

PHYTOTOXICITY TEST OF KEROSENE-CONTAMINATED SOIL USING BARLEY

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ABSTRACT

This study was aimed to determine a phytotoxicity experiment with kerosene as a model of a total petroleum hydrocarbon (TPHs) as Kerosene pollutant at different concentrations (1% and 6%) with aeration rate (0 and 1 L/min) and retention time (7, 14, 21, 28 and 42 days), was carried out in a subsurface flow system (SSF) on the Barley wetland. It was noted that greatest elimination 95.7% recorded at 1% kerosene levels and aeration rate 1L / min after a period of 42 days of exposure; whereas it was 47% in the control test without plants. Furthermore, the percent of elimination efficiencies of hydrocarbons from the soil was ranged between 34.155%-95.7% for all TPHs (Kerosene) concentrations at aeration rate (0 and 1 L/min). The Barley could efficiently encourage the degradation of complete total petroleum hydrocarbons depending to plant growth parameters when the kerosene level in water was up to 1%. A rhizobacteria attached with Barley roots played a major role in biodegradation of Kerosene in contaminated soil when the initial kerosene concentration was 1%. This study also revealed that Barley and rhizobacteria can reclaim hydrocarbon-polluted water in a subsurface flow system.

Keywords: Phytoremediation, SSF, TPHs, Aeration, Flow system, retention time

علي وآخرون

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فحص السمية (Phytotoxicity) للتراب الملوثة بالكربوسين باستعمال نبات الشعير		
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المستخلص

تهدف الدراسة الى اجراء تجربة السمية النباتية مع الكربوسين كنموذج لهيدروكربونات البترول الكلية (TPHs) مثل ملوث الكربوسين بتراكيز مختلفة (1 % و 6%) مع معدل تهوية (0 و 1 لتر / دقيقة) ووقت الاحتفاظ (7، 14، 21، 28 و 42 يوماً) على الأراضي الرطبة للشعير في نظام التدفق تحت السطحي (SSF). وقد لوحظ أنه بعد فترة 42 يوماً من التعرض، كان الحد الأقصى للإزالة 95.7% عند تركيز الكربوسين بنسبة 1% ومعدل التهوية 1 لتر/دقيقة؛ بينما كان 47% في اختبار السيطرة دون النباتات. علاوة على ذلك، تم تحديد النسبة المئوية لفعالية إزالة الهيدروكربونات من الرمال لتكون في حدود ما بين 34,155 % - 95,7 % لجميع تراكيز TPHs (الكربوسين) بمعدل تهوية (0 و 1 لتر/دقيقة). وفقاً لمعايير نمو النبات، يمكن أن يعزز الشعير بشكل فعال تحليل إجمالي الهيدروكربونات البترولية عندما يصل تركيز الكربوسين في الماء إلى 1%. لعبت الرايزوبكتريا المرتبطة بجذور الشعير دوراً رئيسياً في التحلل الحيوي للكربوسين في التربة الملوثة عندما كان تركيز الكربوسين الأولي 1%. كما أظهرت هذه الدراسة أن الشعير والبكتريا في نظام التدفق تحت سطح الأرض لديها القدرة على استصلاح المياه الملوثة بالهيدروكربون.

الكلمات المفتاحية: المعالجة النباتية، التدفق تحت سطح الأرض، مجموع الهيدروكربونات البترولية، التهوية، نظام التدفق، وقت الاحتفاظ

INTRODUCTION

The most causes to the pollution of refined oil is from accidental spills, leakages from containers, pipes, joints and land disposal of petroleum wastes in addition, the extraction, transportation and crude oil refining causes substantial ecological contamination and refining crude oil causes significant pollution of the environment (29). The majority of organic pollutants are in the environment (ranging from C6 to > C50) and are recognized to be toxic to many organisms. The reason of dangerous ecological problems and the rise of many harmful effects on human health like toxicity and carcinogenesis is the excessive usage of synthetic chemical compounds. By releasing strong, liquid and gaseous waste comprising several pollutants, including hydrocarbons, heavy metals, organic solvents, various anthropogenic activities have adversely impacted significant environmental components (air, water, soil, biota) (28). One public health problem is environmental contamination. In fact, the methods of exposure of human to numerous environmental contaminants are many and made by various exposure pathways: particle breathing, ingestion, immediate contact, food chain ingestion (12, 15). It is possible to protect public health by recovering contaminated sites that can also be reused for various future activities. Then, consideration is given to eco-friendly biological methods for soil remediation (7). Bioremediation is described as the process of biologically removing or degrading pollutants to a harmless state under controlled conditions, or to levels less the regulatory limits (14, 20). Phytoremediation is a technique that can be applied to many remediation treatments as phytoremediation has been not overlapped with the ecosystem, so it needs a small amount of work and is, therefore, cheap compared with conventional physical-chemical methods. Temperature of the soil, humidity and dissolved oxygen affect microbial societies and their capability to break down TPH (10, 26). Plant roots increase soil aeration considerably by enhancing porosity and decreasing the concentration of soil moisture. Plants therefore encourage a more vibrant atmosphere for aerobic microbes to break

down hydrocarbons (13). Aerobic microorganisms generate dioxygenase and monooxygenase enzymes that cause the conversion and mineralization of TPH, bacteria utilize oxygen as a reactant in aerobically active soils for metabolic operations. The amount of oxygen in a soil system is directly affected by soil moisture. Low-water concentration with soil systems can influence microbial activity and pollute bioavailability, while elevated water concentrations can generate anoxic conditions with reduced diffused oxygen levels (24). The indigenous plant species were utilized for procedures of phytoremediation for TPH waste (17). Laboratory and greenhouse experiments comparing germination and growth of distinct crops in contaminated soils with TPHs and assisting to choose the most appropriate crops at farm level are therefore crucial for assessing their impacts on levels of pollutants. *Sorghum bicolor* and *Hordeum vulgare* (hereafter referred to as sorghum and barley) were chosen for the phytoremediation of oil-polluted soils around the Isfahan Oil Refinery (Isfahan, Iran) taking into consideration prior oil contamination research and soil characteristics (2,8). Many researchers (34) who tested soils contaminated with 74.12 ± 3.50 g kg⁻¹ D.M. of total petroleum hydrocarbon (TPH) in pot experiment and used the common flax (*Linum usitatissimum*). The results exhibited that, during one vegetative cycle of flax crops, the TPH decrease effectiveness of fly ash treated soils was 56.20-63.25 percent during the 100-day experimental period. After plant harvest, the residual TPH content in soil is 27.0-32.5 g·kg⁻¹ DM. Idowu and Fayinminnu (18) evaluated phytoremediation organically for soil polluted with 0.3 and 6% (W/W) Spent Lubricating Oil (SLO) by *Jatropha curcas* in a pot experiment and at the end of twelve weeks reported that the *J. Curcas* seedlings can remain alive at 0,3 and 6 percent of SLO polluted soil as a phytoremediator. The adverse impact of SLO on growth parameters such as height, stem diameter and amount of leaves depends on dose. Some researchers (33) studied petroleum hydrocarbon-contaminated soils with a primary levels of 40000 ppm in a pot experiment and selected two plants (Sorghum

and common flax). Results showed that contamination with petroleum hydrocarbons considerably lowered the development (growth) of the plants tested. Compared to the control treatment, sorghum and common flax decreased the concentration of TPHs by 9500

and 18500 mg kg⁻¹, respectively. The aim of this research was to determine Kerosene's phytotoxicity impacts on Barley plants and its tolerated and withdrawn hydrocarbons from wastewater.

