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Impact of Kerosene, Diesel and Gasoline Treatments on the Morphology, Growth Indices and Plant Based Component of an Aquatic Macrophyte *Eichhornia Crassipes*

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Abstract

A study was carried out at the screen house of the Department of Plant Biology and Biotechnology, University of Benin, Benin City to determine the growth response of water hyacinth (Eichhornia crassipes) to three refined petroleum products: gasoline, kerosene and diesel oil. The experimental treatments used were 0% (control), 10%, 13%, 15% (v/v) for kerosene, diesel and gasoline respectively. The parameters measured include leaf diameter, stolon girth, root length, chlorophyll content index, plant height, stolon length and biomass. Using randomized complete block design (RCBD) and 3 replicate per treatment, the plant was exposed to treatment for 14 days. The results obtained from the experiment showed reduction in leaf diameter, root length, stolon girth, plant height and chlorophyll content exposed to all concentration of petroleum products for 14 days when compared with the test plant without treatment. The results showed that kerosene had more toxic effect on the plant than diesel and gasoline. Chlorosis and wilting were also observed in abaxial and adaxial surface of the leaves of the test plant at the end of the experiment. The findings from this study show that concentration and duration of exposure are the factors that determine the effect of petroleum products on E. crassipes.

1. Introduction

Oil pollution is the negative polluting effect that oil spills have on our environments and living organisms, including humans [1]. Oil spill may be due to releases of crude oil from tanker, offshore platform, drilling rigs and wells, as well as spills of refined petroleum products and their by-products, heavier fuels used by large ship such as bunker fuel, or the spill of any oily refuse or waste oil [2]. It is a major impact on the ecosystem into which it is released [3]. Spills in populated areas often spread out over a wide area, destroying crops and aquacultures through contamination of the ground water and soils. Refined petroleum products are products derived from crude oil as it is processed in oil refineries [4]. They are derived through processes such as catalytic cracking and fractional distillation. These refined products have physical and chemical characteristics that differ according to the type of crude oil and subsequent refining processes. Aquatic habitat is most prone to oil pollution, which has harmful effects on the water body at different sensitivity levels, depending on the habitat. Many of the chemical substances present in the petroleum products are toxic and when it gets into water body, it alters the physical chemistry including temperature, pH

and conductivity [5]. Some studies have also shown the effects of oil pollution on plant survival and biomass. They include [6], [7], and [8].
This paper evaluates the tolerance rate, growth indices and plant based components of *Eichhornia* cras*sipes* when exposed to kerosene, gasoline and diesel pollution.

2. Methodology

2.1. Study Site

The screen house of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin was used as the study site.

2.2. Plant collection

Water hyacinth plants were collected from Ikpoba River, Edo State Nigeria. The plants were carefully removed to avoid root damage and placed in a plastic bowl containing 4 litres of the river water to avoids excess dehydration before getting to the screen house.

2.3. Experimental design and setup

Different amounts of 0%, 10%, 13% and 15% (v/v) were used for the study. The test plant was thoroughly rinsed with tap water to wash off any particles attached to the leaf surfaces and roots. It was there after transferred to the 36 bowls, (the first twelve bowls for diesel treatment, the second twelve for the kerosene treatment and the third for gasoline treatment).

For kerosene treatment, the bowls were categorized into four places as follows:

- 0% $^{v}/_{v}$ containing 1000ml deionized water as control.
- 10% $^{v}/_{v}$ containing 900ml deionized water and 100ml diesel.
- 13% $^{v}/_{v}$ containing 870ml deionized water and 130ml diesel.
- 15% $^{v}/_{v}$ containing 850ml deionized water and 150ml diesel.

There were three replicates of each category and they were labelled accordingly. The procedure was repeated for the gasoline and diesel treatment. The experimental setup was left for 14 days and readings were taken every day. All the experimental materials were placed under the same environmental conditions, to ensure completeness and accuracy of data.

2.4. Data Recording

Morphological observations of the plants were made to ascertain change in leaf diameter, leaf colour, stolon length, stem girth, chlorophyll content index and root length. The following data were collected during the experiment.

2.5. Leaf diameter measurement

The diameter of the sample plant leaves was measured using a metre rule.

2.6 Stem Girth Measurement

The stem girth of the plants was measured using a digital vernier calliper.

2.7. Chlorophyll content Index Determination

Chlorophyll contents index of the leaves were measured using the Apogee™ chlorophyll content meter. Measurement was done by holding the arm of the chlorophyll content meter in direct contact with the leaf until it made a beep. The chlorophyll content index was displayed on the screen of the device and was recorded before treatment (day 0) and after treatment (day 14).

