Plant communities and potential native phytoremediator species in petroleum hydrocarbon-polluted desert systems

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Abstract

This paper reported the recovery of desert plant communities after twenty years of oil-derived hydrocarbon contamination in desert habitats of Kuwait, caused by the First Gulf War (1990 – 1991). The hypothesis that certain native desert plant species can tolerate weathered oil-polluted soils with oil breakdown products (i.e., polycyclic aromatic hydrocarbons (PAHs)) and have the potential to function as bioindicators and phytoremediator species for oil-polluted soil was tested. A field survey of 200 quadrat sampling plots at seven hydrocarbon-contaminated and unpolluted desert areas in Kuwait was performed that recorded 42 plant species, with Haloxylon salicornicum, Cyperus conglomeratus and Rhanterium epapposum as the most dominant species. Analysis of plant tissues indicated plant uptake and accumulation of some PAHs. H. salicornicum was used as a representative species in a controlled field study that included growth of plants in hydrocarbon-polluted and unpolluted soils in two separate desert areas under similar growth conditions. Results showed a significant decrease in plant biomass in oil-contaminated soil compared to those from the uncontaminated site. However, the plants appeared green and healthy in both sites, and showed no overt stress. The results suggest that some desert plant communities exhibit signs of recovery after severe oil pollution, and that H. salicornicum may serve as a phytoremediator of oil-contaminated desert soils. Our results also demonstrated that some desert plant communities could be cultivated in oil fields to reduce hydrocarbon contamination and provide guide to other ecosystem services through improving soil quality and biodiversity.

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Running head: Native phytoremediators in oil-polluted desert ecosystems

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Author contributions: SA, LIA conceived and designed the research and carried out the field work and experiment; SA, LIA, VKS, MA, and XM analyzed data, write, and edited the manuscript.

Abstract

This paper reported the recovery of desert plant communities after twenty years of oil-derived hydrocarbon contamination in desert habitats of Kuwait, caused by the First Gulf War (1990 – 1991). The hypothesis that certain native desert plant species can tolerate weathered oil-polluted soils with oil breakdown products (i.e., polycyclic aromatic hydrocarbons (PAHs)) and have the potential to function as bioindicators and phytoremediator species for oil-polluted soil was tested. A field survey of 200 quadrat sampling plots at seven hydrocarbon-contaminated and unpolluted desert areas in Kuwait was performed that recorded 42 plant species, with *Haloxylon salicornicum, Cyperus conglomeratus* and *Rhanterium epapposum* as the most dominant species. Analysis of plant tissues indicated plant uptake and accumulation of some PAHs. *H. salicornicum* was used as a representative species in a controlled field study that included growth of plants in hydrocarbon-polluted and unpolluted soils in two separate desert areas under similar growth conditions. Results showed a significant decrease in plant biomass in oil-contaminated soil compared to those from the uncontaminated site. However, the plants appeared green and healthy in both sites, and showed no overt stress. The results suggest that some desert plant communities exhibit signs of recovery after severe oil pollution, and that *H. salicornicum* may serve as a phytoremediator of oil-contaminated desert soils. Our results also demonstrated that some desert plant communities could be cultivated in oil fields to reduce hydrocarbon contamination and provide guide to other ecosystem services through improving soil quality and biodiversity.

Key words: Phytoremediation; Oil pollution; Desert vegetation; Biodiversity; Polycyclic aromatic hydrocarbons

Implications for Practice:

- Native plant communities in oil-polluted desert ecosystems can be useful indicators of the degree of pollution and stage of recovery of impacted sites.
- The use of native plant species as phytoremediators to assist in restoration of oil-polluted desert ecosystems is desirable because of the ease of obtaining seeds and/or growing of plants prior to transplanting into desired locations.
- If phytoremediator species are preferred for grazing by a wide range of animals such as camels and goats, the protection from grazing is necessary for succeeding in phytoremediation.
- A suitable supply of irrigation water during seedling establishment is necessary unless adequate rainfall can be reliably predicted during the plant establishment period.

Introduction

The use of petroleum hydrocarbons for energy and raw materials in various applications is increasing significantly, leading extensive release of hydrocarbon contaminants to affect air, soil, surface water and groundwater. Phytoremediation has been an appealing technology to clean contaminated soil and water as it does not require extensive capital investment and can enhance soil properties. However, studies rarely explored the efficacy of phytoremediation in one of the world's most polluted desert soils, caused by the deliberate destruction of oilfield installations during the First Gulf War in 1990-1991 (Hirschmann 2005; Omar et al. 2009).

In addition to possible previous land degradation and climate change impacts (Berdugo, et al 2020), the soil was contaminated by oil spillage from 798 sabotaged oil-wells. Moreover, formation water (over 10% salt content) and over 6 billion gallons of seawater (4% salt content) used to extinguish the burning oil

wells flowed from the damaged wells and accumulated in the natural depressions of the landscape. The areas were termed 'oil lakes' (CIC, 2003). The damage resulted in contaminating over 40 million m^3 of soil with oil and salt residues, resulting various types of oil contaminations including wet 'oil lakes' dry 'oil lakes', and oil-contaminated soil piles. Although the crude oil was mostly pumped out (Al-Daher et al., 1998), nonetheless the petroleum penetrated down 30 cm or deeper across large swathes of soil. The Kuwait Environmental Public Authority (KEPA: Beatona, Kuwait Official Environmental Portal, 2013) reported that oil lakes changed the soil texture, killed wildlife, and in some cases seeped into the soil layers reaching the only freshwater aquifer in northern Kuwait. This severely contaminated desert ecosystems continue to this day, and in the worst affected areas, it is thought that the pollution would likely persist for decades (Hirschmann 2005).

Over the years, the crude oil further broke down to smaller molecules such as polycyclic aromatic hydrocarbons (PAHs). Most PAHs are hydrophobic, display high stability, and can persist for decades in the environment (Wagrowski & Hites 1997; Hirschmann 2005). The amplified possibility of biomagnification due to their lipophilic nature increases the chance of persistence and trophic-level transfer in impacted ecosystems.

