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Bioremediation of soil contaminated with oil residues using Helianthus annuus and Aspergillus Niger

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***Abstract*---**A study was conducted in the greenhouse / Department of Life Sciences / College of Education / Samarra University on 10/5/2021 and lasted for 4 and a half months. T0P2) The highest conductivity was (4.94) dS. M-1 significantly increased, while the electrical conductivity decreased in treatment T3P2) to (1.93) dS. M-1, and treatment (T3P2) recorded the highest pH value of (7.98), while treatment (T1P1) recorded the lowest value of (7.1), and treatment (T3P0) had the highest percentage of organic matter that reached (3.50) g. kg-1 soil, while the treatment (T2P0) recorded the lowest percentage which is (1.25 gm). kg-1 soil, while treatment T0P1) achieved the highest concentration of dissolved bicarbonate equal to (20.68 cmol. L-1), while the lowest concentration achieved by treatment (T2P1) was (14.40 cmmol. L-1), while the dissolved sulfate was the highest concentration equal to (16.86 cmol. l-1) in treatment (T0P2) and the lowest concentration in treatment (T1P2) is (8.58 cmol. l-1), and the highest concentration of calcium was in treatment (T1P2) equal to (35.37 cmol. l-1). The lowest concentration in the treatment (T2P2) equaled to (23.20 cmmol. L-1) while the highest concentration of dissolved magnesium in the treatment (T1P2) equaled to (32.17 cmmol. L-1) and the lowest concentration in the treatment (T0P2) was (17.90 cmol. L-1). The percentage of leaves of treated plants (T1P1) increased to (23.72) leaves, while the number of leaves of the plant decreased when treatment (T3P0) reached (18.33) leaves, and the highest increase was reached when treatment (T1P0) increased by (98.66) cm, while the height of plants decreased at Treatment (T3P0) reached (75.66) cm, while in treatment (T3P0) the treatment (T1P2) recorded the lowest dry weight percentage, which reached (25) g, while treatment (T1P1) recorded the highest dry weight percentage, which reached (34) g. At the end of the experiment, it was found that the

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sunflower plant and A. niger fungus removed high concentrations of oil residues efficiently, where the treatment (T3P2) excelled in cracking oil residues with a percentage of (100%) micrograms kg-1 soil, followed by treatment (T3P1) and by (99%). %) microgram kg-1 soil, and it was found that the fungus A. niger has the ability to grow and exploit oil residues contaminated with soil. The study confirms the possibility of using these species in biological treatment of soil contaminated with oil. Reducing oil pollution in various ecosystems.

Keywords---biological treatment, oil residues, Helianthus annuus L. sunflower, Aspergillus Niger.

Introduction

Soil pollution is: a defect of a physical, chemical or biological nature, the source of which is a pollution activity that leads to breaking the state of equilibrium between the components of the soil. The interaction of the soil that helps the deposition of heavy elements in it (Zhang *et al.,*2020). Oil is one of the most dangerous sources of soil pollution and its transformation into soil unsuitable for plant and animal life, as it contains harmful compounds in the form of toxic organic or inorganic petroleum pollutants, and it includes many compounds such as: phenolic compounds, cyanide compounds, sulfides, toxic metal ions, and dissolved substances. , suspended, and hydrocarbons that pollute the soil and turn it into unusable soil, and oil residues, especially aromatic ones (Al-Taai *et al.,*2016).

Oil-contaminated soil can be treated in several ways, such as: physical, chemical, and biological. Biological treatment is one of the most promising methods, as it has low cost and lower environmental risks. Many researches have indicated the role of plants in removing pollutants from the environment with a process called phytoremediatio (Kumar and Saxena 2020). Phytoremediation is also known as the green technology by which the problem of pollution is reduced or the concentration of pollutants in the soil is reduced by absorbing them from cultivated plants. It is an excellent way to clean or remove pollutants from the soil. By-products (He *et al.,*2020). Microbial treatment that has been used in the past decades and increasingly to reduce environmental accidents and pollution is to take advantage and get rid of polluting materials, then biological treatment techniques were developed using fungi to get rid of pollutants and toxins in the environment (Quintella *et al.,*2019) interest in fungi has increased for their use in the biological treatment of oil and other environmental pollutants, as they possess a complex enzymatic system that increases their ability to break down or transform a large number of pollutants dangerous to humans and the environment. A thick layer on the surface of the oil as well as the possibility of its growth under harsh environmental conditions (Al-Hawash *et al.,*2018).