2.8. Root length measurement

The length of the root was measured by the use of a metre rule. The root of the test plant was measured before introducing it to the treatment and also measured at the end of the experiment

2.9. Stolon length

The stolon length was measured using meter rule and it was measured before introducing it to treatment, and also at the end of experiment.

2.10. Plant height

The plant height was measured using meter rule. The measurement was taken from the tip of the longest leave to the root cap.

2.11. Fresh and dry weight determination

The fresh and dry weights were determined after fourteen (14) days of treatment. After recording all observations on day fourteen (14), the plants were separated into leaves, stem and root. The fresh weight was obtained after weighing using an electronic sensitive balance. Newspapers were used to package the various plant portions and thereafter labeled accordingly. The dry weight was also obtained by drying the plant parts packaged in the newspaper in a ventilated oven at 65^{0}C for 48 hours, after which dry weight was determined using an electronic sensitive balance.

2.12 Statistical Analysis

The results are the means $\pm$ S.E. of three independent replicates. All obtained data were subjected to statistical analysis using statistical package for social science (SPSS) version 20.0. Analysis of variance (ANOVA) was performed appropriate to the experimental design used. The post-hoc procedure employed was Duncan Multiple Range Test and data were represented in the form of Tables and bar chats.

3.0. Results and Discussion

The results on the effects of kerosene, gasoline and diesel on the leaf diameter of *Eichhornia crassipes* is shown in Tables 1, 2 and 3 respectively. There was a decrease in the mean value for all RPP as treatment concentration increases. Kerosene had more effects on the leaf diameter as compared to gasoline and diesel. From day 7, all the leaves in treatment 10%, 13% and 15% had withered off.

Table 1: Effect of kerosene on the leaf diameter of *E.crassipes*

Treatment % (v/v)		**Days**	**After**	**Treatment**	**(cm)**									
	1	**2**	**3**	**4**	**5**	6	7	8	9	10	11	12	13	14
0	8.71± 0.61^{a}	9.38± 0.37^{b}	9.40± 0.36^{b}	9.66± 0.36^{c}	9.67± 0.36^{c}	9.45± 0.38^{c}	9.38± 0.40^{b}	10.05 ±0.33^{b}	9.95± 0.31^{b}	10.00 ±0.32^{b}	10.00 ±0.32^{b}	10.00 ±0.32^{b}	10.00 ±0.32^{b}	10.00 ±0.32^{b}
10	8.05± 0.20^{a}	7.63± 0.17^{a}	7.51± 0.25^{a}	6.93± 0.43^{b}	6.28± 0.14^{b}	5.40± 0.17^{b}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0 .00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}
13	8.70± 0.35^{a}	8.05± 0.30^{a}	6.87± 0.79^{a}	5.55± 0.37^{a}	4.70± 0.45^{a}	3.99± 0.39^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}
15	8.53± 0.18^{a}	8.25± 0.14^{a}	7.80± 0.20^{a}	7.18± 0.44^{b}	6.66± 0.66^{b}	6.10± 0.64^{b}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}
	N.S	*	*	*	*	*	*	*	*	*	*	*	*	*

Key: N.S = Non significant (P<0.05)   * = Significant (P > 0.05)

Table 2: Effects of diesel treatments on the leaf diameter of *Eichhornia crassipes*

Treatment % (v/v)		**Days**	**After**	**Treatment**	**(cm)**									
	1	**2**	**3**	**4**	**5**	6	7	8	9	10	11	12	13	14
0	8.27± 0.41^{a}	8.57± 0.49^{a}	8.67± 0.49^{b}	8.78±0. 48^{b}	8.80± 0.49^{b}	8.83± 0.47^{b}	8.87± 0.47^{b}	9.25± 0.27^{b}	8.78± 0.33^{b}	8.78± 0.19^{b}	9.02± 0.04^{b}	8.93± 0.12^{b}	8.93± 0.12^{b}	8.97± 0.13^{b}
10	7.88± 0.49^{a}	7.63± 0.17^{a}	7.51± 0.25^{ab}	6.93±0. 43^{a}	6.28± 0.14^{a}	5.40± 0.18^{a}	5.40± 0.18^{a}	3.99± 0.27^{a}	3.69± 0.23^{a}	2.99± 0.11^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}

