Does a Nutrient Gradient in Mud Lake Bog Affect the Leaf Color Polymorphism of Pitcher Plants (*Sarracenia purpurea*)?

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Abstract

Bogs are very important, yet very threatened, ecosystems that provide many ecosystem services such as being carbon sinks, filtering pollutants, and acting as flood barriers. Because bogs have low levels of nutrients some plants in bogs have evolved carnivorous diets in order to supplement growth; the purple pitcher plant (*Sarracenia purpurea*) is an example of a carnivorous plant and is the organism we studied. We conducted our study at Mud Lake Bog in Cheboygan County, Michigan. We wanted to find out if there was a nutrient gradient extending from the lake into the bog, with higher concentrations of nutrients at the edges of the bog and lower nutrients in the middle, and whether the nutrient gradient we expected to find was correlated to pitcher plant redness; we expected to see more red pitchers in areas with lower nutrients because the redder the pitcher is the more insects it catches. We marked a 70 m by 40 m transect and used a 1 m^2 quadrat every 10 m in the transect. We assessed pitcher plant redness and took water samples in each quadrat to assess nutrients. Our results showed that there was a significant nitrate gradient, but no gradient with respect to the other nutrients we measured. There was no correlation between percent redness of pitcher plants and the nitrate gradient. The nitrate gradient is most likely related to trees which are present at the high concentrations of nitrate. Understanding bogs well is critical to protecting them from further threats.

Introduction

Wetlands are important harbors of biodiversity, providing habitats for many unique species. Understanding wetlands is important because of the high biodiversity, which can lead to novel medical compounds, as well as many other ecosystem services such as filtering of pollutants, serving as flood barriers, and as habitats for many unique species. Currently many wetlands are being degraded and destroyed by human activities, though there is considerable scientific inquiry into whether or not regulatory protections for wetlands is necessary and how important those protections are, such as that done by Gibbs (2000).

One of the categories of wetlands is a peatland. Peatlands are areas with a natural accumulation of peat. Peat is a form of detritus made up of plant material that does not decay due to high acidity and anaerobic conditions which makes it difficult for many decomposers to survive. Peatlands can be minerotrophic or ombrotrophic; minerotrophic areas are fed by streams or springs while ombrotrophic areas only receive water from precipitation. Minerotrophic peatlands tend to have higher pH and calcium concentrations than ombrotrophic peatlands, minerotrophic peatlands are also generally fens while ombrotrophic peatlands are generally bogs (Verhoeven 1986). Peatlands are important because peat is a valuable resource and an important carbon sink (Kimmel and Mander 2010). There are several categories of peatland, namely bogs, fens, and conifer swamps, which are differentiated based on vegetation and telluric nutrient status (Schwintzer 1981). Many peatlands are dominated by a certain type of moss called sphagnum, which forms large, floating mats and acidifies the water, creating an inhospitable habitat (Andrus 1986). One of the characteristics types of vegetation in bogs is carnivorous plants, which includes plants such as sundews, pitcher plants, and bladderwort. Low nutrient level is also characteristic of bogs, though the nutrients are cycled to the top of the sphagnum mat instead of sinking down beneath the mat (Damman 1986).

Pitcher plants are a carnivorous plant that grow in bogs, they use insects to supplement their nutrient needs due to the low nutrient levels characteristic of bogs. There are several speculations about how pitcher plants capture their prey; pitcher plants have UV patterns on their leaves, which are possibly used to attract prey (Joel, Juniper, and Dafni 1985). Another possibility is that pitcher plants use nectar to attract their insects (Bennet and Ellison 2009), or they use the red color of their pitchers to lure prey (Effinger 2013). Nitrogen is often a limiting nutrient for pitcher plants and they can shift their phenotype to accommodate their nutrient needs; for example, when there is higher amounts of nitrogen they produce less of the pitchers for capturing insects and more phyllodia, which are a type of leaves they can produce that are more efficient at photosynthesis but unable to capture insects (Ellison and Gotelli 2002). Pitcher plants also exhibit leaf color polymorphism—the ability to change the color of their leaves—which they can use to control how much energy they put into photosynthesis (green is better for photosynthesis) versus how much energy they put into capturing insects (red attracts insects better) (Effinger 2013).

Understanding pitcher plants is important because not only can we determine nitrogen deposition from them (Ellison and Gotelli 2002), but they also form their own detritus-based micro habitats and support numerous other organisms (Gray et al. 2006). We predicted that there would exist a nutrient gradient extending from high concentrations near the lake to lower concentrations further from the lake and then high concentrations again at the end of our transect and hypothesized that the amount of redness of the pitcher plants was correlated with this horizontal nutrient gradient. Since redness increases the pitcher plant's ability to lure insects we predicted that when there were more nutrients available in the water for the pitcher plants the leaves would be greener but in areas with less nutrients the leaves would be more red.

Methods

Mud Lake Bog is a quaking bog in Cheboygan County, Michigan; there is a large floating sphagnum mat with vegetation consisting of mainly of tamarack (*Larix laricina*), black spruce (*Picea mariana*), leatherleaf (*Chamaedaphne calyculata*), pitcher plants (*Sarracenia purpurea*), and sundews (*Drosera intermedia* and *D. rotundifolia*). There are two lines of trees in the bog, between these two tree lines is a large, mostly treeless, expanse of sphagnum. We conducted this study on specimens of pitcher plants in Mud Lake Bog. We marked a transect of 70 m by 50 m and laid a 1 m^2 quadrat every 10 meters, we chose this size of a transect because we wanted it to include both tree lines and the expanse in the middle (Figure 1). We GPS marked each quadrat and took a water sample, which we had analyzed to determine NO_3^-, PO_4^{3-}, and NH^{4+}. We also took pH and dissolved oxygen in each quadrat, which, coupled with the other nutrients, let us determine whether or not there was a nutrient gradient. In order to quantify redness we took pictures of each pitcher plant that fell within our quadrats; we then analyzed these pictures with Adobe Photoshop CS6 (Adobe Systems Incorporated, San Jose, California), taking the amount of red pixels and dividing by the overall amount of pixels which gave us percent redness (Figure 2). We used the program ArcGIS to overlay our nutrient gradients (NO_3^-, PO_4^{3-}, and NH^{4+}) onto an aerial photo of the bog. Our statistical analysis consisted of regressing pitcher plant redness against all the nutrients we collected and with pH level, as well as regressing nutrient levels against distance from the lake. We split the bog into 4 zones, zone 1 was 0-10m, zone 2 was 20m-30m, zone 3 was 40m-50m, and zone 4 was 60m-70m.

