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THE IMPACT OF CHEMICAL ADDITIVES ON THE PROCESS OF BIODEGRADATION OF OIL PRODUCTS

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Abstract. Many modern technologies for treatment of soil polluted with oil products are developed through creation of new and efficient bio-agents that help to degrade oil products. Another trend in development of new technologies aims to speed up the growth of soil microorganisms, this way accelerating biodegradation of oil products without the help of introduced microorganisms. The biodegradation of diesel fuel and heavy fuel oil was tested in the soil using chemical additives (oxidizing agents). The tests aimed to ascertain the impact of H_2O_2, $KMnO_4$ and $MnSO_4$ on residual concentrations of heavy fuel oil and diesel fuel, and the total number of microorganism colonies, as well as the number of oil-oxidizing microorganism colonies in the soil. After the statistical analysis of the data obtained during the experiment, a statistically significant ($p < 0.05$) difference between control samples and samples with introduced chemical additives was obtained both in the soil contaminated with heavy fuel oil and diesel fuel. It was determined that use of $KMnO_4$ as an oxidizing agent in the soil contaminated with heavy fuel oil, resulted in 3 times less statistically significant residual value of the heavy fuel oil concentration than in the control samples; however, no statistically significant difference was found between oxidizing agents (potassium permanganate and hydrogen peroxide). In cases where soil is contaminated with diesel fuel, there is a significant difference between $KMnO_4$ and H_2O_2, which shows that potassium permanganate has a bigger impact on the degradation of diesel fuel than hydrogen peroxide. The residual concentration of diesel fuel in the samples with $KMnO_4$ was 3 times less statistically significant than in the samples with H_2O_2, and 5 times less than in the control samples. The use of both – $KMnO_4$ and $MnSO_4$ – created more favourable conditions for biodegradation of diesel fuel and heavy fuel oil in the soil. A positive growth of microorganisms using Mn of different valence was observed during the entire course of experiment. Various chemical additives could be used in the technological process of biotreatment, when soil is contaminated with oil products.

Keywords: biodegradation, heavy fuel oil, diesel fuel, chemical additives, microbial communities.

1. Introduction

As the amounts of cargo handling via Klaipėda Port are increasing, the risk of accidents is increasing as well. The coastal area is especially sensitive to anthropogenic impact. Oil and oil product spills are possible in all stages of oil extraction, production and transportation. Some compounds constituting the composition of oil or oil products evaporate (Laškova *et al.* 2007; Paulauskiene *et al*. 2009), others contaminate the soil or water (Vasarevičius *et al.* 2005).

There exist a lot of technologies for the decontamination of soil from organic contaminants in the world. All of them may be divided into two types: *in situ* – when contamination is liquidated in the place of pollution – and *ex situ* – when pollutants are collected and transported to the places of treatment or buried in hazardous waste landfills. The first type of technologies are usually cheaper; however, due to the specificity of the port area, the pollutants are collected and transported to the soil treatment sites, as is the case with other industrial objects. Other technologies, such as extraction with solvents, adsorption and chemical processing, have several disadvantages, such as incomplete oil removal, expensive equipment and monitoring system requirements, high reagent or energy requirements and generation of toxic sludge or other waste products that require disposal (Liu *et al.* 2009). Different natural processes may be involved in the hydrocarbon removal of polluted areas, including evaporation, photo-oxidation and microbial degradation. However, among these, the application of biological degradation strategies for this purpose is of particular interest. The use of biological methods for cleaning oil-polluted ground is the most environmentally friendly technology available due to its lower risk, price and exploitation expenses. One of the main reasons this technology is not used more frequently is the length of time required for the biodegradation process to occur in climate with low temperatures, which cause a slowdown in the biodegradation process (Borden *et al.* 1995; Richmond *et al.* 2001;

Salminen *et al.* 2004). Biological cleaning technologies are progressing in two ways: through optimization of various biodegradation influencing factors, i.e. the increase in both quality and quantity of hydrocarbon degrading microorganisms (HDM) (Gallego *et al.* 2001; Atlas 1995; Margesin, Scinner 2001); and production of new effective bioagents (Gallego *et al.* 2006; Mishra *et al.* 2001). As discussed in the literature (Suguira *et al.* 1997; Dai *et al.* 2004), only 10–20% of microorganisms extracted from a polluted environment can effectively break down oil hydrocarbons. Therefore, to clean polluted soil, it is necessary to enrich it with selected strains of microorganisms that have high hydrocarbon degradation activity (Mannisto *et al.* 2001). Chander and Brookes (1993) used complex biocultures for hydrocarbon degradation, which were artificially made of several microorganism species that possess well-know oil oxidation properties. Yet other scientists (Oh *et al.* 1997) used naturally formed microorganism combinations, albeit without identifying the individual elements that composed them.

Despite the fact that biological treatment is the cheapest technology with the lowest environmental impact, it does have certain disadvantages. Microorganisms are active oxidizing agents of oil and oil products only in temperatures above 10 °C (Borden *et al.* 1995; Richmond *et al.* 2001). As this technology is largely dependent on the ambient air temperature, the treatment process continues for about 6 months in our climate zone. Much research is carried out with the purpose to reduce the duration of the treatment process. Most often this includes development of new efficient bioagents and optimization of various technological parameters. Acceleration of the natural process can be accomplished through artificial means, by addition of nutrients, oxygen, or other compounds to stimulate biological activity.

