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BIOREMEDIATION POTENTIAL OF DUCKWEED (*LEMNA PAUCICOSTATA*) ON WASTEWATER FROM A MECHANIC WORKSHOP, PORT HARCOURT, NIGERIA

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ABSTRACT: *Wastewater pollution and its bioremediation remain a major challenge in urban areas. Lemna paucicostata were exposed to wastewater containing some amount of used engine oil in a four-week relative static bioassay. The physicochemical characteristics of the wastewater were determined using standard methods. The results obtained from the experiment showed that L. paucicostata was able to purify and concentrate nutrients from the wastewater. The efficacy of purification was manifested in the results where the total hydrocarbon content reduced from 8.15% - 4.00% over the period. Turbidity also reduced from 66.7% - 23.08%; biochemical oxygen demand reduced from 70.00% - 14.29%. Phosphate and nitrate also reduced from 34.59% - 12.09% and 39.74% - 11.92%, respectively. Chemical oxygen demand and total suspended solids increased from 22.92% - 73.16% and 2.90% - 14.39% respectively, over the periods. The temperature slightly changed from 29.3°C to 29.5°C. The pH also changed slightly from 7.00 – 7.50, making the wastewater slightly alkaline. Slightly alkaline conditions enhance overall hydrocarbon biodegradation in wastewater. However, prolonged exposure of L. paucicostata led to its mortality especially in the higher concentrations of hydrocarbons. Percentage mortality increased with concentrations and period of exposure, from 10% to 60% at the 3rd week and from 20% to 80% at the 4th week. Lethal concentration (LC_{50}) of 14.60ppm was noticed at the 3rd week, while LC_{50} of 12.60ppm was noticed at the 4th week. The effectiveness of this algae, L. paucicostata, on the bioremediation of wastewater is not only of scientific but also of economic and social importance.*

KEYWORDS: Bioremediation, duckweed, *Lemna paucicostata*, Wastewater.

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INTRODUCTION

Pollution is one of the critical existential problems affecting modern society. The industrial revolution of the last century and the dramatic increase in human population over time have resulted in the generation of an unprecedented amount of waste materials and pollutants into the environment. According to the Lancet Commission on Pollution and Health, Air, Water and Soil, pollution was responsible for 16% of deaths worldwide in 2015 and about 92% of such deaths occurred in developing countries with children being at high risk (Landrigan & Fuller, 2017).

Over the past 50 years, different strategies have been introduced for the remediation of polluted air, land and water. Many of these strategies driven by government policies and public opinion favors ecological based treatments as against the conventional methods.

Water pollution is one of the major challenges facing emerging economies. Over a billion people are affected by water pollution issues especially in developing countries (Landrigan & Fuller, 2017). Since the beginning of the anthropocene, humans have treated water with levity. Freshwater resources were largely seen as a receptacle for domestic and industrial waste. Today, water pollution and the growing share of wastewater released into water bodies is a concern for the sustainable development of water resources for society.

Phytoremediation, which is an aspect of bioremediation that deals with the application of plants for the remediation of polluted environments, involves the application of certain plant species to accumulate pollutants in terrestrial and aquatic environments. Plant species selected for phytoremediation have the potential to accumulate a specific or wide range of pollutants (Nwachukwu & Osuji, 2007; Omokeyeke *et al*., 2013; Udeh *et al*., 2013). In some cases, plants known as hyperaccumulators have the potential to bioaccumulate pollutants several times above the plant biomass (Van Epps, 2006). Several reviews have been published within the last 35 years on the application of aquatic macrophytes in phytoremediation of a wide range of pollutants in water (Gupta *et al*., 2012; Vithanage *et al*., 2012; Luqman *et al*., 2013; Halaimi *et al*., 2014).

