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Article

Myrtus Communis is a plant that can be used to clean up hydrocarbon pollution

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Abstract: The current study's aim is to see if phytoremediation can be utilized to treat soil that has been contaminated with crude oil, and it used the Myrtus Communis plant, which was grown in industrially polluted soil, to do so. With four crude oil concentrations: 25, 50, 75, and 100 g/kg The soil was clayey clay with mild alkalinity and acidity, according to the results of the physical and chemical investigation. The findings revealed the impact of crude oil on various soil parameters, including low pH and high total nitrogen, moisture content, organic matter, EC, and the total carbon to total nitrogen ratio. Plant phenotypic and biochemical measurements, such as chlorophyll Super oxide dismutase (SOD), Catalase activity (CAT) measurement, were also included in the study. in addition, sixteen polycyclic aromatic hydrocarbons (PAHs) were identified. As priority pollutants recognized by the US Environmental Protection Agency as naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysine, and benzene [b] in plants by extraction and chromatography utilizing HPLC analysis technique. The results show that majority of these chemicals accumulate in Myrtus Communis including benzo[k]fluoranthene,benzo[a]pyrene,dibenz[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3cd-pyrene]. After a month of testing, in the plant, demonstrating the plant's efficacy in eliminating polyaromatic hydrocarbons (PAH).

Keyword: Phytoremediation, Myrtus Communis, accumulation, HPLC analysis, poly aromatic hydrocarbons (PAH).

1. Introduction

The fast development of the global economy has resulted in an increase in the amount of persistent organic and inorganic pollutants, causing serious environmental pollution [1] A major environmental hazard in today's globe is due to the widespread usage of petroleum products [2]. Saturated hydrocarbons, aromatic hydrocarbons, resins, and asphaltenes are the most common petroleum chemicals [3]. There are several reports that petroleum has a detrimental effect on soil ecosystems, including restricting plant growth and deteriorating the structure and quality of groundwater , Toxic chemicals generated from petroleum pose a threat to the health of humans [4] It has taken a long time and a lot of work to clean up petroleum-contaminated places. Many environmental issues may be remedied via plant-based remediation. To expedite the breakdown and removal of contaminants from polluted soil or groundwater, a biological approach known as phytoremediation is used Eliminating or making harmless environmental toxins by bioremediation (Phytoremediation) is becoming more popular), Pollution in the environment may be efficiently reduced by plants. In order to cure the condition, root secretions are used to increase bacterial growth in the soil while also accumulating pollutants in the tissues. Soil is cleaned with plants since they are ecologically benign, inexpensive, and effective. As a result of the chemicals in sediments, utilizing plants in treatment might put the plant under stress and enhance the toxicity of those pollutants. A large amount of biomass may be generated as a consequence of increasing the plant's reactivity to contaminants [5] .

2. Materials and Methods

2.1. Soil collection

Unpolluted soil was gathered from Hilla city, Babylon Province, and was taken from the upper layer (25-30 cm in depth), dried by air, and sieved (to a particle size of less than 2 mm).

1. Crude oil was obtained from the Iraqi refinery Al-Najaf Petroleum.

2. Adding crude oil to soil that hasn't been contaminated:

Four different concentrations of total petroleum hydrocarbon (TPH) were used in the experiments: 25, 50, 75, and 100 g/kg soil, with 0 g/kg soil serving as a control for plant biomass output. Three kilograms of soil were divided into three pots for each treatment. Each concentration of hydrocarbon was completely mixed with 3 kg of soil on a metal plate to ensure that the soil and hydrocarbon were fully mixed, and then returned to 3 L pots perforated at the base to promote drainage and aeration. Before planting, each pot was suitably classified to identify its individual treatment and left for a week.

3. an examination of the ground Soil properties, both physical and chemical

2.2. Design of the experiment:

Myrtus Communis was chosen and grown in soil contaminated with various concentrations of crude oil (25,50,75,100 g/kg) and a control pot in which a plant was planted in clean soil for the purpose of studying the effects of crude oil on plants, with three replicates and a control pot containing soil Polluted without plants for the purpose of estimating the percentage of volatilization and cracking by microorganisms.

The plant = Myrtus
the family = Myrtaceae
The scientific name = Myrtus Communis
life forms = small evergreen shrubs that grow mostly in moist and shady places.

