Effects of ionic liquids on respiration in freshwater lakes

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BIOS 35502-01
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2008

Abstract:

The effects of green chemicals on aquatic ecosystems have been poorly studied. One example of these poorly studied chemicals is room-temperature ionic liquids (ILs). ILs are designed to replace traditional volatile organic solvents in industrial processes. I studied if 1-butyl-3-methylimidazolium bromide affects the respiration rates in freshwater ecosystems. The concentration of IL studied were Control (no IL), Low (50 mg/L) and High (500 mg/L). The tests were performed at a lake mesocosm that mimics the lake environment. Our results show that these concentrations of IL can significantly alter the consumption rates of oxygen of the bacterial community. This study indicates that ILs can affect the respiration rates of freshwater communities.

Introduction:

New green chemicals are being designed to meet industry and society needs for improving existing processes and higher environmental quality standards. "These chemicals are designed specifically to enhance biodegradability, to reduce toxicity and prevent waste at its source." (Kulacki and Lamberti, 2008). An example of these green chemicals is ionic liquids (ILs). "Room-temperature ionic liquids (ILs) are non-volatile organic solvents being designed to replace volatile organic solvents (VOS), such as benzene and toluene. Ionic liquids typically consist of a bulky cation, often an imidazolium or pyridinium ring; side chains that vary in length, number, and position; and a variable anion" (Kulacki and Lamberti, 2008). The effects of these green chemicals on aquatic ecosystems have been poorly studied, meaning that more research is needed on these chemicals to ensure if they are indeed safe for the environment and humans.

One important process in aquatic ecosystems is the respiration of bacteria and algae. "The reactants and products of respiration, such as oxygen, are key players in the function of the biosphere" (Del Giorgio and Williams, 2005). Respiration can lead to reduced concentrations of oxygen in water. "Streams and rivers that support luxuriant algal growth may experience broad daily ranges in DO as photosynthesis increases oxygen concentration during the day and respiration reduces oxygen concentration at night (Hauer and Hill, 2006). Water needs to have an adequate concentration level of oxygen so that living organisms are not negatively affected. As these ionic liquids are industrial chemicals that are used today, and may someday make their way into aquatic systems, it is important to study if these chemicals can affect respiration in natural aquatic environments.

The objectives of this study are to see if ILs can affect the respiration processes of freshwater lakes. The IL that was studied was 1-butyl-3-methylimidazolium bromide bmimBr; Figure 1). The specific hypothesis being tested is that the IL decreases the respiration processes of freshwater communities. I also predicted that the conductivity is affected by ionic liquids, even if the lake had a high conductivity to begin with.

Materials and Procedure:

Ionic Liquid (IL) Test:

The IL used in this study was 1-butyl-3-methylimidazolium bromide, purchased from Sigma Aldrich (St. Louis, MO). A stock solution (26.82 mg/L) of bmimBr was prepared using deionized water. Laboratory preparation of experimental units consisted of labeling the 500 mL Whirl Pak bags with the concentration and replicate number. Concentrations used were Control (no IL), Low (50 mg/L) and High (500 mg/L). All test concentrations were replicated five times. There were five sample lakes: Brown, Cranberry, Morris, Plum, Roach and a pot: Bog Pot. From each of the lakes, we collected 12 liter of sieved (250 µm) water, oxygen concentration and temperature readings of water with a DO probe (DO 200, YSI Environmental) and Conductivity reading (HI 9033, Hanna instruments). Sample water was taken back to James B. Hank Research facility at UNDERC. Here we prepared 1250mL of lake water treated with the appropriate amount of ILs for our desired concentrations (Table 1): Control (no IL), low (50 mg/L bmimBr), and high (500 mg/L bmimBr). This 1250mL was then distributed evenly among 5 replicate Whirl Pak bags.