13	7.77± 0.18[a]	8.05± 0.30[a]	6.87± 0.79[a]	6.38±0. 60[a]	5.92± 0.77[a]	5.41± 1.02[a]	2.48± 2.48[a]	2.47± 2.47[a]	2.46± 2.46[a]	1.91± 1.91[a]	1.57± 1.57[a]	1.23± 1.23[a]	0.00± 0.00[a]	0.00± 0.00[a]
15	7.08± 0.80[a]	8.25± 0.14[a]	7.80± 0.20[ab]	7.18±0. 44[ab]	6.67± 0.66[a]	6.10± 0.63[a]	0.00± 0.00[a]	0.00± 0.00[a]	0.00± 0.00[a]	0.00± 0.00[a]	0.00± 0.00[a]	0.00± 0.00[a]	0.00± 0.00[a]	0.00± 0.00[a]
	N.S	**N.S**	*	*	*	*	*	*	*	*	*	*	*	*

Table 3: Effects of gasoline treatments on the leaf diameter of *Eichhornia crassipes*

Treatment % (v/v)		**Days**	**After**	**Treatment**	**(cm)**									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	9.37±0 .83[ab]	9.48±0 .54[ab]	9.58±0 .57[ab]	9.78±0. 71[b]	9.85± 0.64[b]	9.88± 0.91[b]	9.92± 0.64[b]	9.95±0 .61[b]	10.02± 0.56[a]	9.93± 0.67[a]	9.98± 0.67[a]	10.00± 0.65[a]	10.02± 0.66[a]	10.05± 0.67[a]
10	8.40±0 .45[a]	8.12±0 .42[a]	7.77±0 .63[a]	7.17±0. 41[a]	7.02± 0.37[a]	6.85± 0.34[a]	8.23± 0.28[a]	8.23±0 .28[ab]	8.27±0 .27[a]	8.10± 0.36[a]	8.13± 0.35[a]	8.03±0 .33[a]	8.10±0 .32[a]	8.07±0 .33[a]
13	8.37±0 .47[a]	8.28±0 .29[a]	8.62±6 .19[ab]	7.40±0. 25[a]	7.16± 0.24[a]	6.76± 0.35[a]	8.31± 0.92[a]	8.28±0 .93[ab]	5.95±3 .04[a]	5.95± 3.04[a]	5.94± 3.04[a]	5.90±3 .01[a]	5.90±3 .01[a]	5.87±3 .00[a]
15	10.47± 0.12[b]	9.97±0 .53[b]	9.97±0 .53[b]	8.17±0. 37[a]	7.83± 0.38[a]	7.35± 0.52[a]	3.40± 3.40[a]	3.47±0 .47[a]	3.37±3 .37[a]	3.35± 3.35[a]	3.34± 3.34[a]	3.34±3 .34[a]	3.34±3 .34[a]	3.27±3 .27[a]
	*	*	*	*	*	*	*	*	**N.S**	**N.S**	**N.S**	**N.S**	**N.S**	**N.S**

The impacts of refined petroleum products kerosene gasoline and diesel on the root length, chlorophyll content index, stolon length, plant height, stolon girth and biomass are shown in Figures 1-8. All growth parameters were adversely altered as concentration of refined petroleum increased. Kerosene had more adverse effects on the parameters measured as compared to gasoline and diesel.

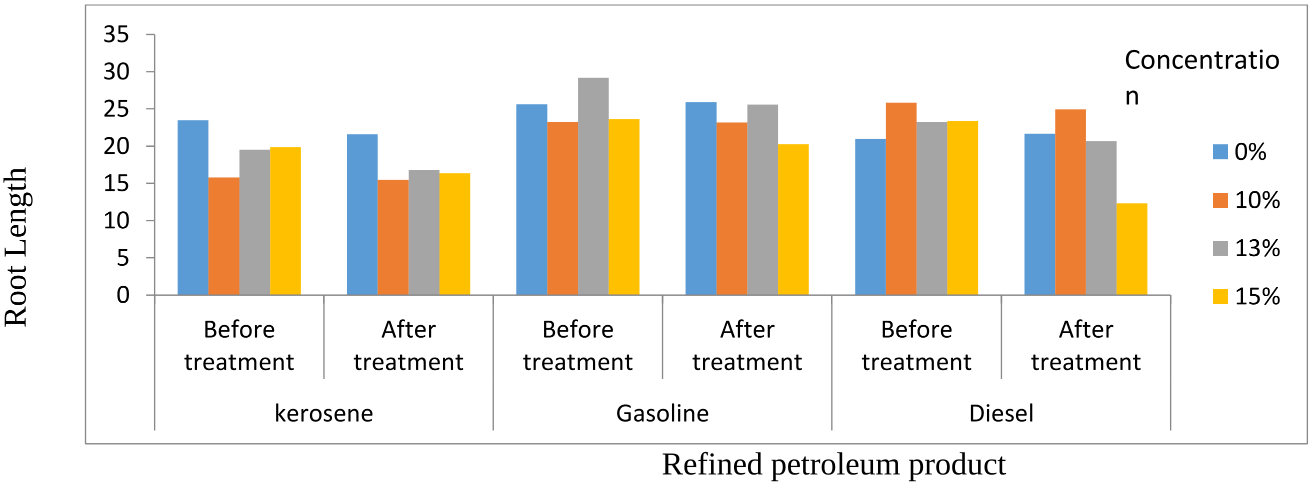


Fig 1: Effects of refined petroleum products on the root length of *Eichhornia crassipes*

