Cantaurus, Vol. 25, 29-33, May 2017 © McPherson College Department of Natural Science

The Effect of Petroleum Fuels and Biofuels on Aquatic Plant Life

Tyler Lambert

ABSTRACT

The demand for an alternative fuel source is increasing. Biodiesel, the leading alternative, is a fuel derived from a variety of fats and oils. In regards to air pollution, petroleum diesel is more toxic. Biodiesel produces far less greenhouse gas emissions, and has the potential to seriously improve air quality around the world. Although the use of biodiesel reduces carbon emissions, the contamination effect it has on other environments still needs consideration. The effect petroleum diesel and biodiesel have on aquatic organisms has been studied, however minimal research has been done on the effect they have on aquatic plant life. In this study, using duckweed as a test organism, the difference between toxicities was investigated. Significant differences between duckweed populations exposed to diesel and B20 biodiesel were exhibited in two different experiments. The initial experiment yielded 100% death rates for both fuels, but was likely influenced by error in experimental design. Updated design and daily recounts in the second round of treatment revealed that biodiesel influenced far more death than the petroleum diesel, every day. This could be due to the differences in chemical composition. Fatty acid methyl esters present in biodiesel influence hygroscopy and miscibility with water by way of emulsion.

Keywords: *Biodiesel, Diesel, Petroleum, Duckweed, Contamination, Toxicity, Aquatic, Ecosystem*

INTRODUCTION

Most people by now are aware of the harm that burning petroleum fuels is doing to our environment and our fossil fuel reserves. Petroleum fuel causes pollution at every stage of its life cycle; this includes drilling, refining, transporting and the actual burning of the fuel. If we as a society are to live sustainably, we must find a safer, alternative fuel source. There hasn't been an alternative yet that has completely solved the problem but one product in particular that is becoming more popular is biodiesel, an alternative transportation fuel. Biodiesel is a renewable diesel fuel substitute that can be made from a variety of natural oils and fats (Canakci 2015, Sheehan 1998). Although biodiesel has been proven to produce far less greenhouse gas emissions, point and non-point source pollution can be a product of both fuels.

Point pollution occurs when any single identifiable contaminant is directly discharged. Diesel fuel effects aquatic life via point source pollution when transportation vehicles wreck, and discharge the fuel into the water. Research has been done on the toxicity of diesel fuel in aquatic environments, including the *Journal of Applied Sciences and Environmental Management,* who tested the effects of diesel on small goldfish. Every single fish in the study died due to an extreme reduction in dissolved oxygen (Dede 2015). Studies have only investigated the indirect effects of biodiesel on aquatic life. It has been found that the production of biodiesel can cause a significant amount of eutrophication, which is nonpoint pollution (Carpenter). This study however, is focused on point source pollution, and there is no pertinent literature on the effect of point pollution via biodiesel.

Duckweed has been used in many studies throughout the years and has proven to be a very successful test organism for aquatic life research, largely due to its small size, rapid growth, and vegetation propagation. In fact, since duckweed has proven to be so successful in labs, it has been said that duckweed can and should be used as indicators of water quality (Caudhary, 2014). By using this organism to represent water quality, this study will answer the question: Do petroleum diesel and biodiesel have an effect on duckweed population growth? The objective of this study is to learn the effects each of these two fuels has on aquatic plant life and ultimately to contribute in the search for a safe alternative fuel source. Even if this study leads to a simple suggestion in the battle against pollution it would be a huge success. This will not only be helpful to oil and natural gas companies in pollution conservation efforts but will also be useful to wildlife conservation organizations and environmental response teams. These two fuels would have a direct effect on aquatic life in scenarios where they have been spilled into the water. Current studies suggest that petroleum diesel is in fact the more toxic contaminant. Cornell University tested a diesel fuel spill site that resulted in almost zero aquatic life and very minimal invertebrate diversity (Lytle 2016). But what if it had been biodiesel? In this study we will certainly find out. In comparison of the two fuels, the Journal of Air and Waste management conducted a study on the acute toxicity of petroleum diesel vs. biodiesel and biodiesel blends. Their results indicate also indicate that petroleum diesel is the most toxic (Khan, 2007). Similar studies were conducted by the Royal Society of Chemistry, with the same results. One thing that none of these projects studied, however, is how the two different fuels effect aquatic plant life. We know that diesel is more toxic to fish and other aquatic organisms, but what about plants? Diesel is not miscible with water, and may not reach the roots of the plants below the surface, while the chemical difference in biodiesel could possibly allow for some absorption by the plants. This particular research project is going to investigate the effect both diesel and biodiesel have on photosynthetic, aquatic organisms.

