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Phytoremediation of Petroleum Contaminated Water Using a Tropical Aquatic Plant (*Pistia stratiotes*) Under a Green-House Condition

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ABSTRACT

The influence of petroleum on the physical response of *Pistia stratiotes* and ability of extraction through the measurement of the total petroleum hydrocarbon per time were investigated in this study. Experiment was conducted in a greenhouse using four different concentrations (0.0%, 0.1%, 0.3% and 0.5%) in a litre of water per concentration. Each concentration was in triplicates in one litre capacity glass jars under a completely randomized design. Petroleum absorption by the plant caused de-coloration of leaves (from green to greenish-yellow to dark brown, and finally light brown, shrinking and coiling of whole plant and mortality as time of exposure progressed or concentration of pollutant increased. Total mortality of plant took three days. A range of absorptions of 44.2 to 161.0 ug/g and 38.7 to 168.9 ug/g at 5^{th}-hour and 10^{th}-hour, respectively, were observed. The 15^{th}-hour witnessed no detection by gravimetric method. Absorption of petroleum from contaminated water per plant was small (0.01% of body-mass) which expressed inefficiency but can be maximized by the invasive and domineering nature of *P. stratiotes* in phyto-technology as first-aid treatment. However, prompt harvest from water is advocated after use to prevent fouling plus mechanical treatment using rollers.

Keywords:
Phytoremediation, *Pistia stratiotes*, Green-house, Petroleum

Introduction

Petrol and kerosene are Refined from crude petroleum and contains a mixture of hydrocarbons [Edorie *et al.*, 2014]. However, petrol is lighter than kerosene fraction at 5 – 10 carbon chain compound compared to kerosene with 11 – 18 carbon atom chains. Hence, petrol is more volatile than kerosene. Consequently, due to increased application of petroleum products on daily basis and multiple use, interest or focus is now on the effects of petroleum products on both terrestrial and aquatic resource plus means of treatment after contaminating land and water ecosystems. Therefore, most studies investigated the toxicity of these products on fishes (Chukwu and Okhumale, 2009).

Pistia stratiotes is a water plant that belongs to the family Araceae. It is otherwise called water cabbage, water lettuce, Nile cabbage or shell flower. It is a tropical plant found in almost all countries of Africa

and some parts of America (Tripathi *et al.*, 2010). It is a sprawling semi-woody shrub that invades fresh water bodies. The green stems are up to 4.6m and leaves can measure between 2.0cm to 15.0cm in length. It resembles a floating head of lettuce and can exist all year round in water (Bachanska, 2019). Also, it has the ability to spread and cover water surface over a large area, hinders water flow in irrigational schemes but can be regulated and serve as food for herbivorous fishes.

The remediation of hydrocarbon polluted water can be achieved by three methods, namely, phytoremediation, Bioremediation and chemical remediation. Therefore, phytoremediation involves the use of plant to reduce the volume, mobility and toxicity of contaminants in soil and water. Phytoremediation operates through different process such as phyto-transfer, Rhizosphere bioremediation, phytostabilization, rhizofilteration and phytoextraction (Kumar *et.al.*, 2018).

Phytoextraction is otherwise called phytoaccumulation where plants or algae remove contaminants from soils, sediments or water and conversion into harvestable plants biomass. This present research, therefore, investigated the ability of a floating aquatic plant (*Pistia stratiotes*)in filtering and extracting petroleum [motor spirit] from contaminated water under a green- house condition and mortality effects.

Methodology

Pistia stratiotes was collected from standing (lotic) water and streams that where tributaries of Ofiki rivers in Ibarapa Central Local Government Area. The average wet-weight of plant was 6.2 ± 0.15 g with a foliage canopy of 4.0 ± 0.2cm. This was translocated in the early morning hours to avoid scorching or de-hydration by the sun. Precisely, rectangular plastic tanks containing about 3 litres of water plus the whole plants were employed during transportation to the green house.

The population of 300 whole plants of *Pistia stratiotes* were first conditioned by their introduction into the resting earthen pond with a pool of water for two weeks. This helped to ascertain their fitness and direct nourishment plus readiness of the green house. Plants were then harvested and transferred to 1 litre capacity glass jars at a population of four (4) plants per glass jar after filling with water from the fish – farm of Oyo State College of Agriculture and Technology at a pH of 6.7. Two (2) gramme of fertilizer (urea) was then added to each glass jar to supply the plants with nutrient and sustain them for three days before experiment. This was followed by a starvation period of 24 hours before the actual experiment by a complete change of water.

