

Occurrence Of Bacteria In Some Contaminated Soil By Diesel Fuel In Baghdad-Iraq

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ABSTRACT: Bacteria *Bacillus apiarius*, *Bacillus laterosporus*, *Bacillus badius*, *Bacillus subtilis*, *Bacillus larvae*, *Pseudomonas*, *Chromatium*, *Azotobacter*, *Staphylococcus*, *Streptococcus*, *Ancalomicrobium*, *Butyrivibrio*, were isolated and identified from eight different contaminated soils sites in Baghdad. Results showed concentrations of COD in contaminated soil by 2110-4000 ppm, TOC by 105-2300 ppm, TN by 63-110 ppm and TP by 36-63 ppm. Bacteria *Bacillus* and *Chromatium* showed occurrence by 100% in all soil samples while, the other bacteria showed occurrence by 37-62%.

Keyword: Contaminated soil, Bacteria, Diesel fuel

Introduction:

In recent years increased the phenomenon of spread of electric generators in Baghdad and provinces that are operating by diesel fuel. Which have been caused soil pollution in populated areas with residues of diesel, grease and black oil. that are contain the residues of hydrocarbon compounds, heavy metals, compounds aromatic and aliphatic ; which have negative effects on the environment and public health In addition to the distasteful smells[1]The residues of diesel fuel and grease scorched of the dangerous pollutants because they contain compounds hydrocarbons and metallic complicated that are causing in the contamination of soil and lead to defect of the ecological balance of soil. The residues contain many of the heavy elements, which cause cancer diseases when enter into the respiratory system through the volatility in air[2]. Proved [3] that these pollutants lead to the killing of microorganisms present in the soil due to lack of their ability on tolerate the high concentrations of hydrocarbon compounds, heavy metals and complicated compounds associated with scorched oil. New species emerged ; that are living in this contaminated environments. The presence of these organic and inorganic pollutants form suitable environment for co-existence and the presence of bacterial species which have ability to decompose of hydrocarbon compounds, Study [3] indicated the presence of bacteria *Pseudomonas* spp., *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Escherichia* spp., *Acinetobacter* spp., *Citrobacter koseri*, in addition to 33 strains of bacteria isolated from the contaminated soil with fuel derivatives of three cities in Turkey. Also showed [4] In their studies on some soils polluted by residues of engine oils the presence of bacteria *Ancalomicrobium adetum*, *Bacillus*, *Pediococcus damnosus* Which is one of the microorganisms that have capability to decompose hydrocarbons compounds. Proved [5] in their study the presence of bacteria *Bacillus*, *Pseudomonas aeruginosa*,

Chromatium with density of high growth in the soil polluted with residues of diesel fuel that is containing of pollutants high sulfide, organic compounds and hydrocarbons complicated This study aims to isolate and identify some bacterial species located in the soils contaminated by residues of diesel fuel and oils near the generators of eight sites in Baghdad (Al-Sadr city, Al-Baladyat, New Baghdad, Zayouna, Adhamiya, Kadhimiya, Abu-Disher (Al-Durra), Saidiya (Al-aalam)).

Material and Method:

1- Sampling: Samples collected from soils polluted with diesel fuel and scorched oil of eight sites in Baghdad (Al-Sadr city, Al-Baladyat, New Baghdad, Zayouna, Adhamiya, Kadhimiya, Abu-Disher (Al-Durra), Saidiya (Al-aalam)). The samples transported to the laboratory by using plastic bags for isolating and identifying bacterial species.

2- Cultivated media: Mineral salt agar, Nutrient agar, Nutrient broth, *Pseudomonas* agar, Blood agar base, Simmon citrate agar, Nitrate broth, Peptone water, Trypton soy broth, Starch agar, Casein agar, Nutrient gelatin, Urea agar, Brain heart infusion agar, Glucose – Phosphate broth.