Materials and Working Methods

Or not: Study site and field experiment design

Two laboratory experiments were conducted, the first included the development of Aspergillus niger fungus as pure isolates in the laboratories of the Department of Life Sciences, College of Education / University of Samarra, and the second experiment included agricultural pots in the spring-summer season for the year 2021, and was implemented in the vegetable greenhouse of the Department of Life Sciences, College of Education / University of Samarra using a design Randomized Complete Blocks (RCBD) to study the possibility and ability of Helianthus annuus and Aspergillus niger to reduce pollution and the accumulation of oil residues in oil-contaminated soils through the absorption of polluting compounds by this plant and reduce their concentration in the soil and A. niger cracking of oil residues.

Secondly: laboratory work

Soil samples were collected from the shoulders of the Tigris River near the Samarra Dam, then samples were taken after removing the impurities and plant residues from them, then left in the laboratory to dry well, then passed through a sieve with holes 2 mm in diameter, then placed the soil samples in sterile plastic boxes It is marked with the date and type of the sample, then it was transferred to the laboratory and the necessary laboratory tests were conducted on it before planting the plant (Khaleel, 2014).

Table of chemical, physical and biological characteristics of the study soil before planting

Adjective	the value	Unit
pH	7.1	
EC	5.22	Desimens. M-1
OM	6.2	kg-1 soil
CO_3^{-1}	0.1	Milli Mall. L-1
HCO_3	12.2	
Ca^{+2}	10.6	
Mg^{+2}	34.02	

Third: The parameters used in the experiment

- First concentration (0%); Not to add the polluting substance (oil).
- second concentration (1%); By adding 10 ml of the pollutant to each (1) kg of soil.
- Third concentration (3%); By adding 30 ml of the pollutant for every (1) kg of soil.

Parameters used in the experiment

Transactions iterators	0 % P 0	1 % P 1	3 % P 2
T 0	soil only	soil only	soil only
T 1	only plant	only plant	only plant
T 2	mushroom only	mushroom only	mushroom only
T 3	plant + mushroom	plant + mushroom	plant + mushroom

Fourth: Preparation of Potato Dextrose Agar The fungus Aspergillus Niger

The medium was prepared by taking (400) g of peeled potato tubers and cut into small pieces and adding to them (500) ml of distilled water, then boiled for (30) minutes in a volumetric flask, then mashed the potatoes and filtered the mixture using a piece of gauze to obtain the filtrate, then thawed (15) gm of agar and (20) gm of dextrose sugar gradually in an appropriate amount of distilled water so as not to agglomerate agar, then add potato filter to it and complete the volume to (1000) ml, then add antibiotic Chloramphenicol by (250) mg in per liter to prevent the growth of bacteria, the medium was then transferred to the autoclave for sterilization for (15) minutes, at a temperature of 121 °C and a pressure of 1.5 bar. At (25) °C, mushrooms spores were harvested at maturity for later use (Khaleel, 2013).

Fifthly: Sterilization and preparation of soil and pots

The soil was sterilized by adding formalin (formaldehyde) at a concentration of 2% before planting. (20) liters of water was prepared with (40) ml of formaldehyde, and according to the dilution laws, the soil was sprayed to the point of wetness, then turned and covered with transparent polyethylene and tightly for (48) hours, Then the cover was lifted and the soil was left to ventilate for three days (Tortor, 2010)) and the soil was then distributed to (36) ready-made sterile plastic pots with a capacity of (12) kg, where (10) kg of sifted and sterilized soil were placed for all of the pots, and divided into three groups by (12) pots for each group and the treatments were distributed randomly according to the randomized complete block design (RCBD). Then, the previously prepared Aspergillus niger spores were added to (18) pots, and the soil was moistened with water to ensure the growth of the fungi.