MATERIALS AND METHODS

In order to test the effect of biodiesel and petroleum diesel on duckweed population growth I took an experimental approach with quick and easy sampling methods that were conducted right here in McPherson College laboratories.

B20 biodiesel was purchased from a general gas station in York, Nebraska. B20 is a biodiesel blend containing 20 percent biodiesel, 80 percent diesel.

I collected my duckweed from the USD 418 outdoor laboratory, and the McPherson wetlands which are both available for public use. I made sure the duckweed stayed moist during transportation back to the laboratory by transporting them with a considerable amount of water. Once there, the duckweed was put into the aquarium with 5 gallons of deionized water, and 5 milliliters of aquatic plant food for nutrition.

Experiment A

For this experiment, I set up three different groups of 50 cups according to treatment group. The groups were labeled C1-50 (Control), B1-50 (Biodiesel) and D1-50 (Petroleum Diesel). After the three-day aquarium period had ended, the rest of the experiment was executed. The cups were separated according to treatment group and filled with 100ml deionized water and 30 microliters of aquatic plant food. 25 individual duckweeds were then placed into each cup using a toothpick. Using pipettes, 100 microliters of biodiesel were inserted into each of the 50 cups within its treatment group, as well as 100 microliters of diesel into all of the diesel treatment cups. The cups were placed randomly in the environmental chamber, where they received 24 hours of light at 75 degrees Fahrenheit for seven 7 days. The total number of duckweed remaining in all the cups were then recorded. One-way ANOVA tests were used to analyze the data, so that we can compare the differences in duckweed population growth between treatment groups.

Experiment B

In the first experiment it was easy to recognize that the duckweed was being effected by factors other than the biodiesel. I noticed that the plastic dissolves when it comes into contact with the two fuels when two cups that I had been using to hold the fuels had completely dissolved in the trash can by the next morning. 100 microliters isn’t a large enough concentration to do that to the cups involved in the experiment, but it is highly possible that it dissolved the plastic enough for residue to enter the water and effect the duckweed. The cups were uncovered, and the lack of ventilation in the environmental chamber was not accounted for before treatment. It was very noticeable that the fumes were trapped inside the chamber and the gaseous form of the two fuels not only affected each other but affected the control group as well. These faults explain the inability for the duckweed in the control group to grow as well as the rapid death of the two treatment groups.

The resolution of the data wasn’t enough to make firm conclusions, so I made some changes in the design of the experiment. In addition to the overall growth rate of the duckweed over seven days, the duckweed was counted every day to observe the differences in the three groups day by day.

72 glass finger bowls were used for the second treatment period. In order to get more data on the how the duckweed react to the fuels, they were exposed to 20, 40, 60, 80 and 100 microliters of their respective treatments. The bowls were labeled according to treatment and concentration. The finger bowls are much bigger than the previously used plastic cups, so 150ml of deionized water were used along with 40 microliters of aquatic plant food. The duckweed was inserted the same way, however in the previous round of treatment, fumes were a major factor even to the control group. In order to prevent the fumes evaporated from the fuels from effecting the duckweed, the bowls were wrapped in saran wrap before being randomly placed in the environmental chamber. For this round of treatment, the duckweed populations were counted, recorded every day throughout the 7-day period to see exactly how many days it took for the duckweed to die.

Statistics used to analyze the data from both experiments were ANOVA, post hoc tukey test to find patterns existing in the data, repeated measures and pairwise comparison tests to compare the differences between treatments and concentrations across repeated measurements over time (7 days) all of which were conducted using JASP statistical analysis software.

