Remediation of Hydrocarbon-Contaminated Soil by Vermicompost Tea

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Abstract
Elutriate was produced from vermicompost under specific conditions – aerated vermicompost tea (ACT). This medium, rich on microorganisms and water soluble nutrients was applied on strongly contaminated site by hydrocarbons for 12 months. Ecotoxicity, hydrocarbons content, phytotoxicity and microbial activity were analysed by different methods. Results showed evident improvements of all analyzed parameters and significant decrease of hydrocarbon content.

Introduction
Vermicompost is compost made by earthworms. It has a rich microbial diversity and contains substances, which are beneficial for plant growth. Water elutriates – Compost tea (Scheuerell, 2004) could be used to enhance plant condition and soil fertility. Aerated vermicompost tea (ACT) with additives is recommended to be produced (Ingham, 2006) to enhance the growth of beneficial and suppress pathogenic microorganisms.
The main objective of this paper is to find out the influence of ACT on remediation of soil contaminated by hydrocarbon. The main assumptions were that microorganisms in ACT would speed up the remediation process.

Materials and methods
Aerated vermicompost tea was produced in an extraction vessel with the volume of 850 litres from 10 litres compost.
Research was performed on soil that was intensively contaminated by hydrocarbons (25 – 33 mg of non-polar extractives per gram of soil). The site was at a disposal site of liquid industrial waste. Waste hydrocarbons from wood impregnation were disposed here many years ago. *(Fig. 1)*
Two experimental sites were marked in the area of 4 m^2. The first site B-1 was watered by tap water (a blank). The second one was in parallel watered by the same volume of ACT – 15 l/m^2. Application of water/ACT was performed overall 7 times, approximately once per 1 – 2 months, from September 2006 to September 2007, during vegetation period only.
Each soil sample was taken from 5 points of the site, from the depth of 5 – 10 cm. An average sample was done subsequently by homogenisation process. Samples were taken before the first application of the ACT/water on 5 September 2006 and after the last application on 17 October 2007.
Ecotoxicity of the samples was measured as an indicator of remediation. Ecotoxicity tests were performed by cress (*Lepidium sativum)*, according to OECD guidelines 208 “Terrestrial Plant, Growth Test”; duckweed (*Lemna minor)* according to ISO/CD 20079 “Duckweed Growth Inhibition Test” and daphnia (*Daphnia magna)* according to OECD guidelines 202 “Daphnia sp., Acute Immobilisation Test”.

Fig. 1 - Contaminated site

Hydrocarbons content was measured by IR spectrometry for quantity and by UV spectrometry for quality, following the norm STN 830540, which is an equivalent to the norm DIN 38409 Teil 18.
Botanical research in situ was measured via species *Carex Hirta* analyzing its number of plants per m^2, number of leaves per plant, leaf length and plant vitality using the bio-indicating tongs KSM-3, model 100 produced by ELSACOn Kolin (CZ) measuring electrical conductivity of plant tissues. At the same time the production of above-the-ground biomass was determined.
Microbial activity was analyzed by membrane filter methodology and via cultivation of extracted soil suspension at nutrient agar, and different bacteria strains were searched for (*Salmonella, Shiggella, Streptococcus, Pseudomonas sp., Mycobacterium sp., Penicilium sp., Rhizopus sp., Mucor sp., Altenaria sp., Cladosporium sp., Citrobacter sp., Bacillus sp., Micrococcus sp., Enterobacteriace, etc.*) following the norm STN ISO 9308 –1, STN ISO 7899-2 and STN 83 0531.

Results

ECOTOXICITY. There is an evident proof of ecotoxicity decrease due to ACT application after the period of 12 months under external conditions at the contaminated sites by hydrocarbons. The ecotoxicity of the contaminated substrate decreased to the non-quantifiable level monitored by *Daphnia magna (Fig. 2 and 3)* and *Lemna minor (Fig. 4 and 5)*. Ecotoxicity determined by *Lepidium sativum* was reduced from EC_{50} = 0,061 to EC_{50} = 0,110.

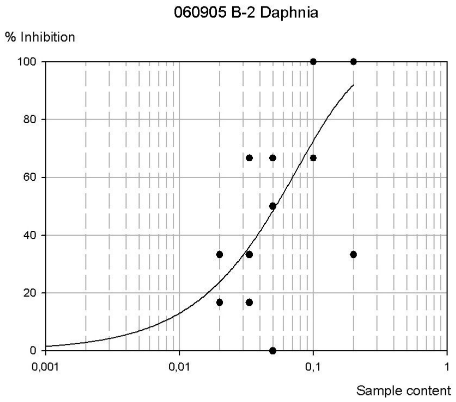

Fig. 2: Ecotoxicity of hydrocarbon-contaminated soil before the first application of ACT, determined by *Daphnia magna*.
EC_{50} = 0.0522

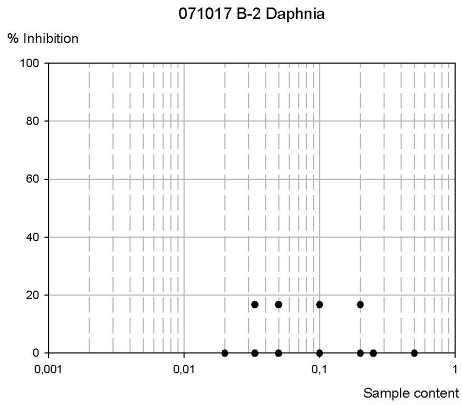

Fig. 3: Ecotoxicity of hydrocarbon-contaminated soil after all applications of ACT, determined by *Daphnia magna*.
EC_{50} = n.d.

