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Effect of remediation strategies on biological activity of oil-contaminated soil - A field study

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ABSTRACT

The effects of oil contamination and different remediation strategies (natural attenuation, biostimulation, and bioaugmentation) on physico-chemical and biological parameters of podzolic soil were studied. The relationships between petroleum hydrocarbons, total organic carbon, nutrients, basal respiration and enzymatic activity (dehydrogenase, catalase and urease) were evaluated in soil over a 9-year period. The principal component analysis indicated that hydrocarbons were mainly responsible for changing metabolic activity for all treatments. Dehydrogenase activity was the most sensitive biological indicator with greater levels in unpolluted soil than those recorded in contaminated soil under all remediation strategies. The activity of urease was not directly correlated with oil degradation, while the relationships of catalase and respiration rate with petroleum hydrocarbons were dependent on method of remediation. Although both biostimulation and bioaugmentation had a positive influence on the biological activity of soil and its physicochemical properties, the considerable part of decontamination could be attributed to degradation activities of indigenous microorganisms. The addition of oil-degrading bacteria (bioaugmentation) enhanced biodegradation rates only temporarily indicating that biostimulation is a better remediation strategy for podzolic soil in the field.

1. Introduction

Oil pollution has become one of the acute global environmental problems. Huge quantities of crude and refined oil that are produced and transported over long distances are associated with increased contamination by petroleum and its derivate products (Wolińska et al., 2016). Oil spills in particular affect large areas of land, rivers, lakes and seas (Kimes et al., 2014; Riveroll-Larios et al., 2015). Petroleum oil enters the environment through different routes including leakages from wellheads, pipelines, and underground storage tanks, incorrect disposal of petroleum wastes, drilling operations and many other ways.

Oil spills on land affect whole ecosystems, changing vegetation, wildlife, microbial processes, soil characteristics and overall soil health. Ecological impact of oil on the functioning of soils is most clearly seen through the change in the activity of soil microorganisms and enzymes (Li et al., 2007; Silva-Castro et al., 2015), thus making soil microbial activity a sensitive biological and biochemical indicator of soil quality (Margesin et al., 2000; Kaczyńska et al., 2015). At the same time, the ability of microorganisms to metabolize petroleum hydrocarbons has successfully been used for the decontamination of oil-polluted areas as one of the most environmentally friendly and versatile approaches (Bento et al., 2005; Silva-Castro et al., 2013).

Numerous studies have demonstrated high efficiency of bioremediation in the purification of oil polluted soils (Zhao et al., 2011; Dias et al., 2012; Souza et al., 2014). Since remediation technologies affect not just the oil concentration, but the enzymatic response (Margesin et al., 1999; Gong, 2012), enzyme activity is a sensitive indicator used for evaluation and monitoring of bioremediation process (Dawson et al., 2007; Medvedeva et al., 2010). Enzymes that were found useful for monitoring hydrocarbon removal include soil dehydrogenases, catalases and ureases (Maila and Cloete, 2005; Polyak and Bakina, 2015; Wu et al., 2016).

The success of bioremediation depends on the conditions that influence oil biodegradation: soil type and characteristics, microbial activity, humidity, temperature, pH, availability of oxygen, nutrients and oil concentration (Suja et al., 2014; Varjani and Upasani, 2017). However, these data come mostly from laboratory experiments, performed under controlled conditions, while long-term field

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bioremediation experiments are rare (Ouyang et al., 2005; Couto et al., 2010; Das and Chandran, 2010).

There are inherent limitations, associated with laboratory studies. The duration of experiment is among the main differences between studies carried out under laboratory and field conditions. Mostly, the former are focused on the short-term effects, seen after a few weeks or even days of contamination, while the study period of several years is uncommon (Li et al., 2007; Colla et al., 2014). The properties of the soil change dramatically during long-term incubation in the laboratory, thus complicating interpretation of any observed effects. Additionally, laboratory experiments cannot reproduce the changes which take place under natural conditions with time. Overall progress in developing effective bioremediation strategies depends on achieving as good results in the field as in the laboratory, which in itself is a complicated problem (Bento et al., 2005).

In this study, a long-term field experiment was performed to evaluate the ecological effects of petroleum contamination on soil biological activity over nine years and to compare two remediation methods. We tested bioremediation by bioaugmentation (introduction of hydrocarbon-degrading microorganisms) and biostimulation (introduction of additional nutrients into the soil, in order to enhance the activity of indigenous degraders), and compared them with natural attenuation. In the present study, we analyzed the interplay of environmental and biological factors involved in decontamination to better understand the response of the soil microbiota to various remediation strategies.

2. Materials and methods

2.1. Study site

The experiment was started in June 2006 and continued until October 2015 at the trial field near St. Petersburg, Russia (59°44′34″N, 30°22′49″E). This area has a humid continental climate with the average annual temperature, precipitation and air humidity of 5.8 °C, 660 mm and 78%, respectively. The soil moisture in the area is mostly high, since evapotranspiration is low due to the cool climate. The soil itself is a loamy podzol (48.1% sand, 30.3% clay, 21.6% silt) with pH_{H2O} of 6.81, 2.9% organic carbon, and 175 and 250 mg kg^{-1} available P and K, respectively.

2.2. Experimental design

Four square plots (8 m^2 each) were established separately in the flattened part of the study area. The first plot served as an uncontaminated control (1), and three were treated with crude oil (10 L m^{-2}) to test: natural attenuation (2), biostimulation by adding nutrients (3) and bioaugmentation in the presence of added nutrients (4). For bioaugmentation we used a commercial bacterial product. For biostimulation, and to supplement bioaugmentation, we added mineral amendments (30 g m^{-2} K_2O, 30 g m^{-2} P_2O_5 и 25 g m^{-2} N_20). Biopreparation and nutrients were added to a depth of 0–20 cm.