Sixthly: The studied characteristics of the vegetative system and some growth indicators in sunflower plants under the influence of oil residues and Aspergillus Niger

- Plant height.
- The total number of leaves in a plant
- Dry weight of the vegetative system

Seventh: Determination of oil from contaminated soils

The method of (Goutx and Saliot 1980) was adopted in extracting oil from the soil, and a weight of (20) g of the dried and sieved sediment was taken and placed in an extraction thimble, and an extraction process was carried out using the intermittent extraction method (extraction Soxhlet Intermittent) to make the sample ready for measurement with a device (Gas Chromatography GC).

Eighth: Chemical and physical analyzes of soil samples after planting.

Chemical analyzes were carried out on potted soil samples after the end of the planting period and the plant harvest, according to the methods that were adopted in estimating the soil before planting. The analyzes were carried out in the central laboratory at the College of Science / University of Baghdad, according to the described method (khaleel, 2019).

Ninth: Statistical Analysis

The results were statistically analyzed by applying the statistical program Minitab Ver.17, according to the ANOVA test, and the arithmetic averages were compared to determine the significant differences using Duncan's polynomial test with a probability level of 0.05 (Al-Rawi 2000), and the graphs were drawn using the Microsoft office excel 2010 program.

Results and Discussion

Chemical, physical and biological characteristics of the cultivated study soil. The electrical conductivity of the soil

Figure (1) shows a difference in the conductivity ratio (EC) of soil treated with different levels of oil residues, which increased with the increase in the level of addition of oil, as the treatment of (T0P2) achieved the highest conductivity and reached (4.94) dS. M-1 significantly increased, while the electrical conductivity of the saturated paste decreased in the T3P2 treatment) to (1.93) dS. M-1, and the results show that there are significant differences between the average levels of P on the one hand and the average levels of T on the other hand. This is due to the fact that some dissolved salts have turned into undissolved forms bound in the soil due to their association with the added oil residues, which worked to reduce the solubility of some salts by closing the pores in the soil and encapsulating the salts, thus poor filtration and ventilation and the concentration of salts on the surface of the soil (Pinto *et al.,*2015).

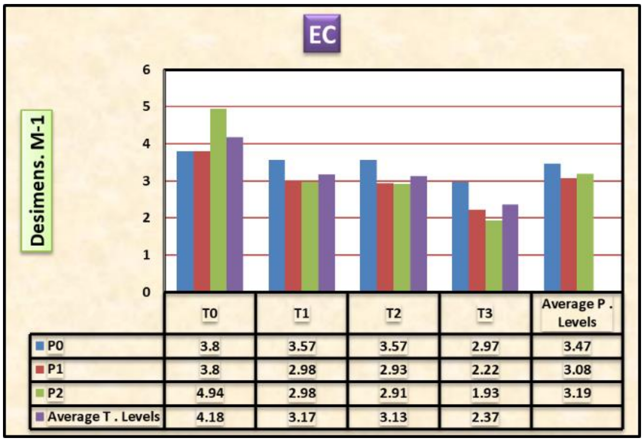

Figure 1. (EC) values

2- pH

The results in Figure (2) showed a significant change in the degree of interaction of the saturated soil paste treated with different levels of oil residues, as the treatment (T3P2) recorded the highest value, which is (7.98), while the treatment (T1P1) recorded the lowest value, which is (7.01). The results indicate that The soil was moderately acidic to weakly alkaline, and the results show that there are significant differences between the average levels of P on the one hand and the average levels of T on the other hand. As the pH is one of the factors that determine the proliferation of disintegrated microorganisms of oil residues, it is also one of the main environmental factors that affect the bioavailability of pollutants, the availability of other nutrients and the activity of biological processes, and thus the overall biological treatment method from polluted crude oil to soil and water (Kumar *et al.,*2018).

