# Evaluation Of Bioremediation Of Agricultural Soils Polluted With Crude Oil By Planting Beans Seeds, Phaseolus Vulgaris

Aboaba, O.A., Aboaba, O.O., Nwachukwu, N.C., Chukwu, E.E. And Nwachukwu, S.C.U.

Department Of Microbiology, University Of Lagos, Yaba, Lagos, Nigeria.
Department Of Pblic Administraton, National Open University, Victoria Island, Lagos, Nigeria
simboaboaba@yahoo.com

**ABSTRACT:** The impact of crude oil on agricultural land, germination, growth and morphology of beans seeds (Phaseolus vulgaris) after bioremediation of agricultural soils polluted with crude oil using selected strains of Pseudomonas putida (PP) was investigated (E). A similar polluted agricultural soil not inoculated with PP served as a control (C). In e, the residual oil concentration decreased from 0.260 at week zero to 0.002g/g soil at week 10 representing about 92% oil reduction. The corresponding values for c were 0.240 and 0.170g/g soil respectively and this is equivalent to about 17% oil reduction. The levels of oil reduction in both E and C were confirmed by the results of gas liquid chromatography which revealed smaller peaks for E. At day 16 during germination experiments, there was 40% germination in E and 20% in C. For C, the mean seedlings height was 5.20 cm and all displayed abnormal morphology such as stunted growth and chlorosis. Thus treatment of oil-polluted agricultural land with PP culture as bioremediating agent can produce soils which can grow healthier plants than where bioremediation has not taken place. In conclusion, beans seeds germination and the general morphology of the seedlings seem to be reliable biological indices for the evaluation of the recovery of crude oil-impacted land after bioremediation protocols using selected microorganisms. [Nature and Science. 2007;5(4):53-60].

**Keywords:** Crude Oil, Pollution, Bioremediation, Pseudomonas Putida, Agricultural Soil And Seed Planting

## INTRODUCTION

Land, a major factor of production and an employer of human resources for agricultural purposes for food production, has become a necessity especially in countries where fertile arable land is scarce (Atlas, 1991; Harley and Englande, 1992). To avoid poor yields of agricultural produce caused by planting in oil-impacted land, the regaining of the soil fertility upon pollution is very important and may involve some waste management such as bioremediation protocols (Atlas, 1991: Achana et al, 2005: Nwachukwu, 2001). This can be achieved using a selected strain of Pseudomonas putida (PP) which was examined as a part of the objective of this study. Even after bioremediation of oil-impacted land, there is the critical need to evaluate and ascertain the recovery of the land in terms of soil structure and fertility before planting. The best method to achieve this is by a biological method. Thus, the effectiveness of the bioremediation programmes put in place in the oil-impacted soils were evaluated by planting bean seeds (Phaseolus vulgaris) as the major aim of this study. Also, the percentage germination and growth profiles of beans seeds as biological evidence of the recovery of the oil-impacted soils were examined.

## MATERIALS AND METHODS

Duplicate oil-impacted agricultural soils (4kg each) to the level of 2.04%w/w pollution collected from Niger Delta area of Nigeria was bioremediated by application of 350ml of the developed culture of Pseudomonas putida (PP) containing 3.80 x 106 cfu/ml in nutrient broth (E). The duplicate setups (C) contained all the materials present in E but without PP culture. At two weeks intervals, inoculation of E with PP culture and C equivalent amount sterile distilled water was repeated for a period of ten weeks. During this period, changes in the residual crude oil concentration by both gravimetric and gas liquid chromatographic methods (Yveline et al, 1997), population density of total bacteria on nutrient agar and PP on selective Pseudomonas medium (Oxoid) and fungi on potato dextrose agar (Oxoid) were monitored. At the end of the bioremediation period (10 weeks), the soil samples were tested to check whether or not the oil pollutant was eliminated by planting selected ten healthy beans seeds (Phaseolus vulgaris) (PV) per replicate of soil E and comparing the morphological characteristics such as percentage seed germination, leaf structure, height, etc of the seedlings obtained with those of C (Nwachukwu et al., 2001). Using Pseudomonas spp as test organisms with a mean generation time (T) in tropical soil ecosystem polluted with crude petroleum as 14.84 days as previously determined (Chukwu et al., 2005), the time required for the environment to recover completely on terms of microbial load was estimated using the model (Dawes, 1969):

$$\frac{\text{Log } N_t - \log N_0}{\text{Log } 2} = \frac{t}{T}$$

Where $N_t$ = highest exponential growth phase population density of PP at time t; $N_0$ = initial exponential growth phase population density at time zero, $t_0$; t = exponential growth phase time; T = mean generation time of PP in oil-impacted tropical soil ecosystem. Thus, in this case, $N_t$ = 1.511 x 10 8 cfu/g, $N_0$ = 1.82 x 10 5 cfu/g and T= 14.84 days.

