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PHYTOREMEDIATION INNOVATIVE TECHNOLOGY (SERIES 2): A SHORT-TERM COMPARATIVE ANALYSIS OF ENHANCED BIODEGRADATION OF CRUDE OIL IN THE SOIL BY MACROPHYTIC NODULATION

N. L. EDWIN-WOSU and P. D. S. KINAKO

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ABSTRACT

A short term comparative ecological study of the use of two agro-forestry species, *Leucaena leucocephala,* Lam De. Wit and *Bauhinia monandra*, Kurz, in bioremediation of oil polluted environment was carried out, focusing on the evaluation and enhancing potential of the macrophytic species for degradation of hydrocarbon (crude oil) in the soil relative to their nodulation efficacy. Results show that *L. leucocephala* had nodules with levels ranging between 1.40 ± 1.40 to 11.60 ± 2.20 plant^{-1} and relative to various pollution levels and 15.10 ± 6.10 plant^{-1} in the control condition. Though *L. leucocephala* experienced pollution depression upon time lag in nodulation, the depression was directly proportional to the intensities of pollution. *Bauhinia monandra* had none at the end of the experiment both in the polluted and controlled conditions. Total hydrocarbon level following treatment of the soil by *L. leucocephala* and *B. monadra* has been deduced in relation to post-pollution concentration, disappearance and net % loss on such treated soil environment. A comparatively high value of 60% total loss of crude oil was indicated in *L. leucocephala* treated soil. This shows that natural biodegradation could be a major and ultimate mechanism for elimination of oil in terrestrial environment. The highest total % loss of crude oil was recorded in *Leucaena leucocephala than Bauhinia monandra* treated soil. *Leucaena leucocephala* showed an enhanced performance than *Bauhinia monandra* at the end of the study. *Leucaena leucocephala* was thus considered more ideal and more promising for remediation work than *B.monandra*

KEY WORDS: *leucaena leucocephala, Bauhinia monandra*, bioremediation, crude oil. nodulation.

INTRODUCTION

Bioremediation by macrophytes is another important environmental modification approach for the remediation of oil-polluted habitat. Although there is little information on the bioremediation potential of macrophytes on crude oil polluted habitats, there appears to be an absolute requirement for environmental reclamation activities using macrophytes. Hence it may take a long term for oil polluted terrestrial habitat to undergo self-rehabilitation through natural processes as noted by Kinako (1981), Studies have shown that such oil polluted habitat which may otherwise be reclaimed using an integrated approach like burning, soil scarification and liming treatment to offset most of the physico-chemical and biological aspects of the problem, could also be reseeded with appropriate oil pollution resilience and tolerant macrophytes (Kinako and Zuofa, 1991).

Hence the direct planting of grown plants have also proved positive at the instance of such species as *Panicum maximum, Luffa* sp and *Zebrina* sp., for the reclamation of oil polluted habitat (Kinako, 1984), there is need to evaluate the potential efficacy of the use of nodulating macrophyte in the enhancement of bioremediation of crude oil polluted habitat. Despite the reported damaging effect of crude oil pollution, there have also been reported cases of improvement in growth in soils contaminated with crude oil due to Nitrogen fixation and nutrient enrichment by nodulation species. The usefulness of tree legumes in farming

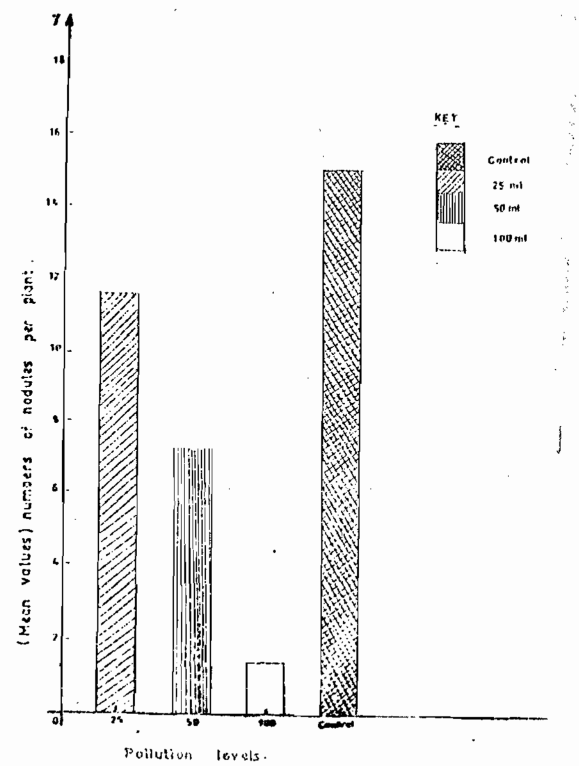

Fig. 1: Effect of crude oil pollution on Nodulation of L. leucocephala grown in crude oil polluted loam soil.

N. L. EDWIN-WOSU, Ecology Unit Dept. of Plant Science & Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria.
P. D. S. KINAKO, Ecology Unit, Dept. of Plant Science & Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria.

