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Effects of two Refined Petroleum Products on the Growth Response, Survival and Mineral Nutrient Relations of *Sacciolepis africana* (Hubb and Snowden)

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Authors' contributions

This work was carried out in collaboration between all authors. Author JFB designed the study. Author IAO managed literature search and also performed the statistical analysis. Author MOE wrote the manuscript. All authors read and approved the final manuscript

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ABSTRACT

This study was carried out to investigate the effect of refined petroleum products (diesel and gasoline, RPPs) on *Sacciolepis africana* (Hubb and Snowden). The parameters measured include number of leaves, plant height, biomass production, chlorophyll content index, leaf area and mineral nutrient content. The refined petroleum concentrations used were 0.2, 0.4, 0.6 and 0.8% (v/w). The results showed that leaf and biomass production, chlorophyll content index and mineral nutrients were significantly reduced (P<0.05) in *S. africana* when exposed to all concentrations of the RPPs for eight weeks. A gradual decrease in height of the test plant was also noticed. Leaf area was reduced as concentration increased. The effects were concentration and medium dependent. RPP of gasoline had more toxic effect on the test plant. The leaves of the test plant also showed signs of chlorosis and wilting. This study has been able to demonstrate that *S.*

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africana can play a role in phytoremediation at low levels of pollution and also that the test plant can act as a bio indicator of polluted water body. It is recommended that proper measures be put in place to prevent release of these products into the environment where it can adversely affect plants thus disrupting the ecosystem balance.

Keywords: Refined petroleum products; Sacciolepis africana; gasoline; diesel.

1. INTRODUCTION

The development of the world today is closely associated with, and it's driven by the discovering of crude oil which provides the basic energy needed in manufacturing, transport, agricultural and other sectors of the economy. The importance of petroleum oils to Nigeria and the world in general cannot be over emphasized. Oil presently accounts for over 90% of the annual revenue in Nigeria [1].

Refined petroleum products are derived from crude oil through a process such as catalytic cracking and fractional distillation. These products have physical and chemical characteristics that differ according to the type of crude oil and subsequent refining processes. Exploration, production, and transportation of petroleum oils and its refined products had the potential to pollute the environment. Oil exploration and production have several consequences for the environment. Oil pollution problems are one of the major problems faced by coastal ecosystems. Oil development activities have led to multiple ecological impacts on wetlands and coastal ecosystems through the various stages which include pipeline installation, spills and clean up and site closure. Oil pollution is the unintentional release of liquid petroleum hydrocarbon into the environment as a result of human activity. Oil pollution which arises mainly from oil spills has serious implication for biodiversity as most biotic habitat is either destroyed or altered making them unsuitable for habitation. The major sources of oil pollution include drilling accidents, transportation accidents affecting tankers, leakages from refineries and storage tanks and leakages from pipelines. Some of these oil production facilities have been in use for decades without replacement. Fifty percent (50%) of oil spill are due to corrosion. [2] stated that between 1976 and 1997, there have been many spillages releasing about 2.8 million barrels of oil into the land, swamps, estuaries and coastal waters of Nigeria. According to [3], oil pollution is the introduction by man directly or indirectly any hydrocarbon materials especially crude oil and its refined products into the environment.

Diesel fuel is a liquid used in diesel engines. Diesel is obtained from fractional distillation of crude oil between 200℃ and 350℃ resulting in a mixture of carbon chains that typically contain between 8 and 21 carbon atoms per molecule. The short term hazards of the lighter more volatile compounds such as toluene, ethyl benzene and xylenes in diesel include toxicity to aquatic life especially in confined areas. Diesel spills on agricultural land generally reduce plant growth [4]. Diesel reduces soil fertility and soil micro flora population [5,6] also reported that the addition of diesel oil to the soil led to a significant reduction of organic content of the soil. Long term effects include contamination of ground water. Diesel fuels could result in potential acute toxicity to some forms of aquatic life.

Gasoline also called petrol or premium motor spirit consists of aliphatic hydrocarbons contained by fractional distillation of petroleum. A typical gasoline consists of hydrocarbons with between 4 and 12 carbon atoms per molecule. The manner in which petroleum acts on plants is complex and involves both direct contact and indirect effects mediated by the interactions of petroleum hydrocarbon with abiotic and microbial components of the habits. Some of the indirect effects of petroleum products on plants include oxygen deprivation.