Plant processing after exposure to the contaminants involves harvesting and separation of plant parts into rhizome and leaf, placement in aluminum foil and drying at room-temperature before grinding into fine particles using pestle and mortar. Packaging and labeling of the processed samples were then ensured using small sample bottles. Samples were then transported to the organic chemistry laboratory at the department of chemistry in the university of Ibadan for the investigation of Total Petroleum Hydrocarbon (TPH). Total petroleum hydrocarbon (TPH) was determined using gravimetric method. This involved three (3) basic steps : Saponification of a quantified sample in methanolic – KOH, extraction of sample or constituents by n-hexane, and separation using a liquid chromatography.

Specifically, a known weight of sample is transferred into a round bottom flask containing. 200ml of methanol and 8.0g of potassium hydroxide (KOH). These flasks are rotated in an attempt to rinse the sides to capture the introduced samples completely; complete mixing and dissolution. Next, the solvent

was transferred to the separating funnel; facilitating extraction by shaking vigorously for two (2) minutes. The formed layers were then allowed to separate, filtration was then ensured through n-hexane layer using a funnel fitted with n-hexane moistened filter paper into a 250ml beaker. This extraction process was repeated using 30ml portion of fresh n-hexane by direct addition in the separating funnel and recollected in a beaker. This was to ensure a complete extraction through further extraction by using 5-10ml of n-hexane and final collection of filtrate in a beaker. Finally the extract filtered is reduced to 5.0ml using a rotary evaporator. Next, the liquid chromatography is applied. Hence a column was packed with silica gel and 5.0ml of the concentrated extract is eluded through the column. Elute was collected through a tarred flask and n-hexane recovered using the evaporator.

Calculation:

TPH ($^{g}/_{g}$) = $\frac{weight\ of\ TPH\ in\ tarred\ flask \times 1000}{weight\ of\ sample}$. (EPA, 1999)

Results

Table 1 captured the behavioural response of plant during exposure to petroleum in experimental chambers under a greenhouse condition. The observed responses include gradual folding of leaves to total collapse, bleaching of leaves and submergence, coiling of rhizome, and mortality at the 4th, 20th,48th and 72nd hour, respectively. These effects increased with increasing concentration of petroleum and time of exposure. Also, Table 2a revealed a general trend of increasing extraction of petroleum from the water-petroleum medium in the experimental glass jars per increase in concentration of pollutant at a specific time of exposure. The increase in extraction (TPH) was evident until the 10th-hour but undetectable at the 15th-hour by the gravimetry. These variations were significant compared to the zero-treatment media. Similarly, Table 2b revealed variation in average values of pollutant absorbed (TPH) by the water plant (*P. stratiotes*) in a specific treatment and changing period of time (hour). It indicated an increase in the amount of total petroleum hydrocarbon extracted by *P. stratiotes* as the time of exposure increased.

	Recorded response to exposure in different concentration		
Duration of Exposure (hours)	**Treatment 1**	**Treatment 2**	**Treatment 3**
4th hour	Partial folding of leaves Zero bleaching of leaves Normal rhizome structure	Intense folding of leaves Partial bleaching of leaves Normal rhizome structure	Total collapse of leaves Partial bleaching of leaves Rhizome appearance normal
20th hour	Partial bleaching from green to yellowish-green Partial cooling of root Zero death of plant	Partial submergence of the whole plant Intense cooling of root Low death (30%)	Intense submergence of the whole plant Bleaching to yellowish-green Moderate death (50%)
48th hour	Bleaching of leaves to yellowish-green	Browning of leaves (intense)	Intense brown coloration of leaves

	(intense) Coiling of rhizome Moderate death (80%) of plant	Coiling and shortening of the rhizome Total death	Coiling and shortening of the rhizome Total death
72nd hour	Moderate bleaching to brown 80% mortality	Bleaching to brownish-white Total mortality	Bleaching to brownish-white Total mortality

Table 2a: Mean values of Total Petroleum Hydrocarbon (TPH) in whole plant samples of *Pistia stratiotes*(µg/g)

	Measured TPH (µg/g) at different concentration			
Duration of Exposure (hours)	**Treatment 0**	**Treatment 1**	**Treatment 2**	**Treatment 3**
5th-hr	4.30^{a}	42.8^{a}	55.1^{c}	165.3^{d}
10th-hr	4.10^{a}	48.5^{b}	57.1^{c}	173.0^{d}
15th-hr	3.96^{a}	Undetected	Undetected	Undetected

Means with the same superscript are not significantly different at P≥0.05 along the same row (time)

Table 2b: Mean values of Total Petroleum Hydrocarbon (TPH) in whole plant samples of *Pistia stratiotes* (µg/g)

	Measured TPH (µg/g) at different concentration			
Duration of Exposure (hours)	**Treatment 0**	**Treatment 1**	**Treatment 2**	**Treatment 3**
5th-hr	4.30^{a}	42.8^{a}	$55.1^{b}16$	165.3^{c}
10th-hr	4.10^{a}	48.5^{b}	57.1^{b}	173.0^{c}
15th-hr	3.96^{a}	Undetected	Undetected	Undetected

