had a close relationship with the color of water since turbidity measures the amount of suspended particles in water, which we can visually observe from the transparency of water. Similarly, in this experiment, the order of water purification was almost the same for the turbidity measurement and the color observation. However, there were also some limitations to the data. First, for pH, DO, and turbidity measurements, there was a rapid change between day 1 and day 2 across all four groups, compared to the change after day 3. The pH increased 0.30 on average from day 1 to day 2, then changed by 0.10 per day afterward. Similarly, dissolved oxygen increased by 1.68 mg/L on average between day 1 and day 2, while it changed by less than 0.54 mg/L per day afterward. Less change in pH and DO level after day 2 indicates that water was in the process of reaching equilibrium—such as solids settling out or adapting to the atmosphere of the lab—from day 1 to day 2. To reduce the error coming from this changing variable, the day 1 measurement was excluded from the analysis in order to identify the trend of equilibrium post-collection. Another limitation is that there was a sign of systematic error in the conductivity data. In its graph, the measurements for both the control group and the experimental group dropped between day 3 and day 4. This drop may be due to incorrect calibration of the measuring tool. Thus, the order was determined by comparing each group to the trend of the control group measurement, which reduces the accuracy of conductivity data. Further, since conductivity measurements for all groups—whether it shows increasing or decreasing trend—were in the permissible range, we decided not to include the order of conductivity when identifying the optimal floating plants for water purification. Another possible error could have been caused by the temperature at which the bacteria were grown on the culture media. In the experiment, the temperature of the incubator was set to 31°C, which may have suppressed the growth of bacteria that have different optimal growth temperatures. This error could be improved through multiple trials with different temperatures, which would allow the researcher to observe the pattern of bacteria growth.

	Conductivity	pH	Turbidity	Dissolved oxygen *trend from day 3 was considered	Number of Colony	Color	Total
Control Group	Acceptable range (273.8μS/cm)	Acceptable range (pH 8.15)	4 (-6.39NTU)	3 (+2.10 mg/L)	4 (715cfu)	4	15
Water hyacinth	Acceptable range (267.8μS/cm)	Acceptable range (pH 8.07)	1 (-14.25NTU)	4 (+1.85 mg/L)	3 (271cfu)	2	10
Duck- weed	Acceptable range (276.9μS/cm)	Acceptable range (pH 8.20)	2 (-13.10NTU)	1 (+2.53 mg/L)	2 (178cfu)	1	6
Azolla	Acceptable range (276.8μS/cm)	Acceptable range (pH 8.14)	3 (-11.67NTU)	2 (+1.96 mg/L)	1 (97cfu)	3	9

Table 2. Compilation of results from various measurements.

Furthermore, dissolved oxygen measurements for water hyacinth had inevitable limitations due to the inability to set up the same environment as an actual pond or river. Cornwell's research suggests that water hyacinth needs a water level of 0.7-1.8 meters to perform photosynthesis effectively (26). Acknowledging that plants need enough space and carbon dioxide to perform photosynthesis effectively, if the experiment is conducted in open-space where it meets those needs, it is possible that an experiment group could perform better on increasing DO. In addition, the absence of water flow may have affected the measurements of dissolved oxygen.

However, despite the limitations this research contains, we expect that the application of a natural treatment system using floating plants will bring a positive impact on water quality, providing better habitat for aquatic life as well as increasing the usefulness of the water. For instance, duckweed can be added to a pond in which wildlife cannot propagate due to contamination from wastewater. This can reduce the peril of wastewater with less cost than physical or chemical treatments. The government can even adopt constructed wetlands, where microorganisms and aquatic plants act as a filter as water slowly enters the wetland; nutrient and pollutant in water naturally breaks down and is taken up by plants and bacteria. This way, the wastewater from agricultural run off or human, industrial waste can be treated with low cost and energy consumption, protecting the environment (27). However, there are some limitations of using duckweed. Duckweed has a high rate of reproduction, however, excess quantity of duckweed can contaminate water quality when it covers the water surface and blocks the sunlight needed for microorganisms that decompose contaminants. In fact, some countries had to undergo disposal of duckweed in sewage systems as duckweed propagated more than they expected (28). Hence, future studies could focus on the relationship between the number of duckweed covering water surface and water purification level.

MATERIALS AND METHODS

Water sample collection

Water samples from the Mithi river were collected in five 20 L plastic bottles. Collected water was directly used in the experiment without any other disinfection or treatment in order to predict the effect of floating plants on water in reality. The water samples were then distributed equally to 16 plastic bowls for 16 set-ups (four set-ups per group).

Group set up

The experiment included one control group and three experimental groups. The control group did not contain any floating plants, but only a water sample. The experimental groups—water hyacinth, duckweed, and azolla—each contained a type of floating plant covering approximately 80% of the surface area of the water. Each group had 4 set-ups to reduce the random error; hence, a total of 16 samples (four groups with four set-ups) were used in this experiment. Observations only happened for seven days due to our inability to replicate the environment of the real river; after seven days, plants started to function less due to space restraints and the water started to be more contaminated due to the absence of its flow. The set-ups were placed indoors, right next to the window, to prevent excess evaporation of water from strong sunlight and high temperatures, which may affect the dissolved oxygen level and turbidity of water. The average ambient temperature of the setting was 29.0°C and the intensity of sunlight was 5.4 kWh m-2 day-1.

Data collection of conductivity, pH, dissolved oxygen, and turbidity

Four types of measurements—pH, turbidity, conductivity, and dissolved oxygen—happened every day. For each test, measurements were taken five times per set-up. pH was measured with pH meter (± 0.01), turbidity was measured with a turbidity meter (±0.01 NTU), conductivity was measured with electrical conductivity meter (± 0.1μS/cm), and dissolved oxygen was measured with dissolved oxygen meter (± 0.1 mg/L). In addition, photos were taken for each set-up every day for the comparison of the water color.

Data collection of the colony forming units

After seven days, water samples were collected for each test subject in order to be used for culture medium. On the agar plate, 1.00 ± 0.01 mL of each sample was sprayed using a pipette. Then, the agar plates were put in an incubator at 31.0 ± 0.1 °C according to the literature for the optimal temperature to grow most of the types of *E. coli* (29). Observation of agar plates happened every 12 hours, and the final number of colonies was counted after 48 hours. This was due to the fact that the number of bacteria surviving on the agar plate started to diminish after 48 hours.

Determine the ranking of water plants that were most effective in water purification

The order of effective water plants to purify water was determined in consideration of six testings (**Table 2**). First, we identified whether pH and conductivity was within acceptable range set up before the experiment. Then we created a table with numbers. which are the rankings of different experimental groups in each of the testnings: Turbidity, DO, FC, and color of water. Then, we summed all the numbers for each group to find the group with the lowest sum of numbers–a group that ranked relatively high in all four testings.

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