2.3. The plant's phenotypic characteristics:

- The length of the stem. Measure the stem length from the soil surface to the top of the stem, as directed by the source (in cm).
- The length of the root According to the source, measure the root's length from its point of attachment to the plant to the end of its expansion in the soil (in cm)
- Wet mass According to the source, the moist weight was measured right after the plant samples were collected and brought to the lab, where they were weighed using a sensitive scale.
- Dry mass The plant samples were dried for 72 hours at 75 ° C in a drying oven, after which they were analyzed

2.4. Plants' biochemical measurements Myrtus Communis is a species of Myrtus

- Total chlorophyll: According to 6 total chlorophyll was extracted with acetone 80 percent by squishing 0.5 gm of fresh leaves, filtering the extract, and diluting it to 10 ml with acetone. The absorbance of 645 and 663 nm wavelengths was measured using a spectrophotometer.
- Super oxide dismutase (SOD): The activity of superoxide dismutase (SOD) was tested in fresh plant leaves
- The activity of catalase (CAT) was determined using the method described [7]

2.5. Plant extraction

Five g of fresh plant were homogenized in 25 mL of extraction solvent (acetonitrile: water: citric acidic (ACN: H2O: CA, 80:19:1, V/V) by mortal and pistil, 2- the homogenized plant was then transferred to 50 mL polyethylene container vortex mixer for 1 minute before being fully soaked for 10 minutes. After that, it was shaken for 20 minutes in a spinning shaker. 3- ultrasonic extraction at 4 C for 30 minutes. 4- anhydrous sodium sulfate (7.50 g) and 1.50 g sodium chloride (Na2SO4:NaCl, mass ratio 5:1) were added, and the mixture was vortex mixed and centrifuged for 10 minutes at 4 C at 8874 g. 5- Using vacuum, the top organic phase was removed and concentrated for 1 mL. 6- A 0.45m filter was used to filter the concentration The sample is then ready for HPLC analysis.

3. Results

Sixteen polycyclic aromatic hydrocarbons (PAHs) were measured as priority pollutants identified by the US Environmental Protection Agency as naphthalene, acenaphthylene, acenaphthene, fluorine, phenthrene, anthracene, fluoranthine, pyrene, benz[a]anthracene, chrysin, and benzene [b]. benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]berylene, and indino[1,2,3cd pyrenes) .(The results indicated the accumulation of some polycyclic aromatic hydrocarbons in the plant at different rates, as the accumulation value depended on the concentration of hydrocarbons, so the highest accumulation value was at a concentration of 100 g / kg, followed by a concentration of 75 g / kg after 30 days from the start of the experiment and this is consistent with what It was found [7] . when he stated that the uptake of hydrocarbons by 12 different types of plants cultivated in polluted soil is dependent on hydrocarbon concentrations in the soil. Because they collect water-soluble contaminants largely through roots and transport, through various plant tissues where they can be digested, stored, or volatilized, plants serve as solar-powered pumping and filtering systems [8]. The most essential portion of the system is the root zone, The rate of petroleum hydrocarbon cracking is controlled by the microbial community of different plant species.

[8] indicated that all these compounds and organic carbon are used to supply energy for microorganisms in the soil, that plants can tolerate organic pollutants, and this is what the current study indicated, as the Acacia glauca plant had a high ability to withstand the high toxicity of petroleum hydrocarbons and its ability to accumulate. Two PAHs, such as naphthalene and benzo(b)fluoranthene, can possess very different chemical properties and behave quite differently in air/water/soil systems. Naphthalene is the most soluble of the monitored PAHs. Naphthalene also has the highest vapors pressure of the 10 PAHs and a characteristic mothball smell. Naphthalene does not adhere strongly to soils or sediments and can pass through sandy soils with relative ease and readily contaminate groundwater supplies [9]. Conversely, benzo(b)fluoranthene has the lowest solubility of the monitored PAHs. Benzo(b)fluoranthene is a non-volatile PAH that adheres very strongly to soil and organic matter. Contrasting the chemically-related parameters of an LMW and HMW PAH demonstrates the difficulty associated with the remed iation of complex mixtures of PAHs, such as creosote goes on to say that PAHs don't accumulate as quickly as other lipophilic chemical molecules like PCBs, Instead they're transformed to more water-soluble forms making them easier to excrete from the body .