Soil samples were collected one week and three month after the contamination, and later on annually at the end of September. Each plot was divided into 4 subplots of 2 m^2 and 10 coring sites were chosen within the subplot with a method of randomized repetitions (Dospehov, 1985). We took samples with a soil corer (20 cm diameter), pooled and sieved (< 2 mm) all ten soil cores, collected from each subplot. This combined sample represented one replicate, so that there were four replications from each plot. The total weight of a combined sample was 900 g.

2.3. Physical and chemical analyses

Chemical and physical characteristics of the collected samples were determined according to Arinushkina (1970). Total petroleum hydrocarbons (TPH) were extracted from soil samples with hexane and measured according to PND F 16.1:2.21–98 (2012). TPH were determined by UV fluorescence spectroscopy by using a FLUORAT-02-3M spectrofluorometer (Nordinkraft-Sensor, Russia). The fluorescence of the samples was measured at 270 nm excitation and at 320 nm emission wavelengths.

2.4. Soil biological activity

We measured soil basal respiration (BR) using alkali absorption method. Moist samples (50 g dry weight equivalent) were adjusted to 60% of field holding capacity and pre-incubated at 25 °C for 14 days. Following incubation each soil sample was spread on the bottom of a 500-ml glass jar and reaction container with 20 ml NaOH (0.02 N) solution was suspended inside the jar, above the soil. After incubation at 25 °C for 24 h 2 drops of phenolphthalein indicator were added into the reaction containers, and then titrated with 0.02 N H_2SO_4. The jars without soil served as the controls. The difference in the consumed volume of H_2SO_4 between the treatment and control was used to calculate the quantity of CO_2 emission from soil microorganisms. The quantity of CO_2 (the ratio of respiration) was calculated as μg CO_2-C g^{-1} h^{-1}.

We determined three types of soil enzymatic activity (dehydrogenase, catalase and urease) using the methods described by Haziev (2005) with little modification. Dehydrogenase activity (DA) was analyzed via the reduction of the 2,3,5-triphenyltetrazolium chloride (TTC) to a red-colored 2,3,5-triphenyl formazan (TPF) with 1% (w/v) glucose solution added as a source of organic carbon. After incubation period of 24 h at 30 °C, we extracted TPF with methanol and measured the optical density of solution at 484 nm (spectrophotometer Genesys 10 UV, Thermo Spectronic, USA). The latter values served to determine the TPF concentration. Dehydrogenase activity was expressed as mg TPF g^{-1} day^{-1}.

The activity of catalase (CA) was determined following Johnson and Temple method (1964) by back-titrating residual H_2O_2 with $KMnO_4$. The mixture of 2 g of dry soil and 0.3% hydrogen peroxide solution was shaken at 120 rpm for 20 min and then 1.5M H_2SO_4 was added. Afterwards the solution we filtered and titrated the liquid using 0.1M $KMnO_4$. The result was expressed as ml $KMnO_4$ g^{-1}.

To measure urease activity (UA) we followed method described by Haziev (2005) with urea used as a substrate. We mixed 5 g of dry soil and 1 ml of methylbenzene in a 150-ml conical flask. After 15 min, 10 ml of 10% urea and 20 ml of citrate buffer (pH = 6.7) were added to the sample. The mixture was incubated at 37 °C for 24 h and filtered. One ml of filtrate was transferred into a volumetric flask, mixed with 4 ml of sodium phenoxide, 3 ml of sodium hypochlorite and diluted to 50 ml. Absorbance was determined at 578 nm using a spectrophotometer Genesys 10 UV (Thermo Spectronic, USA). The urease activity was expressed as μg NH_3-N g^{-1} h^{-1} at 37 °C.

2.5. Data analyses

We compared variables describing soil conditions through the duration of the experiment, as well as across the treatment types. Since empirical distributions of the measured variables were non-normal and displayed pronounced heteroscedasticity, we employed non-parametric methods of statistical testing. Wilcoxon signed rank test was used to compare central tendencies of paired data. For all variable pairs we calcualted Spearman rank order correlation coefficients with Tukey's HSD correction. To study the relationship between biological parameters and soil physicochemical properties, we used principal component analysis (PCA). Differences were considered significant at $p < 0.05$. Statistical analyses were performed using Statistica software (version 10; Statsoft).

Table 1
The main agrochemical properties of oil-contaminated soil throughout the experiment (9 years).