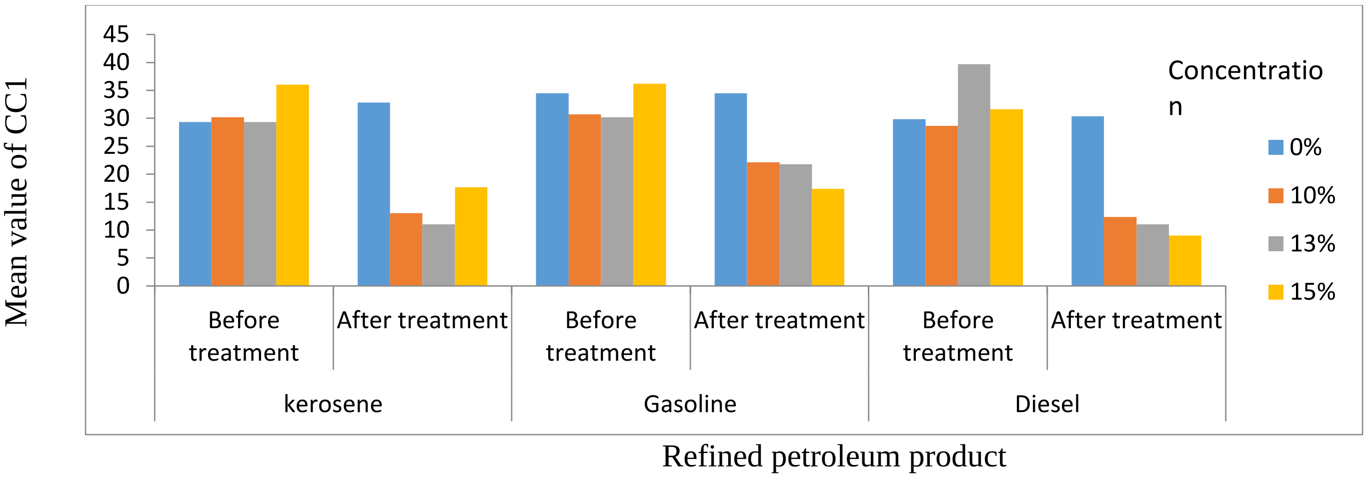


Fig 2: Effects of refined petroleum products on the chlorophyll content index of *Eichhornia crassipes*

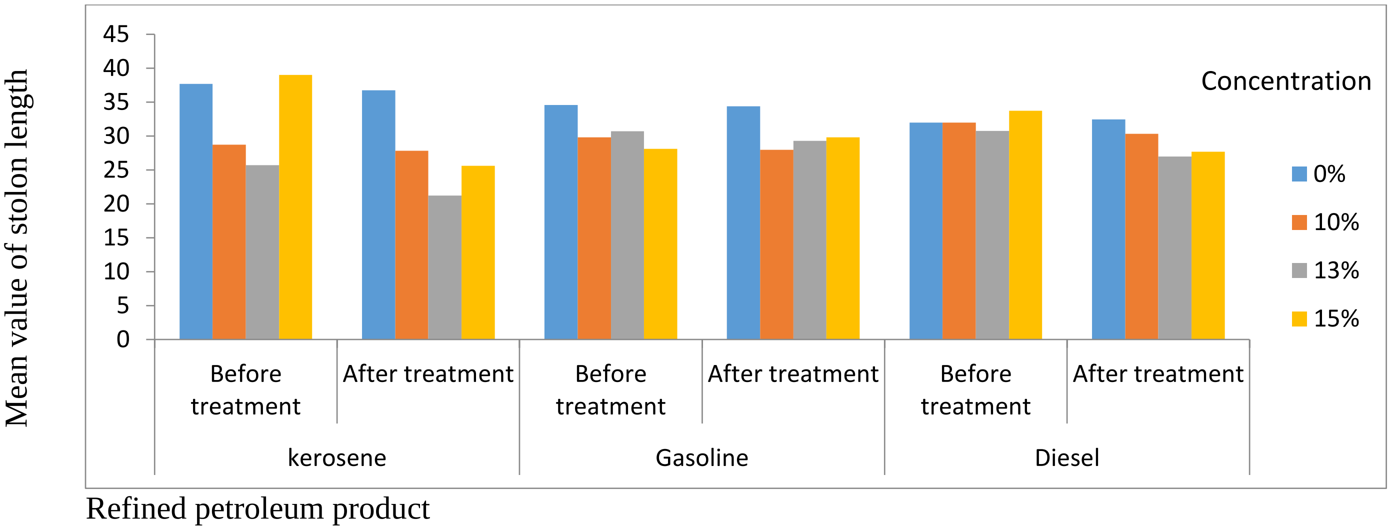


Fig 3: Effects of refined petroleum products on the stolon length of *Eichhornia crassipes*