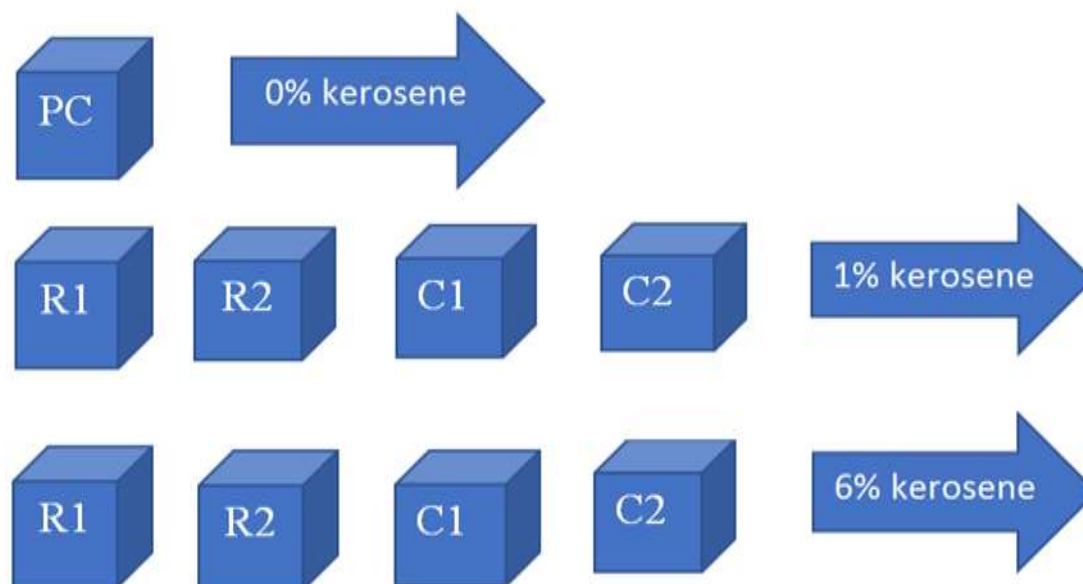


Figure 1. Experimental set-up of the phytotoxicity experiment

MATERIALS AND METHODS

Experimental phytotoxicity test set-up

The phytoremediation experimental research was carried out under the outdoor environment conditions. In this research, nine glass aquariums have been utilized to minimize any Kerosene sticking on the walls. All the aquariums were run in batchwise kerosene. From the bottom to the upper layer, aquarium with dimensions of 30 × 30 × 30 cm had: (1) 8 cm of gravel with a depth of 1–2 cm, (2) 3 cm of gravel with a thickness of 1-5 mm and (3) 10 cm of 0.15-1.18 mm of sieved fine sand. There were two replicates for each concentration (R1 and R2) and an aquarium for plant-free control pollutant (C1 and C2), in addition another aquarium without kerosene pollutant as plant control (PC) Fig. 1. Aeration is provided to enhance the aerobic condition which is expected to increase plant-microbial interaction activity to get better performance in phytoremediation process. Air pipes were designed that extended along the tank, the pipe contains more than (30) holes, aeration is done by using an air pump (pumping speed=1L/min). Healthy Barley plant (7 days old) were cultivated in each glass basin comprising 6 L of synthetic wastewater get ready by blending water with conventional

kerosene collected at separate levels of 1 and 6 percent from the local Al-Dora oil station (VKerosene / VWater). The water level within the sand layer surface was retained to imitate a subsurface flow scheme as generally utilized in a constructed wetland (36). In this study, the observation was done throughout the 42 days and the sampling time was on day 0, 7, 14, 21, 28, 35 and 42. The analyzed parameters are the concentration of TPH in the medium of water and sand, the physical parameters of vegetation including wet weight, dry weight, root length and stem height were also measured.

Determination of water's physicochemical properties: Sampling was performed on days 0, 7, 14, 21, 28, 35 and 42 days. In order to record the changes physicochemical in water, a multi-sampler of IQ 150 (IQ Scientific Instruments, UK) for pH, ORP and temperature measurements (GLI International, Model 63, USA) was utilized to register temperature (°C), pH and oxidation reduction (ORP, mV). The media parameters in water were recorded to observe at the Ministry of Science and Technology in Jadriya.

Water sampling: Total petroleum hydrocarbon (TPH) as an indication of the presence of kerosene by periodically collecting

water samples from the growth medium (100 mL) in clean aquarium containers on sampling days for all remediation. A liquid-liquid extraction method and gas chromatography were used to determine the concentration of TPH in synthetic wastewater (23). The method followed the 3510C method of the Environmental Protection Agency (38). Dichloromethane was utilized as a solvent (Merck, Germany). Wastewater (100ml) sample was transmitted to a separate funnel (1L) after 25 mL of dichloromethane was added and shaken for 2 min. The lower organic layer of sodium sulfate was removed from the flask bottom and then dried (Merck, Germany). In an overhead fume hood, the residuals were placed in a 10 mL vial to evaporate the remaining water for 3-4 days.

Sand sampling

At the Ministry of Science and Technology, the samples were measured. For more data on sand absorption of kerosene, the sand has been extracted using Method 3550C (37) of the Environmental Protection Agency. To analyze the sand, Periodically 10 g of sand from the experimental growth medium was gathered from each aquarium in clean containers on the same sampling days for all (TPH) then the TPH has been ultrasonically determined using a solvent extraction procedure (22). First, by blending with sodium sulfate (Na_2SO_4), sand samples were dried and then 50 mL of dichloromethane utilized as the solvent added in Schott bottle (100ml.), then, in an ultrasonic cleaner (Kwun Wah International Ltd., China) the Schott bottle was placed for 30 minutes at 50°C. After that, the samples were purified through glass wool and the solution was put into a 15 mL vial and left for 3-4 days in the fume hood to dry up any water and dichloromethane traces.

Plant growth

Barley plants exposed to various kerosene levels (1 and 6%) was noticed for 42 days. Plants were harvested after these periods 0, 7, 14, 21, 28, 35 and 42 days from each of four replicates. Plants were washed with tap water entirely and then left to dry at room temperature and after that registered stem height, root size, and wet weight. Then, all the same plants were placed in an oven (Memmert, Germany) at 70 °C for 72h for

drying, and after that dry weight was determined (31).

Analysis and removal of kerosene percentage: Sand extracts were focused to 2 mL in GC vials and analyzed by GC-FID utilizing capillary column gas chromatography (Agilent Technologies, Model 7890A, GC System, UK) with an HP-5 5% phenyl methyl siloxane column (30 m x 0.32 mm i.d. x 0.25 microns) and helium as the carrier gas. The column temperature was detained for 1 min at 50 C and then ramped for 10 min at 15 °C per min at 320°C. The TPH removal percentage for each sample was calculated utilizing Eq.:

$$\% \text{ Removal} = \frac{(TPH_0 - TPHS_t)}{TPHS_0} \times 100$$

Where TPH_0 = total petroleum hydrocarbon on sampling day 0 and $TPHS_t$ = total petroleum hydrocarbon on each sampling day.