Table:1 Concentrations used for IL tests:

Concentration (mg/L)	Volume on flask	Stock bmimBr to put in flask (mL)
50	0.25	0.4661
500	0.25	4.6607
50	1	1.8643
500	1	18.6428

Initial oxygen concentration and temperature in each bag were record with the DO probe, then each bag was carefully sealed without air bubbles present. All of the sealed bags were put into a garbage bag with two ceramic clay tiles. The garbage bag created an environment for only respiration to occur (darkness), while the tiles were included to anchor down the bag. At the wet laboratory at UNDERC, a lake mesocosm was prepared to mimic a natural lake environment. The garbage bag with all the IL bags was deployed in the mesocosm. After 6 hours, oxygen concentration, temperature, and conductivity readings were taken again. All IL-contaminated lake water was disposed in as hazardous waste. The DO probe was cleaned with deionized water after all the readings. These steps were repeated for each of the six sampled water bodies.

Lake Test:

Prior to reaching the lakes, we labeled the Whirl Pak bags with treatment (natural) and replicate number at the laboratory. Each sample was replicated six times. There were six water bodies sampled: Brown lake, Cranberry lake, Morris lake, Plum lake, Crampton lake and Bog Pot. On each of the lakes, an oxygen concentration and temperature reading of water using a DO probe (DO 200, YSI Environmental) and Conductivity reading (HI 9033, Hanna instruments) were taken. The bags were prepared by pouring 250 mL of sieved (250 µm) water from a graduated cylinder into each bag. Oxygen concentration and temperature were recorded with the DO probe and was sealed without air at each bag. These steps were repeated until all the bags (labeled) have the sample. Each of the full bags was putted in a garbage bag with two tiles. The tiles are for making the bag sink and the garbage bag makes the environment so respiration only occur (darkness). The garbage bag was deployed at the water body. After 6 hours an oxygen concentration and temperature with the DO probe were taken at each of the bags. The water of each bag was pour into the water body and the DO probe was clean with deionized water after all the readings. These steps were repeated for each of the six water body studied.

Water analysis:

Samples for chlorophyll a (chl a) analysis were taken for eight different lakes and analyzed on a fluorometer (FM109535, Quantech) using a methanol extraction method (American Public Health Association, 1999). The lakes sampled were: Tenderfoot, Roach, Morris, Brown, Plum, Cranberry, Crampton and Bog Pot. All lakes are on UNDERC property. On the same lakes, water samples were also taken and filtered phosphorus analysis. Replications were only on Morris Lake and Bog Pot. An SRP analysis was performed according to standard procedures (Steinman and Mulholland 2006). A spectrometer (Genesys 2, Spectronic) was used to read the absorbance of each sample, as well as laboratory prepared (known) standards.

Statistical Analysis:

ANOVA analyses were performed to test for significant differences between lakes and IL treatments. All analyses were performed using Systat (Version 12) statistical software (San Jose, CA, USA).

Results:

The presence of IL in some lake samples/treatments did show statistically significant effects on the change of oxygen level (see table 2, 5 & 8). Bog Pot have a p-value of .007 indicating that at low IL concentrations, there was less decrease in oxygen per hour going on the bags than in other treatments (see table 8). Also, there was a significant effect of the high IL treatment on the oxygen consumption/respiration/change in oxygen in Roach Lake (Table 2 & 8). Controls from these two water bodies did have the highest change on oxygen level (see graph 1 & 2). Also the conductivity between each concentration did have a statistically significant difference, meaning that high IL concentration has high conductivity (see table 2, 5 & 8).

Of the natural condition samples (lake site experiment), there were statistically significant differences between change of oxygen levels among lakes (see table 7). Brown, Crampton and Plum are similar, while Cranberry and Morris are similar to each other (see table 7). Also the Weather on the days before sampling also had a significant effect on oxygen changes (raining day before there is more respiration). Also the conductivity of the lake has a statistically significant difference with the weather of the day before (see table 7), it shows that raining the day before make the conductivity higher.

The natural and IL conditions data (union of lake site experiments and IL experiments) shows that the condition had statistically significant difference with the oxygen level on all lakes except Plum (table 9). Plum was the only lake that has an IL and natural condition sample from the same day. Bog Pot on the other hand there were significant differences between the IL treatments and the control/natural but no differences between the control and natural themselves. The others lakes had differences between natural and others conditions, even control (see table 6 & 9).

