GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

Check for updates

Bioremediation of crude oil and its effect of residue in growth of wheat plants

Ihsan Flayyih Hasan AI-Jawhari [1,*], Ahmed Ihsan Flayyih [2], Kadhmiyah Jawad AL-Mansor [3] and Dumue Qasim Mohammed [2]

[1] *Department of Biology, College of Education for Pure Sciences, University of Thiqar, Nasiriyah, Iraq.*
[2] *Ministry of Education, Iraq.*
[3] *Department of Plant Protection, College of Agriculture, University of Basra, Iraq.*

GSC Advanced Research and Reviews, 2022, 13(01), 188–199

Publication history: Received on 21 September 2022; revised on 25 October 2022; accepted on 28 October 2022

Article DOI: https://doi.org/10.30574/gscarr.2022.13.1.0287

Abstract

Three strains of bacteria were studied to bioremediation crude oil in soil and in the laboratory. These bacteria were *Azospirillum spp*., *Azotobacter spp*. And *Rhizobium spp*. The results showed that *Azospirillum spp*. , Rhizobium spp. was more efficiency to bioremediation crude oil than *Azotobacter spp*., also the results showed that the higher number of bacteria was recorded in soil treatment with Rhizobium spp. in presence of crude oil in first week , and the number of this bacteria reached to 22.6 x 108 , but the lowest number of bacteria was recorded in soil without added crude oil (control), and the number reached to 2 x 107.The statistical methods obtained that no significant were recorded between different treatments . Also, the results showed that the numbers of bacteria in soil treatment with crude oil were decreased regularly from second week until ten weeks. The results showed that the height of the aerial parts of wheat plants reached to 73 cm in soil treatment with *Azotobacter spp*. And crude oil after 10 weeks, and the lower length reached to 47 cm. At the same time the results obtained that the higher wet weight of wheat plants was reached to 56 gm in soil treatment with *Rhizobium spp*., and in the presence of crude oil after 10 weeks, but the lower wet weight was reached to 25 gm in control. Also, the results showed that the higher of dry weight of wheat plants was reached to 23 gm in soil treatment with Rhizobium spp. and in the presence of crude oil after 10 weeks, but the lower dry weight was reached to 10 gm in control, however the statistical methods that no significant were recorded between the different treatments. Also, that all these bacteria have ability to removal crude oil in soil and in mineral salts medium.

Keywords: Bioremediation; Hydrocarbons; Soil; Bacteria; Pollution; Fertilizers

1. Introduction

Hydrocarbons can reach the soil from many sources, including pipeline blowouts, road accidents, leaking of underground storage tanks, landfarming fields and non-controlled landfilling. The release of oily wastes containing toxic hydrocarbons and to cause serious damage to natural ecosystem. In the soil, hydrocarbons are subjected to physical processes (evaporation, leaching, adsorption) and biological processes (biodegradation) [1,2]. Residual hydrocarbons may persist for a long time [1], and both hydrocarbons and their metabolites may contaminate the groundwater [3].

The technology commonly used for soil remediation includes mechanical, burying, evaporation, dispersion and washing. However, these technologies are expensive, incomplete decomposition of contaminants, time consuming and less effective [4,5]. Biological methods can treatments and removing oil spill. Bioremediation technology is a safe, economical, more efficient [6,7], and to be promising, practical, also to complete mineralization of hydrocarbons to carbon dioxide and water (Wang et al., 2015) [8]. The principle of bioremediation depends on using microorganisms to

* Corresponding author: Ihsan Flayyih Hasan AI-Jawhari
Department of Biology, College of Education for Pure Sciences, University of Thiqar, Nasiriyah, Iraq.

destroy hazardous contaminant and convert them to harmless products [4,9]. Many microorganisms such as bacteria, fungi and yeast use their enzymatic activity to utilize hydrocarbons as a sole carbon and energy [6].

Bioaugmentation involves the addition of external microbial population (endogenous or exogenous) to the polluted site. Bacteria are the most common bioaugmentation organisms [10]. Bio stimulation involves the addition of appropriate microbial nutrients to a polluted site to increase nutrient and microbial activities of indigenous microbial flora [11,12]. Bioaugmentation and bio stimulation are two approaches to bioremediation geared toward enhancing and speeding up the process [13,14].

Wheat is a global crop that has contributed to the nutritional and economic requirements of all nations, however in the case when a plant is growing in soil polluted with crude oil, efforts should be made to bioremediate the soil.

The objective of this study, therefore, was to investigate the efficacy of bioaugmentation (using bacteria) and bio stimulation (using fertilizing NPK) in bioremediation crude oil - polluted soil. This was monitored through their influence on the vegetative and reproductive parameters of wheat plants.

2. Material and methods

2.1. Samples of crude oil

Crude oil was supplied by AL-Rumella field at AL- Basra governorate (South of Iraq). It was transferred to laboratory in dark bottle closed tightly and kept in a cold and dark place until to use.

2.2. Soil collection

Table 1 Characteristic of soil under study

Parameter	Unit	Value
pH		7.8
EC	$ds.m^{-1}$	4.1
Ca^{++}	mmole/L	12.80
Mg^{++}		11.5
Na^{+}		3.11
K^{+}		0.35
Cl^{-}		14.29
$So_4^{=}$		8.32
Hco_3^{-}	mmole/ L	5.69
$Co_3^{=}$		Nill
N		51.90
P		1.92
O.M	%	0.98
Texture	gm / kg^{1}	
CEC		20.50

The soil samples used in the study were collected from a different site in soil are no recorded, cases of crude oil contaminated. Soil samples were collected randomly in depth 5-20 cm from the field at AL-Jebaish region in Thiqar governorate - South of Iraq. The soil was then analyzed to estimate the moisture, pH, Electric conductivity, texture, Ca^{++}, Mg^{++}, Na^{+}, K^{+}, CI^{-}, $SO_4^{=}$, HCO_3^{-}, $CO_3^{=}$, N, p, organic matter, CEC (Table. 1) after air dried and sieved (2 mm diameter openings). Five kilograms of dry soil were packed into a pot (25 cm diameter). Crude oil from north AL- Rumella field at AL-Basra governorate (South of Iraq) was added to the soil at a rate of 50mL per pot. Soil and crude oil were

thoroughly mixed. This concentration was shown to be only phytotoxic, also the soil was treatment with 2% level from NPK fertilizer to activated microorganism's factor. Immediately after crude oil addition, one replicate of each treatment was collected to measure the initial hydrocarbon concentration in soil. Then after 1,2,4,6, and 10 weeks, three replicates of each treatment were sampled and analyzed.

