Preserving aquatic ecosystems by removing excess amounts of phosphorus using a transgenic plant

Baishali Chaudhuri, Eunchai Kang

BioBuilderClub, Andover High School, Andover, MA, USA

Reviewed on 8 May 2021; Accepted on 28 June 2021; Published on 25 October 2021

Eutrophication is one of the leading causes of the deterioration of many marine ecosystems around the world. It is characterized by excess plant and algal growth that results from the rising levels of chemical nutrients—most notably, phosphorus and nitrogen—from fertilizers washed off of lawns and agricultural lands that have accumulated in bodies of water. The abundance of these nutrients results in the dramatic growth of algae in the ocean. When they decompose, the bacteria that digest the algae—and other dead plants—will exhaust the oxygen in the ocean and produce carbon dioxide, resulting in deficient oxygen levels in the water; this condition is known as hypoxia. It results in the deaths of many wildlife in the ocean as they all depend on oxygen for survival. These ocean areas lacking marine diversity due to hypoxia are commonly called "dead zones," and are a major threat to aquatic ecosystems.

Here, we describe a solution for addressing eutrophication by proposing a genetically modified transgenic floating plant to absorb phosphorus better than native plants. We plan to modify water cabbage (*Pistia stratiotes)*, to express AtPHR1, a gene that has been shown to increase phosphate absorption. *Pistia stratiotes* was chosen for its floating ability, as it allows for easy extraction. The system will consist of a phosphate detecting device and a phosphate regulation device. Our goal is to have the system absorb harmful amounts of phosphorus from the ocean to amend eutrophication. Once a sufficient amount of phosphorus is collected, the floating plant can be easily removed and will not pose a threat of spreading further or dying, which would otherwise lead to further problems. If our system works as intended, it would prevent the formation of large algal blooms and dead zones, thus preserving the biodiversity of the ocean.

Keywords: Eutrophication, algae, hypoxia, AtPHR1, phosphate, algal blooms

Mentors: Baishali Chaudhuri, Eunchai Kang, Lindsey L'Ecuyer

Direct correspondence to bchaudhuri2022@k12.andoverma.us, ekang2022@k12.andoverma.us, lindsey.lecuyer@andoverma.us

Background

Eutrophication is a significant problem in estuaries and coastal waters around the world. In just the contiguous United States, researchers have found that sixty-five percent of coastal waters have been moderately to severely deteriorated by excess nutrients (National Ocean Service, 2017). Excess amounts of nutrients in the environment are detrimental to the ecosystem, as they lead to an overabundance of algae and plants, and their decomposition results in very low levels of oxygen in the ocean—a condition known as hypoxia that can lead to the deaths of many organisms (National Ocean Service, 2017).

In addition to depleting the oxygen in the ocean, the overgrowth of algae blocks sunlight, causing more deaths of marine life. The decomposition of algae and plant matter also leads to the release of large amounts of carbon dioxide, thus lowering the pH of the water and resulting in ocean acidification (National Ocean Service, 2017). Acidification is a threat to marine organisms, like coral and fish, because it can prevent them from forming the outer shells they need to survive. Harmful algal blooms have also been linked to the degradation of water quality, the destruction of fisheries, and public health risks (Chislock et al., 2013).

Eutrophication occurs naturally over centuries as lakes age and collect nutrient-filled sediments (Chislock et al., 2013). However, human activities have significantly increased the speed of this process, leading to fatal consequences for aquatic ecosystems. Scientists have linked eutrophication to activities including industry, agriculture, and sewage disposal—all of which produce the principal nutrients that result in algal blooms: phosphorus and nitrogen (Chislock et al., 2013). Phosphorus is essential for marine life, because it promotes the production of microbes and phytoplankton, which makes up the base of the aquatic food chain (Woods Hole Oceanographic Institution, 2015). However, too much phosphorus can speed up eutrophication, contaminating the water.

To address eutrophication, scientists have proposed several ways in which phosphorus could be removed from the ocean. Using physical methods such as filtration or chemical methods such as phosphate precipitation (Lenntech, 2021) has shown to be effective in removing phosphorus. However, these methods can be a challenge as they require complex, expensive equipment and large amounts of chemicals that can lead to further environmental issues. One biological method, a sludge method known as anaerobic-aerobic-oxic method or A2O (Lenntech, 2021), has been widely used in water and sewage plants, but it has proven to be difficult to apply directly to aquatic environments due to complicated equipment.