Lemna paucicostata (Duckweed) belongs to the family Lemnaceae. It is a small aquatic herb floating on or below the surface of quiet streams and ponds, forming dense homogenous clonal populations, often an indicator of high nutrient load. They are monocots (like grasses and palms) and are divided into five genera and 38 species. The five genera include; *Lemna, Spirodela*, *Wolffia*, *Landoltia*, and *Wolfiella* (Bog *et al*., 2019). Of these genera, *Spirodela* is the largest, while *Wolfiella* is the smallest. The plant body is not differentiated into a stem or leaf but reduced to a fleshy or thallus-like ovoid or flattened structure bearing one or several roots (without root hairs) on the underside. They are about 4mm long and 2mm broad (Correll & Correll, 1972; Rusoff *et al.*, 1980). It produces a considerable number of daughter fronds during its life cycle, but mother fronds die after production of six generations (Ziegler *et al*., 2015). Kutscher and Niklas (2014) dubbed the duckweed family as “Darwinian Demons” due to their ubiquitous reproductive capacity, sporadic development and ability to almost “live forever”.

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This research was aimed at assessing the bioremediation potential of duckweed (*L. paucicostata*) on wastewater.

MATERIALS AND METHODS

Collection and maintenance of specimens: The test specimens (*Lemna paucicostata*) were collected in 2008 from a freshwater pool at the Government Reserved Area (GRA) (latitude 4^{o}46'38.71" N, longitude 7^{o}00'48.24" E), Port Harcourt, Rivers State, Nigeria. They were transported in aerated containers to the Marine Laboratory, Department of Applied and Environmental Biology, Rivers State University, Port Harcourt. The wastewater used in the research was collected from a mechanic workshop located at Elioparanwo (latitude 4^{o}83'35. 01" N, longitude 6^{o}96'41.14" E), Port Harcourt, Rivers State, Nigeria. The specimens were acclimated in a 50l glass container for two days in oxygenated water. Six smaller glass containers of 10l volume each were used for the bioassay.

The wastewater was put in an airtight plastic container and kept in a cool place to avoid escape of volatile components prior to use. Concentrations of wastewater used were 20%, 40%, 60%, 80% and 100%. Tap water was used as the control. Each treatment was replicated in duplicates. Each treatment contained 4000 ml of wastewater and tap water. That is, the solutions were prepared by adding 800ml, 1600ml, 2400ml and 3200ml of wastewater to tap water to make it up to the required volume (4000ml); for 100% concentration, only 4000 ml wastewater was used.

Experimental procedure: A 50l capacity tank was used as the main stock which was open at the top to generate enough oxygen. The total hydrocarbon content of the *L. paucicostata* was determined and recorded before commencing the experiments.

In the set of experiments, 100 algae were picked at random from the stocking tank and put in each of the experimental tanks. Also, the same procedure was adopted for the control which contained 0ml of wastewater. Each treatment was maintained under light conditions. Physicochemical parameters were measured in each of the treatments once every week for four weeks. Also, the algae were observed for physiological changes within this period, and a relative toxicity test was carried out in each of the treatments after the entire period.

Physicochemical parameters: Physicochemical characteristics of the wastewater as well as the freshwater pond from where the algae were collected were recorded. pH, turbidity and temperature were determined using Horiba water checker (model U-10). Dissolved oxygen and biochemical oxygen demand were determined by the use of Winkler's method (APHA, 1998). The chemical oxygen demand was determined spectrophotometrically with the HACH DRB200 COD reactor and DR/2500 spectrophotometer.

Total suspended solid (TSS) was determined graphically using APHA 2540 method (APHA, 1998). The total hydrocarbon content (THC) was determined using the ASTM D3921 extraction/spectrophotometry method (APHA, 1998). Nitrate was measured by ultraviolet spectrophotometric screening method (APHA, 1998). Phosphate was determined using the stannous chloride method (APHA, 1995).

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Biodegradation test: The used engine oil wastewater was subjected to biodegradation by the algae *Lemna paucicostata*. This was done by putting 100 algae in each of the different concentrations of the wastewater polluted with used engine oil, which was allowed to stay for four weeks. During this period, the algae was monitored and parameters, such as total hydrocarbon, biochemical oxygen demand, chemical oxygen demand, turbidity, pH, temperature, phosphate, nitrate and total suspended solids were measured. This was followed by the extraction of residual hydrocarbons using carbon tetrachloride, and then analyzed using gas chromatography.

Determination of LC_{50}: The LC_{50} of the used engine oil was determined by a plot of percentage mortality against concentration of polluted water used, and extrapolating to the concentration at which 50% mortality occurred.