Remediation strategy	Initial time	1 year	2 years	3 years	8 years	9 years
pH						
Control	6.81 ± 0.05	6.25 ± 0.03	6.40 ± 0.05	6.29 ± 0.04	6.41 ± 0.03	6.37 ± 0.02
Natural attenuation	5.86 ± 0.02	5.82 ± 0.02	5.83 ± 0.05	6.02 ± 0.05	6.41 ± 0.02	6.34 ± 0.04
Biostimulation	5.73 ± 0.11	5.82 ± 0.08	5.84 ± 0.04	5.96 ± 0.04	6.36 ± 0.02	6.33 ± 0.02
Bioaugmentation	6.65 ± 0.12	6.57 ± 0.03	6.73 ± 0.03	6.69 ± 0.05	6.51 ± 0.02	6.42 ± 0.03
Hydrolytic acidity (HA), cmol kg^{-1}						
Control	3.4 ± 0.1	2.7 ± 0.1	3.2 ± 0.1	4.4 ± 0.3	3.5 ± 0.0	3.8 ± 0.2
Natural attenuation	3.2 ± 0.1	3.3 ± 0.1	3.7 ± 0.1	5.0 ± 0.2	3.5 ± 0.1	3.6 ± 0.2
Biostimulation	3.0 ± 0.4	3.8 ± 0.2	4.4 ± 0.4	5.0 ± 0.3	3.6 ± 0.2	3.9 ± 0.3
Bioaugmentation	5.7 ± 0.4	1.7 ± 0.1	1.9 ± 0.1	3.3 ± 0.2	2.8 ± 0.2	3.4 ± 0.2
Sum of exchangeable bases (SEB), cmol kg^{-1}						
Control	13.9 ± 0.4	13.6 ± 1.3	14.8 ± 0.8	13.5 ± 0.8	14,4 ± 1,0	14,1 ± 0,8
Natural attenuation	11.3 ± 0.8	12.6 ± 0.4	12.9 ± 0.6	12.7 ± 0.5	15,6 ± 0,6	14,8 ± 0,4
Biostimulation	11.0 ± 0.5	11.3 ± 0.4	12.9 ± 0.4	12.4 ± 0.5	14,9 ± 0,4	13,5 ± 0,6
Bioaugmentation	17.8 ± 0.7	26.1 ± 2.3	24.1 ± 1.7	21.8 ± 2.2	18,5 ± 1,0	15,1 ± 0,9
Total organic carbon (TOC), %						
Control	2.61 ± 0.12	2.76 ± 0.20	2.64 ± 0.18	2.69 ± 0.06	2.70 ± 0.15	2.80 ± 0.21
Natural attenuation	7.49 ± 0.10	6.85 ± 0.41	6.72 ± 0.43	6.11 ± 0.12	5.24 ± 0.07	5.16 ± 0.17
Biostimulation	7.24 ± 0.32	5.98 ± 0.21	5.96 ± 0.33	5.90 ± 0.35	5.14 ± 0.20	5.06 ± 0.23
Bioaugmentation	6.83 ± 0.17	5.90 ± 0.18	5.71 ± 0.11	5.50 ± 0.28	5.23 ± 0.22	5.23 ± 0.09
Available potassium (AK), mg kg^{-1}						
Control	198 ± 8	197 ± 11	174 ± 6	nd	178 ± 10	198 ± 7
Natural attenuation	161 ± 9	154 ± 6	160 ± 5	nd	169 ± 4	184 ± 9
Biostimulation	324 ± 19	204 ± 11	197 ± 13	nd	182 ± 7	184 ± 12
Bioaugmentation	387 ± 21	22,3 ± 10	208 ± 16	nd	186 ± 9	199 ± 11
Available phosphorus (AP), mg kg^{-1}						
Control	143 ± 6	135 ± 7	125 ± 12	nd	118 ± 9	139 ± 12
Natural attenuation	108 ± 4	124 ± 13	115 ± 6	nd	128 ± 13	141 ± 15
Biostimulation	298 ± 28	220 ± 24	164 ± 14	nd	156 ± 15	183 ± 18
Bioaugmentation	345 ± 29	249 ± 11	230 ± 20	nd	161 ± 12	135 ± 15

3. Results

3.1. Soil chemical properties

Dynamics of the main soil characteristics is shown in Table 1. At the beginning of the experiment, control and oil-contaminated soils had pH values of 6.8 and 5.8 respectively (Table 1). There was no difference in pH between soils under natural attenuation and biostimulation (5.73–5.86), while in soils under bioaugmentation pH was significantly higher (6.65, $p < 0.05$). The pH of soil under bioaugmentation remained nearly neutral during the whole experiment (pH range 6.5–6.7). In soils under natural attenuation and biostimulation pH decreased to 5.8 after contamination and stayed in this level over a period of a few years. After 8 years of experiment, all soils showed a similar pH of 6.4.

A limiting condition in biological decontamination is the availability of nutrients, especially of nitrogen and phosphorus. We found that oil contamination itself decreased the concentration of available phosphorus (AP) in the soil by 25% (Table 1). This reduced concentration remained unchanged for the first year, but gradually increased to control values thereafter.

The increase in soil available phosphorus was observed when bioremediation treatments were applied. Available phosphorus concentrations increased from initial value of 143 mg kg^{-1} to 298 and 345 mg kg^{-1} under biostimulation and bioaugmentation, respectively. After two years of experiment the concentrations dropped to 164 and 230 mg kg^{-1}, respectively and reached control values after nine years of experiment.

The unpolluted soil (control) had high concentration of mineral nitrogen ($N\text{-}NH_4$ and $N\text{-}NO_3$). The initial $N\text{-}NH_4$ was 40 mg kg^{-1} in unpolluted soil and polluted soil, and remained relatively stable in soil under natural attenuation during the whole experiment (Fig. 1a). In soils under bioaugmentation and biostimulation, the $N\text{-}NH_4$ content increased by 50%, reaching 60 mg kg^{-1} after three month. However, this increased $N\text{-}NH_4$ concentration was short-lived – it persisted only for the first few months, thereafter decreasing to control values.

The $N\text{-}NO_3$ content of all soil samples was 51–56 mg kg^{-1} at the start (Fig. 1b). This concentration remained at the same level in unpolluted soil, but grew by 250% in soil under bioaugmentation and decreased by 80–85% under natural attenuation and biostimulation. After 9 years of experiment the $N\text{-}NO_3$ content remained the same (58 mg kg^{-1}) in unpolluted soil, but only reached 13–23 mg kg^{-1} in oil-contaminated soils.

3.2. TPH biodegradation

The effect of natural attenuation and two bioremediation strategies on TPH biodegradation is shown in Table 2 and Fig. 2. Over a period of nine years the initial oil contamination of 53.7 g kg^{-1}, expressed in TPH concentration, was reduced to 4.7 g kg^{-1} (Fig. 2), which corresponds to a decontamination of 91.2% (Table 2). The final level of decontamination was similar for all three experimental conditions and was due to biodegradation activity of both the indigenous microorganisms and the added inoculum.