Earlier research has shown that small concentrations of metals may speed up the biodegradation (Zukauskaite *et al.* 2008). Chemical soil treatment methods are normally applied more often when pollutants are resistant to biodegradation (Nazina *et al.* 2008; Ferrarese *et al.* 2008), or when the concentration of a pollutant is very big (Pignatello, Chapa 2009). The use of chemical substances is expensive, and chemical oxidizing agents can destroy the soil microorganisms. However, it is an efficient method, enabling to reduce the concentration of pollutants by approx. 80% within less than 50 days (Xie, Barcelona 2003). Other advantages of chemical oxidation include formation of small amounts of waste during the process and short duration of the process (Goi *et al.* 2006).

Oxygen or some other appropriate electron acceptor must be present for microbial metabolism of organic substrates. H_2O_2, and ozone are the most widely used oxidizing agents (Goi *et al.* 2006); however, other oxidizing agents, such as $KMnO_4$, MgO_2 (Xie, Barcelona 2003; Chaliha, Bhattacharyya 2008) and Fenton's reagent (Pignatello, Chapa 2009; Lu *et al.* 2010) may be used as well.

In attempt to accelerate the process of biodegradation, research was carried out so as to combine biological treatment with chemical oxidation using small concentrations of chemicals. Chemical oxidation increases the speed of biodegradation and the efficiency of treatment as well as reduces the costs of treatment (Sutton *et al.* 2010). Two oxidizing agents – $KMnO_4$ and H_2O_2 – were chosen for the research. Another source of Mn, i.e. $MnSO_4$, was used in attempt to clear out, whether $KMnO_4$ acts as an oxidizing agent, or as a microelement speeding up the biochemical reactions.

2. Materials and methods

The soil for research was sampled from the Botanical Garden of Klaipėda University. Then it was air-dried and stored in the dark.

During the experiments, an impact of $MnSO_4$, $KMnO_4$ and H_2O_2 was analyzed. The Mn concentration was 1000 mg/kg of dry soil in both cases. Chemicals were incorporated into the samples in the form of sterilized salt solutions. The amount of hydrogen peroxide was added so that the amount of oxygen contained therein would be equivalent to the amount of oxygen contained in $KMnO_4$ (1163.6 mg/kg O of dry soil).

5 kg of soil was placed into vessels; each test was made in triplicate. In one case, soil was polluted artificially with heavy fuel oil (30 g/kg), and in another – with diesel fuel (30 g/kg). After 48 h from the beginning of the experiment, chemical substances were added into all samples, except for the control sample. The oil product destruction process in samples was observed each month with the help of IR spectrophotometric analysis. Oil products were extracted from soil with chloroform, and then a chromatographic column filled with aluminium oxide was used to separate other organic compounds. Eluate IR-radial absorption was evaluated under wave number $1/\ = 2930\ cm^{-1}$.

The impact of chemical additives on microbiological activity was studied by analysis of the samples every 30 days in order to detect the quantities of aerobic heterotrophs and HDMs. Samples were taken from several vessel places and layers: from depths of 0–5 and 5–20 cm. 1–2 g of polluted soil samples were taken using sterile tools and stored in sterile utensils. The samples were analysed on the day they were prepared.

The study was performed on aerobic heterotrophs contained in test-tubes under the sterile conditions; and the impact of chemical additives was analysed double-checking all samples. The data were compared with the averages of aerobic heterotroph concentrations in the control sample. In the experiment, two nutritive media were used. The first media consisted of: $Ca(NO_3)_2$*4 H_2O – 1.0 g, KNO_3 – 0.25 g, KH_2PO_4 – 0.25 g, K_2HPO_4 – 0.25 g, $MgSO_4$ *7 H_2O – 0.25 g, and $FeSO_4$* 7 H_2O – 0.005 g in 1 litre of distilled water. The composition of the second media was: $(NH_4)_2SO_4$ – 2.0 g, KH_2PO_4 – 1.6 g, K_2HPO_4 – 0.3 g, $MgSO_4$ 7 H_2O – 1.0 g, Na_2SO_4 10 H_2O – 0.5 g, NaCl – 0.5 g, $CaCl_2$ 6 H_2O – 0.5 g and $FeCl_3$ 1% solution – 0.5 ml in 1 litre of distilled water. The contents of the test-tubes were analysed after 4, 6 and 8 days, by taking 1 ml of the solution for each analysis. Several dilutions were prepared in the physiological saline solution before inoculating MPA (meat-

peptone agar by Carl Roth). Agar plates were incubated in triplicate at 28 °C and colony forming units (CFUs) were counted after 24 h.

To determine the quantity of HDMs, a mineral medium (Zviagincev 1991) consisting of: KCl – 0.5 g, $MgSO_4$, – 0.5 g, K_2HPO_4, – 1 g, $FeSO_4$, – 0.01 g, $NaNO_3$ – 2 g, $CaCO_3$ – 3 g and agar – 20 g in 1 litre of distilled water was used. Sterile heavy fuel oil (1% v/v) was used for a carbon source. Serial dilutions were prepared in the physiological saline solution before inoculating the media. The plates were incubated three times at 28 °C and counted after 48 h. The calculation of aerobic heterotrophs was performed accordingly (Peressutti 2003).

During the experiment, soil moisture was 10% or higher; monitoring was performed by meter KERN MRS 120–3.

Twice each month, the soil was enriched with nutritive substance: ammonium nitrate (NH_4NO_3) – 1.2 g/5 kg of soil, super phosphate ($CaH_2PO_4 \cdot H_2O \cdot 2CaSO_4$) – 0.22 g/5 kg of soil, and potassium chloride (KCl) – 0.12 g/5 kg. Chemical additives were introduced once per month. The experiment was made in natural daylight at room temperature of 18–22 °C. The soil was aerated twice a week.