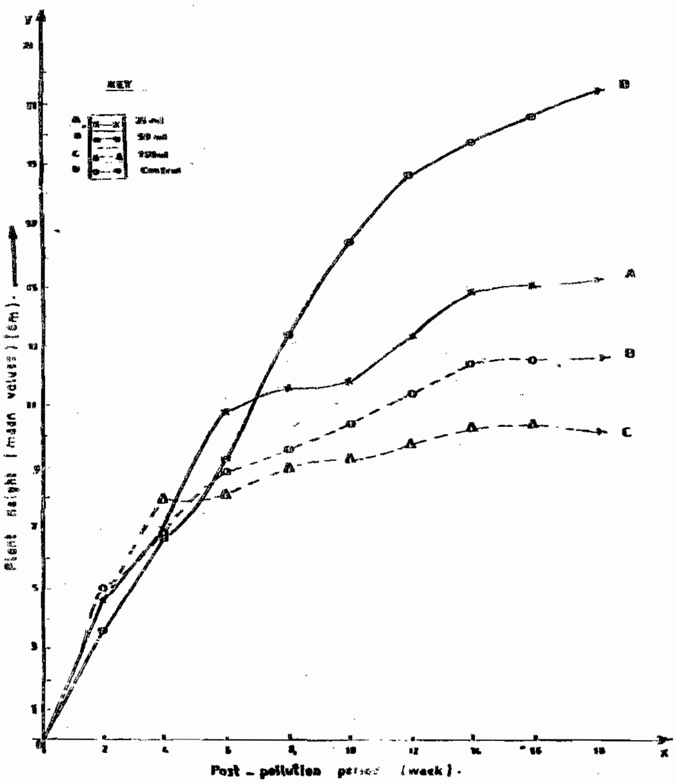

Fig 2a: Effect of oil pollution on the height of L. Leucocephala growth in a crude oil polluted loam soil.

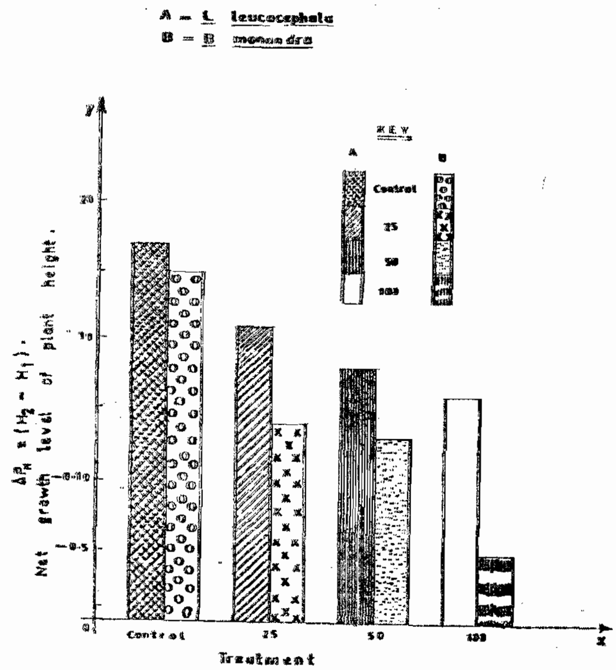

Fig 2c: Net performance of the height of L. Leucocephala and B. Manandra grown in a crude oil polluted loam soil.

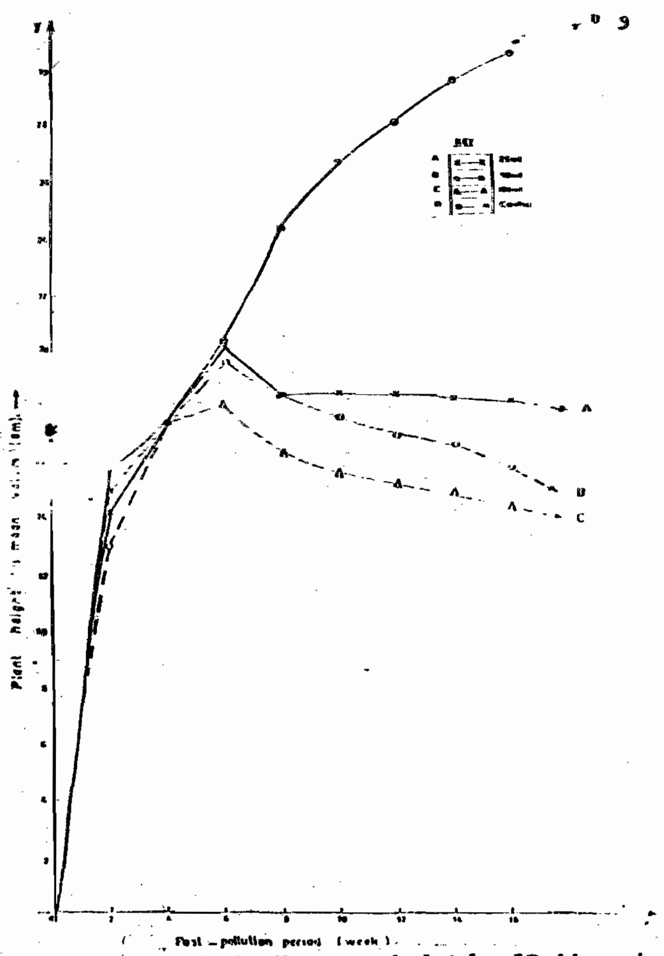

Fig. 2b: Effect of oil pollution on the height of B. Manandra grown in crude oil polluted loam soil

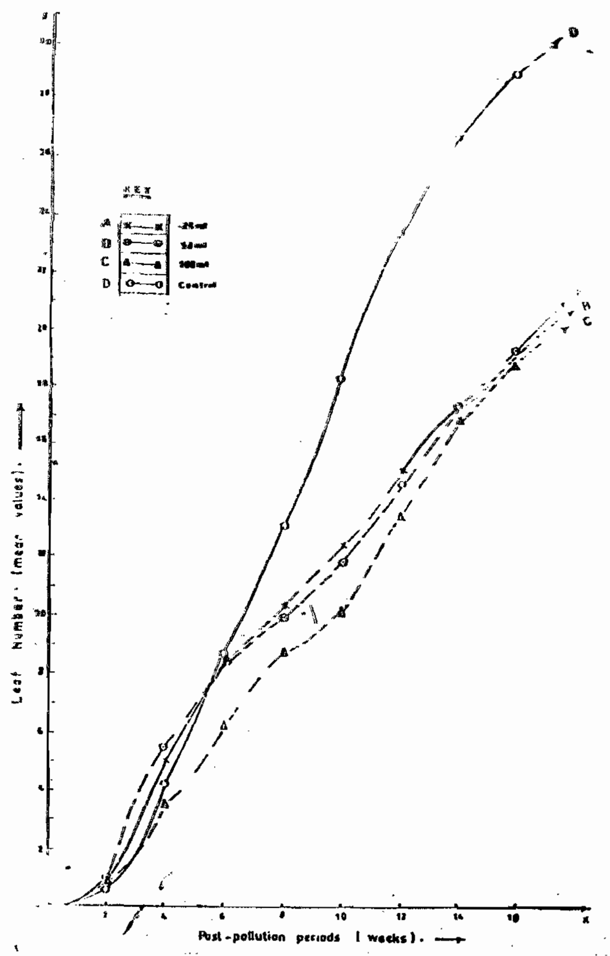

Fig. 3a: Effect of crude oil pollution on leaf numbers of L. Leucocophala grown in loam.