RESULTS AND DISCUSSION

Monitoring of parameters of physical chemistry:

During the phytotoxicity test, physical parameters (T, pH, DO and ORP) were registered Fig.2 and Fig.3. For plants and without plants treatments at kerosene levels 1 and 6% and without and with air at 1L/min flow. The results generally indicate that the mean temperature ranged from (11-25 ° C) over the 42 days with no air; this is suitable for a tropical zone. The aquarium average pH ranged from 6.8 to 7.6 with the absence of air, mentioning that there was no significant difference in pH between the treatments. ORP oscillated between (-35 and +9.3mV) with the absence of air, mentioning that all kerosene levels and plant controls oscillated between aerobic and anoxic conditions (Fig.2). Also, the results revealed that the average temperature oscillated between (11.5-25°C) with air 1L/min over the 42 days; this is suitable for a tropical zone (9). PH is an essential factor in the quality of water and has a major impact on the aquatic system. The aquarium average pH ranged from (6.5 to 7.5) with air 1L/min, mentioning that there was no significant difference in pH between the treatments (Fig. 3), (8). The pH was greater for present of plant than for without plant because bacteria found in the root of was pH- and nutrient-dependent, efficient degradation and raised microbial growth took place between pH (6-9.5). Generally, the effect of pH in

Kerosene degradation is attributed to its role in the solubility of the medium nutritional substances. The pH for the unpolluted soil is within range (5-7), which is considered appropriate for all well agrarian soil. Effective degradation and enhanced microbial development happened between pH (6-9.5). Increased acidity owing to the presence of kerosene is an issue for agricultural soils because very low pH values, indicative of acidity, are associated with negative soil circumstances including decreased microbial activity, enhanced accessibility and toxicity of heavy metals as well as decreased accessibility of plant nutrients. (32, 6). Kerosene consists of a mixture of aliphatic and aromatic hydrocarbons. Both need to be degraded, but they undergo slightly different pathways. Enzymes attack the terminal methyl group and convert the alkanes into alcohol. Further oxidation of the alcohol results in the formation of first aldehyde, and then to fatty acids. Fatty acids are later broken down by β -oxidation to acetyl-CoA (n-alkane with even carbon number) or propionyl-CoA (n-alkane with uneven carbon number). pH of soils can vary considerably from 2.5 (mine soils) to 11.0 (alkaline deserts). Most bacteria survive at near neutral pH and thus soils with extremes of pH are considered to have lower petroleum biodegradation rates. It has been reported that the rate of degradation almost doubles when pH of soil is raised from 4.5 to 7.4. acetyl-CoA (n-alkane with even carbon number) or propionyl-CoA (n-alkane with uneven carbon number) DO and ORP calculations whether aerobic or anaerobic can

distinguish the phytotoxicity test circumstances (30). The results revealed that the treatment environment was aerobic, where ORP oscillated between (-19.2 to +25.4) mV with air (1L/min) Fig.3, to indicate that all levels of kerosene and plant controls were in aerobic range circumstances. Kerosene influenced the rhizosphere treatment environment and induced a decrease in ORP readings, indicating a more anaerobic environment with enhanced kerosene concentration. The decrease in consumed oxygen indicated that the treatment was heading towards anoxic or anaerobic conditions. (16), stated that high ORP promotes aerobic processes while lower ORP promotes anaerobic processes. In this study, supplementary aeration for SSF pilot constructed wetlands was effective at flowrate 1 L/min aeration to increase oxygen transfer in wetland treatment systems. Such findings indicate that oxygen available into SSF via diffusion or root oxygen release can be enhanced with additional supplementary aeration. Oxidation reduction potential (ORP) and dissolved oxygen (DO) can be used to determine aerobic and anaerobic conditions in a constructed wetland reactor (30). Kadlec (19) classified the media conditions according to ORP, aerobic (ORP > 200 mV), anoxic (200 to -200 mV), and anaerobic (ORP < -200 mV). It was noted that the anoxic circumstances were dominated on the non-aerated and aerated wetlands based on the surveillance of ORP and DO.

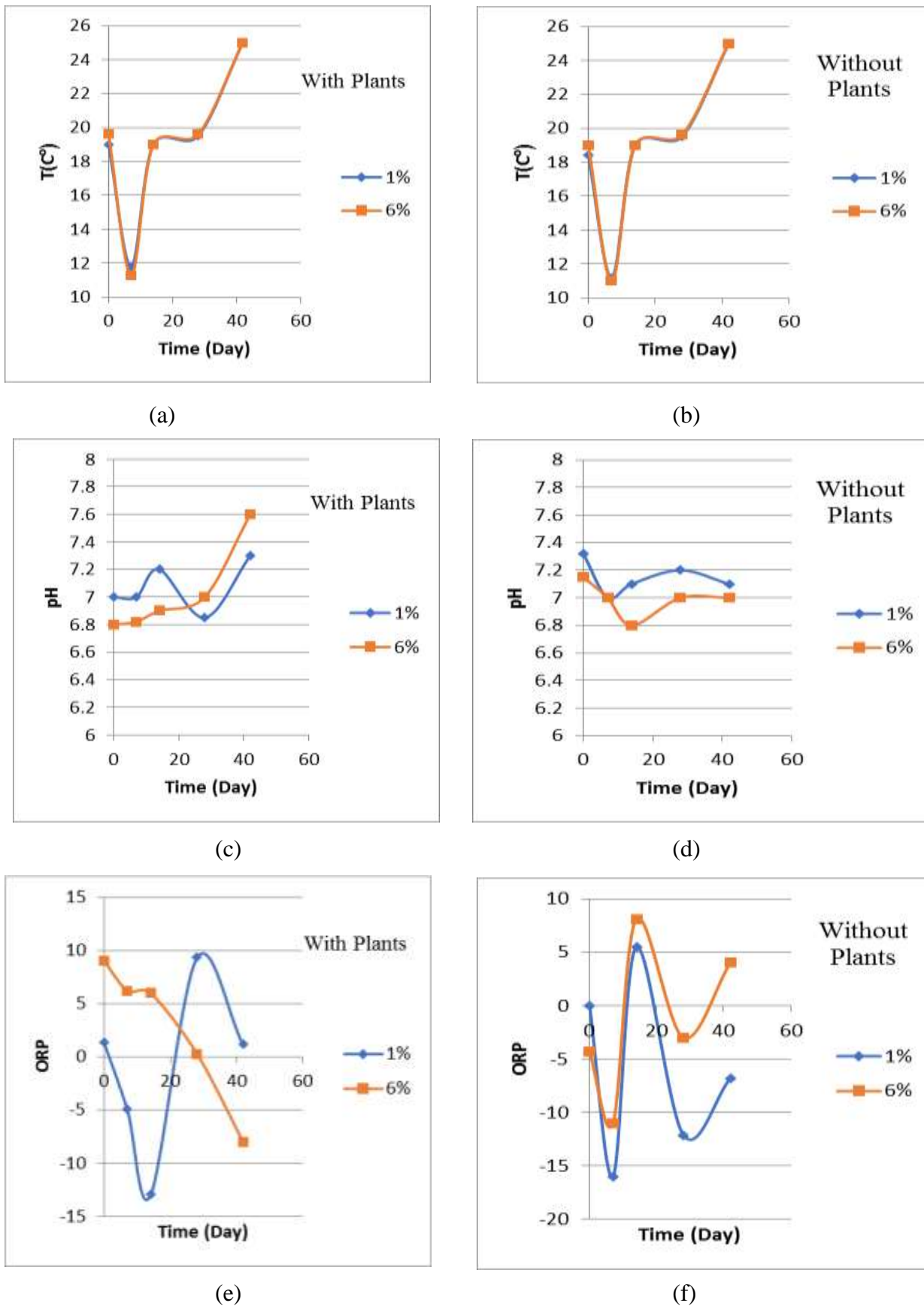


Figure 2. Physical parameters differences in the phytotoxicity experiment with Barley utilizing kerosene as the pollutant (1 and 6%) at (0 L/min): (a and b) temperature, (c and d) pH, (e and f) ORP

