Using *Pistia stratiotes* to Phytoremediate Oil and Grease from Greywater in Georgetown, Guyana

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ABSTRACT

This study sought to investigate whether turbidity can be used as a surrogate variable for oil and grease pollution and to analyze the growth dynamic of *Pistia stratiotes* L. (water lettuce) to phytoremediate oil and grease from water. A quantitative analysis was done using a Completely Randomized Design (CRD) using seven treatments of greywaters with constant concentrations of nutrients (NPK) and emulsifier, and variable concentrations of waste lubricant oil which included a control over a 20-day period. The growth of the *Pistia* plants was monitored using the Leaf Area. The data were then analyzed using a one-way ANOVA. The results showed a significant correlation between turbidity and oil and grease. The greywater that was treated with *Pistia stratiotes* had as high as 85% reduction in oil and grease over a period of 30 days and as low as 65% in 20 days. Consequently, *Pistia stratiotes* was found to be highly efficient in phytoremediation of oil and grease pollution. The results also suggest that turbidity can be used as a surrogate variable for oil and grease pollution.

Keywords: Phytoremediation, pollution, wastewater, oil and grease, turbidity, surrogate variable

RESUMEN

Este estudio buscó investigar si la turbidez puede usarse como una variable sustituta para la contaminación por petróleo y grasa y analizar la dinámica de crecimiento de *Pistia stratiotes* L. (lechuga de agua) para fitoremediar el aceite y la grasa del agua. Se realizó un análisis cuantitativo mediante un Diseño Completamente Aleatorizado (CRD) utilizando siete tratamientos de aguas grises con concentraciones constantes de nutrientes (NPK) y emulsionante, y concentraciones variables de aceite lubricante residual que incluyó un control durante un período de 20 días. El crecimiento de las plantas de *Pistia* se controló utilizando el área foliar. Luego, los datos se analizaron mediante un ANOVA unidireccional. Los resultados mostraron una correlación significativa entre la turbidez y el aceite y la grasa. Las aguas grises tratadas con *Pistia stratiotes* tuvieron una reducción de hasta un 85 % de aceite y grasa en un período de 30 días y tan solo un 65 % en 20 días. En consecuencia, se descubrió que *Pistia stratiotes* es muy eficaz en la fitoremediación de la contaminación por aceites y grasas. Los resultados también sugieren que la turbidez puede usarse como una variable sustituta para la contaminación por petróleo y grasa.

Palabras clave: Fitoremediación, contaminación, aguas residuales, aceites y grasas, turbidez, variable sustituta.

INTRODUCTION

Different types of oils such as derivatives of petroleum, vegetables, or animal fats all share some common physical properties producing environmental effects that can be devastating in water bodies. Among those effects are oxygen depletion, and negative impacts on the health of fish and aquatic birds. Oil and grease are present in wastewater usually in concentrations between 10 to 100 mg/L. It is considered an organic contaminant with a recommended limit of 10.0 mg/L (Mustapha 2018).

In Georgetown, Guyana, the city is crossed by canals where greywater is discharged from residential and commercial areas. These canals can be considered as constructed wetlands as they are inhabited by many aquatic species, native and exotic, as well as fishes, amphibians, reptiles, invertebrates, and aquatic birds (Mitsch and Gosselink 2015) and also receive water from local river basins and reservoirs. The greywaters may contain oils and detergents creating emulsifier solutions (e.g., waste lubricant oil also comes from mechanic shops).

Guyana ("the land of many waters") has a new oil-based economy producing increasing environmental pollution, especially the disposal of waste lubricant oils in the different processes that involve the use of engines in terrestrial, marine, and freshwater ecosystems. The increase in vehicles and machinery, as well as ocean-going vessels, means that there will be an increase in the volume of waste lubricant oil in canals. Waterlilies (*Nymphaea* spp.), Lotus (*Nelumbo nucifera*), and Amazon Water Lily (*Victoria amazonica*) are found in deep canals with good flow dynamics, while several aquatic weeds including Water Hyacinth (*Eichhornia crassipes*), Water Lettuce (*Pistia stratiotes*), and Duckweed (*Lemna minor*) can reach high densities when there is a clear indication of water pollution (personal observations).

Several studies at the University of Guyana have demonstrated the potential of aquatic species such as *Eichhornia crassipes, Nelumbo nucifera*, and *Pistia stratiotes* in removing nitrogen, phosphorous, and heavy metals such as copper and zinc in controlled environments or from wastewaters (Persaud 2013; Persaud 2011; Harnchandra 2008). Contamination resulting from various petroleum oil derivatives are often ignored by such studies. Lubricant oil changes at gas stations, auto repair shops, and mechanic shops are carelessly disposed of and washed into drains

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(Franklin et al. 2012). The greywaters may contain waste lubricant oils and detergents acting as emulsifier agents or surfactants producing an oil and water mix that can negatively impact water quality. Wetland plants may provide a cost-effective alternative biological clean-up mechanism compared to the other methods (Elredaisy 2010).