Biodegradation processes initiated shortly after contamination and continued for nine years. The rate of decontamination was the fastest in the first year, but slowed down later, although both biostimulation and bioaugmentation provided a short-term increase in the rate of TPH degradation. After the first year under natural attenuation TPH concentration decreased to 38.5 g kg^{-1}, which translates to 28.3% of degradation. By contrast, concentration of TPH reached 30.9 g kg^{-1} and 27.9 g kg^{-1} (39.3 and 39.9% degradation) in soils exposed to biostimualtion and bioaugmentation, respectively. The highest rate of degradation of TPH (29%) was observed under bioaugmentation after 3 months of experiment (data not shown).

After the second year biodegradation of TPH reached 61.0% and 55.1% in soils exposed to bioremediation and natural attenuation, respectively (Table 2). From the third year the level of oil degradation was similar in all treatments. At the end of the study 90–91% of initial TPH were biodegraded in all three conditions.

Mass balance of petroleum hydrocarbons was performed to evaluate

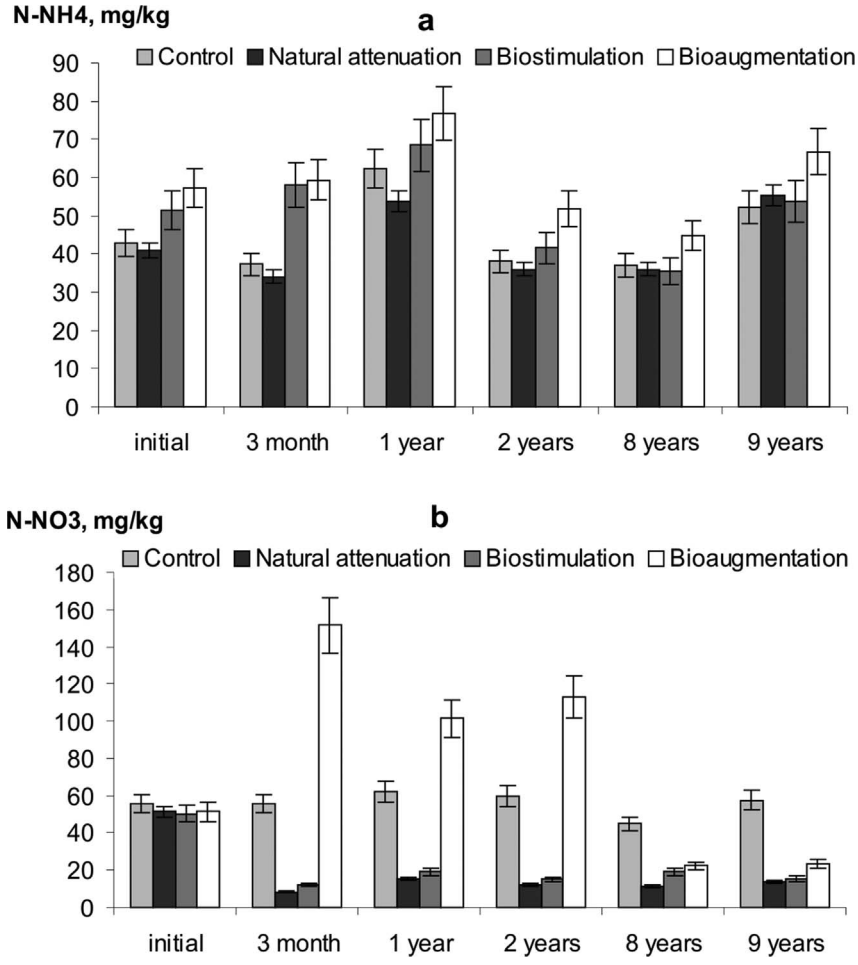

Fig. 1. Changes in nitrogen concentrations of oil-contaminated soil exposed to different remediation strategies: (a) $N\text{-}NH_4$; (b) $N\text{-}NO_3$.

Table 2
The effect of remediation strategies on hydrocarbon movement in soil over time.

Time, years	% of TPH recovered			% of TPH remaining in soil		
	NA	BS	BA	NA	BS	BA
1	28.3	39.3	39.9	71.7	60.7	60.1
2	55.1	61.5	61.0	44.9	38.5	39.0
3	67.6	67.8	69.2	32.4	32.2	30.8
4	74.7	76.4	76.3	25.3	23.6	23.7
5	81.4	82.7	83.2	18.6	17.3	16.8
6	85.1	86.2	86.2	14.9	13.8	13.8
7	88.6	88.4	88.3	11.4	11.6	11.7
8	90.5	90.4	89.0	9.5	9.6	11.0
9	91.2	91.2	89.4	8.8	8.8	10.6

TPH, total petroleum hydrocarbons; NA, natural attenuation; BS, biostimulation; BA, bioaugmentation.

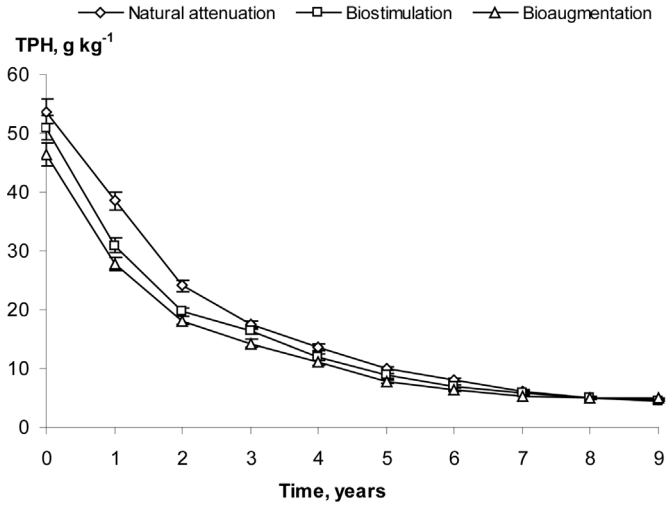

Fig. 2. Biodegradation of total petroleum hydrocarbons in oil-contaminated soil exposed to different remediation strategies.

the fate of TPH during remediation. Hydrocarbon degradation for different remediation strategies was assessed as carbon dioxide production (mineralization), quantity of TPH remaining in soil (TPH_r), and loss of volatile and other compounds due to the combined result of evaporation, photooxidation, and chemical oxidation (TPH_p).