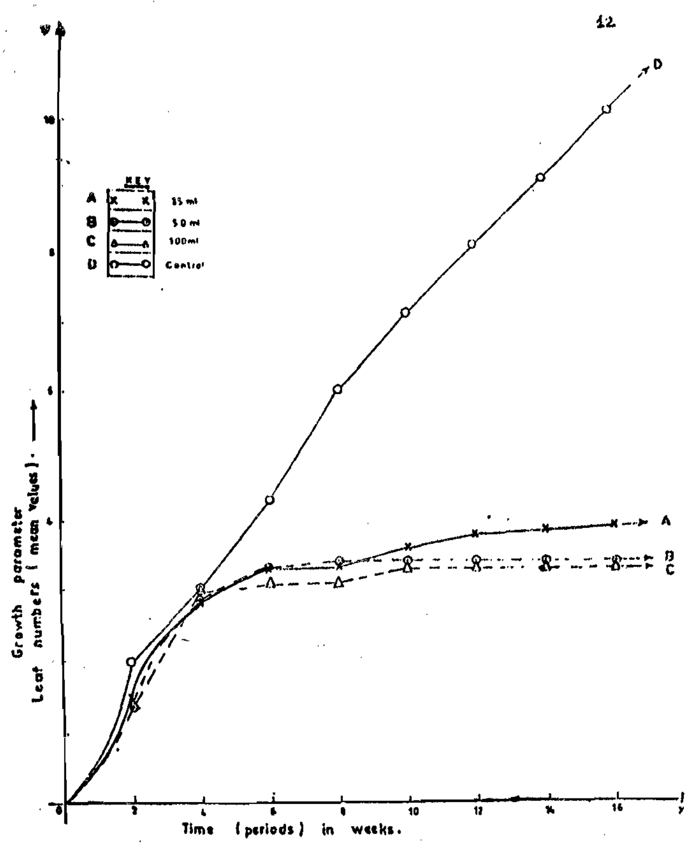

Fig. 3b: Effect of oil pollution on the leaf numbers of B. manandra grown in crude oil polluted loam soil.

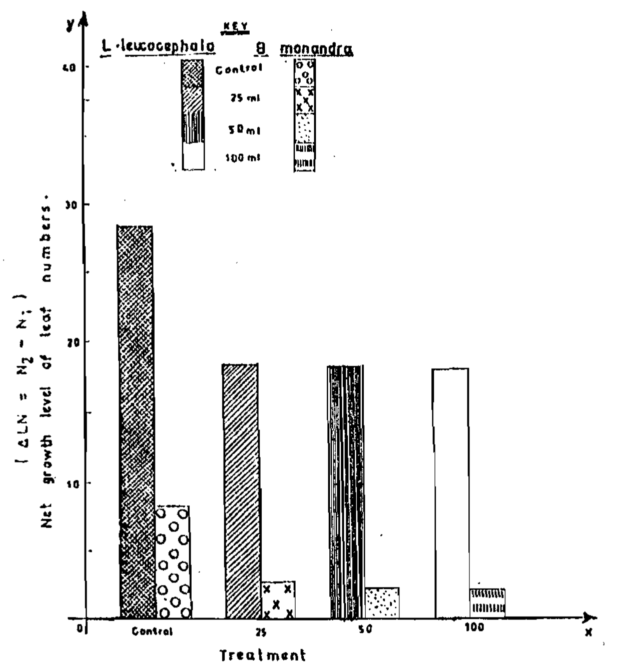

Fig. 3c: Net performance of the leaf numbers of L. leuccocephala and B. manandra grown in a crude oil polluted loam soil.

system have been documented by several authors (Brewbaker *et al*, 1982, Rokoski *et al*, 1982; Sprent, 1986). All these reports appear to conclude that the potentials of these tree legumes in enhancing soil fertility are high and therefore will be useful in soil enrichment, restoration and reforestation programmes in the tropics.

Some groups of Fabaceae plant family nodulate with the *rhizobia* present in most tropical soils. It can thus thrive in Nitrogen poor soils where cereals will grow well, contribute Nitrogen to associated crops and leave residual nitrogen in the soil for subsequent crops to exploit (Mulongoy and Akobundu, 1990). In the tropical rainforest zone, a number of these legumes occur in their wild state and are very useful in restoring fertility during fallow practice in traditional farming system and bioremediation activities in a contaminated soil, particularly a crude oil polluted soil. However, a lot of these species are yet to be assessed for nodulation and bioremediation activities This study was therefore carried out in order to ascertain the growth nodulation and bioremediation potential of these two agro forestry species (*Leucaena leucocephala and Bauhinia monandra*) in a crude oil polluted soil at the seedling stage, with the following objectives.

i. To screen agro forestry species for nodulation in a crude oil polluted soil

ii. Determine the relationship between nodulation and growth as well as the relationship between growth parameters in a crude oil polluted habitat.

iii. Carry out investigation on the use of the two agro-forestry tree species for habitat remediation work, in relation to the degree of nodulation.

