Plant Archives Volume 20 No. 2, 2020 pp. 4913-4921 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

PHYTOREMEDIATION OF SANDY SOIL CONTAMINATED WITH CRUDE OIL BY *PARKINSONIA ACULEATA* L. AND SPRAYING GLUTATHIONE

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Abstract

This study dealt with the phytoremediation of sandy soil contaminated with crude oil with four levels of oil pollution (0, 20000, 40000, 60000 $mgkg^{-1}$) to know the efficiency of phytoremediation of *Parkinsonia aculeata* L. The plants were sprayed with glutathione at concentrations of (0 , 100 mgl^{-1}), The experimental measurements were taken 9 months after planting in the polluted soil. Results indicated that the plant tolerates the crude oil and showed a decrease in the characteristics of vegetative growth, such as plant height, stem diameter, number of leaves and leaf area, as well as decreased biochemical characteristics such as the leaf content of total chlorophyll, carbohydrates and glutathione GSH. As for enzymatic activity for catalase CAT, peroxidase POD and glutathione peroxidase GPX increased by increasing the concentration of crude oil in the soil, with the exception of the activity of the enzyme catalase which was decreased. The results showed that the sprayed plants with glutathione were highly tolerance of crude oil when compared to the non-sprayed plants. The plant showed the highest crude oil removal rate with a concentration of 20000 $mgkg^{-1}$ with GSH sprayed, the removal was 55.5%.

Key words: Phytoremediation, crude oil, Glutathione, Rhizodegradation.

Introduction

The features of nature have changed in large areas of the world due to the organic and inorganic pollutants released into the environment (Rajakaruna *et al*., 2006). Environmental pollution with crude oil may occur due to exploration, production, transportation, storage and refining operations, as well as oil marketing operations (Oberdorster and Cheek, 2000) and Because of human activities, whether intentionally or not, such as improper management of oil waste leaked from pipelines (Oyibo, 2013). Iraq is one of the most important oil-exporting countries in the world and the production and export of oil is one of the most important resources of Iraq, which depends mostly on the annual budget, as 95% of the total financial budget depends on crude oil production in 2014 (Faucon *et al.*, 2014). Crude oil consists of a complex mixture of aliphatic and aromatic hydrocarbon and heterocyclic hydrocarbons (Falkova *et al*., 2016). Pollution affects essential components of the biosphere such as water, air quality, soil fertility, forests, biodiversity, climate changes, and the spread of pests and diseases (Landrigan *et al*., 2018). The most environmentally important compounds are polycyclic aromatic hydrocarbons (PAHs) (Haritash and Kaushik 2009). There are several techniques for treating the environment contaminated with crude oil and choosing the most appropriate technique is crucial to reduce the negative effects these techniques are chemical, physical and biological methods have been developed to clean the soil (Moreira *et al*., 2011). These different physical and chemical mechanisms are usually of great multiplier effects due to their management costs and the harmful changes they cause to the physical and chemical properties of the soil (Joner *et al*., 2002). Phytoremediation is the use of green plants to clean contaminated sites, which is an environmentally friendly, less expensive and also simpler method compared to other technologies (Joner *et al*., 2002). Phytoremediation is a unique treatment strategy (Kvesitadze *et al*., 2006). It has an advantage over chemical and physical technologies in decontamination because it is based on biodegradation oil to treat hazardous pollutants and not harm the environment (Zhang *et al*., 2011). There are five

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techniques of the phytoremediation, Phytoextraction is the absorption of pollutants (mostly heavy metals) by plant roots and accumulate them in the shoot followed by plant harvesting and safe disposal of it (Mahar *et al*., 2016). Phytostabilization is the use of some types of plants to paralyze the movement of pollutants in the soil and groundwater by accumulating pollutants in the roots and adsorption to the roots or sedimentation in the root zone (Ghosh and Singh, 2005). Phytofiltration is mainly the use of aquatic plants to absorb heavy metals and other pollutants from aquatic environments, Phytovolatilization is the use of plants to absorb toxic elements and then convert and release them to less toxic forms into the air (Rajakaruna *et al.*, 2006). Phytodegradation is the absorption of pollutants by the plant and degrading it through metabolic processes in plant cells or the degradation of pollutants outside the plant through the effect of the compounds that the plant produces, such as, enzymes (Mukhopadhysy and Maiti, 2010). Rhizodegradation is the degradation of soil pollutants through the activity of microorganisms in the rhizosphere in the soil (Germida *et al*., 2002). Glutathion is a component of plant and animal cells and acts as an antioxidant, it is the most abundant form of organic sulfur in plants. There are two forms of glutathione, oxidized (GSSG) and reduced form (GSH) (Tandogan and Ulusu, 2006). It contains a thiol group (- SH) acts as a reducing nucleophilic undergo oxidation. This leads to the formation of oxidized glutathione (GSSG) (Eshdat *et al*., 1997).