The values of CO_2 emission (excluding basal respiration due to soil organic matter decomposition) obtained for contaminated soil samples were used to calculate the hydrocarbon loss due to microbial oxidation (TPH_m). The amount of hydrocarbons evaporated and photo- and chemically oxidized (TPH_p) was determined by calculating the difference between the initial hydrocarbon content and the sum of TPH_m and TPH_r.

Fig. 3 shows the removed amounts of petroleum hydrocarbons based on remediation mass balance. Mineralization was the smallest of the fractions shown in mass balance in soil under natural attenuation. For the 3-month period, only 5.3% of TPH loss was due to mineralization, and 8.1% due to combined effect of chemical and physical processes. Biostimulation showed higher oil decontamination due to microbial oxidation (15.7%). In soil under bioaugmentation, the percentage mineralizied was 3.3 times higher (17.5%) than in soil under NA. There was no difference in TPH_p loss between soils under natural

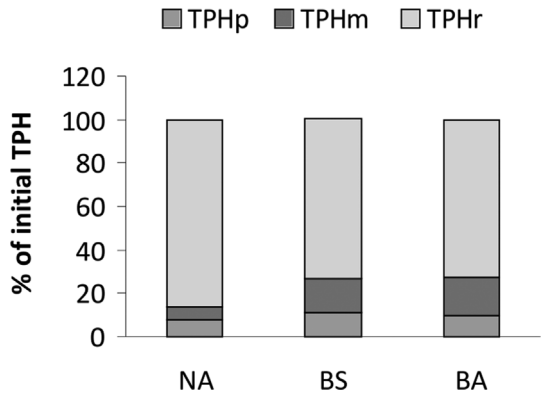

Fig. 3. Mass balance of petroleum hydrocarbons after 3 month of remediation: TPH_p - the combined result of photo-oxidation, chemical oxidation, and evaporation; TPH_m – result of microbial oxidation (mineralization); TPH_r - petroleum hydrocarbons remaining in soil.

attenuation, biostimulation, and bioaugmentation.

3.3. Basal respiration

Fig. 4 presents the data on basal respiration (BR) obtained during the experiment. Within the first week since the introduction of oil, BR increased in all samples. The increase was moderate under natural attenuation (14.5 μg CO_2 g^{-1} h^{-1}; 1.3 times higher than control) and more pronounced in soils under biostimulation and bioaugmentation (51.3 and 70.9 μg CO_2 g^{-1} h^{-1}; 5 and 7 times higher than control). After three months, basal respiration decreased sharply to 17 μg CO_2 g^{-1} h^{-1}, (2 times above control) in soils subjected to biostimulation and bioaugmentation. Soil samples under natural attenuation displayed a more prolonged period of stimulation, with basal respiration growing for the whole first year (up to twice the control level). However, during the second year these samples also showed a downward trend.

In all treatments, basal respiration recovered from stimulative effect, and reached the control level of basal respiration after two years. No significant differences in BR between treatments and control ($p > 0.05$) were found later.

3.4. Soil enzymatic activities

Initial values of DA varied between experimental plots, with the highest level (122% compared to control) found under conditions of bioaugmentation. We found that dehydrogenase activity levels were sensitive to the oil contamination and bioremediation treatments (Fig. 5). At the onset of the experiment, DA abruptly decreased. After the first week the value of DA fell to 68% compared to control in the soil under conditions of natural attenuation and to 43% in the soil under biostimulation. DA continued to drop during the first three month, until its values approached 8–17% compared to control. Later dehydrogenase activity grew steadily. After a year it increased to 20–26% of control values. After two years, soil under bioaugmentation supported the fastest growth (77% compared to control), while in other samples DA levels were more modest (33–48%). At the end of the experiment DA was similar in all contaminated soils, although still significantly lower ($p < 0.05$) than in control (63%).

There was a marked difference in catalase activity response to different treatment types. Natural attenuation showed the least CA: after initial decrease, catalase activity remained at the same 40% level for a period of three years. Later it grew slowly to reach 74% of the uncontaminated level. Bioaugmentation stimulated soil catalase activity (125%) during the first week, but after three month it returned to the control value and remained at this level for the first year. In the following years it fluctuated at about 70% of control value. Under conditions of biostimualtion CA did not experience an initial surge, but here, too, it remained at control levels during the first year and fell to about 60% in the following years. By the end of the experiment, catalase activily somewhat improved, but remained significantly lower than pre-contamination levels in all three treatments ($p < 0.05$).

Activity of the urease in the soil demonstrated a more complex response to the addition of oil. Right after the contamination, its activity grew to 144% compared to control. The addition of nutrients and inoculate led to a further increase of 214 and 496% for biostimulation and bioaugmentation, respectively. However, this initial effect was short-lived. After the first three months UA decreased to 63, 91, and 198% compared to control for natural attenuation, biostimulation, and bioaugmentation, respectively. Urease activity, detected in all three treatment types, remained below control for the second and third year, but stabilized at the pre-contamination levels later. There were no significant differences ($p > 0.05$) between control and all treatments after nine years of experiment.

3.5. Statistical analyses

Matrices of correlations between biological, physical and chemical properties of the samples, one for each of the remediation strategies, are given in the Table 3. Several of the studied physicochemical and biological parameters significantly correlated with the hydrocarbon content. We found strong negative correlation between TPH and dehydrogenase activity, and strong positive correlation of TPH with soil basal respiration. It was particularly high in soils under biostimulation and bioaugmentation (Table 3).