Statistical analysis was made using Statgraphics Plus software. The influence of chemical substances on growth of microorganism, the rate of biodegradation process and oil products concentrations were evaluated using analysis of variance (ANOVA). The significance probability levels of the results are given at the $P < 0.05$.

3. Results and discussion

3.1. The impact of chemical additives on the residual concentrations of oil products

Large concentrations of pollutants have been chosen for testing biodegradation of oil products. When the pollution of soil is 30 g/kg and more, the duration of treatment is no less than 150–180 days. Oil products block the soil pores; it is complicated to ensure aerobic conditions and sufficient amount of microorganisms for biodegradation to occur. Positive effect of $KMnO_4$ was noticed in earlier research tests (Zukauskaite *et al.* 2008), when small amounts of metal additives were inoculated, they had an impact on both growth of HDMs and the speed of biodegradation. On the other hand, Mn salts bear the acceptor characteristics of and electron, so they may oxidize hydrocarbons at anaerobic conditions. Research was carried out using Mn (IV) for oxidation of aliphatic and aromatic hydrocarbons (Villatoro-Monzón *et al.* 2008).

The change in the concentrations of oil products is shown in Figs 1 and 2.

As previously mentioned in the experiment, heavy fuel oil and diesel fuel concentrations were the same in all treatments, i.e. 30 g/kg.

Fig. 1 illustrates that within 30 days, the addition of Mn into soil samples with heavy fuel oil and diesel fuel provide the best biodegradation conditions. The heavy fuel oil biodegradation process succeeded mostly in those samples, where $MnSO_4$ was added. Soil pollution with heavy fuel oil concentrations decreased more intensively in samples with $MnSO_4$. The least residual concentrations of heavy fuel oil were, respectively: with $MnSO_4$ – 14.49 g/kg, with $KMnO_4$ – 16.35 g/kg, while in control samples – 18.11 g/kg. Comparing the change of diesel fuel concentrations using different valences of Mn salts, we can confirm that diesel fuel was intensively decomposed in samples with added $KMnO_4$.

Our results indicate that, when using $KMnO_4$, the effectiveness of diesel fuel degradation after 120 days was 73%, while in the control samples – 62%. After $MnSO_4$ inputs were made, the effectiveness of heavy fuel oil degradation was 52%, compared with control samples with 40%.

In attempt to find out the impact of $KMnO_4$, the oxidizing effect of the material was compared with the oxidizing effect of another oxidizing agent H_2O_2. Hydrogen peroxide is usually used as an oxidizing agent both in a composition with Fenton's reagent (Pignatello, Chapa 2009) and as a separate oxidizing agent (Nazina *et al.* 2008).

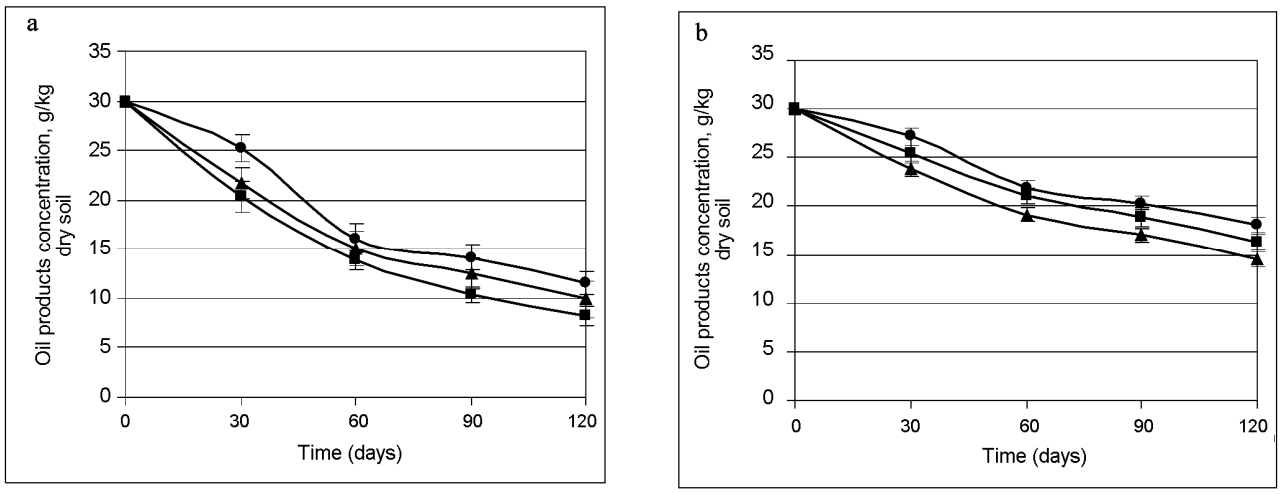

Fig. 1. Change in oil product concentration depending on time (● – control group; ■ – $KMnO_4$; ▲ – $MnSO_4$): a) diesel fuel, b) heavy fuel oil. Error bars indicate standard deviation between replicates (n = 3)