With our design, we aim to provide an easy, low-cost, environmentally-friendly solution—one that does not require as many chemicals that could potentially harm the environment or many equipment—that will extract the excess phosphorus from the water and thus prevent eutrophication. Our solution comes in the form of phytoremediation, a process in which plants are used for the rehabilitation of contaminated soil and water (Van Aken, 2011). As plants absorb phosphorus naturally from its roots, we hope to genetically modify a plant to have the capacity to absorb excess amounts of phosphorus. The plant we have chosen for our design is *Pistia stratiotes*, commonly known as water cabbage, because of its ability to float on water, which provides an easy means of extraction once it collects the amount of phosphorus that needs to be removed.

Systems Level

Ideally, we would modify the *P. stratiotes* with the gene, AtPHR1, which encodes for PHR1 proteins that absorb phosphate. AtPHR1 is an inorganic phosphate (Pi) starvation-related gene found in *Arabidopsis thaliana,* a small flowering plant (Matsui et al., 2013). Researchers who tested the AtPHR1 gene found that it resulted in enhanced Pi absorption in different plant species, thus demonstrating to be a possible pathway to effectual phytoremediation in aquatic environments overwhelmed with phosphorus (Matsui et al., 2013). The system would be designed to detect and absorb the excess amounts of phosphorus in the ocean, which will then be stored in the *P. stratiotes* until it is collected. An area of the ocean would be fenced off with floating booms, so the *P. stratiotes* would be secure in one place and not float around in areas where phosphorus levels were normal or escape into the wild. We choose to use floating booms to contain the *P. stratiotes* because it has good buoyancy and is lightweight, and will not present as many environmental threats as opposed to other barriers, such as the rope from a surrounding net.

Device Level

We chose the host of our design to be *P. stratiotes* as the host for our design because as a floating plant, it gets all of its nutrients directly from the water, making it an excellent candidate for fighting against algae (Spece, 2021). *P. stratiotes* have also been used in the past to combat toxins in polluted water (Woods, 2020). As the concentration of phosphorus is much higher in the coastal regions (National Geographic Society, 2012), we

would have multiple stations along the coastal areas designated for these plants. At these stations, the floating ability of the plant will also allow us to easily extract the plant manually, as it is just on the surface of water. *P. stratiotes* is relatively resistant to salinity (Howard et al., 2021), and with the correct genetic modifications, it should absorb the phosphorus from the ocean.

The main function of our system is the phosphate reducing device, which will involve the gene AtPHR1. The gene's only role will be taking in excess amounts of phosphate from the water, thus storing it in the plant. To test if the AtPHR1 is performing its role, the system will involve a reporter called amilGFP, or yellow chromoproteins. A reporter will be used within the same coding sequence to ensure that our design is working as intended and to confirm that the AtPHR1 gene is being translated. After AtPHR1 translates, amilGFP will translate right after. The presence of yellow chromoproteins—which will be visible—will confirm that AtPHR1 has translated.

The second component of our design is the phosphate regulation device, and it will be responsible for stopping the transcription of AtPHR1 once sufficient phosphorus has been absorbed. This will be done using another promoter that will turn on once low levels of phosphorus have been reached, and will induce the transcription of the AtSPX1, which suppresses AtPHR1 activity. Further discussion of these components will be expanded on in the next section.

Parts Level

Our design features an enhanced cauliflower mosaic virus 35S promoter, an effective promoter for expression of foreign genes (Mitsuhara et al., 1996), which are the AtPHR1 and amilGFP genes. This promoter was chosen because our chassis is a plant and it has demonstrated to be compatible with the AtPHR1 gene (Matsui et al., 2013), ensuring it works within the system. Following the promoter will be a strong ribosome binding site (RBS), BBa_J63003 from the Registry of Standard Biological Parts. An RBS is needed for our design, as it is responsible for initiating translation of our genes. BBa_J63003 was chosen because it works well with a eukaryotic chassis, which is the *P. stratiotes* (Ajo-Franklin, 2006).

The coding sequence of our design will involve the main functional component, the AtPHR1 gene, and the reporter (amilGFP). AtPHR1 encodes the transcription factor that activates the transcription of genes which then responds to Pi starvation (Matsui et al., 2013). Pi-starvation responses are utilized by plants to increase the acquisition of Pi from the outside when there is not a sufficient amount of Pi for sustainment. AtPHR1 is being used because the overexpression of the gene has shown to increase the concentration of Pi in plants, and therefore could theoretically decrease the concentration of phosphorus in the water when implemented in our design.