A number of studies point to the value of phytoremediation for water treatment in Guyana canals and elsewhere. *Eichornia crassipes* and *Spirodela polyrhiza* (Greater Duckweed) could absorb several pollutants (e.g., lead, chloride, sulphate, phosphate, potassium) from the canals in only 15 days according to Nazir and others (2020). Byrne and others (2015) demonstrated the potential for phytoremediation of nutrients and coliforms from wastewaters in the canals using the aquatic plants *Typha dominguensis* (Cattail) and *Spirodela polyrhiza*. A significant decrease in turbidity was found with time in all the experimental chambers with both species. Aquatic plant phytoremediation has the potential to decrease oil and grease pollution in water bodies. Previous experimental data from Tiwari and others (2017) demonstrated that phytoremediation of waste lubricant oils by *Pistia stratiotes* coupled with harvesting, composting, and biogas production can be part of the solution. Dominguez-Rosado and Pichtel (2004) examined and evaluated the degradation of used motor oil in the soil rhizosphere of selected plant species: Soybean (*Glycine max*)/Green Bean (*Phaseolus vulgaris*), Sunflower (*Helianthus annus*)/Indian Mustard (*Brassica juncea*), mixed grasses/maize (*Zea mays*), and mixed clover (Red Clover, *Trifolium pratense*/Ladino Clover, *Trifolium repens*). A pilot experiment with *Pistia stratiotes* was evaluated in its potential to phytoremediate wastewaters and was effective in reducing turbidity (Schwantes et al. 2019). However, an extensive review by Ekperusi (2018) found that although *P. stratiotes* has been successful in the reduction of organic pollutants and heavy metals in greywaters and shows high potential in the phytoremediation of agrochemicals, pharmaceutic and radioactive wastes, nanomaterial and petroleum hydrocarbons, it needs more evaluation in its potential for remediating waste oils. Consequently, research on the remediation of petroleum hydrocarbons by *P. stratiotes* is a promising area of phytoremediation, especially since ecologically based or nature-based solutions are a hot topic.

Our study aimed to provide new data for the phytoremediation of waste lubricant oils in lab-controlled environments resembling the greywaters in canals of Georgetown, capital city of Guyana. This city is drained by a series of canals originally used for the drainage of the swamps that allowed the use of such lands for agriculture and urbanization. Domestic wastewater generated in households, office buildings, and commercial areas enters the canals supposedly without fecal contamination. Sources include sinks, showers, baths, cloth washers, and dishwashers. However, greywater may still have some microorganisms content including microalgae that can also work on the phytoremediation process (Hernandez-Perez et al. 2018). The objective of our study was to evaluate the use of turbidity as a surrogate variable for the reduction of oil and grease water pollution and analyze the dynamics of *Pistia stratiotes* growth in the phytoremediation of variable concentrations of oils and grease.

Figure 1. Picture of canal with P*istia stratiotes* (Water Lettuce) in Plaisance, East Coast Demerara, Guyana, used for collection of the study plants.

STUDY AREA

Georgetown, Guyana (known as "the land of many waters") is located in northeastern South America (6'81 N - 58'12 W). Since the transformation of the original swamp coastal ecosystems to agricultural and urban lands during the 1800s, this capital city has been crossed by canals that take stormwater from the roads and send it to the sea. Canals in front of the residential areas (Figure 1) are common. When the tide rises, the sluices are closed and when the tide is low, the sluices are opened, allowing the water to drain out. The city experiences tropical macrothermic, humid weather with an average precipitation of 2,290 mm concentrated in two rainy seasons between mid-April to mid-August and from December to January.

METHODS AND MATERIALS

Research Design

A quantitative analysis with an experimental design was carried out in the greenhouse of the Faculty of Agriculture and Forestry (FAF) at the University of Guyana (Turkeyen, Greater Georgetown). The experiment followed a Completely Randomized Design (CRD) with four single blanks (To, ToE, ToN, TONE) and three replicates assigned for each of three treatments with different concentrations of waste lubricant oil. The blanks consisted of zero to common concentrations of emulsifier E and a nutrient solution N. The greenhouse received a similar percentage of the total daily irradiance every day so that all the experimental and control treatments can be controlled with minimum external disturbance and under the same conditions thereby allowing homogeneity (Figures 2 and 3). A portion of used waste lubricant oil was collected directly from one car engine provided by a car shop in Georgetown on the October 18, 2019. Twenty-five plastic containers with a capacity of 20 liters were used for the experiment.

