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Cassia glauca is a plant that can be used to clean up hydrocarbon pollution

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ABSTRACT

The aim of this study is to see if phytoremediation can treat soil that has been contaminated with crude oil, and using *Cassia glauca*, which was grown in industrially polluted soil, to do so with four crude oil concentrations: 25, 50, 75, and 100 g kg^{-1}. 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 measurement, were also included in the study. In addition, sixteen polycyclic aromatic hydrocarbons (PAHs) were identified. The pollutants recognized by the US Environmental Protection Agency included 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 *Cassia glauca*, 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).

Key words: Phytoremediation, *Cassia glauca*, Accumulation, HPLC analysis, Poly aromatic Hydrocarbons (PAH).
Article type: Research Article.

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 (Shahsavari *et al*. 2013), A major environmental hazard in today's globe is due to the widespread usage of petroleum products (Cheng *et al*. 2010). Saturated hydrocarbons, aromatic hydrocarbons, resins, and asphaltenes are the most common petroleum chemicals (Liu *et al.* 2014) . 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 (Cai *et al.* 2010; Wang *et al.* 2012). Toxic chemicals generated from petroleum pose a threat to the health of humans *(Anyika et al.* 2015; Han *et al.* 2016). 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 (Hoffman *et al.* 2019). To expedite the breakdown and removal of contaminants from polluted soil or groundwater, a biological approach known as phytoremediation is used (Kamath *et al.* 2004). Eliminating or making harmless environmental toxins by bioremediation (phytoremediation) is becoming more popular (Cunningham & Berti 1993). 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 (Dixit & Dhankher 2011).

MATERIALS AND METHODS

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).

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

3- Adding crude oil to soil with no contamination: Four different concentrations of total petroleum hydrocarbon (TPH) were used in the experiments: 25, 50, 75, and 100 g kg^{-1} soil, with 0 g kg^{-1} 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.

4-An examination of the ground soil properties: Both physical and chemical.

5- Design of the experiment: *Cassia glauca* was chosen and grown in soil contaminated with various concentrations of crude oil (25, 50, 75 and 100 g kg^{-1}) 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.

6. The plant: is shown in Table 1**.**

Table 1. The plant used in the present study.

Properties	***acacia***
Family	***Leguminosae***
Scientific name	***Cassia glauca***
Life form	Evergreen, fast growing, with a small size, its length and weight are about 7 m and 3 m respectively. Its fruit length and width range are 7-10 cm and 1-1.5 cm respectively.

7- The plant's phenotypic characteristics

A-The length of the stem: The stem length was measured (in cm) from the soil surface to the top of the stem, as directed by the source (Ogbo *et al.* 2010).

B- The length of the root: According to the source, the root's length (in cm) was measured from its point of attachment to the plant to the end of its expansion in the soil (Ogbo *et al.* 2010).

C – 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 (Ogbo *et al.* 2010).

D – Dry mass: The plant samples were dried for 72 h at 75 °C in a drying oven, afterward were analyzed (Ogbo *et al.* 2010).

8- *C. glauca* biochemical measurements

A-Total chlorophyll: According to Porra (2002) , total chlorophyll was extracted with 80% acetone by squishing 0.5 g 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.

B- Super oxide dismutase (SOD): The activity of superoxide dismutase (SOD) was tested in fresh plant leaves (Marklund & Marklund, 1974).

C- The activity of catalase (CAT): was determined using the method described by Aebi (1974).

9-Plant extraction

1- Five grams fresh plant were homogenized in 25 mL 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 min before being fully soaked for 10 minutes. Afterward, it was shaken for 20 min in a spinning shaker.
3- Ultrasonic extraction at 4 ℃ for 30 min.
4- Anhydrous sodium sulfate (7.50 g) and 1.50 g sodium chloride (Na_2SO_4: NaCl, mass ratio 5:1) were added, and the mixture was vortex mixed and centrifuged for 10 min at 4 ℃ by 8874 g.
5- Using vacuum, the top organic phase was removed and concentrated for 1 mL.
6- A 0.45 µm filter was used to filter the concentration. The sample was then ready for HPLC analysis.