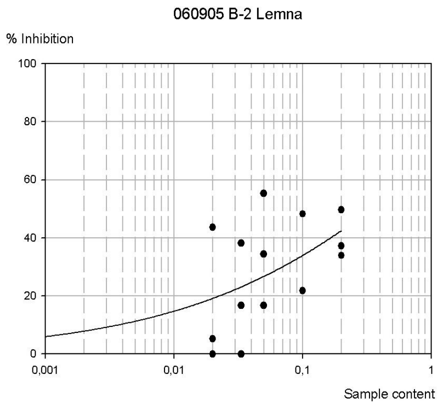

Fig. 4: Ecotoxicity of hydrocarbon-contaminated soil before the first application of ACT, determined by duckweed (*Lemna minor*). EC_{50} = 0.3439

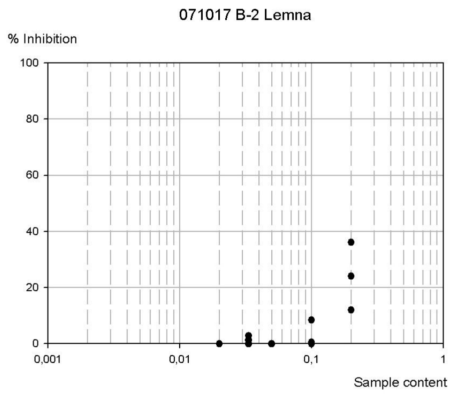

Fig. 5: Ecotoxicity of hydrocarbon-contaminated soil after all applications of ACT, determined by duckweed (*Lemna minor*). EC_{50} = n.d.

Figures 2 – 5: Influence of ACT on the ecotoxicity of the contaminated soil samples determined by *Daphnia magna* and *Lemna minor*.

HYDROCARBONS CONTENT**.** Hydrocarbons were determined by IR spectrometry in 4 analyses during the experiment in dates indicated in the Table 1. Despite natural fluctuation caused mostly by ground water capillarity and rainfalls, there is an evident decrease of hydrocarbon content due to ACT application - 61 % at the end of the experiment (Fig. 6).

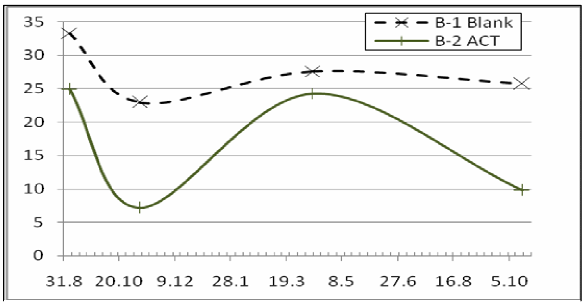

Fig. 6 Hydrocarbons determined in $mg.g^{-1}$ B – 2 ACT are samples taken from the area treated by vermicompost tea.

Date of taking samples	05.09.06	07.11.2006	11.04.2007	17.10.07
B-1 blank	33,32	22,95	27,60	25,74
B-2 ACT	24,90	**7,13**	24,28	**9,80**

Table 1. Quantity of hydrocarbons determined as non – polar extractives in $mg.g^{-1}$

BOTANICAL RESEARCH. Carex hirta is a resistent species of herb, which is tolerant of hydrocarbon contamination of soil. It has grown at every experimental site . At ACT sites (B2 and B4), which were treated by vermicompost tea, Carex hirta had higher increment, larger and longer leaves and also better vitality (Fig. 7).