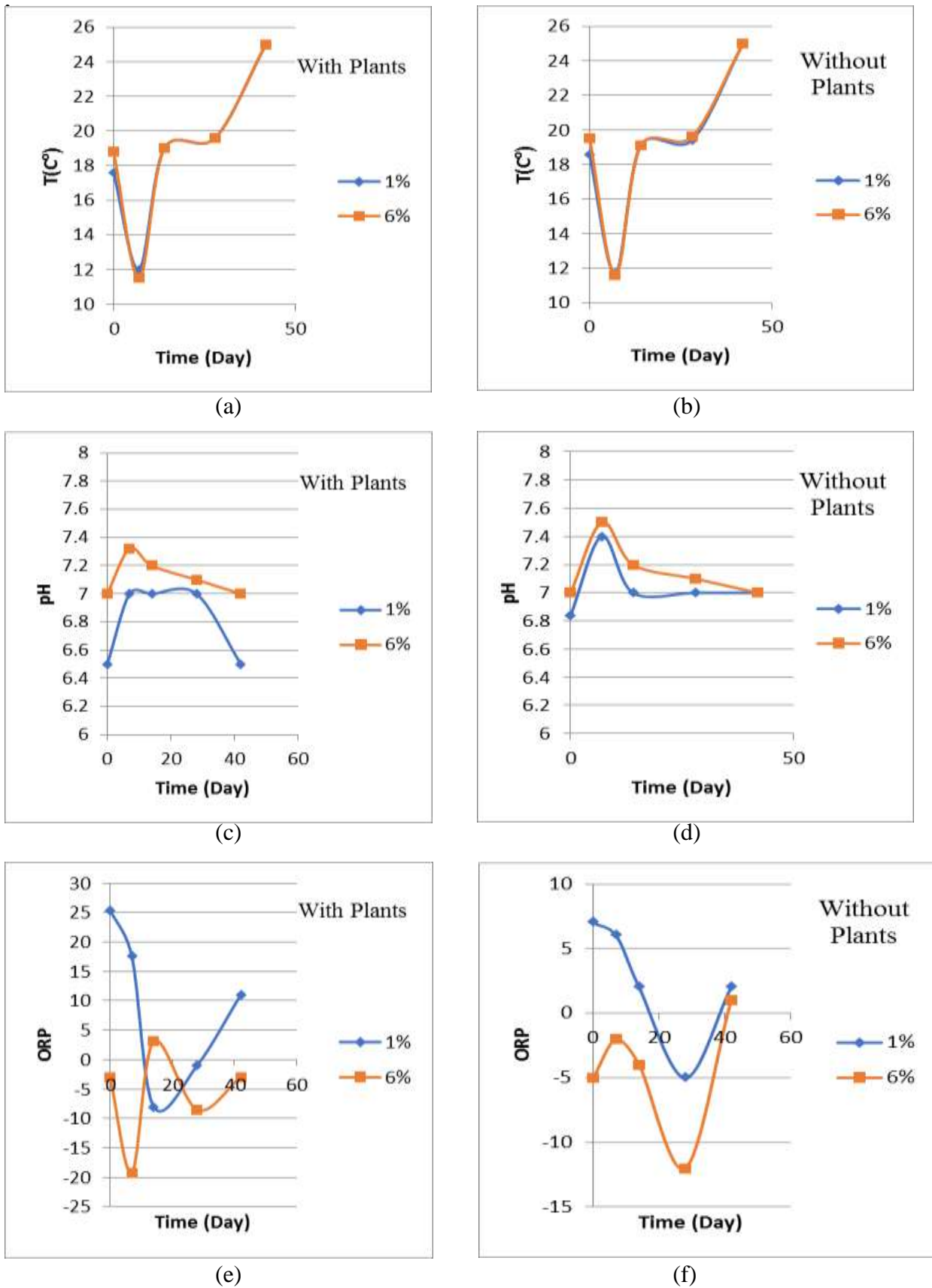


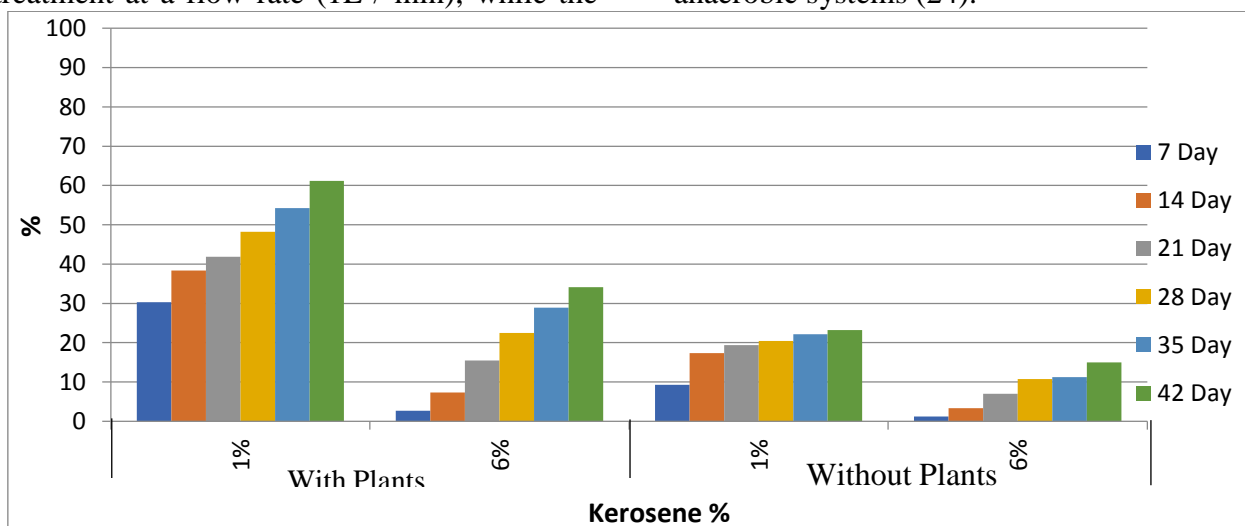
Figure 3. Physical parameters differences in the phytotoxicity experiment with Barley utilizing kerosene as the pollutant (1 and 6%) at (1 L/min): (a and b) temperature, (c and d) pH, (e and f) ORP

Degradation and removal of kerosene from water: The breaking down percentage of total petroleum hydrocarbons (TPH) by Barley plant was registered from the extraction of synthetic wastewater of two kerosene concentrations (1 and 6%) with plants at air (without and with air at 1L/min flow) and the corresponding control pollutant without plants over the 42 day treatment period.