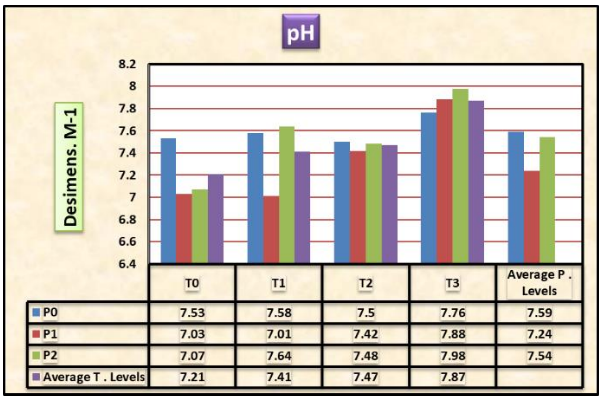

Figure 2. (pH) values

Soil organic matter (OM)

The results recorded in Figure (3) showed that treatment (T3P0) had the highest percentage of organic matter, reaching (3.50) g. kg-1 soil, while the treatment (T2P0) recorded the lowest percentage which is (1.25 gm). Kg-1 soil, while the control coefficient gave values all within the low range as a result of soil contamination with oil residues, which led to a decrease in soil porosity, prevention of ventilation and a decrease in ready nitrogen. The results also show that there are significant differences between the average levels of P on the one hand and the average levels of T on the other hand. As an increase in the soil's organic matter content increases its ability to absorb heavy metals, in other words, the readiness of heavy metals to be absorbed into the soil is positively correlated with the soil's organic matter content (Hamid *et al.*,2020).

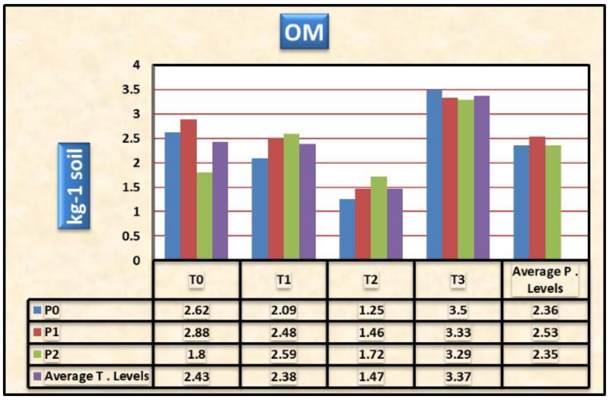

Figure 3. Soil organic matter (OM) values

Concentration of negative dissolved ions (HCO3)

It is clear from Figure (4) that adding oil residues at different levels to the soil led to different levels in the concentration of dissolved negative ions, as the treatment T0P1) achieved the highest concentration of dissolved bicarbonate equal to (20.68) centimol. L-1, and the lowest concentration achieved by the treatment (T2P1) was (14.40 cmol. L-1), the reason may be attributed to the fact that the addition of oil residues to the soil increased the chances of stabilizing the dissolved ions in the soil and turning them into less ready forms of plants (Konur ,2021).

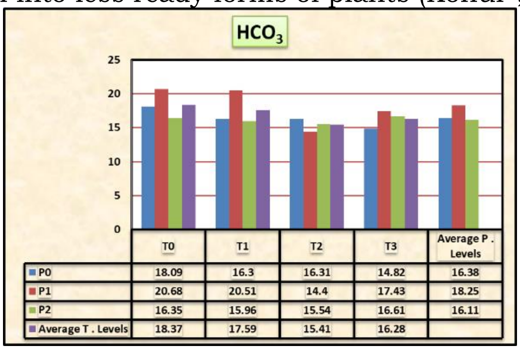

Figure 4. (HCO3) values

Concentration of negative dissolved ions (SO4)

The results shown in Figure (5) indicate a difference in the percentage of dissolved sulfate at different levels, as it increased with an increase in the level of addition of oil, where the highest concentration (16.86 cmol. l-1) was recorded in the treatment (T0P2) and the lowest concentration in the treatment (T3P1) was (8.67 cmol. L-1), as increasing the soil content of negative ion concentration increases its ability to adsorb heavy metals. In other words, the readiness of heavy metals to be absorbed into the soil is positively correlated with the soil content of negative ions (Liu *et al.,*2018).