The phosphorous water analysis and chlorophyll analysis are on tables 3 & 4. The chlorophyll analysis shows us that the chlorophyll concentration were significantly different between lakes (p-value $< .001$).

Discussion:

While most lakes did not show us any statistically significant effect of ILs on respiration, Bog Pot and Roach lake did show differences. I believe that ILs probably kill or otherwise affect the organisms in the water of those water bodies making the respiration happen less. For the other lakes, higher concentrations of ILs would likely be needed to have significant impacts. One possible explanation as to why these lakes did not show impact is because they are better buffered against ILs. The change in respiration rates is different for each lake because the water is not the same sources and might have different microbial communities that may respire faster or better than others. We also saw conductivity increase as the concentration of IL was higher. This is probably due to the Bromide on the IL molecule affecting the water by making ions and conducting better current.

The natural samples tell us that the lakes waters are different from each other, because there is significant difference between changes of oxygen level among lakes. These differences are likely due to different organisms and nutrients present in the different water bodies. The lakes also differed significantly in chlorophyll *a* concentrations. The chlorophyll shows us that there are photosynthetic organisms in the water samples, indicating that both production and respiration are going on the lake. The weather of the day before had a significant effect on the oxygen level changes, indicating that the rainy days cause more respiration going on the water. This could be because the rainwater contributes sediments (due to runoff) and nutrient inputs, making the organisms increase their reproduction rates. The statistical analyses show us that if the day before it was raining then the conductivity was significantly higher. This can be explained by the sediment and nutrients input the water, as more ions get into the water so it's better at conducting current.

When comparing natural (within lake) samples to experimental controls (no ILs), the data shows that the condition had statistically significant effects on the oxygen level at all the lakes except Plum and Bog Pot. Plum was the only lake that has the natural and IL conditions samples taken on the same day, this tells us that the lake mesocosm can mimic very well the lake properties. On Bog Pot also shows us that the natural (within lake) and experimental control (mesocosm test) condition are the similar. For the others lakes sampled, the natural samples were different from the mesocosm controls, indicating that the water was not the same. This suggests that samples need to be collected on the same day so that water does not differ drastically between natural and the IL controls. Some changes that can happen to water are nutrient input because of runoff, higher concentration of oxygen because of rain, increase respiration rates by increase of microbial community

The phosphorous water analysis indicates that there are only small differences between lakes. This could tell us that the internal phosphorous cycles and input rates are similar at all the lakes. These internal phosphorous cycles are from organism that eats algae or sediments from the water and then it excrete phosphorous to the lake (Costello, 2008). The chlorophyll *a* analysis indicates how many organisms can photosynthesis in the water. The lakes had different concentration of chlorophyll *a*, meaning that some lakes may photosynthesize more than others. The lakes that have more chlorophyll may be eutrophic because of high biological production, but may also be depleted of oxygen. On the other hand the lakes that have less chlorophyll could be oligotrophic, having less biological production, but often being well oxygenated (Crowl, 2008).

Conclusion:

Room-temperature ionic liquids are considered to be an improvement to industrial solvents, however these compounds have been shown to be toxic (Kulacki and Lamberti, 2008). This study indicates that ILs can affect the respiration rates of freshwater communities, in this way supporting our hypothesis. Also our alternate hypothesis of ILs affects the conductivity of freshwater lakes is being supported. The conductivity increases even if the lake had a high conductivity in the beginning.

For future studies, we can suggest some changes on the procedure. First, water samples for natural (in lake) and IL tests (at mesocosm) should be taken on the same day to reduce differences in test water. As the paper of Kulacki and Lamberti say there should be also a study about the bioaccumulation vs toxicity of ILs in the freshwater organism. Finally, we have to study not only the effect of ILs on respiration, but also how these chemicals can affect the organisms and the food webs of water bodies.

Acknowledgment:

I will like to thank Megan Schlichte for laboratory and field assistance. Also Konrad Kulacki for teaching and being there for this study. Dave Costello for being there for any question there was. Gary Belovsky, Michael Cramer and Heidi Mahon at UNDERC facilities thank them for providing resources, facilities and help in any way.