RESULTS

Results A

The growth rates of the duckweed differed significantly between our treatments ($F_{2,147}$ = 532.2, P< 0.001; Figure 1). After the first round of treatment exposure the duckweed proved to be very irritable to both fuels. Neither of the two treatment groups contained a single live duckweed. Unexpectedly, the control group also had a negative growth rate. Post hoc Tukey results

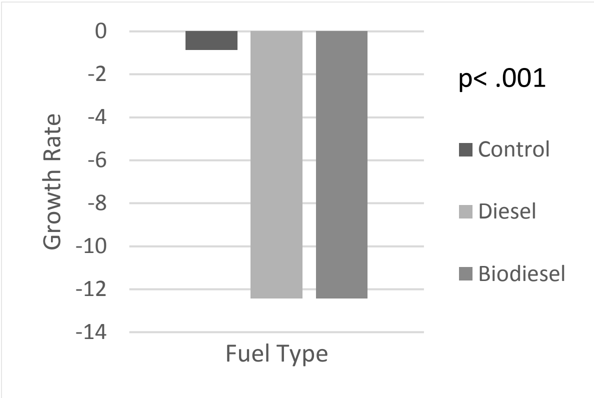

Figure 1. Duckweed growth rates exposed to different fuel types for Experiment A.

indicate that the control group is not the same as the diesel or biodiesel groups, but the two fuel treatment groups are not different (*P* < 0.05).

Results B

The second round of treatment featured much different results. Duckweed population growth was different across all treatment groups (Treatment effect: $F_{2,69}$= 125.2, P =0.001; Figure 2).

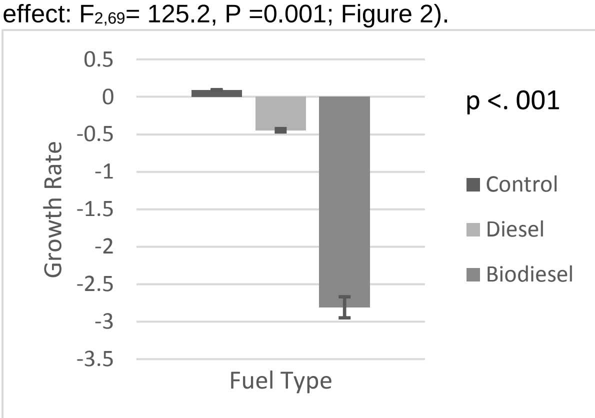

Figure 2. Duckweed growth rates exposed to different fuel types for Experiment B.

The amount of duckweed across all groups changed significantly over time (Time effect: $F_{2,\ 69}$= 125.2, P= 0.001). Not only did they change over time, but the treatment and concentration affected their growth in different ways (Treatment * Concentration * Time Interaction: $F_{6,\ 24}$ = 1.843, P = 0.011; Figure 3A, 3B, 3C).

In this case the control duckweed experienced a fluctuation of survivorship. As you can see in figure 3A, the control saw a large increase in death rates at the beginning of the seven-day period and recovered by the end of the week. Figures 3B and 3C represent duckweed survivorship at each concentration for each fuel. As you can see, the duckweed exposed to biodiesel had far less success. There is a significant