MATERIALS AND EQUIPMENT

Deionized water (DIW) without microorganisms was used in the experiment. A small water pump (marine type), valve, small hose/tube, and nutrients 15:30:15 NPK hydroponic nutrient solution (N), were all bought on October 25, 2019. Sodium lauryl sulfate emulsifier (E) was purchased from Sterling Products on November 28, 2019. Plant rosettes of *Pistia stratiotes* were collected on December 4, 2019 from a canal in a commercial area on Prince William Street, Plaisance, East Coast Demerara (Figure 3). They were rinsed with tap water then with DIW and placed in a big container where they were left in DIW water for seven days so that they could reach stabilization in the new environmental conditions. The presence of dark leaves was used as a criterion to eliminate stressed rosettes.

Experimental Treatments

The number of rosettes and the amount of waste lubricant oil varied in the experiment. The following were the no oil blanks and oil treatments applied: Blanks = control (absolute, To, only NPK ToN, only Emulsifier ToE, only nutrient and emulsifier ToNE); T2 and T3 for 5ml of waste lubricant oil; T4 and T5 for 10ml of waste lubricant oil; T6 and T7 for 20ml of waste lubricant oil.

ToNE: 10L DIW and 15:30:15 (N/P/K) 0.6 mg/l hydroponic nutrient solution + 1mg/l SLS emulsifier.

T1: Four (4) water lettuce rosettes, no waste lubricant oil

Figure 2. The experimental area with the containers in the greenhouse of the Faculty of Agriculture and Forestry, University of Guyana.

Figure 3. Researcher selecting the plant rosettes to be used in the experiments.

plus 15:30:15 0.6 mg/l hydroponic nutrient solution + 1mg/l SLS emulsifier.

T2: Four (4) water lettuce rosettes with 5ml of waste lubricant oil plus 15:30:15 0.6 mg/l hydroponic nutrient solution + 1mg/l SLS emulsifier.

T3: 5ml waste lubricant oil + 15:30:15 (N/P/K) 0.6 mg/l hydroponic nutrient solution + 1mg/l SLS emulsifier but no water lettuce rosettes.

T4: Four (4) water lettuce rosettes with 10ml of waste lubricant oil plus 15:30:15 (N/P/K) 0.6 mg/l hydroponic nutrient solution and 1mg/l SLS emulsifier.

T5: 10ml waste lubricant oil + 15:30:15 0.6 mg/l hydroponic nutrient solution + 1mg/l SLS emulsifier but no water lettuce rosettes.

T6: Four (4) water lettuce rosettes with 20ml of waste lubricant oil + 15:30:15 0.6 mg/l hydroponic nutrient solution and 1mg/l SLS emulsifier.

T7: 20ml waste lubricant oil + 15:30:15 0.6 mg/l hydroponic nutrient solution + 1mg/l SLS emulsifier but no water lettuce rosettes.

Twenty-four (24) water lettuce rosettes of similar size and number of leaves (3) were selected from the container and weighed in the lab to obtain the initial fresh biomass then four (4) water lettuce rosettes were placed in each container replicate of the respective treatments that included *Pistia stratiotes* water lettuce rosettes (T2 5 ml, T4 10 ml, and T6 20 ml). The waterline was marked on the outside of the container and was checked every day. If the water fell below the waterline more DIW was added/poured thereby keeping the water level constant. Also, the containers were aerated every day (except Saturday and Sunday) for 10 minutes each using the water pump. To evaluate leaf area, pictures of the plants growing in the containers with treatments were taken every Monday, Wednesday, and Friday for 4 weeks. Figure 4 show pictures of experimental containers with plants and without plants.

Turbidity
Turbidity was measured every Monday, Wednesday, and Friday. The water collected in the test tube was lightly shaken then poured back and forth into the glass bottle in order to ensure the sample was homogenous. It was then placed in the turbidimeter (2100P ISO Portable Turbidimeter), the range was set to "Auto range" then the turbidity was measured. This was repeated for all twenty-five samples. The bottle was washed and rinsed with distilled water after each sample was read. Turbidity was measured at times 0, 10, and 20 days together with the number of rosettes of *Pistia stratiotes* plants. New rosettes were incorporated at the 20 days to replace the rosettes that had sunk and those that were overshading the others. This was carried out in order to reduce the effects of intraspecific competition among individuals. A similar approach followed by Fonkou and others (2002) in an experimental waste control using populations of *Pistia stratiotes* and is supported by the explanations by Fletcher and others (2020) about community competition in natural experiments with several species.

