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A COMPARATIVE ANALYSIS OF ECO-TOXICITY OF CRUDE OIL POLLUTION ON THE CHLOROPHYLL BIOSYNTHESIS IN TWO AGRO-FORESTRY SPECIES.

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ABSTRACT

A short-term ecological study was conducted to ascertain the degree of envisaged impact of petroleum pollution to biota (macrophyte) in a polluted habitat. The biosynthetic process in chlorophyll content reflects the level of the photosynthetic activities. It was envisaged that such biosynthetic process of pigment formation could become a sensitivity index to any polluted environment. T. this end, this study has elucidated the importance of petroleum on photosynthetic capacity of two agroforestry species (*Leucaena leucocephala* and *Bauhinia monandra*). The relative accumulation of the pigments was used to evaluate the influence of petroleum on the biosynthetic process of the plant species in focus. Results have established (with the variation in the optical transmittance of the pigment) the concentration of the chlorophyll pigment in various pollution intensities. The result showed 4792 mg/g in the control for *L .leucocephala*. 3195 mg/g, 1118 mg/g and 958 mg/g with percentage depression of 58.6%, 68.7% and 76.5% in the respective (25, 50 and 100 ml) pollution levels. *B. monandra* had 1438 mg/g in the control. 1118, 958 mg/g and 479 mg/g) and (%) depression of 59.9 69.0 and 78.1 (%) respectively. The variations in the species pigment content indicate the predicament of both species and consequently their sensitivity potential to a polluted habitat. Hence such index provides a means of determining the importance of petroleum in accounting for photosynthetic rate and yield potential under environmental condition.

KEYWORDS: *Leucaena leucocephala, Bauhinia monandra,* chlorophyll, crude oil toxicity, oil pollution.

INTRODUCTION

Petroleum crude oil is one of the energy sources of global importance. It is subject to the fact that it could be portrayed with the double sword syndrome; hence it is source of economic and foreign earnings to the society and could also engender doom to the society, and its environs. On this note it may be beneficial and also toxic to the environment. Toxicity is the ability of a chemical molecule or compound to produce injury once it reaches a susceptible site or on the body. It could interfere with the cellular or sub-cellular process leading to the disruption of the normal metabolism of biota. The first noticeable eco-toxicological effect of oil spilled across the land surface in any quantity is usually on vegetation. The vegetation is a fragile ecosystem and a major target of toxicity caused by petroleum pollution in the terrestrial habitat. Many plant physiological processes respond to environmental and other external influences like pollution. These physiological processes and external influences could be determinant of plant growth and productivity. They are important in determining the ecological relationship between crops and the environment. Such ecological relationship determines the biosynthetic activities and survival of plants in different environmental conditions (Osmond *et al*, 1987).

Photosynthesis and respiration (a process of chlorophyll and biomass generation in plant) are major physiological processes that determine the growth and productivity of plants in their immediate environment. Chlorophyll content reflects the level of the photosynthetic activities. This process of biosynthesis could be manipulated by both environmental influences such as light, water and oxygen in quality and quantity and also by external factor or agent like pollutant.

Petroleum hydrocarbons manifest their toxic effects by competing with some endogenous metabolites or some pathways of biota. It may or may not be lethal. Though autotrophic plant are directly affected by intensity of light, which drives photosynthesis and provides the necessary ingredients for plant growth and development (Boardman, 1977). The process might also be affected by presence of organic substances such as petroleum, which may be toxic to plant in its growth and development. Also a combination of environmental and biological factors could cause stress and disrupt metabolism in plants and may be expressed as reduction in growth, and yield or value or death of the plant or plant part (Hale and Cutt, 1987). To this end, any alteration in either environmental or physiological trend may disrupt plant metabolic processes either positively or negatively, thus chlorophyll concentration in leaves can also be altered.

Based on the above information, the present study was aimed at determining the importance of petroleum on the photosynthetic efficacy of agroforestry plant species. The relative accumulation of the pigment (chlorophyll) was used to evaluate the degree of influence of petroleum on the biosynthetic process of the plant species *L. leucocephala* and *B. monandra*. This approach provides a means of ascertaining the importance of petroleum in accounting for photosynthetic rate and yield potential under the environmental conditions.

MATERIALS AND METHODS.