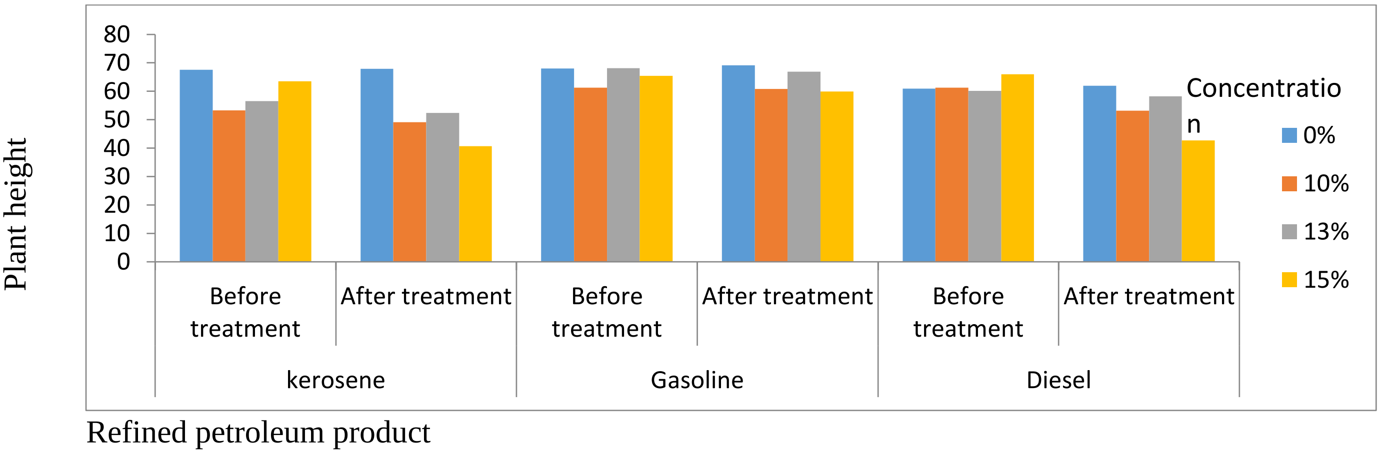


Fig 4: Effects of refined petroleum products on the plant height of *Eichhornia crassipes*

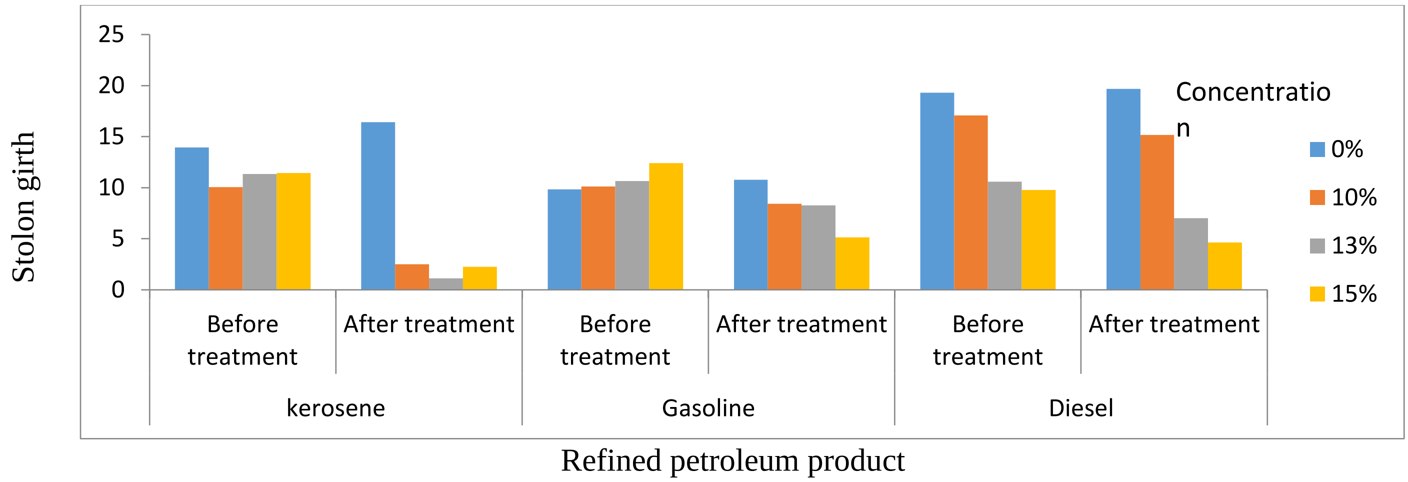


Fig 5: Effects of refined petroleum products on the stolon girth of *Eichhornia crassipes*

