THE MATHEMATICAL IMPLICATION OF Kalanchoe pinnata (Lam.) Pers. LEAVES IN THE EX SITU PHYTO-QUANTIFICATION OF CRUDE OIL SPILLS

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Abstract

This study investigated the mathematical implication of *Kalanchoe pinnata* leaves in the *ex situ* phyto-quantification of crude oil spills. Several methods are employed in the quantification of aquatic spills, but no widely accepted standard method is established for the estimation of spill size in a case of terrestrial spillage. This has made the quantification of the initial amount of spill very difficult cum expensive, and the actual value for compensation almost impossible to determine. This study was conducted to find a cheaper way of estimating the initial amount of crude oil spill in the soil using plant leaves. Investigations were made on the effect of varying amounts of terrestrial crude oil spill (0 ml/g, 0.105 ml/g and 0.211 ml/g which represented 0%, 50% and 100% soil saturation respectively) on the biomass, rate of absorption, chlorophyll content, total petroleum hydrocarbon content of the leaves of *Kalanchoe pinnata*. The results showed decreases in both leaf biomass (-0.75g to -1.9725g) and foliar total chlorophyll content (31.528 mg/L to 4.896 mg/L) while foliar TPH increased (24.50448 ppm to 523.68452 ppm) with increase in the amount of crude oil. It was also observed that the foliar rate of absorption of crude oil (2.5×10^{-5} ml/s) and the fresh leaf mass per unit leaf area (0.08458554946692 ± 0.009478141887493 gcm^{-2}) were not affected by differences in the leaf area. The mathematical relationships of the effect of varying amounts of spills on these parameters were combined to establish the Phyto-Quantification Model, a mathematical formula that, within a 5-day exposure period, implicates the leaf area and the final leaf weight of the leaves of *K. pinnata* in the quantification of the initial amount of crude oil spilled on the soil. The implication of this study has demonstrated that the mathematical model adopted is faster, cheaper and eco-friendly phyto-technology, unlike the other methods which are cumbersome, unreliable and expensive.

Keywords: PhytoQuantification, *Kalanchoe pinnata*, Crude oil pollution, PhytoQuant Model, Niger Delta

INTRODUCTION

Crude oil spills are some of the menaces of Nigeria presently. There have been several reports of the spills of the liquid mineral due to cases of either blatant violation of the standard operational procedures or cases of vandalism. Such occurrences have made the Niger Delta regions of Nigeria a pitiable plight for agricultural and recreational activities. Crude oil spills (petroleum pollution) has been proven to have negative effects on plants, animals, lithospheric aesthetics, humans and agriculture generally: reducing/inhibiting many beneficial plant and animal physiological processes and diminishing the size of farms (Nwilo, 1998; Agbogidi and Ejemete, 2005).

As a problem, therefore, crude oil pollution is a problem that needs to be solved immediately. The first step to providing a solution to any problem, after identifying the problem, is to quantify the problem – to measure the problem. One of the major problems of responding to crude oil spills is found in the estimation of the volume. Huseyin (2013) observed that the determination of the initial amount of spilled oil is an important issue since cleaners overestimate and spillers underestimate the volume. He stated that the volume of the spilled oil is extremely critical for the actions of spill mitigation as well as legal issues and fines to the spillers. Several methods, such as the satellite-assisted Slicker method (Goodman 1994; Salt *et al*. 2012), have been employed in the establishment of crude oil pollution levels, but these are mostly useful for aquatic environments; and in most cases, for freshly spilled petroleum, in the aquatic environment. Also, these methods do not consider the depth of the pollution, but only consider the surface area covered by the pollutant. This assumption could prove obviously very faulty, especially in terrestrial environments such as agricultural farms. And since spills on agricultural farms are rarely reported as at time of happening, the soil samples taken for analyses is usually not having the volatile constituents of the pollutant. From the foregoing, quantification of crude oil spills on land could be a major problem, as most of the established methods for estimating the level of crude oil pollution were devised for the aquatic environment. Several publications citing the Royal Society of Canada have reported that Remote sensing technologies have been tested and applied in oil spill responses for decades with mixed results (Goodman 1994; Fingas, 2011; Salt *et al*. 2012). Their incorporation into oil spill response requires confidence in the robustness and reliability of the information, based on capturing oil spatial patterns consistent with oil spill response processes. Beyond the technical challenges, considerations of cost-effectiveness and difficulties in conducting field trials have limited actual field applications to data processing (Leifer *et al*. 2011).

Other reasons why it is seemingly difficult to estimate the level of crude oil pollution on lands, especially in the Nigerian Niger Delta environment, include (1) lack of access to the pollution sites by scientists due to geographic reasons, (2) denial of access to pollution site by ignorant/corrupt locals and chiefs who often ask for a bribe before access is granted, (3) security risks borne by the scientist during estimating i*n situ*, even when the quantification method requires the physical presence of technical expertise (the scientist) at the site of the spill.

Thus, it is important to establish a means of estimating crude oil pollution on land with the following benefits – easily understandable and practicable; ease of application; and eco-friendly.

It has been severally explained that the environment can affect certain features or characteristics of a plant and effect certain physicochemical changes in the plant (Terek *et al*., 2015; Odiyi *et al*., 2020). It therefore goes further to explain that the changes in the physicochemical properties of the plant due to increasing or decreasing environmental assaults or stress (crude oil pollution, in this case) can be mathematically understudied and a mathematical relationship/model can be established to determine the exact level of pollution in relation to either the amount or rate of change in the plant. This process of using the level of change in the plant (morphological, biochemical or physicochemical) features to quantify the level of pollution in the environment by means of an established mathematical relationship or model is herein defined as Phyto-Quantification.

By means of its ability to survive in crude oil spills and having been described as a hyperaccumulator of heavy metals (Ekwumemgbo *et al*., 2013), one plant that could possibly best serve the purpose of phyto-quantification is *Kalanchoe pinnata*.

Kalanchoe pinnata (syn. *Bryophyllum pinnatum*), also recognized with the common names Miracle leaf, Air plant, Life plant or Goethe plant, is a succulent perennial plant that grows up to 3 – 5 feet tall. It has hollow stems, fleshy dark green leaves that are distinctly scalloped and trimmed in red, and bell-like pendulous flowers (Ghani, 2003). *K. pinnata* belongs to the family Crassulaceae, known to exhibit Crassulacean acid metabolism (CAM) (Milad *et al*., 2014).

The aim of this study is focused on investigating the use of *Kalanchoe pinnata*leaves in the estimation and quantification of crude oil concentrations (or pollution levels) in the soil; and, this is to be achieved by checking the effect of petroleum on the total petroleum hydrocarbon (TPH), the total chlorophyll content, the foliar biomass and the foliar rate of petroleum absorption of *Kalanchoe pinnata* leaves. The changes in these parameters due to crude oil pollution will be mathematically related into a model which could help develop cum provide a modelled simulation for establishing the level of crude oil pollution in agricultural soils. This work will find significance in the estimation of crude oil pollution levels (i.e. concentration of crude oil) in the soil while also helping to address the problem often faced by researchers (including the security risks) in accessing the oil spill sites, especially in the developing nations such as Nigeria and its oil-rich Niger Delta region. The relative low cost of this technology will also help reduce the high cost of estimation of crude oil spills in the Niger Delta, and thus making it budget friendly even for oil firms and regulatory bodies.

MATERIALS AND METHODS

Source of plants and leaves

The plants, *Kalanchoe pinnata*, where obtained from the Faculty of Pharmacy Medicinal Garden, University of Port-Harcourt, UniPark Campus. The collected plants were identified by a taxonomist from the Department of Plant Science and Biotechnology, University of Port-Harcourt.

The leaves were detached, rinsed, and weighed; and those of similar weight were used for the study. The leaf areas of these same leaves were established, and those of similar leaf area were used for the study.

Source of Sterilized Sand

River bed sand collected from the Choba River was sterilized by boiling in water (100◦C) for 15 minutes, and then allowed to cool. This procedure was done to remove external factors such as inherent seed banks and plant disease-causing agents. The pH level was determined using a pH meter, and levelled up to the pH of 7.0±0.3 by adding drops of 1M NaOH.

Establishment of Polluted Soil Treatments and Plant Materials

Nigerian sweet crude was obtained from the NNPC, Onne, Port-Harcourt. This was added to the washed riverbed soil samples to obtain varying concentrations of crude oil pollution: 0.00ml/g, 0.105ml/g and 0.211ml/g which were tagged as 0% (control), 50% and 100% treatments respectively. The 0.211ml/g treatment was determined as the amount of petroleum that covered the entire soil (extreme case of pollution). This was obtained, by pilot experiments, as 40ml of petroleum in 190g of treated soil. Other lesser concentrations were calculated duly.