2.3. Isolation of Nitrogen fixing bacteria

1gm soil was suspension in 9 mL of sterile distilled water in test tube and mixing 5-minute, serial dilutions were made until the dilutions were made up to 10^7. 1mL from 10^7 were pipetted and transferred to petri dishes , then added semi solid nitrogen free malate medium (NFB) [15] to isolate *Azospirillum* , sucrose mineral salts solution (SMS) [16] to isolate *Azotobacter* , and also added yeast extract mannitol agar (YEMA [17] to isolate *Rhizobium* , these media were amended with 250 mgL^{-1} Nystatin to inhibitor added into sterilized(121^oc , 15 min) media to dishes by using poured plate method . The inoculated media plates were covered and allowed to dry. After 30 min, the plates were transferred to darkness incubator. Bacteria population in the different media plates were enumerate by their colony forming unit (CFU). The CFU were determined after 3 days by counting the visible colonies. The total up of the colonies were used to calculate the CFU g^{-1} dry weight of soil using the formula:

$$\text{CFU / g dry weight of soil} = \frac{\text{Colony forming unit x dilution factor}}{\text{Amount of aliquot x dry weight of soil (g)}}$$

2.4. Identification of Bacteria

All isolated of bacteria identified depended on morphology, motility, microscopic characters, and biochemical tests [18].

2.5. Preparation of Standardized inoculums

All isolated of nitrogen fixation bacteria were inoculated on slant of nutrient broth and incubated in 30 c^o to 72h. The inoculums of bacteria were prepared by adding sterilized distilled water to all test tube till the optical density reach to 1.47 by using spectrophotometer with 600 nm wavelength. All bottles contained 1000 mL from the liquid specific medium were inoculated with 1 mL from inoculums of bacteria and incubated in 30 c^o in orbital shaker (100 rpm) until the optical density reached equal to 1.47. Then 50 mL from inoculums were added to all pots. All treatments in this work were triplicate. *Azospirillum spp.*, *Azotobacter spp.* was isolated from a sediment of Abo- Subuat marshe, but *Rhizobium spp.* was isolated from root nodules of *Viccia faba* planted in AL-Jebaish region in Thiqar governorate (South of Iraq).

2.6. Soil treatment with Nitrogen fixation bacteria

50 mL from inoculums 2.6 x 10^7 CFU from bacteria *Azospirillum spp., Rhizobium spp.* and *Azotobacter spp.* Were added with maintained oxygen through continuously mixing soil and added distilled water of water- holding capacity. After two weeks, the soil in all pots were planted with 10 seeds of local species of wheat (*Triticum asetivum*). The soil was supplemented with one level of concentrate super phosphate (16 kg p. $Hectare^{-1}$), K_2SO_4 (35 kg k. $Hectare^{-1}$) preplanned. But the Nitrogen fertilizer (Urea) was added to half (50%) with (120 kg N. $Hectare^{-1)}$ in two treatments, the first was during planted and the second was after one week of planted to measure the growth percentage. All pots were irrigated immediately after sowing. Then the plant growth was observed carefully.

2.7. Estimated the numbers of Bacteria

The number of colony- forming units (CFU) were determined in the soils using three plates per dilution in soil treatment with crude oil and without treatment (control) week after week, until ten weeks. The methods previously described by [19].

2.8. Plant Biomass

After the final period of experiments, the height and wet, dry weight of the aerial parts of wheat plants were measured in soil treatment.

2.9. Total nitrogen in wheat plants

After 10 weeks of growth, total N (Kiel dahl) were determined in wheat plants according to standard methods.

2.10. Biodegradation of crude oil in soil

2.10.1. Extraction of crude oil from soil

The extraction of Total Petroleum hydrocarbon (TPH) from soil was conducted according to the methods used by [20] with slight modified. 10 gm of soil sample (triplicate) was taken from each treatment and transferred into a 50 mL flask and the hydrocarbon content in soil polluted soil was extracted using mixing 10 mL of n- hexane and methylene chloride (5:5V) shaken vigorously on a magnetic stirrer for 30 min and allowed to stand for 10 min until the hexane extract completely separated the oil from the soil sample. The solution was then filtered using a Whatman No.1 filter paper and the liquid phase extract (filtrate) diluted were removed and evaporated on a 70 ℃ water bath BSI (Jeo – Tech- Korea) to approximately 1 mL, and then transferred to determine by Gas chromatography (GC-FID Shimadzu 2014) with a capillary column (length 30 m, id: 0.24 mm). The carrier gas was helium at a constant rate of 1.5 mL^{-1}, with column pressure of 100kpa and interface temperature 280 ℃, where it was maintained for 10 – 20 min. The injection volume was 2 µL.

2.10.2. Biodegradation of Total Petroleum Hydrocarbons (TPH) in Mineral Salts medium

Extraction

The extraction of total petroleum hydrocarbons from mineral salts medium was conducted to the methods used by [21] with slight modified. 2 mL of crude oil from Al- Rumella field at AL-Basra governorate (South of Iraq) as a sole of carbon and energy was added to 98 mL mineral salts medium in 250 mL flasks. The liquid mineral salts medium, which is used as a selective growth medium, with the following compositions: Yeast extract (1g); $MgSO_4$. $7H_2O$ (0.2g); K_2HPO_4 (0.2 g); KH_2PO_4 (0.2 g); $MnCI_2$.$7H_2O$ (0.2 g); NaCI (1.0 g). All the dry chemicals were measured out first and prepared by adding to each a liter of distilled water, the pH was adjusted to (7.2) using (1M) NaOH solution, medium sterilized by autoclave under (121 ℃) and pressure (15) bound / $inch^2$ for 15 minutes, and then inoculated with one loopful from culture of *Asospirillum spp.*, Rhizobium *spp.*, and *Azotobacter spp.* With age 48h separately. The control flasks were not inoculated (only crude oil). All flasks were covered with non – absorbent cotton wool and incubated at 37 ℃ in orbital shaker (100 rpm) to adequate mixing and homogeneity of the contents. The experimental setup was monitored for a period 48h. The mixture was extracted with 10 mL of n- hexane, and the solution was then filtered using a Whatman No.1 filter paper, and the liquid phase extract (filtrate) were removed and evaporated on a 70 ℃ water bath BSI (Jeo – Tech – Korea) to approximately 1 mL and then transferred to determine by gas chromatography (GC- FID Shimadzu 2014).

