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

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**Methods**

To 4 samples of 50g (dry weight) of clean sterilised soil, 250µL of hexadecane was added and flasks were vigorously shaken to evenly distribute the hydrocarbon throughout the medium. All the flasks were then supplemented with 1ml of *Pseudomonas putida*, at a concentration of 2.5x106 CFUg-1 of dry soil, calculated after incubation.

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Working in aseptic conditions, to 4 Eppendorf tubes per sample (30%, 40%, 50%, 60% of field capacity; with and without hexadecane)- a total of 24 Eppendorfs- 900µL of sterile ¼ strength Ringer solution was added. To 8 universal bottles, 9980µL of ¼ strength Ringer solution was added, followed by 120mg of each sample of soil. Each bottle was shaken vigorously by hand for 1min to form the 10-2 dilution. From the 30W universal bottle, a 100µL aliquot of 10-2 soil suspension was transferred to 900µL of ¼ strength Ringer solution in the corresponding Eppendorf and vortex mixed to obtain a 10-3 dilution. Serial dilutions were continued to obtain 10-4, 10-5, and 10-6 dilutions. This procedure was repeated for 40W, 50W, 60W, 30WO, 40WO, 50WO, and 60WO to obtain serial dilutions of each sample. From each sample, spot plates were produced with a spot for each dilution in quadruplicate.

Following incubation of the 24 plates at *T*oC for *H*h, results were observed and colonies were counted. Treating each spot as a sampling unit, *#insert calculations and statistical tests here*

**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.