Plant species have different responses to, and tolerance of, oil contamination. Hence, that plant communities are usually changed by the impacts of such contamination with petroleum hydrocarbon-tolerant species becoming dominant once the initial high-toxic impacts subside to allow re-colonisation. An earlier study (Al-Ateeqi 2010) identified two native plant species growing in oil-polluted soils in Kuwait: *Haloxylon salicornicum* (Amaranthaceae) and*Cyperus conglomeratus* (Cyperaceae). These species, along with a third plant considered as a possible phytoremediator species,*Rhanterium epapposum* (Asteraceae), are widespread in Kuwait (Halwagy & Halwagy 1974; Al-Shehabi & Murphy 2017). The three species are also present in other oil-producing regions of several North African and Middle Eastern countries (www.gbif.org/species/3758958; www.gbif.org/species/2714301; www.gbif.org/species/3110118).

Suitable phytoremediation management procedures require a good understanding of native plant species response to hydrocarbon contamination. Abdullah et al. (2020) assessed the ecosystem resiliency to total petroleum hydrocarbon (TPH) contamination in Kuwait using remote sensing and GIS. This study found that autogenic recovery of native desert plants occurred within a few years as 34% of the TPH contaminated areas were re-colonised with native desert plants. The contamination levels of TPH also changed over time and the variations could be significantly correlated with the soil type, vegetation type, geological substrates, geomorphological features, and annual precipitation. The study shed light on the succession process of vegetation survival and growth over TPH contaminated soils, but did not report the response of specific native desert communities to different TPH contamination at different concentration levels.

The primary goal of the present paper is to test whether indigenous species commonly found in oil-impacted habitats such as *H. salicornicum species* could establish and grow successfully in desert soils contaminated with petroleum hydrocarbons. In our research, we assessed the hypothesis that some native plant communities in Kuwait are able to tolerate conditions in weathered oil-polluted soils containing oil by products (polycyclic aromatic hydrocarbons: PAHs), and that some of their component species may have potential as phytoremediator. The outcome of this study provides a useful low-cost, and ecologically friendly option by delineating a better understanding of utilizing native plant species to assist with clean-up operations in arid regions. These results are of especial importance to the United Nations general assembly declaration in 2019 of its 2021-2030 UN Decade of ecosystem restoration (Willemen et al., 2020).

Methods

Study Area

Kuwait is located at the north eastern part of the Arabian Peninsula, between latitudes 28@30′ and 30@05′ N and longitudes 46@33′ and 48@30′ E, sharing terrestrial borders with the Republic of Iraq to the northwest, and Kingdom of Saudi Arabia to the south. The study was conducted at seven locations in the State of Kuwait covering four hydrocarbon contaminated sites and three uncontaminated sites. The contaminated

sites include Um Al-Aish, Sabriya, Burgan, and Um-Ghadair, and the uncontaminated sites include Bahra, Sabah Al-Ahmad Protected Area, and Um Al-Rros Military Base. The locations of the sampling sites are presented in Fig. 1).

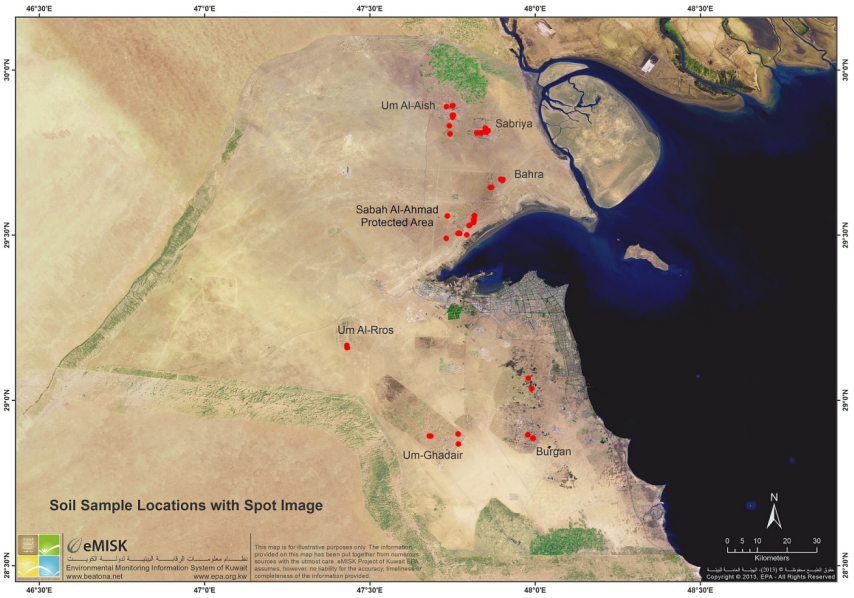

Figure 1. Sampling sites for soil and vegetation survey in Kuwait, 2011 (Base map: (EPA1) 2013).

Methodology

A controlled field experiment was conducted to test the hypothesis following two major approaches. First, we undertook a survey of plant communities and soil conditions in oil-damaged desert habitats in Kuwait. We utilized a multivariate classification approach that has been successfully applied elsewhere in studies of desert plant ecology (e.g., Ali et al. 2000), to determine the vegetation present in relation to quantified total petroleum hydrocarbon (TPH) contents in desert soils. Secondly, we conducted controlled experiments to evaluate the potential to establish native plant species communities on oil-polluted soils, as detailed below.

Plant community and soil PAH pollution survey

Quadrat sampling of desert plant communities (4 m^2quadrat unit size; n = 200 samples) was carried out in 2011 within the seven sampling areas. The selected areas represent a range of geographical locations and intensity of soil PAH pollution throughout Kuwait.

The fieldwork was carried out from January to March 2011. This sampling period was at the end of the rainy season in which the soil seedbank generally germinated after rain events and provided an opportunity to carry out ecological study of desert plant communities (Springuel et al. 1996, 1997; Bainbridge 2007). The number of samples varied at different areas, but each sampling event per area contained at least 10 quadrats. The Universal Transverse Mercator (UTM) geo-coordinates were determined using GPS device (Garmin Etrex: Basic), and minimum of 5 randomly positioned quadrats were sampled around the established sampling site. The percentage frequency of individual species, and mean vegetation height (cm) at each quadrat were collected. The nomenclature of plant species followed the procedure used by Boulos & Al-Dousari (1994). We further confirmed the naming of the species using The Plant List (2013):*www.theplantlist.org.*

Species abundance was assessed using a quadrat-scale frequency method. For each quadrat, the percent frequency of individual species (%F), and the mean vegetation height (cm) were recorded to determine plant

distribution within the selected areas. Species abundance was assessed using the percent frequency at two scales. The first scale was at the sample area scale calculated as:

$$\%FA = 100 \times \frac{\text{number of quadrats with species}}{\text{total number of quadrats sampled per area}}$$

The second scale was at the quadrat scale. for every species present in the sampled quadrat, the $\%F_Q$ was calculated using the following equation:

$$\%F = \frac{number\ of\ hits\ within\ 400\ sub-units}{4}$$

The calculation is based on the fact that each quadrat covers an area of 4 m^2, and the plant species count was conducted by dividing the quadrat into 400 sub-units with 10 x 10 cm in each unit.