RESULTS

The physicochemical parameters of the wastewater and the freshwater pool from where the specimens were collected are presented in Table 1. The total hydrocarbon content was 15.50ppm in the wastewater and 5.63ppm in the freshwater pool. The dissolved oxygen content was 1.22mg/l in the wastewater and 4.06mg/l in the freshwater pool.

Table 1: Physicochemical parameters of the wastewater containing used engine oil and the freshwater pool from where *Lemna paucicostata* were harvested

Content	Wastewater with Used Engine Oil	Freshwater Pool
Total hydrocarbon (THC) (ppm)	15.50	5.63
pH	7.5	7.1
Temperature (^{o}C)	29.4	29.2
Dissolved Oxygen	1.22	4.06
Turbidity (NTU)	67.0	6.0
COD (mg/l)	450.0	780.0
BOD (mg/l)	108.31	25.58
TSS (mg)	12.50	16.40
Phosphate (mg/l)	28.26	20.35
Nitrate (N_2) (mg/l)	72.40	35.38

Results showed that *L. paucicostata* led to a reduction in the total hydrocarbon content of the wastewater as the research progressed from week 1 to week 4. The hydrocarbon content reduced with reducing concentration of wastewater and also reduced drastically as the experimental setup progressed (Table 2).

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Table 2: Effects of *L. paucicostata* on the total hydrocarbon content (THC) of wastewater containing used engine oil, Port Harcourt, Nigeria

Concentration (ml)	Initial THC (ppm)	Week 1	Week 2	Week 3	Week 4
0	0	0	0	0	0
20	13.5	12.4	12.2	11.8	11.4
40	14.0	13.0	12.8	12.6	12.0
60	14.5	13.8	13.6	13.4	12.6
80	15.0	14.7	14.5	14.2	13.2
100	15.5	15.4	15.3	15.0	13.8

Turbidity results (Table 3) showed a reduction as the concentration decreased and equally there was an increase in percentage reduction as the bioassay progressed after the introduction of *L. paucicostata.*

Table 3: Effects of *L. paucicostata* on the turbidity of wastewater containing used engine oil, Port Harcourt, Nigeria

Concentration (ml)	Initial Turbidity (NTU)	Week 1	Week 2	Week 3	Week 4
0	30	25	20	10	10
20	45	40	35	25	25
40	50	45	40	36	36
60	55	50	45	42	40
80	60	55	50	46	44
100	65	60	55	52	50

There was also a general reduction in the Biochemical Oxygen Demand (BOD) of the wastewater. The percentage reduction increased as the concentration reduced. Also, as the week progressed, percentage reduction increased (Table 4). A similar observation was noted for the Chemical Oxygen Demand (COD) (Table 5).

Table 4: Effects of *L. paucicostata* on the biochemical oxygen demand (BOD) of wastewater containing used engine oil, Port Harcourt, Nigeria

Concentration (ml)	Initial BOD (mg/l)	Week 1	Week 2	Week 3	Week 4
0	20	15	10	8	6
20	65	60	55	50	46
40	75	70	65	63	60
60	85	80	75	72	70
80	95	90	85	81	79
100	105	100	95	93	90

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Table 5: Effects of *L. paucicostata* on the Chemical oxygen demand (COD) of the wastewater containing used engine oil, Port Harcourt, Nigeria

Concentration (ml)	Initial COD (mg/l)	Week 1	Week 2	Week 3	Week 4
0	380	645	650	655	658
20	400	636	648	645	645
40	420	632	642	640	638
60	440	626	636	595	593
80	460	620	630	592	592
100	480	610	625	591	590

Lemna paucicostata led to an increase in the total suspended solids (TSS) as the week of exposure progressed (Table 6). Its impact on pH and temperature varied only slightly (Table 7). The phosphate and nitrate levels of the wastewater were reduced by the action of *L. paucicostata* (Table 8 – 9).