Materials and Methods

The experiment was carried out at the College of Agriculture/Basra University for the period from September 2018 to June 2019, using the *Parkinsonia aculeata* L., a tree belonging to the Fabaceae family, which is a local tree and that suits the climatic conditions of Basra governorate.

Soil preparation

A sandy soil was used, brought from southwest of Basra, There are some physical and chemical properties in the table 1. spread the soil under the sunlight until it was completely dried and sifted the soil using a sieve with a diameter of 2 mm and took the weight of 10 kg of soil and put it in a container and contaminated with four levels of pollution with crude oil (0, 20000, 40000, 60000) $mgKg^{-1}$, the soil was mixed with the crude oil well and put in pots with a diameter of 30 cm and a depth of 35 cm. Each pot contains 10 kg of soil contaminated with crude oil and prepared for planting.

Field experiment

The seeds were planted in plastic plates with 200 eyes with dimensions of 2×2×5 cm and after the seedlings reached a height of 10 cm they were transferred to pots containing soil contaminated with crude oil. Filtered water was used in irrigation, especially crude oil. Coverage was used with an open plastic cover on both sides in the winter to keep the crude oil from washing because of the rain. Two concentrations of glutathione were used (0, 100) mgL^{-1}. Plants were sprayed with glutathione every month until the end of the experiment. The experimental measurements were taken 9 months after planting trees in the polluted soil.

Statistical analysis

GenStat discovery edition 3 was used for of the statistical the results evaluation. The experiment was designed as completely randomized with three replicates for each treatment. The result was analysed using the analysis of variance (ANOVA). The differences between means were compared by LSD range at a significance level of $p < 0.05$.

Plant measurement

Vegetative growth

Experimental measurements were taken of three randomly replicates for vegetative growth which were measured by measuring the height of the plant (cm) Using the measurement metal tape and the main stem diameter by feet Vernier caliper (mm), the number of leaves (leaf/plant) and leaf area (m^2 /plant) and the area was measured

Table 1: Some physical and chemical properties of the soil.

Parameters	Value
pH	7.63
Electrical conductivity (EC) dSm^{-1}	2.73
Total nitrogen $mgkg^{-1}$	30
Total phosphorus $mgkg^{-1}$	1.6
Total potassium $mgkg^{-1}$	13.12
Sand %	88.18
Silt %	6.52
Clay %	5.3
Soil Textural	Sandy

Table 2: Description of treatments.

Treatments		code
Conc. Crude oil	Conc. GSH	
0 $mg\ kg^{-1}$	0 $mg\ l^{-1}$	T1
20 000 $mg\ kg^{-1}$	0 $mg\ l^{-1}$	T2
40 000 $mg\ kg^{-1}$	0 $mg\ l^{-1}$	T3
60 000 $mg\ kg^{-1}$	0 $mg\ l^{-1}$	T4
0 $mg\ kg^{-1}$	100 $mg\ l^{-1}$	TG1
20 000 $mg\ kg^{-1}$	100 $mg\ l^{-1}$	TG2
40 000 $mg\ kg^{-1}$	100 $mg\ l^{-1}$	TG3
60 000 $mg\ kg^{-1}$	100 $mg\ l^{-1}$	TG4

by Paper based Image Image software according to the method used by Darwish *et al*. (2014).

Chemical characteristics

Chlorophyll pigment

Total chlorophyll (mgg^{-1}) was measured according to the method of Zaehringer *et al*., (1974) and measured by a Spectrophotometer at two wavelengths (645 and 663) nm.

Carbohydrates

Total soluble carbohydrates in the leaves were estimated using the phenol-sulfuric acid, modified method according to the method of Duboies *et al*., (1956).

