



Resistant bacteria isolated from soil contaminated oils engines for some heavy metals

Atheer S.N.Al-Azaway^{1*}, Wameed A.K.Al-Tae², Hala F.A.Al-Jawahery²

¹Environmental Sciences College/Al-Qasim Green University, Iraq

²Sciences College/Babylon University, Iraq

Abstract : The isolation of Bacteria from oil contaminated soil and its resistant to heavy metal had been studied which represents heavily contaminated soils with used oils by automobile engines. 15 sites were selected, and temperature, pH and electrical conductivity were measured. we used many technique for isolation and resistant tests. The results show the dominant *Bacillus* spp. in current study, and other bacteria were resistant and inhibited in various concentrations of heavy metals.

Keywords: Resistant bacteria, oils engines, heavy metals.

Introduction:

Microorganisms play an important role in food chains, which are an important part of the balance of life¹. Micro-organisms Become significant impact on ecosystems through the normalization of life of many capabilities, making it necessary for other beings on this planet². Soil contaminated by hydrocarbons cause extensive damage local ecosystems by pollutants accumulate in the tissues of animals and plants and may cause death or birth boom³. In Mexico, there are a myriad of sites contaminated as a result of more than 60 years of oil activity in recent years this problem has stimulated research to restore these contaminated sites².

Environmental Protection Agency in the United States defined the oils used in engines that it is derived from crude or industrial oil and as a result of this use, it produces polluted chemical compounds contribute to chronic hazards, including mutations and carcinogens and also cause environmental risks with global ramifications⁴.

Bioremediation became an alternative way to deals with the oil-contaminated sites as it can improve the efficiency of biodegradation either add specific micro-organisms (bacteria, algae, fungi and protozoa), or strengthen existing micro-organisms already⁵, and decompose the wide range of material components to be degradation in the oil sludge^{6,7}.

It has been isolated a large number of strains of *Pseudomonas* bacteria capable of breaking down multiple polycyclic aromatic hydrocarbons present in the soil^{8,9}.

There are also other types of living organisms have the ability to break down petroleum hydrocarbons such as: *Yokenella* spp., *Alcaligenes* spp., *Roseomanas* spp., *Sreanotrophomanas* spp...etc.¹⁰.

Use of petroleum products such as motor oil, gasoline, diesel and kerosene a daily in various forms in the motor repair workshops, these products tend to harden and change the color of the soil, which may be dangerous to the health of technicians and craftsmen indescribably¹¹.

Soil contains a number of heavy elements that differ in the types and concentrations depending on the rocks derivative mother of the soil and the weathering of the rocks as well as the longer the heavy elements of the biggest environmental pollutants as lead continued emitted from various sources (natural and artificial) to increase the concentrations in the atmosphere and water and soil. Heavy elements and includes a large group of them what is necessary for vital events such as iron, copper, some of which is toxic such as mercury, lead, cadmium and nickel, which is highly toxic neighborhoods¹².

Heavy elements grade quality and weight of higher it will be up to (5g/cm^3)¹³, sources of heavy metals in the environment include the Earth's crust and the Biosphere (ground and surface water and marine) and fossil fuels (coal, oil and natural gas), and the burning of the motor fuel mining operations (production of metals from their ores) and smelting raw materials that go into some of the elements in the installation and some agricultural pesticides and adopt the emission of heavy metals into the atmosphere on the geological origin of these elements in different types of raw materials¹⁴, as is the number of Microbiology and effectiveness of metabolic good indicators to determine soil contamination, heavy metals such as cadmium, which has a negative effect on the growth of microorganisms¹⁵.

Materials and Methods:

Fifteen sites were selected for samples collection, which represents heavily contaminated soils with used oils by automobile engines (with a strong character and black color dark) within the study area in the industrial district of the province of Babylon.