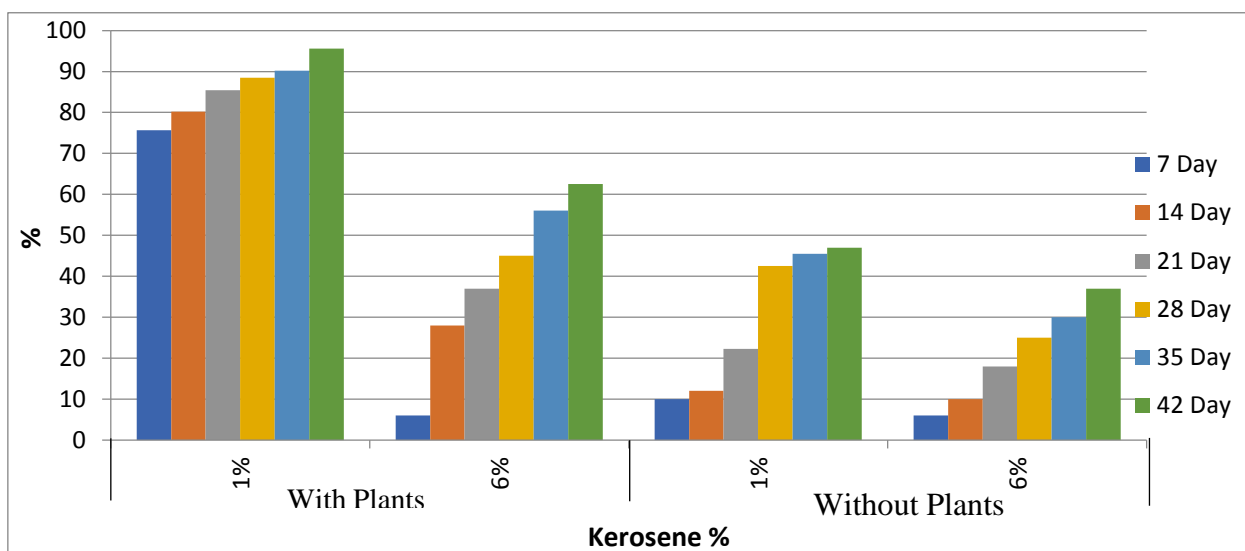
Degradation of kerosene in sand

Sand extraction was performed to acquire more data on kerosene degradation in the substrate. Fig. 4 (A and B) demonstrates 42-day degradation and withdrawal for the two distinct plant and plant-free kerosene levels (1 and 6%) at (without and with air at 1L/min flow), and Fig.8. (a, b, c, d, e, f, g, h) shows GC Curve TPH chromatograms at (1 and 6%) concentration of kerosene at (without and with air at 1L/min flow). In most treatments, the elimination efficiency of kerosene pollutant has significantly different between the two levels and the days of sampling (7, 14, 21, 28, 35 and 42). The greatest elimination of TPH degradation in sand 61.21 percent happened with a concentration of kerosene of 1 percent during 42 days (without aeration), whereas the median elimination was only 23.24 percent in its respective control treatment. Similarly, at 6% kerosene concentration degradation rates were 34.155% (without aeration); While the average removal was 17.16 percent in the respective control treatment, similarly the highest removal of TPH degradation in sand (95.7 percent) happened at a concentration of 1 percent kerosene over the 42 days of water treatment at a flow rate (1L / min); while the

average elimination in the respective control treatment was 47 percent at air (1L / min). Similarly, the degradation levels with 6 percent kerosene concentration were 62.5 percent in the air (1L / min); while the average degradation was 37 percent in water (1L / min) in the respective control treatment (9). Results similar shows Barley's capacity to increase TPH degradation and survive these two levels of kerosene in a subsurface flow system. The hydrocarbons were metabolized because of the interaction between the plants themselves and the rhizobacteria. TPH has been degraded by volatilization, eluviation, and photolysis in the unplanted sands. Sorghum and common flax reduced the concentrations of TPH in contaminated land by 23.63% and 45.97% relative to control (33). With a rise in aeration rate, the TPH elimination from sand and water moved up. It was obviously demonstrated that the elimination of TPH from water improved steadily when the aeration rate increased from 0 to 1L / min owing to enhanced pollutant degradation in the sand medium by giving aeration to accelerate the aerobic biodegradation process. (32, 5). System root of plant increases soil aeration considerably by enhancing porosity and decreasing soil moisture. The root of plants, therefore, enhance a more dynamic hydrocarbon breakdown environment for aerobic microbes (13). To sum up, different microbial communities may use aerobic or anaerobic mechanisms to breakdown hydrocarbons of petroleum in soils. In a bioremediation, aerobic systems were more efficient than anaerobic systems (24).



A



B

Figure 4. Degradation percentage in sand extraction by Barley exposed to kerosene contamination at 1 and 6% (A) with and without plants at (0 L/min) (B) with and without plants at (1L/min)

Plant responses to the pollutant of kerosene

Few of the plants grown in sand irrigated with kerosene-polluted water showed apparent differences in appearance over 42 days of cultivation compared to those found in the control treatment (Barley without the contaminant) Fig. I j, k, l, five and eight. All plants generally revealed enhanced growth after 7 days of subjection to 1 to 6 percent kerosene levels. However, the plants still survived at kerosene concentration of 1% with and without air. The plants were visibly deserted and inhibited after exposure to the kerosene contaminant after 7 days at 0 L / min air flow, for the highest concentration of kerosene 6% ; while the plants died after 14 days at the 1L/min air flow. No plant death with levels of 1% kerosene was reported, with and without air. However, the growth of *Barley* was affected at a kerosene concentrations of 6% v/v, mentioning plant growth inhibition, some of the crops revealed indications of phytotoxicity, such as yellowing leaves, plants died and growth impaired relative to the respective control under all circumstances. These indications agree with the results of (9,33).The roots of Barley are able to uptake TPH up to 2250 ppm TPH at 1% kerosene concentration, respectively after 42 days with air (0 L/min.). While, Barley able to uptake TPH to 3600 mg/kg TPH at 6% kerosene concentration after 7 days with air (0

L/min.).The roots of barley are able to uptake TPH up to 1116mg/kg TPH at 1% kerosene concentration after 42 days with air (1L/min) .While, Barley able to uptake TPH to 1980mg/kg TPH at 6% kerosene concentration after 14 days with air (1L/min.) These signs agree with the results Agamuthu and Azeiz (3). For 1% kerosene concentration the wet and dry biomass was increased with time with and without air. as shown in Fig.6. But for 6% kerosene concentration after day 7 the wet and dry biomass was decreases with 0L/min flow air; but the biomass decreased after 14 days at 6% kerosene concentration with 1L/min flow air. Fig.7.shows the trend of increase in stem and root length throughout 42 days of kerosene exposure for 1 % kerosene concentration with and without air, but for 6% kerosene concentration after day 7 the stem and root length was decreases with 0 L/min air flow; but for 6% kerosene concentration when addition 1L/min air flow after 14 days the stem and root length was decreases. For all treatments the increase in stem length and root length were as control plants without contaminant. Contaminated and control treatments have been contrasted in terms of root and shoot dry weight. Comparisons revealed reductions in root dry matter of about 22 percent and 30 percent and the shoot dry weight of Sorghum and barley in polluted land of 51% and 42% (1,8). Merkl and Infante

(27) Which indicated a significant reduction in the length of the shoot in the presence of 3 and 5% of crude oil. Liste and Felgentriu (21) mentioned a 38.9% reduction in shoot biomass for ryegrass grown over a 95-d period in contaminated soil. Root biomass also declined in their study by 52.6 percent. Petroleum

hydrocarbons, especially low molecular weight hydrocarbons, may inhibit plant growth (27,33).The best results shows the trend of increase in stem and root length; and in wet and dry biomass throughout 42 days of kerosene exposure for 1% kerosene concentration at 1L/min air flow.