Glutathione

Determination of leaf content of glutathione was achieved according to Moron *et al*., (1979) ,glutathione interacts with (DTNB) 5, 5'-dithiobis-2-nitrobenzoic acid, measured by the Spectrophotometer at 412 nm.

Enzymic activity determination

Extract

Plant leaf samples were Extracted according to Luhova *et al*., (2003), in which plant leaf was crushed by 0.1 M phosphate buffer, pH 7, 1:2 (W:V). The extract was filtered through nylon cloth and centrifuged at 12,000 for 30 minutes at 4°C.

Catalase (CAT)

The modified method of Goth (1991) was used to estimate the enzymatic activity of CAT, 0.2 ml of plant sample extract was incubated with 1 ml of substrate (65 µmol per ml hydrogen peroxide in 60 mmol/l sodium-potassium phosphate buffer, pH 7.4) for 4 minutes at about 25^0C, the reaction was stopped by adding 1 ml of ammonium molybdate (32.4 mM) then measured at 405 nm.

Peroxidase (POD)

The method of Angelini *et al*., (1990) was used in estimating the enzymatic activity of peroxidase (POD) using guaiacol, 1 ml of 5 mM guaiacol and 1 ml of 2 mM hydrogen peroxide and 1 ml of 0.1M, phosphate buffer, pH 7 was added to 0.1 ml of the sample extract and then measured at 436 nm for 3 minutes, the reading was recorded every 30 seconds at 30^0C.

Glutathione peroxidase (GPX)

The Flohe and Gunzler (1984) method was applied to estimate (GPX), 0.3 ml of sample extract was put in test tubes and these chemical were added 0.3 ml of 0.1 M phosphate buffer pH 7.4 and 0.2 ml of 0.002 M reduced glutathione and 0.1 ml of 0.010 M sodium azide With 0.1 ml of 0.03 M hydrogen peroxide. Test tubes were placed in a water bath at 37^0C for 15 minutes. 0.3 ml of 5% Trichloroacetic acid (TCA) was added, then the tubes were cooled in an ice bath and placed in a centrifuge at 1500 rpm for 5 minutes, measured at 420 nm.

Determination of total petroleum hydrocarbons in soil (TPHs)

Miles *et al*., (1977) method was adopted to estimate the total petroleum hydrocarbons (TPHs) remaining after remediate. 10 g of dry soil and put in a 250 ml flask and 50 ml of hexane was added, the flask put it in the Rotator for two hours. The extract was passed through filter paper and through a separating column containing glass wool and anhydrous sodium sulfate, then the extract was evaporated and the residual re-dissolved by hexane and measured by Fluorescence Spectrophotometer at 310 nm.

Results

The results in table 3 included the effect of different concentrations of crude oil on the height of the plant. The results showed a significant decrease in plant height recorded by treatment T4 (57.33 cm) as compared to that of T1 (control) 100.67 cm and treatments T2, T3 (89.67, 75.67 cm) respectively and also significantly decreased as compared to the treatment TG4 which was 67.67 cm.

It was also observed in table 3 that stem diameter decreased with the increase of crude oil concentration in the soil. T4 showed the lowest significant stem diameter (5.1 mm) as compared to the control and other treatments. (T4) decreased stem diameter significantly as compared to the treatment TG4 (6.3 mm) which was lower than the control treatment. TG1 and treatments TG2, TG3 which were 8.7 and 7.4 mm respectively.

The number of leaves decreased significantly in treatment T4 which was 31.7 leaves as compared to the T1 (control) treatment which was 90 leaves table 3 also as compared to other treatments T2 , T3 (86 , 66.3 leaves) respectively, the same treatment (T4) decreased significantly number of leaves as compared with TG4 which was 52 leaves and all treatments of TG.

The leaf area decreased significantly in treatment T4 which was 238.9 cm^2 as compared to the T1 treatment 825.2 cm^2 table 3 and to treatments T2 , T3 (786.2 , 529.6 cm^2) respectively, while no significant difference was found between T1 (825.2 cm^2) and T2 (786.2 cm^2), Also T4 decreased leaf area significantly as compared to TG4 (284.2 cm^2) which was decreased significantly when compared with other treatments TG1, TG2, TG3.

