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Germination, physiological and biochemical responses of acacia seedlings (*Acacia raddiana* and *Acacia tortilis*) to petroleum contaminated soils☆

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ABSTRACT

Along the arid Arava, southern Israel, acacia trees (*Acacia raddiana* and *Acacia tortilis*) are considered keystone species. Yet they are threatened by the ongoing aquifer depletion for agriculture, the conversion of natural land to agricultural land, seed infestation by bruchid beetles, and the reduction in precipitation level in the region. In the acacia dominated Evrona reserve (southern Arava), adding to these threats are recurrent oil spills from an underground pipeline. We report here a study of the effects of contaminated soils, from a recent (December 2014) and a much older (1975) oil spills.

The effects of local petroleum oil-contaminated soils on germination and early growing stages of the two acacia species were studied by comparisons with uncontaminated (control) soils from the same sites. For both acacia species, germination was significantly reduced in the 2014 oil-contaminated soils, whereas delayed in the 1975 oil-contaminated soil. There was no significant effect of oil volatile compounds on seed germination. At 105 days post transplanting (DPT), height, leaf number, stem diameter, and root growth were significantly smaller in the oil-contaminated soils. While photosynthetic performance (quantum yield of photosystem II) did not differ considerably between treatments, reductions of chlorophylls content and protein content were found in seedlings growing in the contaminated soils. Significant increases in superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities were found in roots of seedlings growing in oil-contaminated soils. These results demonstrate that seed germination and seedling growth of both acacia species were strongly restricted by oil contamination in soils, from both recent (2014) and a 40-year old (1975) oil spills.

Such long-term effects of oil spills on local acacia seedlings could shift the structure of local acacia communities. These results should be taken into account by local authorities aiming to clean up and restore such polluted areas.

1. Introduction

Within the arid Negev and Arava deserts, *Acacia raddiana* and *Acacia tortilis* trees are considered keystone species (Munzbergova and Ward, 2002) that perform several important ecological services. These trees provide vital forage (leaves and pods) and water sources (leaf water content) throughout the year. Through their shade and shelter creating canopy, nitrogen-fixing qualities and their ability to act as hydraulic pumps that bring water closer to the surface, they improve soil conditions for other nearby plant species, increasing plant biodiversity under the tree canopies (Milton and Dean, 1995; Munzbergova and Ward, 2002; Abdallah et al., 2012) compared to the nearby surrounding areas.

The ongoing mortality of acacia trees in the region continues to be of major concern (Groner et al., 2015; Isaacson et al., 2017). Acacias are threatened by human activities such as road-building and ongoing aquifer depletion for agriculture (Ward and Rohner, 1997; Shrestha et al., 2003), the conversion of natural land to

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agricultural land. Other threats to these trees include seed infestation by bruchid beetles (Rohner and Ward, 1999; Or and Ward, 2004), and the reduction in precipitation level in the region (Ginat et al., 2011). Loss of acacia trees in arid regions is associated with the loss of much of the biota and ecosystem services associated with these trees, as well as enhanced erosion of soils (Ward and Rohner, 1997; Shrestha et al., 2003).

The Evrona reserve is a hyper-arid saline and sandy nature reserve in southern Israel, that is dominated by *A. raddiana* and *A. tortilis*, with tree densities of up to 200 trees per km^2 (Golan et al., 2016). In Evrona, adding to the threats mentioned above are the unknown effects of oil-contaminated soils, the result of both relatively recent (December 2014; Groner et al., 2015) and much older (July 1975; Braster, 1975) crude oil spills. The recent spill (3rd December 2014) of 5000 m^3 crude oil in the Evrona reserve was well documented (Groner et al., 2015) (https://www.youtube.com/watch?v=q7QSkRVwXbA; Fig. 1). However the older oil spill that occurred in 1975 (Braster, 1975) was rediscover only while surveing that current spill by the Israeli Nature and Parks Authority (INPA) some 1 km south of the 2014 event (Nothers et al., 2017). This forty-years-old oil spill was twice the amount of oil the 2014 event (10,000 m^3) but, surprisingly, this event did not draw much attention and had never been treated and the oil effects have been mostly ignored (Golan et al., 2016; Nothers et al., 2017). Both oil spills were caused by damage to local underground oil pipes.

The potential long term effects of oil spills on their surroudning ecosystems are important for decision makers regarding the remedation efforts after such events (Nothers et al., 2017). Most of the post-spill studies have focused on marine environments (Martínez-Gómez et al., 2010) (Supplementary materials Fig. S1A), while not much is known about the effects of oil spills in terrestrial environments, and even less in plants growing in terrestrial arid ecosystems (Nothers et al., 2017; Supplementary materials Fig. S1B).