Results

We ran a regression of phosphate levels (µg-P/L) versus distance from Mud Lake (m) (Figure 3); the results were not significant (p-value=0.544, R^2=0.0097), but we did find several hot spots of phosphates when we used ArcGIS to overlay phosphate levels onto an aerial photo of Mud Lake Bog (Figure 4). We also found that, after running a regression of ammonium levels (μg-N/L) versus distance from Mud Lake (m) (Figure 5), that there was no ammonium gradient (p-value=0.474, R^2=0.0136); although there was, again, several hotspots that we found after using ArcGIS to overlay ammonium levels on the aerial photo (Figure 6). However, there was a statistically significant nitrate gradient present (μg-N/L vs. m from lake) (p-value<0.0001, R^2=0.8416), with lower levels close to the lake and increasing steadily as distance from the lake increased (Figures 7 and 8). We ran an ANOVA on the zones that we created, which showed a statistically significant difference between zones 3 and 4 (F=22.304, p-value<0.001, df=28) with 1 and 2 showing no statistically significant difference from each other, though they were different from zones 3 and 4 (Figure 9). A regression of percent redness of pitcher plants against concentration of nitrate (μg-N/L) showed no statistical significance (p-value=0.552, R^2=0.015) (Figure 10). pH levels were quite stable, they ranged from 3.21 to 4.27, but dissolved oxygen levels were quite variable, ranging from 1.04 mg/L to 8.51 mg/L. We ran a regression of DO (mg/L) versus distance from the lake (m) and found it to be statistically insignificant (p-value=0.322, R^2=0.026) (Figure 12).

Discussion

Bogs provide numerous ecosystem services that are invaluable; they are important carbon sinks, they filter pollutants out of water, they serve as a source of peat, they help to prevent flooding, and they serve as a habitat for numerous unique organisms. Understanding bogs better will help with attempts to protect them. We wanted to see if there was a nutrient gradient extending from the lake with high nutrients near the lake, decreasing nutrients toward the middle of the bog, and higher levels at the second tree line. We also wanted to see if there was a correlation between the expected nutrient gradient and percent redness of pitcher plants. There was no phosphate or ammonium gradient in the bog, though our prediction for the nutrient gradient with respect to nitrate was confirmed. Our hypothesis, that redness of pitcher plants was correlated with the nutrient gradient, was not supported.

Our phosphate analysis showed that there was no horizontal gradient of phosphates, but we did discover that there were certain phosphate hotspots. These hotspots tended to be centered on juvenile trees. The two tree species in Mud Lake Bog were tamarack and black spruce, a possible explanation for the phosphate hotspots is that the trees the hotspots were centered on were black spruces, which is important because the leaves of black spruces leach phosphates during rainfall (Tyrrell and Boerner 1986). Another possibility is because in bogs root size increases as they go down in depth (Kellogg and Bridgham 2003), which can harbor large microorganism activity. Because some microorganisms recycle phosphates, large microorganism populations could lead to higher phosphate concentrations.

Ammonium regressed against distance from the lake also did not show a horizontal gradient. A possible explanation for this is lack of flooding and the presence of black spruce. When there is a lack of flooding black spruce preferentially uses ammonium over nitrate (Islam and Macdonald 2009), which could mean that the ammonium is being constantly taken up by the black spruce which would keep ammonium levels low in areas with high amounts of black spruce.

Our data showed a statistically significant nitrate gradient extending from the lake, it was low in concentration near the lake and increased at the second tree line. There are several possible explanations for the nitrate gradient. In the first tree line nitrate levels were low, whereas in the second tree line they were high. Mineralization, the process of microbes turning chemical compounds in organic matter into forms that are accessible to plants, is high in tamarack monocultures; however, when tamarack is paired with black spruce mineralization decreases substantially (Dijkstra et al. 2009). If the first tree line is a combination of black spruce and tamarack then it would be expected to have a low nitrogen mineralization rate, which corresponds to what we saw with our nutrient gradient. In addition, if the second tree line was mostly or exclusively tamarack we would expect it to have a much higher nitrogen mineralization rate, which also would correspond with the gradient we saw. Another possibility relates to the fact that the roots of trees in bogs get larger increase in size as they go deeper into the mat (Kellogg and Bridgham 2003), the higher surface area of the roots can support large microbe populations. Bogs host many different species of ammonifying, denitrifying, and nitrogen fixing bacteria (Williams and Crawford 1983). The combination of large microbial populations in association with tree roots and many microbes associated with nitrogen cycling being present in bogs could explain why, when the bog becomes tree-dominated, the nitrate concentration increased so much.

In order to fully understand the nitrate gradient and phosphate hotspots that we observed there are several different types of research that could be done. An analysis of microbial communities would be necessary to discover if the nitrate gradient is caused by microbes. In addition future studies should look at tree species in the tree lines to discover if those had an effect on the nitrate gradient. Measuring leaching of phosphates off the leaves of the trees could determine whether that caused the phosphate hotspots that we observed.

Our data showed an insignificant relationship between percent redness of pitcher plants and nutrient levels. These data could indicate that redness of pitcher plants is not actually the way that pitchers attract insects. Likely ultraviolet patterns, as Joel, Juniper, and Dafni (1985) proposed, or nectar production, as Bennett and Ellison (2009) proposed, could be more important factors in insect catching than redness. Another possibility is that the pitcher plants are unable to uptake the excess nitrogen because of competition with other plants (Chapin and Pastor 1994), and so they still need to be red even in areas with high nitrogen. Yet another possibility is that there were microhabitats smaller than our quadrats showed that were associated with redness of pitcher plants, or that variables we did not measure, such as litter type, species of sphagnum, litter type, or degree of humification of peat affected redness (Belyea 1996). Future studies should take more frequent quadrats and analyze those other variables in order to determine what is correlated with redness.