The socio-economic aspects of this study involved interviewing some people from Niger Delta area of Nigeria as regards their demand for land as an important factor of productions, the other possible ways of augmenting their income and the government policy and implementation strategies about their environments grossly polluted with crude oil. To achieve this, 200 male and 200 female adults in the Niger Delta area were interviewed through the use of questionnaires.

**RESULT AND DISCUSSION**

Figure 1 shows the growth profiles of Pseudomonas putida (PP) during inoculum development. The broth inoculated with PP showed increases in optical density over the period. Thus, the culture was at its exponential stage when it was applied as a bioremediating agent to the agricultural soil samples polluted with crude oil. In contrast, the control broth showed little or no increase in optical density over the period suggesting the absence of microbial contaminants in the broth systems during inoculum development. Thus, any observed differences between C and E could be attributed to the culture inoculated into E. generally, the microbial populations increased persistently for the first few weeks and this was much more remarkable in E than observed for C (Fig.2). The predominant microorganisms identified for both E and C replicates included Bacillus spp, Lactobacillus spp (bacteria); Candida spp and Penicillium spp (fungi). In addition, Pseudomonas spp occurred abundantly in E and this could be attributed to the inoculated culture into E as a bioremediating agent. In E, the residual oil concentration (ROC) decreased from 0.026 to 0.002 g/g soil at week 10. The corresponding values for C were 0.024 and 0.017 g/g soil respectively (Fig.3). Moreover, GC profiles of ROC showed remarkable reductions in peaks and the values for biomarkers namely nC17/ pristane and nC18/ phytane ratios were much more pronounced in E (Fig.4) supporting the gravimetric values observed for ROC. Furthermore, the percentage seed germination in E was 40% at day 12 with the seedlings reaching a mean height of 7.75cm at day 16. The equivalent values for C were 10% AND 5.50cm respectively giving the difference of about 48% in seedling heights (Fig.5). Generally the growth profiles in PV were poor in C with pronounced abnormal morphology when compared with results obtained for E (Fig.6). This is in line with our previous reports although cress seeds (Lepidium spp) were used (Nwachukwu et al., 2001). When the values obtained for $N_t$, $N_0$ and T were fitted into the above model, the time probable (t) required for the oil-impacted agricultural soils (E) to recover completely in terms of microbial load from the day of putting in place the bioremediation protocols was estimated at 26.14 weeks. Consequently, it would take the polluted soil ecosystem (E) approximately 26 weeks from the day of sampling to return to the normal conditions obtainable in the equivalent unpolluted agricultural land in the Niger Delta region. This estimation is valid only on the condition that no crude petroleum or petroleum products would be further introduced into the environment. This is because chronic addition of oil pollutant as a result of imminent oil spills due to the activities of oil industries located in the area or to natural blow-outs from oil deposits could increase the recovery time (Atlas, 1991). From the responses of the people in the Niger Delta area interviewed, 344 (86%) were farmers involved in practices such as intensification of agricultural production with shortening of traditional rotation methods and permanent monocropping without sufficient time for replenishment of nutrients. This is further worsened by the chronic crude petroleum pollution in the area with attendant high degree weathering of the environment and poor yield. Incidentally, the government policies about the high attendant socio-economic problems confronting the farmers as a result of the pollution of their agricultural land are not clearly defined. For the control (C), the recovery time could be longer than observed for E but was not estimated using the model. This is because the population density of bacteria in C had not reached its exponential peak to allow the application of the model in order to estimate the recovery time for C (Dawes, 1969).

