



# **Bioremediation of Hydrocarbon Contaminants, Measured by the Dissolved** Oxygen (DO) Level, in Khlong Saen Saep Using Azolla sp. versus Salvinia sp.

### 1. <u>BACKGROUND</u>

Bioremediation is an upcoming environmentally-friendly process that utilizes plants, fungi, algae, etc, to treat contaminated settings. Since this study focuses on plants, the scope is limited to the subject of phytoremediation in Bangkok. Phytoremediation is the ability of plants to "reduce the concentrations or toxic effects of contaminants in the environment" (Greipsson, 2011, p. 1).

Azolla and Salvinia were chosen as the plants of focus since both are free-floating aquatic ferns that thrive in the natural environment of Bangkok, working within the limitations of the experiment. Both plants will be associated with their genus level because the individual species hold nearly indistinguishable characteristics.

Azolla was chosen due to its diverse qualities. For example, it has rapid growth, quick desiccation, and a symbiotic relationship with a diazotrophic cyanobacterium, Anabaena azollae, that allows it to fix free nitrogen from the atmosphere.

Salvinia is another plant that has been investigated extensively due to its versatility. For instance, Salvinia is known for its superhydrophobicity, environment-dependent and reversible feature of folding leaves, and quick growth.

Both the plants will conduct bioremediation in the water of Khlong Saen Saep, a heavily polluted canal in Bangkok. This canal was chosen as the focus because the aim is to find a bioremediator that can improve the quality of wastewater that is polluted with petroleum hydrocarbon. Khlong Saen Saep has been polluted due to industrial and residential waste, and thus, it can be safely assumed that it contains petroleum hydrocarbon.

### 4. <u>DATA ANALYSIS</u>

#### Q-Q plot

- Test for DO data's normality
- An approximately straight line was displayed for both the Azolla and the Salvina graph
- Thus, T-tests were used for further analysis



Figure 3a (left) and 3b (right). Q-Q plot for Azolla and Salvinia.

#### T-test for 1<sup>st</sup> Experiment

- Independent T-test
- The same variable was compared for 2 groups
- This tested the difference (final DO level initial DO level) of the DO level that was collected every day for both plants
- The T-test between the difference of DO of Azolla and the difference of DO of Salvinia generated a p-value of 0.0321

With the result of this research along with supplementary improvements, the NCPO and ministry ○ 0.0321 < 0.05</li> could work together to create a plan about conducting in-situ bioremediation on the Khlong to • There is a statistically significant difference between *Azolla* and *Salvinia's* ability in increasing the DO level detoxify it at a low cost and a time-efficient manner.

- **T-test 2<sup>nd</sup> Experiment**
- Paired T-test
- 2 different variables for the same group were being compared
- The T-test was between the difference of DO on day 1 and the difference of DO on the day bioremediation was conducted in the 1<sup>st</sup> experiment (day 12 for Azolla and day 7 for Salvinia)
- <u>Salvinia</u>

• P-value =  $1.725 \times 10^{-6}$ 

• <u>Azolla</u>

• P-value =  $7.249 \times 10^{-6}$ 

- Both the p-values are less than the significance level (0.05), both reject the null hypothesis
- There is a statistically significant difference between the DO level on the 1<sup>st</sup> day and the DO level on the day an aspect of bioremediation occurred, which is the day that the DO turned positive

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## 2. OBJECTIVE

The objective is to assess the difference between 2 aquatic ferns, Azolla and Salvinia, in increasing the DO levels in Khlong Saen Saep. The results for this can give an indication for which plant is more effective in decreasing petroleum hydrocarbon.



Salvinia is more successful as a bioremediator in terms of increasing the DO level, relative to Azolla, in Khlong Saen Saep. Since Salvinia is able to effectively increase the DO level, it can be said that it will be able to decrease the petroleum hydrocarbon more effectively as well.

#### 5. <u>RESULTS</u>

The produced data shows that there is a significant difference between Azolla and Salvinia's ability in increasing the DO level and between the 1<sup>st</sup> day DO level and the DO level the day that one aspect of bioremediation occurred.

The data analysis also suggests that the DO level increases quicker in Salvinia than Azolla.

## 6. IMPLICATIONS

The NCPO hopes to make Khlong Saen Seap clean so it can be "Bangkok's beautiful tourist attractions," as reported by the Bangkok Post (2015).

This study's contributions could potentially go towards conserving the underwater flora & fauna and making the environment clean.

Beginning with this primary knowledge, the heavily polluted canals in Bangkok may start becoming cleaner.

#### 7. ACKNOWLEDGEMENTS

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Azolla and Salvinia were separately rinsed with tap water followed by distilled water to ensure that all the accumulated debris and adhering mud particles have been removed.

49 grams of the respective plants were placed onto the surface of the respective containers that contained 4 liters of water from Khlong Saen Saep.

The containers were left for 4 days without any tests being conducted to allow the system to achieve equilibrium.

Subsequent to this, a sample of 100 mL wastewater from each container, one from the Azolla and the one from the Salvinia container, was inputted into a plastic bottle to be tested for the DO.