Table 6: Effects of *L. paucicostata* on the total suspended solids (TSS) of wastewater containing used engine oil, Port Harcourt, Nigeria

Concentration (ml)	Initial TSS	Week 1	Week 2	Week 3	Week 4
0	5.42	5.80	6.12	618	6.20
20	7.50	7.82	8.26	8.28	8.30
40	9.66	9.88	10.20	10.22	11.24
60	11.24	11.45	11.54	1.36	11.58
80	13.50	13.62	13.80	13.90	14.00
100	15.50	15.61	15.72	15.96	15.95

Table 7: Effects of *L. paucicostata* on the Temperature (°C) of wastewater containing used engine oil, Port Harcourt, Nigeria

Concentration (ml)	Initial pH (Temp °C)	Week 1	Week 2	Week 3	Week 4
0	7.00 (29.3)	7.10 (29.5)	7.10 (29.5)	7.20 (29.5)	7.10 (29.5)
20	7.10 (29.3)	7.20 (29.5)	7.20 (29.5)	7.21 (29.5)	7.21 (29.5)
40	7.20(29.3)	7.20(29.5)	7.20(29.5)	7.30(29.5)	7.30(29.5)
60	7.15(29.3)	7.30 (29.5)	7.30 (29.5)	7.30 (29.5)	7.30 (29.5)
80	7.31 (29.3)	7.40 (29.5)	7.40 (29.5)	7.40 (29.5)	7.40 (29.5)
100	7.50 (29.3)	7.40 (29.5)	7.40 (29.5)	7.41 (29.5)	7.41 (29.5)

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Table 8: Effects of *L. paucicostata* on the phosphate level of wastewater containing used engine oil, Port Harcourt, Nigeria

Concentration (ml)	Initial phosphate (mg/l)	Week 1	Week 2	Week 3	Week 4
0	24.46	22.24	20.36	18.20	16.00
20	26.28	4.20	22.40	20.42	19.35
40	28.64	26.42	24.28	24.20	24.22
60	30.52	28.33	26.45	26.41	26.40
80	32.34	30.18	28.60	28.55	28.50
100	34.58	32.45	30.44	30.42	30.40

Table 9: Effects of *L. paucicostata* on the nitrate level of wastewater containing used engine oil, Port Harcourt, Nigeria

Concentration (ml)	Initial nitrate (mg/l)	Week 1	Week 2	Week 3	Week 4
0	62.00	50.89	46.62	41.28	37.25
20	64.20	55.65	50.50	46.36	44.36
40	66.45	59.76	59.32	52.20	51.15
60	68.36	64.22	60.58	58.65	57.23
80	70.15	67.58	62.42	60.20	59.15
100	78.25	76.20	72.31	68.94	68.92

Concentrations of the hydrocarbon in the 100% stock solution of the wastewater was 15.0ppm at week 3 and 13.8ppm at week 4. Concentrations in the dilute solutions (20% - 80%) ranged from 11.8ppm to 15.0ppm for the third week of exposure, and from 11.ppm to 12.5ppm at the fourth week of exposure. The lethal concentration (LC_{50}) was computed as 14.6ppm during week 3. For week 4, the LC_{50} was 12.6ppm. The percentage mortality of L. *paucicostata* at Weeks 3 and 4 are shown in Figures 1 and 2.

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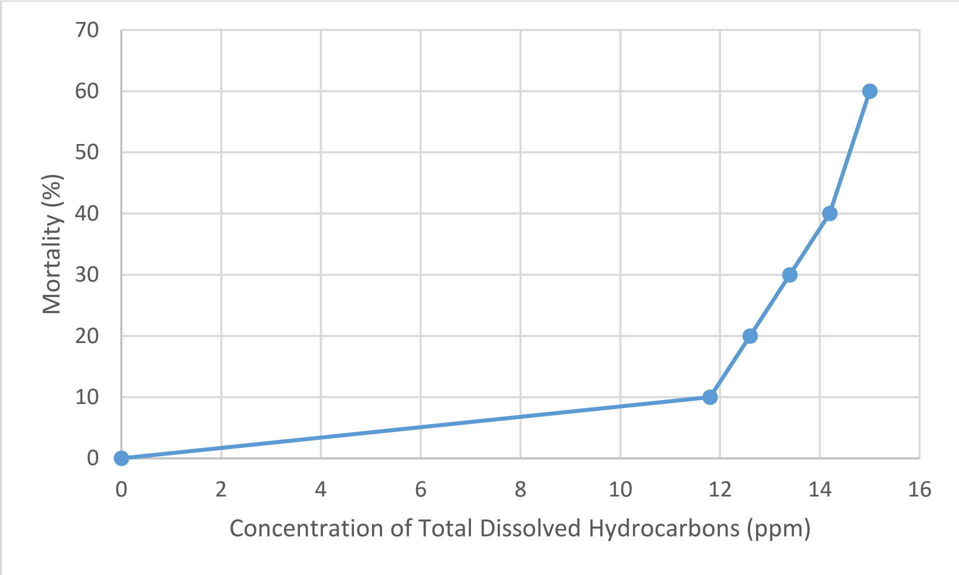