At the time 0 of the experiment a sample of water was collected in 300ml bottles to determine the concentration

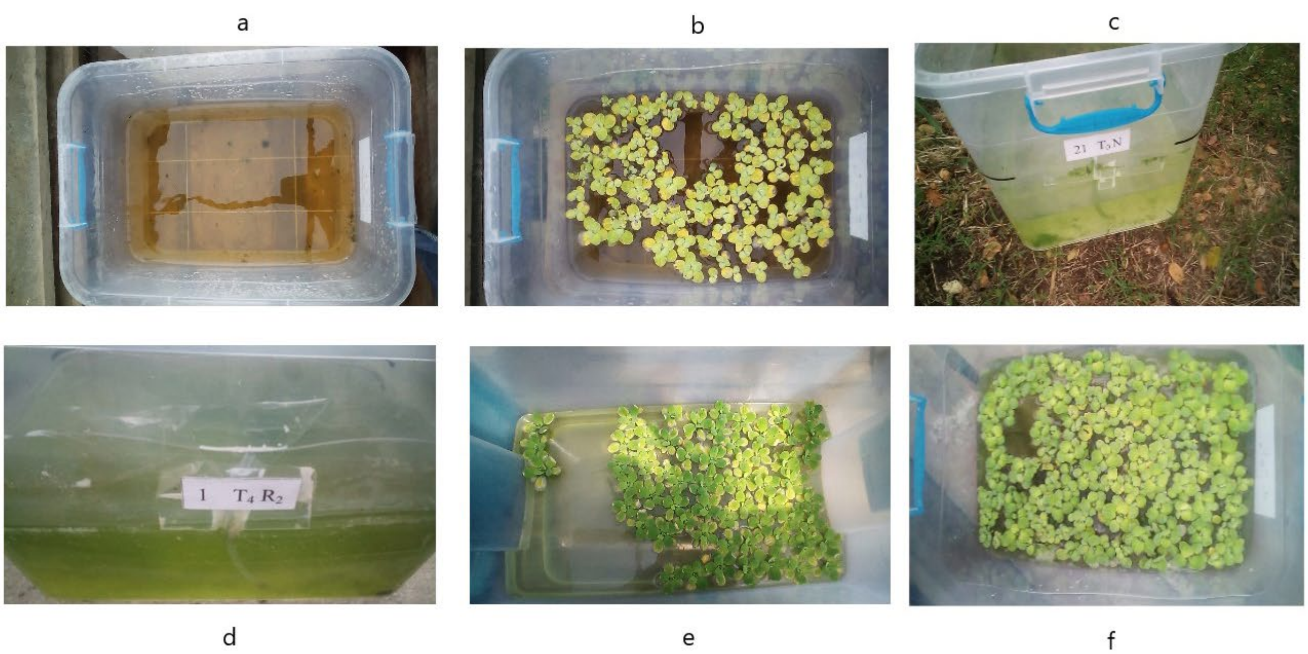

Figure 4. The containers in the experiment (a=10 ml waste lubricant oil and no *Pistia* plants; b=20 ml of waste lubricant oil and *Pistia* plants; c=blank T0N no plants, DWI with Nutrients; d,e, f = evolution 0, 10, 20 days of treatments with *Pistia* plants, 5 ml waste lubricant oil in solution of DWI with Nutrients and emulsifier.

of oil and grease and the correlation with turbidity was calculated. Turbidity was then used as a surrogate of oil and grease concentrations.

Oil and Grease Concentrations
The Institute of Applied Science and Technology (IAST) lab determined oil and grease concentrations using the trichloromethane (chloroform) colorimetric method (Cirne et al. 2016). The first water sample (500ml) was collected and taken to IAST on December 11, 2019 for initial oil and grease content to be determined, with a final determination performed on January 14, 2020.

Determination of Leaf Area
Pictures were taken every Monday, Wednesday, and Friday using a smartphone. The phone was positioned at a similar height and angle each time to record the entire container. The pictures taken were copied to the computer where they were opened in ImageJ app to measure leaf area.

Determination of Wet and Dry Weight
On January 2, 2020, the water lettuce plants were weighed, the number of leaves counted, and length of roots measured and then placed in the respective containers. At the end of the experiment, the plants were scooped up with a strainer and placed in labeled bags then taken to the lab at FAF where they were rinsed with tap water followed by distilled water. They were placed on Bounty tissue to remove excess water. Plants were immediately weighed whole then divided into roots and leaves and weighed separately. The plants were then placed on A4 paper, labeled then pictures were taken. This was repeated for each of the treatments with plants. The plants were left in the lab to dry from January 15, 2020, weighed on the 17th then again on the 20th of January 2020.

Data Analysis
Statistical analysis was performed using the SPSS 20.0 for Windows. Normality tests were made for turbidity and ln transformations were carried out. All the general descriptions of media and standard deviation of the different variables were processed, and also one-way ANOVA ($p= 0.05$) in order to determine a potential difference between the means. Post-hoc analysis using Least Significant Deviation (LSD) tested Ho whether the means are equal, or if the H1 alternative hypothesis is rejected and the means are different ($p=0.05$). A correlation between turbidity and oil and grease concentration was carried out at the beginning of the experiment and a percentage of reduction of turbidity was selected as a surrogate variable to measure the success of the phytoremediation. The percentage of reduction in turbidity was calculated at 10 and 20 days.