Significance and impact of study:

This study confirms that treatment of oil-impacted agricultural land with a selected culture of Pseudomonas putida can produce soils which are capable of growing healthier plants than where bioremediation and bioaugmentation has not taken place. Moreover, from the results of this study, planting of PV seeds seems to be a good biological index that can be used in evaluating the level of recovery of agricultural land upon pollution with crude oil. This is because of the low oil concentration of 0.002 and 0.017g/g soil in E and C respectively at week 10 was probably responsible for the low percentage germinations and the gross biological damages usually observed in crude oil-impacted ecosystems. Thus, a selected culture of P. putida isolated from tropical ecosystems (Nwachukwu, 2001) seems to be a suitable bioremediating agent of oil-impacted agricultural land while P. vulgaris seeds are highly sensitive to crude oil toxicity; its germination efficiency is therefore, a good biological index of evaluation of the rehabilitation of land upon pollution with crude petroleum or petroleum products.

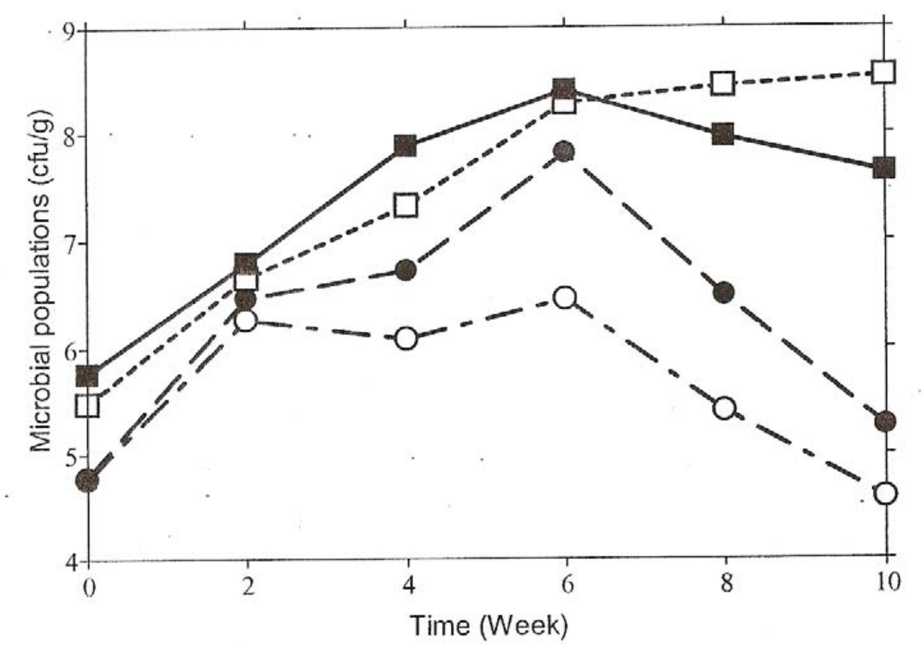
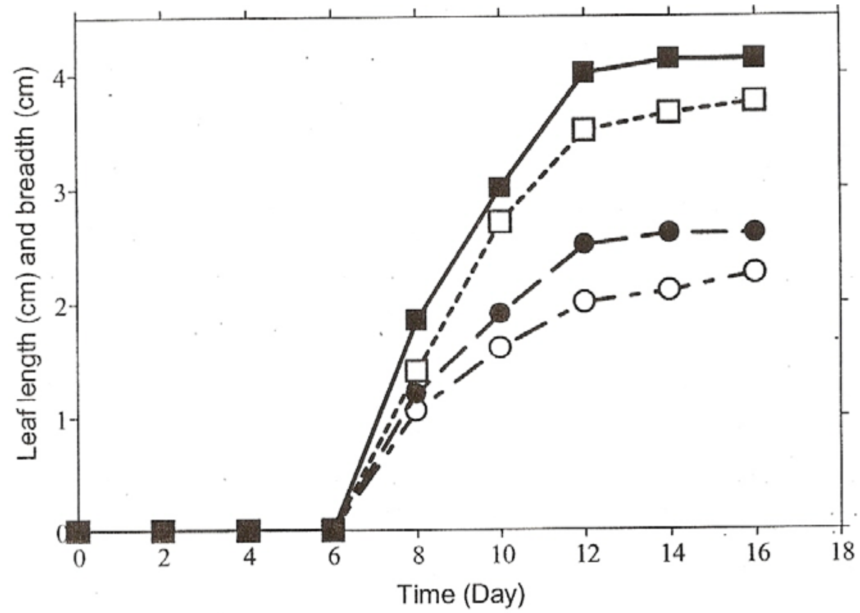



Fig. 2: Mean changes in the total microbial populations in both treated and untre

Figure 1. Micobial populations




Mean leaf length and leaf breadth of bean seedlings planted in oil polluted ural soils after bioremediation. ■ , leaf length in treated and; □ , untreated soil; ● adth in treated and; ○ , untreated soil samples.