Under these treatments, basal respiration was positively correlated with available phosphorus and potassium ($p < 0.05$). In soil under bioaugmentation, urease activity also had a strong positive correlation with AP and AK, whereas in soils under biostimulation this correlation was weak.

In samples under natural attenuation we found a different set of

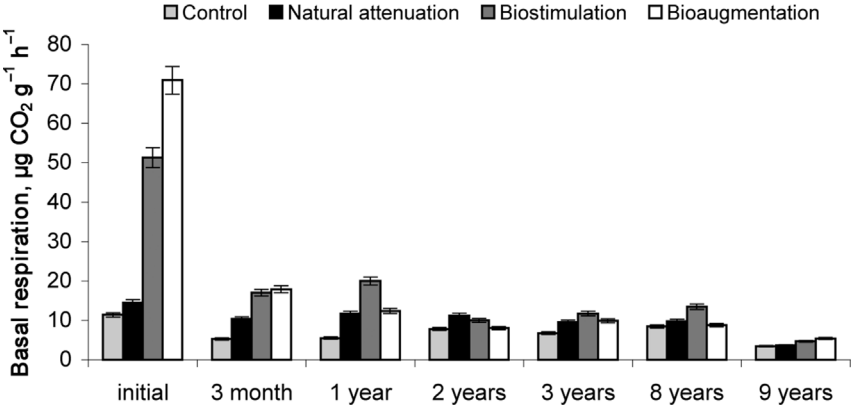

Fig. 4. Basal respiration in oil-contaminated soil exposed to different remediation strategies.

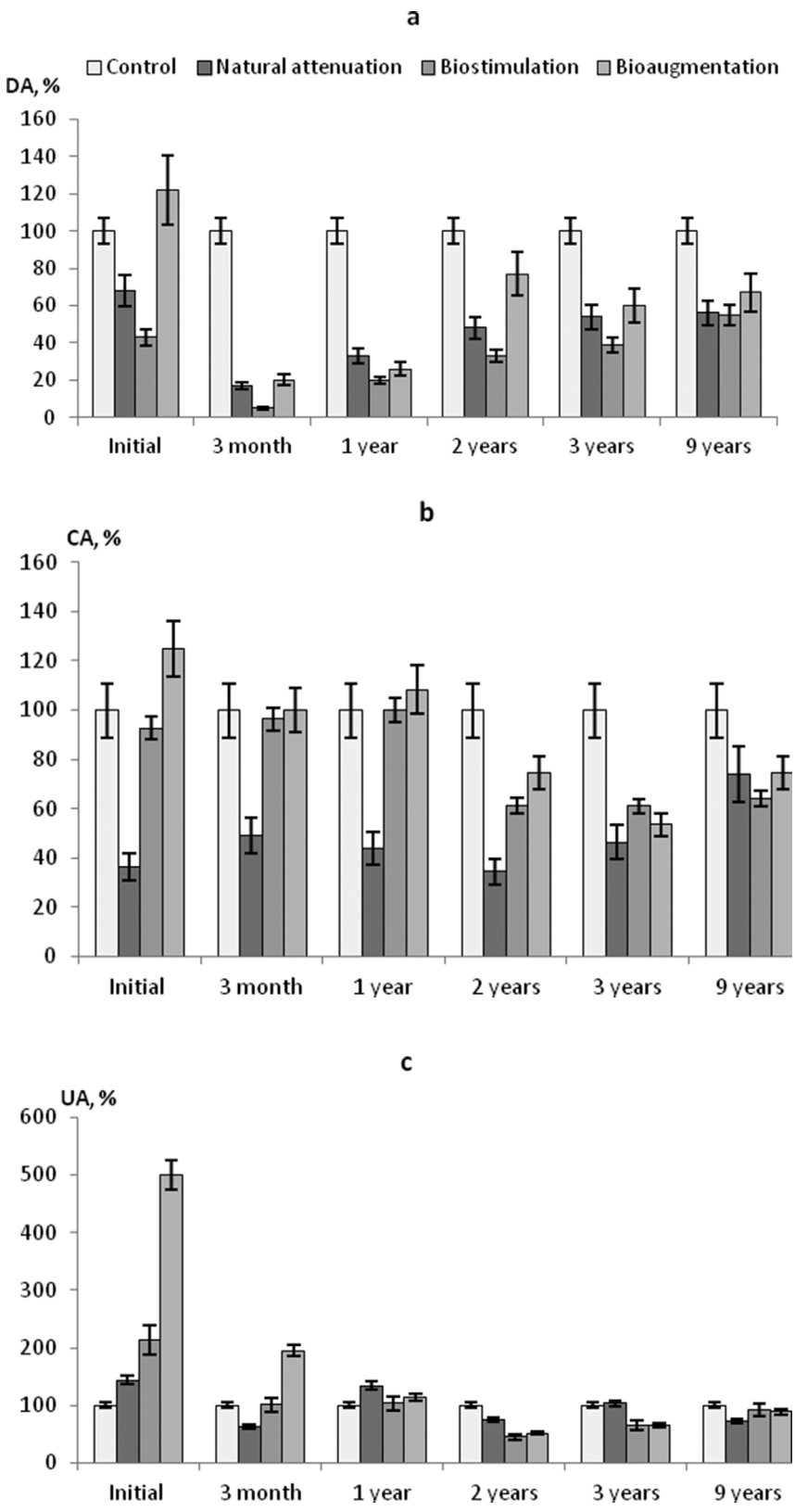

Fig. 5. Enzymatic activities in oil-contaminated soil exposed to different remediation strategies (natural attenuation, biostimulation, bioaugmentation): dehydrogenase (a), catalase (b), urease (c).

relationships. Soil N-NO_3 had a strong positive correlation with BR (0.86), but strong negative relation with soil dehydrogenase (−0.89). Dehydrogenase activity was positively correlated with available phosphorus and potassium in soils under natural attenuation ($p < 0.05$). By contrast, there was no relationship between biological parameters and N-NO_3 in samples subjected to bioremediation.

