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Phytoremediation of Crude Oil-Polluted Soil by Maize (*Zea mays*) and Sunflower (*Helianthus Annus*)

Farag Abu Drehiba¹, Abubaker Edkymish², Abdurrazzaq Braydan¹, Otman Ermithi¹, Mohamed Mukhtar¹ and Elmundr Abughnia¹

¹Libyan Biotechnology Research Center Department tissue culture plant.

²Libyan Authority for Scientific Research, Tripoli, Libya.

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Recent studies on Phytoremediation of Crude Oil-Polluted Soil gave positive results in both efficiency and cost. The purpose of this study was to evaluate the effectiveness of Maize (*Zea mays*), Sunflower (*Helianthus Annus*) in the biodegradation of total hydrocarbons of soils contaminated with crude oil. The experiment was conducted at the experimental station of The Libyan Center for Biotechnology Research (Tripoli, Libya) to test the ability of the selective plants in stimulating the microbial decomposition of soil pollutants - particularly Crude Oil- decreasing or eliminate these pollutants. The experiment was designed according to Randomized Complete Block Design (RCBD), and the selective plants were (maize and sunflower) planted in pots containing soil treated with different crude oil concentrations (0%, 1.25%, 2.50%, 3.75%, and 5% w/w) for 90 days to test and compare the ability of the studied plants in reducing the pollution in the presence of microbial activity. By the treatment of 3.75% (37,500 ppm) crude oil concentration, the total hydrocarbon concentration decreased to 86.10 ppm by maize and to 77.47 ppm by sunflower, while, by the control of treatment was 188.48 ppm. The total number of the aerobic bacteria at the end of the experiment didn't show significant differences in comparison to zero time except for the 5% pollution treatment by which the total number was 313.23x10⁴ CFU by maize, 164.92x10⁴ CFU by sunflower and 2200.17x10⁴ CFU by the control treatment.

1 Introduction

Today, organic pollutants are generally one of the most important topics in regard to negative effects on environment and human health, especially by crude oil, and organic compounds -such as benzene and polyaromatic hydrocarbons- (Ebadi *et al.*, 2018). Certain hydrocarbons are carcinogenic to people and animals, causing genotoxicity, reproductive toxicity, immunological toxicity, and cancer (Kuppusamy *et al.*

2020). Therefore, removing of hydrocarbons from polluted environments is vital for both ecological stability and human health (Alegbeleye *et al.*, 2017). Worldwide, Libya is one of the most important oil-producing countries, and according to activities related to the extraction, transportation, refining, and storage of oil, a lot of infiltration of harmful compounds infiltrate deep aquifers -water reservoirs- through soil layers. Therefore one liter of petroleum compounds can

contaminate a million liters of groundwater, and thus exposing human beings to dangers by drinking polluted water or absorbing it through the skin when using it for recreational purposes (ATSDR. 1988).

Phytoremediation is one of the most important biological methods used for treatment of oil wastes, by which certain plants are used that have the ability to reduce pollution levels by seizing, removing or decomposition of various pollutants (Singh, 2006). The symbiotic relationship between plant roots and soil microorganisms stimulates decomposition of stable organic pollutants. It remains difficult to increase phytoremediation efficiency. The impact of soil physical and chemical characteristics and microbial activity on the success of phytoremediation of hydrocarbon has been thoroughly established (Ye *et al.*, 2017). According to a preliminary study, phytoremediation may be more effect than using only microorganisms. Also, many studies observed that growing plants in hydrocarbon-contaminated soil increased the decomposition of hydrocarbons compared to uncultivated soils (Siciliano, 1998). To clean up soil pollution, many different methods have been used, including physical, chemical, and biological ones. By using plants and associated soil bacteria, phytoremediation is a low-cost, environmentally friendly process for minimizing the abundance, mobility, or toxicity in soil and water (Arslan *et al.*, 2017).

2 Materials and Methods

This study was conducted by using sandy soil taken from the experimental station of Biotechnology Research Center (BTRC). Used soil were dried, sieved with a 2 mm sieve and then analyzed for determining the physical and chemical properties. The soil was synthetically polluted. Five different crude oil contamination levels (0%, 1.25%, 2.50%, 3.75% and 5%) in 4 replications were used to get 20 experimental plots. Pots were filled with 2 Kg soil and planted with either Maize or sunflower, in addition to control pots without plants, then fertilized with one dose of Ammonium phosphate (120 kg/H). The irrigation was controlled according the field capacity. The total hydrocarbons in the soil and plants, and also the numbers of bacteria in the rhizosphere were estimated at the end of the experiment to assess the ability of the tested plants in treating pollution (Tab. 2). The analysis

of variance ANOVA ($\alpha = 0.05$) and Duncan's multiple range test (Duncan 1958) were performed to evaluate the effect of studied factors.