2.11. Statistical analysis

The present work conducted an Anova (analysis of variance) which was performed on all the treatments and done using the SPSS (version 10.0) package to determine whether, a significance differences.

3. Results and discussion

3.1. Wheat growth

Fig.1 showed that the height of the aerial parts of wheat plants reached to 73 cm in soil treatment with *Azotobacter spp.* , and crude oil after 10 weeks , and the lower length was calculated in control without added crude oil and Nitrogen fixation bacteria , the height reached to 47 cm , however the statistical methods showed that no significant were recorded between the different treatments , these results was agreed with [22] which showed that better growth in seed germination and stem heights in *Cowpea* seedlings in soil treatment with crude oil when compared with control (treatment soil with petroleum hydrocarbons).

Also Fig.2 showed that the higher wet weight of wheat plants was reached to 56 gm in soil treatment with *Rhizobium spp.,* and in the presence of crude oil after 10 weeks, but the lower wet weight was reached to 25 gm in control , however the statistical methods showed that no significant were recorded between the different treatments.

Fig. 3 showed that the higher of dry weight of wheat plants was reached to 23gm in soil treatment with *Rhizobium spp.,* and in the presence of crude oil after 10 weeks , but the lower dry weight was reached to 10 gm in control , also however the statistical methods showed that no significant were recorded between the different treatments .

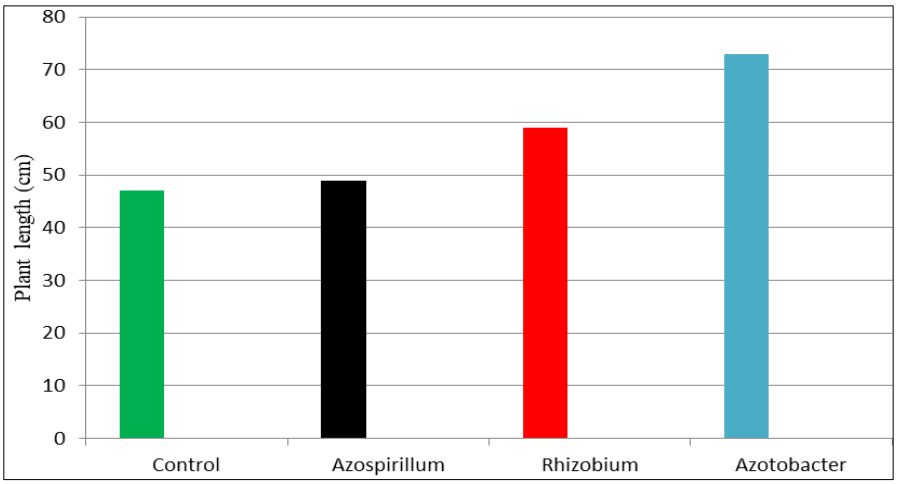

Figure 1 Effect of crude oil with addition *Azospirillum spp., Rhizobium spp., Azotobacter* spp. in length of wheat plant

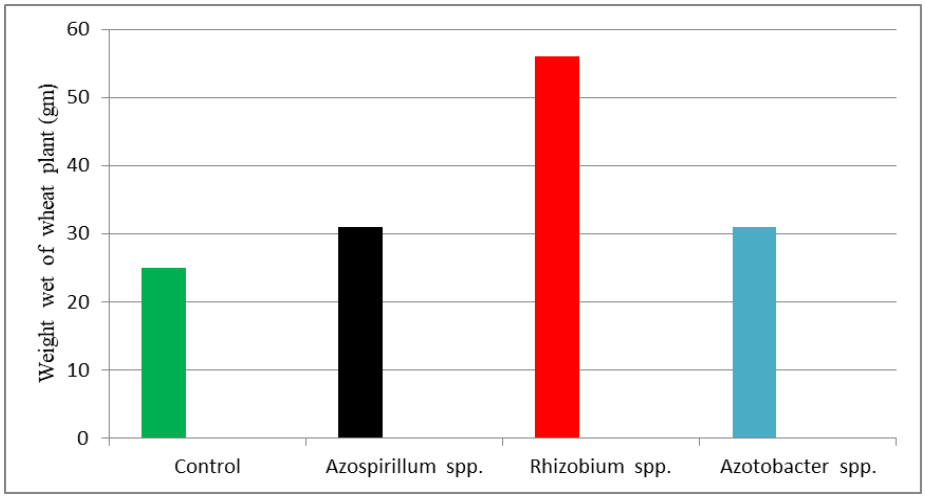

Figure 2 Effect of crude oil with addition *Azospirillum spp.*, *Rhizobium* spp.*, Azotobacter spp.* in weight wet of wheat plant

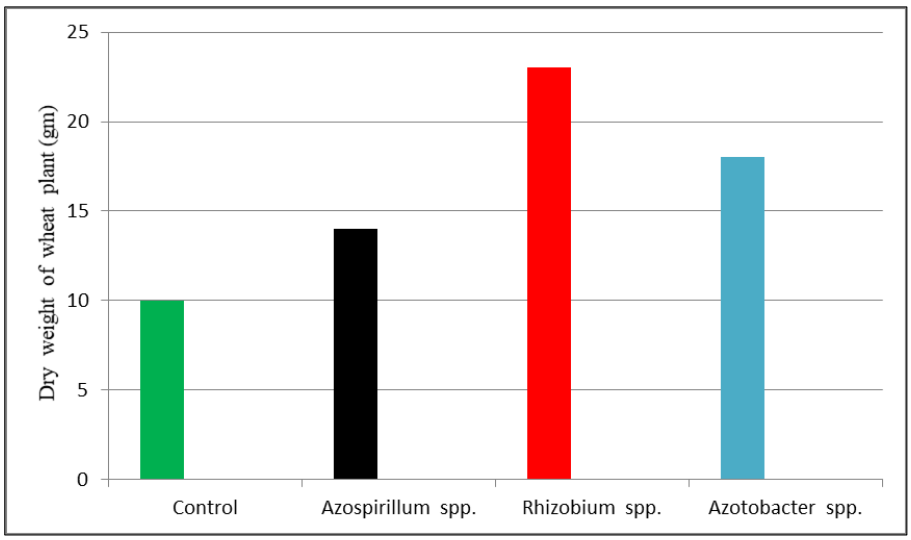

Figure 3 Effect of crude oil with addition *Azospirillum spp., Rhizobium spp.*, *Azotobacter spp.* in dry wet of wheat plant

3.2. Total Nitrogen in wheat plants

Fig.4 show that the highly percentage of total nitrogen was recorded in wheat plants planted in soil treatment with *Rhizobium spp.,* and in the presence of crude oil after 10 weeks, the percentage of total nitrogen reached to 3.36 %, but the lowest percentage of total nitrogen also recorded in wheat plants planted in soil without treatment with bacteria (control), the percentage of total nitrogen reached to 0.84%. The statistical methods also showed that no significant were recorded between different treatments.