Table 1. Comparison of means between final concentrations of waste lubricant oil with and without plants of *Pistia stratiotes* using ChiSquare. It should be noted that the treatment with 20 ml of waste lubricant oil resulted in a significantly lower concentration value at the end of the experiment.

Treatment With Pistia	Treatment Without Pistia	ChiSquare	Sig.
T2 (Pistia, 5 ml waste oil)	T3 (no plant, 5 ml waste oil)	0.04	0.53
T4 (Pistia, 10 ml waste oil)	T5 (no plant, 10 ml waste oil)	1.23	0.27
T6 (Pistia, 20 ml waste oil)	T7(no plant, 20 ml waste oil)	4.29	0.04*

Table 2. Correlations between Ln turbidity and oil and grease concentration at time 0. The correlation is significant and given that, it was possible to use turbidity to evaluate the phytoremediation success of using *Pistia stratiotes* plants.

		Ln Turbidity	Oil & Grease
Ln Turbidity	Pearson Correlation	1	0.95**
	Sig. (2-tailed)		0.00
	N	18	18
Oil & Grease	Pearson Correlation	0.95**	1
	Sig. (2-tailed)	0.00	
	N	18	21

**. Correlation is significant at the 0.01 level (2-tailed).

RESULTS

Turbidity and oil and grease concentration values at the beginning of the experiment (Tables 1 and 2) showed significant differences between the means of the different treatments. Higher turbidity was observed in the treatments with higher oil and grease concentrations. Turbidity decreased in all the oil and grease treatments with *Pistia* plants (Figure 5). On the other hand, there was a slight increase of turbidity in the blanks especially the ones with nutrients and emulsifiers which was reflected in the observation of green color (Figure 6). There was a significant correlation between the Ln of Turbidity and the Oil and Grease concentration values (Table 3). The analysis after 30 days for the treatment with 20 ml of oil and grease resulted in an average reduction of 83% in the concentration of oil and grease compared with the initial value (Figure 7). It had a consistent reduction above 70% of the concentration with the presence of populations of *Pistia stratiotes* and was statistically significant.

Leaf area increased during the first 10 days of the experiment when some of the rosettes of *Pistia stratiotes* were harvested, and a new cohort of plants was added to exploit the continuous effect of phytoremediation (Figure 8). On the other hand, in all treatments weight values of dry leaves and dry roots fluctuated and increased as the process of phytoremediation proceeded. Foliar and root dry weight were higher in the treatments with oil and grease of 10 and 20 ml (Figure 9).

DISCUSSION

This experiment suggests the water renovation value of harvesting *Pistia stratiotes* every 10 days in the management of water canals draining the city of Georgetown. Fonkou and others (2022) in wastewaters indicate a replacement every 15 days, although in our lab experiment the growth efficiency of *Pistia* might be faster and that is why we replaced sunk rosettes at 10 days and not at 15 days as they did. Oil and grease are organic contaminants with a recommended limit of 10.0 mg/L (Mustapha 2018). In this experiment, all concentrations were below the recommended limit. It can be demonstrated that starting with a local population of 4 rosettes per 15 l in a 20 cm depth water body there will be at least a 70% reduction in the concentration of oil and grease and a production of 6kg/150,000 liters of greywater polluted with waste lubricant oils as a potential for use in composting. Green microalgae and other microorganisms such as bacteria may also be responsible for some of the remediation of waste lubricant oil based on the observation of increasing green color and turbidity in the water. The emulsifier acting as surfactant can also release additional nutrients from the oils which may add a source of nutrients to be used by microorganisms (i.e., the growth of microalgae). The increase in turbidity in the blank with only nutrients and emulsifier and a darker green color on the last

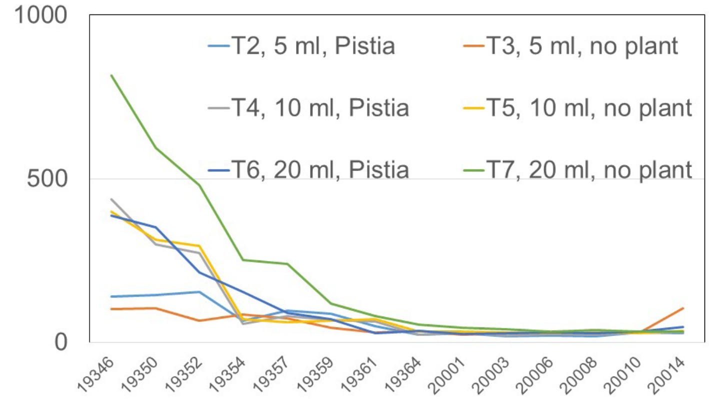

Figure 5. Variation in turbidity with time in each of the treatments with waste lubricant oil (5 ml of oil, 10 ml of oil, 20 ml of oil). All experiments showed a reduction in turbidity indicating that there was an effective process of bioremediation. The experiments with the plants *Pistia stratiotes* were more efficient in reducing the turbidity and hence the oil and grease concentrations especially T6,the treatment with 20 ml of oil, while the lower percentage reduction was noticed in the 20 ml and 5 ml experiments.