Plastic pots were filled with 190g of the different concentrations of polluted river bed sand (0.00ml/g, 0.105ml/g and 0.211ml/g). The detached leaves of *K. pinnata* (of known mass [4.72 ± 0.58g] and leaf area [55.96± 4.59cm^2]) were inserted into the soil by means of the petiole. The experiment was laid out in a completely randomized design (CRD) with four replicates. The leaves were allowed to stay and mop up the petroleum in the soil for 5 days.

At the termination (5 days after mop up), the leaves were removed, rinsed with tap water to remove adhering soil, and excess water removed with paper towel. Data were taken on foliar biomass, rate of absorption, leaf area, total chlorophyll content, total petroleum hydrocarbon (TPH) content.

Experimental Models

The following experiments were conducted in line with the general aim of the study:

Effect of Leaf Area on the Rate of Absorption of Crude Oil

To establish the rate of absorptivity of petroleum by *K. pinnata* leaf, the petiole of the leaf of different leaf areas (small = 24.24 ± 1.85cm^2; medium = 40.998 ± 6.84cm^2; and, big = 60.375 ± 3.21cm^2) were dipped into a measured amount (10ml) of petroleum for five (5) days.

The rate of absorptivity was calculated as the quotient between the difference of initial volume of crude and the final volume per time taken. This experiment was conducted four times and the mean taken. Mathematically, this is represented as:

RoA = Vol. Absorbed/Time (ml/s)

Determination of the Fresh Leaf Mass per Area

Twenty (20) leaves of *K. pinnata* were obtained from the Faculty of Pharmacy Medicinal Garden,, UniPark Campus, University of Port-Harcourt. The fresh weights of each of the leaves were obtained using a digital weighing balance. To obtain the value of the Fresh Leaf Mass per Area for *K. pinnata*, the mass per unit leaf area of each leaf was determined by dividing the value of the weight of the fresh leaf by the leaf area, and the average was taken as the Fresh Leaf Mass per Area (FLMA).

$FLMA = \sum(\text{foliar fresh mass/leaf area})/N$

Where N is the total number of leaves sampled.

The leaf area (LA), and the fresh weight (FW) were determined according to the method adopted in Uzoma *et al.* (2019), with slight modifications. The leaf area was determined by means of Image-J software by applying the Threshold function.

Effect of Crude Oil on Leaf Weight

Different concentrations (0.00ml/g, 0.105ml/g and 0.211ml/g) of the pollutant were used as treatment for the leaves (of the same weight = 4.72 ± 0.58g). The leaves were left in the respective treatments for five days (120 hours), after which the leaves were weighed again to investigate the effect of the crude oil treatment on the weight of the leaves.

The initial fresh weight (iFW) and final fresh weight (fFW) were determined according to the method adopted in Uzoma *et al.* (2019), with slight modifications.

Effect of Crude Oil on Foliar Chlorophyll Content

Different concentrations (0.00ml/g, 0.105ml/g and 0.211ml/g) of the pollutant were used as treatment for the leaves (of the same weight = 4.72 ± 0.58g). The leaves were left in the respective treatments for five days (120 hours), after which the chlorophylls content of the leaves were measured to investigate the effect of the crude oil treatment on the chlorophylls content of the leaves of *K. pinnata*.

The total chlorophyll content (TCC) was determined using the method adopted in Uzoma *et al*. (2018) with some modifications.

Treated and untreated plant samples were oven-dried at 45 °C, pulverized and weighed; and 2g of pulverized plant sample was macerated in 10 ml of Methanol for 15 minutes at room temperature (28 ºC). The solution was filtered, and the solution mixture was analyzed for Chlorophyll-a and Chlorophyll-b contents using a spectrophotometer by measuring the absorbance at 652.4 nm and 665.2 nm. The equations used for the quantification of Chlorophyll-a, Chlorophyll-b and Total Chlorophyll Content (TCC) are shown below:

$$\text{Ch-a} = 16.72A665.2 - 9.16A652.4$$

$$\text{Ch-b} = 34.09A652.4 - 15.28A665.2$$

$$\text{TCC} = \text{Ch-a} + \text{Ch-b}$$

Determination of the Effect of Crude Oil on Foliar TPH

Different concentrations (00.00ml/g, 0.105ml/g and 0.211ml/g) of the pollutant were used as treatment for the leaves (of the same weight = 4.72 ± 0.58g). The leaves were left in the respective treatments for five days (120 hours), after which the total petroleum hydrocarbon of the leaves (foliar TPH) were determined and used to investigate the effect of the crude oil treatment on the foliar treatment.

The total petroleum hydrocarbon (TPH) of the leaves was obtained using the method as defined in Okop and Ekpo (2012). The determination of hydrocarbons in the leaf was performed on the samples and standards using a Varian model BV CP 3800 GC-FID equipped with a split/splitless injection port and Combi PAL auto sampler. All samples were taken into 2 mL chromatographic vial, injected and separated on a Varian Chrompack capillary column CP 5860 with 95% methyl and 5% phenyl-polysiloxane phase, (oven max tempt 350°C), WCOT fused silica, 30 m x 0.25 mm id and 0.25 μm film thickness with CP-Sil 8 CB low bleeds/MS coating. Carrier gas was helium 26 cm sec-1. Temperature profile during the chromatographic analysis was 50°C for 3 minutes; 8°C/min to 320°C hold 15 minutes and detector at 320°C.

Data Analysis

Data were analyzed with Analysis of Variance (ANOVA) using statistical analyses system package 9.1 (2002). Means were separated using Least Significant Difference (LSD) at 5% level of probability.

Model Curve

The foliar biomass and the relative abundance of the total petroleum hydrocarbon (TPH) of *K. pinnata* leaves will be used to derive a model curve or graph (or equation) that will be used to establish the total petroleum content (TPC) [amount of petroleum] in the environment any *K. pinnata* is grown. This model can be represented in the form of an equation.

RESULTS

Effect of Crude Oil Pollution on Leaf Biomass

The biomass (difference between the final weight and the initial weight) of the leaves decreased with increase in the amount of crude oil pollution, thus showing an inverse mathematical relationship. This decrease showed that lowest mean value for the leaf biomass was -1.9725g which was recorded for 0.211 ml/g of crude oil pollution (100% treatment), while the highest mean value for the leaf biomass was -0.75g which was recorded for 0ml/g of crude oil pollution (control treatment) (Figure 1). The difference in their means was shown to be statistically insignificant at a 5% probability of error.

Since the line graph had a slight disobedience to the y=mx+b standard for a straight line, the line graph was straightened using the mean values of the slopes of the two straight lines starting from an independent control (0) x-value, and cutting through either of the two other values of biomass for the 50% (0.105ml/g) and 100% (0.211ml/g) pollution treatments (Figure 2). The result was a straight line graph with the slope/gradient (m) = -6.01596705032724, and a y-intercept (b) = -0.75 (Figure 3). The mathematical relationship between the total petroleum in the soil (TP_s) and the leaf biomass is represented using the straight line equation ($y = mx + b$) as:

$B_L = (Tp_s \times -6.01596705032724) + (-0.75)$

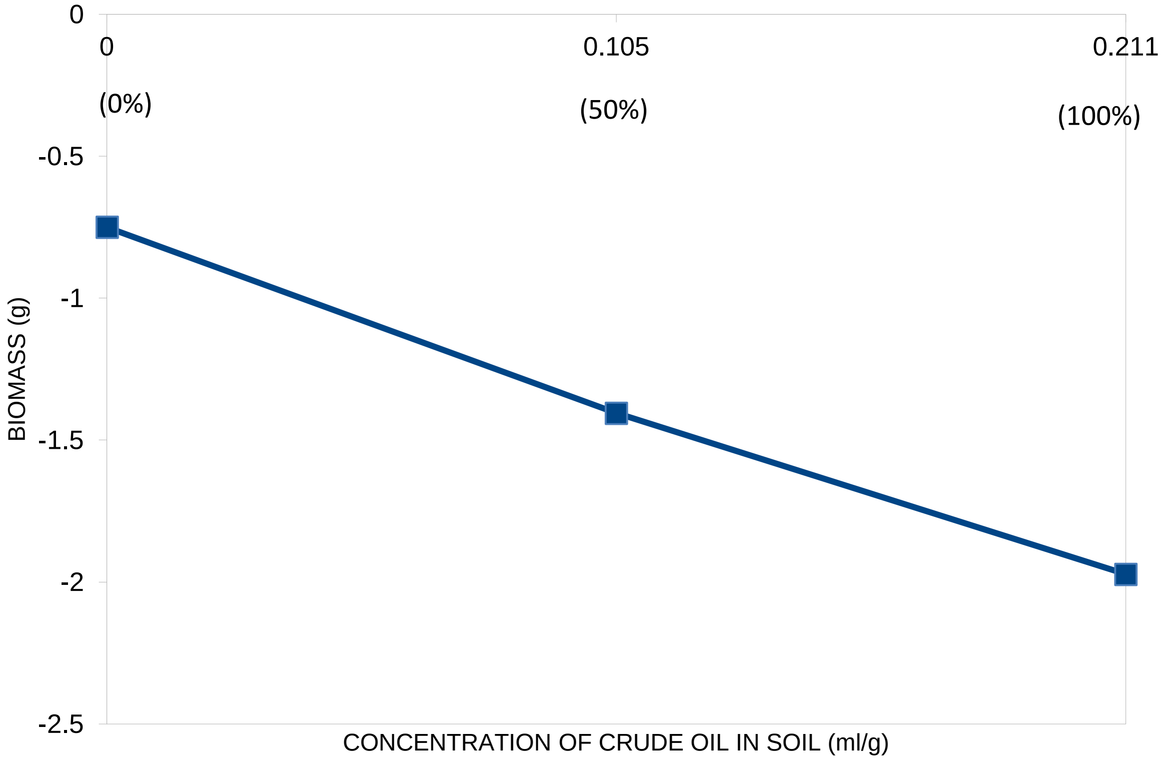

Figure 1: Mean values of the effect of crude oil on foliar biomass depicting a decline in biomass with increasing concentration of crude oil

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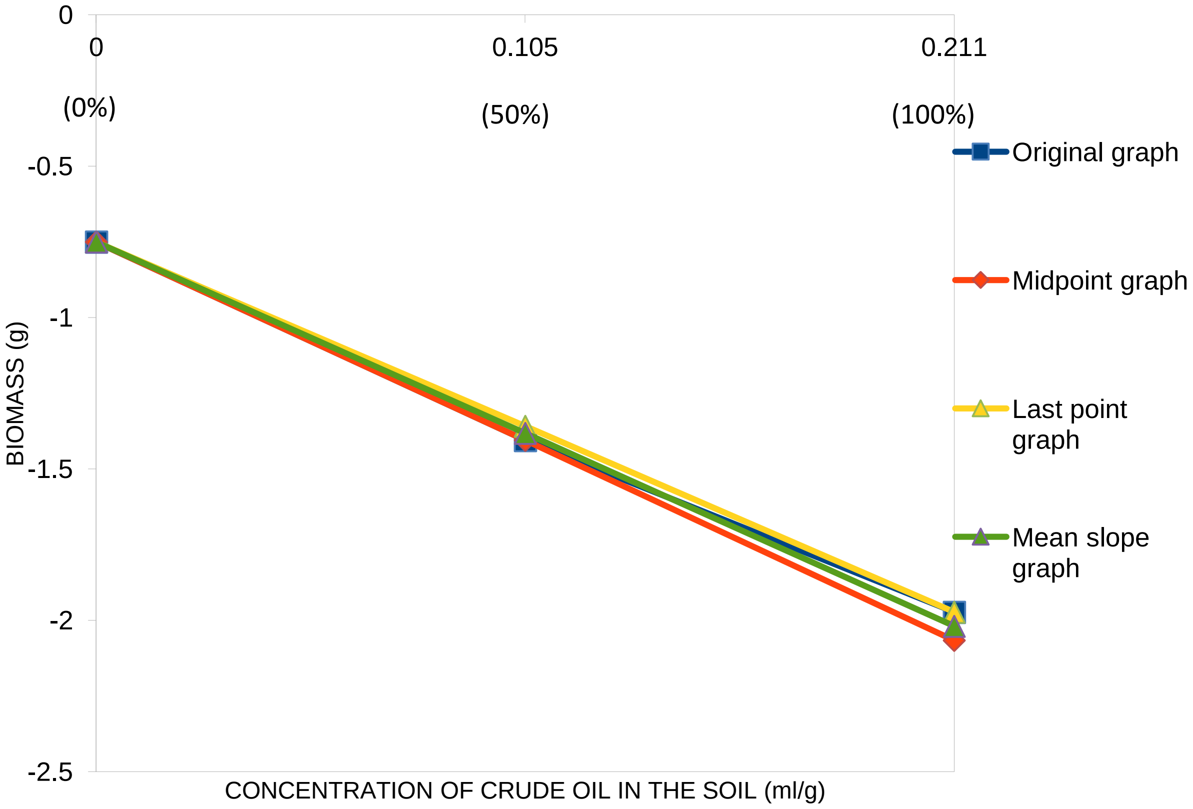

Figure 2: A derived graph of straight lines passing through the mean points of the original graph (Figure 1) of the mean values of the effect of crude oil in the soil on foliar biomass to obtain a mean straight line slope.

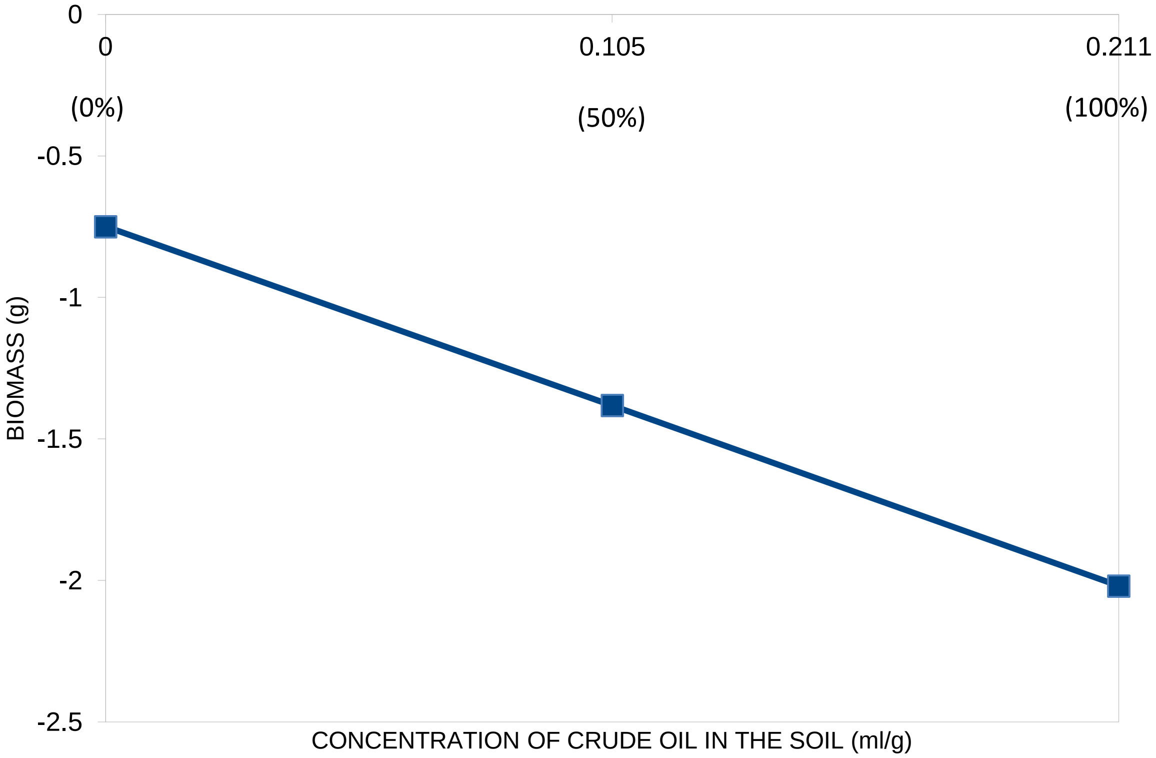

Figure 3: A derived graph of the effect of crude oil in the soil on foliar biomass obtained by a mean straight line slope.

Effect of Leaf Area on the Foliar Rate of Absorption

The rate of foliar absorption was not influenced by the area of the leaves, as it remained constant despite the increase or decrease in the leaf area. The rate of foliar absorption, which was determined as a quotient of the amount of crude oil absorbed per unit time, did not change or trend as was evident in a horizontally linear line graph with a value of $2.5 \times 10^{--5}$ ml/s. The result was a straight line graph with the slope/gradient (m) = 0, and a y-intercept (b) = $2.5 \times 10^{--5}$ (Figure 4).