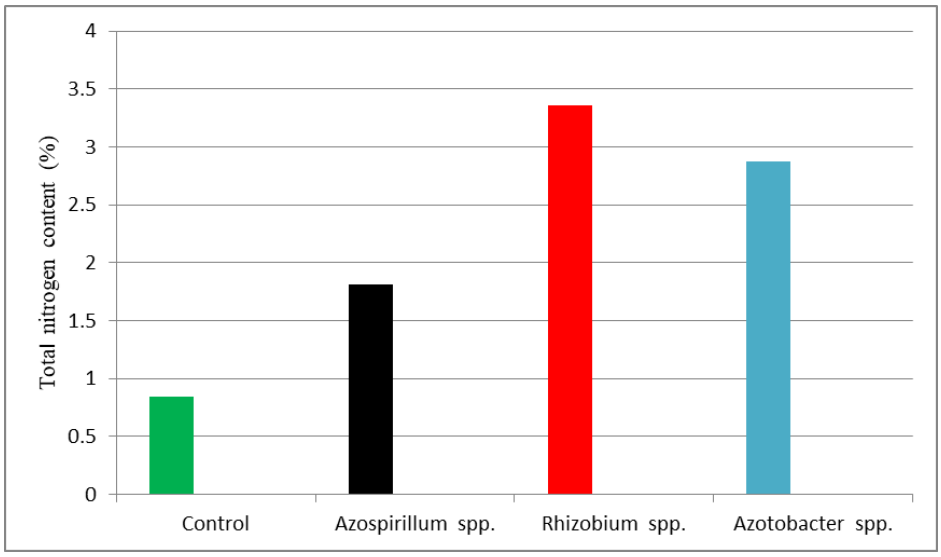

Figure 4 Effect of crude oil with addition *Azospirillum spp., Rhizobium spp., Azotobacter spp.* in total nitrogen content in wheat plant

In the present study, fertilizer (NPK) has supply nitrogen and phosphorus to the soil, these elements were important to plant growth and productivity. The increased nitrogen supplied only was affected the vegetative and reproductive parameters, these results are like the work of [23] who reported that crude oil, kerosene, and petrol significantly inhibited the germination, growth, and productivity of soybean. [11] Also reported that crude oil effected the growth and productivity at *Zea mays*. [24] Suggested that the reduction in yield because of petroleum product pollution was due to interference by adding fertilizer might have been absorbed by wheat plant for growth and productivity. Similar observations were reported by [25] who stated that the increased nitrogen in the consortium, the bacteria might have degraded the crude oil and encouraged atmospheric nitrogen fixation by microorganisms.

3.3. Bacterial counts

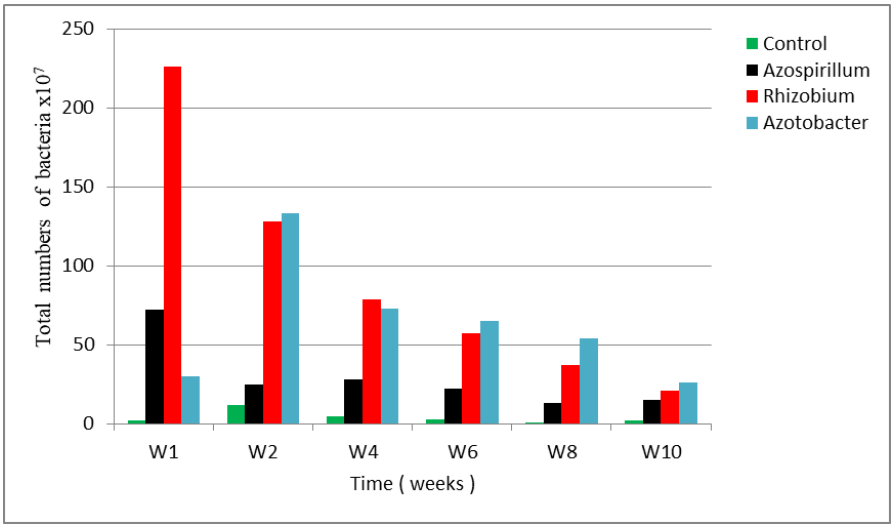

Figure 5 Effect of addition crude oil in soil in total numbers of nitrogen fixing bacteria

Fig.5 show that the higher number of bacteria was recorded in soil treatment with Rhizobium *spp.,* with presence of crude oil in first week, and the number of these bacteria reached to 22.6 x 10^8, and the lowest number of bacteria was recorded in soil without added crude oil (control), and the number reached to 2 x 10^7. The statistical methods obtained that no significant were recorded between different treatments. Also Fig.5 show that the number of bacteria in soil treatment with crude oil were decreased regularly from second week until ten weeks.

These results due to may be to the toxic effect of hydrocarbon fractions in the crude oil on microorganisms, and the differences in response of these bacteria to crude oil components in soil environment and may be due to genetic differences [26]. The results in present work were agreed to the findings of [27] which show that the population of bacteria reached a maximum between 20 and 40 days, then decreased regularly in soil treatment with hydrocarbons. In spite the growth conditions available in the present work were different from those present in the natural environment therefore, it was difficult to interpret the counts in the term of natural situation. However, the microbial counts were a direct indicator of petroleum biodegradation activity [28]. Also, the results obtained in present work was like the findings of (John et al.,2011) [29] which showed that the relation between the densities of nitrogen fixing bacteria and total hydrocarbons content was negative. However [29] refer that when leguminous plant is planted on a crude oil-contaminated soil, the activities of the nitrogen fixing bacteria may be retarted, and the oil also inhibits the action of the enzyme nitrogenase and the increase in the level of pollution result in decrease the number of nitrogen fixer and nitrifiers bacteria with time. However, the decrease in the pH of the soil caused by oil pollution would result in a reduction in number of nitrifying bacteria. As well as the decrease of the numbers of all bacteria in this work may be due to the diversity of hydrocarbon degrading microbes in soil can suppress these bacteria during the competition exists between these microorganisms [30]. However, the results obtained in present work were not agreed with [31] who observed that highest population of nitrogen fixing bacteria in the end of treatment after 28-day incubation. These differences may be due to differences in microbial ecology of the soil or characteristics of the experimental soil) [32].