Several vegetation parameters were also measured including the mean vegetation height averaged across the quadrat (cm), species diversity as number of species present per quadrat sample ($S_Q m^{-2}$), and number of species per sampling area (S_A). Other variables determined for each sample unit were latitude and longitude (°N; °E), semi-quantitative Oil Damage Score (ODS: on a 1 – 3 scale, with 1 = no visible damage, to 3 = major visible evidence of oil damage to soil), and concentrations of polycyclic aromatic hydrocarbons (PAHs: μg kg^{-1} dry weight of soil or plant tissue) in soil.

Plant leaves and soil samples were collected to assess the degree of soil hydrocarbon pollution, and to determine the uptake of petroleum hydrocarbons by the chosen dominant plant species. A total of 63 plant tissue (leaf) samples, from three oil-polluted areas (Um Al-Aish, Sabriya, and Um-Ghadair Oilfields) and one uncontaminated area (Sabah Al-Ahmad Protected Area) were collected in sealed glass jars (frozen) and subsequently analysed at the Central Environmental Laboratory (CEL) (ISO 17025), Kuwait University. These samples included 32 *H. salicornicum* samples, collected from the Um Al-Aish Oilfield, and 18 specimens of *C. conglomeratus* (16 from the Sabriya Oilfield, one from Sabah Al-Ahmad Protected Area, and one from the Um-Ghadair Oilfield). The other 13 specimens were *R. epapposum* , from the Sabriya Oilfield.

In carrying out the soil sample analysis, 184 soil samples were analyzed at CEL in March 2011, comprising 35 soils from Um Al-Aish, 30 from Sabriya Oilfield, 26 from Bahra, 7 from Um Al-Rros, 45 from Sabah Al-Ahmad Protected Area, 21 from Burgan Oilfield, and 20 from Um-Ghadair Oilfield. The moisture contents of soil and plant tissue samples were determined, and the samples were then analysed for 16 priority PAHs, identified by the US Environmental Protection Agency (US-EPA) in oil polluted soils. These 16 compounds were acenaphthylene (ANAY); acenaphthene (ANA); anthracene (ANTH); benzo(a)anthracene (B[a]ANTH); benzo(a)pyrene (B[a]P); (benzo(g,h,i)perylene (B[ghi]PERY); benzo(b)fluoranthene (B[b]FLAN); benzo(k)fluoranthene (B[k]FLAN); chrysene (CH); dibenzo(a,h)anthracene (D[ah]ANTH); fluoranthene (FLAN); fluorene (FL); indeno(1,2,3-cd) pyrene (I[123-cd]PY); naphthalene (NA); phenanthrene (PH) and pyrene (PY). In this study, these hydrocarbons fractions were used to assess the total Petroleum Hydrocarbon (TPH: mV.sec) content of oil-polluted soils. The concentrations of each compound are reported as μg kg^{-1} of dry soil or dried plant tissue. These compounds were quantified using semivolatiles gas chromatography/mass spectrometry (GC/MS) method (Smith & Lynam 2014).

Survey data analysis

The survey data were analysed using a multivariate approach. Specifically, the polythetic divisive classification procedure Two-Way Indicator Species Analysis (TWINSPAN: Hill 1979; Gauch 1982) was applied to simultaneously group samples, and species present in the samples. This strategy allowed classifying samples based on similar vegetation types and identified species that tend to occur in the same sample, producing a species classification that identifies the main assemblages present in the dataset. Moreover, the analysis

distinguished indicator species which characterize sample-groups produced by the classification (Ali et al. 2000; Al-Shehabi & Murphy 2017).

Then, ANOVA tests with mean separation by Tukey's least significant difference test (significant outcomes only: $P<0.05$) were applied to examine differences between plant and environmental variables, both between geographical areas, and between sets of samples making up sample-groups identified by TWINSPAN classification. Datasets of both the plant-related data, and environmental data were tested for normality (Ryan-Joiner test) and $\log_{10}$-transformed if necessary prior to the use of ANOVA. In cases that the data could not be normalized by transformation, the non-parametric Kruskal-Wallis test was applied instead of ANOVA.

Field experiment: growth of *Haloxylon salicornicum* in oil-polluted soils

A controlled field experiment was carried out in in 2013 with the aim to: (1) determine the tolerance of *H. salicornicum* to desert soil contaminated with petroleum hydrocarbons, (2) assess factors important for growing native plant species in contaminated desert soils such as ease of handling for operational purposes, and (3) determine requirements for environmental amendment(s) to assist plant establishment and growth.

Experimental plots were located at two of the areas where oil-polluted soils were found during an earlier vegetation survey. One site was located in north of Kuwait (Bahra) and the other in south (Burgan). Two-year old *H. salicornicum* plants (grown in a greenhouse of the Public Authority of Agriculture and Fisheries Resources (PAAF) Kuwait) was utilized as a representative native desert plant species. In both locations, plants were grown according to an identical experimental setup that comprised ten randomly located plots (Hurlbert 1984) within a fenced area of weathered contaminated soil (in an old dry oil lake). Three replicates were used per plot. As a control, a second set of plants were similarly planted at a nearby similar-sized fenced area of clean soil at both locations. Plants were planted in a 20-30 cm deep hole (depending on specimen size). Fencing was required to keep out grazing animals, especially camels and goats. Each plot was watered prior to planting, and a 1.0 litre supply of "Driwater" (http://driwater.com/what-is-driwater/) was installed per plot. "Driwater" is a cellulose gum source of water that dissolves at a constant rate and when installed into the soil adjacent to a plant. It releases water to the root system for up to 70 days.