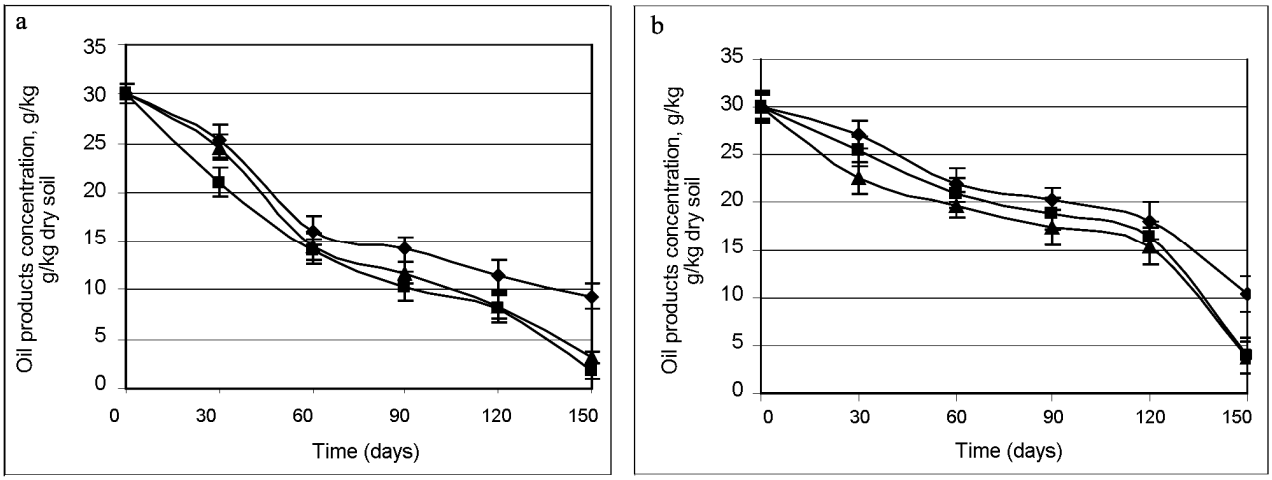

Fig. 2. Change in oil product concentration depending on time (♦ – control; ■ – $KMnO_4$; ▲ – H_2O_2): a) diesel fuel, b) heavy fuel oil. Error bars indicate standard deviation between replicates (n = 3)

The change of concentrations of diesel fuel in soil is presented in Fig. 2a, showing that concentration of diesel fuel in control samples and samples containing hydrogen peroxide was changing similarly up to day 60, and where potassium permanganate was used as an oxidizing agent, much quicker removal of pollutant from soil can be observed. The results are correlated with research carried out by other authors (Tsai, Kao 2009), who state that free OH radicals from hydrogen peroxide form better in the presence of metal. The process of degradation became slower after 90 days: the degradation of diesel fuel was almost the same in samples with oxidizing agent. However, the efficiency of potassium permanganate for the degradation of pollutants remained higher than that of hydrogen peroxide. Compared with the initial concentration of diesel fuel, we can see that the residual concentration of diesel fuel using hydrogen peroxide is 3.11 g/kg, while using potassium permanganate – 1.74 g/kg, while the value in control samples is 9.38 g/kg.

When soil is polluted with diesel fuel, a statistically significant difference ($p < 0.05$) between the control sample and samples with oxidizing agents occurs in 60 days. A statistically significant difference was determined between $KMnO_4$ and H_2O_2, which shows that potassium permanganate has a greater impact on the degradation of diesel fuel than hydrogen peroxide. The residual concentration of diesel fuel in samples with $KMnO_4$ was 3 times less statistically significant than in samples with H_2O_2, and 5 times less than in control samples.

When comparing the decrease of heavy fuel oil concentration using oxidizing agents in the course of time (Fig. 2b), it was noticed that a statistically significant difference ($p < 0.05$) occurs between control samples and samples with added oxidizing agents. Residual concentrations of heavy fuel oil were almost 3 times less statistically significant in samples with oxidizing agents than in control samples. However, there is no statistically significant difference between the oxidizing agents used (potassium permanganate and hydrogen peroxide).

A particular effect of oxidizing agents on the decrease of heavy fuel oil concentration was observed during the last month of research. If the initial concentration of heavy fuel oil was 30 g/kg, residual concentrations were, respectively: with hydrogen peroxide – 3.64 g/kg, with potassium permanganate – 3.95 g/kg, while in control sample – 10.29 g/kg (Fig. 2b).

When assessing the efficiency of degradation of oil products in different samples, it was determined that diesel fuel was degraded the fastest in samples with potassium permanganate, where efficiency of degradation was 94%, and 90% in samples with H_2O_2, when efficiency in control samples was only up to 69%. Heavy fuel oil was degraded the best in samples, where H_2O_2 was added: efficiency of degradation was up to 88%. In samples with $KMnO_4$, efficiency of degradation was 87%. In control samples – 66%.

3.2. The impact of chemical additives on the growth of microorganisms

The aim of the experiment was to identify the impact of chemical additives on both the total amount of microorganisms (aerobic heterotrophs) in soil and hydrocarbon degrading microorganisms. When analyzing the effect of Mn of different valence on the total number of microorganisms in soil, attempts were made to find out, whether the used concentrations of chemical substances do not reduce the number of microorganisms, as not only the number of HDM, but also the total vitality of microorganisms in soil affect the process of oxidation of oil products.

Fig. 3 shows that different influence of Mn salt on the increase of microorganisms in soil with heavy fuel oil is observed on day 60, and in soil with diesel fuel – on day 30.

Moreover, on day 60, in the samples of soil polluted with heavy fuel oil impacted with $KMnO_4$, the amount of aerobic heterotrophs was increasing and reached its max of 338.56 mln. col./g; a sudden decrease of the amount of microorganisms amounting to 50.59 mln. col./g was noticed in control samples and 30.52 mln. col./g in samples with $MnSO_4$. Soil polluted with diesel fuel with samples

