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Reclamation of soil hydrophobicity and bioremediation enhancement of oil-contaminated soil in Evrona nature reserve

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Observations of oil-spills in 2014 and 1975, in the Evrona basin (Israel), demonstrate the central role soil hydrophobicity has in vegetation damage and site remediation. We conduct incubation experiments to investigate the persistence of oil spill induced hydrophobicity in Evrona arid soil. We also isolate biosurfactant-producing bacteria from the oil-contaminated soil and evaluate their ability to reduce surface tension. This study aims at assessing the decrease of the degree of hydrophobicity under different environmental conditions, the relationship between the content and composition of hydrocarbons and the severity of hydrophobicity, and the presence of in-situ bacteria capable of enhancing oil removal. Incubation experiments were conducted with soils polluted by the 2014 and 1975 crude oil spills. Soils were incubated with the addition of different combinations of water, nutrients, and surfactant. The changes in the degree of soil hydrophobicity were quantified by three measures: (i) water drop penetration time (WDPT), (ii) molarity of ethanol droplet (MED), and (iii) initial advancing contact angle (CA) of a sessile drop. The results of the incubation experiment showed a concomitant decrease in hydrophobicity and hydrocarbon content in soils to which water or water and nutrient and surfactants were added. The fastest degradation was observed in the soil which was amended with both nutrients and surfactants, while in the water only treatment, degradation and hydrophobicity reduction were very slow. Overall, during the one-year incubation, the total petroleum hydrocarbon (TPH) of the soil treated with nutrients and water decreased by 65% in soil from the 2014 spill and by 70% in the 1975 spill. The observation shows that the addition of water and nutrients is sufficient to trigger substantial bioremediation which is accompanied by a hydrophobicity decrease. Presence and quantification of oil-degrading bacteria show that alkanes and aromatics degraders exist in the soils of all treatments, even in the clean soil. Twenty-five bacterial strains were isolated and fourteen strains were screened for surfactant production to select the most promising ones. Production of possibly an anionic biosurfactant in these strains was tested using agar plates containing methylene (MB) and cetyl trimethylammonium bromide (CTAB), bacterial adhesion to hydrocarbons (BATH) assay, drop collapse assay, oil spreading assay and surface tension measurement. Seven strains found with positive drop collapse and oil spreading results showed a significant reduction (30∼41%) in pure water surface tension (72 mN/m) of cell-free culture broth. The clear blue halo areas (178∼206mm2) in the MB-CTAB plates indicated the abundance of anionic biosurfactant. The selected isolates were identified by 16S rRNA gene sequencing and were
found to belong to the *Pseudomonas* genus. While oil-degrading bacteria indicate a direct potential for oil degradation, surfactant producing bacteria increase hydrocarbon bioavailability and their degradation rates, thus enhancing oil and hydrophobicity attenuation. Overall, these findings have implications for the oil removal and remediation in Evrona arid oil-contaminated soil.