Oil pollution has negative impact on plant survival. Floating oil covers the water surface and prevents oxygen from dissolving in water. Crude oil and its refined products also contain toxic compounds which interfere with nutrient uptake, photosynthesis and other biochemical processes resulting in poor growth or outright mortality of plants [7,8]. Oil degrading organisms also compete with plants for mineral nutrients [9]. Oil pollution adversely affects the availability of the mineral by encouraging the rapid growth of soil microorganism which immobilizes soil mineral nitrogen and this may cause yellowing of the leaves [10]. Studies have also indicated that oil pollution have negative impact on plant survival and biomass production [11]. Defoliation and mortality of mangroves trees have been observed in swamps affected by oil spills. Seedlings of red mangroves have been reportedly killed while the pneumatophores or lenticels in their prop roots are completely smothered by oil resulting in oxygen depletion [12]. According to [13], the degree of oil impact on plants depends on various factors such as the type and amount of oil, the extent of oil coverage, the plant species, the season of spill and the habitat composition. The application of different concentrations of diesel oil into the soil resulted in the reduction in seed germination and primary root length of peanut, cowpea (*Vigna unguiculata*), sorghum (*Sorghum bicolor*) and corn (*Zea mays*) as observed by [14,15] reported that waste pit soil from drilling waste dumps in Kutchalli oil drilling area of Nigeria completely inhibited the germination of maize and bean seeds. Plant parameters such as plant height and number of leaves were reduced compared to control. [16] made similar observations on growth and anatomical feature of *Chromolaena odorata* grown in soil treated with crude oil. [17], noticed reduction in chlorophyll and protein levels in *Amaranthus hybridus* grown in soil contaminated with engine oil. [18] observed that diesel oil inhibited the growth of cereals causing chlorosis and dehydration. [19] also noticed a reduction in the leaf area of *Paspalum vaginatum* in soil contaminated with crude oil in both oil pretreated and post treated experiments. Adverse effects of aquatic pollution include loss of species diversity, loss of habitat, destruction of breeding grounds of aquatic organisms and sometimes death of organism including man.

Sacciolepis africana is a glabrous perennial grass up to 2 m high, with rhizomes and spongy culms that root profusely from the lower nodes. It reproduces from seeds. The leafy blade is about 15-30 cm long and 0.5-1.5 cm wide, and narrowed at the base. It belongs to the family Poaceae [20]. The objective of this research was to access the effects of two refined petroleum products on the growth and mineral content of *S. africana* and also to access the potential of *Sacciolepis africana* being used for phytoremediation.

2. MATERIALS AND METHODS

2.1 Study Site

The study was carried out in the screen house of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria. *S. africana* of the same height collected from Ikpoba hill river, Benin City, Edo State were planted in a bowl filled with loam soil. These were planted in five replicates for the two treatments (diesel and gasoline). The treatment concentrations consist of 0% which served as control, 0.2, 0.4, 0.6 and 0.8% (v/w) each of gasoline and diesel. The plants were watered and grown for eight weeks after which the experiment was terminated.

Sources of gasoline and diesel fuel: Gasoline and diesel fuel used for this experiment was obtained from total filling station along Mission road Benin City, Nigeria.

Data recording: The following data were collected during the experiment.

Number of leaves: The leaves of the plant were counted weekly.

Height of plant: The height of the plants was measured weekly using a meter rule.

Length and breadth of leaves: The length and breadth of the leaves were measured using a measuring tape.

2.2 Fresh and Dry Weight Determination

The fresh and dry weights were determined after 8 weeks of treatment following the method of Hunt, [21]. At harvest the root system was retrieved by immersing in water and washing carefully. The plants were separated into roots, stem, leaves and fruit. Medium sized brown envelopes were purchased from the market for packaging the various plant portions. One (1) envelope from the pack was first weighed in the electronic balance to determine the weight of the envelope. The fresh weight was obtained after packaging the fresh portions in envelops and weighed thereafter. The dry weight was obtained by drying the plant parts packaged in envelops in a ventilated oven at 70℃ for 24 h, after which dry weight was determined using a sensitive electronic weighing balance.

Determination of leaf area: The leaf area was measured for each leaf per plant per pot. This was done by using a measuring tape to measure the length and breadth of each leaf. Leaf area was calculated as 1/2 BXH.

2.3 Chlorophyll Content Index Determination

Chlorophyll content index of the leaves was measured using the Apogee chlorophyll content meter CCM-200 plus. Measurement was done by holding down the arm of the sample head on the intact leaf until a beep was heard. The chlorophyll content was displayed on the screen of the device.

2.4 Elemental Analysis

Elemental analysis was carried out at the Central Analytical Laboratory, Nigerian Institute for Oil Palm Research, NIFOR to determine the level of N, P, K, Ca, Mg, Na, and Fe in the leaves of *S. africana.* Ca, Mg, and Fe, content were determined with atomic absorption spectrometer, bulk Scientific VGP 210. Na and K was determined using flame photometer while P and N were determined using colorimetric method.