Total chlorophyll pigment decreased significantly in

T4 treatment (0.931 mgg^{-1}) as compared to the treatment control (T1) that was 1.177 mgg^{-1} table 3 and also T4 decreased significantly as compared with the two treatments T2, T3 which were 1.079, 1.078 mgg^{-1} respectively. Also the same treatment T4 decreased total chlorophyll significantly as compared to the TG4 (1.105 mgg^{-1}). also TG4 decreased total chlorophyll significantly as compared to other treatments TG1, TG2 (1.189, 1.066 $mg.g^{-1}$) respectively.

T4 recorded significant decrease in total soluble carbohydrates (83.51 mgg^{-1}) as compared to the other treatments (T1, T2),and with TG4 which was 93.78 mgg^{-1}. Also TG4 decreased significantly total soluble carbohydrates as compared to TG1 , TG2 , TG3 which were 116.18, 109.33, 103.50 mgg^{-1} respectively table 3.

Results in table 3 showed that GSH content in leaves increased significantly for the TG4 treatment (61.07 $\mu molg^{-1}$) as compared to the treatments TG1, TG2, TG3 which were 46.87, 57.75, 54.19 $\mu molg^{-1}$ respectively. Also GSH increased treatment for T4 (56.95 $\mu molg^{-1}$) as compared to the treatment control (T1)which was 45.38 $\mu molg^{-1}$.

Results in the Fig. 1 indicated a significant decrease in the enzymatic activity of CAT for treatment T4 which was 5.9 Ug^{-1} as compared to treatments T1, T2,T3) 36.6, 20.29, 14.31 Ug^{-1} (respectively. Also the same treatment T4 decreased the activity significantly as compared to the TG4 treatment (12.5 Ug^{-1}) and (TG4) decreased the activity significantly as compared to the TG1, TG2, TG3.

Fig. 2 showed that the enzymatic activity of POD increased significantly for the T4 treatment (73. 4 Ug^{-1}) as compared to other treatments T1, T2, T3 (33.5, 44.0, 54.4 Ug^{-1}) respectively.

As shown in Fig. 3, the enzymatic activity of GPX increased significantly for the TG4 treatment (42.8 Ug^{-1}) as compared to the TG1 treatment (34.78 Ug^{-1}), but no significant difference was found between the treatments TG2 and TG3 (45.63, 47.72 Ug^{-1}) respectively.

The Fig. 4 showed the percentage of removal of crude oil from the soil which was increased significantly for TG2 treatment (55.5%) as compared to TG4 (48.5%). Also the treatment T2 (53.4%) increased the percentage significantly as compared to the T4 treatment (45.3%), while no significant difference was found between the T2 (53.4%) and T3 (51.7%). Also no significant difference was found between the TG2 (55.5%) and TG3 (52.5%).

Discussion

Results showed in table 3 indicated that soil contamination by crude oil led to a decrease in the height of the plant and may be attributed to a change in the chemical properties of oil contaminated soil (Balasubramaniyam and Harvey 2014). Thus, the nutrients decreased with increasing oil concentration in the soil (Odjegba and Atebe 2007) and low available phosphorus and total nitrogen (Benka-Coker and Ekundayo, 1995). The effect of crude oil on the plant depends on both the type of hydrocarbons (molecular weight) and on the total concentration of hydrocarbons in the soil and because hydrocarbons are hydrophobic compounds, they hinder the plant's absorption of water from the soil (Kirk *et al*., 2005). The reason for the increase in plant growth with spraying of glutathione is that it consists of three amino acid, glycin, glutamic, cycteine and these amino acids change the osmotic potential which leads to a reduction in the cell's water potential and thus increases the cell's ability to take up water and dissolved nutrients, and then leads to the increased shoot growth of the plant (Claussen , 2004; Amini and Ehsanpour, 2005). These results are in accord with Ramos *et al*., (2009), Who studied the phytoremediation of contaminated soil with crude oil using *Sebastiania commersoniana*, and also with Merkl *et al*., (2004) for phytoremediation of contaminated soil with

Table 3: Effect of crude oil on some vegetative growth and some biochemical characteristics of plants.