RESULTS AND DISCUSSION

Table 1. Concentrations of PAHs (µg g^{-1}) in *Cassia glauca* after one month at 25°C.

Sample	PAHs	100 g kg^{-1}	75 g kg^{-1}
1	Naphthalene	N.D.	N.D.
2	Acenaphthylene	3.3271507	1.6760009
3	Acenaphthene	0.58471632	0.13150836
4	Fluorene	N.D.	N.D.
5	Phenanthrene	0.06272676	0.0248643
6	Anthracene	0.1429641	0.0642347
7	Fluoranthene	1.34508432	0.34628976
8	Pyrene	0.1988551	0.1577249
9	Benz[*a*]anthracene	0.05476512	0.027432
10	Chrysene	2.63497486	0.8584841
11	Benzo[*b*]fluoranthene	0.0953425	0.0339967
12	Benzo[*k*]fluoranthene	0.05367882	0.01742292
13	Benzo[*a*]pyrene	0.0874074	0.0667518
14	Dibenz[*a*,*h*]anthracene	N.D.	N.D.
15	Benzo[*ghi*]perylene	N.D.	N.D.
16	Indeno[1,2,3-*cd*]pyrene	0.0467419	0.0229621

Note: *N.D. = Not Detectable

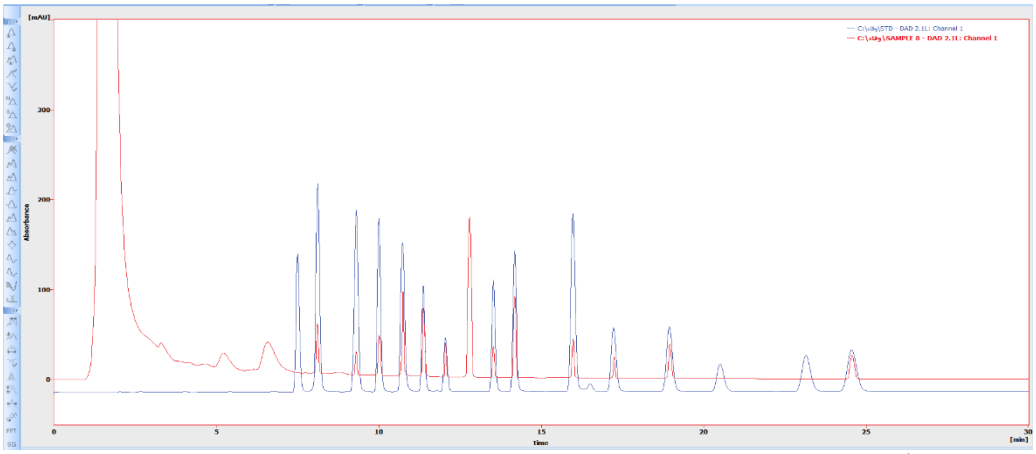


Fig. 1. After one-month growth in soil contaminated with crude oil at a concentration of 75 g kg^{-1}, HPLC measurement of accumulated polyaromatic hydrocarbons in *Acacia glauca* (mg g^{-1}).