2.5 Statistical Analysis

The results are the means±S.E. of at least 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.

3. RESULTS

The results on the effects of refined gasoline and diesel oil on the number of leaves and plant height of *S. africana* is shown in Figs. 1 to 4. There was a decline in number of leaves and plant height as concentration of the refined product increased. There was a gradual decline in the number of leaves and plant height of *S. africana* treated with diesel oil compared to that treated with gasoline oil.

The effects of refined diesel and gasoline oil on the fresh and dry weight content of the leaves, roots and stem of *S. africana* is presented in Tables 1 to 6.

The effects of refined gasoline and diesel oil on the chlorophyll content of *S. africana* are shown in Figs. 5 and 6. A decrease in the value of chlorophyll content was noticed as concentration of refined oil increased.

The effect of refined gasoline and diesel oil on the leaf area of *Sacciolepis africana* is shown in Fig. 7. The leaf area of the test plant was affected more by gasoline.

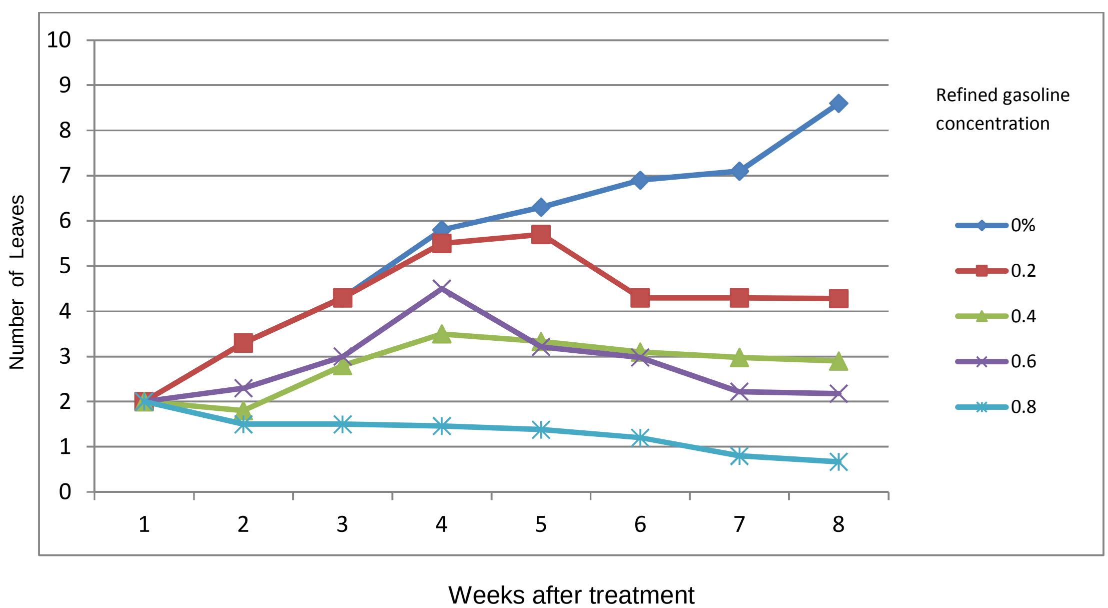
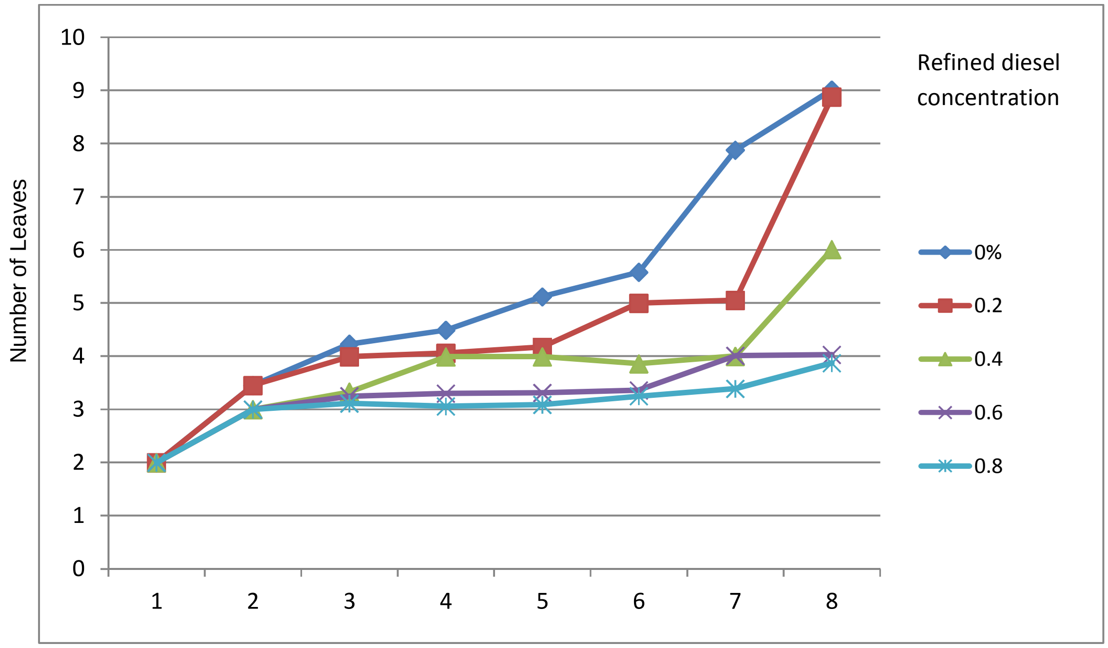
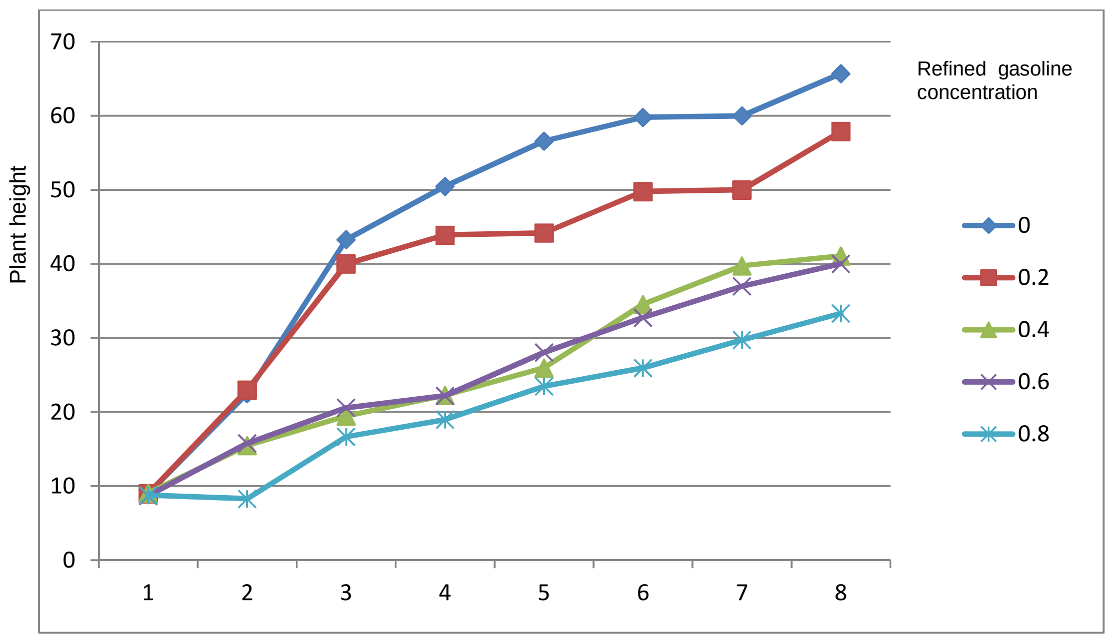