N-NH_4 content had a strong positive relationship with urease activity in unpolluted samples (0.83), but not in other types of treatment. By contrast, in soil under bioaugmentation, it was negatively correlated with DA (0.80).

When enzymatic activities were compared with each other, the only significant correlations were found between the catalase and urease in

Table 3
Spearman Rank correlations between soil biological and physicochemical properties.

	pH	HA	SEB	C	AK	AP	N-NH_4	N-NO_3	DA	CA	UA	BR
Control												
pH		0.257	0.371	**−0.700***	0.100	0.205	−0.300	−0.600	−0.429	0.543	−0.086	0.429
HA			−0.257	−0.200	−0.500	−0.462	**−0.800****	**−0.900****	0.543	0.257	−0.086	−0.143
SEB				−0.086	−0.515	**−0.662***	**−0.696***	−0.319	−0.257	0.143	**0.771***	0.126
TOC					0.205	−0.100	0.500	0.300	0.200	0.200	0.500	−0.529
AK						**0.794****	**0.638***	0.232	**−0.900****	−0.100	−0.600	0.116
AP							**0.754***	0.406	−0.359	−0.462	**−0.975****	−0.029
N-NH_4								**0.829****	−0.200	−0.700	**0.800****	−0.314
N-NO_3									0.100	**−0.900****	−0.500	−0.257
DA										−0.429	0.029	−0.404
CA											0.257	0.086
UA												−0.086
Natural attenuation												
TPH	−0.551	−0.429	**−0.929****	**1.000****	**−0.657***	**−0.886****	−0.348	0.257	−0.580	0.147	0.203	−0.406
pH		0.174	0.406	**−0.700***	**0.821****	0.205	−0.289	−0.103	−0.429	0.543	−0.086	0.429
HA			0.314	**−0.700***	−0.500	−0.300	**−0.872****	**−0.900****	0.371	−0.232	**−0.829****	−0.543
SEB				**−0.886****	0.600	**0.829****	0.145	−0.371	**0.771***	0.174	−0.029	**−0.643***
TOC					**−0.700***	**−0.900****	−0.154	**0.700***	**−0.900****	−0.359	0.300	**0.886****
AK						**0.771***	0.551	0.257	**0.700***	0.200	**0.900****	−0.486
AP							**0.667***	0.143	**0.900****	0.600	0.300	**−0.829****
N-NH_4								**0.754***	0.000	−0.300	0.600	0.257
N-NO_3									**−0.886****	−0.261	0.200	**0.857****
DA										−0.029	−0.200	−0.512
CA											0.058	−0.464
UA												0.600
Biostimulation												
TPH	**−0.943****	−0.086	**−0.786***	**1.000****	**0.943****	**0.829****	0.143	0.203	**−0.886****	0.029	0.493	**0.857****
pH		−0.200	0.543	**−1.000****	**−0.900****	**−0.900****	−0.500	0.205	**0.943****	−0.086	−0.406	**−0.714***
HA			0.257	−0.400	−0.100	−0.100	0.600	**−0.975****	−0.029	−0.257	−0.493	−0.314
SEB				**−0.886****	**−0.943****	**−0.886****	−0.371	−0.319	0.371	0.200	−0.319	−0.643
TOC					**0.900****	**0.700***	0.000	0.616	**−0.900****	−0.100	0.300	**0.771***
AK						**0.943****	0.314	0.116	**−0.900****	−0.100	0.564	**0.829****
AP							0.371	0.174	**−0.900****	−0.100	0.564	**0.771***
N-NH_4								−0.406	−0.500	−0.500	−0.462	0.029
N-NO_3									0.205	0.051	0.500	0.348
DA										−0.314	−0.551	−0.552
CA											**0.812****	0.600
UA												**0.899****
Bioaugmentation												
TPH	0.143	0.086	0.464	**0.986****	**0.943****	**1.000****	0.086	0.543	**−0.657***	0.429	**0.657***	**0.893****
pH		0.143	−0.029	0.200	0.400	0.400	0.000	0.500	0.543	−0.543	−0.429	−0.200
HA			**−0.771***	0.100	0.300	0.300	−0.500	−0.500	0.314	0.371	0.314	0.371
SEB				0.377	0.314	0.429	0.143	**0.771***	−0.486	−0.429	−0.200	0.357
TOC					**0.975****	**0.975****	0.359	0.564	−0.600	0.400	0.400	**0.783***
AK						**0.943****	0.314	0.600	−0.600	0.400	**0.700***	**0.829****
AP							0.086	0.543	−0.600	0.400	**0.700***	**0.943****
N-NH_4								0.257	**−0.800****	−0.200	0.100	0.029
N-NO_3									−0.400	−0.600	−0.200	0.314
DA										−0.543	**−0.714***	−0.466
CA											**0.943****	0.543
UA												**0.714***

TPH, total petroleum hydrocarbons; HA, hydrolytic acidity; SEB, sum of exchangeable bases; TOC, total organic carbon; AK, available potassium; AP, available phosphorus; DA, dehydrogenase; CA, catalase; UA, urease; BR, basal respiration.
Figures in bold type defines values significant correlations (level of statistical significance: **$p < 0.01$, *$p < 0.05$).

soils under biostimulation and bioaugmentation (Table 3). The latter hydrolytic enzyme also displayed strong positive correlation with the basal respiration of the soil.

Principal component analysis demonstrated that the first two principal components (PCs) accounted for only 64.5% of the variance of data (PC1: 36.9%, PC2: 27.6%, Fig. 6a) in control. In polluted soils the amount of explained variance was higher: 85.4% under biostimulation (PC1: 62.0%, PC2: 23.4%, Fig. 6c), 71.8% under bioaugmentation (PC1: 48.3%, PC2: 23.5%, Fig. 6d) and 77% under natural attenuation (PC1: 55.1%, PC2: 21.9%, Fig. 6b).

