MICROBIAL ACTIVITY OF SOIL INFLUENCED BY DIFFERENT LEVELS OF CRUDE OIL HYDROCARBONS CONTAMINATIONS

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Abstract: Bioremediation is a method of reviving the environment through natural processes. These processes may be faster and more effective thanks to modern technology. This diploma thesis deals with the topic of microbial activity of soil influenced by different levels of crude oil hydrocarbon contamination and observation of microbial consortia activity in contaminated, non-contaminated and sterile soil. The initial chapter deals with crude oil contamination and bacterial metabolism which is able to remove this contamination. A container trial was executed in the experimental part of the thesis. The plants were planted into different types of modified soil (crude oil application, sterilization etc.). The production of biomass was compared and several conclusions from the results were drawn. The basic fact is that the soil microorganisms which occur in oil soil can design a life strategy in this environment and can also prosper, which is reflected in the production of biomass. The container trial was determined as the most exact method of soil activity valuation because it most approximates the real soil proportion. Analyses of storage soil were performed after finishing the container trial. These results brought similar conclusions; however the cultivation in the culture medium and cultivation in the soil as such are incomparable. The storage soil underwent a watercress trial. This trial confirmed toxic effects of crude oil but it also showed the fact that crude oil is a natural substance and microorganisms can adapt to it. The mineralization of soil was measured with help of ionic measurements.

Key Words: Microbial activity, crude oil, bioremediation, soil respiration

INTRODUCTION

On the one hand, crude oil substances lie at the foundations of our prosperity; on the other hand they cause environmental pollution, involving various procedures ranging from its extraction to the final processing (Urcová 2012). Next to contamination of soil, water and air, there is the issue of carbon, which has been accumulating in terrestrial ecosystems for millions of years (Hou et al. 2015, Van Hamme et al. 2003) and whose sudden release contributes to the global warming (Pacala, Socolow 2004). Although independence of crude oil is light years away, it is an organic compound that is a source of energy and structural units for microorganisms (Carrera-Martinez et al. 2011). Potential decomposing agents are abundant in every type of common soil and if favorable conditions are set, they may efficiently remove these pollutants which are dangerous for humans (Woodruff 2001). This can also be achieved by physical and chemical methods; however the question is whether the soil exposed to high temperatures or chemical substances is still a soil (McKinley et al. 2005).

Microorganisms represent an incredibly rich source for various remediation technologies. Thanks to an enormous numbers of species which densely populate every inch of healthy soil, mixtures of substances can be processed, metabolites can be exchanged and thus even such a complex combination of substances as crude oil can be almost entirely degraded in the end (Mukherjee, Bordoloi 2011). It is a difficult task to set suitable conditions for biodegradation: soil is a live and rich ecosystem having its specific needs which should be understood and respected. Although bacteria are classified as simple organisms, they have complex metabolism whose ability to adapt to its environment has not been entirely explained (Head et al. 2003, Margot et al. 2000).

MATERIAL AND METHODS

Experimental soils

Two sites contaminated with crude oil were selected in cooperation with Moravské naftové doly (MND Group). The agreement with the company included the provision about non-disclosure of the exact position of one of the sites. A reference sample of soil with similar properties as the comparative sample was taken simultaneously.

Sample No. 1 Contaminated site – CS

The sample was taken in the area which was exposed to oil leak in the past. The sample was stored in the refrigerator under 7°C for one month. The determined concentration of oil substances was 0.022 $kg.kg^{-1}$ of soil.

Sample No. 2. Oil tank – OT

The second sample was taken from the backfill surrounding the oil tank. The amount of crude oil in this sample was 0.005340 $kg.kg^{-1}$ of soil.

Figure 1 Sample No. 2. Oil tank

Figure 2 Sample No. 3. Non-contaminated soil

Sample No. 3. Non-contaminated soil – R

This sample was taken in the site close to the sites where sample No. 1 and sample No. 2 were taken. It did not contain any oil contamination and served as a reference soil. Sample No. 3 was not close to any agricultural work or road.

All the samples were homogenized, supplied equal degree of humidity and sieved through 2-mm mesh sieve.

The design of container experiment

The container trial was established on 19 March and 112 lettuce seeds (*Lactuca sativa*) were sowed. Lettuce was grown in small pots of baked clay, diameter 0.06 m, 7 different substrates, see Table 1. Four lettuce seeds were placed into each pot. Each substrate variant had 4 repetitions; 28 pots were prepared altogether. The production of aboveground and underground biomass was compared in different substrate variants.