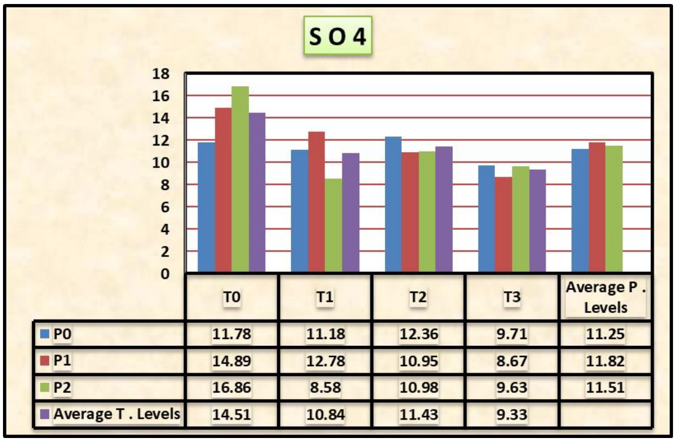

Figure 5. values for (SO4)

Concentration of dissolved positive ions (Na)

The results of Figure (6) indicate an increase in the sodium content of the used soil. The highest concentration of sodium was equal to (56.90 cmol. l-1) in treatment (T1P1), while the lowest concentration was in treatment (T3P1) equal to (32.75 cmol. l-) 1), as the results show that there are significant differences between the average levels of P on the one hand and the average levels of T on the other hand. The reason for the disturbance in the sodium content in the plant tissues is due to the presence of heavy elements that cause osmotic disturbances and consequently disturbances in the absorption of nutrients. These differences in the concentration of sodium in the vegetative parts of the plant may be due to the great damage caused to the plasma membranes as a result of treatment with oil residues. (Rehman *et al.,*2021).

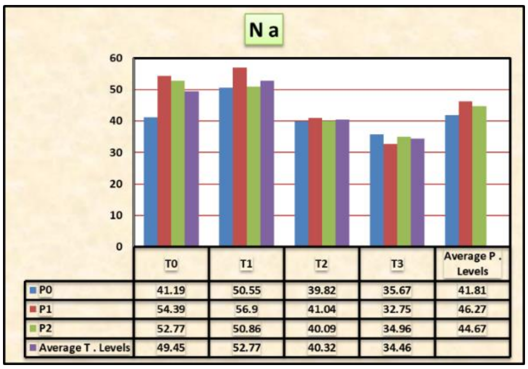

Figure 6. Values of Positive Dissolved Ions (Na)

Concentration of dissolved positive ions (Ca).

The results of Figure (7) showed a difference in the proportions of calcium ions, where the highest concentration in the treatment (T1P2) equal to (35.37 cmol. l-1) and the lowest concentration in the treatment (T2P2) equal to (23.20 cmol. l-1), as the results show. There are significant differences between the average levels of P on the one hand and the average levels of T on the other hand. The cause of the disturbance in calcium concentration is attributed to the concentration of heavy elements, which in turn leads to severe crowding out in the absorption area between ions and heavy elements, which leads to a decrease in calcium concentration in plant tissues due to competition between them, as well as the direct impact of these elements on the absorption process and the transfer of nutritious minerals. In plants (Kopittke and Menzies 2017).