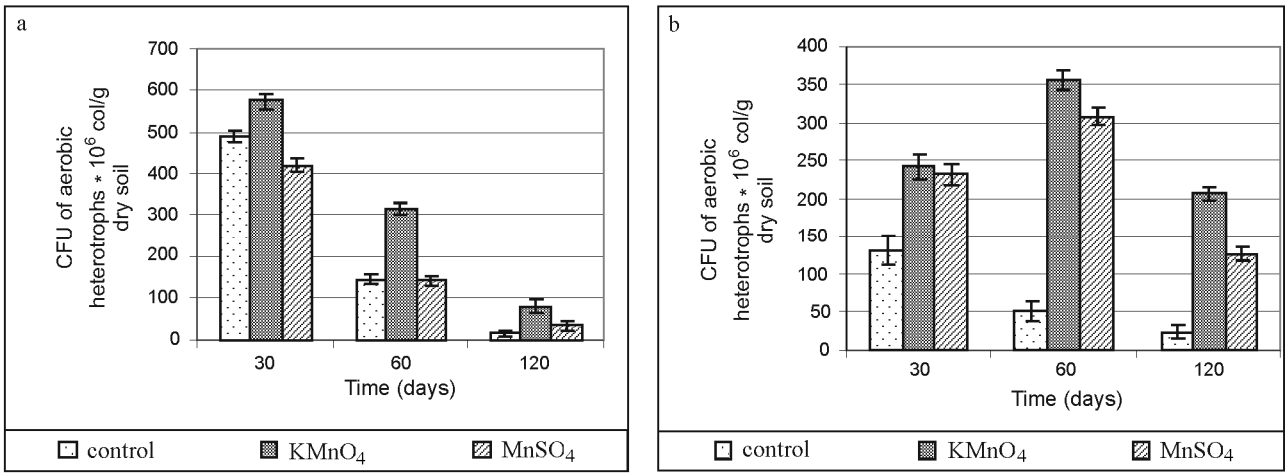

Fig. 3. Change in amounts of aerobic heterotrophs depending on time: a) diesel fuel, b) heavy fuel oil. Error bars indicate standard deviation between replicates (n = 9)

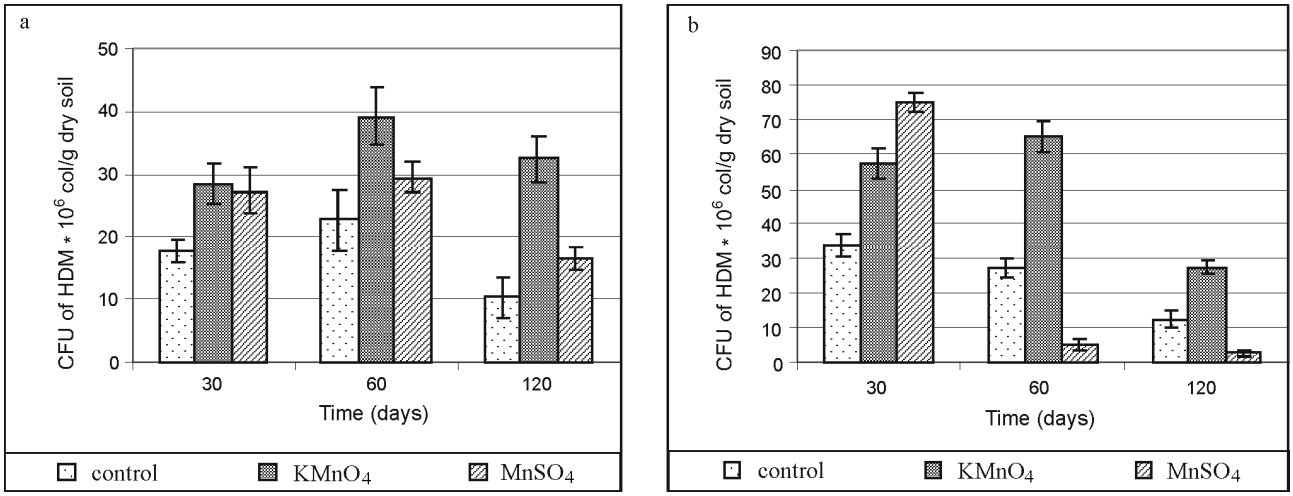

Fig. 4. Change in HDM amounts depending on time: a) diesel fuel, b) heavy fuel oil. Error bars indicate standard deviation between replicates (n = 9)

of $KMnO_4$ (on day 30 of the experiment) reached 515.24 mln. col./g. and with samples of $MnSO_4$ – 418.96 mln. col./g. However, on day 60, the higher amount of microorganisms was in the samples with $MnSO_4$. During the entire course of the experiment, positive growth of microorganism colonies was observed, when Mn salts were used, especially $KMnO_4$. Wünsche *et al.* (1995) reported that change in hydrocarbon content in soil resulted in characteristic shifts of the substrate utilization patterns by the microorganisms. Furthermore, the altered pattern of substrate utilization corresponded with similar changes in the abundance of hydrocarbon-utilizing bacteria and the occurrence of specific bacterial groups in the soils.

The concern related to the use of microorganisms as bioindicators is that changes in bacterial numbers might indicate a stimulated biodegradation process, but they do not necessarily represent an accurate measurement of the actual biodegradation (Maila 2005).

Fig. 4 shows that after 30 days in the samples of soil polluted with heavy fuel oil impacted with $MnSO_4$, the highest amount of HDM is approx. 75.20 mln. col./g; however, the amount of microorganisms decreased suddenly during the remaining time of the experiment. The biggest amount of HDM – about 64.84 mln. col./g – was indicated with $KMnO_4$ on day 60. The minimal amount of microorganisms was in the control samples. In case of soil polluted with diesel fuel, a stable increase of HDM was perceptible in all samples, especially with Mn salts. However, the amount of HDM was increasing the most effectively in samples with $KMnO_4$, just as in case with soil polluted with heavy fuel oil.

Higher growth rate of HDM with the help of Mn salts allows us to conclude that Mn acted not only as a hydrocarbon oxidizer, but also as a promoter of biodegradation.