1. Temperature, pH and E.C. were measured.
2. Isolation of Bacteria from oiled contaminated soil by using Bushnell-Hass medium¹¹
3. Diagnosis of isolated bacteria by using morphological examinations of the colonies as well as the nature of their response to Grams stain in addition to the biochemical tests for this bacteria¹⁶
4. Isolation and diagnosis the bacteria that appear the resistance to different concentration of some heavy metals (Cr, Cu and Cd).
5. Determination the Minimum Inhibition Concentration (MIC) for bacteria that isolated from polluted soils by used oil engine .

Results: and Discussion:

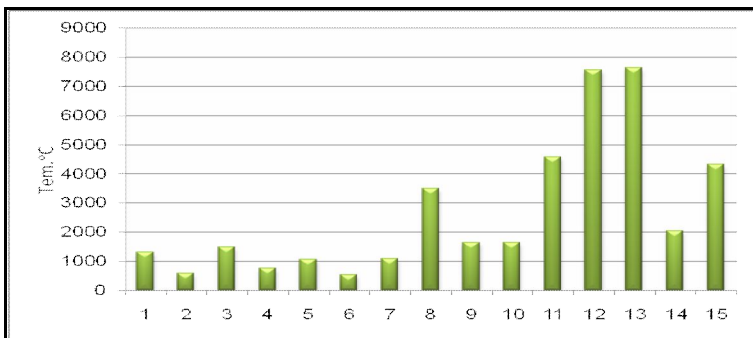
Physical and chemical characteristics of the contaminated soils:

From the results obtained can be observed were clear effects of the oil previously used in the engines of vehicles on the physical and chemical characteristics and bacteriological contaminated soil with those oils¹⁷. Causing low bacterial diversity due to toxic compounds to those oils contaminated soils¹⁸.

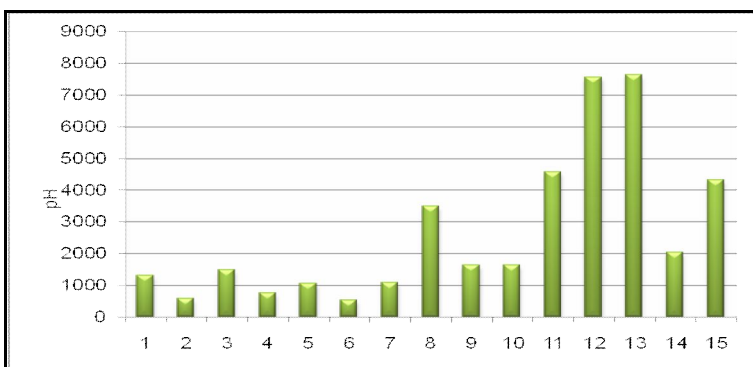
Physical and chemical properties of contaminated soils are shown in Table 1. It showed that the lowest value of the temperature was (10°C) in the sample (7) and highest value was (26.6°C) in the sample (11) during the study period and recorded the lowest value of pH (pH) during the study period in the sample (3) it was (7.8) and the highest value was recorded in the sample (6) was (8.4). The Electrical Conductivity ranged from (525- 7630) $\mu\text{s/cm}^2$ samples 6 and 13 respectively.

Table(1) show the Physical and Chemical Properties For contaminated soil during the study period

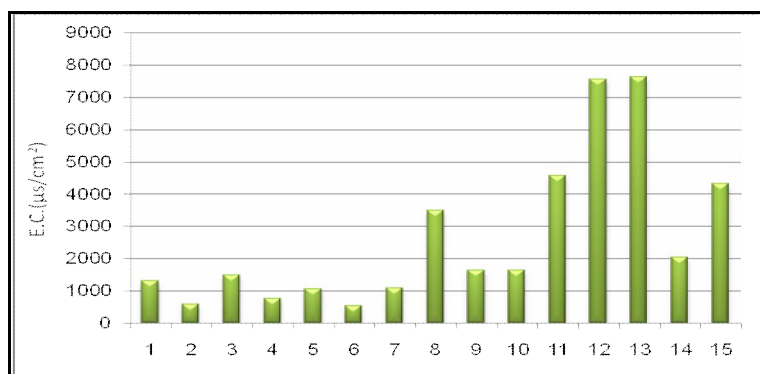
E.C ($\mu\text{s}/\text{cm}^2$)	pH	Temperature ($^{\circ}\text{C}$)	Properties
			Samples
1304	8	14.2	1
588	8	15	2
1480	7.8	14.5	3
768	8.2	14.1	4
1058	8	15.2	5
525	8.4	21.1	6
1087	8.11	10	7
3480	7.95	15	8
1629	8.17	16.1	9
1630	8.15	15.5	10
4580	8.04	26.6	11
7550	8.06	25.5	12
7630	7.95	25	13
2030	8.20	22.2	14
4310	8.30	24.4	15



