**Determining the efficacy of *Pseudomonas putida* to degrade hexadecane in soils at different moisture levels**

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**Methods**

Eight samples of 50 g (dry weight) of clean, sterilised soil were prepared. Hexadecane (0.25 g)

was added to four of the samples, and the flasks were vigorously shaken to ensure even distribution of the hydrocarbon throughout the soil, achieving a final concentration of 0.5% w/w (based on dry soil weight). All flasks were then inoculated with 1 mL of Pseudomonas putida suspension at a concentration of 2.5 × 10⁶ CFU g⁻¹ dry soil, as calculated post-incubation. Each soil sample was supplemented with 6.11 mL, 8.15 mL, 10.19 mL, or 12.23 mL of sterile water to create final soil moisture levels of 30%, 40%, 50%, and 60%, respectively — with and without hexadecane, forming eight treatment groups in total. Under aseptic conditions, 24 Eppendorf tubes were each prepared with 900 µL of sterile ¼-strength Ringer's solution. To eight universal bottles, 9.98 mL of ¼-strength Ringer's solution was added, followed by 120 mg of soil from each treatment. Bottles were shaken vigorously by hand for 1 minute to produce a 10⁻² soil suspension. From each universal bottle, 100 µL of the 10⁻² suspension was transferred to the corresponding Eppendorf and vortexed to produce a 10⁻³ dilution. Serial dilutions were then performed to obtain 10⁻⁴, 10⁻⁵, and 10⁻⁶ suspensions. Spot plates were prepared, with quadruplicate spots for each dilution. Plates were incubated at 25 °C for 24 hours, after which colony counts were recorded.

The entire procedure was repeated three times. Statistical analysis was performed using the Scheirer-Ray-Hare test, followed by post hoc Dunn's tests, treating each spot as a replicate. To confirm the identity of the bacterial growth as *Pseudomonas putida*, a combination of diagnostic methods was used. These included oxidase testing using oxidase reagent strips, light microscopy, culturing on selective media, and PCR followed by gel electrophoresis. All procedures were carried out in accordance with the BABS2 Soil Microbiology Strand – Hydrocarbon Degrader Practicals: Practical 1a protocol (University of York, 2025).

**Hypotheses**

* *P. putida* can degrade hydrocarbons and utilise them as a carbon source, so without any other carbon sources, there will be significantly more CFUs per unit volume in the hydrocarbon-contaminated samples than the non-hydrocarbon-contaminated samples at the same moisture level.
* Previous research suggests that *P. putida* degrades hydrocarbons and proliferates at optimum soil moisture levels of 40% field capacity, so there will be significant differences between samples at different moisture levels, with the greatest colony count at 40%.

**Reason**

Previous research demonstrated that select strains of *P. putida* degrade naphthalene, a polycyclic unsaturated aromatic hydrocarbon, at an optimum soil moisture level of ~40%. However, aromatics only make up ~15%wt of liquid petroleum oil spills, with alkanes and naphthenes making up 30% and 49%, respectively. Knowing how *P. putida* can bioremediate saturated hydrocarbons- including hexadecane- at varying soil moisture levels will aid in understanding the bacteria’s potential to deal with oil spills in a variety of climates with varying humidities.

**Aim**

Obtain count of CFUs per gram of soil for samples with and without hexadecane contamination, at 30, 40, 50, and 60 % of field capacity, with sample size of 18 for each sample.