Soil samples were collected from both oil-polluted plots (n = 10 per sampling area) and were analysed using a standard GC analytical procedure, which was carried out at the Kuwait University Laboratory for Bioremediation Research. The field experiment began 31 January 2013 in Bahra and ended 28 March 2013 with 9 field trips to collect data. In Burgan the start date was 5 February 2013, and ended 3 April 2013 after 10 sampling trips. In both cases, sampling was undertaken during 7 - 9 day intervals and plant height was measured to determine incremental growth rate. At the end of the experiment, the plants were carefully removed from the soil and transported to the lab for measuring their biomass (shoot, root and total dry weight per plant). The TPH in plant tissues were quantified using a procedure described above.

Results

The distribution of plants and hydrocarbon contamination

The semi-quantitative Oil Damage Score (ODS), shown in Fig. 2, indicates substantial variations between the examined sites in pollution (Kruskal-Wallis test, $P<0.004$). Um Al-Aish, Sabriya, Burgan, and Um Ghadair oil fields were highly impacted by massive oil pollution, whereas Bahra and Sabah Al-Ahmad Protected Area are less influenced, and Um Al-Rros Military Base was generally unaffected. The variation in hydrocarbon contamination was strongly associated with the geographical occurrence of the three dominant species (*Haloxylon salicornicum, Cyperus conglomeratus,* and *Rhanterium epapposum), seen in* the survey. All three species were found in both hydrocarbon contaminated areas (often in oil-polluted soils) and undamaged areas (Table. 1, Fig 3).

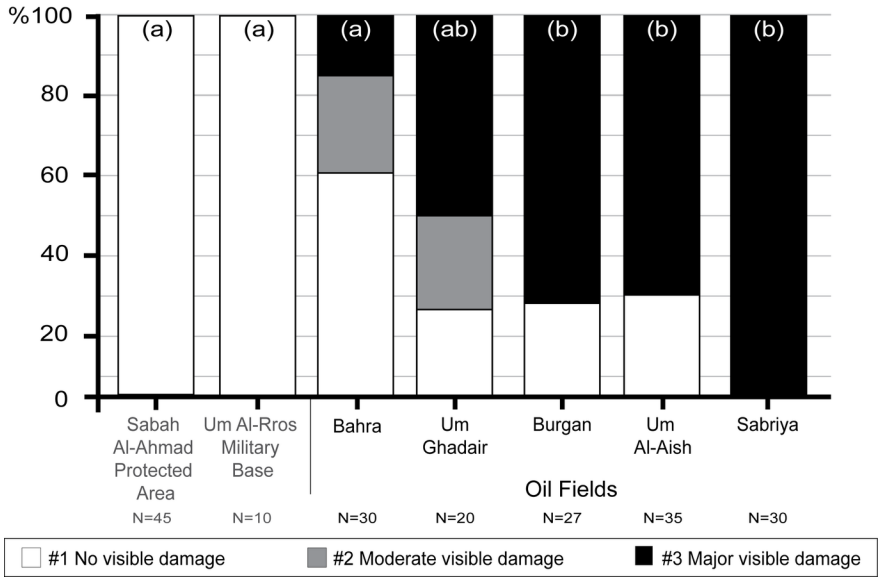

Figure 2: Oil Damage Score (ODS) for quadrat samples collected from the experimental areas. A semi-quantitative Oil Damage Score scale was developed to quantify the degree of damage in all seven sampled areas. N is the number of quadrats per area. The results indicate substantial variations between sites (Kruskal-Wallis test, P<0.004). Bars sharing a lowercase letter are not significantly different from each other.

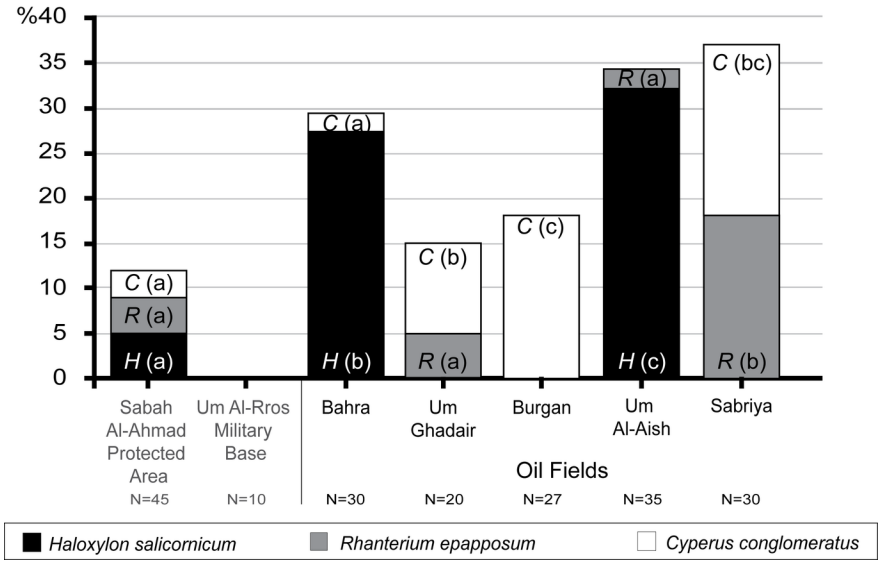

Figure 3: Distribution and abundance (%F) of the dominant species at the sampling areas. Vertical axis shows the proportion of quadrat samples in which each species occurred for each area. The results indicate significant differences in the geographical occurrence of the three dominant species recorded from the field survey (One way ANOVA, P<0.001). Bars, per species, sharing a lowercase letter in common are not significantly different from each other.

Geographical comparison of soil and vegetation variables between sampling areas

Vegetation variables (vegetation height; quadrat-scale plant alpha-diversity, S_Q; abundance of three dominant species: Table.1) showed significant differences between the seven sampling areas, though no consistent pattern between oil-contaminated areas and unaffected areas was apparent. Our survey results suggested that *Haloxylon salicornicum* tended to be abundant at sampling areas located in northern Kuwait, and was absent from southern sampling areas. The other two species occurred in both northern and southern areas and showed high frequency values at individual areas in both regions of Kuwait.

A similar pattern of significant differences in soil moisture (though all values were very low in absolute terms), Table 1, was seen between the sampling areas, again apparently unrelated to the presence or absence of oilfield installations.

In contrast, the TPH and individual PAHs in soil samples from the seven areas showed a significant correlation to the presence of oilfield installations within or close to the sampling areas (Table 1). The semi-quantitative Oil Damage Score (ODS) indicates the severity of oil-pollution in soils of impacted areas (Um Al-Aish, Sabriya, Burgan, and Um Ghadair Oilfields), with greater ODS indicating more severe contamination and lower ODS for the less impacted areas (Bahra, Sabah Al-Ahmad Protected Area) and Um Al-Rros Military Base).