Figure(1).The Temperature values during the study period



Figure(2).The pH values during the study period

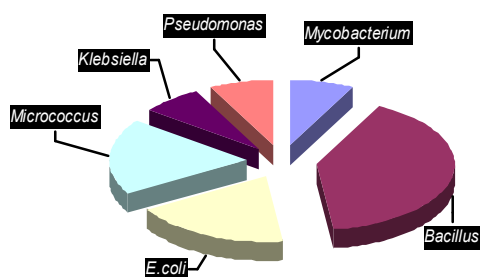


Figure(3).The E.C. values during the study period

Isolated Bacterial species from contaminated soil:

Some studies have pointed to the dominated the Gram Positive bacteria in the soils contaminated with crude oil ¹⁷, seven types of bacteria have been diagnosed in soils that contaminated by used oils in automobiles are:

Pseudomonas spp., *Bacillus* spp., *Klebsiella* spp., *Micrococcus* spp. and *E.coli* (Fig.4). *Bacillus subtilis* can break down types of Kirkuk crude oil average, but it's the most efficient from the *Pseudomonas* bacteria, which is also an analyst for Hydrocarbons¹⁹, the results of the current study showed the dominance of Gram Positive bacteria which is *Bacillus* spp. that represent the prevalent in this soil through the analysis of hydrocarbon compounds in oils contaminated soils ^{20 21 22}, in this study the rate of appearance of the *Bacillus* spp. bacteria was the highest among the other species(40.74%) while the *Micrococcus* spp. and *E.coli* (18.51%), finally the rest of the species were (7.40%) for each of them,



Figure(4).The appearance percentage for the isolated bacteria during the study area

Resistance of Bacteria for Some Heavy Metals:

Microbial Resistance to heavy metals return to a variety of mechanisms for removing toxins developed by resistant microorganisms such as the composition of the complexes by Exopolysaccharides which is a polymer with a molecular weight high component sugars excreted to the periphery by the micro-organisms which are associated with the bacterial cell envelope, reducing of minerals, metals and put out of the cell, sometimes these mechanisms can be encrypted in the genes of the plasmid and facilitate the transfer of charge from one cell toxic metals resistance gene to other²³

From the current study, *E.coli* had been inhibited at concentrations (100,250)µg/L for Cu,Cr and Cd, but *Mycobacterium* spp. was resistant to each concentrations for all heavy metals used in this study. *Bacillus* spp. was resistant to concentration(100 µg/L) and inhibited at concentration(250 µg/L) for Cd and Cu, but resistant to each concentrations(100,250)µg/L for Cr. *Micrococcus* spp. Was resistant for each concentrations for all heavy metals used in this study and *Klebsiella* spp. was inhibited at each concentrations for all heavy

metals used in this study, but *Pseudomonas* spp. was resistant to each concentrations for all heavy metals used in this study.

