EVALUATION OF BIODEGRADATION CAPABILITY OF CONTAMINATED SOIL FOR *IN SITU* TREATMENT

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EXPERIMENT

Soil sampling for the experiment was performed according to the developed protocol.

Treatments for experimental soils (~10 kg in a bucket):

1. No treatment
2. Aeration
3. Fertilization (NPK 23-8-3)
4. Aeration and fertilization (NPK 23-8-3)

Incubation 4 months.
Sampling + analysis 3 x

	Experimental soils contaminated with petrol, diesel and/or fuel oil				Clean soil
Characteristics	A	P1	P0	Y	V
Soil depth (m)	0.4-2.5	0.4-1.0	1.0-1.5	0.3-1.0	0-0.2
Soil type	moraine silt	moraine silt	moraine silt	moraine sand	sandy clay
pH	5.5	5.3	5.2	5.5	6.3
EC	0,70	0,16	0,43	0,11	0,45
LOI %	1.3	1.2	1.1	2,8	5,8
P (mg/kg)	438	400	382	321	1060
Hydrocarbons (mg/kg)					
C10-C21	1000	2300	970	5700	< 20
C22-C40	41	< 20	< 20	840	23
C10-C40	1100	2300	990	6600	< 40
Aliphatic	820	1580	616	5102	nd
Aromatic	140	472	180	1056	nd

EC = soil electricity conductivity, LOI = Loss of ignition, nd = not determined

Three soils (A, P1, P0) were collected from former oil harbour and one soil (Y) from former petrol selling village shop. Soil A had been excavated already 3.5 months earlier and stored in a windrow before the experiment.

METHODS TESTED FOR EVALUATION

Soil is analysed for:

1. Soil properties
2. Biomass in soil
   a) Carbon content
   b) Loss of ignition
   c) Chloroform-fumigation extraction (microbial mass)
3. Toxicity of soil
   a) Flash test (Bioluminescent bacterium)
   b) Sperm test (motility of boar spermatozoa)
   c) Growth and reporduction of kidney cells (BHK-21)
   d) Growth inhibition of *Phacelia tanacetifolia* plant
4. Enzyme (lipase) activity
5. Presence of oil degrading and bacterial genes
   a) nahAC coding for naphthalene dioxygenase Fe-S large subunit protein
   b) alkB coding for alkane 1-monooxygenase involved in the pathway alkane degradation
   c) 16S rRNA

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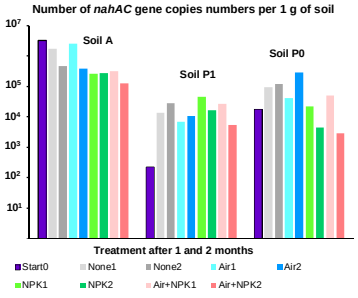
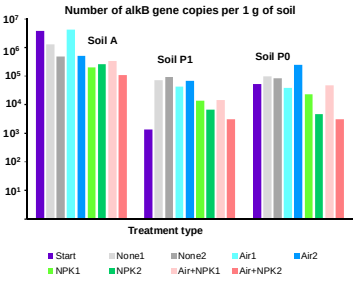
Sampling the soil

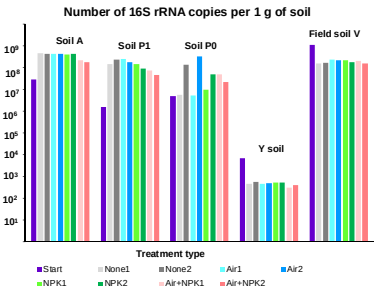
PURPOSE

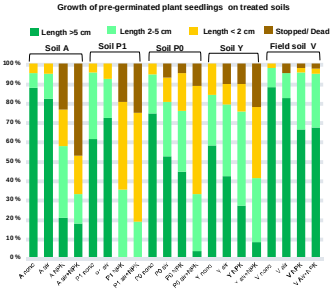
For contaminated soil treatment *in situ* technologies should be favoured according to principles of sustainable development.

- *In situ* and on site treatment technologies mostly rely on the microbiological processes in soil
- Currently, no commercial method exists for the evaluation of the biodegradation capability of the microbial population in soil
- Our aim is to develop a test package for evaluation of soil for the status of microbiological degradation capacity. Test results help with the decision for implementation of *in situ* or on site treatment.









RESULTS

Quantitative PCR method of oil degrading genes *nahAC* and *alkB* (figures) was applied to find out whether bacteria with oil degrading capacity live in the experimental soils, and how soil treatments affect the gene copy numbers:

- The A soil (3.5 mo in windrows) had the highest copy numbers for the both genes (appr. $3x10^6$), but there was a decreasing trend during incubation of two months
- In P1 soil the both copy numbers were clearly increased during incubation from low starting values and *nahAC* gene in P0 soil increased as well, but the starting level for P0 was significantly higher
- No oil degrading genes was found in Y and V (clean) soil

Gene 16S rRNA analysis (see fig.) was used as a standard for PCR methods in general, but it also gives estimate of the bacterial population in soil. Amount of bacterial copy numbers were low in Y soil, which probably explains the lack of oil degrading genes in this soil. Y and V soils contained plant roots, and decreasing bacterial copy numbers can be explained by disappearance of root associated bacteria. Bacterial numbers in soils A, P0 and P1 increased varying amounts according to the treatment.

All the contaminated soils were toxic according to toxicity tests, except Y soil in Flash test and A soil in plant test. Otherwise the differences in toxicity were small at the beginning of the experiment. The treatments, however gave varying results: fertilization disturbed strongly the plant growth (see fig.) in all contaminated soils, but seemed to stimulate motility of sperm cells.

The reliability of the test method is dependent on representative samples. In order to achieve this, a sampling protocol was written to accompany the test kit. Sampling protocol includes a concise version for experts, and a thorough step-by-step version for non-experts. The protocol takes into account different soil types, and ensures that the sample number is sufficient for overcoming variety deriving from soil heterogeneity.

Buckets were covered with gauze to minimize drying

CONCLUSIONS

Microbial methods are important for evaluation of biodegradation capability

Several analysis are needed together.

The project is still ongoing – the most relevant methods can be selected when all the results are available.