MATERIALS AND METHODS

The microplots of study were loam soil sample, collected from the park of the University of Port

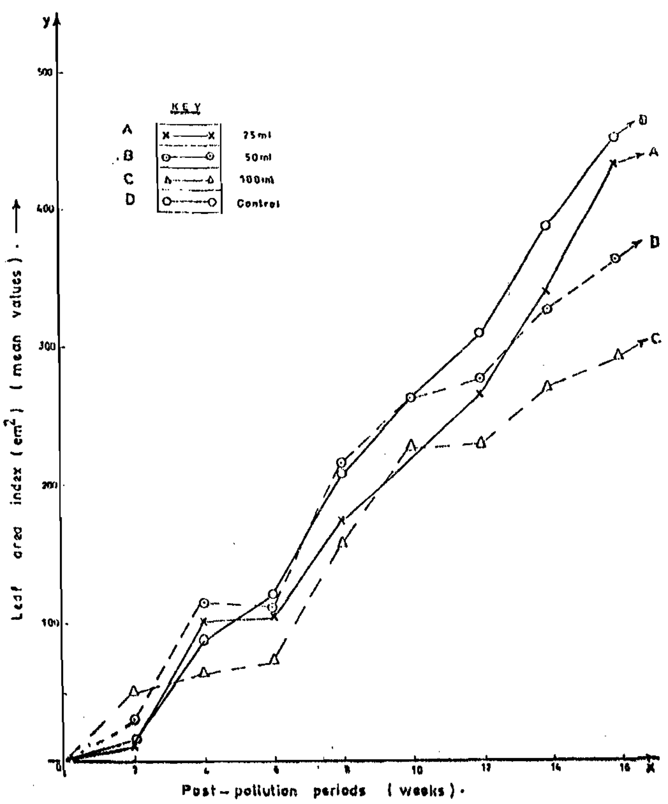

Fig. 4a: Effect of oil pollution on the leaf area of L. leucocephala grown in crude oil polluted loam soil.

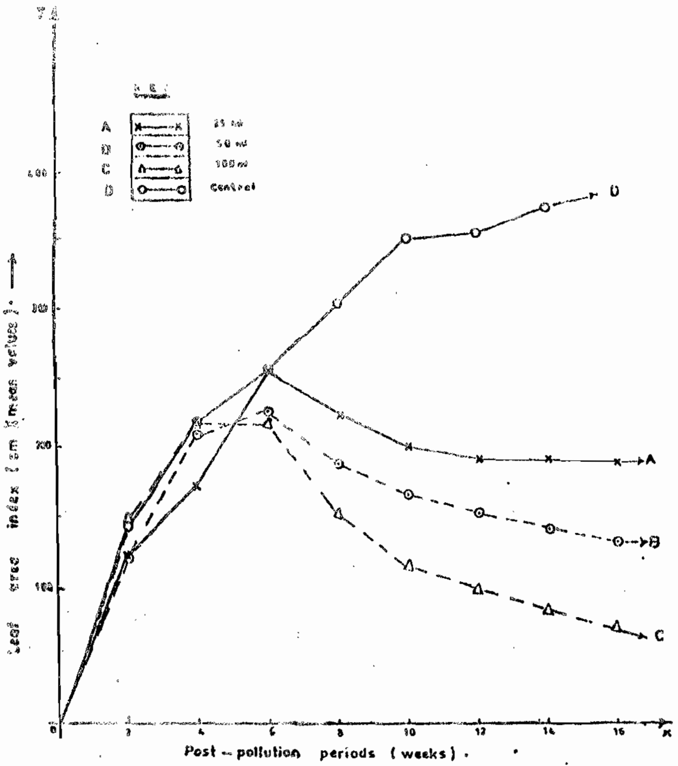

Fig. 4b: Effect of oil pollution on the leaf area of B. manandra grown in crude oil polluted loam soil.

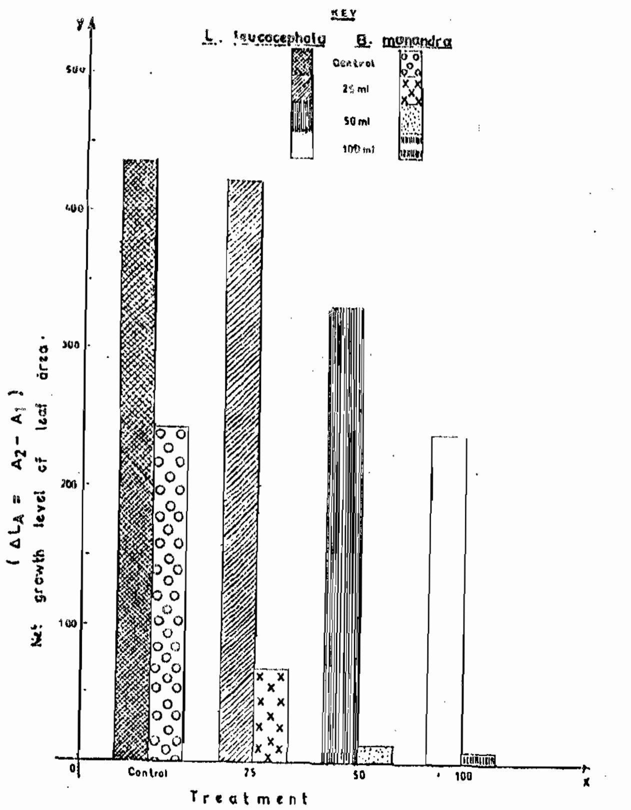

Fig. 4c: Net performance of leaf area of L. leucocephala and B. manandra grown in a crude oil polluted loam soil.