3- Isolation and Cultivation of Bacteria: One gram of contaminated soil was dissolved in nine ml of sterile distilled water to make soil suspension (10^{-1}). Serial dilution was carried out for getting several dilutions (10^{-2} , 10^{-3}), 0.1ml of each dilution transferred and spread on nutrient agar plates (three replicates). Plates incubated at 30 C for 7 days. After the colonies were appeared on plates, gram staining used to identify of bacterial species. Colonies were purified by re culturing in nutrient agar for getting pure colonies [7]. One gram of contaminated soil was dissolved in 10 ml of sterile media containing 0.005 g / L peptone, 0.005 g / L yeast extract, 20 ml / L Mineral salt (three replicates) and incubated at 25C for 2 weeks, after then, one milliliter took and put in nine ml of sterile distilled water to make suspension (10^{-1}). Serial dilution was carried out for getting several dilutions (10^{-2} , 10^{-3}), 0.1ml of each dilution transferred and spread on nutrient agar plates (three replicates). plates incubated at 30 C for 7 days[8]

4- Identify of Bacteria: Bacteria *Streptococcus*, *Chromatium*, *Azotobacter*, *Pseudomonas*, *Staphylococcus*, *Ancalomicrobium* and *Butyrivibrio* identified according to [9], [10]. Bacteria *Bacillus* identified according to [11].

5- Biochemical test: Tests such as LV (egg yolk) reaction, Citrate utilization, V-P reaction, Nitrate reduction, Indole production, Growth in 7% NaCl, Starch hydrolysis, Casein hydrolysis, Gelatin hydrolysis, Urease activity, Haemolysis

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(blood agar), Catalase, Motility, Oxidase were carried out to detect the enzymatic activity of isolated organism

6- Calculation the rate of appearance Bacteria: The rate of appearance = The number of appearance times bacteria / the number of sites × 100.

7- Chemical Tests: Concentration of oxygen demand (COD), total organic carbon (TOC), total phosphorus (TP) and total nitrogen (TN) were measured in wastewater sample before and after treatment, adopted on a standard methods of analysis[12].

Result and Discussion:

Table (1) Showed diagnostic tests for bacteria Bacillus isolated and diagnosed six species (Bacillus filicolonicus, Bacillus apiarius, Bacillus laterosporus, Bacillus badius, Bacillus subtilis and Bacillus larve) in eight sites of the soils contaminated with residues of diesel fuel and oils in Baghdad.

Table (1) Biochemical tests for diagnosis of Bacillus species Bacillus larve was catalase (-)

Bacteria Test	apiarius	filicolonicus	laterosporus	badius	Subtilis
LV (egg yolk)	-	-	+	-	-
Citrate	+	-	-	-	+
V-P reaction	-	-	-	-	+
Nitrate	+	+	+	-	+
Indole	-	-	+	-	-
Growth in 7% NaCl	+	+	-	+	+
Starch hydrolysis	+	+	-	-	+
Casein hydrolysis	+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	+
Urease	+	-	-	-	+
Haemolysis	+	+	-	-	+
Catalase	+	+	+	+	+

Bacillus is one of microorganisms that degradable for organic compounds, hydrocarbons and oil in soil contaminated. In a study [13] was diagnosed 18 strains of bacteria Bacillus in soils contaminated by petroleum waste and oils of cars, these bacteria has the ability to secrete Protease and Catalase, which work to break down of organic compounds[14]. Table (2) shows Bacterial species isolated and the rate of appearance in the soils contaminated in areas (Al-Sader city, Al-Baladyat, New Baghdad, Zayouna, Adhamiya, Kadhimiya, Abu- Disher (Al-Durra), Saidiya (Al-aalam)). Bacillus and Chromatium and Pseudomonas found in each study sites ; which recorded a rate of appearance 100% compared to other species of bacteria. While, bacteria of Ancalomicrobium existed with the rate appearance of 62.5% for the site 1, 4, 5, 6 and 8. Azotobacter appeared in the sites 1, 2, 4 and 5. Whilst, Staphylococcus was appeared in the sites 4, 6, 7, 8 at rate of 50% respectively, Butyrivibrio was appeared in 3, 5 and 8 and Streptococcus in 1, 2 and 3 at the rate of 37.5%, respectively.

Table (2) shows Bacterial species isolated and the rate of appearance in the soils contaminated in areas (Al-Sader city, Al-Baladyat, New Baghdad, Zayouna, Adhamiya, Kadhimiya, Abu- Disher (Al-Durra), Saidiya (Al-aalam)).