3.4. Biodegradation of crude oil in soil

The results showed that the axenic cultures of bacteria were degrading in soil. Fig. 7,8,9 showed disappearance of large number of bands when compared with control (un inoculated) Fig.6.

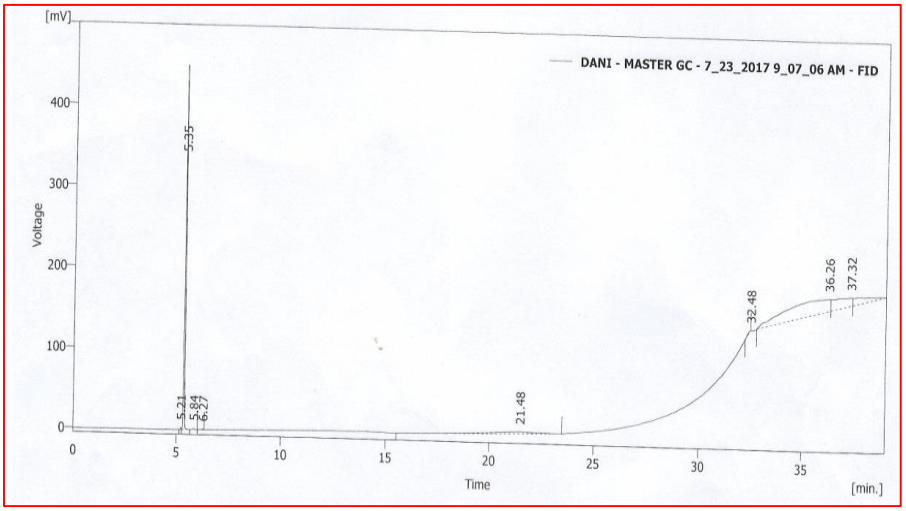

Figure 6 GC chromatogram of crude oil in soil (un inoculated) with any bacteria (Control)

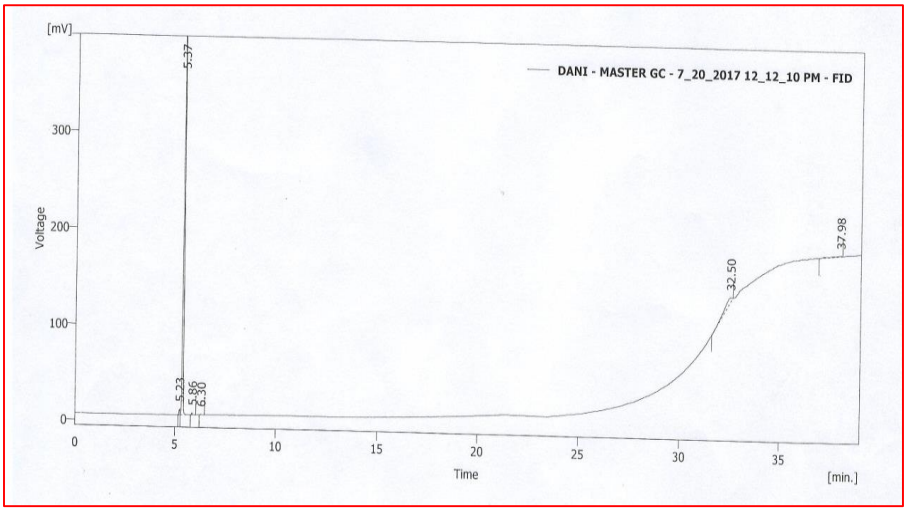

Figure 7 GC chromatogram of crude oil removal after 10 weeks in soil treatment with *Azospirillum spp.*

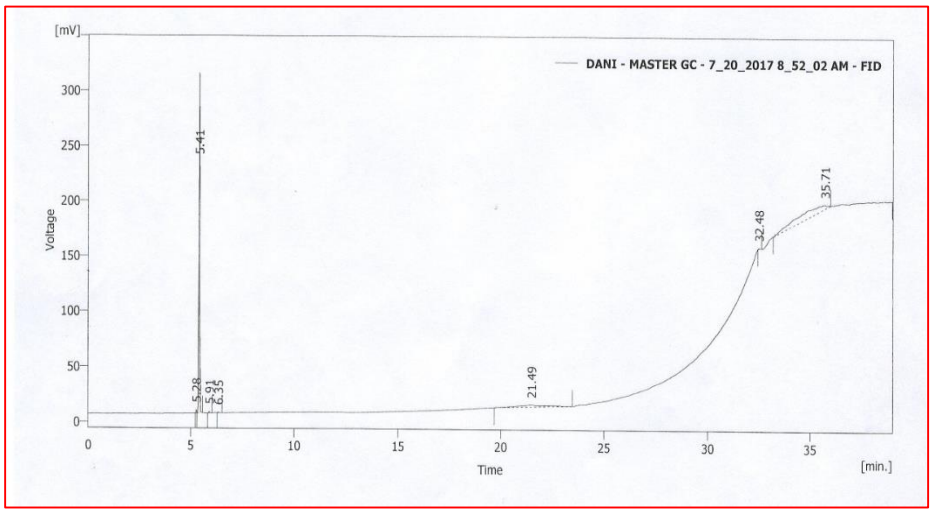

Figure 8 GC chromatogram of crude oil removal after 10 weeks in soil treatment with Azotobacter spp.