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Tables and Figures

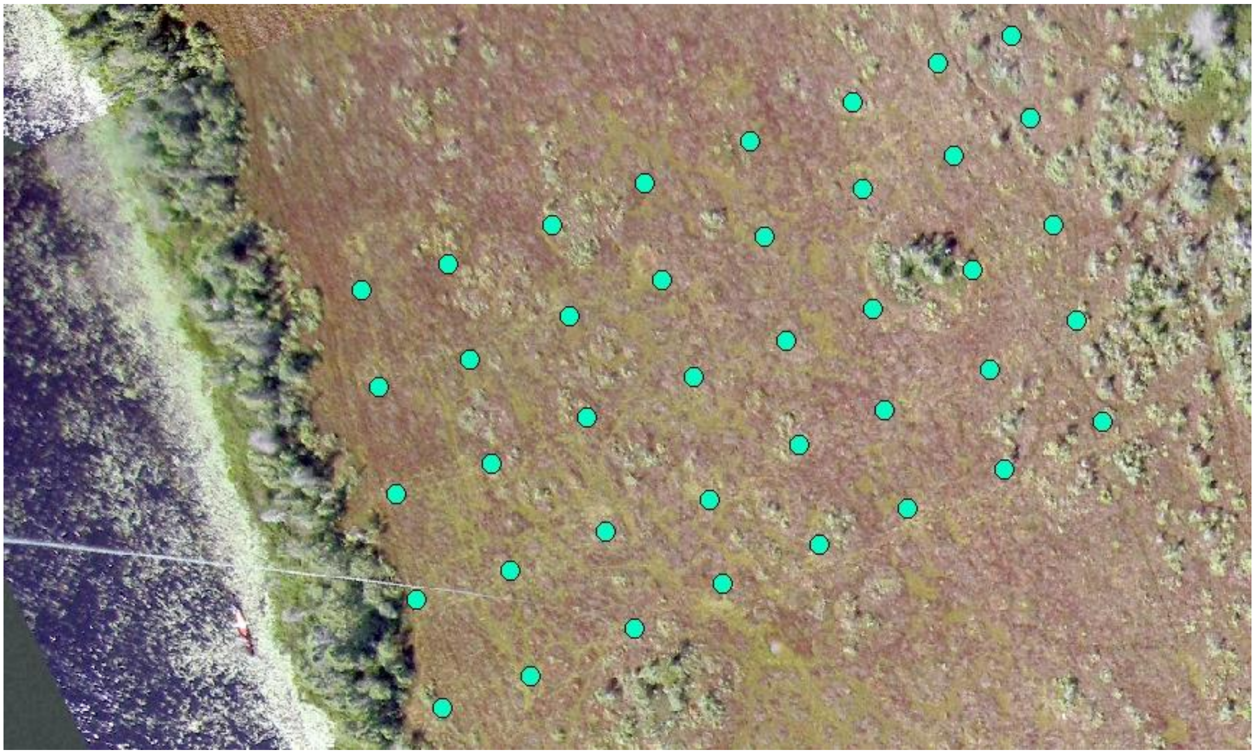

Figure 1: Aerial photograph of our 70m by 40m transect, green dots are locations of our quadrats

Figure 2: Pixel analysis of pitcher plants

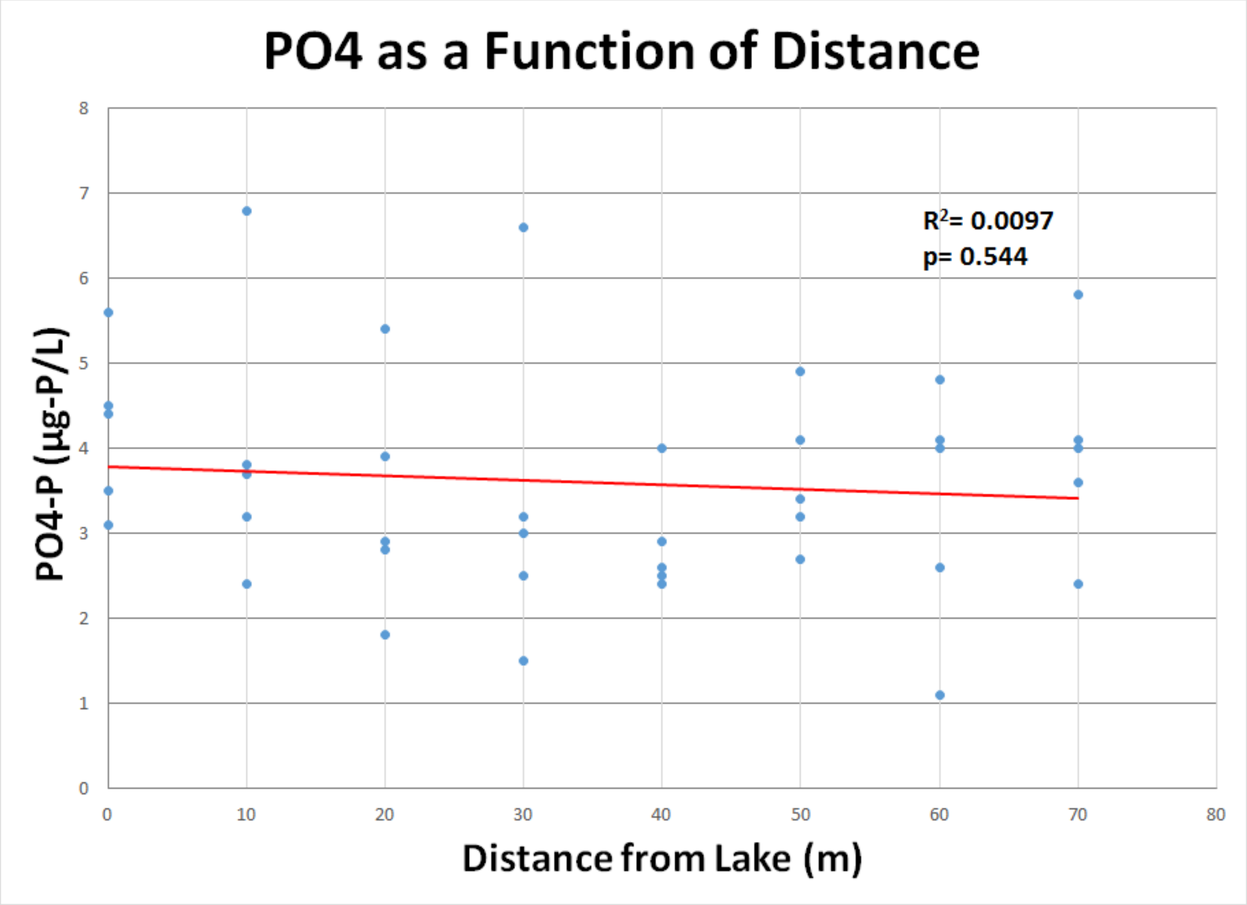

Figure 3: Regression of phosphate level in micrograms of phosphorus per liter (y-axis) against the distance from the lake in meters (x-axis).