Fig. 1. Effects of refined gasoline oil on the number of leaves of *S. africana*



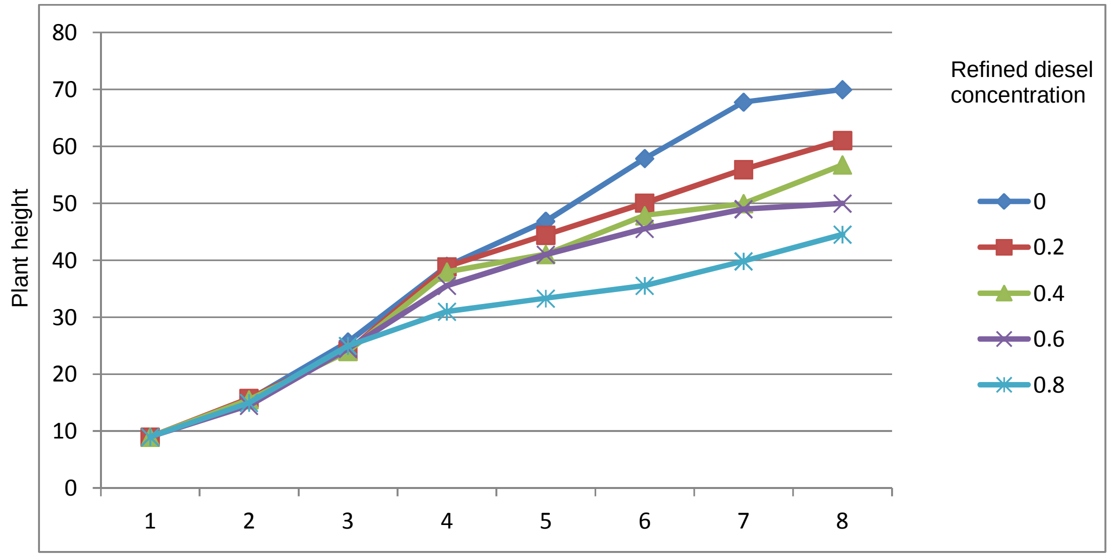
Weeks after treatment

Fig. 2. Effects of refined diesel oil on the number of leaves of *S. africana*



Weeks after treatment

Fig. 3. Effects of refined gasoline on the height of *S. africana*



Weeks after treatment

Fig. 4. Effects of refined diesel on height of *S. africana*

Table 1. Fresh weight and dry weight of *S. africana* leaves after diesel treatment

Treatment % (v/w)	Fresh weight (g)	Dry weight (g)
0.0	6.70±1.78[a]	1.11±0.38[a]
0.2	3.86±0.37[a]	0.70±0.05[a]
0.4	3.50±0.11[a]	0.65±0.22[a]
0.6	3.38±0.33[a]	0.10±0.42[a]
0.8	3.32±0.42[a]	0.07±0.28[a]
	N.S	N.S

Key: Each value is a mean±standard error of five replicates. Means within the same column followed by the same letter are not significantly different at (P>0.05) from each other using New Duncan Multiple Range Test.

Table 2. Fresh weight and dry weight of *S. africana* leaves after gasoline treatment

Treatment % (v/w)	Fresh weight (g)	Dry weight (g)
0.0	4.67±0.90[a]	1.09±0.31[a]
0.2	3.51±0.60[a]	0.83±0.14[a]
0.4	2.10±0.34[a]	0.74±0.17[a]
0.6	1.58±0.357[a]	0.64±0.44[a]
0.8	0.95±0.19[a]	0.30±0.18[a]
	*	*

Key: Each value is a mean±standard error of five replicates. Means within the same column followed by the same letter are not significantly different at (P>0.05) from each other using New Duncan Multiple Range Test.

Table 3. Fresh weight and dry weight of *S. africana* stem after diesel treatment

Treatment % (v/w)	Fresh weight (g)	Dry weight (g)
0.0	5.59±0.78[a]	3.02±1.25[a]
0.2	5.75±0.63[a]	2.63±0.61[a]
0.4	4.56±0.30[a]	2.48±0.38[a]
0.6	4.53±0.70[a]	1.42±0.23[a]
0.8	3.83±0.69[a]	1.35±0.22[a]
	N.S	N.S

Key: Each value is a mean±standard error of five replicates. Means within the same column followed by the same letter are not significantly different at (P>0.05) from each other using New Duncan Multiple Range Test

Table 4. Fresh weight and dry weight of *S. africana* stem after gasoline treatment

Treatment % (v/w)	Fresh weight (g)	Dry weight (g)
0.0	4.67±0.90[a]	1.09±0.31[a]
0.2	3.51±0.60[a]	0.83±0.14[a]
0.4	2.10±0.34[a]	0.74±0.17[a]
0.6	1.58±0.357[a]	0.64±0.44[a]
0.8	0.95±0.19[a]	0.30±0.18[a]
	N.S	N.S

Key: Each value is a mean±standard error of five replicates. Means within the same column followed by the same letter are not significantly different at (P>0.05) from each other using New Duncan Multiple Range Test

Table 5. Fresh weight and dry weight of *S. africana* root after diesel treatment

Treatment % (v/w)	Fresh weight (g)	Dry weight (g)
0.0	10.10±1.62[b]	4.33±1.10[b]
0.2	6.31±1.27[b]	3.00±0.65[ab]
0.4	5.91±2.44[a]	1.97±1.19[ab]
0.6	4.85±0.45[a]	1.67±0.19[a]
0.8	3.15±1.05[a]	0.92±0.32[a]
	*	*

Key: Each value is a mean±standard error of three replicates. Means within the same column followed by the same letter are not significantly different at (P>0.05) from each other using New Duncan Multiple Range Test

Table 6. Fresh weight and dry weight of *S. africana* root after gasoline treatment

Treatment % (v/w)	Fresh weight (g)	Dry weight (g)
0.0	6.11±0.84[b]	1.72±0.50[a]
0.2	5.05±1.04[ab]	1.34±0.47[a]
0.4	4.43±1.14[ab]	1.32±0.57[a]
0.6	2.30±0.80[a]	0.67±0.25[a]
0.8	1.95±0.13[a]	0.35±0.13[a]
	*	N.S