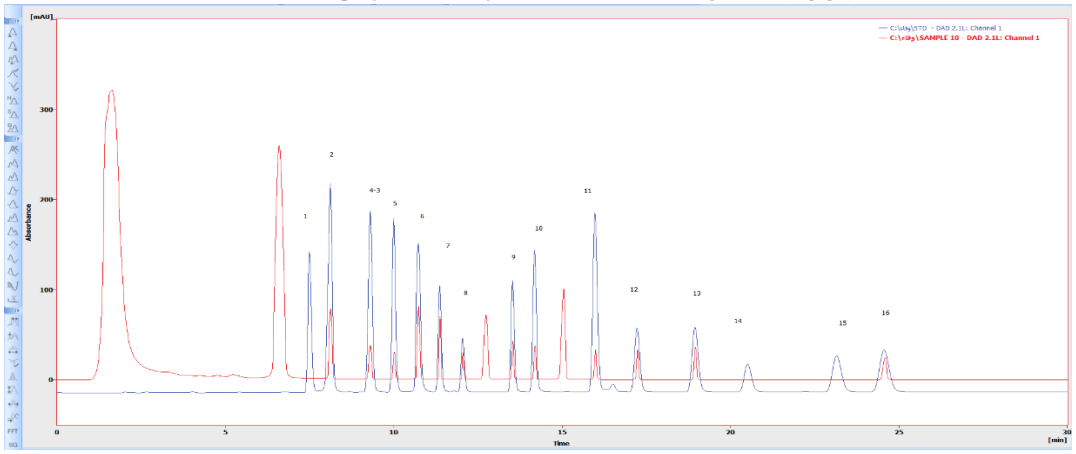


Fig. 2. After one-month growth in soil contaminated with crude oil at a concentration of 100 g kg^{-1}, HPLC measurement of accumulated polyaromatic hydrocarbons in *Acacia glauca* (mg g^{-1}).

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 (Arun *et al.* 2011). The results indicated the accumulation of some polycyclic aromatic hydrocarbons in the plant at different rates. Since the accumulation value depended on the concentration of hydrocarbons, so the highest accumulation value was 100 g kg^{-1}, followed by 75 g kg^{-1} after 30 days from the start of the experiment, consistent with what results obtained by Chen *et al.* (2004) who reported that the uptake of hydrocarbons by 12 different types of plants cultivated in polluted soil is dependent on the hydrocarbon concentrations in the soil. Since 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 (Greenberg *et al.* 2006). 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 (Shirdam *et al.* 2008).

Chaudhry *et al.* (2005) indicated that all these compounds and organic carbon are used to supply energy for microorganisms in the soil. These plants can tolerate organic pollutants, similar to the current study which indicated that *A. glauca* 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. It also has the highest vapours 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 (CDC 2009). 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 remediation of complex mixtures of PAHs, such as creosote (Van Zuydam 2007). Pickering (1999) goes on to say that PAHs do not accumulate as quickly as other lipophilic chemical molecules like PCBs. Instead they are transformed to more water-soluble forms making them easier to excrete from the body

Biochemical measurements of *C. glauca*

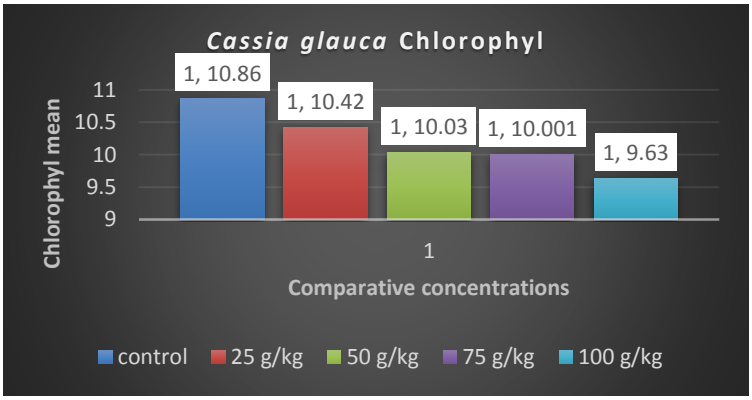


Fig. 4. *Cassia glauca* Chlorophyl.

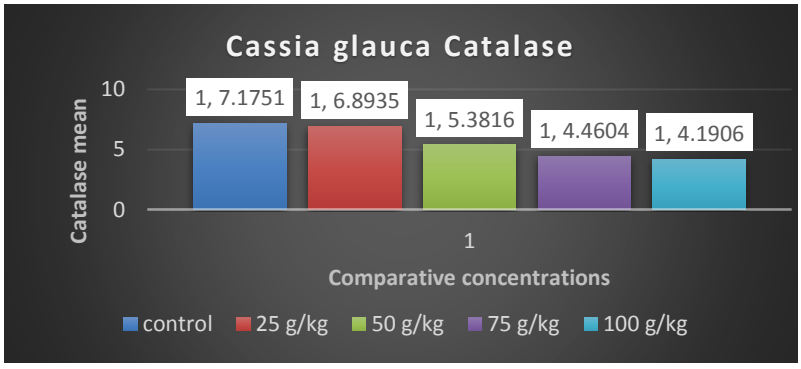


Fig. 5. *Cassia glauca* catalase.