Table 1. Concentrations of PAHs (µg/g) in Myrtus Communis after one month at 25°C

Sampl	PAHs	75 g/kg	100 g/kg
1	Naphthalene	N.D	**N.D**
2	Acenaphthylene	N.D	**1.4508826**
3	Acenaphthene	0.55041282	**0.19739376**
4	Fluorene	N.D	**N.D**
5	Phenanthrene	N.D	**0.0399582**
6	Anthracene	0.0302663	**0.1952193**
7	Fluoranthene	0.90860016	**1.64644656**
8	Pyrene	N.D	**N.D**
9	Benz[a]anthracene	N.D	**0.4409296**
10	Chrysene	0.06535242	**0.19182844**
11	Benzo[b]fluoranthene	0.1139263	**0.1337653**
12	Benzo[k]fluoranthene	N.D	**N.D**
13	Benzo[a]pyrene	0.0129786	**0.0547287**
14	Dibenz[a,h]anthracene	N.D	**N.D**
15	Benzo[ghi]perylene	N.D	**N.D**
16	Indeno[1,2,3-cd]pyrene	N.D	**N.D**

[1]*N.D = Not Detectable

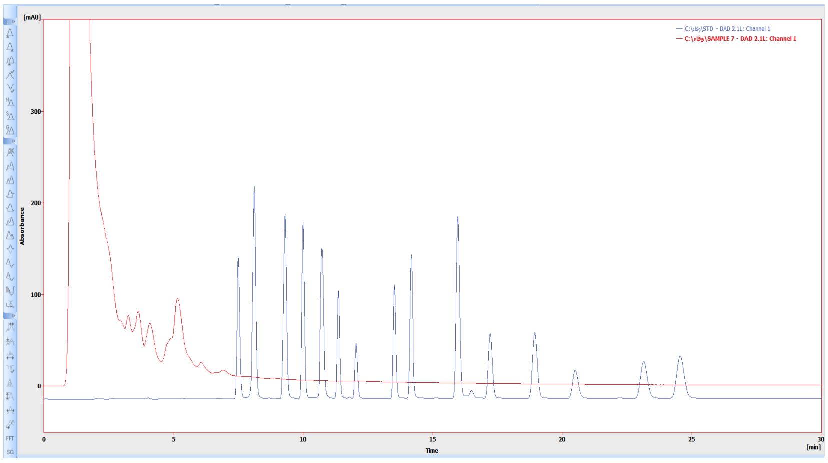

Figure 1. After one month of growth in soil contaminated with crude oil at a concentration of 75 g/kg, HPLC measurement of accumulated polyaromatic hydrocarbons in Myrtus Communis (mg/g).

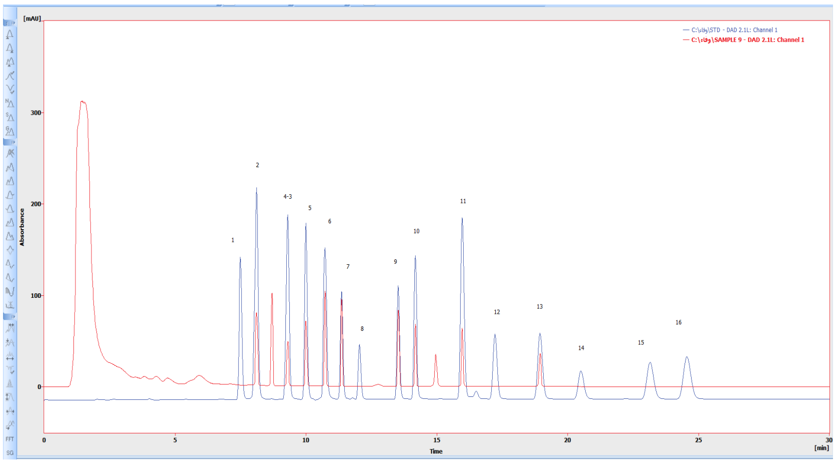

Figure 2. After one month of growth in soil contaminated with crude oil at a concentration of 100 g/kg, HPLC measurement of accumulated polyaromatic hydrocarbons in Myrtus Communis (mg/g).