PAH concentration in soils and plant tissue

Out of the 16 PAH component chemicals analysed from soil samples, measurable concentrations were detected for 14 PAHs (Table 1: fluorene (FL) and acenaphthene (ANA) were below levels of detection in all soil samples). There was considerable variation in both the composition and concentrations of PAHs making up the total PAH load in samples from each area, with values for individual PAHs varying from 0.1 μg kg^{-1} (e.g. benzo(g,h,i)perylene (B[ghi]PERY) in a sample from Sabah Al-Ahmad Protected Area), to 1010.0 μg kg^{-1} of chrysene (CH) and phenanthrene (PH) in a soil sample from Sabriya Oilfield. Chrysene (CH) was usually the predominant PAH, especially in Sabriya Oilfield (where it was found in 30 samples). Next commonest were phenanthrene (PH) and pyrene (PY) in soils collected from different conditions (fresh/dry oil lakes, and deposit piles of contaminated soil at several locations). Other PAHs were generally either present in lower concentrations or were below detection levels.

The highest values of total PAH contents in soils were seen in samples from two northern areas (Sabriya Oilfield and Bahra, the latter being located close to two major oilfields and strongly affected by oil flows and hydrocarbon deposition in 1991). Elevated total PAH values (though much lower than the values seen in Sabriya soils) were also found in soil samples from two other oilfield areas (Burgan and Um Al-Aish, with some individual samples exceeding 200 μg kg^{-1} soil dry weight for total PAH), but not in the Um-Ghadair Oilfield, where the total PAH in soil was close zero to very low values usually detected in unpolluted areas such as Sabah Al-Ahmad and Umm Al-Rros.

PAHs in plant leaf tissues collected from the two areas with the highest total PAH soil contamination (Um Al-Aish and Sabriya Oilfields: Table 2) showed a different pattern from the soils. There were significant between-area differences in values for some individual PAHs (e.g. fluorene) but there was no significant difference in total PAH content, with high values found in leaf samples collected from both areas. Mean values in plant tissues were also higher than in soils for some individual PAHs, suggesting substantial uptake by the plants. Phenanthrene (PH), one of the most abundant PAHs in soils, also showed the highest mean concentration in plant tissues, with the highest concentration detected in *Cyperus conglomeratus* . Suprisingly, fluorene (FL), which was detected in high concentrations in leaf samples taken from Sabriya, and to a less extent in samples from Um Al-Aish, was never detected in soil samples. Other individual PAHs with moderate to high leaf tissue concentrations were fluoranthene (FLAN), naphthalene (NA) and pyrene (PY). The remaining PAHs showed relatively low concentrations or were not detected in plant tissues. It is interesting to note that despite the high concentrations of chrysene (CH) found in soils, this PAH compound showed very low uptake by plants. Similar phenomenon was also observed for pyrene (PY). There was a small but significant difference in plant tissue moisture content between leaves sampled from plants growing in the two areas (Table 2).

Comparison of environmental and vegetation variables between TWINSPAN sample groups

A total of 42 species (**Supplementary File 1**) were observed from the sample-sites, with the highest area diversity (S_A) found in the Sabah Al-Ahmad Protected Area, followed by the Um Al-Aish Oilfield area. The results of multivariate classification of the samples x species matrix provide strong evidence (Table 3) that variation in the vegetation present was not solely associated with geographical location. Seven vegetation sample groups supporting vegetation (labelled Groups A – G) were identified by TWINSPAN at level 2 of the sample classification, and an eighth group (H) contained samples entirely lacking vegetation. Sample groups tended to be separated with high eigenvalues in the TWINSPAN analysis, indicative of substantial between-group differences in terms of plant assemblage present in their component samples.

Indicator species were identified for each sample group supporting plants (other than the three major focused plants). Results of one-way ANOVA comparisons (or non-parametric Kruskal-Wallis Test, for non-normal variables) between TWINSPAN-defined sample groups for individual environmental and vegetation variables (Table 3) provided evidence that Groups C and D had significantly elevated levels of soil PAHs compared with the remaining sample groups, whilst Group B had an intermediate PAH loading. Samples making up these groups also tended to be allocated high ODS values. The soil samples making up these two groups was very dry, and the mean species diversity of the two groups also tended to be low. However, there was no evidence for suppression of vegetation height in Groups C and D, with mean values falling in the intermediate range of the eight sample groups. These three groups were characterised by the presence of one or more species of *Haloxylon salicornicum* ,*Cyperus conglomeratus* and *Rhanterium epapposum* , whilst*Zygophyllum qatarense* (Zygophyllaceae), *Salsola imbricata*(Amaranthaceae) and *Stipagrostis plumosa* (Poaceae) were also indicators for elevated PAH soil conditions in at least one of these three sample groups.

Field experiment results

Two-way ANOVA for the growth rate data (Table 4) showed no significant differences due to location or exposure to TPH, although there was a tendency for plants in oil-polluted soils to elongate slightly more quickly than in clean soils, and to grow faster in the South than in the North (Burgan: 0.3 – 0.4 cm day^{-1}; Bahra 0.15 – 0.2 cm day^{-1}). Analysis of the final dry weight for shoots alone, roots alone and whole plant biomass datasets (Table 4) showed a significant treatment effect in every case, with plants grown in oil-polluted plots showing significantly reduced biomass when compared with plants in clean soils. However, location was not a significant factor influencing either growth rate or final plant biomass.

As expected, there was quite considerable variation in the degree of TPH pollution across the polluted experimental plots (in the range 40 – 350 mV.sec), but the difference between the two locations was not significant (Table 4), and overall, the data are indicative of moderate TPH pollution in the oil-contaminated treatment plots established in both areas.

Discussion

The absolute values of PAH concentrations and TPH found in Kuwaiti desert soils polluted by a one-off (though catastrophic) hydrocarbon pollution event, which occurred 20 years prior to sampling, are relatively low compared to values seen in some chronically long-term hydrocarbon-polluted industrial sites (e.g. Smith et al. 2006). Nevertheless, the results observed from both our field survey data, and the experimentally assessed response of *H salicornicum* plants to*in situ* weathered oil-polluted soils suggest that the levels of PAH pollution found in Kuwaiti desert soils in 2011 still have an adverse effect on plant survival and growth. This was reflected in the distribution of individual species and plant assemblages across the studied areas.