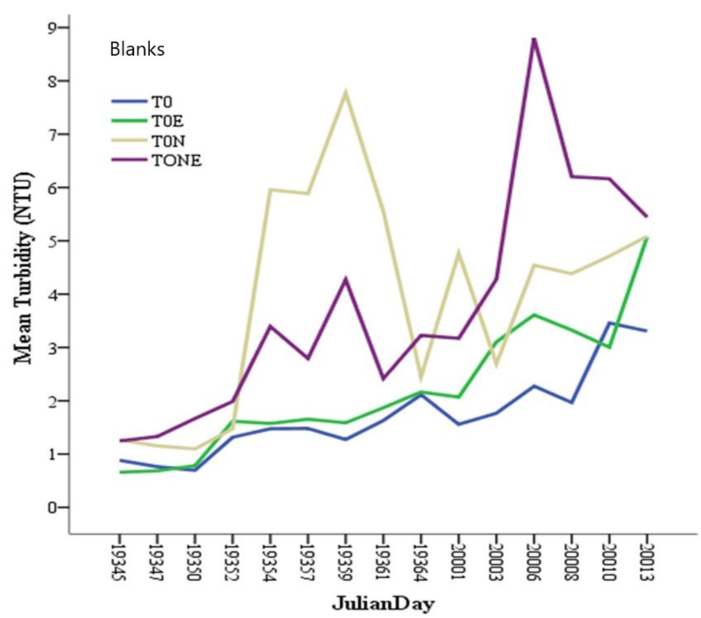

Figure 6. Variation in turbidity with time in the blanks without waste lubricant oil nor plants of *Pistia stratiotes* (water lettuce). T0= absolute blank only DWI deionized water, T0E= DWI+Emulsifier, T0N=DWI+Nutrients NPK, T0NE=DWI+Nutrients+Emulsifier. This figure shows potential existence of microorganisms adding its effects to the process of bioremediation. It can be noticed a slight increase in turbidity that was related to the growth of microorganisms.

Table 3. Comparison of the reduction of turbidity at 10 and 20 days in the different treatments. It can be noticed that at day 10, the highest phytoremediation percentage in terms of the surrogate variable turbidity was for the 20 ml with Pistia stratiotes plants. The differences with the treatments without plants however are not very large indicating that other sources of bioremediation can be present.

Treatment	Turbidity T=0	%	Turbidity T=10d	%	Turbidity T=20d
T0 absolute blank	0.9	68.2	1.5	5.4	1.6
ToNE	1.3	123.2	2.8	13.6	3.2
T2 (Pistia, 5 ml waste oil)	139.4	-31.6	95.4	-72.2	26.5
T3 (no plant, 5 ml waste oil)	131.4	-36.3	83.7	-59.2	34.2
T4 (Pistia, 10 ml waste oil)	438.0	-81.6	80.6	-63.0	29.8
T5 (no plant, 10 ml waste oil)	398.9	-84.5	62.0	-47.4	32.6
T6 (Pistia, 20 ml waste oil)	602.7	-74.5	153.6	-76.6	35.9
T7 (no plant, 20 ml waste oil)	815.3	-70.6	239.7	-81.2	45.0

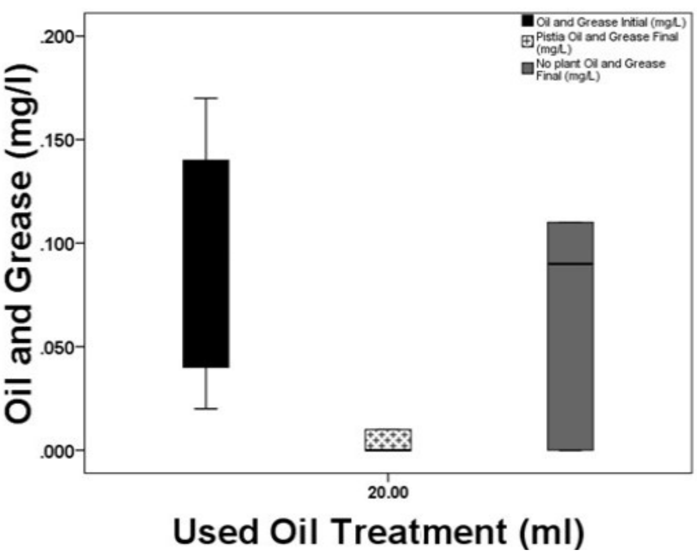

Figure 7. Oil and grease concentrations at the beginning (black) and 30 days at the end of the experiment (cross-hatched – with plants; gray – no plants). 20 ml of waste oil). An effective phytoremediation of the oil and grease concentration is clearly a result of the presence of the plants of *Pistia stratiotes*, however another potential bioremediation factors could be added with the presence of microorganisms.

day indicate that the presence of microalgae can also be participating in the phytoremediation process as observed by Hernández-Pérez et al. (2018).