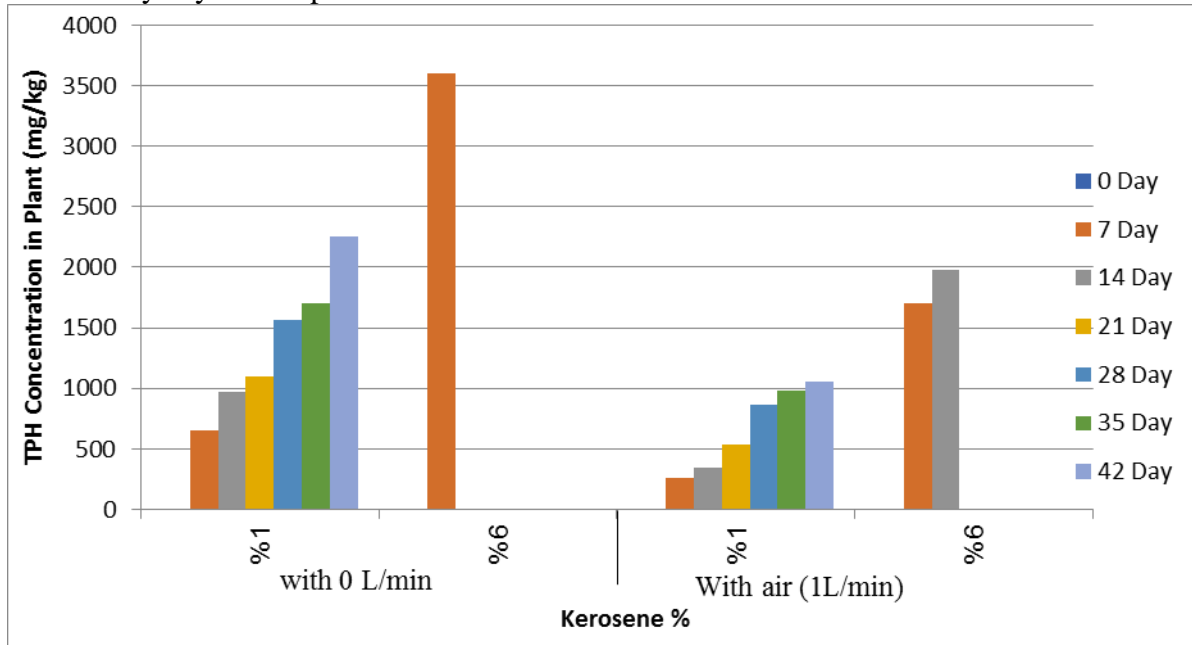


Figure 5. Concentration of kerosene in plants of Barley at 1% and 6% kerosene contaminant with 0L/min air flow and with 1L/min air flow

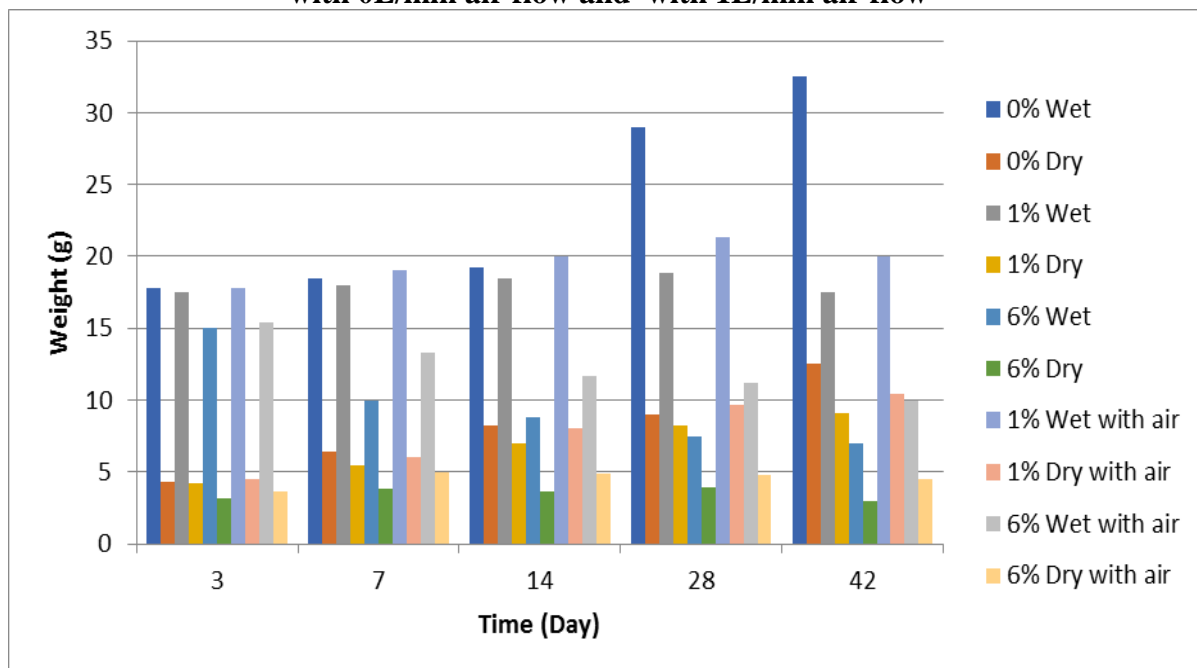
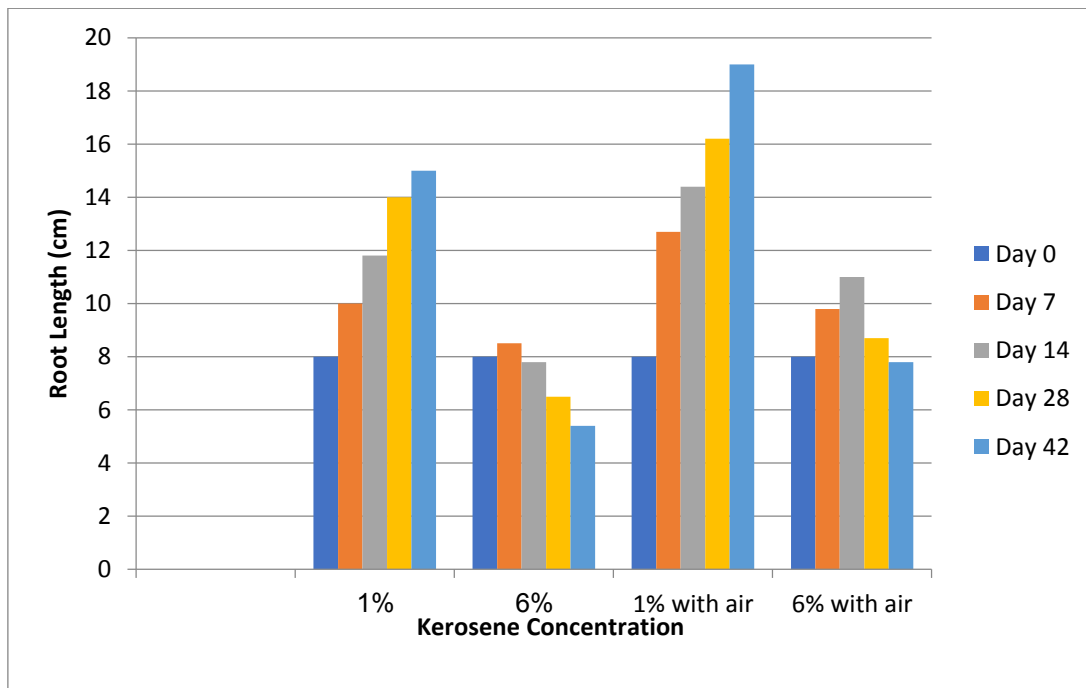
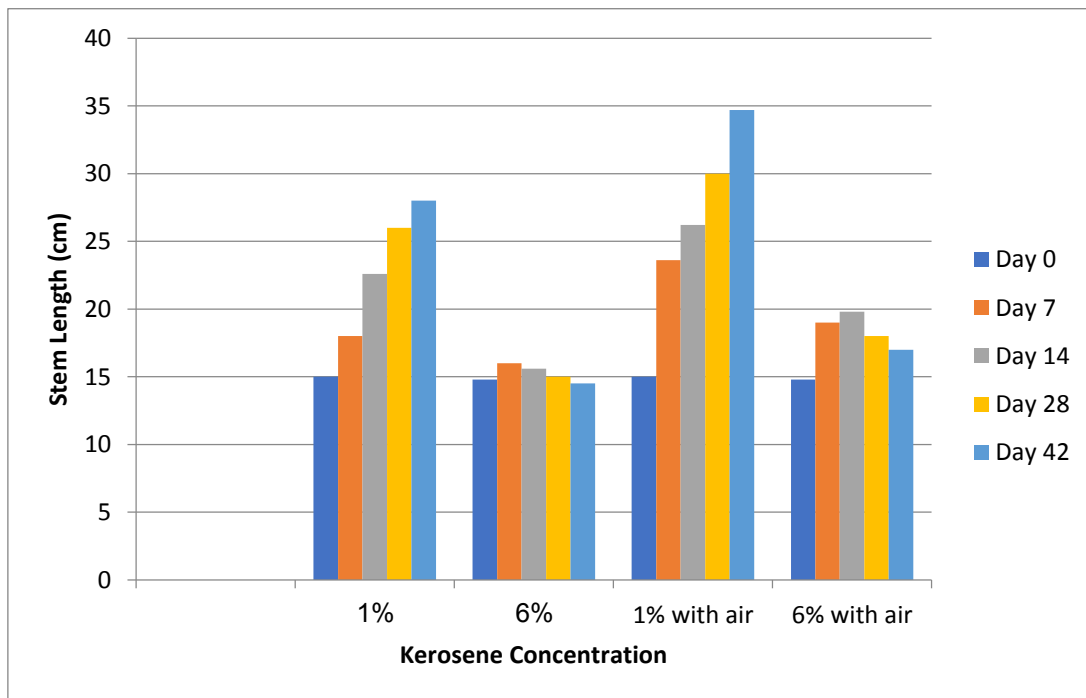