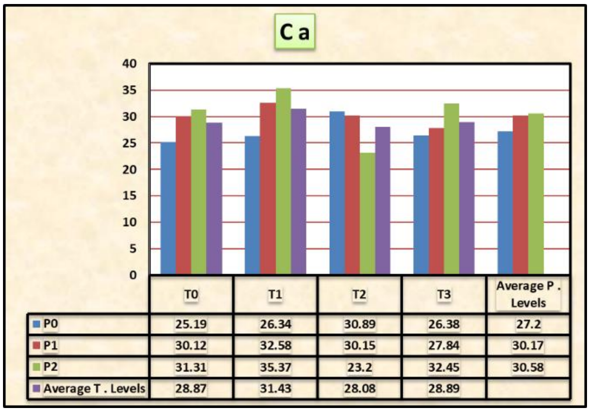

Figure 7. Values of the positive dissolved ions (Ca)

The effect of oil residues on the number of plant leaves.

We notice from Figure (8), which represents the number of plant leaves during the growth period, that there are apparent differences in the number of plant leaves between treatments that show the negative impact of the concentrations of oil residues and heavy metals on the number of leaves of plants. The percentage of leaves of treated plants (T1P1) increased to (23.72) While the number of plant leaves decreased when treated (T3P0) to reach (18.33). The results show that there are significant differences between the average levels of P on the one hand and the average levels of T on the other hand. As the soil polluting oil prevents air from entering the soil and leads to suffocation of plants and thus causes their weak growth due to the absorption of heavy elements from the plant (Artiola *et al.,* 2019).

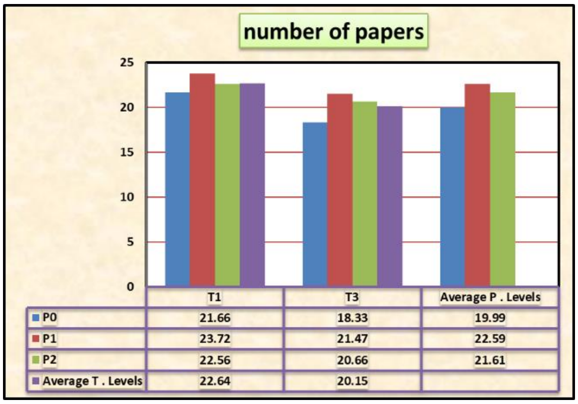

Figure 8. Number of Leaves

Effect of oil residues on plant height

The results of Figure (9) showed that there were significant differences in the characteristics of the height of the plants by the effect of the treatments, but they showed the negative effect of the concentrations of oil residues and heavy metals on the height of the plants.) to reach (75.66) cm, and the results show that there are significant differences between the average levels of P on the one hand and the average levels of T on the other hand. As the addition of oil residues at different levels to the soil led to a significant decrease in the rate of plant height and to a significant degree by increasing the level of addition(Konur, 2021).

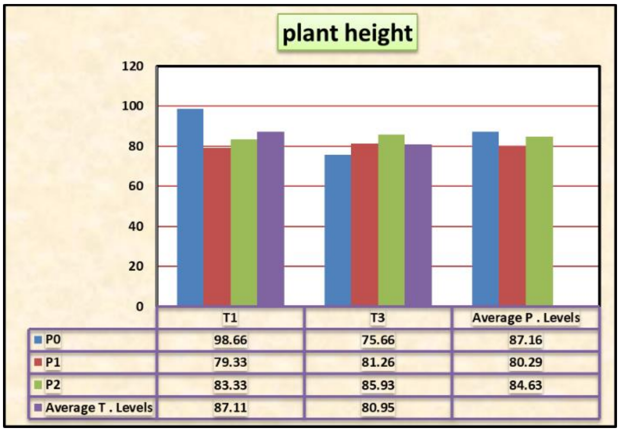

Figure 9. Plant height

Dry weight of the vegetative system

The results of Figure (10) showed a decrease in the dry weight of the vegetative total with an increase in the concentration of oil residues. The treatment (T1P2) recorded the lowest dry weight percentage, which reached (25) g. The reason for the decrease in the dry matter yield of plants growing in oil-contaminated soils may be due to the lack of absorption Plants for water from oiled strata or strata below (Odebode *et al.*,2021).