Lu and others (2010) utilized *Pistia stratiotes* to phytoremediate nutrients from eutrophic stormwaters in constructed water detention systems. They found that there was an improvement in the water quality as seen from the reduction of turbidity by 65.54% compared with the control. Our study also demonstrated the potential of *Pistia* to reduce turbidity plus there was a marked difference in oil and grease content between the planted and unplanted control as was seen in Figures 5 and 7. The efficiency in the growth can be seen in the growth dynamics of both the leaf areas and the leaves and roots dry weights as seen in Figures 8 and 9.

CONCLUSION AND RECOMMENDATIONS

This study showed that there is a high potential that turbidity can be used as a surrogate variable to monitor oil and grease pollution in water. Wastewater that was phytoremediated with *Pistia stratiotes* had a significant reduction in oil and grease contamination with phytoremediation efficiency ranging from 65% to 83% over a 30-day period. To fully make use of the phytoremediation property of *Pistia stratiotes*, we decided that periodic resupplies would have to be conducted since plants that reach their biological growth peak may have reduced phytoremediation efficiencies and slow the wastewater treatment process. That was observed in Figure 8 and it would fit the replacement value that is demonstrated by Fonkou and others (2022) in wastewaters. Fletcher and others (2020) also explain the competitive effects of wetland communities with different plant species and phytoremediation is carried out based on the

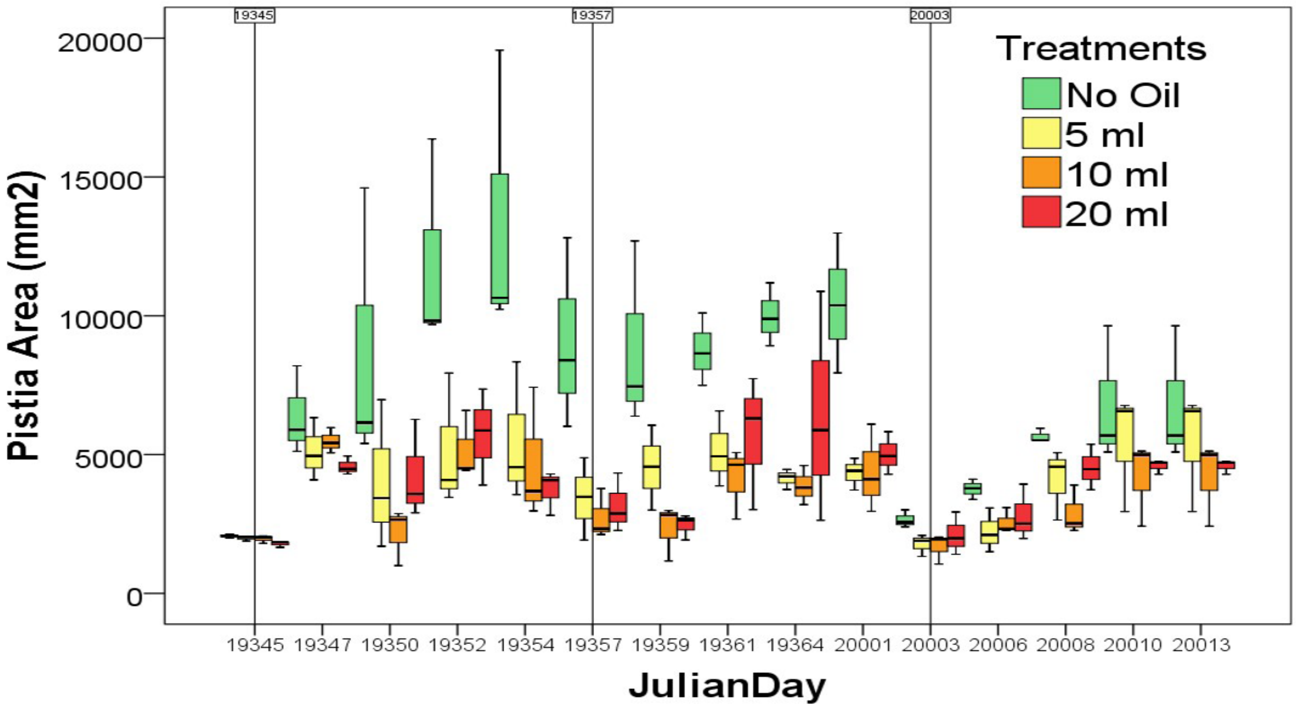

Figure 8. Temporal dynamics in the of leaf area growth of *Pistia stratiotes* with zero and three different concentrations of the waste lubricant oil (5 ml, 10 ml, 20 ml). This figure show how in T1 the presence of *Pistia stratiotes* without oil and grease concentrations increase and further is reduced given the overshading establishing competition among the individuals. The largest growth is produced in the 20 ml concentration but after the 10 days there is a replacement of those rosettes that were overshaded and the same pattern of growth is repeated.

temporal growth dynamics of the individuals. Intraspecific competition among individuals of *Pistia stratiotes* would also need to be taken into account.