3 Results and Discussion:

Soil properties and Concentrations of hydrocarbons:

Some parameters of physical and chemical properties of used soil were investigated at BTRC laboratory according (Tab.1).

Table.1: Physical and chemical properties of used soil

pH	EC Mmhos /cm	CaC O3 %	O. M. %	Macro nutrients			Texture %		
				K pp m	P pp m	N %	Cl ay	Si lt	Sa nd
7. 95	2.04	5	0. 33	5. 3	0. 06	0. 46	1. 1	5	93. 9

Concentrations of total hydrocarbons have been determined in the soil, roots, and shoots of both studied plant species. During the experiment sunflower plants did not tolerate the highest pollution treatments, total hydrocarbons up took by sunflower plants in the 1.25% pollution treatment - 420.98 ppm- was about twice as much as by maize. By maize plants, the concentrations ranged between 230 ppm and 245 ppm in the three lowest pollution treatments, while the highest concentration was 749.38 ppm (Fig. 1).

The concentrations of hydrocarbons in planted samples of Maize were lowering than in unplanted - control-samples because of increased microbial activity in the rhizosphere induced by the root system. The concentrations of total hydrocarbons were (28.38, 44.77, 76.52, 86.10, and 78.84 ppm) respectively.

Maize plants were selected due to their global availability, high germination rate, fibrous roots, and versatility (Khan; *et al.*, 2018). In our study hydrocarbon compounds were effectively decreased by maize (fig 2). Similar results were also reported by Zand *et al.*, 2010 at 3.5% pollution level. Murotova, *et al.*, 2003 mentioned that success of phytoremediation of hydrocarbon contaminated soils is related to plant ability in improving microbial activity in the rhizosphere. Microorganisms create a wide range of enzymes to control oxidation reactions that govern the degradation pathways, which cause the mineralization

of hydrocarbons to produce CO₂ and H₂O (Cui, 2020), and (Ali, 2020).

The ability of sunflower plants to endure all levels of pollution for an extended period of time was lacking; for instance, at a 5% pollution level, the plants only perished after two weeks. Even though it had a positive

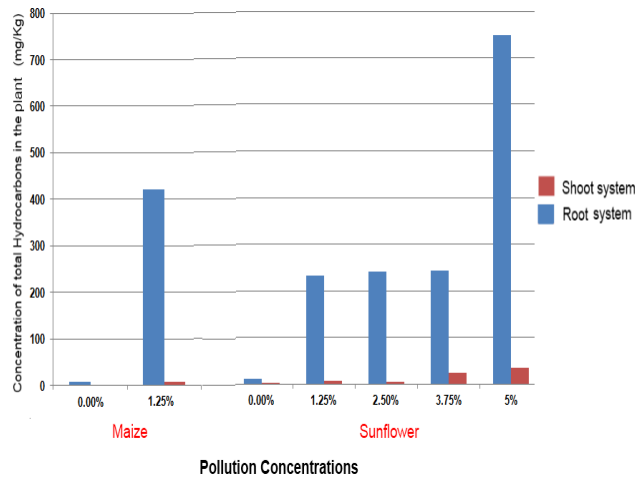


Figure.1: Concentration of total Hydrocarbons in the plant (ppm).

• **Numbers of bacteria in the rhizosphere:**

The injection of crude oil caused the pH of the soil to decrease from 8.00 to 6.50. That was still within the range of growth for bacteria, though. A smaller change in pH values was observed in the soil planted with maize as opposed to the soil planted with sunflowers. According to (Baruah et al. (2013), the pH of soil will decrease as a result of the accumulation of organic acids brought on by the degradation of crude oil.

Relating to the number of bacteria, the results of this study showed a clear increase in the number of bacteria just by the highest pollution level (5%), and this was also found by (Radwan et al., 2005). It also showed that the maize plants had a stimulating effect on the microbial activity in crude oil-contaminated soils, and this was compatible with the study referred to (Norino et al., 2004) that the maize roots had a positive effect on the activity of microorganisms in the contaminated soil compared to the uncontaminated. After while, as demonstrated in (Fig. 3) the unplanted soil having 5% pollution seemed to have the highest level of microorganisms. There were not any significant differences between the contaminated and uncontaminated sunflower plants.

effect on reducing hydrocarbons compared to unplanted soil, for example, 66% of all hydrocarbons were reduced at the 5% pollution level (Fig. 2), this point was confirmed by (Dominguez and Pichtel, 2004), who found that using sunflower plants at a 1.50% pollution treatment reduced the crude oil content by roughly 67%.