Figure 2. Mean leaf and leaf breadth of bean seedling

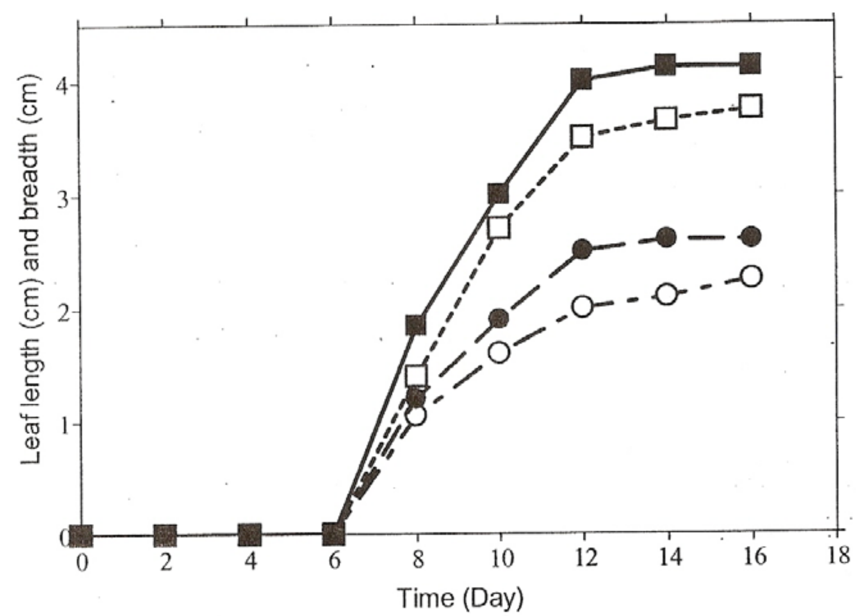



Figure 3. Leaf length and leaf breadth

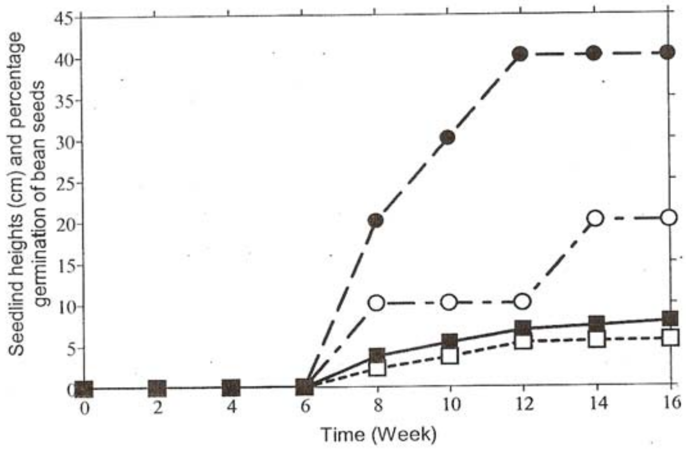



Figure 4. Seedling heights

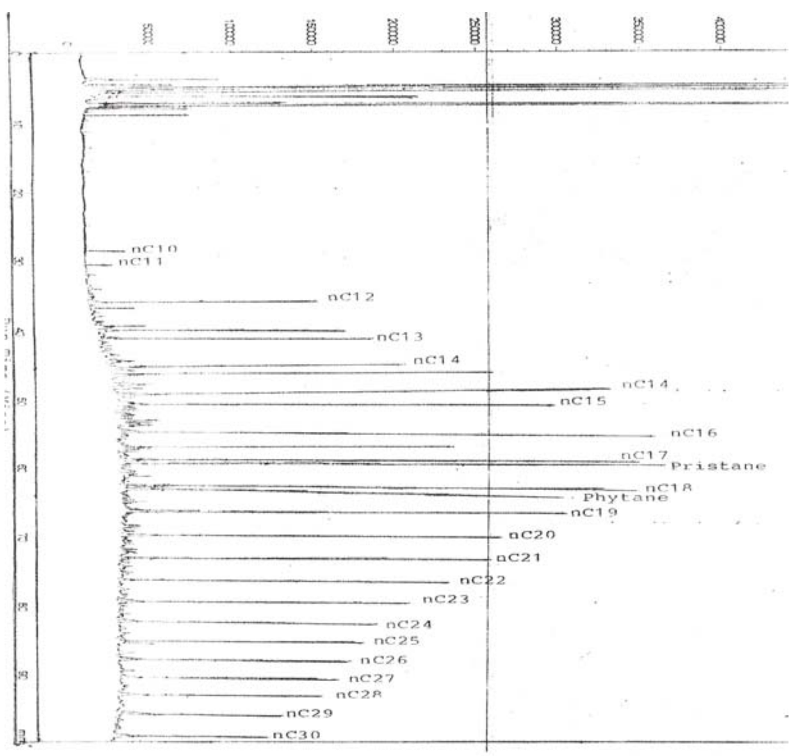



Figure 5. Chromatography

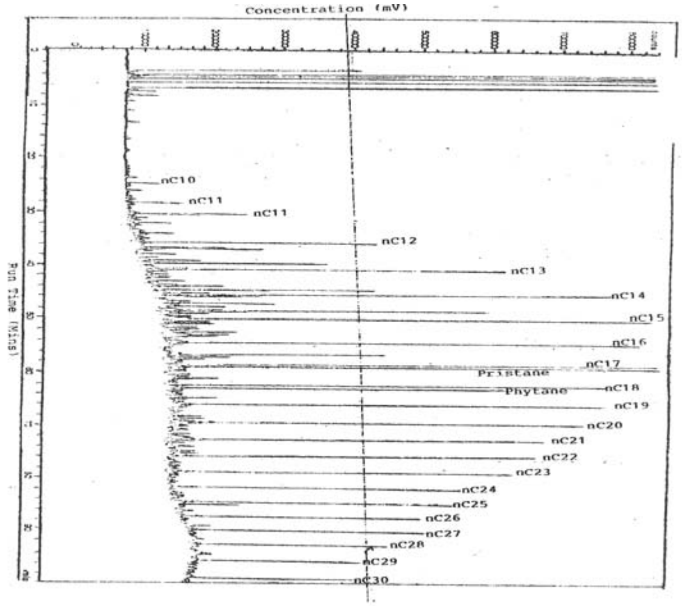



Figure 6. Chromatography

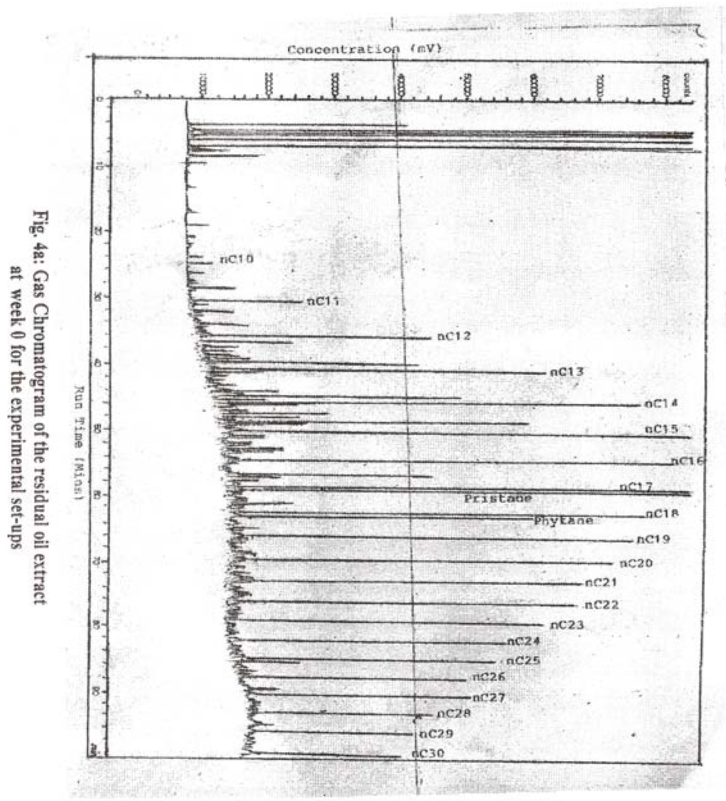



Figure 6. Gas Chromatography

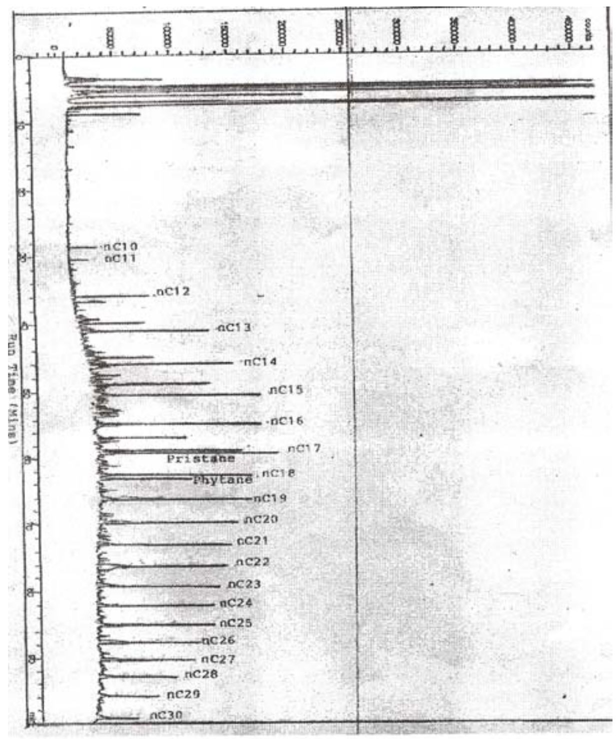



Figure 6. Gas Chromatography

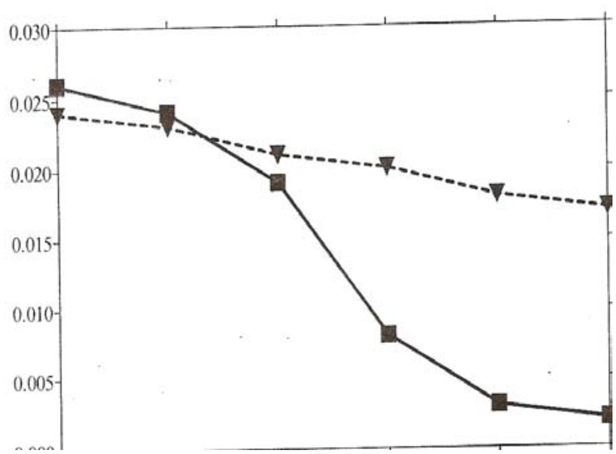



Figure 7.

## CONCLUSION

From the results of this study, rehabilitation agricultural land polluted with crude petroleum at the Niger Delta area or elsewhere could be facilitated by inoculation with a selected culture of Pseudomonas putida. The poor percentage seed germination in both the