

## **Uptake of Phosphorus and Iron in Duckweed**

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## INTRODUCTION

The Macatawa Watershed consists of wetlands, streams, and a lake, which have continuous runoff forming non-point source pollution from the communities within the watershed (The MACC 2012). The use of chemicals in agricultural practices, waste disposal, and other sources has caused dispersal of excess nutrients into the environment. This runoff has led to increased concentrations of plant limiting nutrients, and has caused hypereutrophication to occur in some areas. The Holland community is deeply invested in maintaining a healthy watershed and it is important to investigate methods of cleanup for our surroundings.

Some heavy metals in low concentrations are required by plants to maintain some physiological functions (Hansch and Mendel 2009), but an excessive accumulation of heavy metals can interfere with processes such as photosynthesis. Based on the different heavy metals, each plant's toxicity depends on different factors (Shaw and Rout 2002) and the rates at which some of these heavy metals are absorbed occur on a logarithmic scale (Uzun and Güzel 2000).

The central concept of compound's toxicity is that it is dose-dependent. Anything can be toxic when in excess or in a deficiency. Specifically, iron is necessary for plants to make chlorophyll, which is critical to photosynthesis, yet in high concentrations iron becomes toxic to plant species (Foy, Chaney and White 1978). These high concentrations of iron are often associated with waterlogged environments such as wetlands (Wheeler, Al-Farraj, and Cook 1985).

Excess phosphorus is also a concern because it is the limiting factor in plant growth and concentrations must be decreased in the environment to reduce the potential of hypereutrophication (Öbek and Hasar 2002). Duckweed harvesting has been used in treatment sites in order to reduce these concentrations by absorbing the nutrients from the surrounding water. Extensive studies in the upper Mississippi offer more support for the use of duckweed in taking out nutrients (Sullivan and Giblin 2012). Duckweed is an extremely effective resource in nutrient absorption due to its relatively fast growth. However, because of duckweed's rapid growth and short life span, it is necessary to harvest the plants frequently in order to successfully remove the nutrients from the environment (Öbek and Hasar 2002).

The main objective of this study is to determine the extent of uptake of phosphorus and iron and how effective it could be as a water treatment plan to remove iron and phosphorus from the watershed through harvesting. It is assumed that the duckweed would be harvested after absorbing the nutrients so that they would not reenter the environment.

## METHODS

### Duckweed collection and sterilization

Duckweed was collected from Pine Creek on the north region of the Macatawa Watershed and stored in a 25 gallon tub with some of the local water from Pine Creek. On the same day of collection, the duckweed was sterilized with a prepared 20% bleach solution using pure bleach and Millipore ultrapure water of approximately 18.2 MΩ·cm at 25 °C from a Milli-Q filter. The sterilization process was adapted from the Missouri Botanical Gardens and included

transferring small batches of duckweed into the 20% bleach solution for 30 seconds, then rinsed in ultrapure water for 1.5 minutes, rinsed a second time in ultrapure water for 1.5 minutes and then placed in a 25 gallon tub filled with ultrapure water (Cross 2012). This process was repeated for 4 additional small batches of duckweed to get a substantial amount of sterilized duckweed for testing. A second trial of sterilization was prepared by adjusting the amount of time in the bleach solution to one minute and maintaining analogous rinse intervals in purified water. The tubs were all placed under artificial light set on a timer for twelve hours of light a day.

### Solution Preparation and Sample Collection

An approximate 12-13 ppm iron solution was prepared from 2.35 g of iron (II) sulfate heptahydrate in 10 gallons of ultrapure water and allowed to equilibrate overnight. The phosphate solution was prepared from ~44.0 g of monopotassium phosphate and then the pH was fixed at 6.5, measured by using a pH electrode and adding prepared concentrated NaOH solution. After the solutions were divided amongst a control tub and a tub to place duckweed, ruler sticks were placed to record the change in height over time. Water samples and a small amount of duckweed were collected separately in 7 mL glass vials every twelve hours. At the time of sampling, water height was also recorded to account for evaporation in addition to adding ultrapure water to the iron control and iron with duckweed tubs to avoid precipitation. The samples were stored at 4°C until analyzed.