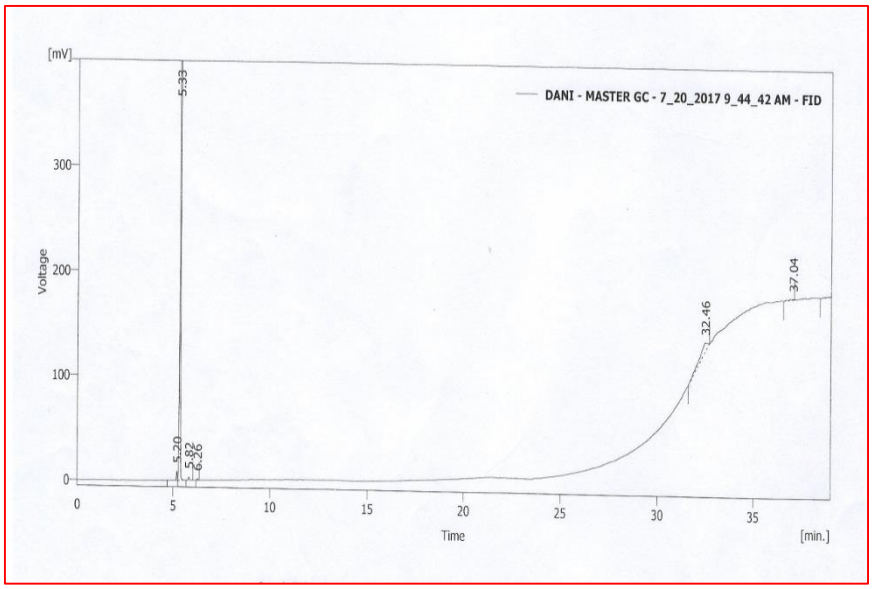

Figure 9 GC chromatogram of crude oil removal after 10 weeks in soil treatment with *Rhizobium spp.*

These results were like the findings of [32] which showed that high utilization of hydrocarbon in the soil amended with different hydrocarbon utilizing bacteria such as *Pseudomonas spp., Bacillus sp.*, *Corynbacterium sp.*, *Achromobacter sp.*, *Micrococcus sp., Nocardia sp.,* and *Klebsiella sp.,* and the highest net percentage loss of TPH was observed at day 14 in soil. At the same time [33] refer that the reduction in petroleum hydrocarbons in spent oil may not only be due to the biodegradation processes induced by nutrient additions, but other processes such as volatilization, adsorption to organic compounds and other abiotic factors.

3.5. Biodegradation of crude oil in Mineral Salts medium

The results showed that the axenic cultures of bacteria were also degraded crude oil in mineral salts medium. Fig 11,12,13 showed disappearance of large number of bands when compared with control (un inoculated) Fig.10.

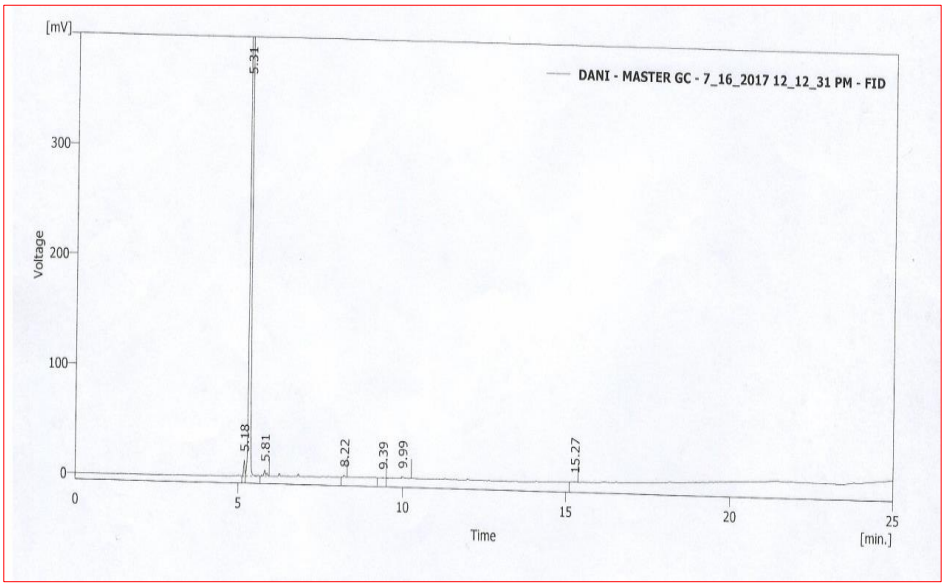

Figure 10 GC chromatogram of untreated crude oil in mineral salts medium (Control)

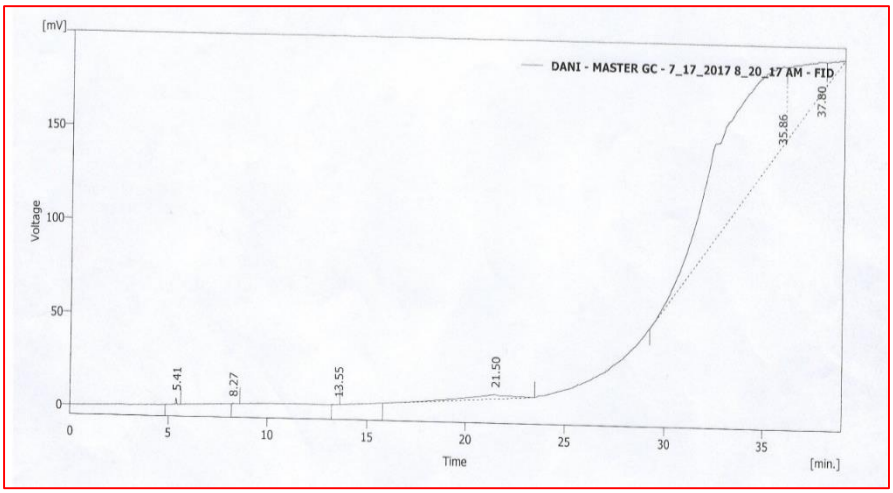

Figure 11 GC chromatogram of crude oil after 48h exposure to a pure culture of *Azospirillum spp.* in mineral salts medium