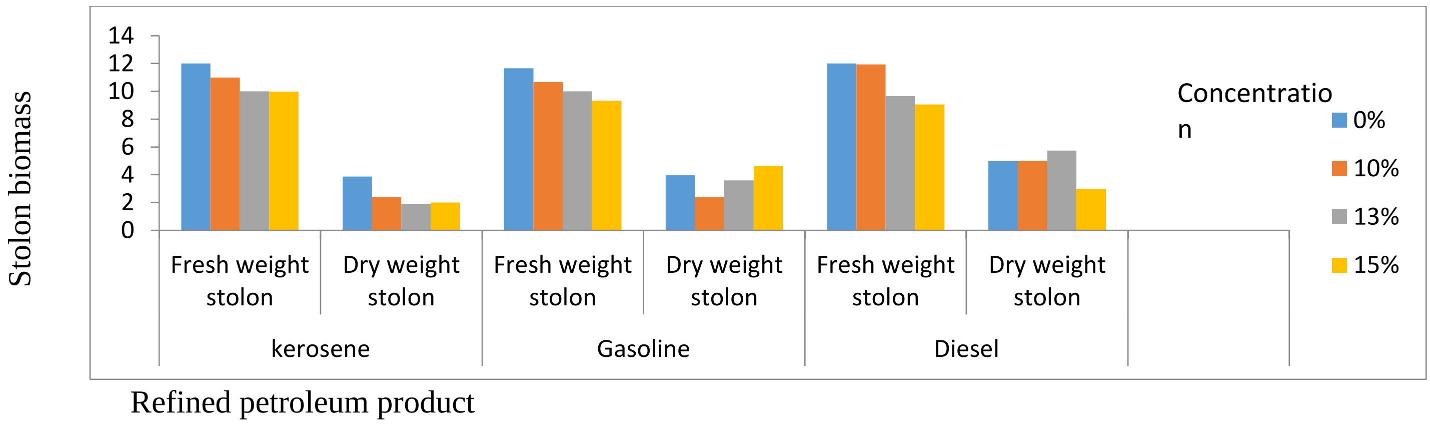


Fig 6: Effects of refined petroleum product the stolon biomass of *Eichhornia crassipes*