### Biogeochemical Cycle

According to Figure 1, iron and or phosphorus has four different reservoirs it could be found. Thus the amount of phosphorus or iron present in the plant at a specific time could be represent by the following equations

$$P_{\text{plant}} = P_i - P_{\text{water}} - P_{\text{atm}} - P_{\text{container}} \quad \text{and} \quad Fe_{\text{plant}} = Fe_i - Fe_{\text{water}} - Fe_{\text{atm}} - Fe_{\text{container}}$$

where  $P_i$  and  $Fe_i$  are the amounts of phosphorus and iron present in the water. Thus, by using a control system for both phosphorus and iron, the amount of each lost to the atmosphere and container can be determined. To determine the amount absorbed by the plant, the concentration present in the water and amount of water will need to be measured.

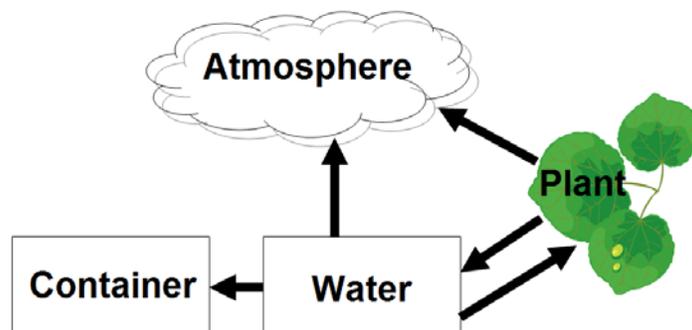


Figure 1. Biogeochemical Cycle for Iron and Phosphorus in the System

## Solution Analysis

The concentration of phosphorus present in the control tub and duckweed filled tub were determined using an Auto Analyzer 3 using external standards of 20, 50, 100, 200, 300, 450 ppb solutions to create a calibration with a correlation coefficient of 0.9985. The Auto Analyzer procedure used was from a standard Hope College guideline. The samples collected were diluted down to the ppb range from ppm using micropipettes and  $M_1V_1=M_2V_2$  to bring the sample within the sensitivity range of the Auto Analyzer 3. Then using the external standards calibration curve, the concentration present in the water in the control tub and in the tub with duckweed over the course of time was determined.

The concentration of the iron present in the water samples collected from the control and from the tub with duckweed was determined using atomic absorption (AA) spectroscopy. The AA hollow cathode lamp position, fuel flow rate, and flame height were optimized using a 50 ppm iron solution prepared from 44.5 mg of iron(II)chloride tetrahydrate in 250 mL ultrapure water and a few drops of concentrated 18 M nitric acid to ensure complete ionization. A calibration curve was created using external standards of 5, 10, 15, 20, and 25 ppm prepared using volumetric glassware.

The water samples had one drop of 18M concentrated nitric acid added to the vials and were shaken up to remove any iron stuck to the cap or walls of the vial. The samples were then individually aspirated to record the absorbance of iron present in each vial at a 248.3 nm wavelength. The atomic absorption spectrometer was zeroed with purified water between samples. Using the calibration curve for iron, the amount of concentration of iron present in the control or duckweed tub could be determined.

## Duckweed Analysis

The harvested duckweed were weighed in crucibles and burned using a muffle oven at 500°C for a total of 5 hours. The resulting ash and crucible was then weighed and the difference in mass was recorded. To the ash, 50 mL of 0.30 M HCl was added to digest the phosphate present (Rehcgil and Payne 1989). The solution was filtered to remove any remaining ash and the samples were diluted to bring them within the range of the Auto Analyzer calibration curve. The diluted samples were run using the same calibrants as for the water analysis.

## RESULTS

### *Iron:*

Table 1 and Figure 2 show the results from the analysis of the iron concentrations in the control solution and solution with duckweed using atomic absorption. The atomic absorption gave absorbances for collected water samples, which were converted into concentrations using a Beer's Law calibration curve from the prepared external standards.