3.1.The results of biochemical tests:-

Myrtus Communis cultivated in crude oil polluted soil showed significant decrease of total chlorophyll content and catalase enzyme in comparison with control A significant decrease of Super Oxide Dismutase enzyme in comparison with control was observed as in Figure (3) (4) (5)Plant development in crude oil-contaminated soil has a considerable negative impact on the production of chlorophyll pigments [8]. Chlorophyll synthesis in plants may be inhibited by crude oil contamination of the soil..

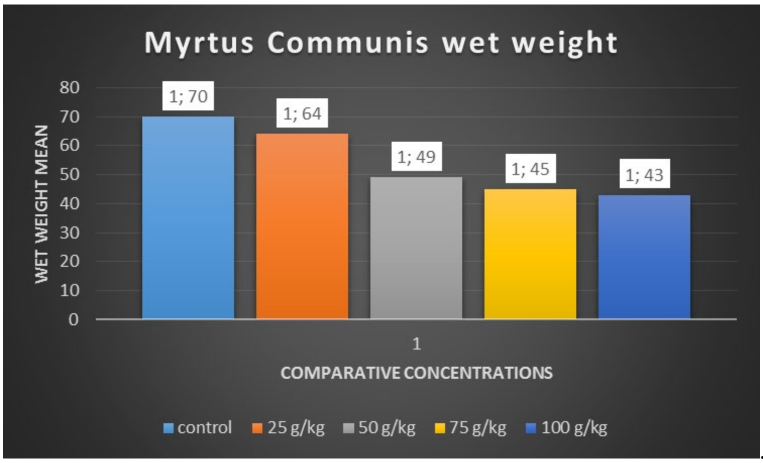

Figure 6. wet weight of Myrtus Communis.

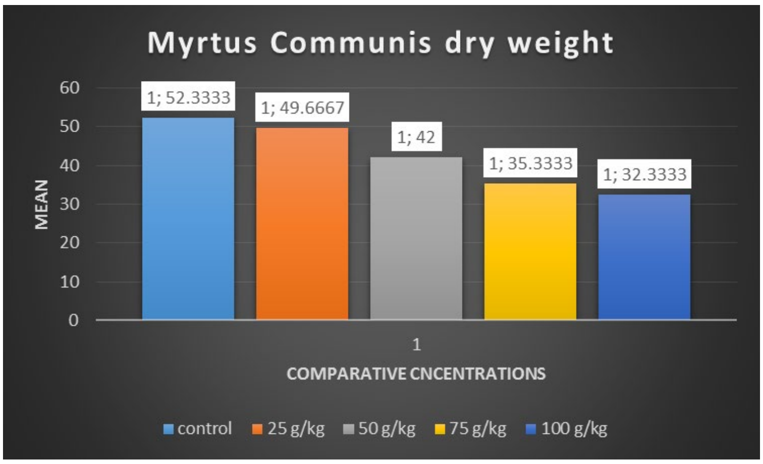

Figure 7. Dry weight of Myrtus Communis.

The root length of the Myrtus Communis fluctuates between pots containing different amounts of hydrocarbons, according to the current study. Because of the toxicity caused by petroleum hydrocarbons on the roots of plants grown in contaminated pots, the root length in the control pots was the longest, while the root length in the pots with different concentrations of petroleum hydrocarbons had a lower root length, which is consistent with what was found[12] which showed that the toxic effect of hydrocarbons had a severe effect It also agrees with the findings of[6] where it was found that crude oil inhibits root growth by preventing water from entering the roots and thus reducing water absorption[12] explained that the acute toxicity of petroleum hydrocarbons led to the inhibition of plant growth, especially the growth of the root group.

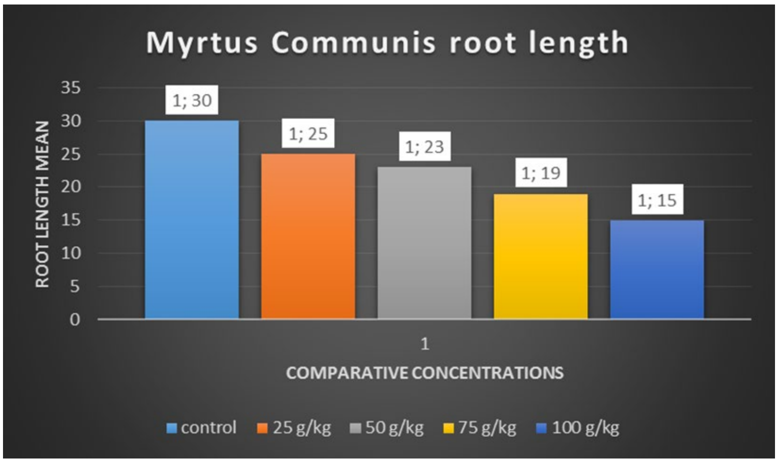

Figure 8. Myrtus Communis root length.