The yellow chromoprotein, amilGFP, will be the second part of the coding sequence. It exhibits a bright yellow color (Sun, 2011), which will be easily observable and can clearly indicate whetherAtPHR1 is functioning. As AtPHR1 is translating alongside the reporter gene, yellow chromoproteins should be secreted as the plant absorbs phosphorus from the water. The amilGFP will only be used to test the effectiveness of AtPHR1; when actually implementing the solution, the reporter will be taken out as we do not want the water to turn bright yellow.

The second sequence of our design will involve the use of the inducible promoter, PphoA, or BBa_K1139200. The promoter is derived from the alkaline phosphatase gene

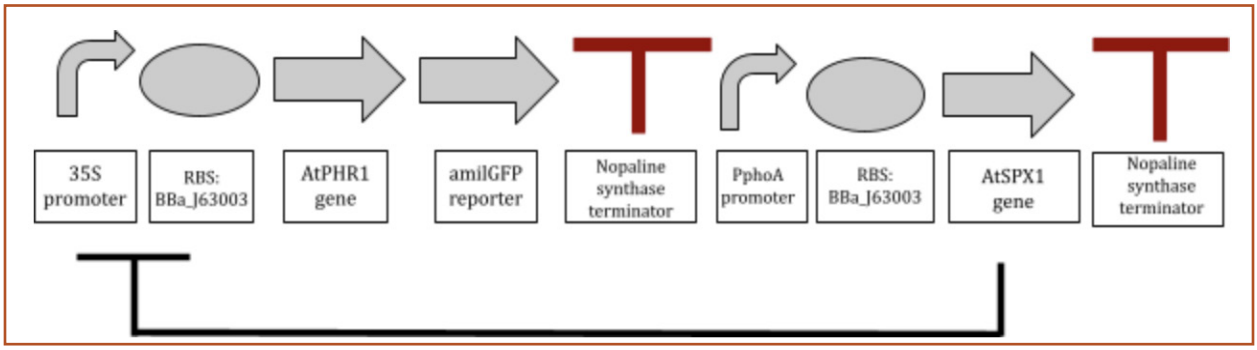

Figure 1. *Construct is composed of 2 sequences with multiple parts, further explained under "Parts Level."*

(phoA), and has been shown to be drastically repressed at high phosphate concentration levels (Ogino, 2013). When the transgenic plant is first placed in the water, PphoA will be turned off, and AtPHR1 would do its work in accumulating phosphorus. Once sufficient phosphorus has been absorbed and there are only low concentrations of it left in the water, PphoA will be turned on, and will turn on transcription of AtSPX1. AtSPX1 suppresses phosphate-starvation induced (PSI) genes—or the genes that play a role in the Pi-starvation response, meaning it inhibits them from collecting phosphorus (Qi et al., 2017). AtSPX1 binds to AtPHR1, and tightly controls the regulation of PSI gene expression. Once AtSPX1 begins transcription, the plant will no longer absorb phosphorus from the water.

To conclude the design, a nopaline synthase terminator compatible with plants will be used. This terminator signals the end of gene expression and is commonly used with the cauliflower mosaic 35S promoter (Mitsuhara et al., 1996), making it an effective choice for a successful terminator.

Safety

The imbalance of chemical nutrients in the water can result in a significant disruption of the ecosystem as seen in dead zones, which result from the rising levels of phosphorus and nitrogen. Thus, it is critical to test our method without drastically altering the concentration of chemical nutrients in the water. In order to safely test our device, our device will be constructed and tested in a laboratory. Laboratory equipment such as a humidifier will be used to recreate the environment as close as possible to that in which our device would be used. In addition, the concentrations of chemical nutrients in dead zones will be taken multiple times to determine the average concentration that the device will be first applied to.

As our device's main purpose is to reverse the effects of the dead zones in the warm southern sea of the United States, the testing room will maintain a very close temperature to that of the southern sea of the United States, in order to make sure that our device is able to function as expected at such temperatures. All experiments will be conducted in a contained environment, and once we can assert that our device can decrease the concentration of excess phosphorus and nitrogen in the water, we will test it on actual water bodies. To predict how the *P. stratiotes* will act in the water, the device will first be placed in a closed, controlled region of water where it will be critically monitored. The *P. stratiotes* will be carefully watched to determine how invasive or disruptive it may be to other organisms. If the *P. stratiotes* is found to be more harmful to the marine ecosystem, it will be removed immediately and further testing will be done to address how to prevent the damages presented.