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Tables

Table 2: Average of data by lake

Lake	Date	Weather day before	Condition	ΔO2 (mg/L)	Time in Bag (min)	pH	Conductivity (µS/cm)	ΔO2 / h
Roach	6/7/2008	sunny	Control	-2.194	435	.	.	-0.303
Bog Pot	7/1/2008	sunny	Control	-0.726	390	.	.	-0.112
Morris	7/3/2008	Overcast	Control	-0.962	380	7.7	105	-0.152
Cranberry	7/4/2008	sunny	Control	-1.004	390	4.38	18.82	-0.154
Plum	7/13/2008	Overcast	Control	-0.192	360	7.4	65.18	-0.032
Brown	7/14/2008	sunny	Control	-0.754	365	8	156	-0.124
Roach	6/7/2008	sunny	High	-1.814	435	.	.	-0.250
Bog Pot	7/1/2008	sunny	High	-0.612	390	.	.	-0.094
Morris	7/3/2008	Overcast	High	-1.016	380	7.72	342.75	-0.160
Cranberry	7/4/2008	sunny	High	-1.084	390	4.14	311.6	-0.167
Plum	7/13/2008	Overcast	High	-0.158	360	7.46	293.5	-0.026
Brown	7/14/2008	sunny	High	-0.810	365	7.94	405	-0.133
Roach	6/7/2008	sunny	Low	-1.918	435	.	.	-0.265
Bog Pot	7/1/2008	sunny	Low	-0.440	390	.	.	-0.068
Morris	7/3/2008	Overcast	Low	-0.926	380	7.72	128.46	-0.146
Cranberry	7/4/2008	sunny	Low	-1.040	390	3.96	85.12	-0.160
Plum	7/13/2008	Overcast	Low	-0.170	360	7.36	85.45	-0.028
Brown	7/14/2008	sunny	Low	-0.768	365	8	177.3	-0.126
Cranberry	6/6/2008	Overcast	Natural	-0.545	390	.	.	-0.084
Bog Pot	6/30/2008	Overcast	Natural	-1.170	375	5.2	11.9	-0.187
Morris	6/30/2008	Overcast	Natural	-0.220	370	6.7	104	-0.036
Bog Pot	7/2/2008	Overcast	Natural	-0.718	390	5.2	11.7	-0.111
Morris	7/2/2008	Overcast	Natural	-0.777	370	6.7	103	-0.126
Brown	7/12/2008	Overcast	Natural	-0.153	370	8.2	152.1	-0.025
Plum	7/13/2008	Overcast	Natural	-0.127	370	6.9	64	-0.021
Crampton	7/14/2008	sunny	Natural	-0.163	390	5.4	13.5	-0.025

Table 3: Chlorophyll Concentration

Lake	date	mL filtered	chlorophyll (ppb)	Chlorophyll/mL
Bog Pot	6/2/08	25	49.8	1.99
Bog Pot	6/2/08	50	138.04	2.76
Bog Pot	6/2/08	100	244.33	2.44
Bog Pot	6/30/08	25	74.87	2.99
Bog Pot	6/30/08	50	135.03	2.70
Bog Pot	6/30/08	100	235.3	2.35
Bog Pot	7/1/08	25	50.8	2.03
Bog Pot	7/1/08	50	59.83	1.20
Bog Pot	7/1/08	100	158.09	1.58
Brown	7/12/08	100	187.17	1.87
Brown	7/12/08	200	270.4	1.35
Brown	7/12/08	300	353.62	1.18
Crampton	7/14/08	100	34.76	0.35
Crampton	7/14/08	200	69.86	0.35
Crampton	7/14/08	300	103.95	0.35
Cranberry	7/4/08	100	93.92	0.94
Cranberry	7/4/08	200	157.09	0.79
Cranberry	7/4/08	300	248.34	0.83
Morris	6/30/08	100	65.85	0.66
Morris	6/30/08	200	119.99	0.60
Morris	6/30/08	300	154.08	0.51
Morris	7/2/08	100	71.86	0.72
Morris	7/2/08	200	144.06	0.72
Morris	7/2/08	300	191.18	0.64
Morris	7/3/08	100	118.99	1.19
Morris	7/3/08	200	229.29	1.15
Morris	7/3/08	300	308.5	1.03
Plum	7/13/08	100	40.78	0.41
Plum	7/13/08	200	186.17	0.93
Plum	7/13/08	300	259.37	0.86
Roach	7/6/08	100	22.73	0.23
Roach	7/6/08	200	62.84	0.31
Roach	7/6/08	300	89.91	0.30
Tenderfoot	7/6/08	100	48.8	0.49
Tenderfoot	7/6/08	200	129.02	0.65
Tenderfoot	7/6/08	300	194.19	0.65