The soil samples used for this study were collected from the proposed botanical garden site at the University Park of the University of Port Harcourt, (UNIPORT) Rivers State. The microplot of polybags and seeds of *Leuceana leucocephala* plant were obtained from the Rivers State Agricultural Development Programme (ADP) Head-quarters, Rumuodomanya, Port Harcourt. The *Bauhinia monandra* was obtained from the premises of the Faculty of Social Sciences, UNIPORT. The crude oil used was obtained from Shell Petroleum Development Company (SPDC), Port Harcourt. All chemical reagents, glasswares and equipment employed in this study were of analytical grade while some chemical regents were purchased from Welly International (Nig) Co. (scientific / Hospital and chemical supplies), few others, the

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TABLE 1: Spectrophotometry at 660 nm absorbance of 100% concentration and dilution series of chlorophyll content in *B. monandra* and *L. Leucocephala*.

	Crude oil (ml)							
	Bauhinia monandra **absorbance** ~~absorbance~~				***Leucaena leucocephala***			
Chlorophyll Concentration (ml)	**Control**	**25**	**50**	**100**	**Control**	**25**	**50**	**100**
Normal (5ml) concentration	1.578	0.812	0.551	0.345	3.685	1.520	0.793	0.698
7	1.162	0.547	0.390	0.304	1.379	0.748	0.604	0.408
9	1.145	0.435	0.354	0.300	1.272	0.613	0.535	0.395
11	1.003	0.383	0.347	0.200	1.251	0.451	0.432	0.333
13	0.882	0.286	0.260	0.158	1.242	0.380	0.364	0.290
15	0.781	0.165	0.128	0.125	1.026	0.364	0.355	0.195
Mean	**1.092**	**0.438**	**0.338**	**0.239**	**1.643**	**0.679**	**0.514**	**0.387**
SD (±)	**0.28**	**0.23**	**0.14**	**0.09**	**1.01**	**0.44**	**0.17**	**0.17**

SD (±) = Standard Deviation

Table 2: Summary of chlorophyll absorbance of two agroforestry plants grown on crude oil polluted loam soil.

Plant species	Variance – ratio	Probability
Leuceena leucocephala	6.13	*P< 0.05 (3.10)
Bauhinia monandra	25.46 * *	* Significant

TABLE 3 – The Optical Density (OD) and total chlorophyll content of the Spp. in a polluted terrestrial habitat.

Species	***B. monandra***	***L. leucocephala***	***B. monandra***	***L. leucocephala***
Crude oil (ml)	**Optical density (g)**		**Total chlorophyll (mg/g)**	
control	0.009	0.03	1438	4792
25	0.007	0.02	1118	3195
50	0.006	0.007	958	1118
100	0.003	0.006	479	958

glass wares and equipment were obtained from the Departments of Chemistry, Biochemistry, Plant Science & Biotechnology and College of Health Sciences, UNIPORT respectively. At 7days after seedling emergence in the nursery, the seedlings were transferred to the polluted micro plot of loam soil, surface area 78.5 cm^2. Levels of pollution were 0 ml, 25 ml, 50 ml and 100 ml per 78.5cm^2 soil simple polybags The growth performance of the species in terms of photosynthetic analysis of the chlorophyll pigment was done after harvesting and drying of the species.

Total Chlorophyll Content Analysis.

The Comar and Zcheille (1942) and Goodwin (1965) methods were adopted for the analysis. Fresh leaves were used and stored in refrigerator at 20°C to avoid degradation of coloured pigment. Fresh leaves were finely cut, and mixed thoroughly while dried leaves were ground and passed through 0.5 mm sieve. Each of these samples was macerated and homogenized once with a (20 ml) of acetone and then filtered into volumetric flask using glass funnel. After filtration, some quantity of water was added to adjust to 85% concentration through the separating funnel. Filtration was repeated by adding a 20ml of 85% prepared acetone until the washings are colourless. The filtrates were transferred to a suitable volumetric flask. A normal 85% acetone extract and series of dilutions of this extract using (7, 9, 11, 13, and 15 mls) 85% acetone solvent were made and passed through spectrophotometer. At (660 nm), the chlorophyll absorbance (wavelength) was determined, then optical density (OD), and total chlorophyll content determined. Optical density (OD)

Table 4 Summary of t-Test analysis for chlorophyll Optical Density and content of two species grown in crude oil polluted loam soil

Species	OD t-ratio	Chlorophyll t-ratio	Probability table-ratio
L. leucoucephala			$p<0.05$ (2.78)
	3.02*	2.71	* Significant
B. monandra			

measurement was made on the peak of absorption curve through the wavelength calibration. By knowing the gradient of the highest peak, curves of absorbance, optical density (OD) and total chlorophyll content against chlorophyll concentration were made.