Time	Iron Solution with Duckweed		Iron Solution Control	
	Concentration (ppm)	Mass (mg)	Concentration (ppm)	Mass (mg)
0	12.93	77.56	13.73	82.37
12	11.37	68.23	14.72	88.30
24	10.99	64.66	15.85	94.46
36	12.97	74.23	12.83	74.43
48	12.22	73.80	10.85	64.25
60	9.82	58.51	15.24	90.81
72	9.53	57.58	12.22	72.83
84	9.06	53.87	14.10	82.37
96	10.85	65.12	12.64	75.36
108	8.64	52.17	13.21	78.73
121	8.50	49.83	13.40	78.78
133	9.53	56.82	13.54	80.69
145	8.97	53.31	13.87	80.45
157	10.10	59.23	13.26	79.01
170.5	8.50	50.64	14.20	82.93

Table 1. Concentrations (ppm) and mass (mg) of iron with duckweed and control samples

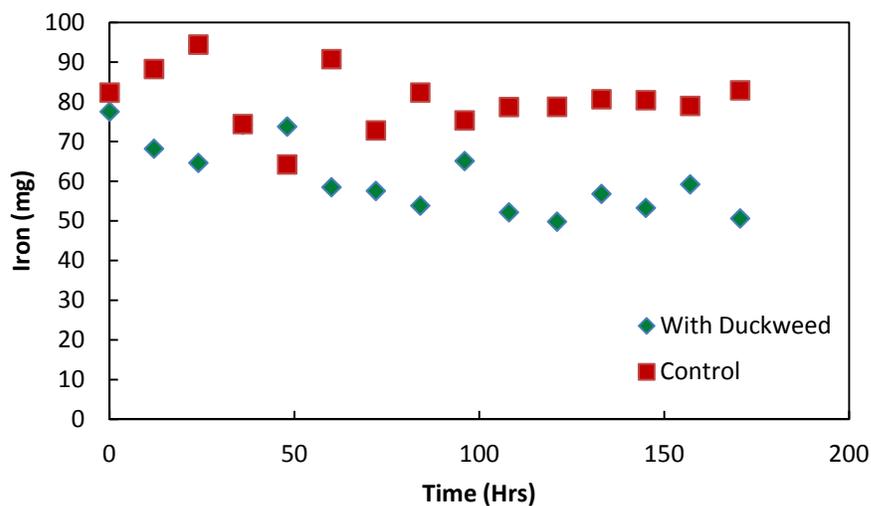


Figure 2. Iron mass in control and in the presence of duckweed over time

Since the amount of iron present in the control solution remained relatively constant, there was no iron moving into the container or atmosphere reservoir. Therefore, any decrease in iron concentration in the solution with duckweed would be due to absorption of iron by the duckweed. Initially, there was a noticeably rapid absorption of iron by the duckweed and then it leveled off around 100 hours.

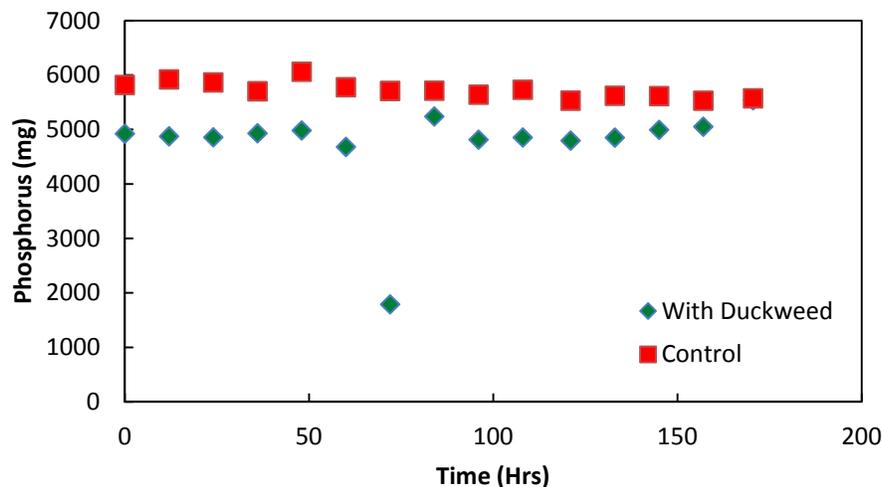
*Phosphorus:*

The following analytical results given in Table 2 were found for the collected samples every twelve hours. The total phosphorus masses were determined using the concentrations of phosphorus present, the evaporation gauge heights, and surface area for both containers.