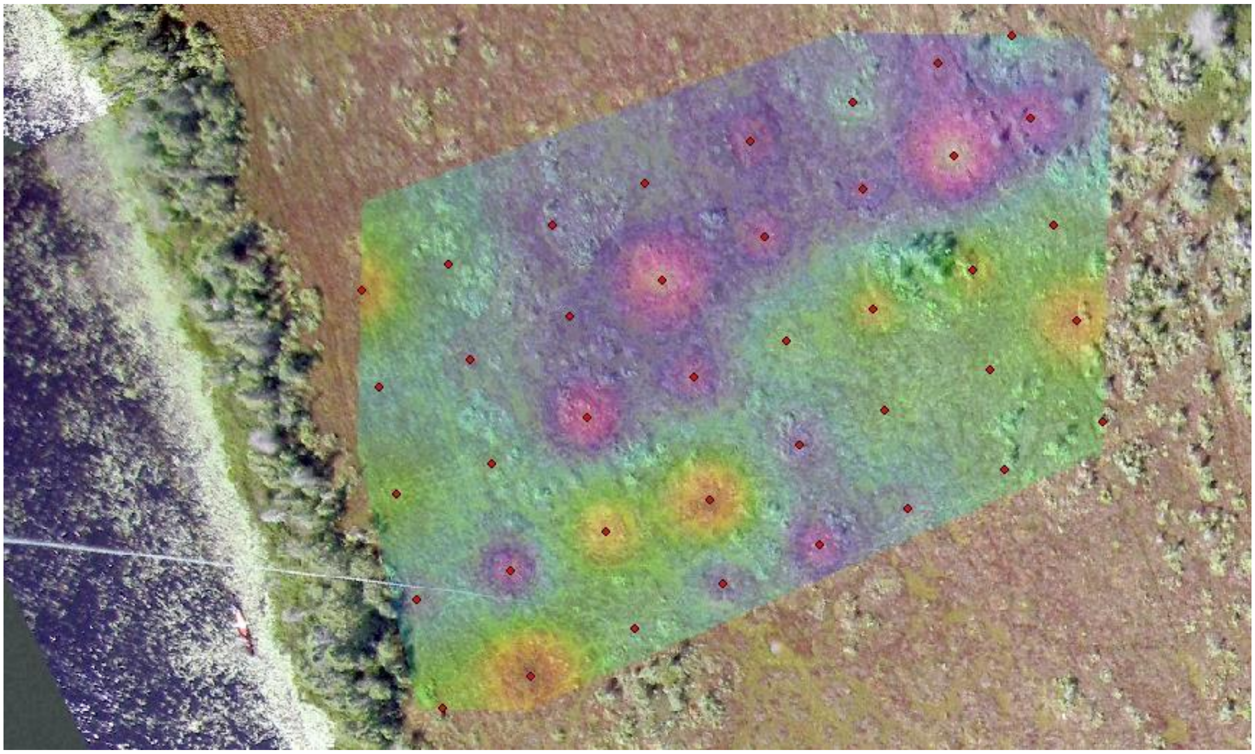

Figure 4: ArcGIS heat map of phosphate concentration. Purple is low and red is high.

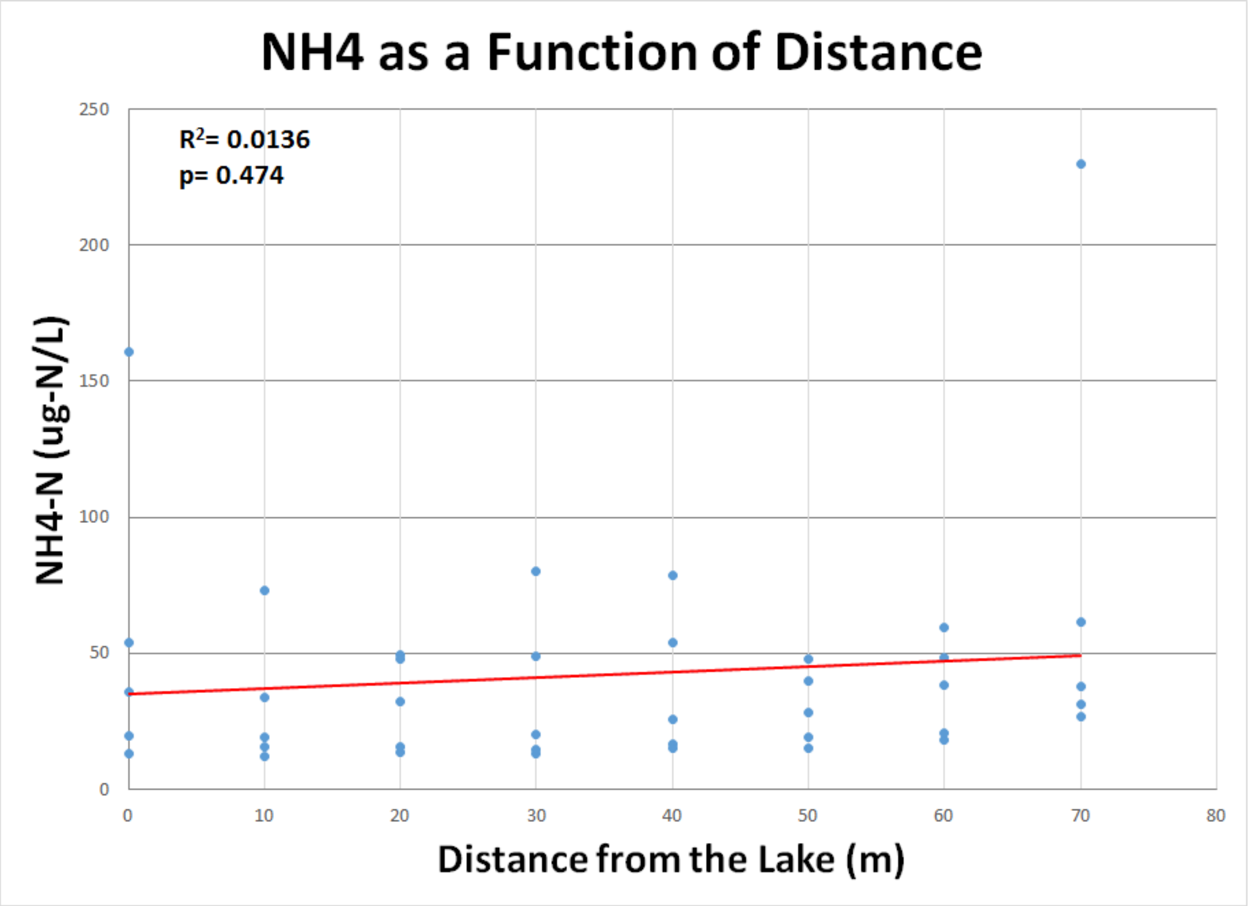

Figure 5: Regression of ammonium concentration in micrograms of nitrogen per liter (y-axis) against distance from the lake in meters (x-axis).