The differences in the mean rates of foliar absorption was shown to be statistically insignificant with a p-value of 1 for all leaf sizes. The mathematical relationship between the rate of absorption (RoA) and the leaf area is represented using the straight line equation (y = mx + b) as:

$RoA = (0 \times LA) + 2.5 \times 10–^{5}$

$RoA = 2.5 \times 10^{--5}$

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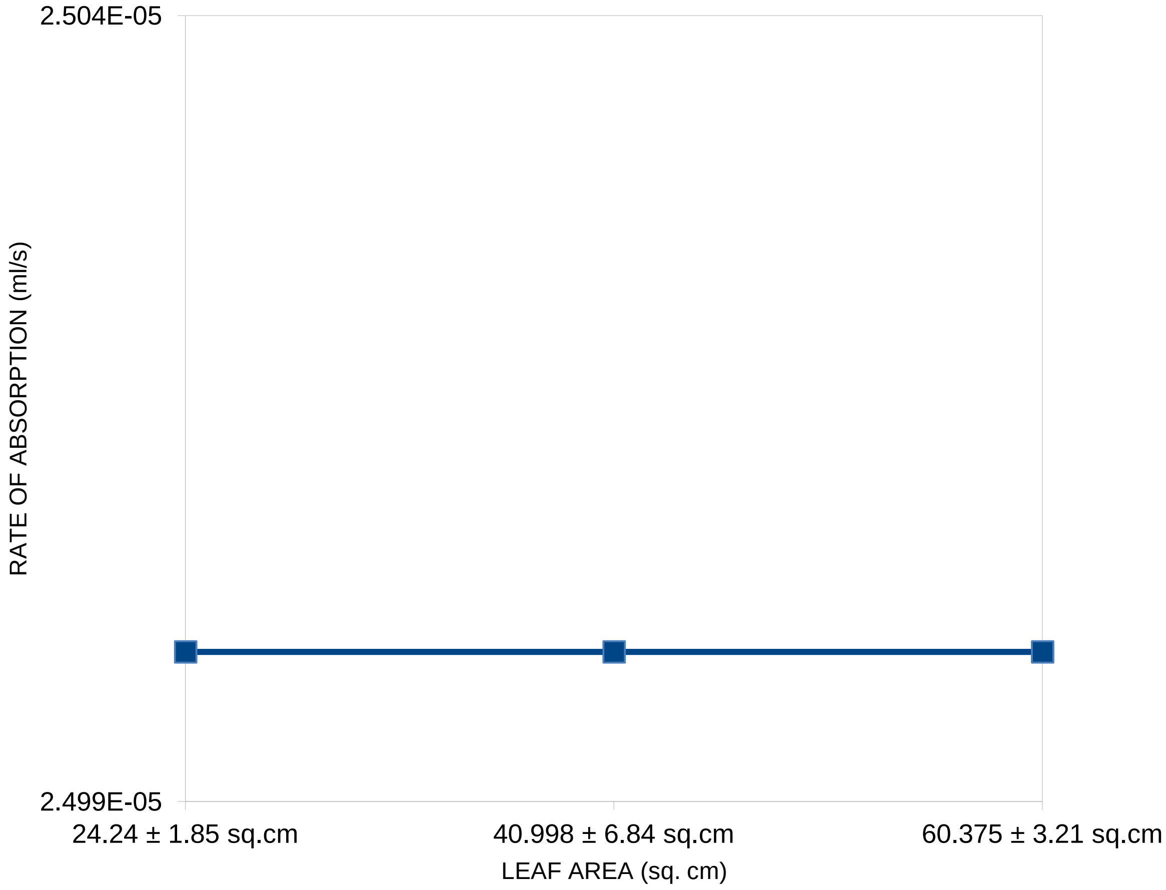

Figure 4: Effect of leaf area on the foliar rate of crude oil absorption depicting a constant rate of absorption with increasing leaf area

Effect of Crude Oil Pollution on Total Petroleum Hydrocarbon (TPH) of Leaf

The total petroleum hydrocarbon of the leaves increased with increase in the in the amount of crude oil pollution, thus showing a direct mathematical relationship. This increase showed that the leaves subjected to the 0.211ml/g treatment absorbed the highest amount of crude oil (24.50448 ppm) compared to those subjected to the 0.105ml/g treatment (364.40167 ppm) and 0.00ml/g treatment (24.50448 ppm) (Figure 5). The difference in their means was shown to be statistically significant at a 5% probability of error.

Since the line graph had a slight disobedience to the y=mx+b standard for a straight line, the line graph was straightened using the mean values of the slopes of the two straight lines starting from an independent control (0) x-value, and cutting through either of the two other values of TPH of the leaf for the 0.105ml/g and 0.211ml/g pollution treatments (Figure 6). The result was a straight line graph with the slope (m) = 2801.44914, and a b-value = 24.50448 (Figure 7).

The mathematical relationship between the Total Petroleum Hydrocarbon (TPH) and the amount of petroleum spilled in the soil (TP_s) is represented using the straight line equation (y = mx + b) as:

$TPH = (2801.44914 \times TP_s) + 24.50448$

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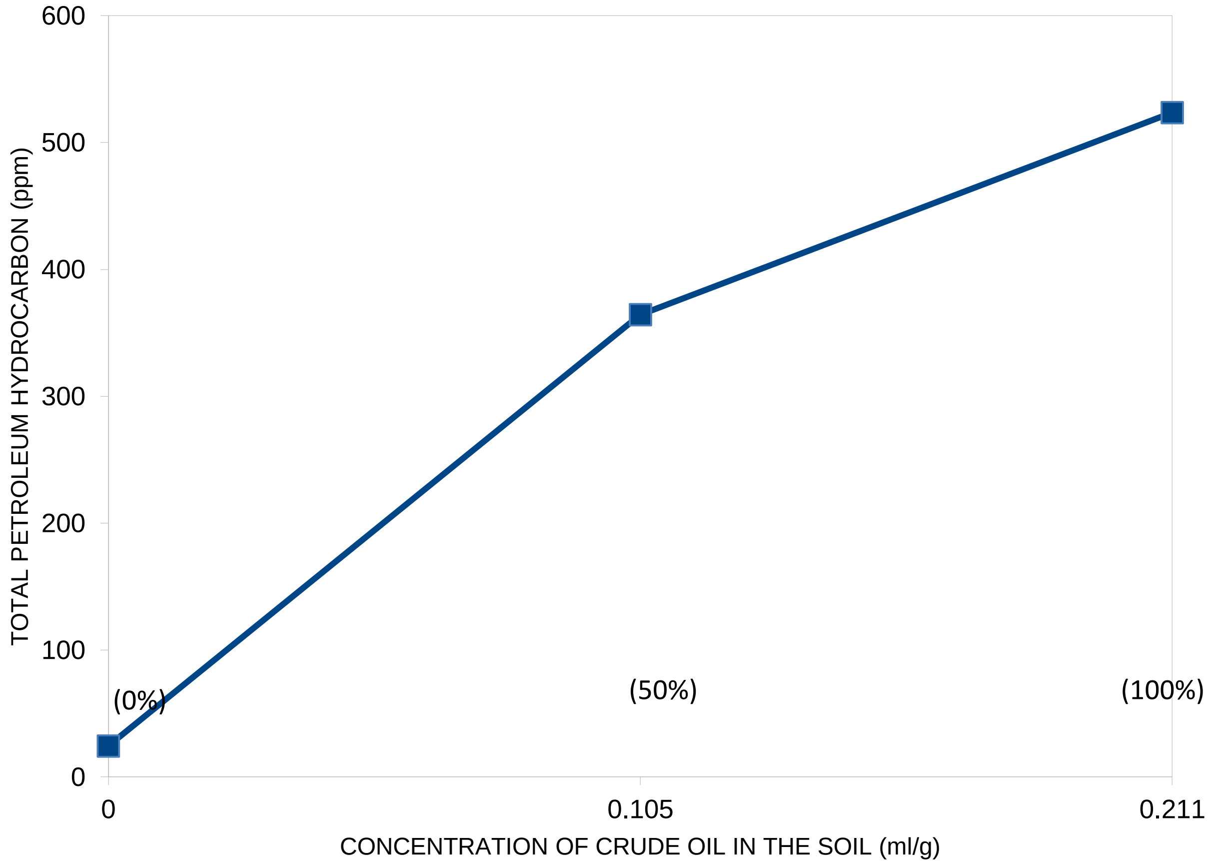

Figure 5: Mean values of the effect of crude oil on the total petroleum hydrocarbon of the leaves depicting an increase in the total petroleum hydrocarbon content of the leaves with increasing pollution level in the soil