Key: Each value is a mean±standard error of three replicates. Means within the same column followed by the same letter are not significantly different at (P>0.05) from each other using New Duncan Multiple Range Test

Table 7. Nutrient accumulated in the leaves of *S. africana* exposed to refined gasoline and diesel oils

RPP	Conc of RPP (v/w)	N(%)	P(%)	K (%)	Ca(%)	Mg(%)	Na(%)	Fe(ppm)
Gasoline	0.0 (control)	0.125	0.049	2.18	0.67	0.58	0.16	1.20
	0.2	0.089	0.039	1.34	0.62	0.27	0.13	0.60
	0.4	0.041	0.016	1.28	0.46	0.16	0.09	0.90
	0.6	0.040	0.011	1.21	0.43	0.13	0.08	0.60
	0.8	0.039	0.011	1.10	0.24	0.09	0.10	0.47
Diesel	0.0	0.182	0.049	2.11	0.68	0.30	0.14	1.10
	0.2	0.152	0.025	1.47	0.64	0.27	0.11	1.10
	0.4	0.126	0.021	1.41	0.62	0.19	0.10	1.00
	0.6	0.050	0.019	1.34	0.57	0.17	0.09	1.00
	0.8	0.040	0.018	1.20	0.55	0.17	0.07	0.60

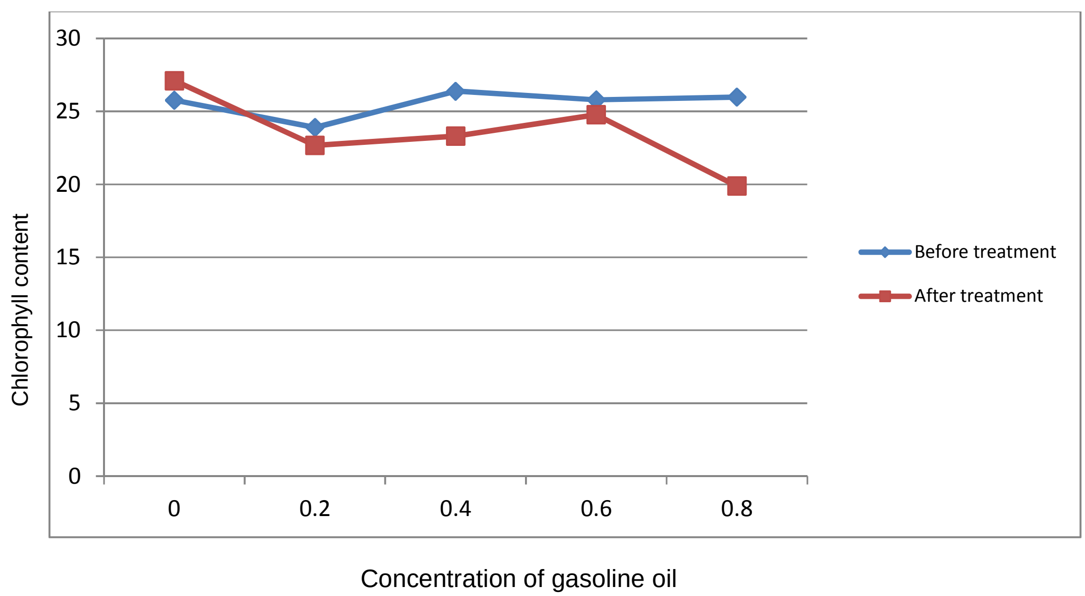


Fig. 5. Effects of refined gasoline oil on the chlorophyll content index of *S. africana*