In the study by (Chander *et al.* 1997), the fluctuation of microorganism biomass in sludge enriched with metals was discussed. Also, experiments with each of microelements (Zn, Cu, Ni, Cd) and without them were made. It was determined that higher than permissible metal concentrations resulted in negative increments of microorganism biomass.

Such results coincided with (Riis *et al.* 2002), where a negative influence of metals (Cu was among them) on microorganisms was observed. This difference was possibly observed due to different salt or metal concentrations.

Comparing the data of soil polluted with diesel fuel with the data of soil polluted with heavy fuel oil, it becomes apparent that the amount of aerobic heterotrophs in soil with diesel fuel was several times higher than the amount of aerobic heterotrophs in soil polluted with heavy fuel oil.

The obtained results demonstrate that comparing different oxidizing agents (Figs 5 and 6), $KMnO_4$ has a positive impact on the amount of aerobic heterotrophs and number of HDM.

As provided in Fig. 5, the biggest amounts of aerobic heterotrophs in all samples (in soil with diesel fuel and in soil with heavy fuel oil) were determined on day 30 of the experiment, while the number of microorganisms suddenly decreased in all of the samples on day 120.

Subsequent to statistical analysis of the data obtained during the experiment, a statistically significant difference ($p < 0.05$) was determined both in soil polluted with heavy fuel oil and soil polluted with diesel fuel, between the control samples and samples, to which oxidizing agents were added. When comparing the total growth of aerobic heterotroph colonies in samples with different oxidizing agents, a statistically significant difference ($p < 0.05$) between the oxidizing agents was also determined.

When soil is polluted with diesel fuel, a statistically significant difference between the oxidizing agents was determined on the day 30 of the experiment. Fig. 5 shows that the biggest amount of microorganisms (550.51 mln. col./g) was determined in samples, where $KMnO_4$ was added. Also, a bigger amount of microorganisms in samples containing hydrogen peroxide compared with control samples during respective periods was observed.

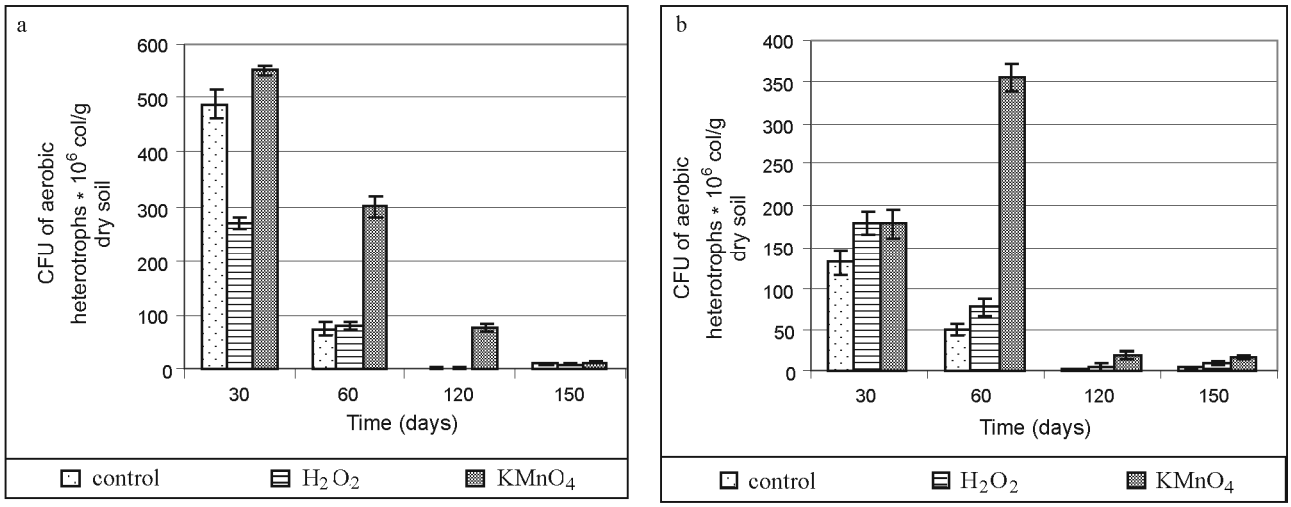

Fig. 5. Change in amounts of aerobic heterotrophs depending on time: a) diesel fuel, b) heavy fuel oil. Error bars indicate standard deviation between replicates (n = 9)