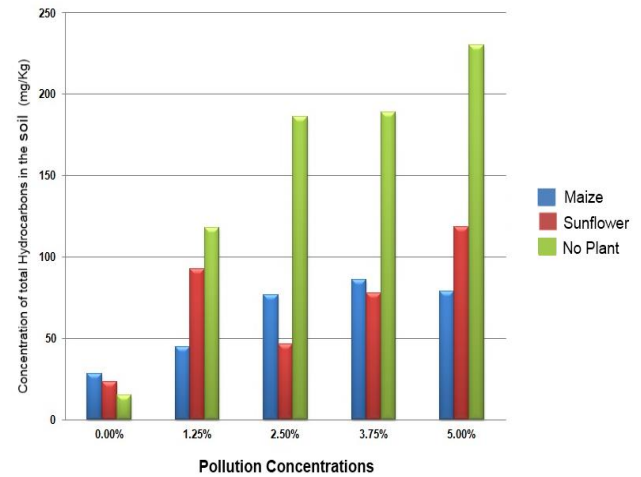


Figure.2: Concentration of total Hydrocarbons in the soil (ppm)

Table.2: Numbers of bacteria in the rhizosphere at zero time and end of experiment

Treatments	Zero Time	End of Experiment		
		Maize	Sunflower	No Plant
%0.00	3.8x10 ⁴ a	2.94x10 ⁴ b	1.73x10 ⁴ a	11.29x10 ⁴ b
%1.25	3.6x10 ⁴ a	4.14 x10 ⁴ b	3.90x10 ⁴ a	8.45x10 ⁴ b
%2.50	4.8x10 ⁴ a	4.45x10 ⁴ b	1.542x10 ⁴ a	10.57x10 ⁴ b
%3.75	2.8x10 ⁴ a	3.71x10 ⁴ b	3.60x10 ⁴ a	12.08x10 ⁴ b
%5	4.1x10 ⁴ a	313.23x10 ⁴ a	164.92x10 ⁴ a	2200.17x10 ⁴ a

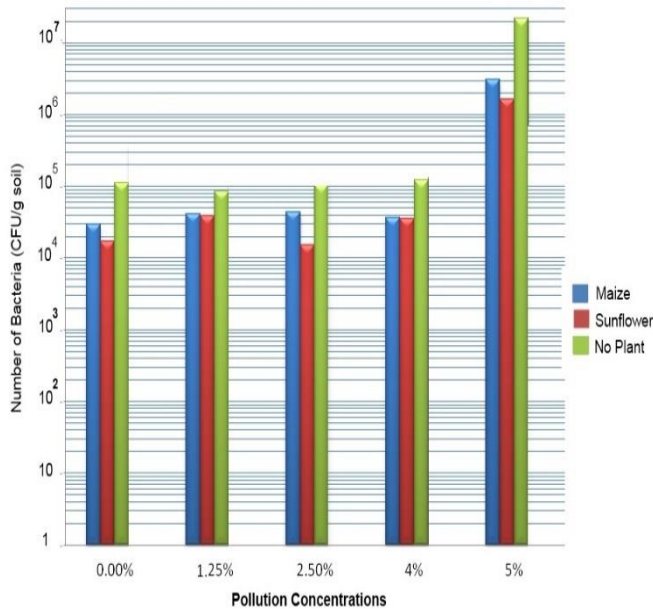


Figure.3: Number of Bacteria (CFU gm/soil) in the soil at the end of experiment.

4 Conclusion

According to the findings, as microorganisms promote plant growth and spread over broad polluted areas, the cooperation between microbes and plants eventually results in the complete removal of pollutants. It is apparent that **Phytoremediation** is a successful method for removing toxins from the soil.

Conflict of Interest: The authors declare that there are no conflicts of interest.

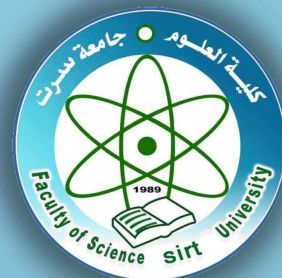
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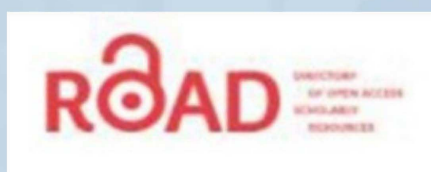
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