Figure 6. Growth response parameters wet weight and dry weight in the phytotoxicity test of Barley with kerosene concentration 1% and 6% with air (1L/min) and without air

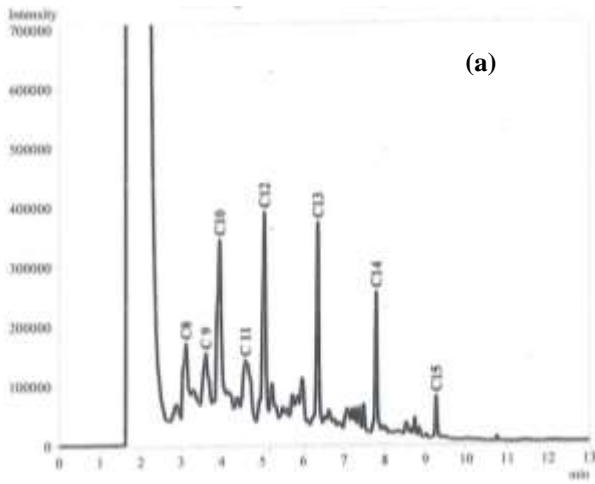


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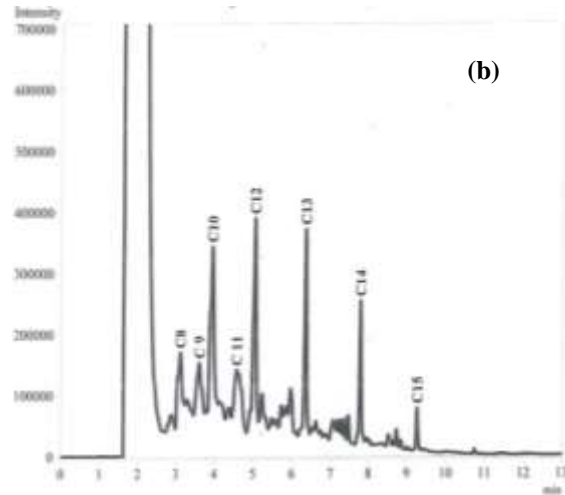


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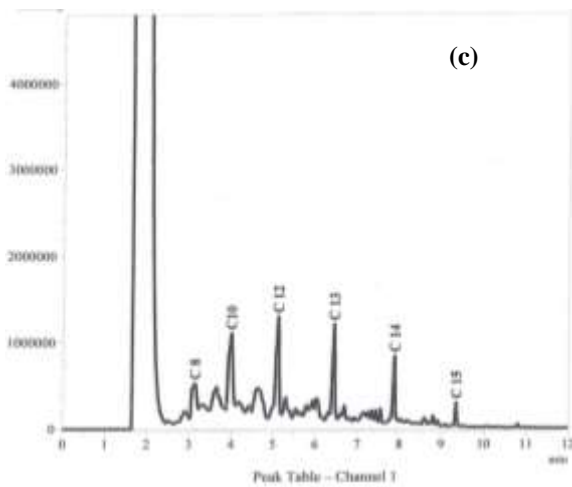
Figure 7. Growth response (A) root and (B) stem length in the phytotoxicity test of barley with kerosene concentration 1% and 6% with air (1L/min) and without air



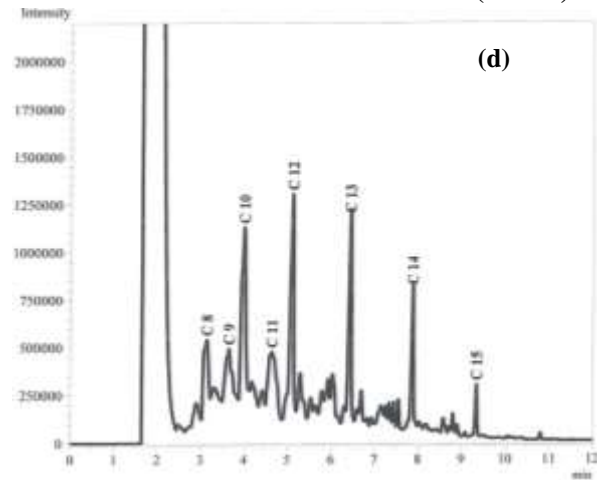
GC curve TPH concentration at initial concentration in Sand 1% with air 0 (L/min)



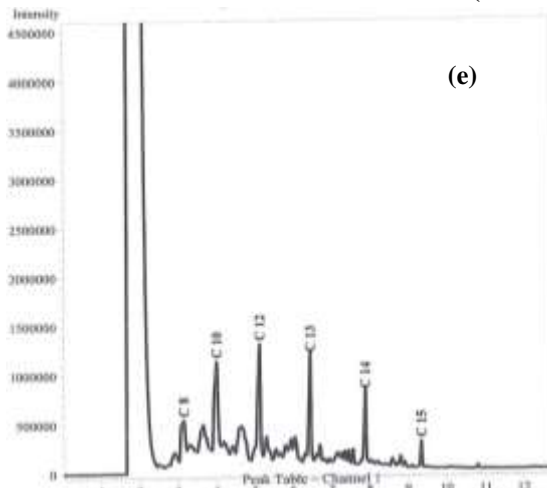
GC curve TPH concentration at initial concentration in Sand 6% with air 0 (L/min)



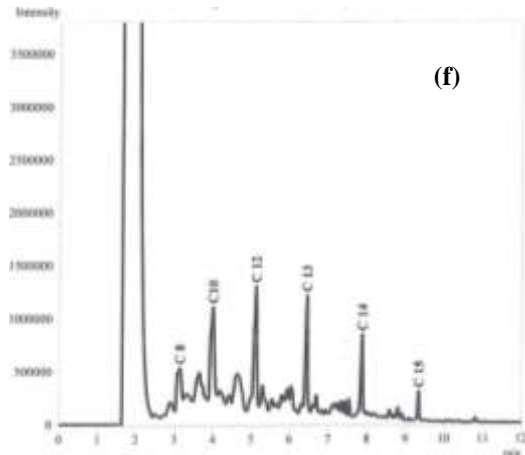
GC curve TPH concentration at Final Concentration in Sand 1% with air 0 (L/min)