One tool that has been used in terrestrial pollution event studies has been eco-toxicity assessments (Hentati et al., 2013). Oil-contaminated soils were shown to reduce seed germination, plant growth and yields of various crop species at different levels of oil contaminations (Agbogidi and Eshegbeyi, 2006; Bona et al., 2011) (Supplementary Table S1). The negative effects of oil-contaminated soils on seed germination was also shown in mangrove species *Aegiceras corniculatum* and *Acanthus ilicifolius* grown in sandy and muddy sediments, contaminated by both fresh and used lubricating oil (Zhang et al., 2007; Ke et al., 2011). Studies have demonstrated the negative effects of oil contamination on above-ground biomass in grains of cereals, legumes and other crops (De Jong, 1980; Ekundayo et al., 2001; Merkl et al., 2004; Issoufi et al., 2006; Kisic et al., 2009). Negative effects of oil pollution were also shown for the plant's belowground compartment, caused by the changes in root structure, reduction in root length, density, surface area of the finest diameter root class and root biomass of many terrestrial species such as *Cyperus aggregatus* (Merkl et al., 2005a), ryegrass (*Lolium perenne*) (Kaimi et al., 2006; Kechavarzi et al., 2007), corn and wheat (Ekundayo et al., 2001; Tang et al., 2011), and mangroves (Cui et al., 2016). The application of petroleum oil emulsions either as insecticides on plants or through accidental oil spills, were shown to reduce photosynthesis and respiration rate in citrus species (Wedding et al., 1952; Rosso et al., 2005), and *Salicornia virginica* (Rosso et al., 2005). Biochemical responses such as decreases in protein content (Odjegba and Sadiq, 2002; He et al., 2005; Achuba, 2006; Fazeli et al., 2007), and increases in the activity of the antioxidant enzyme superoxide dismutase (SOD) (Zhang et al., 2007; Ke et al., 2011; Cui et al., 2016) were also reported (Supplementary Table S1).

Most of these studies were related to marine, tropical, or crop plants, with relatively little experimental work on the effects of oil pollution on desert plants (however, see Golan et al., 2016).

The importance of acacia trees in the arid desert in general and in the Evrona nature reserve in particular, alongside a large number of studies demonstrating negative effects of oil-contaminated soils on a range of other plant species, were the main motivators to

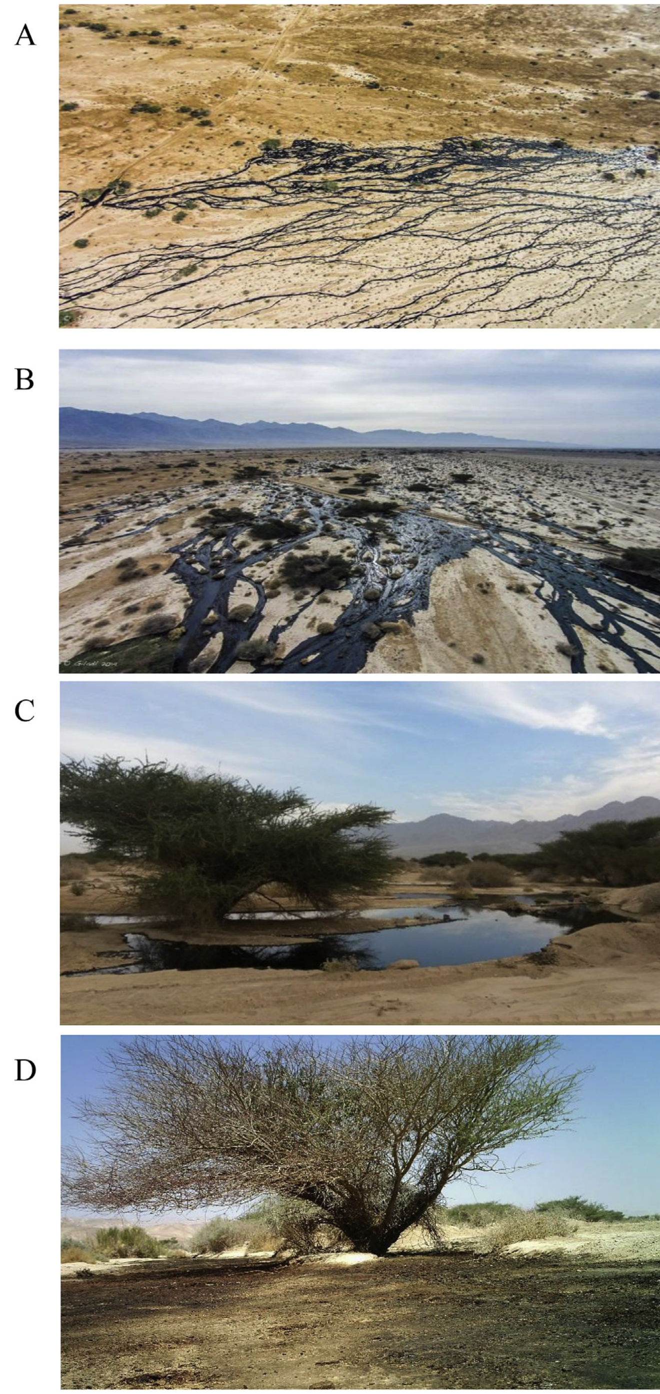

Fig. 1. Aerial (A, B) photographs of the December 2014 petroleum oil spill in the Evrona nature reserve, southern Israel. Photographed were taken on 4th Dec 2014. Shown also are ground photographs of *Acacia tortilis* sitting in a pool of oil (C). Most of this oil has been removed, but there is still a 30–50 cm layer of oil-contaminated top soil in the site (D). A similar oil-contaminated top soil exists in the 1975 polluted site (not shown). Photograph credits: A-B were taken Dr. Roi Talbi – The Israeli Nature and Parks Authority (INPA), C - taken by Nitzan Segev, D - by Gidon Winters.