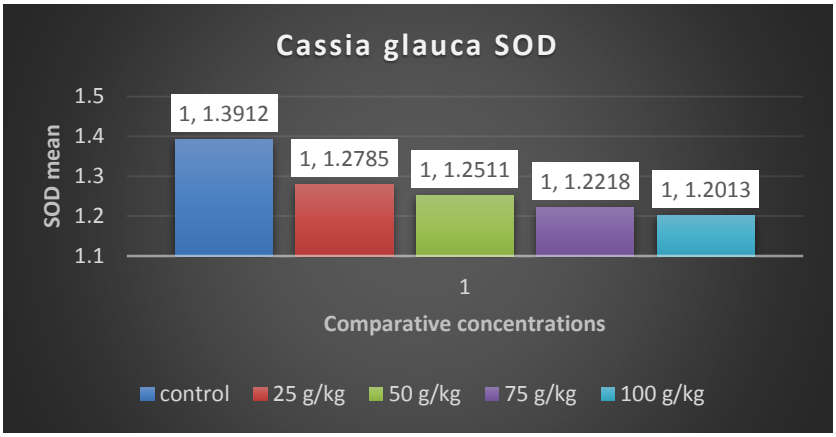


Fig. 6. *Cassia glauca* SOD.

Results of biochemical tests

A. glauca cultivated in crude oil polluted soil exhibited significant decrease in total chlorophyll content and catalase enzyme in comparison with control. A significant drop was observed in super oxide dismutase (SOD) in comparison with control, as shown in Figs. 4, 5 and 6. Plant development in crude oil-contaminated soil has a considerable negative impact on the production of chlorophyll pigments (Baruah *et al.* 2014). Chlorophyll synthesis in plants may be inhibited by crude oil contamination of the soil. The alkaline state induced by the dissolving the compounds in the oil inside the cell sap that were responsible for the breakdown of chlorophyll. It is most likely to blame for the decrease in total chlorophyll content in the leaves (Anthony 2001). Crude oil is a mixture of compounds that inhibit enzymes needed for chlorophyll formation while also lowering leaf area (Al-Hawas *et al.* 2012). Contamination with crude oil creates stress conditions that have a variety of consequences on plants, including oxidative stress caused by an abundance of reactive oxygen species (ROS) (Rao *et al.* 2006). 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 (Desikan *et al.* 2005) . Oxidative stress is caused by the creation of reactive oxygen species (ROS), which causes oxidative damage to proteins, DNA, and lipids (Gill & Tuteja 2010). Plants use antioxidant enzymes like catalase and superoxide dismutase to protect cells and cellular subsystems from the harmful effects of these active oxygen radicals (Fu & Huang 2001). During stress, catalase is vital for H_2O_2 elimination, since it catalyses H_2O_2 dissociation events in the detoxification of H_2O, O_2, and ROS (Gill & Tuteja 2010). The first enzyme for detoxification activities, superoxide dismutase, is a metallic enzyme that catalyses the conversion of an O_2 radical into H_2O_2 and O_2. Dekock *et al.* (1960) 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 *A. glauca* grown in crude oil-contaminated soil revealed a considerable reduction in superoxide dismutase content, which is consistent with what was discovered by Yordanova *et al.* (2004). The effect of immersion of barley (*Hordeum vulgare* L. 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 photooxidative damage to barley leaves as a result of root oxygen deprivation.