Harcourt, Abuja campus. The two plant species of interest (*Leucaena leucocephala* and *Bauhinia. monandra)* were obtained from the Rivers State Agricultural Development Programme (ADP) Headquarters Rumuodamanya, Port Harcourt and the premises of the Faculty of Social Sciences, University of Port Harcourt respectively. Crude oil was obtained from Shell Petroleum Development Company (SPDC), Port Harcourt. Following the germination test carried out in the plant Taxonomy and Biosystematic laboratory after seven days and seven days in the nursery, the seedlings were transferred to replicate micro plots of soil that have been polluted at various intensities with crude oil. The pollution was done in dozes of 25ml, 50ml 100ml, and control and each dose replicated 10 times. The growth performance of thee seedlings and nodulation were monitored for 16 weeks and was used as a measure of their tolerance in the polluted habitat. Post-pollution rejuvenation of the polluted soil was accessed by means of comparative analysis of the physicochemical and other edaphic parameters of the polluted soil by the nodulation phenomenon. After four months of study, and following the termination of the experiment, nodulation study was done. Roots of plant species were harvested by scattering loose the replicate and carefully washed with water to remove all adhering

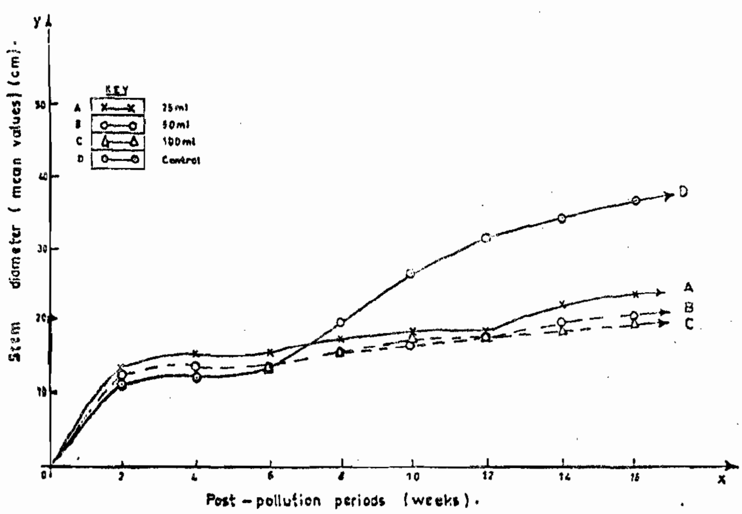

Fig. 5a: Effect of oil pollution on the stem diameter of L. leucocephala grown in crude oil polluted loam soil.

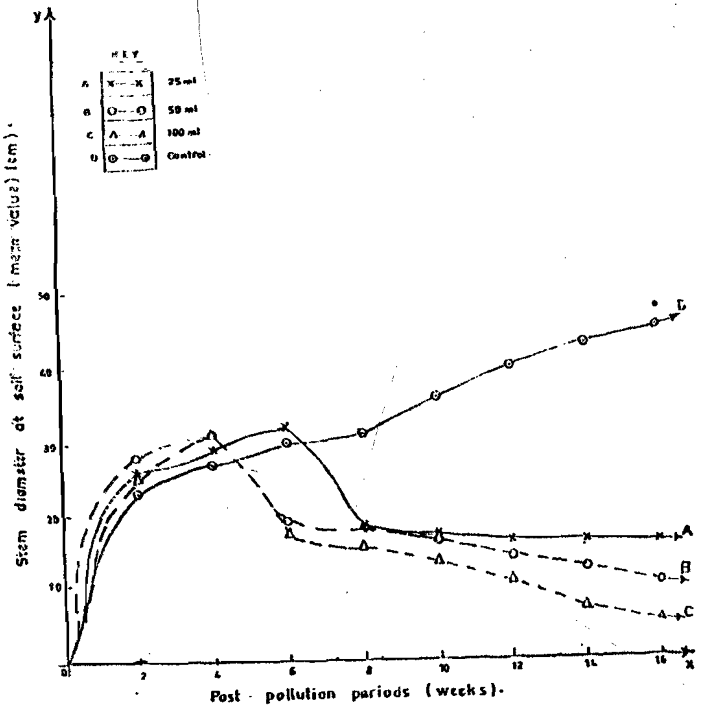

Fig. Effect of oil pollution on the stem diameter of B.manandra grown in crude oil pollute loam soil.

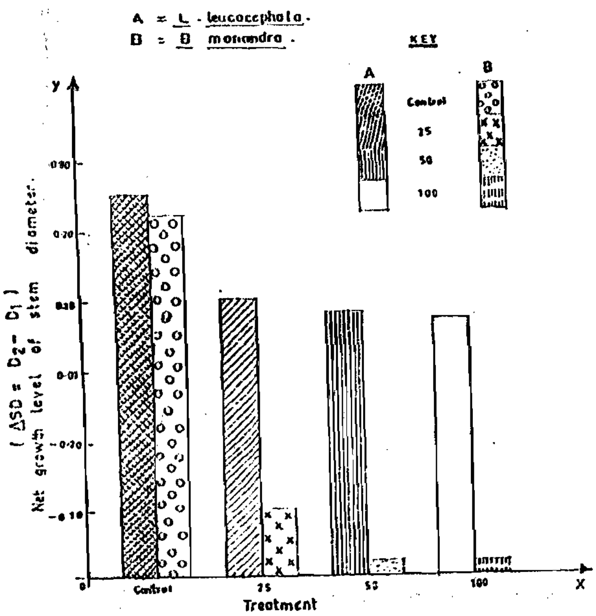

Fig. 5c: Net performance of stem diameter of L.leucocephala and B.manandra grown in a crude oil polluted loam soil.

soil particles. The roots were then dried with filter paper. The nodules were carefully removed from the roots and counted.