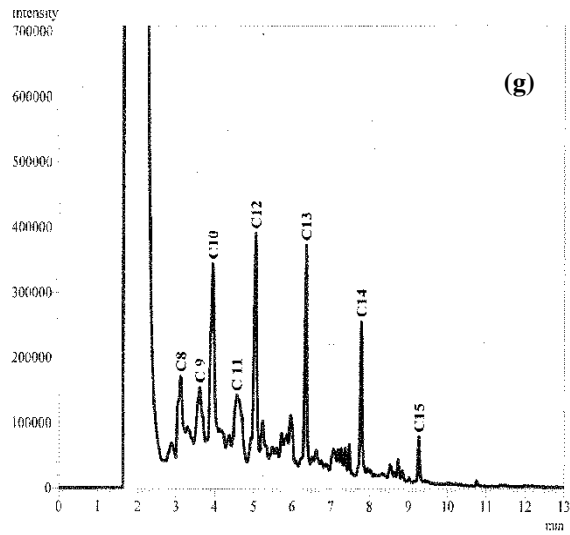
GC curve TPH concentration at Final Concentration in Sand 6% with air 0 (L/min)



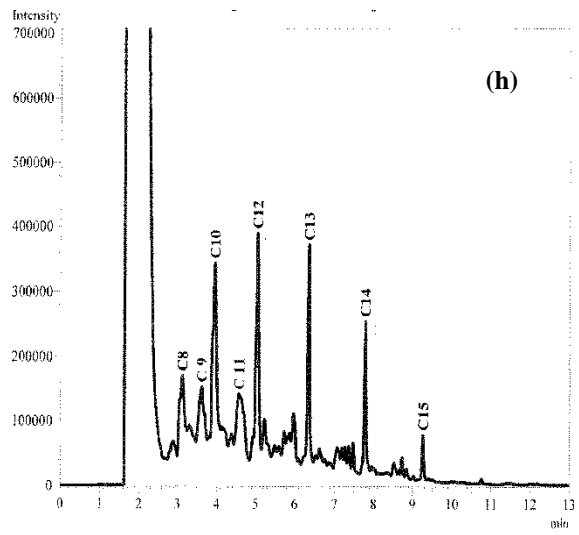
GC curve TPH concentration at Final Concentration in Plant 1% with air 0 (L/min)



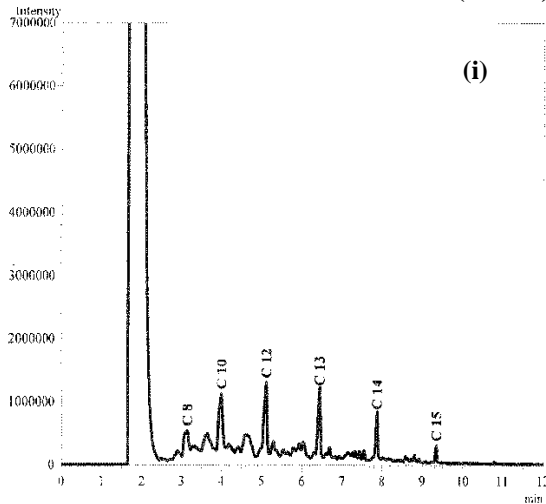
GC curve TPH concentration at Final Concentration in Plant 6% air 0 (L/min)



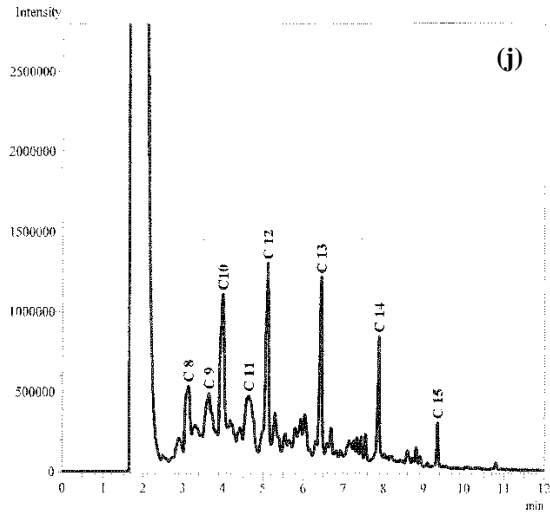
GC curve TPH concentration at initial concentration in Sand 1% with air 1 (L/min)



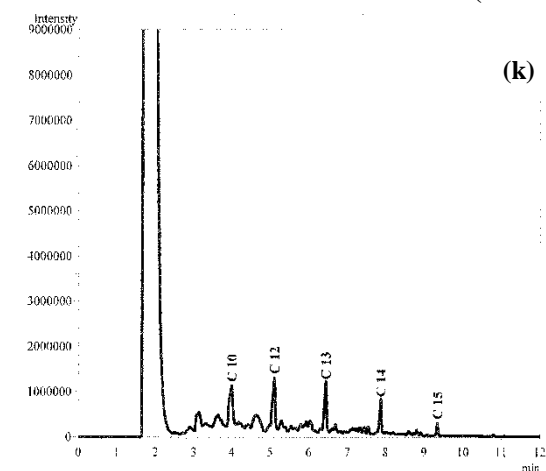
GC curve TPH concentration at initial concentration in Sand 6% with air 1 (L/min)



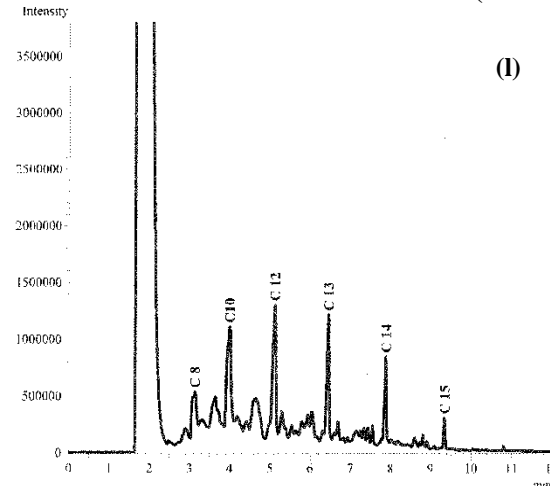
GC curve TPH concentration at Final Concentration in Sand 1% with air 1(L/min)



GC curve TPH concentration at Final Concentration in Sand 6% with air 1(L/min)



GC curve TPH concentration at Final Concentration in Plant 1% with air 1(L/min)



GC curve TPH concentration at Final Concentration in Plant 6% air 1(L/min)

Figure 8. GC Curve TPH chromatograms at different concentration of kerosene (1% and 6%)

In this study of phytotoxicity testing on Barley plant was examined. The plant of barley has the ability to remain alive and give excellent conditions for hydrocarbon degradation with air flow (0L / min and 1L / min) at all studied levels of kerosene 1 and 6 percent. This causes plants inhibition because kerosene was attached to plant tissues and roots. The plants quickly died and bacteria flocks developed around the dead remnants of the aquatic plants that enhanced the kerosene degradation. The highest TPH removal of 95.7% was obtained at a kerosene concentration of 1% and aeration rate 1L/min that was caused by physical, chemical and biological process.

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