Fig. 1: Percentage mortality of *L. paucicostata* (Week 3) at various concentrations of total dissolved hydrocarbons in wastewater containing used engine oil

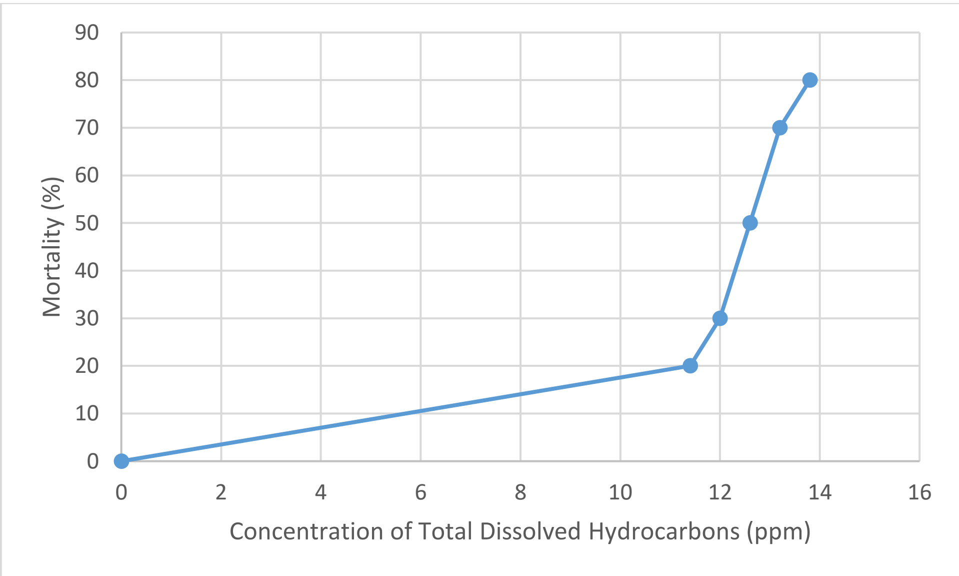

Fig. 2: Percentage mortality of *L. paucicostata* (Week 4) at various concentrations of total dissolved hydrocarbons in wastewater containing used engine oil

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DISCUSSION

Physicochemical and natural degradative measures enable polluted terrestrial and aquatic environments to recover from several exposures to oil pollution (Ekundayo & Obuekwe, 1997). However, the bulk of polluting oils are degraded in the environment by microbial actions since various microorganisms including bacteria, yeast, molds and algae have been reported to possess the capabilities for utilizing petroleum hydrocarbons (Bartha & Atlas, 1997).

Lemna paucicostata (duckweed) used in this study has shown some bioremediation potentials to fresh water pollution. From the results obtained, *L. paucicostata* absorbed some quantity of the hydrocarbon present in the polluted water before mortality was noticed after long exposure. These findings are in accordance with the works of Bartha and Atlas (1997) who observed that some algae have the capability for utilizing petroleum hydrocarbons. The turbidity and biochemical oxygen demand (BOD) reduced when *L. paucicostata* was introduced. On the other hand, Chemical Oxygen Demand (COD) and Total Suspended Solids (TSS) increased. Enhancement of COD was important since it determines the amount of oxygen per liter of wastewater which is consumed during the oxidation of organic matter. The implication of this is that with the addition of *L. paucicostata*, almost all the organic compounds in the wastewater were oxidized. Increase in TSS result agrees with the findings of Solar *et al*. (1991) who studied temporal changes in physicochemical parameters and photosynthetic microorganisms in a self-depuration wastewater lagoon, where some phytoplankton including microalgae played important role in the biotreatment or stabilization.