RESULT

As indicated in Fig. I below *Leucaena leucocephala* had nodulation capabilities in the polluted habitats unlike *Bauhinia monandra* that lack such potential. *Leucaena leucocephala* had an average level of nodulation of 11.60 ± 2.20, 7.20 ± 1.60 and 1.40 ± 1.40 plant^{-1} in the respective levels of light, medium and heavy pollution as

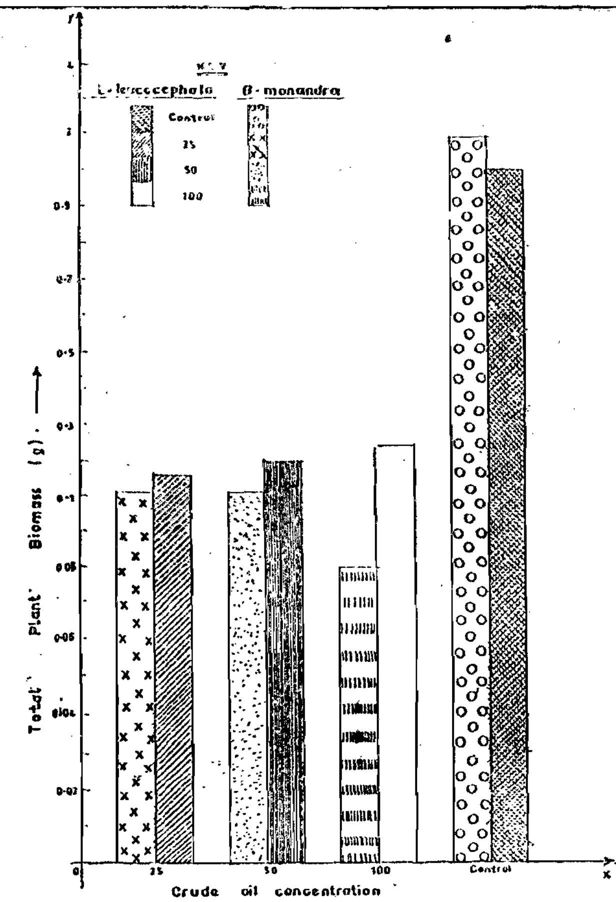

Fig. 6: Effect of oil pollution on the total plant biomass (g) of L.leucocephala and B.manandra grown in a crude oil polluted loam soil.

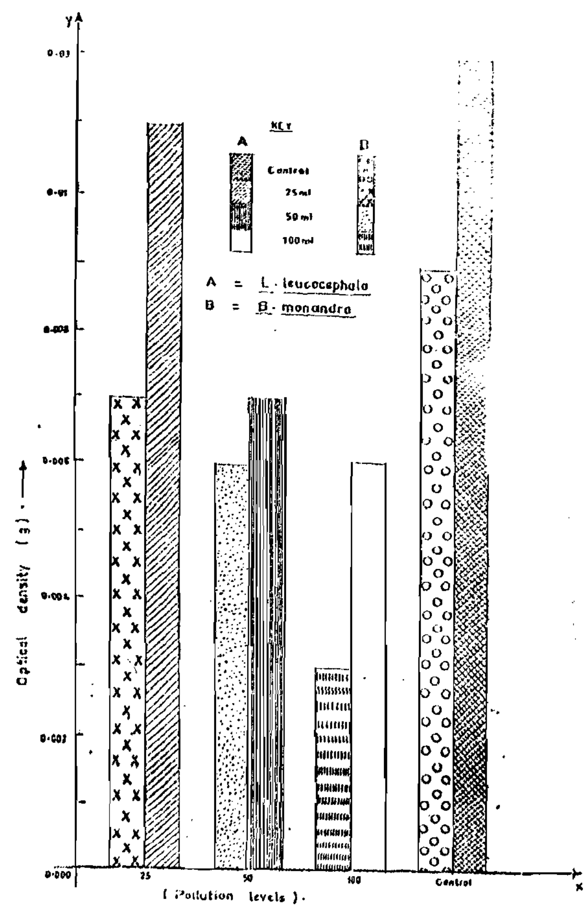

Fig. 7a: Optional density (od) of chlorophyll content of L.leucocephala and B.manandra grown in various levels of crude oil polluted loam soil.

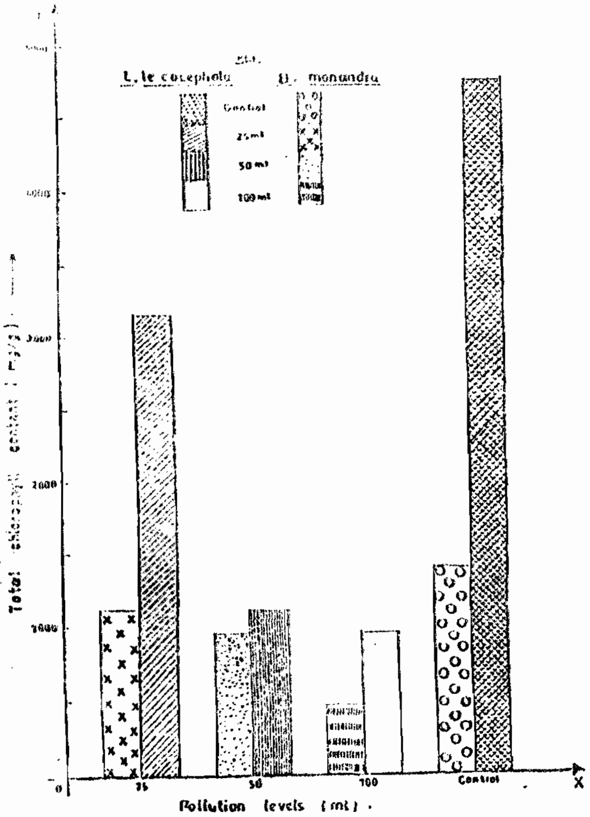

Fig. 7b: Total chlorophil content of L.leucocephala B.manandra grow in various levels of crude oil polluted terrestial habitat.