The results of the current study showed that petroleum hydrocarbons had an effect It is evident along the stem length of Myrtus Communis plant, as the stem length in the control pots was longer than the other pots, as it was found that the stem length is inversely proportional to the concentration of hydrocarbons .This is consistent with what [13] Petroleum hydrocarbons cause phytotoxicity either directly by damaging the plasma membrane and limiting photosynthesis or indirectly by altering the physical and chemical qualities of the soil in which the plant develops, affecting the entire plant and shortening the stem length And [14]pointed out that when oil concentrations are 10 g/kg or greater in soils contaminated with oil, the stem length drops and shortens, which suits our results because the concentrations were higher .

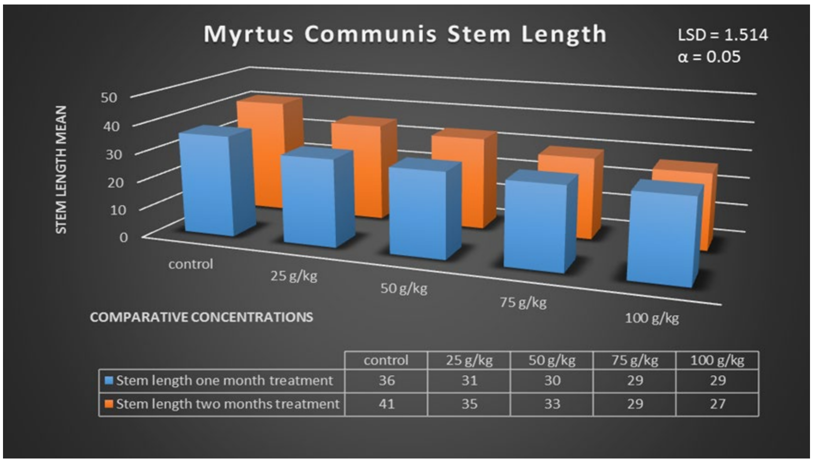

Figure 9. Myrtus Communis Stem length.

4.Discussion

The alkaline state induced by the dissolving of the compounds in the oil in the cell sap that were responsible for the breakdown of chlorophyll is most likely to blame for the decrease in total chlorophyll content in the leaves [9] Crude oil is a mixture of compounds that inhibit enzymes needed for chlorophyll formation while also lowering leaf area[1] Contamination with crude oil creates stress conditions that have a variety of consequences on plants, including oxidative stress caused by an abundanceof reactive oxygen species (ROS) [10] Many cellular components produce reactive oxygen species in a variety of ways as a result of regular metabolic activities and the creation of environmental stress [14-15]. Oxidative stress is caused by the create of reactive oxygen species (ROS), which causes oxidative damage to proteins, DNA, and lipids[10] Plants use antioxidant enzymes like catalase and superoxide dismutase to protect cells and cellular subsystems from the harmful effects of these active oxygen radicals .During stress, catalase is vital for H2O2 elimination because it catalyzes H2O2 dissociation events in the detoxification of H2O, O2, and ROS The first enzyme for detoxification activities, superoxide dismutase, is a metallic enzyme that catalyzes the conversion of an O2 radical into H2O2 and O2 established a link between the amount of catalase and chlorophyll in the soil and the availability of soil nutrients, particularly iron, which is a key component of both molecules.Biochemical experiments on Acacia Glauca grown in crude oil-contaminated soil revealed a considerable reduction in superoxide dismutase content, which is consistent with what was discovered [11-16] .The effect of immersion of barley plant (Hordeum vulgare cv. Alfa) in soil was studied, The reduction in SOD activity was ascribed to the steady decrease in iron-containing SOD activity found in the chloroplasts after soil immersion for 72 to 120 hours,The increased formation of active oxygen species produced photoxidative damage to barley leaves as a result of root oxygen deprivation

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