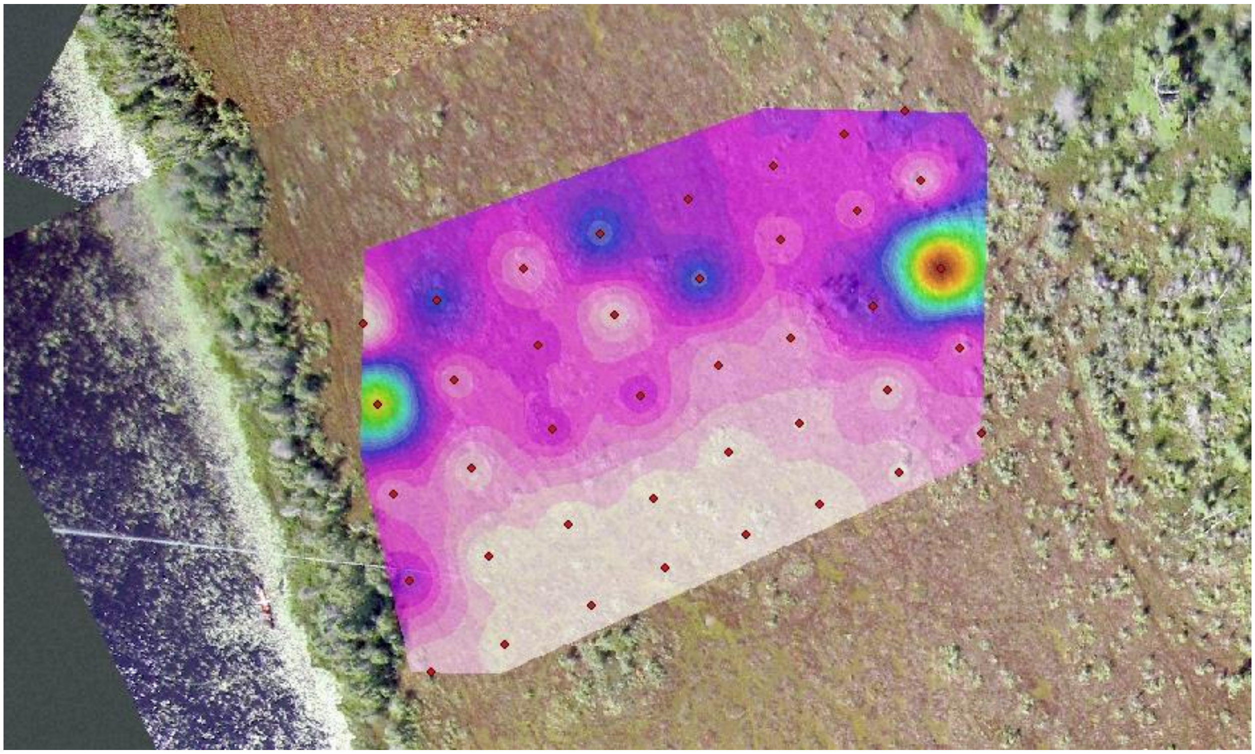

Figure 6: ArcGIS heat map of ammonium concentration. Purple is low and red is high.