The results of the classification exercise (Table 3) showed that desert vegetation in Kuwait responds strongly to the presence of PAHs in the soil with a general shift in species presence towards oil-pollution indicator species at historically oil-damaged sites. This provides evidence that the native vegetation present in oil-damaged desert ecosystems can act as a good indicator of the degree of contamination and stage of recovery of the ecosystem from oil-pollution.

There was substantial uptake and accumulation of some PAH compounds by plants growing in polluted

soils. Phenanthrene concentrations in the leaf tissues of plants sampled in two polluted areas were up to 25% higher on average than the mean values in soils for this PAH. The difference was even more marked for fluorene, which was present in mean concentrations exceeding 60 μg kg^{-1} plant dry weight in leaf tissues sampled from one oilfield area, but was below the limit of detection in soils sampled in all survey areas. Whether this observation reflects substantial long-term (over a 20s period) removal of fluorene (and to a lesser extent, phenanthrene) by plants from these polluted soils, or is due to some other factors, requires further investigation, but the results are broadly in agreement with similar observations made in other PAH-polluted ecosystems (Meng et al. 2011; Wagrowski & Hites 1997).

The use of suitable phytoremediation management procedures, such as utilising native plant species and their associated root microflora, has been shown elsewhere to provide a useful low-cost, ecologically-friendly option to assist with pollution clean-up operations (e.g. Ramos & Maranhao 2009). According to Abdallah et al. (2020), some native plants can grow directly over contaminated soils. Evidence from heavy metal pollution studies, also, suggests that the use of multispecies mixtures (such as naturally occur in native plant communities) can improve the removal of contaminants from soil, compared to using monocultures (Burd et al. 2000; Belimov et al. 2001; Sheng & Xia 2006; Wu et al. 2006; Wenzel 2009). However, relatively little is known about the details of phytoremediation processes and uptake pathways involved in plant removal of PAHs from soils by desert plants (e.g., Slaski & Archambault 2000; Meng et al. 2011),

A major practical advantage of using native plant species as phytoremediators is the ease of obtaining seed and growing the plants prior to transplanting into desired clean-up locations. However, given that some native species such as *Haloxylon* are preferred species for camel grazing (Halwagy & Halwagy 1974: Halwagy et al. 1982), protection from grazing will be necessary for the success of a planting programme for phytoremediation purposes. In addition, a suitable supply of irrigation water during seedling establishment is probably a necessity, unless sufficient rainfall can be predicted during this period.

The experimental and field-survey evidence from this study supports the hypothesis that several native species in Kuwait, including*Haloxylon salicornicum, Cyperus conglomeratus* and*Rhanterium eppaposum* , can survive and grow successfully in multispecies communities on desert soils polluted by weathered oil and the associated mixture of PAHs. The presence of different plant communities, though partially influenced by geographical factors, can provide a good indication of the degree of damage and the stage of recovery of the system from pollution. The three primary target species examined here exhibit high capability to take up, and (to a varying extent) bioconcentrate some, but not all, of the PAH compounds present in these contaminated soils; once again providing evidence of their potential for phytoremediation purposes Halophytes in particular (*Haloxylon salicornicum* is one of them) have been studied before as a potential bioaccumulator (Shaygan et al, 2018)

Further research may include examining the physiology of the three primary target plant species (and other species with potential for oil-pollution phytoremediation usage, in North Africa and the Middle East) in order to ascertain in more detail the uptake mechanisms, pathways and fates of petroleum hydrocarbons and their breakdown molecules in these plants (Edwards 1986; Volkering et al. 1992; Harms et al. 2003). The study is also needed to examine the role of root-associated rhizosphere microflora (e.g., Juhasz & Naidu 2000; Binet et al. 2000; Wenzel 2009) in the target species as a means of enhancing phytoremediation impact.

Finally, the resulted reported herein may have implications to other arid and semi-arid desert areas of North Africa and the Middle East in which oilfields are located, and which are vulnerable to oil-pollution incidents. . Our data clearly demonstrate that native desert plant communities are useful indicators of the degree of damage, and recovery from such pollution. Furthermore, some native phytoremediator species appear to have good potential to assist in the restoration of oil-damaged desert ecosystems.

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Declaration of Conflict of Interest

The authors declare no conflict of interest.

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Table 1 . Vegetation (a) and environmental (b) data for seven sampling areas located in northern (N) and southern (S) Kuwait. Data for variables are mean values (± standard error). Units for PAHs: μg kg^{-1} soil dry weight. Significance testing for 14 individual PAHs (see methods for abbreviations: acenaphthene (ANA) and fluorene (FL) not shown: concentrations below limit of detection in all samples) was carried out only if at least one area mean value exceeded 20.0 μg kg^{-1} soil dry weight. Per variable means sharing a superscript letter in common do not significantly differ (ANOVA with Tukey's post-hoc mean separation test). For variables where data could not be normalised (ODS data) non-parametric Kruskal-Wallis testing was used and mean separation was not attempted. Values for total PAH content are the sum of all component PAHs measured. Outcomes: NS: P<0.05; *: P<0.05; **: P<0.01; ***: P<0.001; - : no determination made.

	Sampling area	Sampling area	Sampling area	Sampling area	Sampling area	Sampling area	Sampling area	Samplin area
	Bahra	**Sabah Al-Ahmad Protected Area**	**Burgan Oilfield**	**Um Al-Aish Oilfield**	**Um Al-Rros Military Base**	**Sabriya Oilfield**	**Um-Ghadair Oilfield**	
(a) Vegetation variables	(a) Vegetation variables	(a) Vegetation variables	(a) Vegetation variables	(a) Vegetation variables	(a) Vegetation variables	(a) Vegetation variables	(a) Vegetation variables	p
Vegetation height (cm)	8.4^{b} ± 1.9	11.9^{b} ± 2.4	13.5^{ab} ±1.9	20.0^{a} ± 2.6	1.3^{c}± 0.3	17.6^{ab} ± 1.9	9.8^{b} ± 0.9	<0.001'
Plant diversity (S_Q)	1.8^{bc} ± 0.2	2.4^{ab} ± 0.2	1.7^{bc} ± 0.2	3.1^{a} ± 0.3	1.1^{c} ± 0.1	2.4^{ab} ± 0.2	1.4^{c} ± 0.1	<0.001'
Haloxylon salicornicum (%F)	12.9 ± 2.8	3.3 ± 1.9	0	35.9 ± 5.0	0	8.8 ± 3.9	0	<0.001'
Cyperus conglomeratus (%F)	0.8 ± 0.6	0.1 ± 0.1	7.6 ± 1.6	0	0	10.8 ± 2.0	18.5 ± 3.3	<0.001'
Rhanterium epapposum (%F)	0	1.5 ± 0.9	0	0.1 ± 0.1	0	19.0 ± 4.5	6.8 ± 2.8	<0.001'