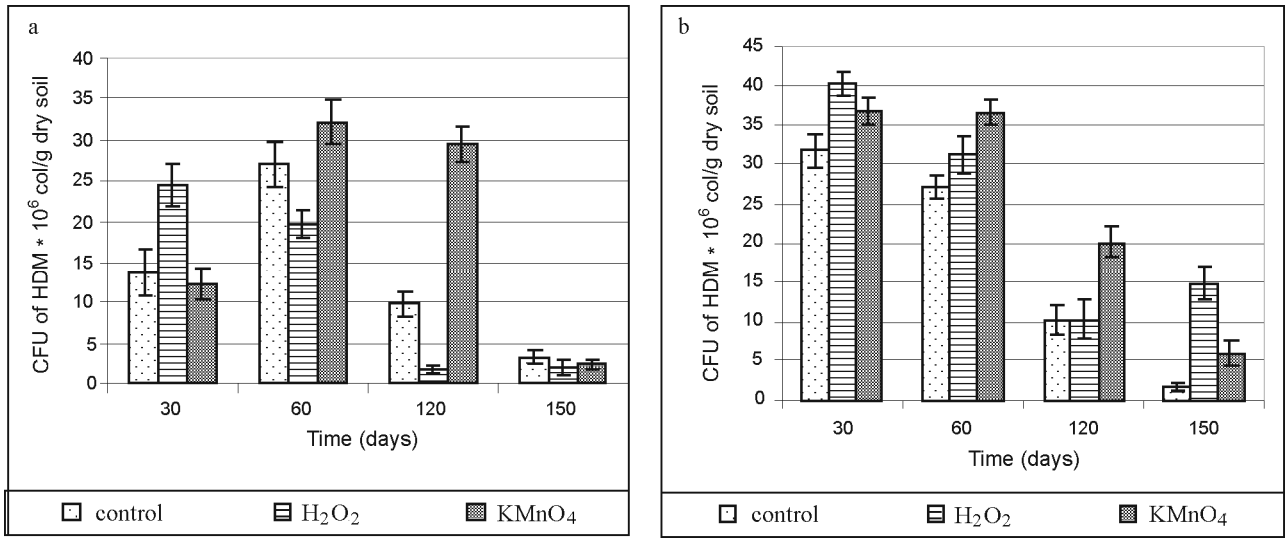

Fig. 6. Change in amounts of HDM amount during time: a) diesel fuel, b) heavy fuel oil. Error bars indicate standard deviation between replicates (n = 9)

A positive effect on the growth of microorganism colonies in the samples of soil polluted with heavy fuel oil and $KMnO_4$ additive was observed during the entire course of the experiment (Fig. 5b). Also, better results were obtained in samples with hydrogen peroxide, when comparing them with control samples. The maximum amount of microorganisms was determined on day 60 in the samples with added $KMnO_4$, and it was up to 355.13 mln. col./g. At this moment, a statistically significant difference emerges between the samples containing different oxidizing agents. It was determined that, when using $KMnO_4$ as an oxidizing agent, the value of the total amount of microorganisms on day 60 of the experiment was 4 times more statistically significant than in samples with added H_2O_2.

The results of the experiment show that potassium permanganate acted not only as an oxidizing agent, but also as a microelement having a positive effect on microorganisms.

Differently than the amount of aerobic heterotrophs, the amount of hydrocarbon degrading microorganisms grew in all samples and decreased significantly only on day 120 (150), as nutrients became insufficient for the total amount of microorganisms, so they began to die and decrease in the number of microorganisms (Fig. 6).

When researching the impact of oxidizing agents on the growth of hydrocarbon degrading microorganisms, a positive effect was observed using both potassium permanganate and hydrogen peroxide. Having made a statistical analysis of results, a statistically significant difference between control samples and samples containing $KMnO_4$ was observed.

On day 30 of the experiment, a greater amount of HDMs was determined in samples with added H_2O_2 both in soil with heavy fuel oil and diesel fuel, and a positive effect of $KMnO_4$ on the growth of microorganisms was observed during the remaining time of the experiment.

The greatest amount of HDMs in the soil polluted with diesel fuel amounting to 32.11 mln. col./g was observed in samples with added $KMnO_4$, while the greatest amount in control samples was 16.97 mln. col./g during the same time. In the soil polluted with heavy fuel oil, maximum amounts of HDMs in samples with $KMnO_4$ were 36.61 mln. col./g. The effect of H_2O_2 on the number of hydrocarbon oxidizing microorganisms was not observed after day 60.

The present research allows making a conclusion that oil products are oxidized by both $KMnO_4$ and H_2O_2. However, Mn also has an effect on the number of HDMs and acts as a catalyst in enzymatic reactions.

4. Conclusions

The experiment performed by the authors has indicated that:

1. During 120 days, it was determined that use of $KMnO_4$ as an oxidizing agent in soil polluted with heavy fuel oil results in 3 times less statistically significant residual value of heavy fuel oil concentration than in control samples; however, there is no statistically significant difference with regard to which oxidizing agent (potassium permanganate or hydrogen peroxide) should be used.

2. When soil is polluted with diesel fuel, there is a statistically significant difference between use of $KMnO_4$ and H_2O_2, which shows that potassium permanganate has a greater effect on the degradation of diesel fuel than hydrogen peroxide. After 150 days in samples with $KMnO_4$, the residual concentration of diesel fuel was 3 times less statistically significant than in samples with H_2O_2 and 5 times less than in control samples.

3. When assessing the oil degradation efficiency in different samples, it was determined that diesel fuel was degraded at the highest rate in samples with potassium permanganate, with degradation efficiency of 94% with $KMnO_4$ and 90% with H_2O_2, when the efficiency of degradation in control samples was only up to 69%. Heavy fuel oil was degraded best in samples with added H_2O_2 with the efficiency of decomposition of up to 88%. In samples, where $KMnO_4$ was added, the efficiency of decomposition was 87%. In control samples – 66%.

4. Chemical additives had a positive impact on both the total number of microorganisms and the number of oil degrading microorganisms. The positive effect of chemical additives was different dependent on the period of biodegradation. A statistically significant difference ($p < 0.05$) between control samples and samples with added oxidizing agents was determined in both soils: soil polluted with heavy fuel oil and soil polluted with diesel fuel. A statistically significant difference ($p < 0.05$) between the used oxidizing agents was also determined, when comparing the total growth of microorganism colonies in samples with different oxidizing agents.

5. In cases of considerable pollution with oil products, it is appropriate to use chemical additives, as the duration of treatment may be reduced, and it is a very significant factor in the seasonal climate areas. Different chemical additives should be considered depending on the type of pollutant.