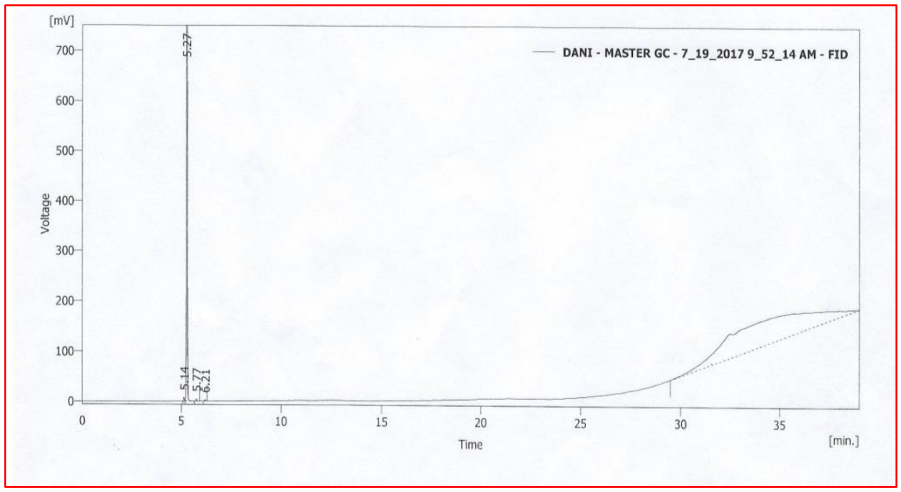

Figure 12 GC chromatogram of crude oil after 48h exposure to a pure culture of *Azotobacter spp.* In mineral salts medium

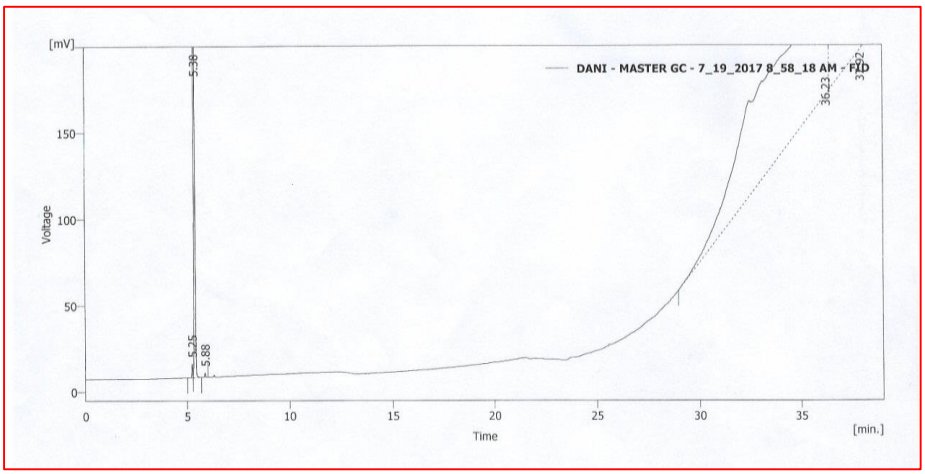

Figure 13 GC chromatogram of crude oil after 48h exposure to a pure culture of *Rhizobium spp.* In mineral salts medium

These results refer that the bacteria under study have ability to utilized crude oil and converted to other compounds. At the same time [6] refer that many microorganisms such as bacteria, fungi and yeast have enzymatic activity to utilize hydrocarbons as a sole carbon and energy. As well as the results in present study were like the findings of [29] which showed that the bacteria *Clostridium pasteuianum, Bacillus polymyxa, Azotobacter sp.*, and *Pseudomonas aeruginosa* have a strong ability to degraded hydrocarbon within the first 10 days of exposure and effectively grew and utilize crude oil as the sole source of carbon and energy. However, studies of [34], [35] it is clear that very low salt concentrations reduce hydrocanonoclastic activity and the optimum biodegradation results are reached within moderate salinity ranges.

The results obtained in present study were like the findings of [36] which showed that the reduction of PAHs and TPH was increased by an increase in the reaction's duration from 1 day to 10 days. Also [37] refer that the reduction of TPH was increased by an increase temperature, pH, NaCI concentration and reaction time in wastewater treatment with mixed of *Pseudomonas aeruginosa and Escherchia coli* during 48h, and the percentage removal of TPH reached to 99.98% when compared with control. However [38] explained that the first stage from metabolism of aromatic compounds were converted or breakdown groups attached on benzene ring and reduction to aliphatic chain with produced compounds reducing one or two carbon atoms.

4. Conclusion

The results of the present study revealed that the crude oil may be considered as an efficient source of carbon and energy for the growth of nitrogen fixing bacteria and obtained that the residue of crude oil was no effected in high of

the aerial parts, wet weight, and dry weight of wheat plants. The three bacteria strain *Azospirillium spp., Rhizobium spp.* and *Azotobacter spp.* have ability to remediation of hydrocarbon contaminated soil through nitrogen fixation. As a future prospect of this work, these three bacterial isolates can be screened for their hydrocarbon degrading capacity to help the bioremediation as well as biofertilization of crude oil contaminated land. Rehabilitation of oil contaminated soil and wastewater by the culture of axenic and mixed bacteria *Azospirillium spp., Rhizobium spp.* and *Azotobacter spp.* Were promising as it can reduce the oil pollution to acceptable levels for reuse of land and water within a short period.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

References

[1] Atlas RM. Microbial hydrocarbon degradation- bioremediation of oil spill. J.Chem .Tech . Biotech., 1991.52. 149 – 156.

[2] Leahy JC, RR Colwell RR. Microbial degradation of hydro- carbon in the environment. MicrobioI.Rev.,54(3), 305-315. 1990.

[3] Cozzarelli IM, Herman JS, Baedecker M. Fate of microbial metabolltes of hydrocarbons in a coastal plain aquifer. The role of electron acceptors. Environ. Sci. TechnoI., 29. 458 – 469. 1995.

[4] Das N, Chandran P. Microbial degradation of petroleum hydrocarbon contaminated: an overview. Biotech. Res. Int., 1-13.2011.

[5] AG Parreira, MR Totolla, GN Jham, SL Silva, AC Borges. Microbial bioremediation of aromatic compounds in a soil contaminated with gasohol. Brit.Biotech. J., 2011.1(2). `8 – 28.

[6] Hidayat A, T Sanro T, Itoh K. Determination of chrysene degradation under saline conditions by Fusarium sp. F092, a fungus screened from nature. J.Fun .BioI , 2012.116(6), 706 – 714 .

[7] Thapa B, Ajay- Kumar KC, Ghimire A. A review on bioremediation of petroleum hydrocarbon contaminated in soil. Kathmandu University J.Sci.Eng.TechnoI., 2012.8(1) . 164 – 170.

[8] Wang X, Chen J, Zhoo J. Adsorption – synergic biodegradation of disel oil in synthetic seawater by acclimated strains immobilized on multifunctional materials. Mar.Poll. BuII., 2015.92(1-2). 195 – 200.