For future work, we recommend:

1. Repeating this experiment with different sizes of plants at lower concentrations of oil and conducting the experiment for a longer period.
2. Identification of green algae and bacteria as potential co-workers in the process of wastewater remediation.

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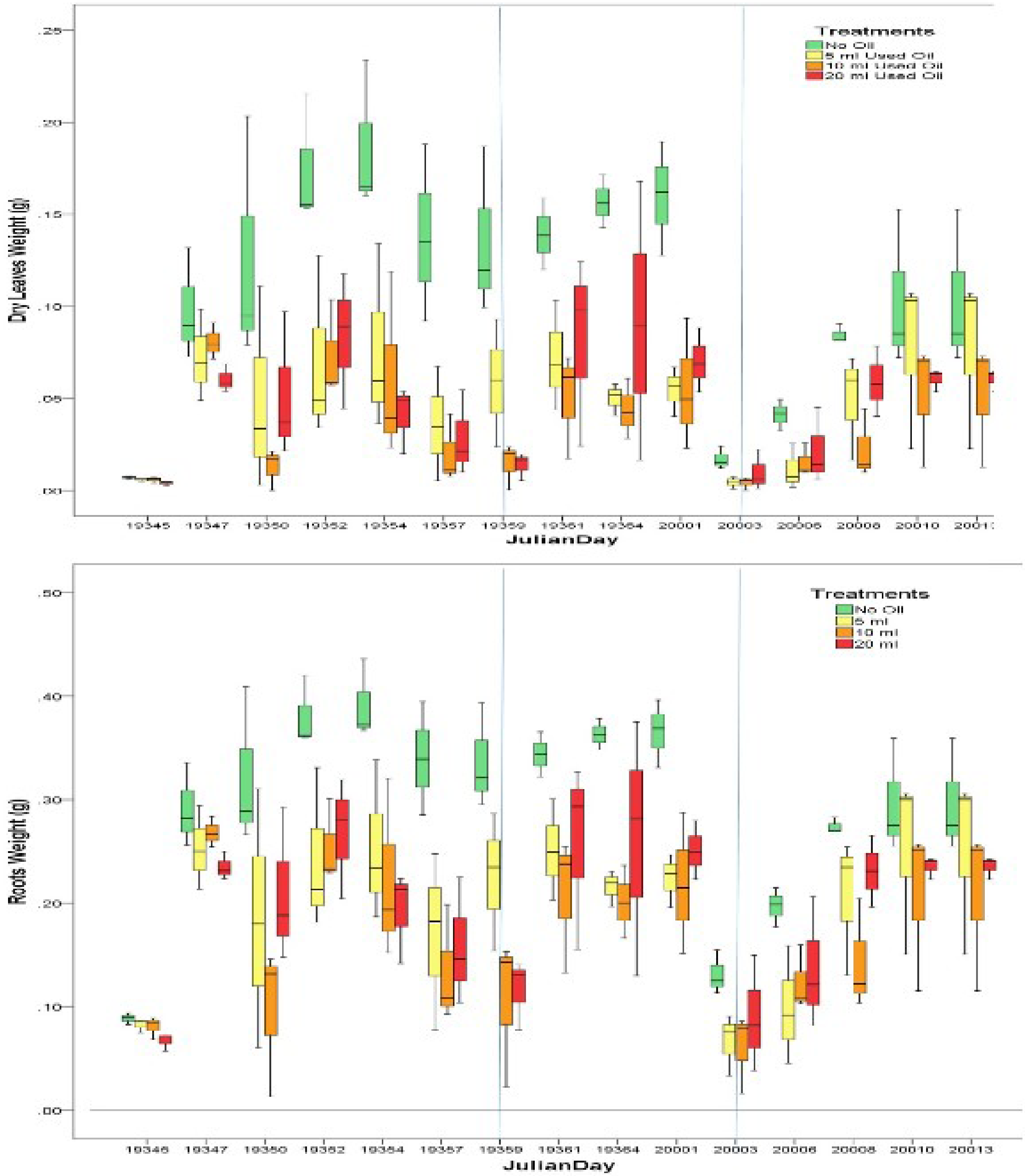

Figure 9. Temporal dynamics of *Pistia stratiotes* leaves weight in the graph above and roots weight in the graph below with zero and three different concentrations of the waste lubricant oil (5 ml, 10 ml, 20 ml). This figure shows how the productivity of *Pistia stratiotes* without oil and grease concentrations increase and further is reduced given the overshading establishing competition among the individuals. The largest carbon uptake is produced in the 20 ml concentration.

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