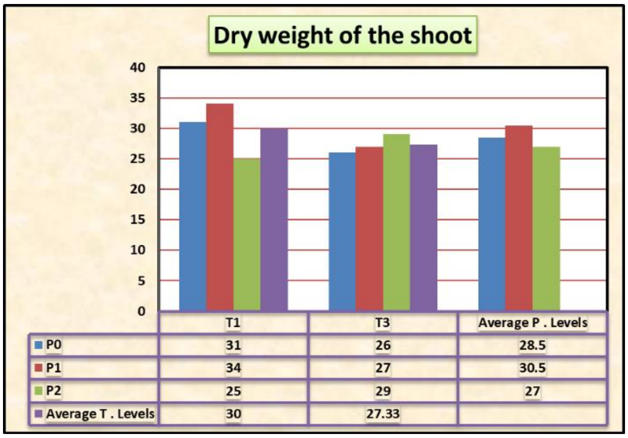

Figure 10. Dry weight of the vegetative system

Cracking of oil waste

The results in Table (11) indicate that the treatment (T3P2) recorded results with high efficiency in the biological decomposition and bio-cracking of oil residues, as it excelled in cracking oil residues by (100%) micrograms kg-1 soil, followed by treatment (T3P1) and by (99%). Microgram kg-1 soil, as shown in Figure (4-11), due to the biodegradation carried out by the fungus Aspergillus niger in cooperation with the sunflower plant, as the oil in the soil is exposed to evaporation photooxidation and microbial activity.

Table 11
Oil compounds diagnosed in polluted soil

	Compound name	sample name							
		T_3P_2	T_3P_1	T_2P_2	T_2P_1	T_1P_2	T_1P_1	T_0P_1	T_0P_2
1	Iso butane	—	—	—	—	—	—	*	—
2	n-Butane	—	—	—	—	—	—	—	—
3	Iso pentane	—	—	—	—	—	—	—	—
4	n- pentane	—	—	—	—	—	*	—	*
5	2,5-Di methyl butane	—	—	—	—	—	—	—	—
6	2- methyl pentane	—	—	—	—	—	—	—	—
7	3- methyl pentane	—	—	—	—	—	—	—	—
8	n- hexane	—	—	—	—	—	—	—	—
9	2,4-Di methyl pentane	—	—	—	—	—	*	*	*
10	benzene	—	—	—	—	—	—	*	*
11	methyl hexane-2	—	—	—	—	*	*	*	*
12	methyl hexane-3	—	—	—	—	—	—	*	*
13	2,2,4-Tri methyl pentane	—	—	—	—	—	—	*	*
14	n-heptane	—	—	—	—	—	—	*	*
15	2,5-Di methyl hexane	—	—	—	—	—	—	*	*
16	2,4-Di methyl hexane	—	—	—	—	—	—	—	*
17	2,3,4-Tri methyl pentane	—	—	—	—	*	*	*	*

18	toluene	—	—	—	—	*	*	*	*
19	2,3-Di methyl hexane	—	—	—	*	*	*	*	*
20	Ethyl benzene	—	—	—	—	—	—	—	*
21	m-xylene	—	—	—	—	—	—	—	—
22	P-xylene	—	—	—	—	—	—	—	*
23	O-xylene	—	—	—	—	—	—	*	*
24	1-methyl -3- ethyl benzene	—	—	—	—	—	—	*	*
25	1,3,5- Tri methyl benzene	—	—	—	—	—	—		*
26	1,2,4- Tri methyl benzene	—	—	—	—	—	—	*	*
27	1,2,3- Tri methyl benzene	—	—	—	—	—	—	*	*
28	naphthalene	—	*	—	—	—	*	*	*
29	2-methyl naphthalene	—	—	*	*	*	*	*	*
30	1-methyl naphthalene	—	—		—	—	—	—	—
31	Di methyl naphthalene	—	—	*	*	*	*	*	*

Integrated oil cracking (-)
It means the oil is not broken.(*)

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