Substrate variants
R - reference soil
CS - soil under long-term contamination
OT - soil surrounding the oil tank
S - sterilized reference soil
X - non-contaminated soil with oil addition (0.0055 $kg.kg^{-1}$ of soil)
S+O - sterilized soil + non-contaminated soil with oil addition (0.0055 $kg.kg^{-1}$ of soil)
P - variant R+CS 15:1

Table 1 The design of respiration experiment

Variant	Oil amount (kg)
Non-contaminated soil	-
x/2	1.55×10^{-5}
x	3.1×10^{-5}
2x	6.2×10^{-5}
4x	12.4×10^{-5}
8x	24.8×10^{-5}
Control	-

The design of respiration experiment

Another set of samples was prepared in order to examine the effect of oil addition on respiration; see Table 1. The measurements were conducted according to Keith and Wong (2006).

Prepared variants of soil were placed in a vessel and over the surface was placed in a container with Soda Lime. Soda Lime served as a sorbent CO^2. These containers were sealed gas-tight and kept in the dark for 24h. After 24h weight of Soda Lime was measured and calculated the amount of CO^2.

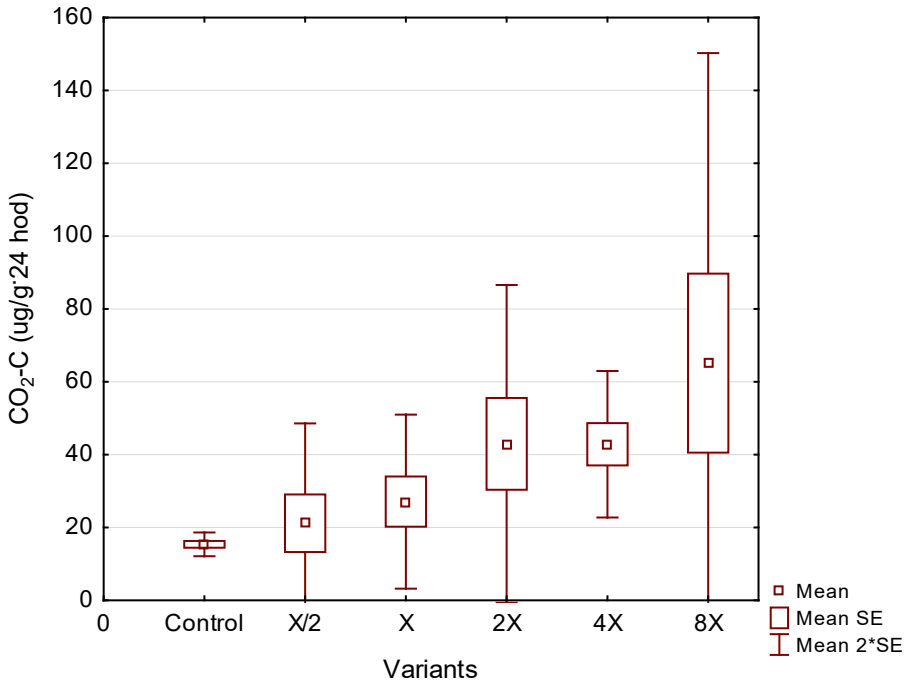
RESULTS AND DISCUSSION

Respiration

Respirations tests were performed in soils contaminated with different amounts of oil substances. Crude oil affected the production of carbon dioxide in all the samples and the results clearly show that the soil activity increases with the increased amounts of the oil contaminant.

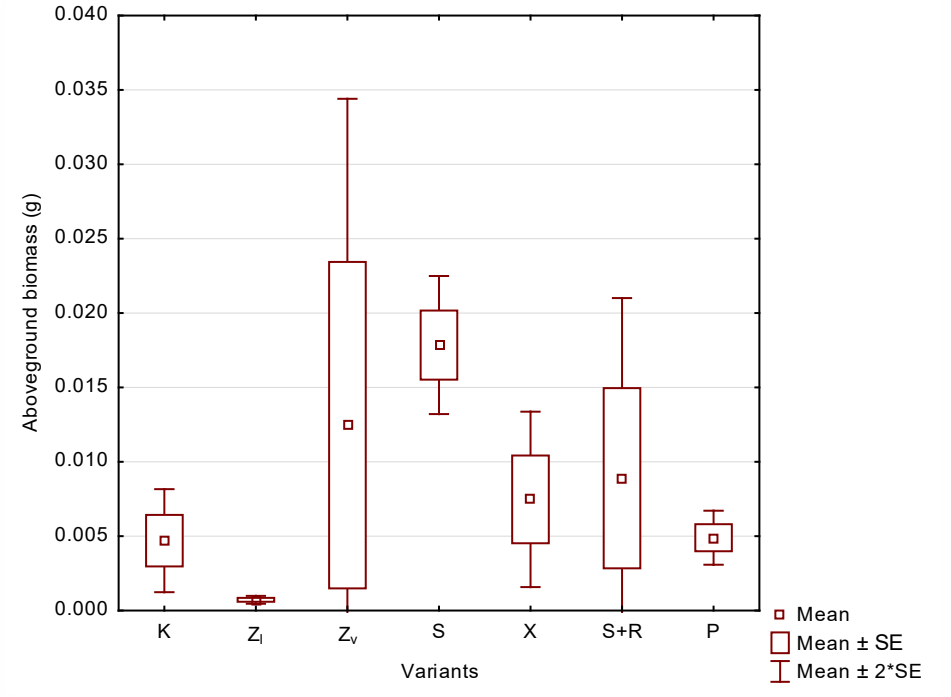
Although microorganisms utilize crude oil as a source of energy and structural units (Alexander, Orbach 1982), the increase in CO_2 production need not be related to their reproduction. The raised CO_2 production may be the response of microorganisms to stress (Haimi, Huhta 1987). Another possible explanation is that the oil killed a part of microbial populations and those surviving have, apart from oil hydrocarbons, also carbonaceous substances and energy from lysed microbial cells (Ramanand et al.1993). The measured values were substituted to the above mentioned relation and summarized in the table and chart below.