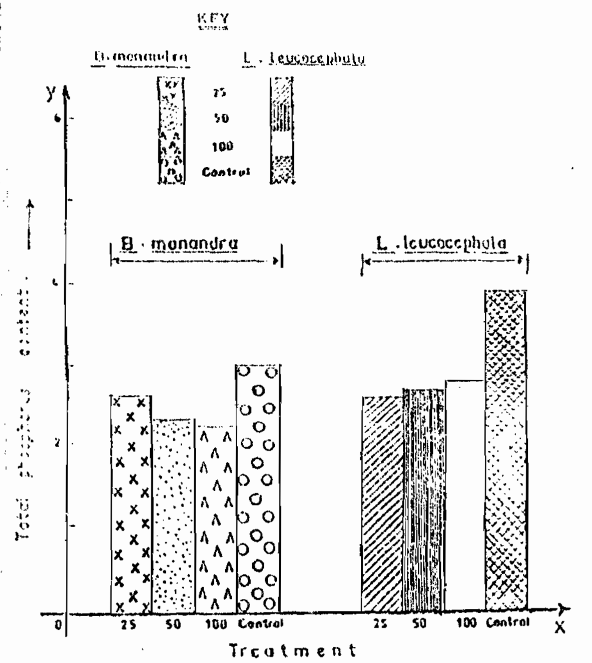

Fig. 8: Total phosphorus content of B.manandra and L.leucocephala grown in crude oil polluted loam soil.

against 15.10 ± 6.10 Plant in the control. Pollution depressed nodulation in the nodulating species. The depression was directly proportional to the intensities of pollution. *Bauhinia monandra* had none at the end of the experiment both in the polluted and controlled conditions

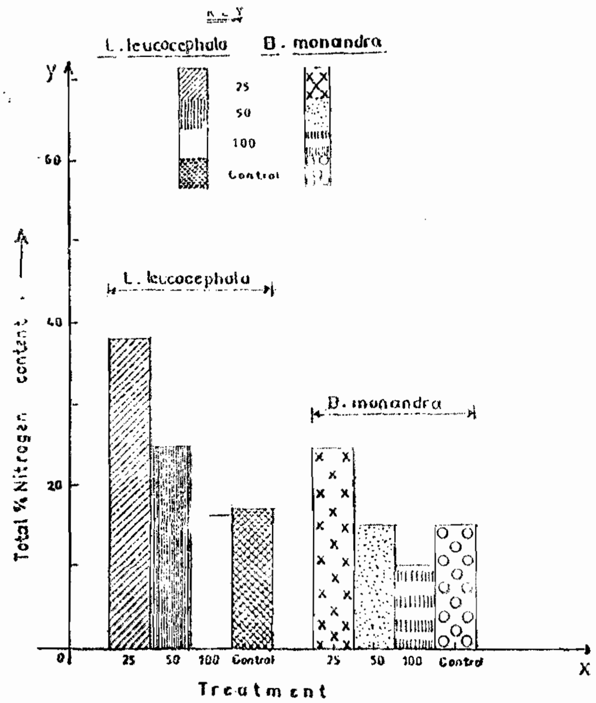

Fig. 9: Total Nitrogen content of L.leucocephala and B.manandra grown in crude oil polluted loam soil.

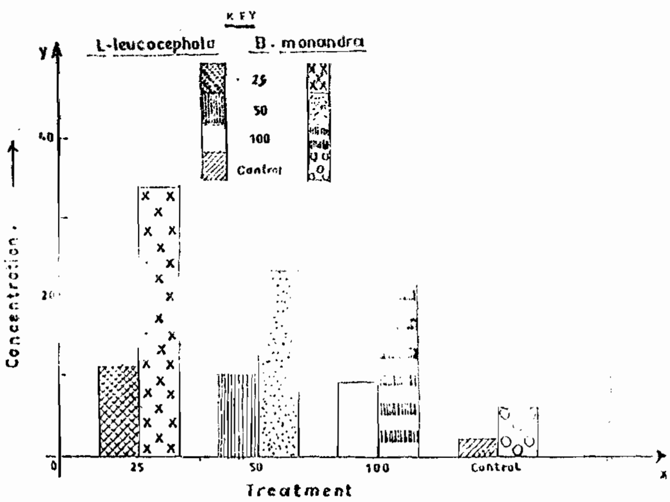

Fig. 10: Post-pollution oil concentration of crude oil polluted loam soil treated with L.leucocephala and B.manandra.

DISCUSSION

The utilization of the nodulating potential of Nitrogen fixing plants in the enhancement of bioremediation of crude oil polluted soil was evaluated. Such growth and nodulation potential of the species concerned is also a reflection of their biological performance and their capabilities to rejuvenate the physicochemical factors of the polluted edaphic environment as well as enhancing hydrocarbon loss as indicated in Figures 1 to 12 below. Atlas and pramier (1990) observed that the use of biodegradable capabilities show how natural biological process can be used to minimize environmental contamination. Unlike the *B. monandra,* the loss of crude oil recovered in *L. leucocephala* treated soil of high pollution agrees with

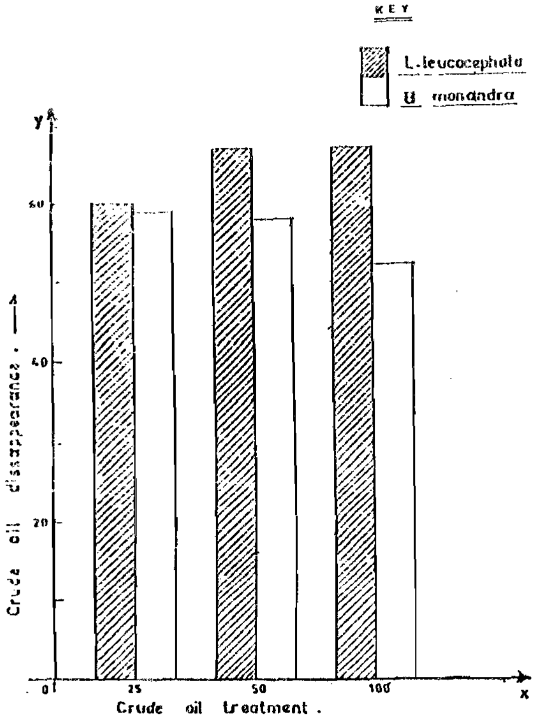

Fig. 11: Crude oil aissappearance of a polluted loam soil treated with L.leucocephala and B.manandra.