Bacterial species	Sites								The rate of appearance
	1	2	3	4	5	6	7	8	
Ancalomicrobium	+	-	-	+	+	+	-	+	62.5
Azotobacter	+	+	-	+	+	-	-	-	50
Butyrivibrio	-	-	+	-	+	-	-	+	37.5
Bacillus laterosporus	+	+	+	+	+	+	+	+	100
Bacillus apiarius	+	+	+	+	+	+	+	+	100
Bacillus filicolonicus	+	+	+	+	+	+	+	+	100
Bacillus badius	+	+	+	+	+	+	+	+	100
Bacillus larve	+	+	+	+	+	+	+	+	100
Bacillus subtilis	+	+	+	+	+	+	+	+	100
Chromatium	+	+	+	+	+	+	+	+	100
Pseudomonas	+	+	+	+	+	+	+	+	100
Staphylococcus	-	-	-	+	-	+	+	+	50
Streptococcus	+	+	+	-	-	-	-	-	37.5

(1) Al-Sadr city (2) Al-Baladyat (3) New Baghdad (4) Zayouna (5) Adhamiya (6) Kadhimiya (7) Abu- Disher (Al-Durra) (8) Saidiya (Al-aalam)

Table (3) shows the chemical test of factors COD, TOC, TN and TP for the sites study. The concentrations of COD value oscillated from 2110-4000 ppm, Whereas, TOC value was from 105-2300 ppm, While, TN value was ranged from 63-110 ppm and TP value fluctuated from 36-63 ppm. Demonstrated [15] in their study petroleum products leads to contamination of soil. Changes in soil properties due to contaminated with petroleum-derived substances can lead to water and oxygen deficits as well as to shortage of available forms of nitrogen and phosphorus. Crude oil is a complicated mixture of hydrocarbon and organic compounds of sulphur, nitrogen, oxygen and a certain quantity of water which varies in composition from place to place [16]. Pollution of the soil environment can also limit its protective function, upset in metabolic activity, unfavorably affect its chemical characteristics, reduce fertility and negatively influence plant production [17].

Table (3) shows chemical test for the sites study

Test	The concentration in sites (ppm)							
	1	2	3	4	5	6	7	8
COD	3200	2110	3000	2600	3100	3100	4000	2700
TOC	1320	600	105	310	1100	390	2300	800
TN	80	110	90	80	75	63	102	78
TP	45	62	56	2600	36	42	60	38

(1) Al-Sadr city (2) Al-Baladyat (3) New Baghdad (4) Zayouna (5) Adhamiya (6) Kadhimiya (7) Abu- Disher (Al-Durra) (8) Saidiya (Al-aalam)

In a study [18] proved that the bacteria Bacillus has ability to produce enzyme hydrogenase, which works to break down hydrocarbons in contaminated soil. That has been isolated and diagnosed the bacteria Chromatium; as these

bacteria found within the soils contaminated with materials sulfur and hydrocarbon. Which produce of enzyme Hydrogenase who works on the oxidation of organic and inorganic compounds especially sulfur and iron compounds [19], as well as proved [20] hydrogenase enzyme are capable on the oxidation of organic compounds, hydrocarbon, sulfur, metals; which converts them into simpler compounds and release of hydrogen gas. The bacteria *Azotobacter* and *Pseudomonas* isolated of soil that implanted with corn containing on urea and phosphate in a study [21] was isolated the bacteria from some sites of soils contaminated with residues fertilizer industry as active microorganisms in breaking down nitrogen and phosphate materials. the bacteria *Azotobacter* have ability to secrete enzyme Nitrogenase which works to break down proteins and nitrogen compounds [22]. as well as the bacteria *Pseudomonas* was effective in reducing phosphorus compounds because it has enzyme hydroxybutyrate ;which converts these compounds into another structure that enables the bacterial cell to take advantage of them in the processes of growth [23]. As also, *Pseudomonas* have ability to produce the enzyme nitrate reductase, which works to break down nitrogen compounds and convert them into simpler compounds for use in growth and reproduction [24] Bacteria need essential elements (macroelements) such as, carbon, phosphorus, nitrogen and sulfur; as well as, need microelements like calcium, iron, potassium, sodium, magnesium and some metals (cobalt, manganese, nickel and zinc). Bacteria degrade long chains from the organic matter to get the carbon element, releasing oxygen and hydrogen for consumption. The nitrogen component is gotten through ammonium compounds, nitrates, nitrites and ammonia; and is gotten phosphorus from phosphate compounds in chemical formula $H_2PO_4^-$, HPO_4^{2-} , PO_4^{3-} [25] and [26].

Conclusion:

Bacteria *Bacillus* and *Chromatium* showed occurrence by 100% in all soil samples while, the other bacteria showed occurrence by 37-62%. Results showed concentrations of COD in contaminated soil by 2110-4000 ppm, TOC by 105-2300 ppm, TN by 63-110 ppm and TP by 36-63 ppm.

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