Concerning the pH and temperature values, there was only a slight difference. Increase in temperature is accompanied by increase in biochemical reaction. Slight increase in pH shows that slightly alkaline pH favors a higher number of microorganisms involved in biodegradation. This is in line with the reports of Vanloocke *et al*. (1975), that there is evidence that overall hydrocarbon biodegradation is higher under slightly alkaline than acidic conditions. In general, the slightly alkaline condition of pH 6 – 8 is regarded as a favorable chemical factor affecting bioremediation (Rogers *et al*., 1993).

Phosphate and Nitrogen showed an increase in percentage reduction at different concentrations and over the period. The result on nutrient removal (N and P) by *L. paucicostata* agrees with the findings of De La Noue and De Pauw (1988) on bio-treatment with microalgae. They observed that microalgae had photosynthetic capabilities of converting solar energy into useful biomass and incorporating nutrients such as nitrogen and phosphorus which cause pollution and eutrophication. This also agrees with the results of Shelef *et al*. (1980) who found that the efficiency of removal of a particular nutrient (for e.g. N or P) by microalgae will also depend on whether or not these nutrients are limiting in the wastewater to be treated. In practice, they found that with adequate stirring more than 90% nitrogen or phosphorus can be removed. However, there was delayed nutrient uptake during the 3rd and 4th week, especially in the higher concentrations of hydrocarbons as the hydrocarbons could have entered into unicel or into intercellular spaces of algal filaments interfering with water/ nutrient uptake and gaseous exchange of the cells resulting in physiological drought and gradual suffocation. These observations are in accordance with the findings of Baker (1970) and Amakiri and Onofeghara (1983) for higher plants.

Percentage mortality was noticed after two weeks of exposure. Mortality increased with concentration, for instance, at 11.8ppm (20% conc.), mortality was 10% while at 15.0ppm

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(100% conc.), mortality was 60% during the third week. Also, at 11.4ppm (20% conc.), mortality was 20% while at 13.8ppm, mortality was 80% during the 4^{th} week. Lethal concentration (LC_{50}) of 14.60ppm was estimated for the 3^{rd} week as shown in Figure 1 whereas lethal concentration (LC_{50}) for the 4^{th} week was estimated to be 12.60 (Figure 2). Mortality of the test organism noticed after prolonged exposure (more than 2 weeks) agrees with the findings of Baker (1970) who observed that petroleum products of high concentration had negative effects on the root system of plants, where plant parts were drastically reduced on soils polluted with petroleum products. Mortality occurred due to the interference of chemical contents in the petroleum products with the photosynthesis and transpiration processes possibly by clogging the stomata which results in reduction of photosynthetic rates. Used engine oil at a higher concentration has a large reduction in redox potentials by plants by interference with the uptake of water by the roots. This is in line with the works of Esenowo and Umoh (1996) while investigating the effects of used engine oil pollution of soil on the growth and yield of *Arachis hypogea* and *Zea mays*. Mortality may also be due to low availability of oxygen in wastewater. The oil film on the water surface reduced the inter-phase with the atmospheres resulting in loss of oxygen in water. The low oxygen in water increased physiological stress on the organisms which eventually led to their death. The longer the exposure of oil (hydrocarbon), the higher the mortality on the algae. Mortality was higher during the 4^{th} week of exposure.

CONCLUSION AND RECOMMENDATIONS

The study has shown that *Lemna paucicostata* has a great potential in wastewater purification. Thus, polluting oils can be degraded in the environment by biological actions which include algae. Biological remediation technology is increasingly gaining acceptance in environmental pollution control. As seen from this study, *Lemna paucicostata* (duckweed) reduced the total hydrocarbon and other objectionable qualities of the polluted water.

The findings of this study have shown the usefulness of this alga in remediation of wastewater in an effective way, the technique is cheap and simple and their application in environmental wastewater management is recommended. Where these algal forms are to be used for remediation purposes, it is recommended that it should be for a short-term exposure (of not more than 2 weeks). As the findings of this study indicates that long term or prolonged exposure would lead to increased mortality. Also, technicians at mechanic workshops should be advised on proper/alternative means of disposing of their waste/used oils rather than channeling it to nearby streams and other water bodies to avoid freshwater pollution.

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