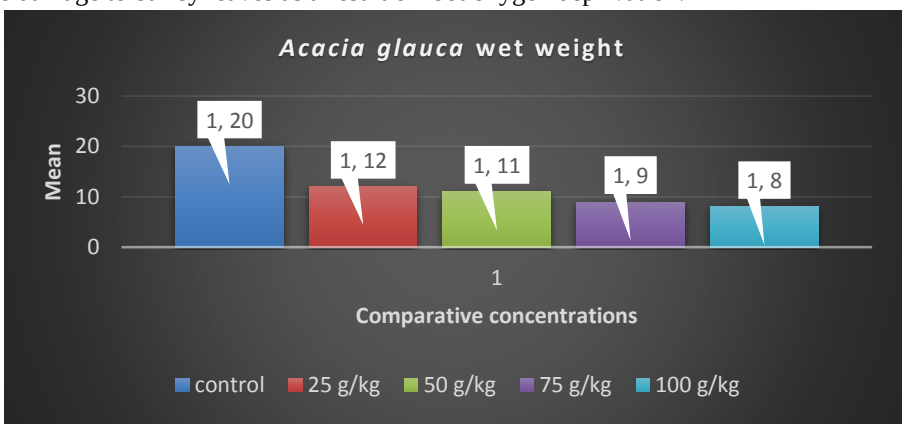


Fig. 7. Wet weight of *A. glauca*.

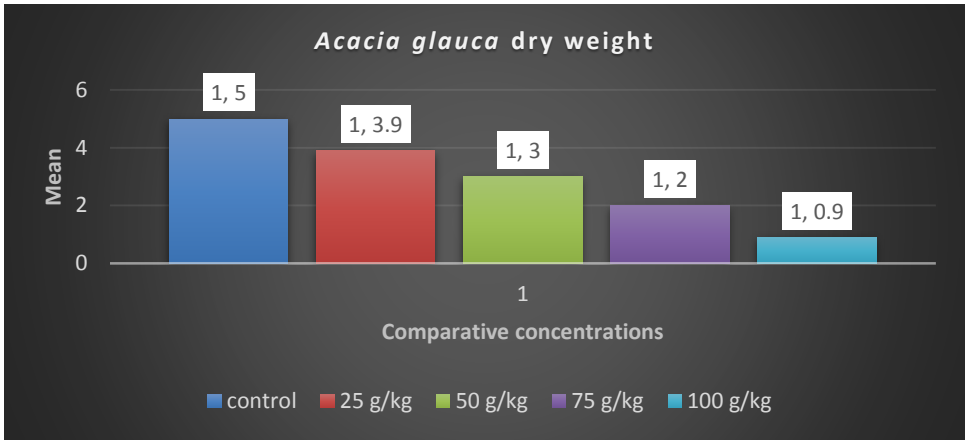


Fig. 8. Dry weight of *A. glauca*.

A. glauca, which grew in soils high in hydrocarbons, exhibited the lowest weight, since it was discovered that the hydrocarbons had a toxic effect on the plant biomass, affecting the photosynthesis process and decreasing the plant's food production, whereas the plants that grew in control pots, displayed the highest weight. The absence of hydrocarbons accounts for the majority of the weight, which is consistent with the conclusion obtained by Reilley *et al*. (1996), who explained that PAHs have indirect damage to plants, since they affect the abundance of water and nutrients for plants that grow in soil contaminated with PAHs, leading to a drop in biomass. Kisic *et al.* (2009) indicated that the oil pollutants showed a negative impact on the biomass of the plant, and Tran *et al.* (2018) reported that the biomass of the plant decreases by the elevation in the oil pollutants, in accordance with the present study.