Treat-ment	Height (cm)	Diameter (mm)	Number of leaves	Leaf area (cm^2Plant^{-1})	Total Chlorophyll (mgg^{-1})	Total Carbohydrate (mgg^{-1})	Total Glutathion ($\mu mol\ g^{-1}$)
T1	100.67	8.2	90.0	825.2	1.177	111.74	45.38
T2	89.67	7.8	86.0	786.2	1.079	95.39	53.22
T3	75.67	6.5	66.3	529.6	1.078	89.39	52.05
T4	57.33	5.1	31.7	238.9	0.931	83.51	56.95
TG1	106.33	8.9	90.7	973.7	1.189	116.18	46.87
TG2	98.33	8.7	89.0	897.3	1.066	109.33	57.75
TG3	85.00	7.4	73.0	591.1	1.139	103.50	54.19
TG4	67.67	6.3	52.0	284.2	1.105	93.78	61.07
L.S.D. at $p<0.05$	3.257	0.76	3.180	44.17	0.060	7.072	2.582

crude oil using tropical plants, six types of which are from the legume family. The results showed a significant decrease in the height of the plant. The results showed in table 3 a decrease in the number of leaves, and the growth of plants may be inhibited because the plant absorbs toxic hydrocarbons of small molecular weight that cause damage to the integrity and permeability of cellular membranes (Reis, 1996). Low molecular weight hydrocarbons are more harmful to plants because they are high availability. On the other hand, short-chain compounds with low boiling points (150-275°C) are more dangerous (Jonker *et al*., 2006). The chemical and physical properties of the soil often overlap to determine the level of toxicity of hydrocarbons (Wu *et al*., 2013). The reason for the increased stem diameter may be that some enzymes use glutathione as an auxiliary material, which are small oxidizing and reducing molecules that have a role in the formation of salicylic acid and plant protection signals (Rouhier *et al*., 2008). These results are in accord with Ramos *et al.*, (2009). Also, soil contamination by crude oil led to a decrease in the leaf area, as in table 3. Contamination of soil with crude oil reduces soil aeration by blocking air spaces and thus creates a state of air and causes stress on plant roots, which leads to reduced leaf growth (Shukry *et al*., 2013). Glutathione also scavenges toxic hydrogen peroxide (Noctor and Foyer, 1998). Because glutathione is an antioxidant, it protects cells from breakdown by free radicals and also helps the cells to remain active (Mamdouh, 1995). The results showed a decrease in the leaves content of total chlorophyll as in table 3. The reason for the decrease in the content of chlorophyll in the tissues of the leaves due to the availability of nutrients and environmental stress, such as soil pollution with crude oil (Onwurah *et al*., 2007). Also, crude oil is a mixture of aliphatic and aromatic compounds and is one of the organic compounds with high molecular weights that inhibit the enzymes needed for the synthesis of chlorophyll (Anthony, 2001). This according with Arellano *et al*., (2017) When comparing tropical trees in Amazon regions, it was found that trees have less chlorophyll content in polluted areas compared to non-polluted areas. It was also in accords with Al-Hawas *et al*., (2012) that increasing the concentration of crude oil reduces the leaf content of chlorophyll A, chlorophyll B, carotene and all pigments in the plant. The results showed a decrease in carbohydrates in the leaves as in table 3 because the reduced leaf area in plants due to oil exacerbates the problem in the rate of photosynthesis in the plant, which leads to faltering plant functions (Agbogidi *et al*., 2007). Also, the reduced connectivity of the stomata, which is a mechanism that the plant performs under conditions of drought stress in order to avoid losing more water (Li, 1991). This leads to a decrease in the CO_2 concentration, which results in a decrease in the rate of photosynthesis, which is attributed to a decrease for two reasons, either a decrease in stomatal connecting or a decrease in the effectiveness of mesophyll cells (Farquhar and Sharkey 1982). In environmental stress conditions such as drought and pollution of various kinds, the plant has defensive systems against the harmful effects of this tension on the plant, including enzymes such as CAT, which is necessary for scavenging H_2O_2 produced in peroxisomes due to photorespiration (Noctor *et al*., 2000). Fig. 1 showed a decrease in the CAT enzyme, due to changes in chemical structures and enzymes. It is one of the hypotheses observed in plants growing in soils contaminated with