Once the data was received, a homogenous mixture of green algae and fertilizer NPK was deposited into the bottles.

the DO level.

		-			22		AZOLI	LA						
DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14
M1 (mg/L)	4.3	4.5	4.4	4.4	4.6	4.7	4.6	4.8	5	5.1	5	5.3	5.3	5.4
M2 (mg/L)	3.2	3.6	3.4	3.7	3.2	3.9	3.5	4	4.3	4.5	4.2	5.9	5.7	6.1
Diff (mg/L)	-1.1	-0.9	-1	-0.7	-1.4	-0.8	-1.1	-0.8	-0.7	-0.6	-0.8	+0.6	+0.4	+0.7
						SA	LVINI	A					19	
DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14
M1 (mg/L)	4.7	4.6	4.8	4.6	4.9	5	5.3	5.2	5.2	5.4	5.4	5.3	5.4	5.5
M2 (mg/L)	3.4	3.2	3.5	3.3	3.3	3.7	6.7	6.8	6.8	6.9	6.7	6.9	6.8	6.8
Diff (mg/L)	-1.3	-1.4	-1.3	-1.3	-1.6	-1.3	+1.4	+1.6	+1.6	+1.7	+1.3	+1.6	+1.4	+1.3

	22	-		-	-	1	ZOLI	LA			<u> </u>			
DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14
M1 (mg/L)	4.3	4.5	4.4	4.4	4.6	4.7	4.6	4.8	5	5.1	5	5.3	5.3	5.4
M2 (mg/L)	3.2	3.6	3.4	3.7	3.2	3.9	3.5	4	4.3	4.5	4.2	5.9	5.7	6.1
Diff (mg/L)	-1.1	-0.9	-1	-0.7	-1.4	-0.8	-1.1	-0.8	-0.7	-0.6	-0.8	+0.6	+0.4	+0.7
						SA	LVINI	A						
DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14
M1 (mg/L)	4.7	4.6	4.8	4.6	4.9	5	5.3	5.2	5.2	5.4	5.4	5.3	5.4	5.5
M2 (mg/L)	3.4	3.2	3.5	3.3	3.3	3.7	6.7	6.8	6.8	6.9	6.7	6.9	6.8	6.8
Diff (mg/L)	-1.3	-1.4	-1.3	-1.3	-1.6	-1.3	+1.4	+1.6	+1.6	+1.7	+1.3	+1.6	+1.4	+1.3

Table 1a (top) and 1b (bottom). The tables depict the DO level of each plant the day the sample was acquired (M1) and the DO level 2 days later of each of the previously acquired samples (M2). The difference between the 1<sup>st</sup> and 2<sup>nd</sup> measurement (diff) is the result.

Samples of the Khlong water from each of the plant's containers were obtained on the day that bioremediation occurred for the first time in the first experiment.

#1 Setup					#	2 Setu	ip di	#3 Setup					#4 Setup		
(mg/L)	Day 1	Day 7	Day 12		Day 1	Day 7	Day 12	Day 1	Day 7	Day 12		Day 1	Day 7	Day 12	
AZOLLA M1	4.4	-	5.5		4.3	-	5.3	4.4	-	5.4		4.5	-	5.4	
AZOLLA M2	3.5	÷	6.1		3.3		6.0	3.2	-	5.9		3.6	-	5.9	
DIFF	-0.9	-	+0.6		-1	-	+0.7	-1.2	-	+0.5		-0.9	-	+0.5	
SALVINIAM1	4.6	5.4	-		4.6	5.3	-	4.5	5.5	-		4.8	5.3	-	
SALVINIA M2	3.3	6.5	-		3.4	6.6	-	3.3	6.6	-		3.5	6.4	-	
DIFF	-1.3	+1.1	-		-1.2	+1.3	-	-1.2	+1.1	-		-1.3	+1.1	-	

Table 2. All four setups are depicted in this table. The DO level of each plant the day the sample was obtained (M1) is provided along with the DO level of the same sample two days later (M2). Like Table 1, the difference between the two measurements is also provided (DIFF) These measurements are provided for only day 1 and 12 for Azolla and day 1 and 7 for Salvinia. "-" means no data

Figure 2a (left) and 2b (right). Difference of DO level in Azolla and Salvinia in five trials, the fifth one being data from the first experiment. The blue column represents the difference of the DO level on the 1<sup>st</sup> day of obtaining the sample. The red columns represent the difference of the DO level on the 12<sup>th</sup> day for Azolla and the 7<sup>th</sup> day for Salvinia.



#### 3. <u>METHODOLOGY</u>

The bottles were then tightened and left in the sun. 2 days later, the two samples were once again tested for

This process was repeated every day for 14 days.



Figure 1. Difference of DO level in Salvinia and Azolla ove a 14 day period. The X-axis represents the days and the Y-axis represents the difference of DO measured in mg/L.

A 2<sup>nd</sup> experiment one was conducted to verify the results from the 1<sup>st</sup> experiment.

4 more of the same setups were created for each plant.