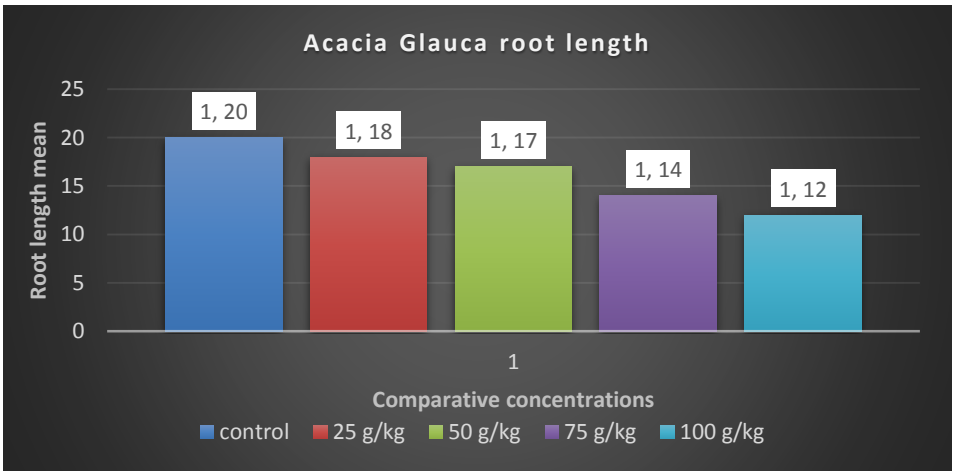
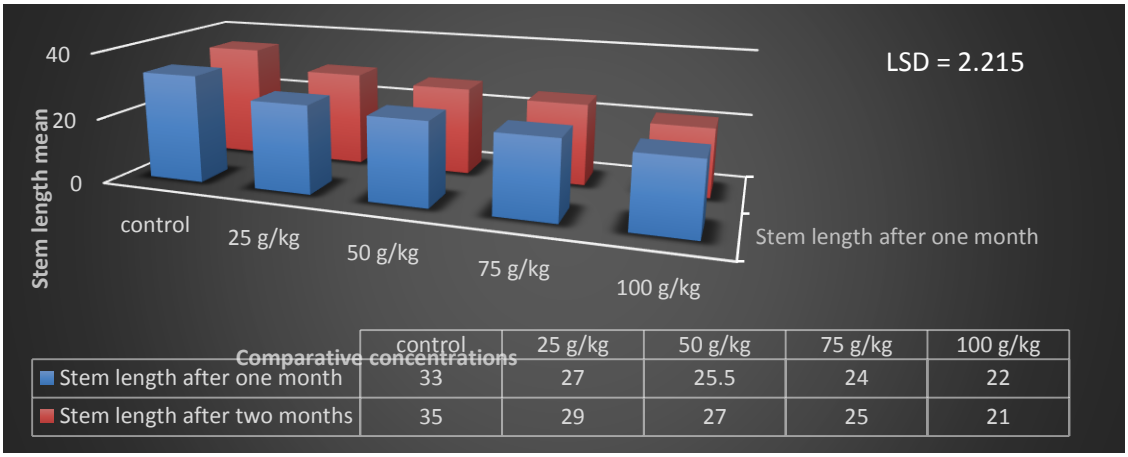


Fig. 9. *A. glauca* root length.



Comparative concentrations	control	25 g/kg	50 g/kg	75 g/kg	100 g/kg
Stem length after one month	33	27	25.5	24	22
Stem length after two months	35	29	27	25	21

Fig. 10. *A. glauca* stem length.

The root length of the *A. glauca* 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, consistent with results found by Visioli *et al.* (2016) who showed that the toxic effect of hydrocarbons had a severe effect. It is also in agreement with the findings of Njoku *et al.* (2009) who found that crude oil inhibits root growth by preventing water from entering the roots and thus reducing water absorption. Thompson *et al.* (2008) explained that the acute toxicity of petroleum hydrocarbons led to the inhibition of plant growth, especially the growth of the root group. According to the results of the current study, the petroleum hydrocarbons exhibited an effect on the plant. It is evident along the stem length of *A. glauca*, as the stem length in the control pots was longer than the other pots, so that, it was found that the stem length is inversely proportional to the concentration of hydrocarbons, consistent with Kabata-Pendias & Sztek (2015). 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. Kuo *et al.* (2014) pointed out that when oil concentrations are 10 g kg^{-1} or greater in soils contaminated with oil, the stem length drops and shortens, which suits our results, since the concentrations were higher.

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