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CHEMINIŲ PRIEDŲ ĮTAKA NAFTOS PRODUKTŲ BIODEGRADACIJOS PROCESUI

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S a n t r a u k a

Daugelis šiuolaikinių užtersto naftos produktais dirvožemio valymo technologijų vystosi kurdamos naujus ir efektyvius, naftos produktus skaidančius bioagentus. Kita naujų technologijų vystymosi kryptis – neįnešant mikroorganizmų, pagreitinti dirvožemyje jau esančių mikroorganizmų vystymąsi, paspartinant naftos produktų biodegradaciją. Dyzelino ir mazuto biodegracija buvo tirta dirvožemyje, naudojant cheminius priedus (oksidatorius). Buvo tirta H_2O_2, $KMnO_4$ ir $MnSO_4$ įtaka mazuto ir dyzelino liekamosioms koncentracijoms, bendram ir naftą oksiduojančių mikroorganizmų skaičiui dirvožemyje. Atlikus statistinę eksperimento metu gautų duomenų analizę, tiek grunte su mazutu, tiek su dyzelinu buvo nustatytas statistiškai reikšmingas ($p < 0,05$) skirtumas tarp liekamųjų naftos produktų koncentracijų tiek kontroliniuose bandiniuose, tiek bandiniuose, kuriuose buvo įterpti cheminiai priedai. Mazutu užterštame grunte nustatyta, kad kaip oksidatorių panaudojus $KMnO_4$ likutinė mazuto koncentracijos reikšmė buvo tris kartus statistiškai reikšmingai mažesnė nei kontroliniuose bandiniuose, tačiau nėra statistiškai reikšmingo skirtumo, kurį oksidatorių (ar kalio permanganatą ar vandenilio peroksidą) naudoti. Kai gruntas užterštas dyzelinu, tarp $KMnO_4$ ir H_2O_2 naudojimo yra statistiškai reikšmingas skirtumas. Tai rodo, kad kalio permanganatas turi didesnę įtaką dyzelino degradacijai negu vandenilio peroksidas. Bandiniuose su $KMnO_4$ liekamoji dyzelino koncentracija buvo tris kartus statistiškai reikšmingai mažesnė negu bandiniuose su H_2O_2 ir penkis kartus mažesnė nei kontroliniuose bandiniuose. Tiek bendrą, tiek naftą oksiduojančių mikroorganizmų skaičių teigiamai paveikė cheminiai priedai. Tiek grunte su mazutu, tiek su dyzelinu buvo nustatytas statistiškai reikšmingas ($p < 0,05$) skirtumas tarp mikroorganizmų kiekio kontroliniuose bandiniuose ir bandiniuose, į kuriuos buvo įterpti oksidatoriai. Tiek $KMnO_4$, tiek $MnSO_4$ naudojimas sudarė geresnes sąlygas biodegraduoti dyzelinui ir mazutui dirvožemyje. Viso eksperimento metu stebėtas teigiamas mikroorganizmų augimas, naudojant skirtingo valentingumo Mn (druskas). Įvairūs cheminiai priedai galėtų būti naudojami biovalymo technologiniame procese, kai dirvožemis yra užterštas skirtingais naftos produktais.

Reikšminiai žodžiai: dirvožemio valymo technologijos, biodegradacija, mazutas, dyzelinas, cheminiai priedai, mikrobiologinis aktyvumas.

ВЛИЯНИЕ ХИМИЧЕСКИХ ДОБАВОК НА ПРОЦЕСС БИОДЕГРАДАЦИИ НЕФТЕПРОДУКТОВ

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Р е з ю м е

Большинство современных технологий по очистке грунта от нефтепродуктов развиваются в направлении создания новых, более эффективных биоагентов, способствующих деструкции нефтепродуктов в грунте. Альтернативное направление развития данных технологий заключается в ускорении размножения микроорганизмов, присутствующих в грунте, и тем самым ускорении биодеградации нефтепродуктов. Исследование биодеградации дизельного топлива и мазута в грунте проводилось при использовании химических добавок (оксидаторов). Проводилось исследование влияния H_2O_2, $KMnO_4$ и $MnSO_4$ на изменение конечной концентрации дизельного топлива и мазута, а также на изменение общего количества нефтеусваивающих микроорганизмов. Статистический анализ экспериментальных данных показал, что в грунте с мазутом, так же, как и в грунте с дизельным топливом, существует статистически значимая разница ($p < 0,05$) между концентрациями нефтепродуктов в контрольных пробах и в пробах грунта с содержанием химических добавок. Установлено, что в грунте, загрязненном мазутом, во время использования оксидатора $KMnO_4$ значение конечной концентрации нефтепродукта было в 3 раза статистически менее значимое, чем в контрольных пробах, однако нет статистически значимой разницы, какой оксидатор использовать – H_2O_2 или $KMnO_4$. В случае загрязнения грунта дизельным топливом было установлено, что между $KMnO_4$ и H_2O_2 существует статистически значимая разница, что позволяет утверждать, что $KMnO_4$ имеет большее влияние на процесс деградации нефтепродукта, чем H_2O_2. В пробах с $KMnO_4$ статистически значимая разница конечной концентрации дизельного топлива в грунте была в 3 раза меньше, чем с H_2O_2, и в 5 раз меньше, чем в контрольных пробах. Химические добавки положительно воздействовали и на общее количество микроорганизмов, и на количество нефтеусваивающих микроорганизмов. Использование $KMnO_4$ и $MnSO_4$ способствовало улучшению условий биодеградации дизельного топлива и мазута в грунте. Во время всего эксперимента, используя соли Mn разной валентности, наблюдался постоянный рост количества микроорганизмов. На основании результатов данного исследования можно рекомендовать различные анализированные добавки к использованию в технологическом процессе биологической очистки грунта от различных нефтепродуктов.

Ключевые слова: технологии по очистке грунта, биодеградация, дизельное топливо, мазут, химические добавки, размножение микроорганизмов.

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