Time (Hrs)	Phosphorus Solution with Duckweed		Phosphorus Solution Control	
	Concentration (ppm)	Mass (mg)	Concentration (ppm)	Mass (mg)
0	274	4923	293	5819
12	274	4876	301	5921
24	276	4858	300	5865
36	288	4932	297	5704
48	295	4983	318	6061
60	279	4681	305	5777
72	107	1790	304	5709
84	322	5240	310	5714
96	300	4814	308	5640
108	305	4854	319	5730
121	306	4797	308	5531
133	313	4853	318	5621
145	325	4995	320	5614
157	339	5052	321	5529
170.5	374	5539	325	5574

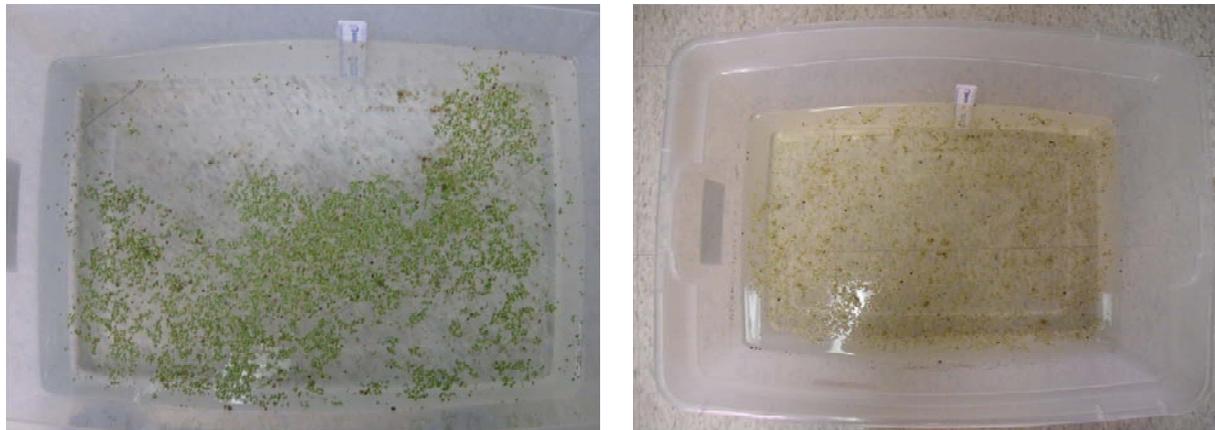
*Table 2.* Concentrations (ppm) and mass (mg) of phosphorus with duckweed and control samples

The change in total mass of phosphorus was depicted in Figure 3. It can be seen that the concentration does not vary over time for both the container with duckweed and without duckweed. Using the geochemical cycle in Figure 1, the constant concentration of phosphorus present in the control indicates that no phosphorus was lost to the container or the atmosphere reservoir. However, since the amount of phosphorus did not decrease or increase over time, then there was no change in the phosphorus present in the duckweed. The variance in total mass of phosphorus control container and the phosphorus container with duckweed was due to the initial difference in volume of solution. The low phosphorus concentration with duckweed point at 72 hours is due to error in micropipetting when performing dilutions.



*Figure 3.* Phosphorus mass in control and in the presence of duckweed over time

After observing the duckweed over the course of the experiment, there was a noticeable change in the vigor of the duckweed. The plants changed from a dark vibrant green to a light yellow-brown and some fell to the base of the container demonstrated by the before and after photos in Figures 4. Our results demonstrate this effect at 121 hours, where the phosphorus mass in the present of duckweed shows a slight increase. This indicates that the duckweed dying is releasing phosphorus back into the water reservoir.



*Figure 4.* Initial duckweed (left) and final duckweed (right) for phosphorus solution

The concentrations of the water samples were ambiguous in comparison to the detection level of the Auto Analyzer and required dilution from parts per thousand to ppb. It was then hypothesized that any of the phosphorus taken up by the duckweed was within the uncertainty of the determined concentrations due to the error in the dilutions and Beer's Law plot. In addition to the water samples, the harvested duckweed was analyzed using the dry ash method as another means of evaluating whether duckweed absorbed any phosphorus and given in Table 3 and Figure 5.