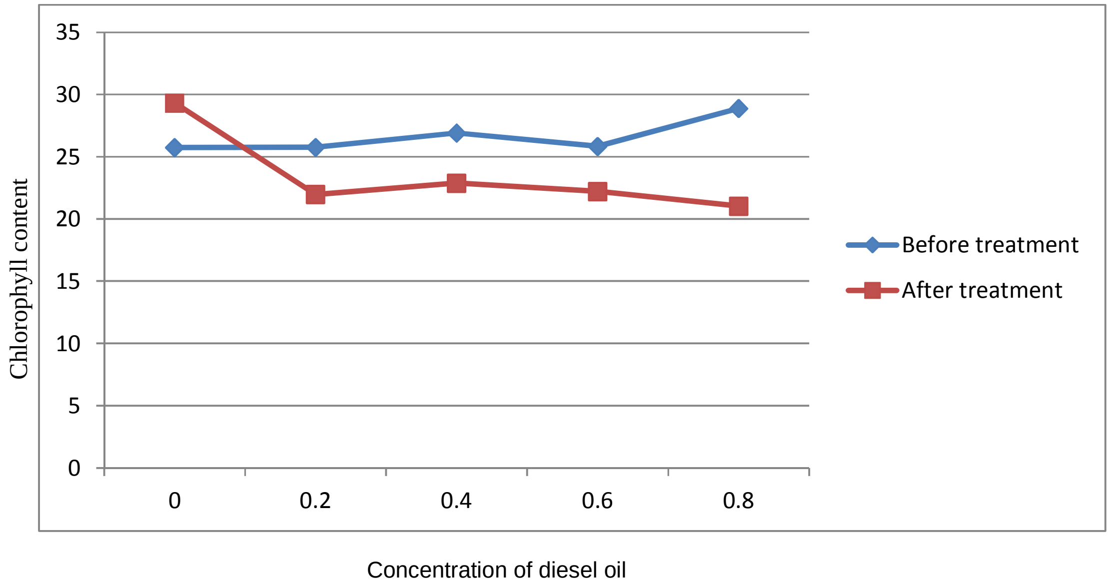


Fig. 6. Effects of diesel oil on chlorophyll content of *S. africana*

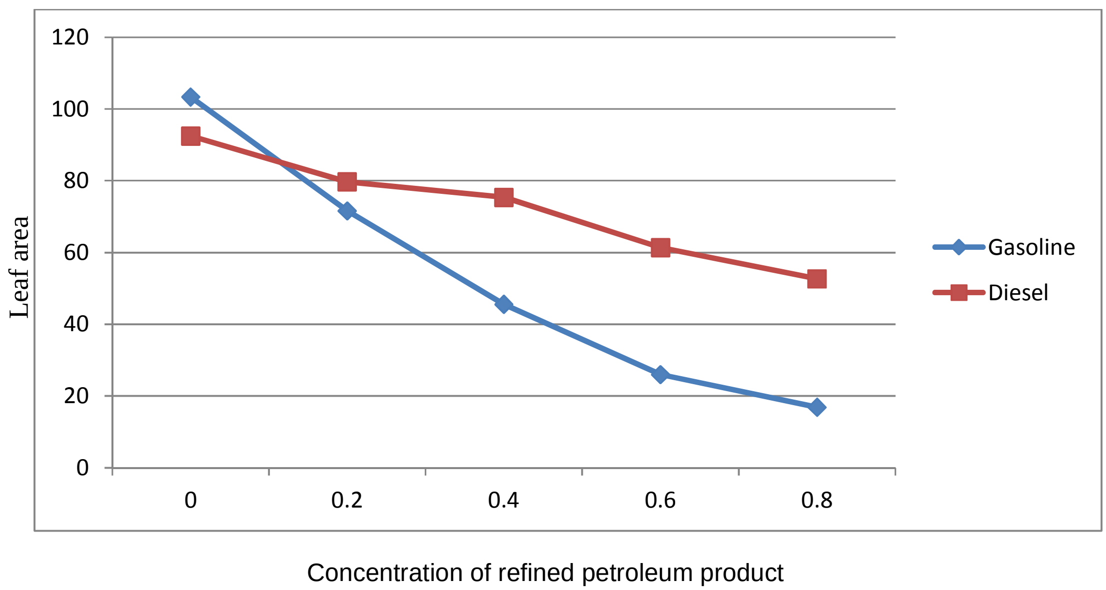


Fig. 7. Effects of gasoline and diesel on leaf area of *S. africana*

4. DISCUSSION

The introduction of refined products such as gasoline and diesel into a wetland environment or ecosystem can change the chemical and physical characteristics of such ecosystems [22,23,24]. Gasoline diesel fuel mixture like the other petroleum products adversely affects the growth and performance of plants as indicated in the results. The differences between the plants exposed to diesel and gasoline shows that the different petroleum products affect plant growth in different ways. It also shows that they though products are phytotoxic, their phytotoxicity vary. The result of the present study shows that refined petroleum products of diesel and gasoline had concentration dependent effects on *S. africana*. When exposed to concentrations of 0.2, 0.4, 0.6 and 0.8% (v/w) of gasoline and diesel products, there was a reduction in the number of leaves (Fig. 1) when compared to control. However, there was no significant difference between the treatments. Leaf number was greatly affected by gasoline treatment as compared to diesel treatment. There was a gradual reduction in the number of leaves treated with diesel from week 1 to week 8 as compared to a rapid reduction in the number of leaves treated with gasoline (Figs. 1 and 2). At weeks 7 and 8 concentration of 0% and 0.2% leaves exposed to diesel treatment had almost the same value of 9.01 and 8.88 respectively. This corresponds with the work [7] which stated a negative interaction was observed in *Ischaemum rogosum* between plants parameters measured and the level of gasoline applied to the soil. This also supports the work of [25] who studied the effects of gasoline diesel mixture on the growth and germination of *Vigna unguiculata.*