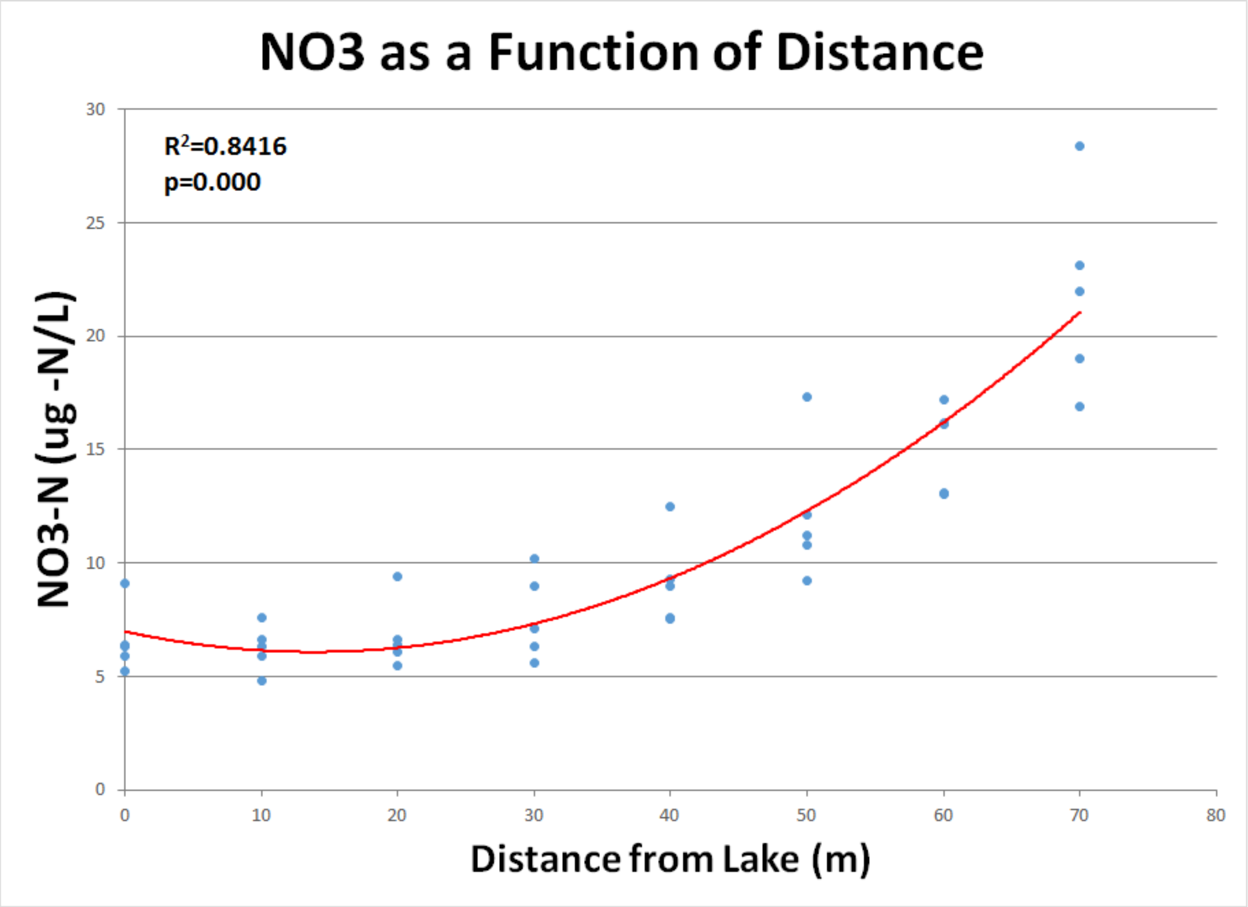

Figure 7: Regression of nitrate concentration in micrograms of nitrogen per liter (y-axis) against distance from the lake in meters (x-axis).

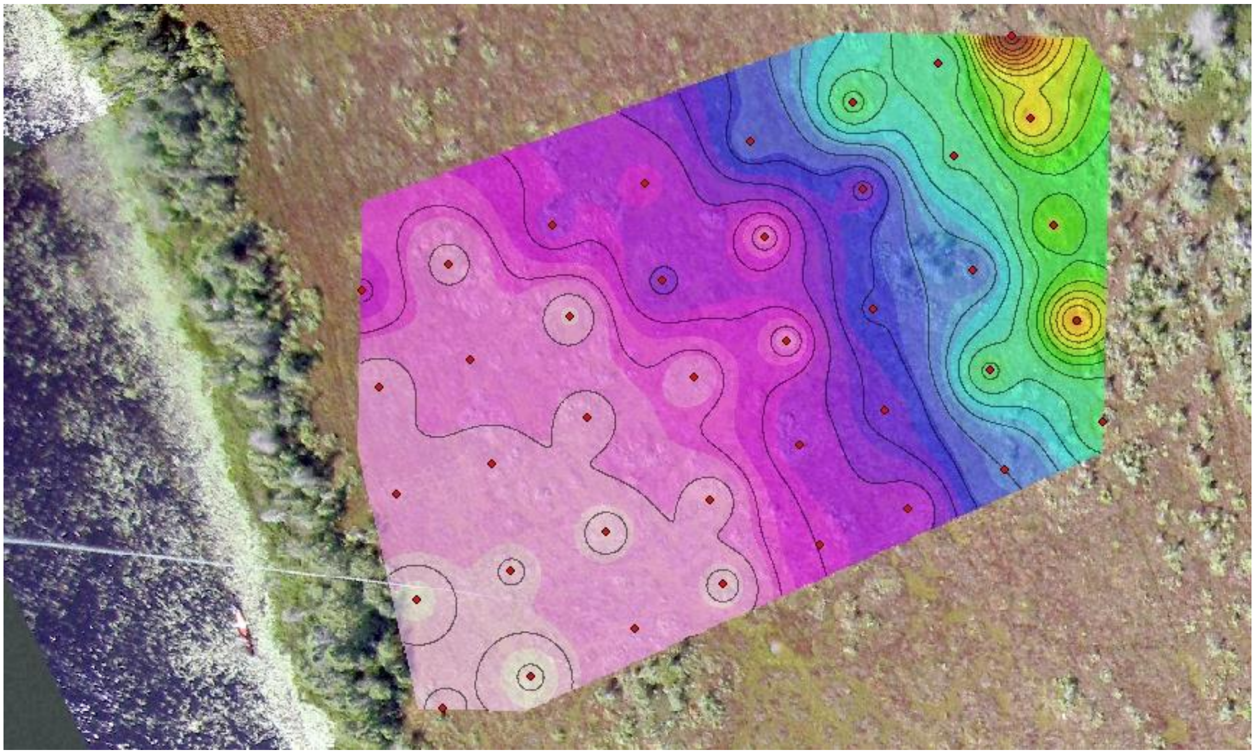

Figure 8: ArcGIS heat map of nitrate concentration. White is low and red is high.