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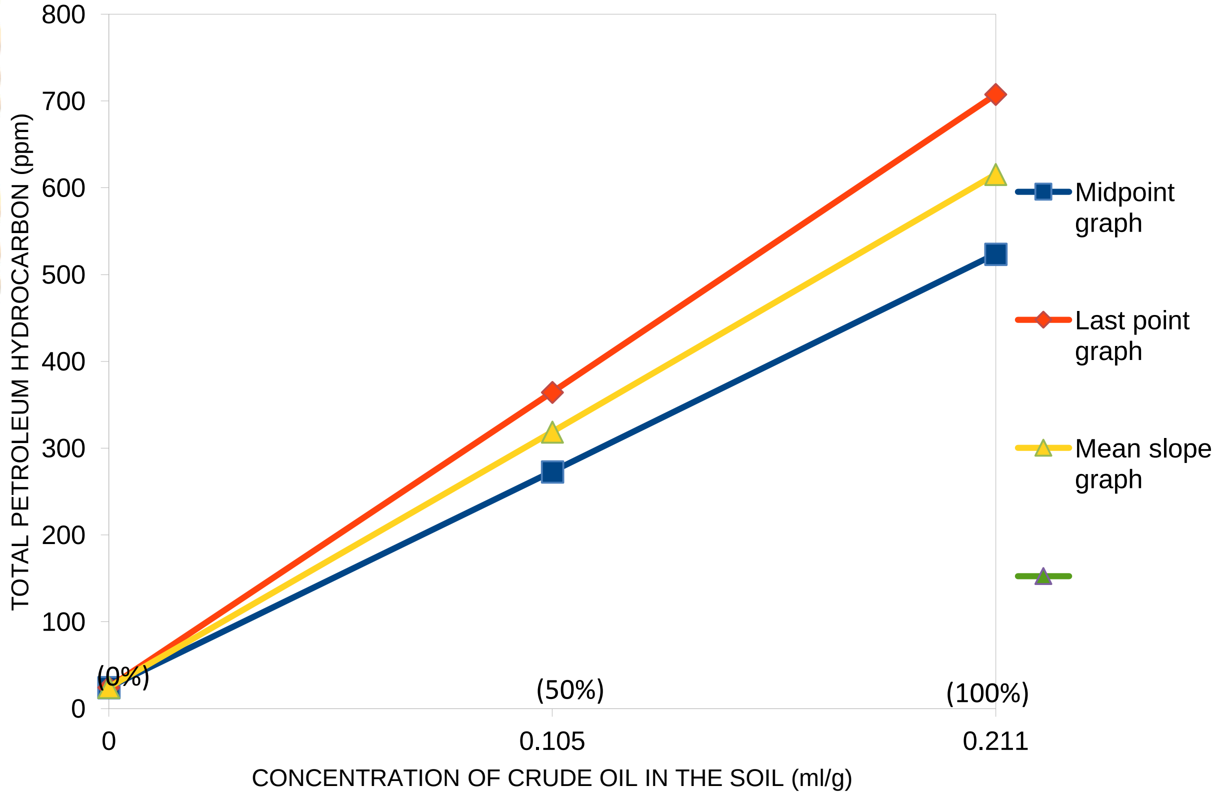

Figure 6: A derived graph of straight lines passing through the mean points of the original graph (Figure 5) of the mean values of the effect of crude oil in the soil on the total petroleum hydrocarbon to obtain a mean straight line slope

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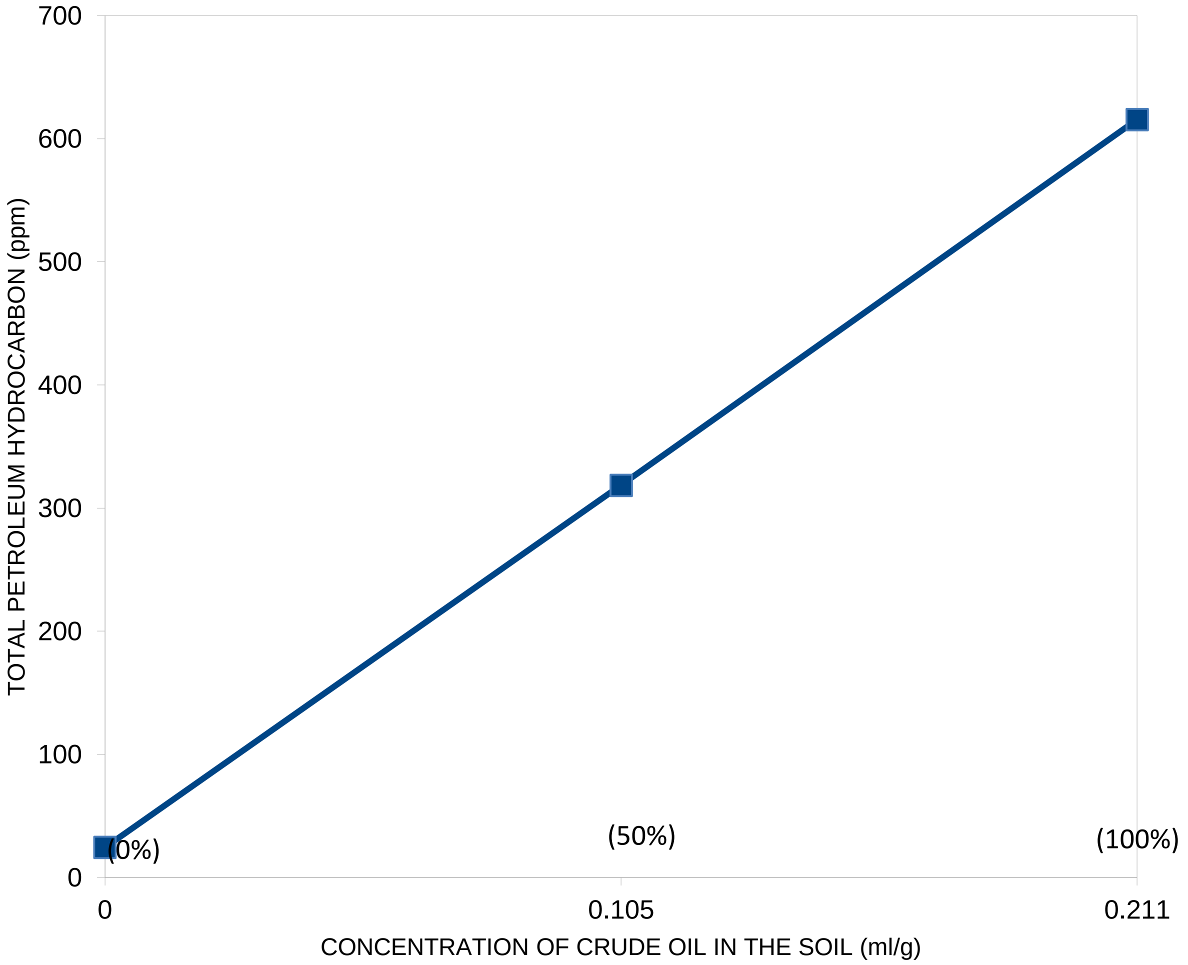

Figure 7: Derived graph depicting the effect of crude oil in the soil on the total petroleum hydrocarbon of the leaves obtained by a mean straight line slope.

Effect of Crude Oil Pollution on Total Foliar Chlorophyll Content

The total chlorophyll content of the leaf were negatively influenced by crude oil pollution: the total chlorophyll content of the leaf decreased with increase in the amount of crude oil in the soil, thus showing an inverse mathematical relationship. This decrease showed that lowest mean value for the total chlorophyll content of the leaf was 4.896 mg/L which was recorded for 0.211 ml/g of crude oil pollution (100% treatment), while the highest mean value for the total chlorophyll content of the leaf was 31.528 mg/L which was recorded for 0ml/g of crude oil pollution (control treatment) (Figure 8). The difference in their means was shown to be statistically significant at a 5% probability of error.