Figure 3 Statistic analysis – Soil Respiration



The respective variants of the experiment did not show a statistically significant difference in the CO_2 production. Moreover, it was proved that the variability of the measured values markedly grows with the increasing amount of added crude oil hydrocarbons, and thus the heterogeneity of the soil environment, or more precisely of various microhabitats in test soils, increases.

Figure 4 Statistic analysis - Production of aboveground biomass ±



The largest increase of the aboveground biomass was observed in the sterilized (S) soil. The sterilized soil represents a highly attractive energy source for microorganisms. After the sterilization, the soil is enriched with cytoplasm released from the dead microorganism cells which serves as a suitable source of carbohydrates for microorganisms that will get to the sterilized soil from air and during later handling. The inanimate component of the soil is not significantly affected by the sterilization. (Drenovsky et al. 2005).

The chart shows that the biomass production in S soil markedly exceeded the others. If we disregard this option, we can see that the second most suitable substrate was the contaminated model soil X. The concentration of crude oil was 5 $g.kg^{-1}$ of soil in this substrate. The increase of biomass in OT soil (backfill around the oil tank) is twice as high as in S+O soil (sterilized soil with the model contamination by crude oil), although the concentration of oil substances is very similar. Thus it may be derived that the sterilized soil with new microorganisms is not able to utilize crude oil to the extent of soil where microorganisms have adapted to the contamination. Many authors have found similar results (Marquez–Rocha 2011, Trindade et al. 2005, Kuiper et al. 2004, Alisi et al. 2009). On the other hand, the Zl soil (from the contaminated site) was the least favorable for the growth of plants. As has been said, the reason for the slow growth may be the necessity to adapt to the new conditions. Although the seeds did eventually germinate in this soil, the germination occurred at the time when the container trial had to be finished. When we compared the increase of aboveground biomass in soil P (mixture of sterilized and contaminated soil and crude oil) and soil S+O (sterilized soil with the model contamination by crude oil), it was apparent that in the absence of microorganisms with suitably set metabolism, the plants prospered less.

CONCLUSION

The experiment results have proved that native microflora of soils that have been contaminated for a long time has adapted to the presence of crude oil hydrocarbons. Several variants of substrates were applied in the container trial: non-contaminated soil (C), contaminated (CS, OT) and sterilized

contaminated (S+O) and sterilized non-contaminated (S). Further variants included contaminated model soil (X) and a trial represented by the mixture of crude oil, sterilized soil and contaminated soil (P). Sterilized non-contaminated soil (S) demonstrated the highest production of biomass, the possible reason being that the dead biomass is an attractive source of energy and carbon for r-strategists. These microorganisms colonize the soil very quickly and only after a time the soil starts to be populated also by K-strategists, whose numbers are fewer. The fast colonization by r-strategists probably caused the large growth of biomass. However, the production ability of the sterile soil, simulated in this way, is short-lived and could disappear after a time. In case crude oil was added to the same, i.e. sterilized soil in the amount of 5 $g.kg^{-1}$ of soil (S+O), the biomass production sharply dropped. On the contrary, the soil formed by the mixture of crude oil, sterilized soil and soil taken from the site permanently burdened with crude oil (P) produced almost twice as much biomass than the S+O type. Equal amounts of crude oil were used in both cases. The proportion of non-contaminated and permanently burdened soil was 15:1. It follows from what has been said above that it is sufficient to inoculate experimental soil with a small number of decomposing agents with their metabolism adapted to crude oil, and the soil shall efficiently cope with the contamination.

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