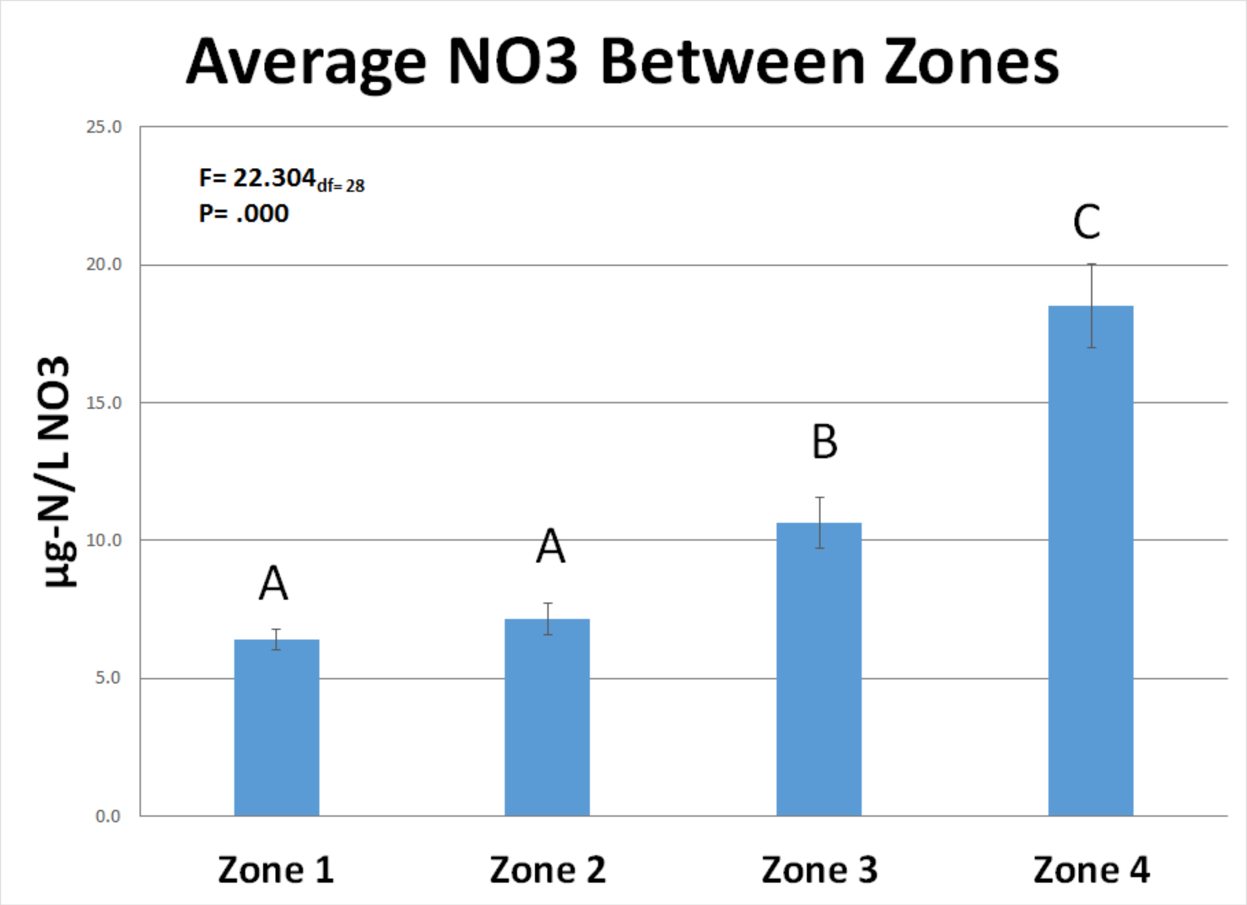

Figure 9: ANOVA test of average nitrate levels in micrograms of nitrogen per liter for each of the 4 zones.

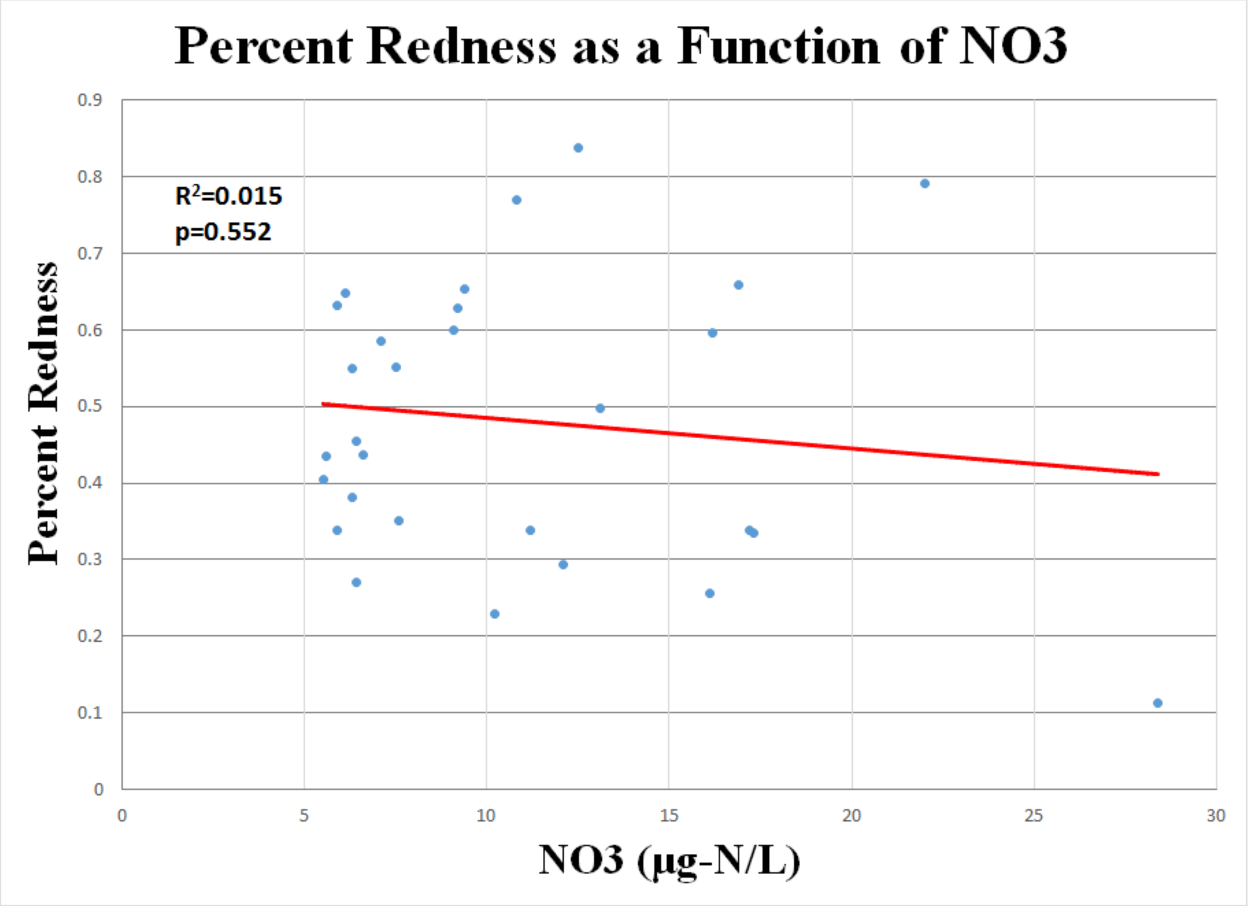

Figure 10: Regression of percent redness (y-axis) against nitrate concentration in micrograms of nitrogen per liter (x-axis).