Time (Hrs)	Duckweed (g)	Phosphorus (g)	Phosphorus/duckweed (g/g)
0	0.003	0.000000342	0.000114
12	0.003	0.000000305	0.000102
24	0.000	0.000000444	-
36	0.004	0.000000238	0.000059
48	0.001	0.000000185	0.000185
60	-0.002	0.000000319	-0.000159
72	0.007	0.000000303	0.000043
84	0.007	0.000000490	0.000070
96	0.003	0.000000463	0.000154
108	0.002	0.000000159	0.000079
121	0.003	0.000000186	0.000062
133	0.004	0.000000176	0.000044
145	0.003	0.000000207	0.000069
157	0.003	0.000000147	0.000049
170.5	0.003	0.000000080	0.000027

Table 3. Mass of phosphorus per mass of ashed duckweed over time

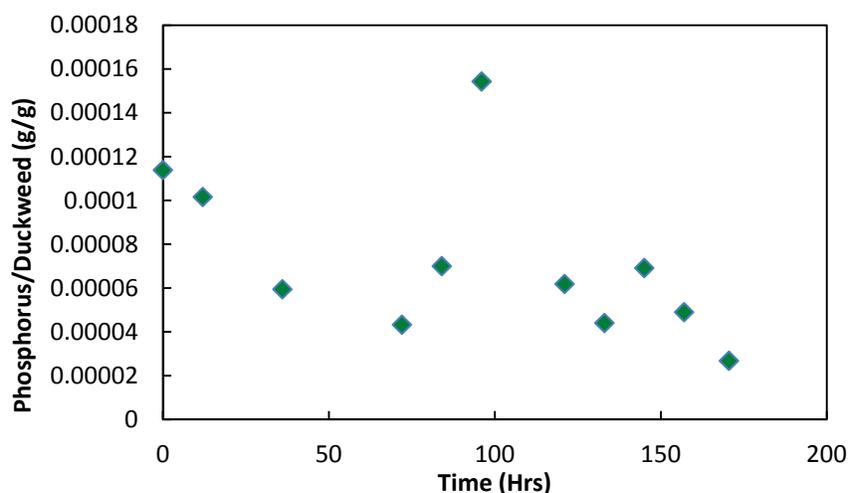


Figure 5. Mass of phosphorus per mass of ashed duckweed over time

## DISCUSSION

During initial trials, hematite precipitation was observed when using a higher iron concentration solution and under direct artificial sunlight. These trials were discarded because the exact precipitation mass could not be determined throughout sample collection. To avoid this problem in the final trial, concentrations were lowered significantly, the solutions were stored away from direct artificial sunlight, and constant solution volumes were maintained. The results showed some variation in concentrations of the samples collected towards the beginning of the final trial. This variation could be due to remaining iron present on the vials or caps that

was not removed effectively by the concentrated nitric acid. Even with the initial discrepancy in concentrations, it was observed that the iron solution in the presence of duckweed deviates noticeably away from the control solution and then becomes parallel to the control.

After a week of sampling, the duckweed in the presence of the iron solution was noticeably healthier and more vigorous than the duckweed in the presence of the phosphorus solution. However, observations for another week showed that the duckweed began to die more rapidly after harvesting was completed. While the duckweed initially absorbed iron quickly, the duckweed would require more frequent harvesting to successfully remove the iron before it would reenter the environment by dying.

The phosphorus concentrations were decreased over the course of three trials from 5000 ppm to 500 ppm due to observed negative effects on the duckweed's health. This decrease was most likely not substantial enough as the duckweed continued to lose vigor at the end of the final trial. Due to the high concentrations of phosphorus used and the propagation of error from dilutions of the collected water samples, the harvested duckweed samples were used for further support. The dry ash method provided the results in Table 3 and all samples with a mass of 0.002 g or less were discarded in the preparation of Figure 5 due to the uncertainty of weighing to be  $\pm 0.001$  g. Since the ash samples had such a low mass, the weighing uncertainty provided a high relative standard deviation for each measurement. The weighing error is shown by the sporadic data points present in Figure 5. Thus, the concentration of phosphorus present in duckweed remained constant or decreased over time. A more precise scale or drying the harvested duckweed before ashing could reduce some of the uncertainty present.

Although previous studies have shown the absorbance of phosphorus by duckweed, this was not observed for our experiment, possibly due to the concentrations used. Based on our results, it was not conclusive whether vigorous duckweed would absorb phosphorus effectively from the environment. However, many studies in the upper Mississippi and in waste water treatment plants use duckweed harvesting as a method of reducing phosphorus concentrations in the effluent water.

## **CONCLUSION**

Overall, the duckweed was found to rapidly absorb iron out of the water. However, the duckweed did not absorb the phosphorus. If duckweed was to be used as part of a water treatment plan, then it would require harvesting as it was observed that the duckweed lost vigor over the course of this experiment. By harvesting the duckweed, it would prevent either the iron or phosphorus from reentering the environment.

## **ACKNOWLEDGEMENTS**

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