Table 4: Phosphorous Concentration

Lake	Date	Concentration Phosphorous (µg/L)
Tenderfoot	6/6/2008	4.9077
Roach	6/6/2008	2.0664
Bog Pot	6/30/2008	3.0996
Morris	6/30/2008	3.22875
Bog Pot	7/1/2008	3.3579
Bog Pot	7/2/2008	3.6162
Morris	7/2/2008	2.19555
Morris	7/3/2008	5.03685
Brown	7/12/2008	6.1992
Plum	7/12/2008	2.71215
Cranberry	7/14/2008	2.3247
Crampton	7/14/2008	4.1328

Table 5: All Ionic liquids days:

Dependent	Independent	p-value
Oxygen per Hour	Conditions	0.786
Oxygen per Hour	Lakes	0.001
Oxygen per Hour	Weather day before	0.001
Conductivity	Conditions	0.001
Conductivity	Lakes	0.05
Conductivity	Weather day before	0.35

Table 6: Average of IL and natural conditions day:

Dependent	Independent	p-value	Notes:
Oxygen per Hour	Conditions	0.309	
Oxygen per Hour	Lakes	0.001	
Oxygen per Hour	Weather day before	0.028	Sunny days had higher oxygen per Hours than Raining days.
Conductivity	Conditions	0.001	High concentration was the different one
Conductivity	Lakes	0.372	
Conductivity	Weather day before	0.444	
Conductivity	Time of bags	0.387	

Table 7: Natural conditions days analysis:

Dependent	Independent	p-value	Notes:
Oxygen per Hour	Lakes	0.001	Brown, Crampton and Plum are similar. Cranberry and Morris are similar
Oxygen per Hour	Weather day before	0.028	Raining the day before there is more respiration
Conductivity	Lakes	0.001	The lakes are different in conductivity
Conductivity	Weather day before	0.007	Trends: Raining the day before the conductivity is higher