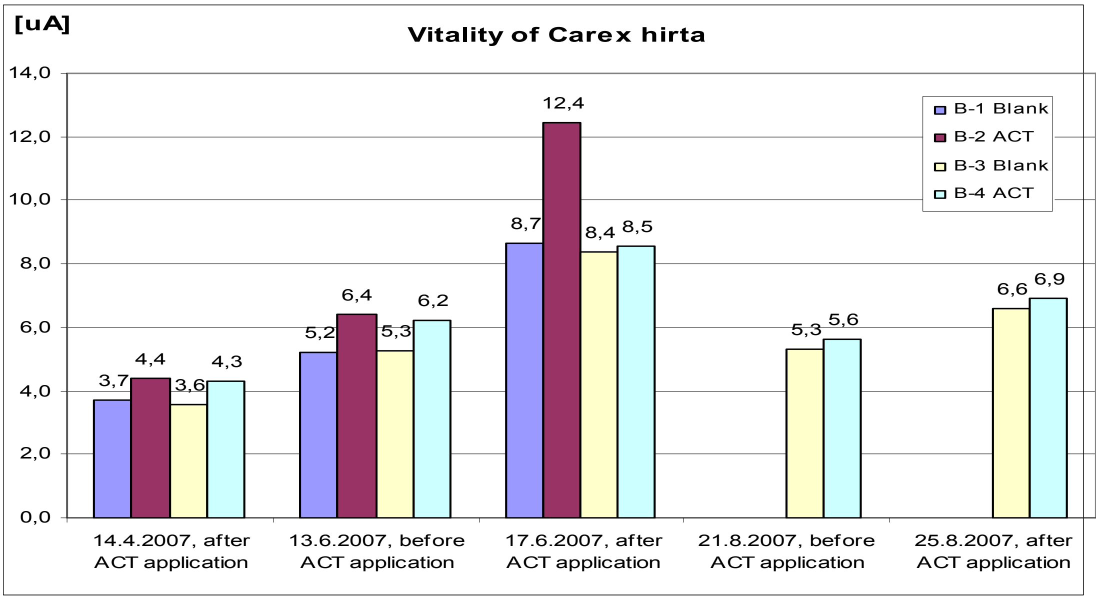

Fig.7 ***Carex hirta*** - plant vitality measurements in situ. B-2 and B-4 are treated by vermicompost tea.

MICROBIAL ACTIVITY**.** As mentioned above in the methodology, there was analyzed number of different bacteria species to define microbial activity at treated and at non-treated sites. Results show significant difference in favour of treated sites where we can find much higher microbial activity (Fig 8). At ACT fields, these bacteria strains were identified: *Pseudomobas sp., Acinetobacter sp., Flavobacterium sp., Corynebacterium sp., Bacillus sp., Micrococcus sp.*, as well as micromycetes, including *Penicilium sp., Aspergillus niger, Aspergilus versicolor, Rhizopus sp., Mucor sp., Altenaria sp., Cladosporium sp.*

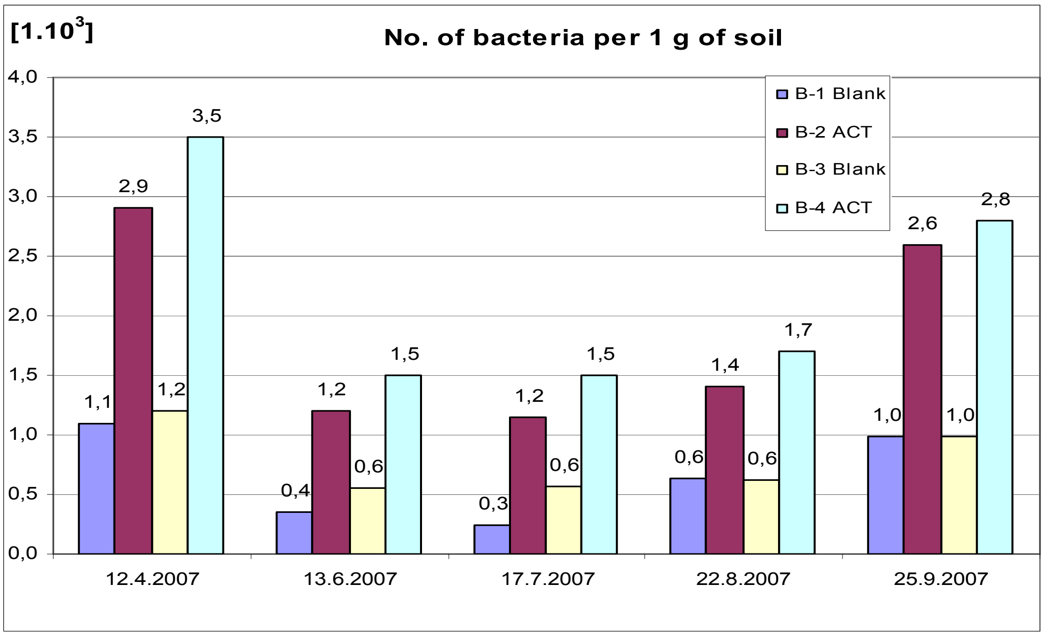

Fig. 8 Amount of bacteria at compared sites B-2 and B-4 were treated by vermicompost tea.

Conclusions

Aerated vermicompost tea (ACT) has proved its positive impact in the remediation process of soil site contaminated by specific hydrocarbons pollutants with high concentration. All observed criteria – hydrocarbons content, ecotoxicity, phytotoxicity in situ, microbial activity had significantly better values at treated sites. Results were reached under external conditions, thus they are applicable in broad practice. However, it is necessary to prove or specify a positive impact of ACT on the different (hydrocarbon) contaminations and it seems to be necessary to develop also the methodology enabling transport of ACT for longer time or distances. Therefore we strongly advise to follow up this research by investigating the answers for both problems opened above and looking for broader application of ACT in practice.

Acknowledgements

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