	Sampling area	Sampling area	Sampling area	Sampling area	Sampling area	Sampling area	Sampling area	Sampling area	Sa are
	Bahra	**Bahra**	**Sabah Al-Ahmad Protected Area**	**Burgan Oilfield**	**Um Al-Aish Oilfield**	**Um Al-Rros Military Base**	**Sabriya Oilfield**	**Um-Ghadair Oilfield**	

	Sampling area	Sampling area	Sampling area	Sampling area	Sampling area	Sampling area	Sampling area	Sampling area	Sa are
(b) Environmental variables: soil	(b) Environmental variables: soil								p
Mean oil damage score (ODS)	1.6 ± 0.1	1.6 ± 0.1	1.0 ± 0.0	2.3 ± 0.2	2.7 ± 0.1	1.0 ± 0.0	2.7 ± 0.1	2.2 ± 0.2	<0
Soil moisture content (%)	1.9^{b} ± 0.2	1.9^{b} ± 0.2	3.8^{ab} ± 0.5	2.00^{b} ± 0.6	7.1^{a} ± 0.7	2.5^{b} ± 0.6	3.2^{b} ± 0.4	1.6^{b} ± 0.3	<0
ANAY	0.2 ± 0.1	0.2 ± 0.1	0	0	0	0	0	0	-
ANTH	0.1 ± 0.1	0.1 ± 0.1	0	0.1 ± 0.1	0	0	0.1 ± 0.1	0	-
B[a]ANTH	1.3 ± 0.8	1.3 ± 0.8	0	0	0	0	6.3 ± 2.4	0	-
B[a]P	0.1 ± 0.1	0.1 ± 0.1	0	0.4 ± 0.4	0	0	2.1 ± 1.3	0	-
B[b]FLAN	1.2 ± 0.8	1.2 ± 0.8	0	1.1 ± 1.1	0.3 ± 0.2	0	13.4 ± 5.5	0	-
B[ghi]PERY	2.2 ± 0.9	2.2 ± 0.9	0.01 ± 0.01	0.7 ± 0.6	1.7 ± 0.6	0	19.8 ± 7.1	0.1 ± 01	-
B[k]FLAN	0.9 ± 0.6	0.9 ± 0.6	0	0.4 ± 0.4	0.3 ± 0.2	0	0.3 ± 0.2	0	-
CH	5.5^{b} ± 3.4	5.5^{b} ± 3.4	0	2.3^{b} ± 2.2	6.1^{b} ± 2.2	0	82.4^{a} ± 32.3	0.6^{b} ± 0.3	0.0
D[ah]ANTH	0.9 ± 0.5	0.9 ± 0.5	0	0.1 ± 0.1	0.1 ± 0.1	0	0.1 ± 0.1	0	-
FLAN	0.2 ± 0.1	0.2 ± 0.1	0	1.0 ± 1.0	0.2 ± 0.2	0	19.3 ± 9.5	0	-
I[123-cd]PY	2.2 ± 0.8	2.2 ± 0.8	0.1 ± 0.1	0.5 ± 0.5	0.6 ± 0.3	0	9.1 ± 3.9	0.1 ± 0.1	-
NA	2.9 ± 0.8	2.9 ± 0.8	1.9 ± 0.06	2.8 ± 1.2	4.6 ± 0.3	0	4.1 ± 0.8	2.0 ± 0.2	-
PH	15.9^{ab} ± 12.8	15.9^{ab} ± 12.8	0.1^{b} ± 0.1	2.2^{b} ± 1.9	1.3^{b} ± 0.6	0	70.4^{a} ± 37.2	0.2^{b} ± 0.1	0.0
PY	4.3^{b} ± 2.9	4.3^{b} ± 2.9	0	0.7^{b} ± 0.6	0.6^{b} ± 0.3	0	53.2^{a} ± 21.0	0	0.0
TOTAL PAH	37.9^{bc} ± 24.1	37.9^{bc} ± 24.1	2.1^{c} ± 0.1	12.3^{c} ± 10.1	15.7^{bc} ± 3.9	0	280.7^{ab} ± 116.2	2.9^{c} ± 0.4	<0

Table 2 . Plant tissue moisture content and concentrations of 16 individual PAHs (see methods for ab-

breviations) in leaf samples from two oil-polluted areas. Units for PAHs: μg kg^{-1} soil dry weight. Means compared by t-test (for each variable with value greater than zero in both locations). Units for PAHs: μg kg^{-1}plant dry weight. For further information see caption to Table 1.

Plant tissue	Um Al-Aish Oilfield	Sabriya Oilfield	*p*
Leaf tissue moisture content (%)	51.8 ± 2.4	69.8 ± 0.9	<0.001***
ANA	3.4 ± 0.1	1.1 ± 0.2	>0.05^{NS}
ANAY	0	0	-
ANTH	1.8 ± 0.4	5.3 ± 1.1 (	0.004**
B[a]ANTH	1.3 ± 0.5	8.4 ± 2.0	<0.001***
B[a]P	0	0	-
B[b]FLAN	0	0	-
B[ghi]PERY	0.7 ± 0.4	0	-
B[k]FLAN	0	0	-
CH	6.1 ± 0.6	6.8 ± 0.5	0.018*
D[ah]ANTH	0	0	-
FL	11.5 ± 0.9	67.6 ±16.8	<0.001***
FLAN	37.0 ± 1.5	47.8 ± 1.8	>0.05^{NS}
I[123-cd]PY	0.2 ± 0.2	0	-
NA	20.5 ± 0.5	30.7 ± 1.9	>0.05^{NS}
PH	100.1 ± 3.5	91.2 ± 6.3	>0.05^{NS}
PY	22.0 ± 0.7	26.3 ± 1.0	>0.05^{NS}
TOTAL PAH	204.5 ± 4.2	276.8 ± 19.3	>0.05^{NS}