For extra precautions, a special netting could be used to surround and cover the roots of the *P. stratiotes* that would allow water, phosphorus, and other elements to flow through, but not the seeds of the plant. This would enable the *P. stratiotes* to absorb the phosphorus in its natural environment without spreading extensively to other uncontrolled bodies of water.

How to prevent the spread of *Pistia Stratiotes*

Another aspect to consider is when to take the transgenic *P. stratiotes* out of the water. Before removing the transgenic *P. stratiotes*, we would compare the phosphorus concentration in the water from before the plants were used to after they were used with our lab experiments which tells us the amount they could absorb which determines whether or not the *P. stratiotes* have absorbed phosphorus to its full ability. The *P. stratiotes* should stop absorbing phosphorus once low levels have been reached, due to the phosphate regulation device. Once the measured concentration levels of phosphorus is at an appropriate level, the *P. stratiotes* will then be manually taken out of the water. If the measured concentration in the water is too low—meaning the device is absorbing too much phosphorus from the water—then it will be removed immediately and testing will be done to assess the problem.

However, if the transgenic *P. stratiotes* were to die before we are able to extract them from the water surfaces, they could potentially release the phosphorus they have stored in them. To prevent this from happening, we would keep a constant watch on these plants, so that we can take the plants out of the water before they die and decompose. A common indicator would be the yellowing or browning of its leaves.

Discussions

Phytoremediation, one of the more natural ways to remove environmental contaminants and has several advantages that the transgenic *P. stratiotes* can implement. Since we are using a plant and just enhancing one of its functions (phosphorus absorption), the risk of exposing pollutants to the ecosystem will be reduced (Yan et al., 2020). Phytoremediation is also a passive technique because the *P. stratiotes*, which

can be found in the environment, will not change the ecosystem drastically, leading to a safer and more sustainable solution of reducing phosphorus levels in the water. In addition, the transgenic plant will be economically feasible; as plants only rely on solar power, very little additional maintenance will be required. As the remediation will be done right in the ocean when the plants are placed within the closed-off region bordered by the floating booms, transportation and off-site processing would not be necessary, thus saving about $200 to $600 per ton (Gupta et al., 2000).

Another advantage of our solution is its ability to be applied over a large scale (Yan et al., 2020). Since not much maintenance nor energy will be needed to make the transgenic plant work, the *P stratiotes* can be used to recover a wide area of an ocean from excess amounts of phosphorus. Dead zones are increasing in size, varying from 5,000 square kilometers to 22,000 square kilometers (National Geographic Society, 2012), and the applicability of the *P. stratiotes* will be an important advantage. The *P. stratiotes* will also be easy to dispose of as it is a floating plant, it can be readily picked up from the surface of the water.

However, there are disadvantages that should be considered. While phytoremediation is a more natural way to address eutrophication, the process is very slow and can take up to three to five years (Bruni & Mcleskey, 2013). It can take a while for the transgenic *P. stratiotes* to be able to absorb the excess amounts of phosphorus, and the efficiency of this system will have to be later tested in a controlled environment. Another aspect to consider is the cost of extracting the *P. stratiotes*.

While phytoremediation is less expensive than other methods, extraction could still be somewhat costly. However, the transgenic *P.stratiotes* should still be a low-cost solution compared to alternate methods that require additional instrumentation and maintenance. One factor that should also be considered is the potential threat that the *P. stratiotes* could grow extensively. Water cabbage is an invasive species (Woods, 2020), and therefore presents the risk of overpopulating, which can endanger the ecosystem. To address this, extensive tests will be done to determine how the transgenic *P. stratiotes* will grow while absorbing the phosphorus in the water. The population of the *P.stratiotes* must be heavily monitored to prevent it from covering a majority of the surface water, which would inevitably block the sunlight from marine life. The overgrowth of the plant could also result in the blockage of irrigation systems which could potentially lead to flooding (Department of Primary Industries and Regional Development's Agriculture and Food, 2020).

More research is needed to determine if a genetic part or a coding sequence could be added to monitor the growth of the *P. stratiotes.* However, despite *P. stratiotes* being considered an invasive plant, its natural habitats are lakes or slow moving rivers, and therefore applying it to the ocean where the salinity and pH level are much different might result in a change in its invasive behavior. More testing will need to be done to evaluate how these factors may impact the *P. stratiotes* in terms of its invasive behavior.