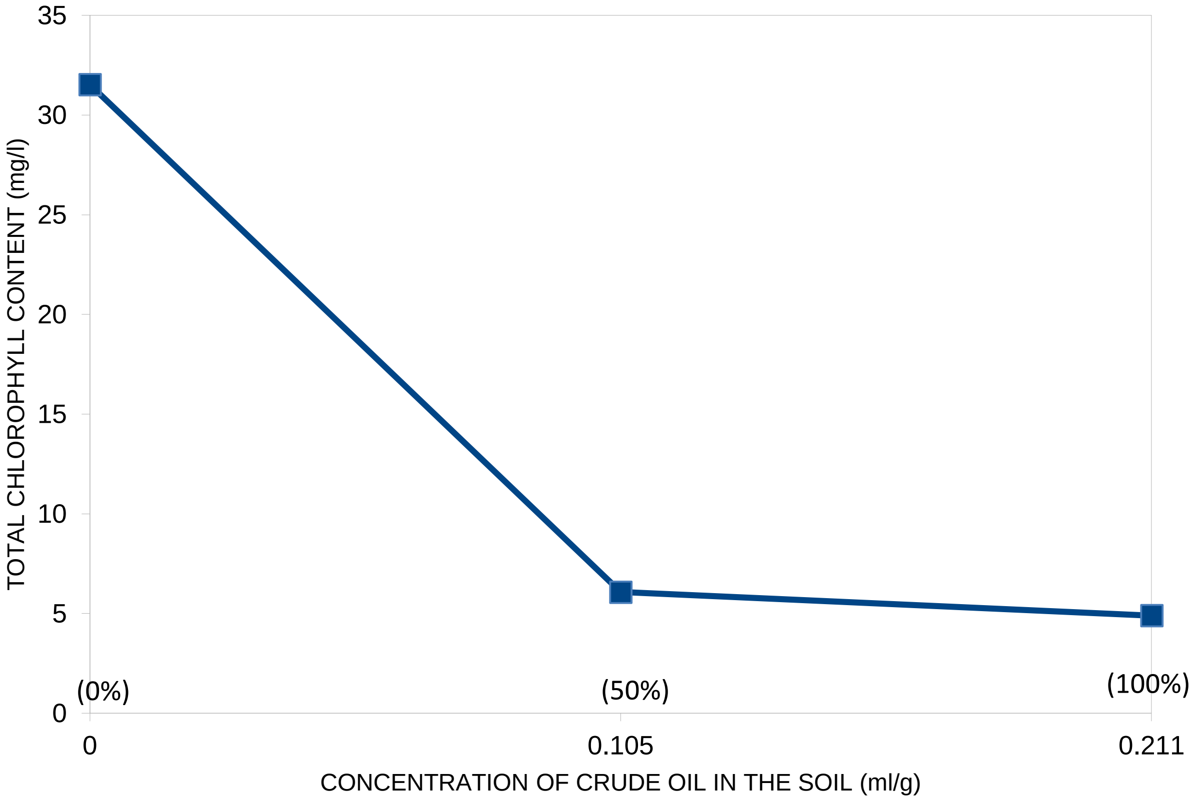

Figure 8: Effect of crude oil on the total chlorophyll content of the leaves.

Relationship between the Leaf Area and the Weight of the Leaf

The fresh leaf mass per area of the leaves were not influenced by the leaf area or the fresh leaf mass: the fresh leaf mass per area was established to be constant. This is so as the increase in the fresh mass of the leaf came with a concomitant increase in the leaf area, thus maintaining the fresh leaf mass per area (FLMA) as a constant. The values of the fresh leaf mass per area ranged from a minimum value of 0.070401083093586 gcm^{-2} to a maximum of 0.099391480730223 gcm^{-2}. The actual FLMA constant was established as the mean value of the total FLMA of all the leaves studied. This constant (mean ± st. deviation) was valued at 0.08458554946692 ± 0.009478141887493 gcm^{-2}. The fresh leaf mass per area (FLMA), which was determined as a quotient of the mass of the fresh leaf per unit leaf area, did not change or trend as was evidently represented in a horizontally linear line graph with the slope/gradient (m) = 0, and a mean value for y-intercept (b) = 0.08458554946692 gcm^{-2}. (Figure 9).

The differences in the mean fresh leaf mass per area (FLMA) was shown to be statistically insignificant with a p-value of 0.914 for all leaf sizes. The mathematical relationship between the fresh leaf mass per leaf area and the fresh leaf mass-leafarea quotient is represented using the straight line equation (y = mx + b) as:

Ʉ = [0 × (FLM/LA)] + 0.08458554946692 gcm^{-2}.

Ʉ = 0.08458554946692 gcm^{-2}.

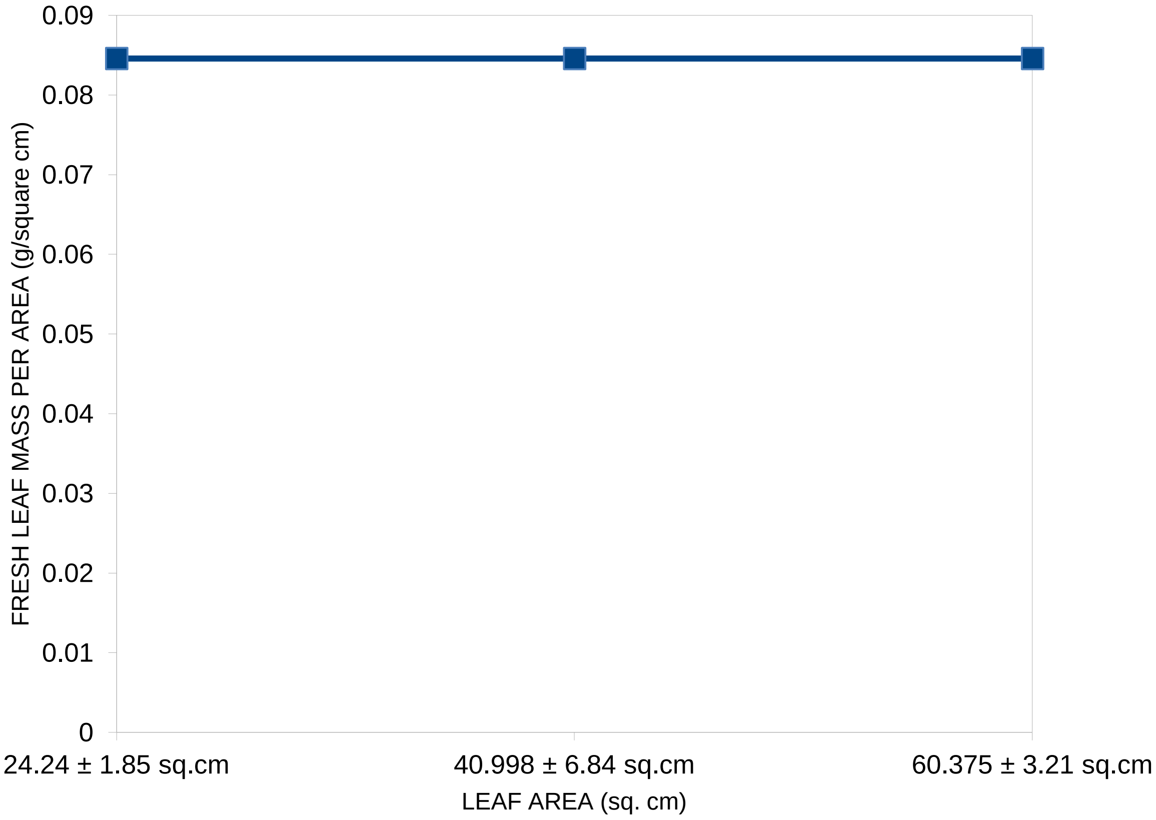

Figure 9: Effect of leaf area on the fresh leaf mass per area of *K. pinnata* leaves.

DISCUSSION

Effect of Crude Oil Pollution on Leaf Biomass

Evidence from the results (Figure 1) showed that the biomass (difference between the final weight and the initial weight) of the leaves decreased with increase in the in the amount of crude oil pollution, thus showing an inverse mathematical relationship. This decrease, though statistically insignificant, showed that the lowest mean value for the leaf biomass was -1.9725g which was recorded for 0.211 ml/g of crude oil pollution (100% treatment), while the highest mean value for the leaf biomass was -0.75g which was recorded for 0ml/g of crude oil pollution (control treatment). The relationship between the amount of petroleum in the soil and the leaf biomass is represented as:

$$Biomass = m_{biomass} \times TP_{soil} + b_{biomass}$$

This relative decrease in the leaf biomass of the *K. pinnata* with increase in the amount of crude oil in the soil could be traceable to the decline in the chlorophyllase activities, reduced respiration cum reduced transpiration that comes with the increased presence of hydrocarbon compounds in the plant (Osuagwu *et al*., 2013). These could also be attributable to the plausible immobilization of nutrients within the plant or leaf, thus resulting to the rapid loss of biomass in the leaves compared to the control treatment (Odiyi *et al*., 2020). These generally could culminate to the rapid reduction of the biomass of the leaves subjected to the crude oil pollution. Though there was a dearth of literature pointing out specifically on the particular effect crude oil pollution has on the leaf of a plant that is directly inserted into a polluted soil, there are a plenitude of literature reporting the negative effect of crude oil on the biomass of whole plants and their leaves. Researchers like Merkl *et al*. (2004), while studying the effect of petroleum on the weeds *Centrosema brasillianum* and *Panicum maximum*, reported reduction in biomass. The efforts of Molina-Baharahona *et al*. (2005) also corroborate this assertion. Another group of workers, Ogbo *et al*. (2009), reported similar results. Working with *Paspalum scrobiculatum*, a common Nigerian weed, the workers tried to investigate the phytoremediation prospects of the weed. They reported that the different levels of crude oil contamination resulted in significant reduction in the leaf area, fresh weight and the plant height. The negative effect of the crude oil pollution on the leaf area of the weed indirectly implies a reduction in the leaf biomass of the weed. This is in line with the assertions made from the results of this study.