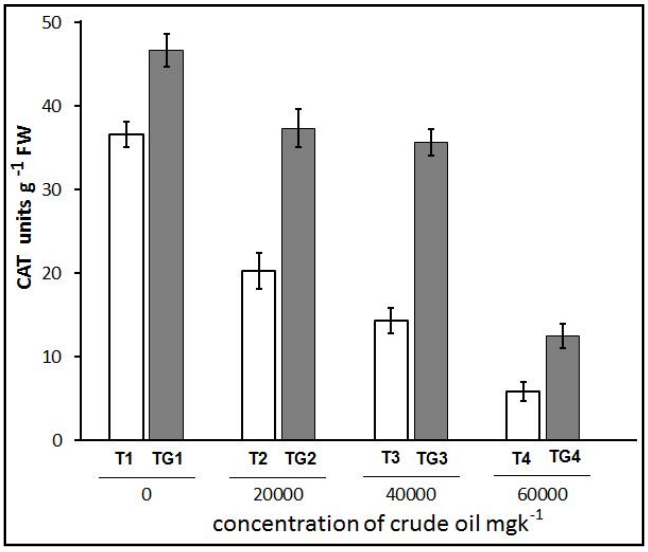

Fig. 1: Effect of crude oil on the CAT enzymatic activity of the leaf.

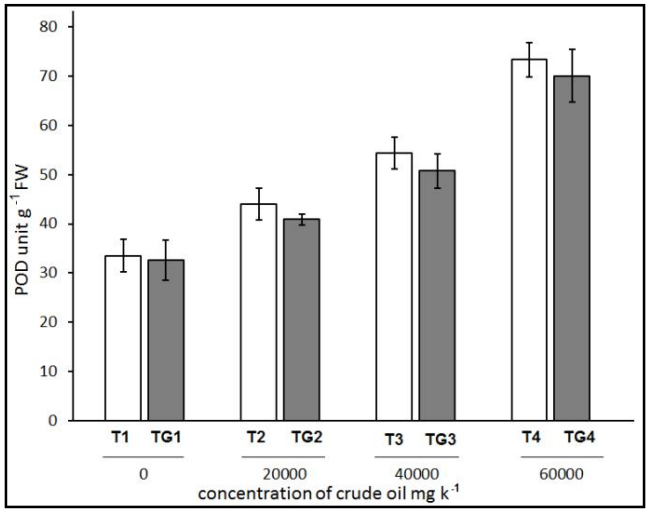

Fig. 2: Effect of crude oil on the POD enzymatic activity of the leaf.

crude oil (Eriyamremu *et al*., 1999). The treatments that sprayed with GSH have an increase in the enzyme activity of the CAT and POD. The reason is that GSH is an antioxidant that protects cells and keeps cells active, so it leads to increased activity of enzymes (Mamdouh, 1995). Fig. 2 showed an increase in the POD enzyme, in accordance with Adeyemi (2020), who observed that oil pollution caused an increase in POD enzymes for *Phaseolus vulgaris* and the reason that soil contaminated with crude oil impeded the availability of water, air and nutrients to plant roots, creating drought conditions that led to oxidative stress on plant. Glutathione has an important role in resisting biotic stress and abiotic stress, and it has a role in the glutathione - ascorbate cycle that reduces toxic hydrogen peroxide (Noctor and Foyer, 1998). Fig. 3 showed increase of enzymic activity of GPX in leaves of *Parkinsonia aculeata* L. due to is abiotic stress conditions (water deficit, metal stresses, and photooxidation), also induce modifications of the Gpx levels in plants (Navrot *et al*., 2006). Glutathione Peroxidase GPX is an antioxidant enzyme that is ubiquitously in plant cells in cytosol, chloroplasts, mitochondria, peroxisome and apoplast, and catalyze the reduction of H_2O_2 and organic and lipid hydroperoxides directly employ GSH and thus protect cells from oxidative damage (Anjum *et al*., 2010). GPX functions provide protection as an antioxidant and salicylic regulator and target free radicals ROS (Chang *et al*., 2009).