Although our device could potentially decrease the excess of phosphorus concentrations in the water and help restore our ecosystems, we must keep in mind that utilizing an invasive species, even if we are using them in its natural environment, could have a negative impact on other organisms. As it is impossible to recreate the exact environment in which our device will be utilized in a laboratory environment, it is necessary to test it on an actual dead zone, and the precautions for such testing are laid out in the "Safety" section.

Once the *P. stratiotes* has done its part in absorbing the phosphorus and has been removed, we hope to reuse the plant by taking out the phosphorus it had absorbed earlier, and reengineering the plant with its genetic components if necessary. Research will need to be done to determine if taking out the phosphorus from the chassis is possible, and if the *P. stratiotes* would be able to survive another round of absorbing phosphorus in the ocean. The cost, amount of time, and the amount of resources needed to conduct this process must be considered to determine whether it is worth the effort. If reusing the *P. stratiotes* proves to be more expensive and less efficient, the *P. stratiotes* should be safely disposed of instead and another plant can be modified in its place.

If it is possible to take out the excess phosphorus from the *P. stratiotes* effectively, a potential use for the phosphorus taken out is to utilize it as a fertilizer. If there is a method to purify the phosphorus the plant has absorbed from the water, it is then possible to use appropriate amounts of it in fertilizers for farmers to use on crops. However, more consideration must be dedicated to this proposal, because too much phosphorus in the fertilizer can find its way into the ocean again, which feeds into the problem of eutrophication that our *P. stratiotes* aims to solve. Research will also be needed to determine how the phosphorus can be purified.

As we become more aware of the harmful effects human activities have on our environment, it is crucial that we do the best of our ability to undo the damage before it is too late. Although dead zones may seem insignificant as

it only affects a small part of our ecosystem, it can have lasting consequences. If more dead zones are created as the result of human activities, more damage will be done to our ecosystems as all of our ecosystems are connected to one another; the fall or one will result in the fall of another.

Next Steps

After coming up with the design, the next step would be to develop an experiment that tests the phosphorus absorption capacity of AtPHR1. We would assemble only the first sequence of our system, which involves the 35S promoter, the strong ribosome binding site (BBa_J63003), AtPHR1, amilGFP, and the nopaline synthase terminator. Before assembly, it is likely that the AtPHR1 gene needs to be amplified by PCR—polymerase chain reaction. PCR will allow us to produce many copies of the gene inexpensively and efficiently (National Human Genome Research Institute, 2019). As we will be testing many different variables using different controls each time, it will be highly beneficial for us to have the genes ready for testing as needed. The primary goal of this experiment would be to test the function of AtPHR1; amilGFP will only be used to ensure that the gene is translating. Once the plasmid has been constructed and AtPHR1 has been translated, positive strains can be chosen using RT-PCR.

To set up the experiment, plants would be placed in different buckets of water with varying phosphorus concentrations. The first bucket would be the control, only having distilled water. The second bucket would have low concentration of phosphorus, and the third bucket would have a high concentration of phosphorus. Before placing the plants in the water, the concentrations of the phosphorus would be measured and recorded. One plant with the AtPHR1 system will be placed in each bucket. Every half hour, or another suitable time interval, the phosphorus concentration in the water will be recorded.

Once this trial is over and a few more trials are conducted, analysis of the data and reflection can help determine the workability of the device. This experiment will be repeated multiple times, each time varying the control. The purpose of using distilled water is to see how much phosphorus the plant can absorb without any outside interference; in the following experiments, we would use different samples of water such as rain water, as our control to see how conditions similar to an aquatic environment may affect the plant's ability to absorb phosphorus.

Author Contributions

B.C. and E.K. did extensive research regarding eutrophication and conceived the goal of the study. B.C. gathered the project's requirements and wrote the manuscript, while E.K. developed the application of the system and assisted with the design's write-up. Both authors contributed to research and analysis.

Acknowledgements

We would like to thank our mentor Mrs. L'Ecuyer for guiding us throughout our project. We were able to connect with her many times to have discussions whether it was about possible genes that could be used or talking about general synthetic biology, which allowed us to think more creatively.

This project was accomplished through participation in the BioBuilderClub, an after-school program organized by BioBuilder Educational Foundation. BioBuilderClub engages high school teams around the world to combine engineering approaches and scientific know-how to design/build/test their own project ideas using synthetic biology.