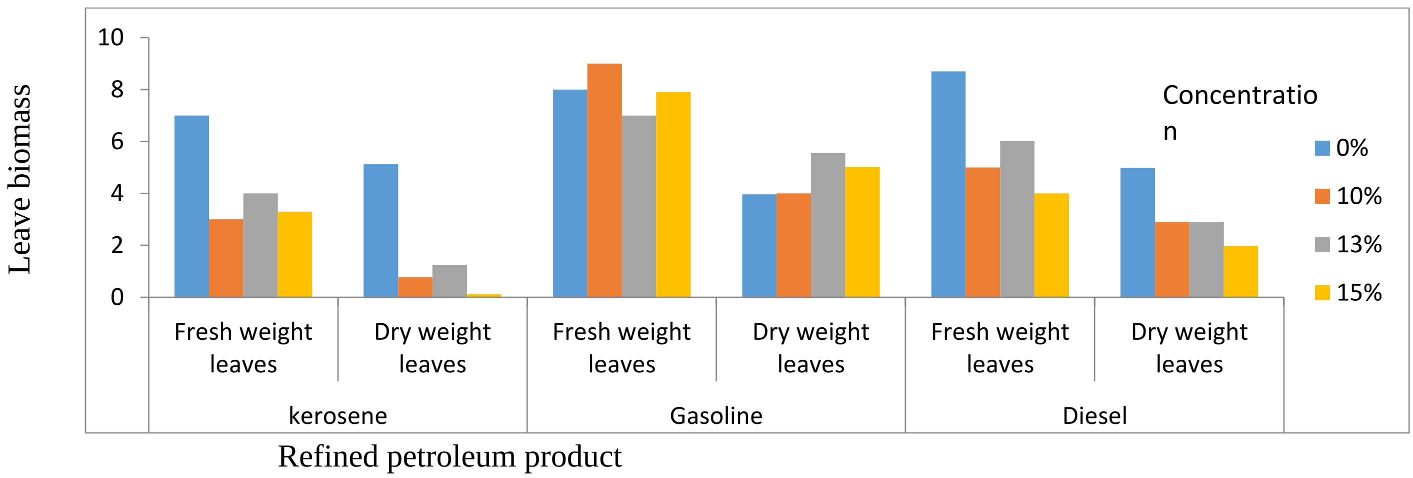
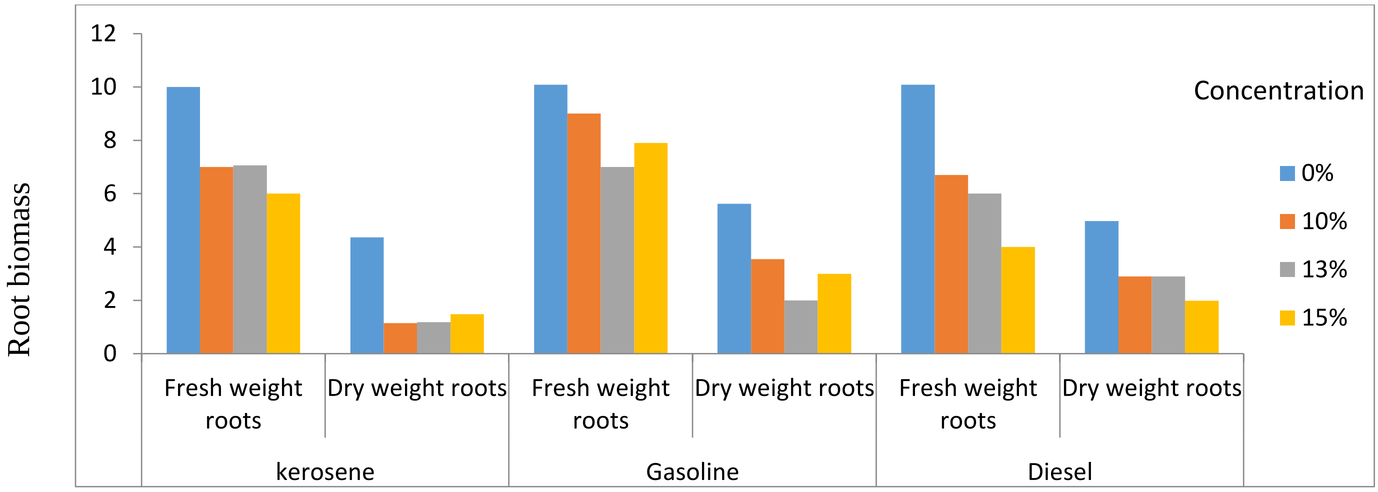


Fig 7: Effects of refined petroleum product the leave biomass of *Eichhornia crassipes*



Refined petroleum product

Fig 8: Effects of refined petroleum product the root biomass of *Eichhornia crassipes*

Table 4: Morphological changes observed in *Eichhornia crassipes* in different refined petroleum products

Treatments	Days duration	Observations
Control	Day 3	Plant growth remain healthy
	Day 6	Healthy plant with viable green leaves
	Day 9	Plant growth continued. New plants formed
	Day 12	New plants formed.
	Day 14	Formation of more plants, healthy and green in colour
Kerosene	Day 3	Plant green in colour, weakness observed at the point of attachment of leaves.
	Day 6	Change in colour of leaves without noticeable growth. Chlorotic spots noticed on leaf surface.
	Day 9	Detachment of some leaves and chlorosis was noticed on the adaxial surface of the leaves. Death of leaves were noticed
	Day 12	Death of leaves continued
	Day 14	Degradation death and chlorosis, necrotic spots noticed on leaves, plants became stunted and leaves were all withered.

Gasoline	Day 3 Day 6 Day 9 Day 12 Day 14	Slight change in terms of formation of new leaves change noticed in plants Decrease in growth but more green coloured leaflets observed. Chlorotic spots observed on leave surface but plant did not die Decrease in plant growth was observed and chlorotic spots seen on some older leave surface Chlorotic spots observed on leaf surface.
Diesel	Day 3 Day 6 Day 9 Day 12 Day 14	No noticeable change in plant growth Stunted growth noticed and chlorotic lesions observed. Necrotic spots noticed on leave surface Decrease in plant growth noticed and leaf abscission was noticed. Some older leaves died and newer leaves had necrotic spots Leaf detachment continued and plants became more stunted.