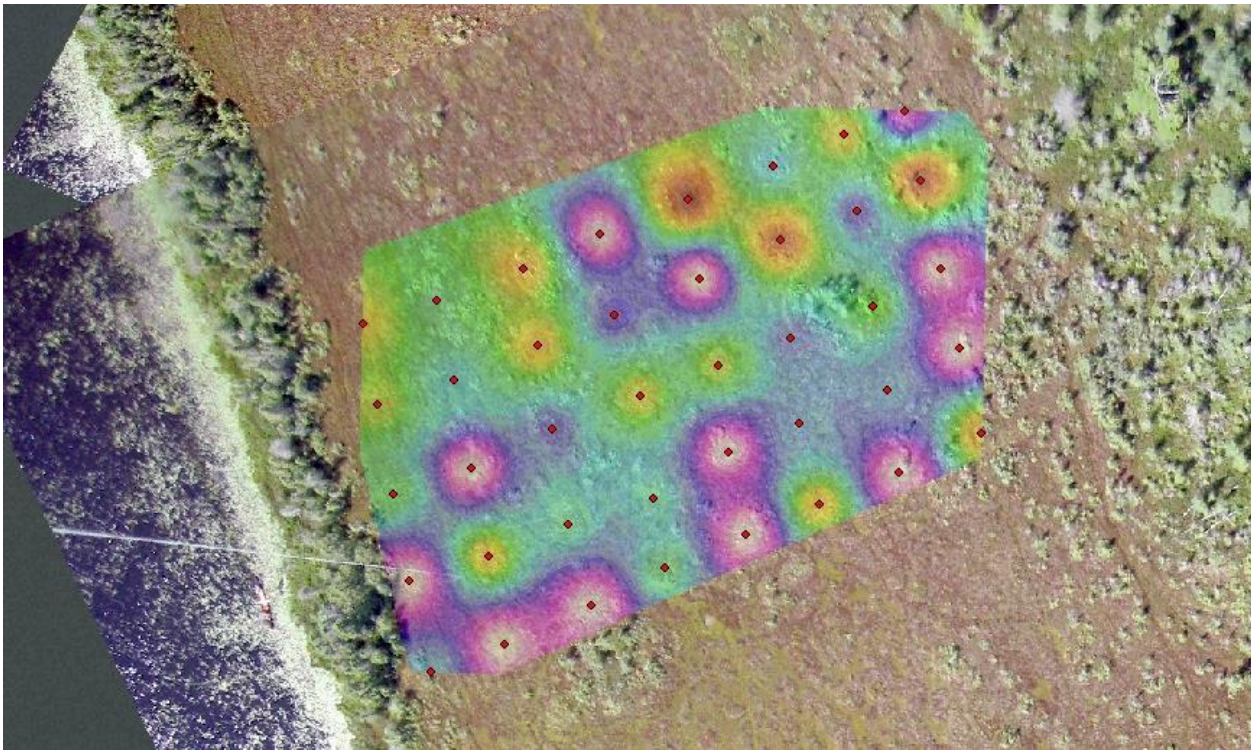

Figure 11: ArcGIS heat map of percent redness of pitcher plants. White are plots with no pitcher plants, red is high percent redness.

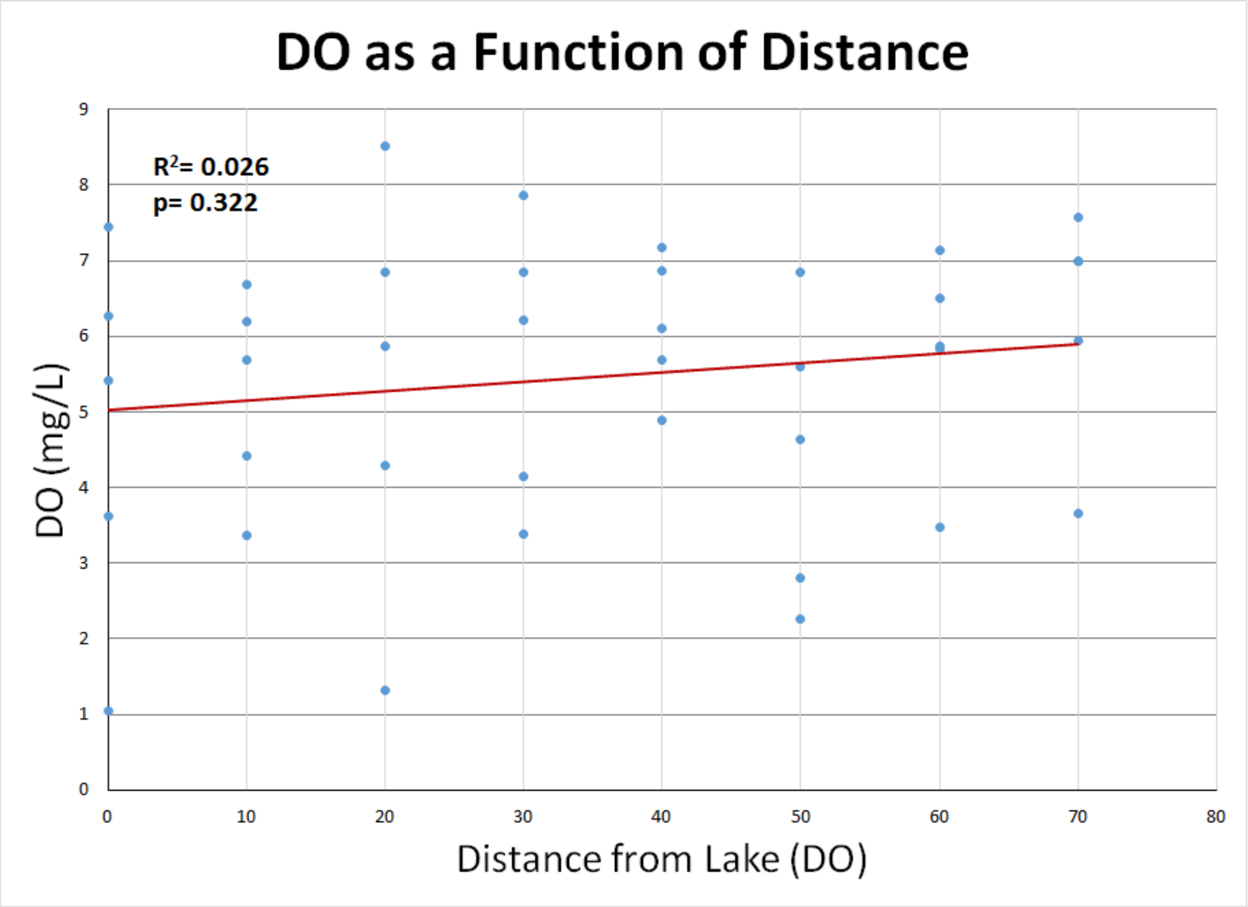

Figure 12: Regression of dissolved oxygen in mg per liter (y-axis) against distance from the lake in meters (x-axis).