References:

1. Madigan MT, Martinko J, Parker M, Brock J. *Biologia de los Microorganismos*, 1998, 8, P. 726
2. Saval S. *Biocnologia*, 1998, 3, P. 71
3. Alvarez P, Vogel M. *Biodegradation*, 1991, 2, P. 43
4. Blodgett WC. Water-soluble mutagen production during the bioremediation of oil contaminated soil, *Floria Sci.*, 2001, 60(1): 28-36.
5. Hagwell I.S, Delfino LM, Ras JJ, Partitioning of Polycyclic Aromatic Hydrocarbon from oil into water. *Environ. Sci. Technol.*, 1992, 26: 2104-2110.
6. Barathi S, Vasudevan N. Utilization of petroleum hydrocarbons by *Pseudomonas fluorescens* isolated from petroleum contaminated soil. *Environ. Int.* 2001, 26: 413-416.
7. Mishra SJ, Joyat RC, Kudad B. Evaluation of inoculum addition to stimulate in situ bioremediation of oily sludge contaminated soil. *Appl. Environ. Microbiol.* 2001, 67: 1675-1681.
8. Johnson HA, Pelletier DA, Spormann AM. Isolation, characterization of anaerobic ethylbenzene dehydrogenase novel mo-fe enzyme. *J. Bacteriol*, 1996, 183: 4536-4542.
9. Kiyohara H, Torigoe S, Kaida N, Soki TA, Lido T, Hayashi H, Takizawa, N (). Cloning and characterization of a chromosomal gene cluster, Pah, that encodes the upper pathway for phenanthrene and naphthelene utilization by *Pseudomonas putida* S82, *J. Bacteriol*, 1994, 176: 2439-2443
10. Antai SP. Biodegradation of Bonny light crude oil by *Bacillus* specie and *Pseudomonas* species, *Waste Manage*, 1990, 10: 61-64.
11. Udeani TKC, Obroh, AA, Okwuosa, CN, Achukwu PU, Azubike N. Isolation of bacteria from mechanic workshops soil environment contaminated with used engine oil, *African Journal of Biotechnology* 2009, 8 (22), pp. 6301-6303.
12. Aubert H, Pinta M. *Trace Elements in Soils*. 1977 Elsevier Science Ltd.
13. Kruss P., Robertson DA, McMellin LA, Effect of Ultrasound on the cementation of cobalt and zinc, *ultrasonics*, 1991, Vol 29, 370-375.
14. Aydyn AA, Aydyn A. *Handbook of Combustion: Part 2. Combustion Diagnostics and Pollutants*, 2016, Wiley-VCH Verlag GmbH & Co.
15. Ghorbani NR, Salehrastin N., Moeini A. Heavy metals affect Microbial population and their activities, 2002, 17th WCSS. Thailand.
16. Atlas RM. *Handbook of Microbiological Media*, 1994, Pp 175. L.C. Parks. CRC Press.
17. Ugoh SC, Moneke LU. Isolation of Bacteria From Engine Oil Contaminated Soils In Auto mechanic workshops in Gwagwalada, Abuja, FCT-Nigeria. *Academia Arena.*, 2011, 3(5).
18. Akoachere JTK, Akenji TN, Yongabi NF, Nkwelang G, Ndip NR. Lubricating oil-degrading bacteria in soils from filling stations and auto-mechanic workshops in Buea, Cameroon: occurrence and characteristics of isolates. *African Journal of Biotechnology*, 2008, 7 (11): 1700-1706.
19. Abbas Kh, Indian AI, Abdul Hussain N, Rahman RH. The diagnosis of local isolates of the bacterium *Pseudomonas* disjointed hydrocarbon and productive life of the emulsions first country scientific conference in the pollution of the environment and methods of Protected it October, 2008, 5-6. (In Arabic).
20. Ijah UJJ. (1998). Studies on relative capabilities of bacterial and yeast isolates from tropical soil in degrading crude oil. *Waste Management*, 18, 293-299.
21. Bento FM, Camargo FOA, Okeke BC, Frankenberger WT. Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresource Technology*, 2005, 96, 1049-1055.
22. Das K, Mukherjee AK. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* & *Pseudomonas aeruginosa* strains isolated from petroleum oil contaminated soil from North-East India. *Bioresource Technology*, 2007, 98, 1339-1345.
23. Silver S. Bacterial resistance to toxic metal ions-a review. *Gene*, 1996, 179, 9-19.