Total chlorophyll is extrapolated using the formula:

$$\text{Total chlorophyll (\%)} = \frac{C\ (\text{mg l}^{-1}) \times \text{acetone extract (ml)}}{10^{-4} \times \text{samples wt (g)}}$$

C = (mg l^{-1}) chlorophyll obtained from the graph of (660 nm)

C = 17.6 x 0.D (660nm) -2.81 x 0D (643 nm)

OD is obtained from the graph

The data were subjected to statistical analysis of variance (one-way ANOVA), and t- Test analysis to ascertain the significant level of impact on the absorbance, optical density, and chlorophyll content of both species of plant.

RESULTS AND DISCUSSION

The results obtained from the ecological studies were the absorbance, optical density and chlorophyll content of the species in relation to the polluted habitats. The chlorophyll content reflects the level of the photosynthetic activities. The chlorophyll biosynthesis in the leaves of *L. leucocephala* and *B. monandra* was sensitive to the crude oil polluted habitat. *Bauhinia monandra* was much more susceptible to the pollution than *L. leucocephala* (Tables 1 to 4). There was reduction in wavelength, absorbance, optical density, and total chlorophyll content with increase in pollution level for both species. The impact was more on *B. monandra*.

In the control condition, the optical density and total chlorophyll content for *L.leucocephala* and *B. monandra* were 0.03 (OD), 4792 mg/g and 0.009 (OD), 1438 mg/g respectively. The light pollution of 25 ml crude oil had 0.02 (OD), 3195 mg/g amounting to 58.9% reduction and 0.007(OD), 1118mg/g, amounting to 59.9% reduction for *L.leucocephala* and *B.monandra*. The medium pollution of 50ml had 0.007; 1118mg/g with 68.7% reduction and 0.006 (OD), 958mg/g with 69.0% reduction were recorded for *L. leucocephala* and *B.monandra*. A 0.006(OD), 958mg/g with 76.5% reduction were recorded for *L. leucocephala* and *B.monandra* in heavily polluted habitat (Tables 1 and 3). All these indicate the high susceptibility of *B.monandra* and *L. leuccocephala* to the polluted habitat and this was more pronounced with increase in the intensity of the pollution.

The pollution effect on the chlorophyll absorbance of *B. monandra* was highly significantly different from that of *L. leucocephala* chlorophyll absorbance (Table 2).

The pollution on the OD of both species is however significantly different but not highly significant on their Chlorophyll content (Table 4.)

The observed reduction in the chlorophyll (plant biomass production), yellowing of the leaves (chlorosis), cessation of growth and eventual 'necrosis' of *B. monandra* and *L. leeucocephala* could be attributed to abnormalities induced by the oil pollution in the physical and physiological activities of the plant. The reduction in the chlorophyll content of *B. monandra* caused by the oil pollution undoubtedly affected the photosynthetic activities. Similarly, Wedding *et al* (1952) and Riehl *et al* (1959) reported the reduction in the photosynthetic rate *in citrus* plants subjected to simulated crude oil pollution. Inhibition of photosynthesis by crude oil pollution will equally affect the plant productivity, thus a reduction in total biomass observed. While Ezeala, D.O. (personal communication) observed 79% loss in the photosynthetic chlorophyll of *Pistia stratoites* induced by crude oil pollution, Odu (1977, 1981) had from field and laboratory reports revealed that oil on touching the leaves of plant penetrates the leaves and interferes with the physiological functioning (transpiration and photosynthesis) of the leaves.

CONCLUSION

In conclusion, the result from the study has shown that chlorophyll is known to enhance efficient light capture, thus serve as an adaptive feature in plants. Its interaction with an unconducive environment could cause a metabolic disruption in plant. Apart from the fluctuation in physiological and environmental factors that may affect the biosynthesis of chlorophyll in these plants *Inter alia* a combination of biotic stress that may disrupt metabolic processes, it was discovered that accumulation of photosynthetic pigments was also affected by the various intensity of crude oil polluted habitat. Therefore the plants species can serve as a sensitivity index for oil spillage in the habitat that it occurs.

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