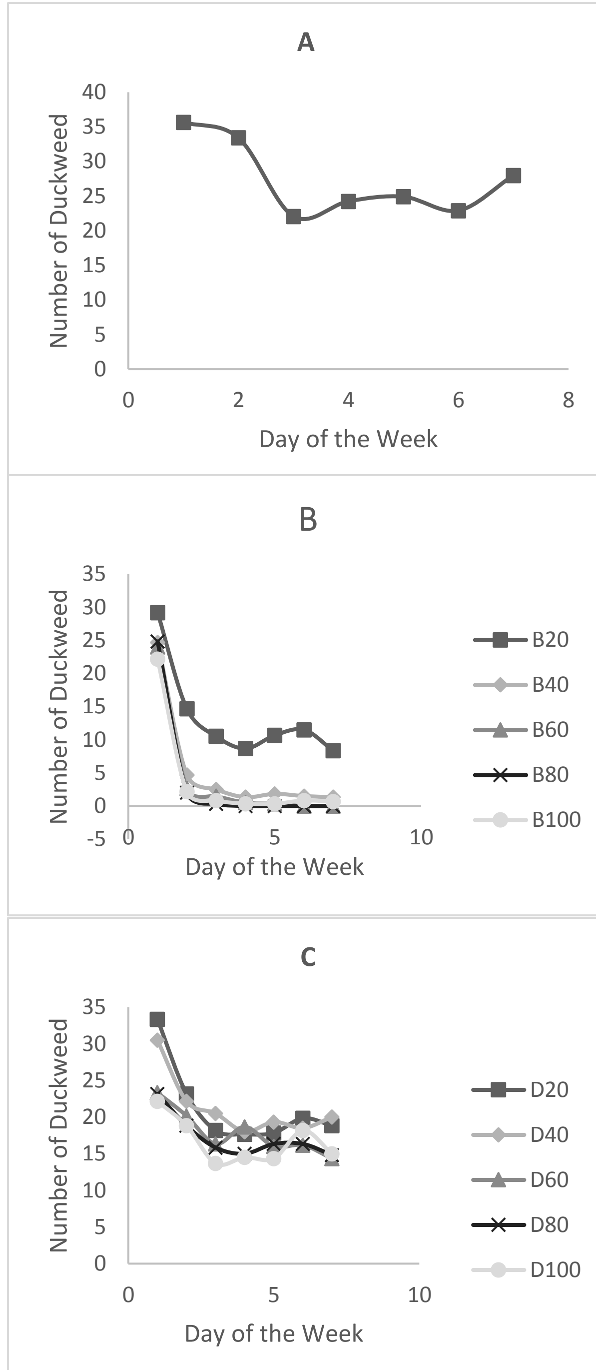

Figure 3. Number of duckweed across the week of Experiment B for the (A) Control Group, (B) Biodiesel Group, and (C) Diesel Group.

difference (P= 0.001) between all treatment groups. These survivor ship curves correlate with the post hoc Tukey analysis that days 1, 2 and 3 are all significantly different than days 4- 7(P< 0.05) After day 3 the number of living duckweed remaining does not experience much growth or death.

The duckweed exposed to biodiesel were mostly dead by day 3, and the remaining live duckweed weren't healthy enough to reproduce. However, the duckweed exposed to petroleum diesel were starting to reproduce more and more as day 7 approached. The 5 different concentrations of petroleum diesel yielded results as expected. Concentrations of 20 and 40 microliters allowed the duckweed to reproduce for a day before succumbing to exposure. 60, 80 and 100 microliter concentrations yielded results similar to each other throughout the week. The 20 microliter concentration of biodiesel was the only concentration that didn't nearly wipe out the entire population.

DISCUSSION

This study shows that petroleum fuels and alternative fuels are detrimental to aquatic environments. Specifically, we found that biodiesel has a much more negative effect on aquatic plant life than that of the petroleum diesel. There is a significant difference between the effects the two fuels had, which is due to the chemical composition of each fuel. Biodiesel contains properties that allow it to be miscible with water, allowing it to be absorbed more easily by the test organism, duckweed, resulting in a much more negative effect.

The significant difference between the control group and both fuel treatments were expected. But the death experienced by the control group was not. This is likely due to the fumes that the duckweed was exposed to. It is difficult to recognize a difference between the diesel and biodiesel treatment groups because the duckweed in them all died so quickly.
The data is dissatisfying due to the experimental error that occurred.

The updated design of the experiment proved to be much more successful and gives a more accurate representation of the difference between the effect of petroleum diesel and B20 biodiesel blend.

The duckweed in the control group experienced a booming increase in productivity during the first day but then saw a large decrease in population before day 3, and then became moderately more productive throughout the week. This is most likely due to the duckweed overusing the nutrients provided by the aquatic plant food. The sudden fall in survivorship before day 3 is likely due to over population resulting in increased competition and diminishing nutrients in the water. After the population had settled back down, it is visible in figure 3 that the duckweed was able to find a sustainable level of production after 3 days.