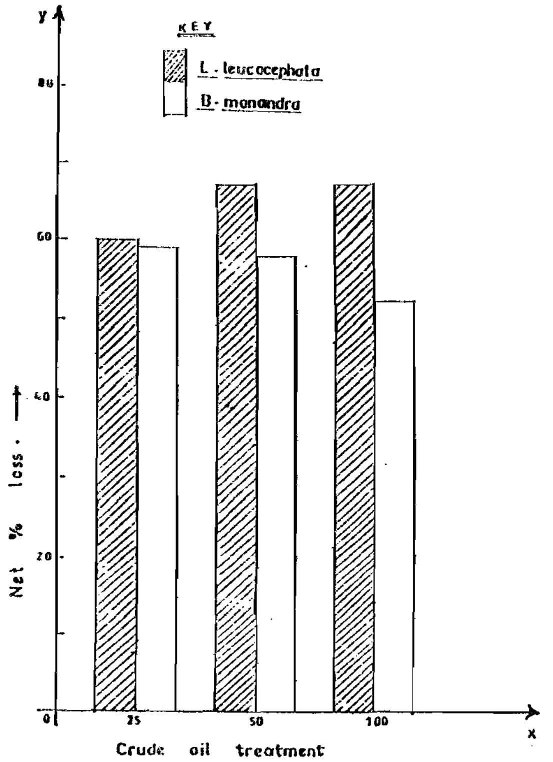

Fig. 12: Net (%) loss of crude oil from polluted loam soil treated with L.leucocephala and B.manandra

the assertion by Ekundayo and Omokaro (1987), that natural biodegradation is the major and ultimate mechanism for elimination of oil especially in aquatic environment, which may also apply to soil environment. Such potential could also be a reflection of nodulating capabilities of the plant species and thus reflect their biotic performances (Figures 1 to 12) in relation to the various levels of pollution intensities. *Leucaena leucocephala* recorded increased values of biotic growth performance in height, leaf numbers, leaf area and stem diameter, biomass, chlorophyll content and abiotic factors like nitrogen content, phosphorus content comparative to *B. monandra* (Figs.1 to 9). The highest total % loss of crude oil was recorded in *L. leucocephala* treated soil of highest pollution. This could be due to adequate aeration and nodulation by the plant roots, thus it implication for nitrogen fixing (Fig. 9) unlike the non-symbiotic effect of the *B. monandra*. The drop in total hydrocarbon level in *L. leucocephala* treated soil (Figures 10 to 12) noticeably may represent the era of their utilization by the Phytomicrobial associated population of the rhizospheres, which lacks in *B. monandra*. However in the polluted nature of the loam soils, *B.* monandra lacks the natural efficacy for nodulation, since with respect to the control replicates, it did not nodulate even in the presence of the necessary environmental factors. It lacks the potential for nitrogen fixation, hence no symbiotic association with the bacteria, *Rhizobium*. The fact that the proportion of hydrocarbon utilizer in the population increases with the introduction of hydrocarbons into an environment (Jones and Greenfield, 1991), accounts for the depletion of nitrogen and phosphorous in the polluted soil treated with *L. leucocephala*. In biodegradation report show that plants have series of effects on the microbial population in the rhizosphere, the immediate area surrounding the root. There is some symbiosis, which occurs between a plant and its microbial neighbours. Microbial populations have been reported to be two orders of magnitude higher in the soil of root zone than in adjacent unplanted soil. The limiting factors for most aerobic microbial consortium growth include oxygen, nutrient and water. The roots of many plants provide these requirements to the microbial zone as a byproduct of normal plant growth.

As root penetrates the soil, there is passive aeration as the roots loosen the soil, and active aeration as the roots release oxygen as part of normal plants of normal plant respiration. As a natural function of reacting to change environment conditions, part of tree roots die off during seasonal water and temperature fluctuations (Anderson, 1993). These abandoned or sloughed roots and root hairs become a nutrient source to the rhizosphere microbial community. These nutrients may serve as cometabolites, sustaining microbes that incidentally degrade contamination as part of their metabolism. Plant also enhance microbial communities by drawing water into the near surface root zone. Dropping leaves and sloughing roots adds to the organic matter content of the soil, which adds to the soils ability to retain water (Steve, 2004). Similarly in such degradation the plant itself could also degrade the contaminant. This may occur as metabolism of a contaminant with the plant, or by transforming or mineralizing it to a less toxic form through exudates. Various plants produce different. Enzymes, many of which are useful in the destruction of contaminants. Nitroreductass, dehalogenase, peroxidase and others have been found to be exuded by some plants (Schnoor, 1995). These enzymes can either detoxify a contaminants or render it vulnerable to microbial consumption. Similar trend of action could be attributed to *L. leucocphala* Because of its potential for microbial habitation (hence its inbuilt phytomicrobia symbiotic association), it enhanced the impact of nitrogen and phosphorous utilization for hydrocarbon degradation, suggesting that it would enhance soil recovery in polluted soil and promote better soil structure than the *B. monandra* treated soil.

The result suggests the possibility of ploughing back nitrogen fixing nodulating plants into the soil for a meaningful contribution to the enhancement of bioremediation. It is inferred that this enhances hydrocarbon degradation, producing carbon, water and Co_2 in presence of phosphorous, nitrogen nutrient and other environmental variables. Though the pollution depressed nodulation in the nodulating species, it was directly proportional to the intensities of pollution. The result indicates that *L. leucocephala* is tolerant and resilient to oil pollution habitat among the legumes used. This remediating quality is attributed to its nodulating and crude oil degrading ability. On the basis of these results, the *L. leucocephala* showed a more enhanced performance than *B. monandra* at the end of the study. *Leucaena leucocephala* was thus considered more ideal and more promising for remediation work than *B. monandra*.

CONCLUSION

By holistic assessment, the study has shown that the application of *Leucaena leucocpehala* has a positive effect on bioremediation. At the seedling level, there was apparent percentage crude oil loss in the *B. monandra* for bioremediation. It was known to promote enhanced good soil structure at the seedling level, thus hold good promise for bioremediation. On the basis of the result it has been possible to determine their suitability or otherwise for agro forestry bioremediation work.

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