The physical and chemical characteristics of such an aquatic habitat can be altered when there is an introduction of refined petroleum products into such habitats. The result of this study shows that refined petroleum products of kerosene, diesel and gasoline have adverse effects on *Eichhornia crassipes*. The difference among the plants exposed to diesel, kerosene and gasoline from the results shows that the different petroleum products affect plant growth in different concentrations. It also shows though the petroleum products are phytotoxic but their toxicity varies.
E.crassipes exposed to different concentration of kerosene, diesel and gasoline shows a significant decrease in leaf diameter as shown in Table 1, 2 and 3. The leaf diameter was greatly affected by kerosene and diesel compared to gasoline. High concentration (15%) of kerosene, diesel and gasoline cause decrease in the leaf diameter of *E.crassipes* indicating their degree of sensitivity to oil pollution; however, at 0%, there was increase in the leaf diameter suggesting that control condition enhance the growth of *E. crassipes*. The reduction in the leaf diameter recorded corresponds with the finding of [8] who observed that exposure of this species to Urucu crude oil concentration between 0.08 and 15.89 $L.m^{-2}$ reduce the number and size of leaves. This also agrees with the work of [9], which stated that the exposure of *Ischaemum rogosum* to gasoline caused reduction of plant growth characters; and this could be attributable to a reduction in carbon fixation consequent upon oxygen tension. The result shows that there was varied plant mortality from day 7, which agrees with the work of [10] who also observed a significant reduction of individuals of *Spartina rapatens* after they were exposed to $2lm^{-2}$ of South Louisiana crude oil. The growth of *E.crassipes* is retrogressively affected by kerosene, diesel and least by gasoline. This effect is dependent on the concentration. For instance, at 10%, 13% and 15%, concentration with kerosene,

the leaves of the test plants from day 7 had necrotic spots. This is as compared to diesel where the leaves all withered from day 11 for 10% and day 7 for 15%. The leaves of the test plant for gasoline only showed chlorotic spots but death of leaves did not occur. All physical parameters measured (such as root length, chlorophyll content index, stolon length, plant height, stolon girth, and biomass production) declined during growth in the presence of kerosene, diesel and gasoline control excluded. This observation agrees with the work of [11] who showed that plants growing in oil polluted soil were generally retarded and showed chlorosis of leaves. They attributed some of the effects to dehydration and general water deficiency. Retardation of growth at high levels of oil pollutant was observed by [12] although using terrestrial plants. This also agrees with the work of [13] who observed that a little dose of crude oil from 10ppm was inhibitory to the growth of *Pistia stratiotes*. According to [14], phytotoxicity of a contaminant depends on the uptake potential, biochemistry reactivity and exposure dose which may correspond to different degrees of internal dose in different species or individuals according to rates of entry, distribution within the plants, environmental conditions and many other factors. All the test plants in the treatments with diesel, kerosene and gasoline at different volume concentrations were observed to be changing in both morphology and physical appearance after some days. Changes was first noticed in the leaf with kerosene treatment from day 3 but more intense change was seen in the leaf with kerosene treatment at day 7and this was most detrimental to the test plant as intense wilting, chlorosis and necrotic spots were noticed on the adaxial surface of the leaf.

There was a significant reduction in the chlorophyll content in the leaves of the test plant. The apparent chlorosis which is as a result of the reduced chlorophyll content may be an implication of heavy metals absorbed by the plant. This corresponds to the work of [15] who worked on phytoremediation potentials of *E. crassipes* in crude oil polluted water. This also agrees with the work of [4], who studied the survival of *E crassipes* exposed to two refined petroleum products. This is also in accordance with the work of [7]. They studied the effects of two refined petroleum products on the growth response, survival and mineral nutrient relations of *Saccioleis africana*. Fresh and dry weight biomass was also affected by other treatments as compared to control. This also agrees with the work of [4]. According to them, a decrease in chlorophyll content can affect the biomass of plants when they are exposed to refined petroleum products. This also agrees with the work of [3] who studied the effects of crude oil on the morphological characteristics of *E. crassipes*. *E crassipes* plants introduced in control treatment had the highest values in all growth variables considered and they were significantly ($p \geq 0.05$) greater than those exposed to other treatments.

4.0. Conclusion

In conclusion, the finding of this study shows that concentration and duration of exposure are the factors that determine effect of petroleum products on *E. crassipes*. The study indicates that high concentrations of kerosene, diesel and gasoline have detrimental effects on *E.crassipes*. Kerosene has more effect on the test plant than diesel and diesel has more effect than gasoline. Although the test plant was able to survive to moderate concentration at short period of exposure, conditions exceeding this concentration and duration will affect the plant growth as well as high mortality. Proper measures should be put in place to prevent the intentional release of these refined products in to the environment.

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