experimental (40%) and control (10%) replicates is, therefore, an indication of crude oil phytotoxicity and the broad range of ecological damage associated with oil spillage in natural ecosystems.

**Correspondence to:**
Aboaba, O.A., Nwachukwu, S.C.U.
Department Of Microbiology,
University Of Lagos,
Yaba, Lagos, Nigeria
simboaboaba@yahoo.com

## REFERENCES

1. Atlas, R.M (1991) Microbial hydrocarbon degradation: Bioremediation of oil spills J. Chem Tech. Biotech. 52:149-156
2. Archana D. D, Sopan T, I, Sanjay B.A and Nilesh D.W,(2005).Ecofriendly approach of urban solidwaste management: A case study of Jalgaon city, Maharashta. J.Environ. Biol.26 (40:747-752.
3. Chukwu, L.O. and Nwachukwu, S.C.U (2005). Impact of refined petroleum spills on water quality, macro-invertebrate and microbial communities of a tropical aquatic environment. J. Environ. Biol. 26(3): 449-458.
4. Dawes, E.A (1969). Quantitative problems in Biochemistry, 4th edition. (Edinburgh and London: E & S. Livingstone Ltd).
5. Hartley, W.R and Englande, J.A.J (1992). Health risk assessment of the migration of unleaded gasoline, a model for petroleum products. Water Sci. Technol. 25: 65-72.
6. Nwachukwu, S.C.U (2001). Bioremediation of sterile agricultural soils polluted with crude petroleum by application of Pseudomonas putida with inorganic nutrient supplementations. Current Microbiol. 42: 231-236.
7. Nwachukwu, S.C.U., James, P and Gurney, T.R (2001). Impacts of crude oil on the germination and growth of cress seeds (Lepidium spp) after bioremediation of agricultural soil polluted with crude petroleum using “adapted” Pseudomonas putida. J. Environ. Biol. 22(1): 29-36.