Means with the same superscript are not significantly different at P≥0.05 along the same column (concentration)

Discussion

The present study recorded a general increase in petroleum absorption (phytoextraction) capacity of *Pistia stratiotes* from the polluted water as the time of exposure and concentration of petroleum increased (Table 2). These effects were similarly reported by Lopes *et al*. (2014) where the survival of water hyacinth (*E. crassipes*) were mainly dependent on concentration (dose) of petroleum oil and time of exposure under an experimental study. However, changes in plant colour from green to greenish - yellow to dark brown and finally light brown were observed in the leaves of *Pistia stratiotes* in the current study. This bleaching effect was faster at higher concentrations (0.3% and 0.5%) of exposure while the lower concentrations also witnessed substantial discolouration as time progresses from the 5th to 72nd-hour. Bleaching of leaves of *Pistia stratiotes* was subsequently followed by a degree of leaf mortality and shortening or intense curvature of the rhizomes. Precisely, total plant death took three (3) days (72 hours) as shown in Table 1. Mortality observed could be explained, partly, due to interruptions of shoots well-being; Since gas (CO_2) transport was limited by the blockage of stomata-pore and surface areas of leaves which led to death in the current study. This agreed with the report of Pezeshri *et al*.

(2000) where alterations directly linked with the sensitivity of each plant species resulted into modifications in water. Precisely, 80% mortality at 0.1% rate of application compared to 100% mortality at 0.3% and 0.5% at the 72-hours (Table 1). The final death of *Pistia stratiotes* could therefore cause accumulation of organic matter plus decomposition (decay) or poisoning of water depending on the chemical property and other factors or interactors in water as similarly reported by Lopes *et al*. (2014).

The ability of *Pistia stratiotes* to extract petroleum was monitored through the measurement of "Total Petroleum Hydrocarbon" in the whole plant per time in this present study. That is Petroleum Hydrocarbons (P.C.H) are noticeable organic contaminants in either water or soil environment and are used as a measure of pollution. However, petroleum hydrocarbons are made up of three groups of compounds; namely: alkanes, alkenes and aromatics. Precisely, the major components are the alkanes (Junk*et al.*, 1989) and present in many refined petroleum products (motor spirit, petroleum/gasoline, diesel fuel, kerosene, Jet fuel and heating oil). Generally, in the present study, the phytoextraction ability of *Pistia stratiotes* tends to increase as the concentration of pollutant increased in the experimental chambers (glass jar) at a particular time. These variations or increase in extraction were significantly different compared to the control and directly proportional to bleaching potential and mortality of *Pistia stratiotes*. This agreed with the report of Baker *et al*.(2003) that reported damage in cell membranes, blockage of intercellular spaces and destruction of vascular systems in plants polluted with oils. However, beyond 15th-hour the extracted petroleum as pollutant could not be detected during investigation. This could be a result of high volatility of petroleum and high ambient (environmental) temperatures (25-27°c) within the greenhouse where the experiment was conducted. This agreed with the report of Baker *et al.* (2003) that reported petroleum to be a very volatile hydrocarbon escaping into air, water and solids as a point release or non-point sources. Furthermore, the efficient uptake of petroleum and kerosene could be explained due to the high solubility of aromatic hydrocarbons that are blessed with lower molecular weight; solubility up to 220 mg/L (Adeniji, 2017).

Phytoremediation ability of *Pistia stratiotes* as a biotechnology is low in this study. This is because a range of absorptions of 44.2 to 161.0 ųg/g and 38.7 to 168.9 ųg/g at 5th hour and 10th hour, respectively. These observations agreed with the findings of NTP (2016) where remediation technologies recorded an incomplete restoration of sites or ecosystems. However, the use of *Pistia stratiotes* as a phytoremediation could serve as a 'First Aid Technique' to petroleum contamination because of an insignificant extraction observed in the course of this experiment.

Conclusion

Water lettuce could serve as first aid treatment to reduce the accidental discharge of petroleum in freshwater ecosystems. However, it is not an efficient candidate for remediation of water contaminated with petroleum because the uptake was 0.01% of the body-mass of a plant; using a universal mean of 102 (µg/g) for TPH (extractable mineral oil and grease). Therefore, other potential aquatic plants need to be screened for efficiency in phytoremediation. Mechanical methods may be preferred which employs foam-rollers during spillage in controllable water ecosystems. Also, a plant density of about 96 to 100 plants per 1.5m^2 is recommended; using a foliage (plant umbrella) of 4.0 diameter.

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Plate1: Set-up

Plate 2: T1 at 72-hr

Plate 3: Control at the end of 72-hr

Plate 4: Processing of samples

Plate 5: Final packaging before laboratory investigation

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