References

Ajo-Franklin, C. (2006, October 11). *Part:BBa_J63003*. Registry of Standard Biological Parts. http://parts.igem.org/Part:BBa_J63003

Bruni, S., & Mcleskey, D. (2013, January 29). *Phytoremediation*. Geoengineer.Org. https://www.geoengineer.org/education/web-class-projects/cee-549-geoenvironmental-engineering-winter-2013/assignments/phytoremediation

Chislock, M. F., Doster, E., Zitomer, R. A., & Wilson, A. E. (2013). Eutrophication: Causes, Consequences, and Controls in Aquatic Ecosystems. *Nature Education Knowledge*, *4*(4), 10. https://www.nature.com/scitable/knowledge/library/eutrophication-causes-consequences-and-controls-in-aquatic-102364466/

Department of Primary Industries and Regional Development's Agriculture and Food. (2020, May 4). *Water lettuce: declared pest*. https://www.agric.wa.gov.au/declared-plants/water-lettuce-declared-pest

Gupta, D., Singh, L. K., Gupta, A. D., & Babu, V. (2012). Phytoremediation: An Efficient Approach for Bioremediation of Organic and Metallic Ions Pollutants. *Bioremediation and Sustainability*, 213–240. https://doi.org/10.1002/9781118371220.ch5

Howard, V., Baker, E., Li, J., & Alsip, P. (2021). *Pistia stratiotes L.* U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL, and NOAA Great Lakes Aquatic Nonindigenous Species Information System, Ann Arbor, MI. https://nas.er.usgs.gov/queries/greatlakes/FactSheet.aspx?Species_ID=1099&Potential=Y&Type=2&HUCNumber

Lenntech. (2021). *Phosphorus removal from wastewater*. https://www.lenntech.com/phosphorous-removal.htm

Matsui, K., Togami, J., Mason, J. G., Chandler, S. F., & Tanaka, Y. (2013). Enhancement of Phosphate Absorption by Garden Plants by Genetic Engineering: A New Tool for Phytoremediation. *BioMed Research International*, *2013*, 1–7. https://doi.org/10.1155/2013/182032

Mitsuhara, I., Ugaki, M., Hirochika, H., Ohshima, M., Murakami, T., Gotoh, Y., Katayose, Y., Nakamura, S., Honkura, R., Nishimiya, S., Ueno, K., Mochizuki, A., Tanimoto, H., Tsugawa, H., Otsuki, Y., & Ohashi, Y. (1996). Efficient Promoter Cassettes for Enhanced Expression of Foreign Genes in Dicotyledonous and Monocotyledonous Plants. *Plant and Cell Physiology*, *37*(1), 49–59. https://doi.org/10.1093/oxfordjournals.pcp.a028913

National Geographic Society. (2012, October 9). *Dead zone*. https://www.nationalgeographic.org/encyclopedia/dead-zone/

National Human Genome Research Institute. (2019, March 9). *Polymerase Chain Reaction (PCR) Fact Sheet*. https://www.genome.gov/about-genomics/fact-sheets/Polymerase-Chain-Reaction-Fact-Sheet

National Ocean Service. (2017). *What is eutrophication?* https://oceanservice.noaa.gov/facts/eutrophication.html

Ogino, S. (2013, September 9). *Part:BBa_K1139200*. Registry of Standard Biological Parts. http://parts.igem.org/wiki/index.php/Part:BBa_K1139200

Qi, W., Manfield, I. W., Muench, S. P., & Baker, A. (2017). AtSPX1 affects the AtPHR1–DNA-binding equilibrium by binding monomeric AtPHR1 in solution. *Biochemical Journal*, *474*(21), 3675–3687. https://doi.org/10.1042/bcj20170522

Spece, J. (2021). *Plant Profile: Water Lettuce*. In The Country Garden & Gifts. http://www.inthecountrygardenandgifts.com/articles/plants/pond_plant_water_lettuce.php

Sun, L. (2011, September 18). *Part:BBa_K592010*. Registry of Standard Biological Parts. http://parts.igem.org/Part:BBa_K592010

van Aken, B. (2011). Transgenic Plants and Associated Bacteria for Phytoremediation of Organic Pollutants. *Comprehensive Biotechnology*, 223–237. https://doi.org/10.1016/b978-0-08-088504-9.00330-5

Woods Hole Oceanographic Institution. (2015, May 14). *Revealing the Ocean's Hidden Fertilizer*. https://www.whoi.edu/press-room/news-release/phosphoruscycle/

Woods, R. (2020, June 4). *Water Lettuce*. Fishkeeping World. https://www.fishkeepingworld.com/water-lettuce/

Yan, A., Wang, Y., Tan, S. N., Mohd Yusof, M. L., Ghosh, S., & Chen, Z. (2020). Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted Land. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.00359