[9] Malik ZA, Ahmed S. Degradation of petroleum hydrocarbons by oil field isolated bacterial consortium. Afr.J. Biotech., 2012.11(3).650 – 658.

[10] Okoh AI. Biodegradation of bonny light crude oil in soil microcosm by some bacterial strains isolated from crude oil flow stations saver pits in Nigeria. Afri.J.Biotech . ,2003.1(3). 45 – 48.

[11] Ogboghodo IA, Erebor LB, Osemwota IO, Isitekhale HH. The effects of application of poultry manure to crude oil polluted soils on maize (Zea mays) growth and soil properties. Environ.Mon. Assess., 2004.96(1-3). 153- 161.

[12] Nwadinigwe AO, Ezeamaman NC. Combating the effects of crude oil pollution on Senna obtusifolia using fertilizer, poultry manure and Penicillium. Nig.J. Botany., 2007.20(1). 133.

[13] Odokuma LO, Dickson AA. Bioremediation of a crude oil – polluted tropical rain forest soil., Glob.J.Environ.Sci., 2003. 2. 29-40.

[14] Okolo JC, EN Amadi EN, Odu CT. Effect of soil treatments containing poultry manure on crude oil degradation in a sandy loam soil. APPI.Eco.Environ .Res ., 2005.3(1). 47 – 53.

[15] Krieg NR, Dobereiner J. Genus Azospirillum In: Krieg, NR. and Holt, JG (eds). Bergey's manual of systematic Bacteriology.1984. VOI.1. PP. 94 – 104. Williams and Wilkins Baltima. London.

[16] Sherma AK. Biofertilizers for sustainable agriculture. Agro – Bios. India.2003. PP.300.

[17] C Barnah FC, HP Barthakur HP. A textbook of soil analysis. Vikas Publishing House PVT LTD. New Delhi. 1999.

[18] Brenner DJ, Krieg NR, Staley JT. Bergey's manual of systematic Bacteriology. Second Edition: New York. USA. PP.304. 2005.

[19] Nichols TD, Wolf DC, Roger HB, Beyrouty AC, Reynolds CM. Rhizosphere microbial population in contaminated soils. Water. Air, Soil. Pollut., 1997.95. 165 – 178.

[20] Adesodun JK, Mbagwu JS. Biodegradation of waste lubricating petroleum oil in a tropical alfisol as mediated by animal droppings. Biores. TechnoI.,2008. 99(13). 5659 – 5665.

[21] Mittal A, Singh P. Studies on biodegradation of crude oil by Aspergillus niger. South Pac.J.Nat.Sci.,2009. 27. 27-60.

[22] Omotayo AE, Egbomeade LO, O Taiwo O, Oyebamij OO, Ilori MO. Hydrocarbon degradation by free – living nitrogen – fixing bacteria., J.Sci.Res.Dev., 2013.14. 75 – 84.

[23] Nwadinigwe AO, Onwumere OH. Effect of petyroleum spills on the germination and growth of Soybean (Glycin max (L.) Merr). Nig.J.Botany ., 2003.16. 76.

[24] Ayotamuno JM, Kogbara RB. Determining the tolerance level of Zea mays to a crude oil polluted agricultural soil. Afri. J.Biotech., 2007.6(11) . 1332 - 1337.

[25] Nwadinigwe AO, Onyeidu EG. Bioremediation of crude oil- polluted soil using bacteria and poultry manure monitored through Soybean productivity., PoI.J.Environ . Stud., 2012. 21(1). 171 – 176.

[26] Greenwood PF, Wibrow S, George SJ, Tibbet M. Hydrocarbon biodegradation and soil microbial community response to repeated oil exposure. Org. Geochem, 1996.40:293 – 300.

[27] Chaineau CH, Morel JL, Oudot J. Biodegradation of fuel oil hydrocarbons in the rizosphere of Maiz. J.Environ .QuaI.2000.29.569 – 578 .

[28] Aeckersberg F, Rainey FA, Widdel F. Growth, natural relationships, cellular fatty acids, and metabolic adaptation of sulfate-reducing bacteria that utilize long-chain alkanes under anoxic conditions. Arch.MicrobioI.170, 1998.361- 369.

[29] John RC, Itah AY, Essien JP, Ipke DI. Fate of nitrogen fixing bacteria in crude oil contaminated wetland ultisol. BuII.Environ.Contam .ToxicoI.,2011. 87. 343 – 353.

[30] Fauzi M, P Suryatmana P. Bioremediation of crude oil waste contaminated soil using petrophilic Consortium and Azotobacter sp., J. Deg.Mini Land. Manag., 3(2)., 2016.521 – 526.

[31] Ogugbue CJ, Soloman L, Olali IN. Enhanced biodegradation of petroleum hydrocarbons in polluted soil augmented with nitrogen – fixing bacteria., Life Sci.J.,2017. 14(1). 82 – 91.

[32] Omoni VT, Aguoru CU, Edoh EO, Makinde O. Bio stimulation of hydrocarbon utilizing bacteria in soil contaminated with spent engine oil using banana and plantain agro – wastes. Acad.J.,2015. 6(8). 225 – 233.

[33] Onuoha SC. Stimulated biodegradation of spent lubricating motor oil in soil amended with animal droppings., J.Nat. Sci. Res.,2013.3(12). 106 – 116.

[34] Abed RM, AI-Thukari A, DeBeer D. Bacterial diversity of a cyanobacterial mat degrading petroleum compounds at elevated salinities and temperatures. FEMS. MicrobioI. EcoI. J., 2006.57(2) ,290 – 301.

[35] Diaz MP, Boyd KG, Grigson SG, Burgess JG. Biodegradation of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M, immobilized onto polypropylene fibers., Biotech. Bioeng., 2002.79(2). 145 – 153.

[36] Najafi M, Kashi G. Removal of poly aromatic hydrocarbons and total petroleum hydrocarbons by using bio – enzyme: a batch study., Med and Bioengi. J., 1(1), 2012.42 -47.

[37] AI-Jawhari IF, Noor JM, Saher AA. Efficiency of Peudomonas aeruginosa and Escherchia coli to treatment of effluent petroleum wastewater from AI-Nasiriya refinery- Iraq.Int.J.Basic APPI . Sci., 2015.4(4).404-412.

[38] Alexander M. Introduction in soil microbiology. Second Edition. John willy house. New Yourk. 1982