The duckweed exposed to the B20 biodiesel blend had little to no survivorship, compared to the duckweed that were exposed to petroleum diesel, which experienced much less death and actually had begun to reproduce by the end of the week. This is due to the chemical differences between diesel and biodiesel. Petroleum diesel is completely immiscible with water. Biodiesel is composed of fatty acid methyl esters (Blair, 2011) and is hygroscopic, meaning that it absorbs water, and is slightly miscible (two liquids will mix with each other at any proportions) with water. It is hygroscopic due to left over mono and diglycerides from incomplete reactions. Mono and diglycerides also act as emulsifiers. Emulsion is the mixture of two liquids that are not normally miscible. Methanol is soluble in biodiesel, but miscible in water. So when biodiesel comes into contact with water, the methyl esters choose the water over the biodiesel (ChmlTech, 2012). This explains why the duckweed, with roots below the surface were able to absorb the biodiesel, and not the petroleum diesel, which only sits on top of the surface of the water.

Another possible explanation for the results is the soap present in biodiesel. Soap is a byproduct of biodiesel production and although it is attempted to remove all soap from the fuel, some still remains (Blair, 2012). The soap from the biodiesel was likely absorbed by the duckweed in my experiment, influencing negative growth rates.

There wasn't much difference in growth between the different concentrations of either fuel except for the 20 and 40 microliter concentrations of petroleum diesel and the 20 microliter concentration of the b20 biodiesel blend. In order to find the precise concentration of fuel that the duckweed can handle it would require much more precise concentrations, such as 41, 42, 43 microliters and so on.

In conclusion, the data recorded proved to be very useful in an effort to compare the effects these two fuels have on aquatic plant life. Fuel spills are very common and detrimental to aquatic life at every level. It is not common for biodiesel spills, due to its lack of popularity. But as the demand for an alternative fuel source increases, so will the use and transportation of biodiesel, as well as accidents that result in a direct discharge into aquatic environments. The data recorded above suggests that if biodiesel or biodiesel blends come into contact with an aquatic ecosystem, is only a short matter of time before the plants in that aquatic ecosystem are completely dead. In this particular example, a response team would have only had 3 days to remove the biodiesel from the water before the total death occurred.

ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. Dustin Wilgers for all of his help with the design and execution of this research, as well as Dr. Jonathan Frye and the rest of the natural science department for their guidance and input throughout the entire process. I would also like to thank Cody Schicktanz for his assistance in the lab.

LITERATURE CITED

Blair, Graydon "Biodiesel Basics 101 - Utah Biodiesel Supply, 2011. Web. <http://www.utahbiodieselsupply.com/biodieselbasics.php>.

Canakci, M., and H. Sanli. "Biodiesel Production from Various Feedstocks and Their Effects on the Fuel Properties." Springer- Verlag, 2008. Web. 14 Oct. 2015.

Carpenter, S. R, N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith 1998. "Nonpoint Pollution of Surface Waters With Phosphorus and Nitrogen." Ecological Applications 8:559–568.

Chaudhary, Ekta, and Praveen Sharma. "Duckweed as a Test Organism for Eco Toxicological Assessment of Wastewater." International Journal of Science and Research.

ChmlTech. "Biodiesel Production - Reaction." Biodiesel Engineering. ChmlTech, 2012. Web. <http://www.chmltech.com/biodieseltech.htm>.

Dede, EB, and HD Kaglo. "Aqua-toxicological Effects of Water Soluble Fractions (WSF) Of Diesel Fuel On O. Niloticus Fingerlings" Journal of Applied Sciences and Environmental Mana Dominguez

Faus, Rosa, et al. "The water footprint of biofuels: A drink or drive issue?." Environmental Science & Technology 43.9 (2009): 3005-3010.gement. 2001. Web. 14 Oct. 2015.

Khan, Nalissa, Mostafa A. Warith, and Grace Luk. "A Comparison Of Acute Toxicity Of Biodiesel, Biodiesel Blends, And Diesel On Aquatic Organisms." Journal Of The Air & Waste Management Association (Air & Waste Management Association) 57.3 (2007): 286-296. Academic Search Premier. Web. 6 Apr. 2016.

Lytle, David A., and Barbara L. Peckarsky. "Spatial And Temporal Impacts Of A Diesel Fuel Spill On Stream Invertebrates." Freshwater Biology 46.5 (2001): 693-704. Academic Search Premier. Web. 6 Apr. 2016.

Sheehan, J. et al. "Life Cycle Inventory of Biodiesel and Petroleum Diesel for Use in an Urban Bus." Final Report. United States: N. p., 1998. Web. doi:10.2172/658310.