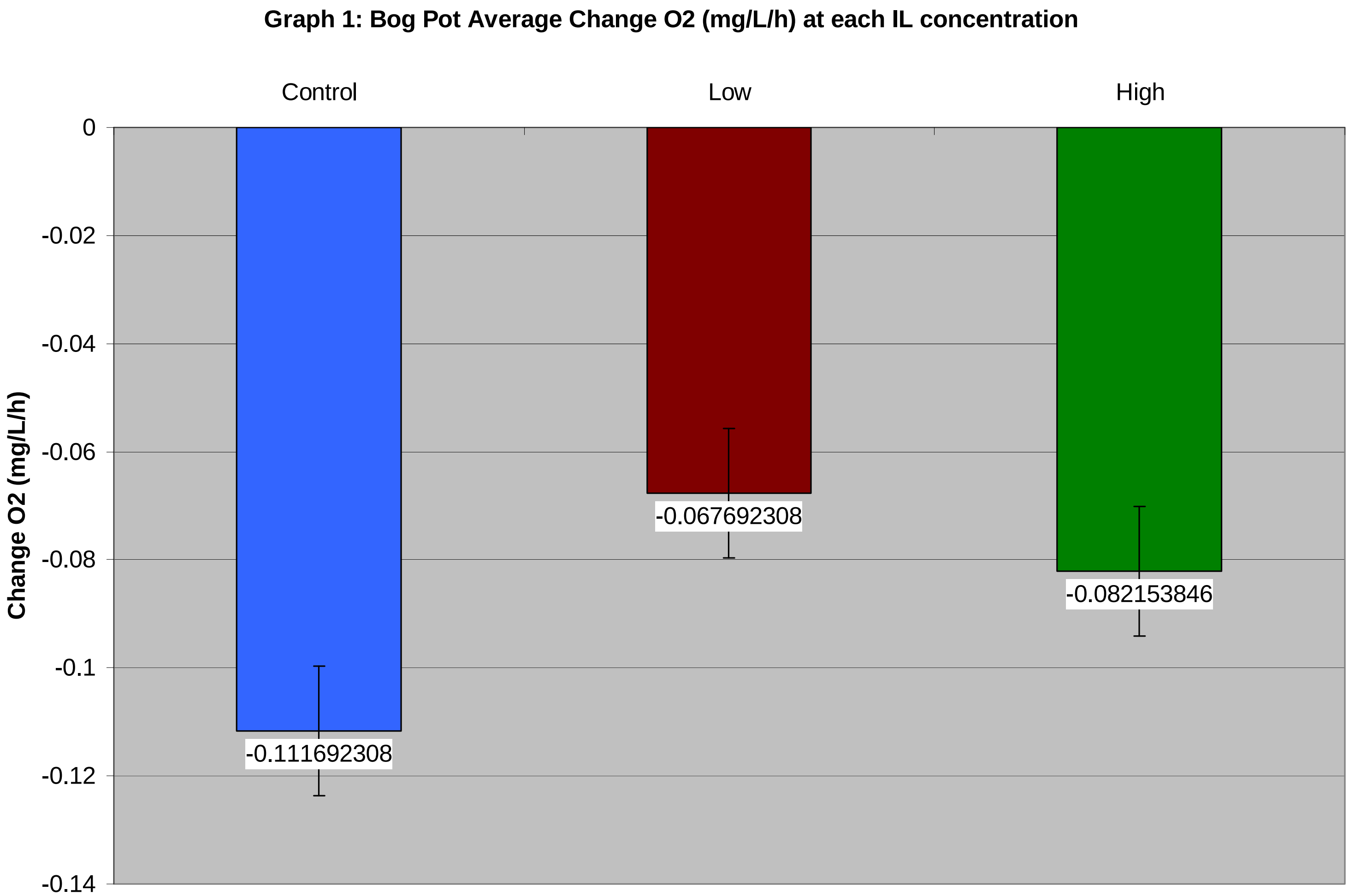
Table 8: IL analysis by lake

Lake	Dependent	Independent	p-value	Notes:
Bog Pot	Oxygen per Hour	Conditions	0.007	Low concentration has less respiration going on. Control has more respiration.
Bog Pot	Conductivity	Conditions	0.001	High concentration was the highest.
Brown	Oxygen per Hour	Conditions	0.317	
Brown	Conductivity	Conditions	0.001	High concentration was the highest.
Cranberry	Oxygen per Hour	Conditions	0.79	
Cranberry	Conductivity	Conditions	0.001	High concentration was the highest.
Morris	Oxygen per Hour	Conditions	0.478	
Morris	Conductivity	Conditions	0.001	High concentration was the highest.
Plum	Oxygen per Hour	Conditions	0.835	
Plum	Conductivity	Conditions	0.001	High concentration was the highest.
Roach	Oxygen per Hour	Conditions	0.001	High concentration has less respiration going on. Control has more respiration.
Roach	Conductivity	Conditions	0.001	High concentration was the highest.