The effect of refined petroleum products of gasoline and diesel on *S. africana* also resulted in the decrease in fresh and dry weight production as shown in Tables 1 to 6. This may be due to disruption in the plant's photosynthetic rate and hence its rate of dry matter accumulation as reported by [7]. This result is also in accordance with the observation of [26] who demonstrated that the biomass of *Spartina patens* was significantly reduced with increasing oil levels in soil. It also in line with the work of [27] who stated that there is a decline in fresh and dry weights of *E. crassipes* when exposed to increased concentrations of refined petroleum products. This also corresponds with the work of [28] which showed that treatment of soils with crude oil, automotive gasoline oil and spent engine oil significantly affected the time of germination, percentage germination, plant height, leaf production and biomass of *V. unguiculata* delaying germination and growth rate. [29] made similar observation when they studied the effect of diesel fuel on the growth of *Nerlum oleander*, beach naupaka, false sandalwood, common ironwood, kou, milo and kiawe. Diesel oil contaminated soil sample shows a negative impact on the fresh weight of the *S. africana*. This maybe that the contaminated soil affects the uptake of minerals and water from the soil as the concentration of the diesel oil increase, thereby affecting the fresh weight of the plant.

Wilting and chlorosis of leaves were also one of the visible effects of gasoline and diesel on *S. africana*. There was significant reduction in chlorophyll content as concentration of refined products increased as shown in Figs. 5 and 6. The apparent chlorosis, a result of the reduced chlorophyll content index may be an implication of heavy metals absorbed by the plant [30]. This observation is also in line with the findings of [31] and [27]. The effect of diesel and gasoline on the leaf area of *S. africana* shows that with increasing concentration there was a reduction in the leaf area of the plant. This corresponds with the work of [25] that noticed a decrease in leaf area, leaf area ratio and relative growth rate of *V. unguiculata* due to gasoline/diesel mixture pollution of soil. This indicates that the mixture interrupts with the growth of the plant. According to [32] the leaf surface area determines in large part the amount of carbon gained through photosynthesis and the amount of water lost through transpiration and ultimately the crop yield. Therefore, the reduction of the leaf area and the leaf area ratio as was observed in this study implies that there would be low a photosynthetic efficiency of the plant as much of the solar energy emitted by sun would not be absorbed by plant for photosynthesis

The effect of refined petroleum products on the mineral nutrient content of leaves of *S. africana* was determined at termination of the experiment. The elemental analysis result (Table 7) reveals loss of nutrient from plant leaves as concentration increased. Nutrient such as Nitrogen (N), Potassium (K), Magnesium (Mg), Phosphorus (P), Iron (Fe) and Calcium (Ca) were reduced when compared to control. According to [33] nitrogen is an essential macronutrient needed by all plants to thrive. It is an important component of many structural, genetic and metabolic essential macronutrients needed by all plants to thrive. It is an important component of many structural, genetic and metabolic compounds in plant cells. It is also one of the basic components of chlorophyll, the compound by which plants use sunlight energy to produce sugar during the process of photosynthesis. The control plant had the highest nitrogen availability which indicates that there was high photosynthetic activity compared to the plant with 0.8% concentration in which the nitrogen composition was the lowest, it shows that photosynthetic ability was reduced. Available Fe in the leaves shows a positive increase for the control plant and Iron is needed to produce chlorophyll, hence its deficiency causes chlorosis, and leaf chlorosis will compromise the photosynthetic capability of plants. This is in agreement with the work of [27]. This also explains the decline in chlorophyll content of treated plants compared to control (Figs. 5 and 6) [34]. Showed that the presence of oil in the soil significantly decreased the available forms of phosphorus and potassium to plants. These nutrients (nitrogen, phosphorus, potassium and oxygen) are essential to plat growth and development hence reduction in their bioavailability will lead to reduced plant growth.

5. CONCLUSION

The findings of this study indicate that high concentrations of refined gasoline and diesel have detrimental effects on *S. africana*. The effects were also concentration dependent. The test plant is more susceptible to gasoline than diesel. Wetland plants are very important because of their eco system functions and they play a major role in phytoremediation at low levels of oil pollution. Delay and inefficient methods of cleaning refined products spills increases the proportion component that remains in the wetland ecosystem with severe toxic effects on wetland plants. It is recommended therefore that proper measures be put in place to prevent release of these products into the environment where it can adversely affect plants thus disrupting the ecosystem balance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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