Fig. 4 showed the removed of crude oil from the polluted soil and the reason is due to the plant roots secrete a variety of chemicals that can act as biological agents that stimulate metabolism enzymes for microorganisms and increase co-metabolism, which means that the microbial enzyme breaks down organic pollutants (Singer *et al*., 2003). Also, plants have the ability to absorb organic pollutants and induce their metabolism or modify them to less toxic metabolites once the organic chemicals are absorbed and transported under three types of transformations: Chemical modification (oxidation, reduction, hydrolysis, etc.). Conjugation with (carbohydrates, glutathione, amino acids, etc.), compartmentalization in cell wall and vacuoles (Burken, 2003; Kvesitadze *et al*., 2006). Hultgren *et al*., (2009) and Rezek *et al*., (2009) found similar results in their studies to treating PAHs in contaminated soil. Rezek *et al*., (2009) studied phytoremediation of contaminated soil by PAHs using two types of trees *Betula pendula* and *Morus rubra*, the results showed that after one year of treatment, the PAH concentration was removed to 50%. Ramos *et al*., (2009) found similar results in his study to treating crude oil in the soil. Fig. 4 showed an increase in the removal of crude oil from the soil when sprayed with glutathione, the reason for the increased removal of crude oil is that glutathione binds to molecules and then the enzymes bind to glutathione, or perhaps because some enzymes use glutathione as a catalyst in the glutaredxion process (Rouhier *et al*., 2008).

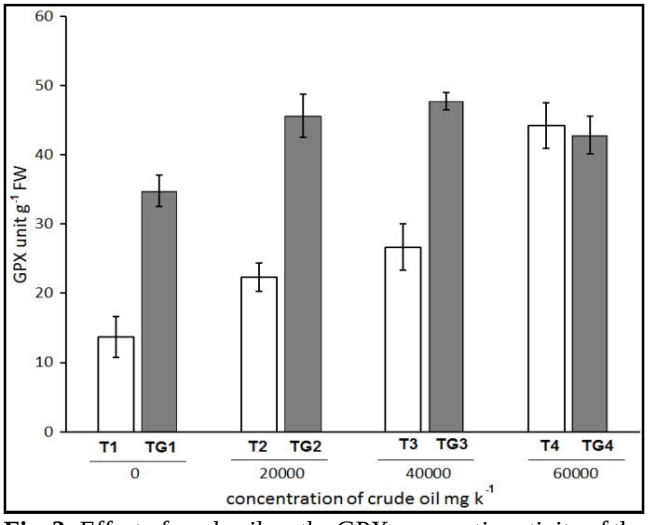

Fig. 3: Effect of crude oil on the GPX enzymatic activity of the leaf.

Conclusion

It is observed in this study that the plant (*Parkinsonia aculeata*) tolerates the different concentrations of crude oil in the soil through measurements of vegetative growth and biochemical characteristics such as chlorophyll pigment and carbohydrates and mechanisms of tolerance and resistance through a system of enzymes that include anti-oxidation enzymes, and it is also observed that the plant has the ability to remove crude oil from the contaminated soil. This ability was enhanced after spraying it with glutathione, and the plant showed greater tolerance to abiotic stress. Also, it showed greater

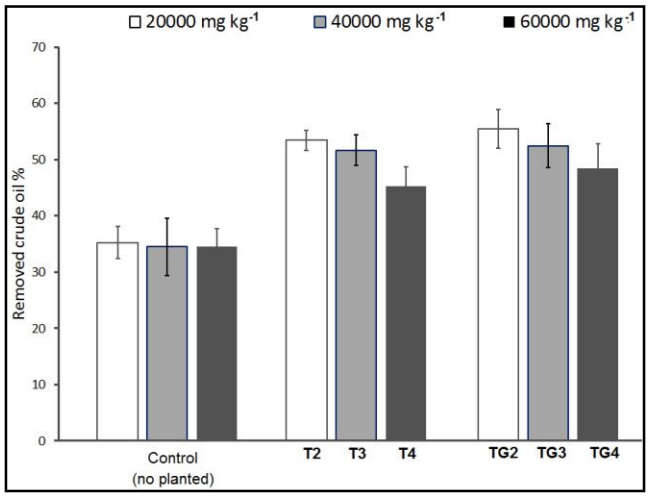

Fig. 4: Removed crude oil in the soil.

removal of crude oil from the soil. It was observed that the plant's content of glutathione increased in the treatments of glutathione spray, and played a role in increasing the plant's efficiency with phytoremediation contaminated sandy soil with crude oil.

Acknowledgment

This study was carried out in the private field of the Medicinal Plants Unit. We appreciate that and for the laboratories of the collage of Agriculture at Basra University.

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