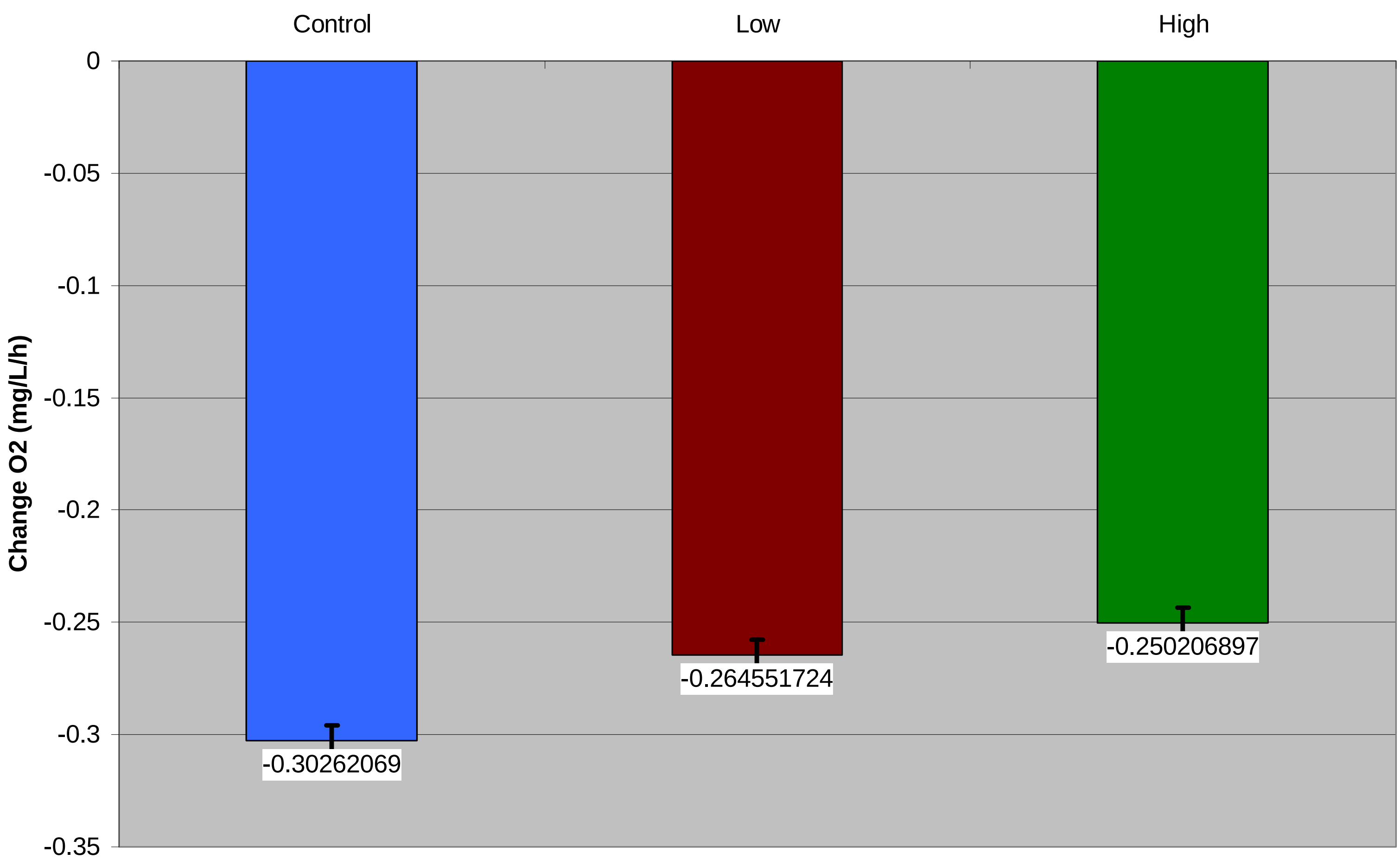
Table 9: IL and Natural conditions analysis by lake:

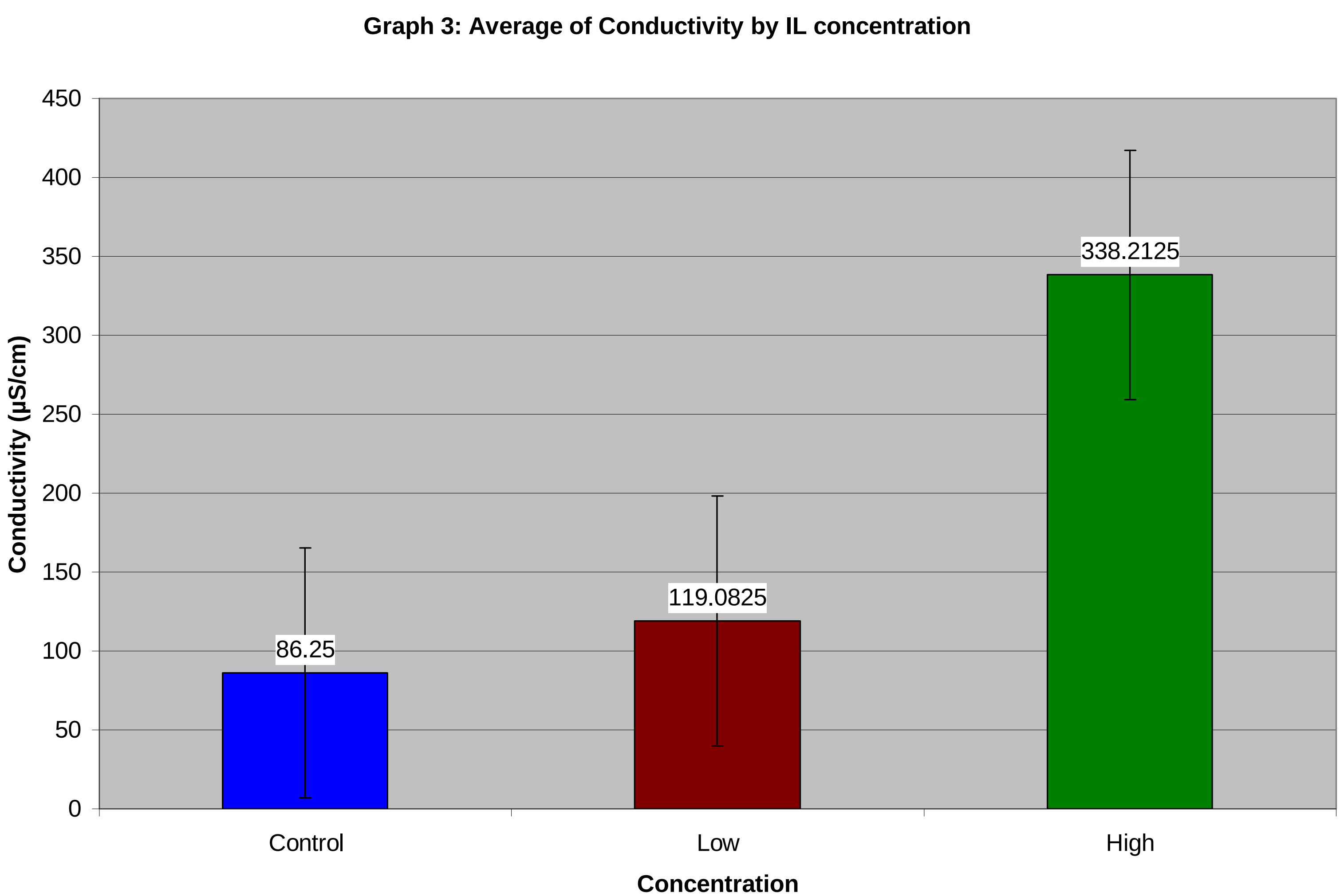
Lake	Dependent	Independent	p-value	Notes:
Bog Pot	Oxygen per Hour	Conditions	0.007	Natural is different than High and Low concentration. Is not significant difference with IL controls.
Brown	Oxygen per Hour	Conditions	0.001	Natural is different than others conditions, even control.
Cranberry	Oxygen per Hour	Conditions	0.001	Natural is different than others conditions, even control.
Morris	Oxygen per Hour	Conditions	0.001	Natural is different than others conditions, even control.
Plum	Oxygen per Hour	Conditions	0.542	Was done the same day

Graphs:


Graph 1: Bog Pot Average Change O2 (mg/L/h) at each IL concentration
Control
Low
High
0
-0.02
-0.04
-0.06
-0.08
-0.1
-0.12
-0.14
Change O2 (mg/L/h)
-0.111692308
-0.067692308
-0.082153846

Graph 2: Roach Lake Average Change O2 (mg/L/h) at each IL concentration





Figures 1: Structure of the ionic liquid used in this study, 1-butyl-3-methylimidazolium bromide.