Effect of Leaf Area on the Foliar Rate of Absorption

Evidence from the results (Figure 4) showed that the rate of foliar absorption was not influenced by the area of the leaves, as it remained constant despite the increase or decrease in the leaf area. With no statistical significance and a p-value of unity (1) for all leaf sizes, the rate of foliar absorption, which was determined as a quotient of the amount of crude oil absorbed per unit time, did not change or trend as was evident in the horizontally linear line graph with a value of 2.5×10^{-5} ml/s and a gradient (m) equal to zero. Several reports have it that the leaf area of a plant positively influences the rate of transpiration (Prijono and Laksamana, 2016); and, the rate of transpiration influences the rate of absorption (Maylani *et al*., 2020). But, evidence from this study is against the logical expectation that the bigger leaves should have a higher rate of absorption; and, therefore suggests that the mechanism of absorption of the crude oil by the leaves of *K. pinnata* is not linked to the surface area. Crude oil is not transported the same way as water, and is not evaporated or transpired by plants, and as such, the area of the leaf is not of any key influence on the rate of absorption of crude oil by the leaf. It is also noteworthy that the study done by Han *et al*. (2016) had shown that the presence of crude oil decreases the stomatal conductance and the rate of transpiration of *Amorpha fruticosa* seedlings. This information account to why the rate of absorption of crude oil does not depend on the area of the leaf, but by the quantity of crude oil in the soil.

This is quite rational as, having the same rate of absorption of crude oil irrespective of the leaf size or area would only mean that the small leaves would reach the maximum capacity/tolerance level and die faster than the bigger leaves which can go on absorbing more crude oil before they reach their maximum capacity or tolerance level. This would otherwise not have been obtainable if the rate of absorption varied with the leaf area. That would have implied that the bigger the leaf, the higher the rate of absorption; and, at the end of the study period, the leaves would have all died at the same time. But, this is never the case, as what we know about the *K. pinnata* leaves is that, when subjected to the same amount of pollution over time, the smaller leaves die before the bigger leaves; thus, suggesting a fixed rate of absorption, but different volumetric cum spatial capacity and tendency to withstand the pollutant over time.

Effect of Crude Oil Pollution on Total Petroleum Hydrocarbon (TPH) of Leaf

Evidence from the results (Figure 5) showed that the total petroleum hydrocarbon of the leaves significantly increased with increase in the in the amount of crude oil pollution, thus showing a direct mathematical relationship. This increase showed that the lowest mean value for the total petroleum hydrocarbon of the leaf was 24.50448 ppm (mg/kg) which was recorded for 0 ml/g of crude oil pollution (control treatment), while the highest mean value for the total petroleum hydrocarbon of the leaf was 523.68452 ppm (mg/kg) which was recorded for 0.211 ml/g of crude oil pollution (100% treatment). This is explainable by the fact that plants have the ability to absorb and sometimes accumulate non-water liquids cum pollutants such as crude oil in their cellular vacuoles; and, the extent of this accumulation is captured when the total hydrocarbon content of the plant is captured.

Workers like Rao *et al*. (2007), while working with *Vicia faba*, have established that plants have the ability to uptake petroleum hydrocarbons into their roots upon exposure to the soil that was polluted with petroleum hydrocarbons. This assertion was corroborated by the works of Lotfinasabasl *et al*. (2013) who worked with Mangrove species and established that the plants have the ability to absorb petroleum hydrocarbons and translocate same to the leaves. It is also noteworthy that the study done by Han *et al*. (2016) had shown that the presence of crude oil decreases the stomatal conductance and the rate of transpiration of *Amorpha fruticosa* seedlings. In their study, Han *et al*. (2016) suggested that the plants respond to crude oil pollution in a similar way they would respond to a situation of water limitation (drought): shut down transpiration by influencing stomatal conductance. This information account to why the rate of absorption of crude oil does not depend on the area of the leaf, but by the quantity of crude oil in the soil. Inasmuch as the rate of absorption of crude oil may have been directly related to the area of the leaves, that was not the subject under investigation. Since only leaves of approximately equal leaf area were used to investigate the effect of crude oil pollution on the TPH, the only significant variable was the amount of crude oil in the soil. This therefore brings to a logical premise that, if all other parameters are fixed, the Total Petroleum Hydrocarbon (TPH) of the leaves is directly proportional to the amount of crude oil in the soil. This statement can be mathematically represented as: $TPH = m_{TPH} \times TP_{soil} + b_{TPH}$.

Effect of Crude Oil Pollution on Total Foliar Chlorophyll Content

Evidence from the study (Figure 8) showed that the total chlorophyll content of the leaf were negatively influenced by crude oil pollution: the total chlorophyll content of the leaf significantly decreased with increase in the amount of crude oil in the soil, thus showing an inverse mathematical relationship. This decrease showed that lowest mean value for the total chlorophyll content of the leaf was 4.896 mg/L which was recorded for 0.211 ml/g of crude oil pollution (100% treatment), while the highest mean value for the total chlorophyll content of the leaf was 31.528 mg/L which was recorded for 0 ml/g of crude oil pollution (control treatment). This result goes in line with the findings of Osuagwu *et al*. (2013) who studied the effect of crude oil pollution on the chlorophyll content of *Dioscorea bulbifera*. In their study, they reported that crude oil caused a significant reduction in the leaf area of the plant. They attributed this reduction in leaf area to [decrease in stomatal or nutrient] conductance. They posited that, since the leaf is the primary site of photosynthesis, a reduction in the leaf area will consequently bring about a reduction in photosynthesis and chlorophyll content. Working with *Zea mays*, another group of workers (Odiyi *et al*., 2020) explained that the reduction in chlorophyll content of the plant was due to a possible interference of the plants ability to absorb some key mineral nutrients due to the presence of the crude oil in the soil. They stated that the presence of the crude oil could prevent the plant roots from absorbing minerals like magnesium, boron, manganese and iron which are essential for chlorophyll synthesis.

But, since this study was done using treated soil and does not report a decrease in the area of the leaves used for the study, it is pertinent to state that the decrease in total chlorophyll content of the leaf could be attributable to certain key factors, some of which include a possible reduction in stomatal conductance which will occur concomitant with a decrease in cellular carbon dioxide levels, this will reduce the rate of photosynthesis and eventually lead to the drastic reduction of total chlorophyll with increase in the amount of crude oil absorbed by the plant. Some authors have asserted that plant response to crude oil pollution is similar to plant response to drought, which comes all as an attempt to reduce the rate of transpiration in the plant (or leaf, in this case). These claims are supported by the works of Han *et al*. (2016) who studied the photosynthesis response of *Amorpha fruticosa* seedlings to increasing levels of crude oil in the soil. These workers reported a significant decrease in the stomatal conductance, transpiration rate and photosynthetic rate in the seedlings. They concluded that the decline in the photosynthetic rate results from the decreased photosynthetic activity of the mesophyll cells and decline in intercellular CO_2 levels; all of which point to one line of thought – a decline in stomatal conductance.

Relationship between the Leaf Area and the Weight of the Leaf

Evidence from the study (Figure 9) showed that the fresh leaf mass per area of the leaves were not influenced by the leaf area or the fresh leaf mass: the fresh leaf mass per area was established to be constant. This is so as the increase in the fresh mass of the leaf came with a concomitant increase in the leaf area, thus maintaining the fresh leaf mass per area (FLMA) as a constant. The values of the fresh leaf mass per area insignificantly ranged from a minimum value of 0.070401083093586 gcm^{-2} to a maximum of 0.099391480730223 gcm^{-2}. The actual FLMA constant was established as the mean value of the total FLMA of all the leaves studied. This constant (mean ± st. deviation) was valued at 0.08458554946692 ± 0.009478141887493 gcm^{-2}. This shows, to some extent, that the leaf content per unit area of a *K. pinnata* leaf is constant, and this constant may be used as a morphological feature to characterize a species, as leaves of different species are not expected to have the same fresh leaf mass per area value. This can also be used as a diagnostic tool to characterize plants or leaves that are diseased or are subject to external environmental influence. This constant value of the fresh leaf mass per area value of *K. pinnata* may be attributable to the constant rate of absorption of non-water substances by the leaves, as was evident in the results of this study which showed a constant rate of absorption of crude oil by the leaves. Without removing the possibility of genetic instructions, this constant rate of absorption must have resulted to a linear progression of the length and width of the leaf which also comes with a concomitant increase in the weight (mass) of the leaf; thus, maintaining the leaf mass-leaf area quotient as a constant. This goes in line with the findings of Awal *et al*. (2004), who studied the specific leaf area (SLA) of oil palm crops. These workers asserted that the specific leaf area (SLA), which is the ratio of leaf area to the leaf mass, is the most important determinant of oil palm growth, and could be used in the monitoring of oil palm and many crop simulation models. They reported that the specific leaf area (SLA) remained constant during the plants development, and that there was no significant variation with time. As an explanation, they stated that the leaf area increased because the leaf length and the leaf thickness also increased, which also comes with an increase in mass. They also explained that while the specific leaf area appeared to be constant with increasing leaf area or leaf dry weight, this value varied slightly with environmental factors like light and temperature. De la Riva *et al*. (2016), who studied the foliar anatomy of 34 Mediterranean woody species, explained that the constant leaf mass per area (LMA) of leaves were determined by the anatomical tissues and the chemical composition of the leaf. They iterated that, albeit being a morphological trait, the LMA and its inverse (specific leaf area, SLA) are related to the rate of photosynthesis, the growth potential and rate of decomposition of the leaves. They explained that, the LMA is simply determined by two key factors, as it is merely a product of the leaf density (LD) and the leaf volume-area ratio (or thickness) (LVA). This therefore explains that, since the leaf-volume area ratio and the leaf density are both determined by the composition of the different anatomical tissues (mesophyll, epidermis and vascular tissues and air spaces), the leaf mass per area value will fairly remain constant.