Table 3 . Characteristics of TWINSPAN sample groups (A – G) and sample-group devoid of vegetation (H): (a) Classification statistics and vegetation variables; (b). Environmental variables. Data for variables are mean values (± standard error where applicable). Per variable, means sharing a superscript letter in common do not significantly differ (p <0.05: ANOVA with Tukey's post-hoc mean separation test (vegetation height; total PAH), or Kruskal-Wallis tests (all other variables): tests only applied for individual PAHs where at least one TWINSPAN group mean value exceeded 20.0 μg kg^{-1} soil dry weight). Variables were log_{10}-transformed where necessary to normalize the data. Values for total PAH content are the sum of all component PAHs measured. Units for PAHs (see Methods for abbreviations): μg kg^{-1} soil or plant tissue dry weight. For further information see caption to Table 1.

	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group
	A	**B**	**C**	**D**	**E**	**F**	**G**	**G**	**H**
(a) Classification outcomes	(a) Classification outcomes	(a) Classification outcomes	(a) Classification outcomes	(a) Classification outcomes	(a) Classification outcomes	(a) Classification outcomes	(a) Classification outcomes	(a) Classification outcomes	(a) Classification outcomes
Eigenvalue for sample group production	0.790	0.790	0.828	0.828	0.583	0.583	0.716	0.716	-

	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sample group
Indicator species	*Convolvulus cephalopodus*, *Pulicaria undulata*, *Moraea sisyrinchium*, *Trigonella strigata*	*Haloxylon salicornicum*, *Stipagrostis plumosa*	*Cyperus conglomeratus*, *Rhanterium epappposum*	*Zygophyllum qatarense*, *Salsola imbricata*	*Fagonia bruguieri*	*Pennisetum divisum*	*Heliotropium bacciferum*, *Cistanche tubulosa*	*Heliotropium bacciferum*, *Cistanche tubulosa*	no vegetation present
Vegetation variables	Vegetation variables	Vegetation variables	Vegetation variables	Vegetation variables	Vegetation variables	Vegetation variables	Vegetation variables	Vegetation variables	Vegetation variables
Vegetation height (cm)	$7.7^{b} \pm 1.5$	$13.7^{b} \pm 1.8$	$14.8^{b} \pm 1.6$	$16.6^{ab} \pm 4.9$	$3.1^{c} \pm 0.7$	$16.8^{ab} \pm 7.8$	$23.7^{a} \pm 12.5$	-	-
Plant diversity (S_Q)	2.2 ± 0.7	2.3 ± 0.2	1.7 ± 0.1	1.3 ± 0.3	2.6 ± 0.3	2.9 ± 0.4	1.7 ± 0.3	-	-
Haloxylon salicornicum frequency ($\%F_Q$)	0	25.6 ± 5.0	0	0	0	0	0	-	-
Cyperus conglomeratus frequency ($\%F_Q$)	0	1.8 ± 0.7	13.3 ± 3.7	0	0	0	0	-	-
Rhanterium epappposum frequency ($\%F_Q$)	0	0.1 ± 0.1	15.0 ± 3.1	0.1 ± 0.1	0	0	0	-	-

	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sam
	A	**B**	**C**
(b) Environmental variables: soil	(b) Environmental variables: soil	(b) Environmental variables: soil	(b) Environmenta
Mean oil damage score (ODS)	1.0 ± 0.0	1.6 ± 0.1	2.7 ± 0.1
Soil moisture content (%)	3.6 ± 0.2	2.2 ± 0.7	1.8 ± 0.4
ANAY	0	0.11 ± 0.06	0
ANTH	0	0.02 ± 0.02	0.08 ± 0.05

	TWINSPAN sample group	TWINSPAN sample group	TWINSPAN sam
B[a]ANTH	0	0.31 ± 0.16	4.07 ± 2.45
B[a]P	0	0.01 ± 0.01	1.54 ± 1.29
B[b]FLAN	0	0.24 ± 0.13	7.23 ± 3.51
B[ghi]PERY	0	0.78 ± 0.30	9.07 ± 4.34
B[k]FLAN	0	0.18 ± 0.09	0.87 ± 0.52
CH	0	1.32 ± 0.64	43.07 ± 21.89
D[ah]ANTH	0	0.21 ± 0.12	0.50 ± 0.43
FLAN	0	0.14 ± 0.06	10.14 ± 7.12
I[123-cd]PY	0.05 ± 0.04	0.81 ± 0.23	3.96 ± 2.25
NA	2.07± 0.09	2.68 ± 0.25	3.73 ± 2.91
PH	0	1.60 ± 0.82	42.48 ± 27.33
PY	0	0.76 ± 0.44	29.10 ± 15.34
TOTAL PAH	2.12^{b} ± 0.12	9.17^{bc} ± 2.91	155.84^{a} ± 78.76

Table 4 . Results of t-test comparisons, for individual variables measured (biomass data as mean values per plant), at sampling sites between treatments and locations (in northern (Bahra: N) and southern (Burgan: S) Kuwait). Values are given as mean and standard error. Kruskal-Wallis testing was used for soil TPH data.; NS: outcome not significant (P>0.05).

	Treatment	Treatment	Treatment	Location	Location	Location
	Unpolluted Soil	**Oil-polluted soil**	p	**Bahra (N)**	**Burgan (S)**	p
Max. growth rate (cm day^{-1})	0.30 ± 0.14	0.25 ± 0.07	$>0.05^{NS}$	0.18 ± 0.06	0.37 ± 0.15	$>0.05^{NS}$
Shoot dry weight (g)	6.65 ± 0.19	5.79 ± 0.18	<0.001***	6.39 ± 6.05	6.05 ± 0.81	$>0.05^{NS}$
Root dry weight (g)	2.17 ± 0.31	1.26 ± 0.08	<0.001***	1.75 ± 0.16	1.68 ± 0.35	$>0.05^{NS}$
Total dry weight (g)	8.82 ± 0.35	7.05 ± 0.24	<0.001***	8.14 ± 0.16	7.73 ± 0.49	$>0.05^{NS}$
Soil TPH content (oil-polluted sites) (mV.sec)	-	-	-	200.35 ± 35.20	210.27 ± 9.31	$>0.05^{NS}$