The mathematical relationship between the leaf weight and the leaf area is represented as:

$$Freshleafmassperarea(Ʉ) = \frac{Freshweight(g)}{LA(cm^2)}$$

The Phyto-Quantification Model for Estimating the Amount of Crude Oil Spilled

The mathematical formula for the rate of absorption is established as:

Rate of Absorption (RoA) = $\frac{Volume(ml)}{Time(s)}$…………………..(Equation 1)

The mathematical constant relating the fresh weight of *Kalanchoe pinnata* leaves and the leaf area is established as:

$Freshleafmassperarea(Ʉ) = \frac{Freshweight(g)}{LA(cm^2)}$……………...(Equation 2i)

If the Fresh weight is made the subject of the formula, then:

$Freshweight = Ʉ \times LA$……………………………………..(Equation 2ii)

Following the straight line graph (y=mx + b) of the Total Petroleum Hydrocarbon (TPH) in the leaves, and the Total Petroleum in the soil (TP_{soil}), the relationship can be represented using the formula:

$TPH = m_{TPH} \times TP_{soil} + b_{TPH}$……………………………………...(Equation 3i)

Making TP_{soil} the subject of the formula:

$TP_{soil} = \frac{TPH - b_{TPH}}{m_{TPH}}$…………………………………………..(Equation 3ii)

Following the straight line graph (y=mx + b) of the leaf biomass and the Total Petroleum in the soil (TP_{soil}), the relationship can be represented using the formula:

$Biomass = m_{biomass} \times TP_{soil} + b_{biomass}$………………………...(Equation 4i)

Making TP_{soil} the subject of the formula:

$TP_{soil} = \frac{Biomass - b_{biomass}}{m_{biomass}}$……………………………………….(Equation 4ii)

Since both equations (3ii) and (4ii) are formula for TP_{soil}, they can be combined. Combining equations (3ii) and (4ii) gives the formula:

$\frac{TPH - b_{TPH}}{m_{TPH}} = \frac{Biomass - b_{biomass}}{m_{biomass}}$…………………………..(Equation 5i)

Multiplying both sides of equation (5i) by m_{TPH} to give equation (5ii):

$TPH - b_{TPH} = \frac{(Biomass - b_{biomass}) \times m_{TPH}}{m_{biomass}}$………..………..…….(Equation 5ii

Making TPH the subject of the formula by adding b_{TPH} to both sides of the equation:

$TPH = \frac{(Biomass - b_{biomass}) \times m_{TPH}}{m_{biomass}} + b_{TPH}$………………………...(Equation 5iii)

To get the mathematical relationship between the TP_{soil}, the leaf biomass and the TPH, substitute equation (5iii) into equation (3ii):

$TP_{soil} = \frac{\left(\left(\frac{Biomass - b_{biomass} \times m_{TPH}}{m_{biomass}}\right) + b_{TPH}\right) - b_{TPH}}{m_{TPH}}$….……………………. (Equation 6)

The result of the substitution is the same as equation (4ii):

$$TP_{soil} = \frac{Biomass - b_{biomass}}{m_{biomass}}$$

This goes to show that equation (4ii) is the actual mathematical relationship between the TP_{soil}, the leaf biomass and the TPH.

But, the biomass of the leaf is obtained by subtracting the initial (or fresh) weight of the leaf from the final weight of the leaf. This is represented mathematically as:

$Biomass = Leafweight_{final} - Leafweight_{initial}$….……………………….(Equation 7)

Since our initial leaf weight is the same as the fresh weight of the leaf as represented in equation (2ii); then, the formula for initial leaf weight is:

$Leafweight_{initial} = Freshweight = \text{Ʉ} \times LA$……………………………….(Equation 8)

If equation (8) is substituted into equation (7); then, the mathematical relationship is established as:

$Biomass = Leafweight_{final} - (\text{Ʉ} \times LA)$…………….…………………..(Equation 9)

If the value of biomass in equation (9) is substituted into equation (4ii) (the actual equation that relates TP_{soil}, Biomass and TPH); then, the relationship can be stated as:

$TP_{soil} = \frac{(Leafweight_{final} - (\text{Ʉ} \times LA)) - b_{biomass}}{m_{biomass}}$……….…………...(Equation 10)

When the actual values of $b_{biomass}$ and $m_{biomass}$ (Figure 3) are input, into equation (10); then, the formula (The PhytoQuant Model) is formed:

$TP_{soil} = \frac{(Leafweight_{final} - (0.08458554946692 \times LA)) - (-0.75)}{-6.01596705032724}$………………(Equation 11[1])

Since the only two variables in The PhytoQuant Model are the final leaf weight (Leaf weight_{final}) and the leaf are (LA), it is noteworthy to state that: with just the values of the leaf area and the final weight of the leaf after exposure to pollution, the value of the initial amount of fresh spill in the soil can be estimated.

5. CONCLUSION

The study of the effect of crude oil pollution on the leaves of *Kalanchoe pinnata* showed a decrease in foliar biomass and chlorophyll content of the leaves with increasing concentration of crude oil in the soil. These inverse relationships were attributed to the inhibition of chlorophyllase activity in the leaves, thus resulting to a decline in photosynthesis and leaf biomass. This inverse relationships followed a mathematical straight line expression (y=mx + b), and represented by the equation $Biomass = m_{biomass} \times TP_{soil} + b_{biomass}$, for the TPH-foliar biomass relationship.

As a sharp contrast to the relationship of crude oil pollution with the foliar biomass and total chlorophyll content, the increase in the concentration of crude oil in the soil increased the total petroleum hydrocarbon (TPH) of the leaves. This increase in the total petroleum hycrocarbon in the leaves showed evidence that the leaves merely accumulated the pollutant and not break it down or volatilize it. This was attributed to a reduction in photosynthesis and stomatal conductance. The relationship between the crude oil in the soil and the increase in the total petroleum hydrocarbon in the leaves followed a straight line graph represented by the equation $TPH = m_{TPH} \times TP_{soil} + b_{TPH}$.

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[1] The unit of $m_{biomass}$ is g^2ml^{-1} (as taken from figure 3.3).

The study to investigate the relationship between the leaf area and the rate of crude oil absorption showed that the rate of absorption of crude oil by the leaves was constant, and therefore not influenced by the leaf area. Another constant was established when a study was conducted to investigate the relationship between the fresh leaf mass and the leaf area. It was established that the quotient of the fresh leaf mass and the leaf area (FLMA) was pegged at a mean value of 0.08458554946692 ± 0.009478141887493 gcm^{-2}.

These relationships were combined to form the PhytoQuant Model for the estimation of crude oil spills in the soil. The PhytoQuant Model was established to be $TP_{soil} = \frac{\left(Leafweight_{final} - (0.08458554946692 \times LA)\right) - (-0.75)}{-6.01596705032724}$. The PhytoQuant Model renditions as a possible eco-friendly, economical and simple way of estimating the initial amount of terestrial crude oil spill at a site. Compared to other methods of estimating initial spill, the PhytoQuant Model can easily be learned by locals, and the results from this phyto-technbology can be used to commence legal action against defaulters. Employment of this model will reduce the cost of measuring initial spill for the monitoring agencies and oil firms. It is also worthy of mention that the utility of the PhytoQuant Model in the quantification of crude oil spills will increase the applicability of Plant Science from just phyto-remediation to phyto-quantification; and, further engrave the footmark of botanists in the field of oil spill research.

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