Studied variables provided dissimilar loadings on two main axes in unpolluted and polluted soils. In uncontaminated samples soil pH, SEB, total organic carbon, N-NO_3, available phosphorus, potassium, TPH, dehydrogenase activity and basal respiration were all significantly correlated with PC1 scores. Hydrolytic acidity, catalase and urease activities were significantly correlated with PC2 scores.

In soil under bioaugmentation total organic carbon, available phosphorus, potassium, TPH, urease activity and basal respiration were major contributors to PC1 while SEB and N-NO_3 were major contributors to PC2. Available phosphorus, potassium, N-NH_4, N-NO_3, and urease activity were significantly correlated with PC1 scores while soil pH, total organic carbon, dehydrogenase activity and basal respiration were significantly correlated with PC2 scores under conditions of biostimulation. Finally, in samples under natural attenuation soil pH, SEB, total organic carbon, available phosphorus, potassium, TPH, catalase activity and basal respiration were major contributors to PC1

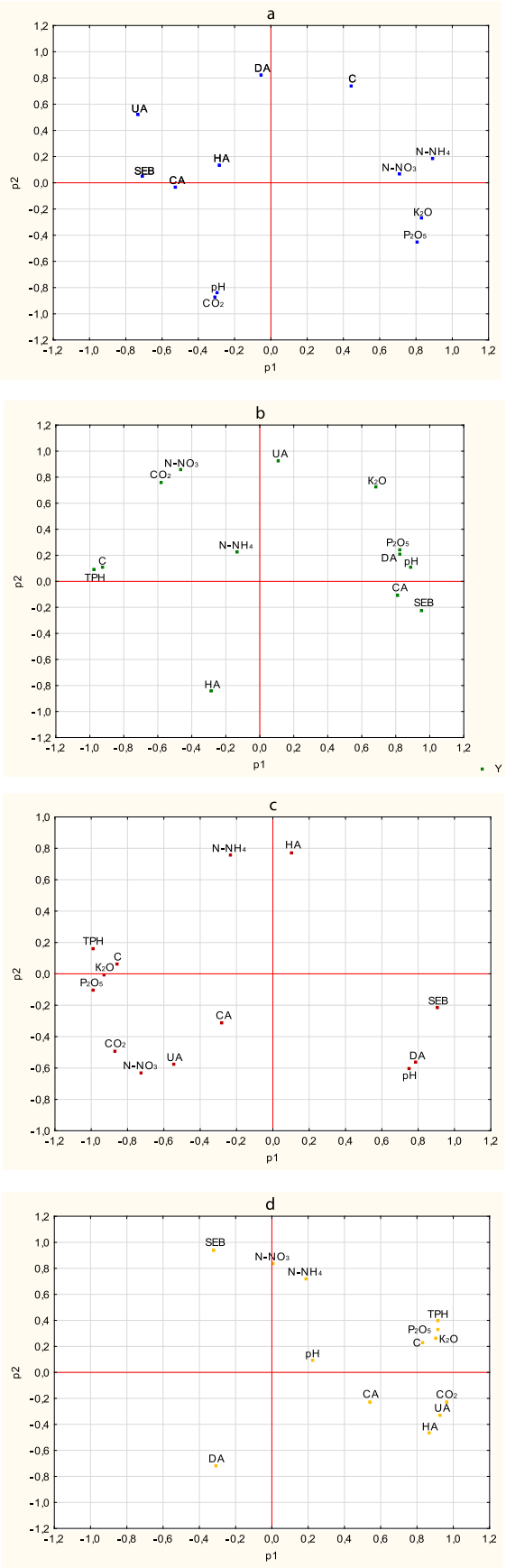

Fig. 6. Principal component analysis biplots of biological parameters and physicochemical properties in oil-contaminated soil exposed to different remediation strategies: control (a), natural attenuation (b), biostimulation (c), bioaugmentation (d).

while hydrolytic acidity, N-NO_3, and urease activity were major contributors to PC2.

In unpolluted soil all enzymatic activities appeared in the same quadrant, but widely dispersed. TOC, N-NH_4, and N-NO_3 were in a neighboring quadrant, while AK and AP appeared in the opposite quadrant, probably because of the inverse relationship between them. In soil under natural attenuation DA, UA, and AK, AP were in the same quadrant, suggesting close relationships between enzymatic activities and nutrients. Mineral nitrogen and basal respiration were also close, and appeared in the same quadrant, while in unpolluted soil N-NH_4, and N-NO_3 occupied a different quadrant other than basal respiration. Available phosphorus and potassium appeared in the different quadrants with BR both in soil under natural attenuation and in control.

The distribution of the variables was similar in soils under biostimulation and bioaugmentation and differed from data on conditions of natural attenuation and control. In soils under biostimulation and bioaugmentation basal respiration, urease and catalase activity were grouped in the same quadrant. BR was closer to urease, and catalase activity was the most distant variable. Dehydrogenase activity appeared opposite to TPH, showing the negative influence of the pollutant on soil microbiota.

TPH and TOC were grouped close together regardless of the remediation strategy. However, in the case of natural attenuation, both TOC and TPH had less distance from basal respiration.

The distribution of the physicochemical parameters was also markedly different between polluted and unpolluted soil. In unpolluted soil pH was very close to basal respiration, and dehydrogenase activity was situated in a different quadrant. With oil contamination, in case of both natural attenuation and biostimulation, pH was close to dehydrogenase activity, in a different quadrant from BR. In soil under bioaugmention no relationships between pH and any of biological parameters were found.

Similarly, hydrolytic acidity was not related to biological parameters in unpolluted soil, while in the case of both natural attenuation and biostimulation strong negative correlation was observed. Sum of exchangeable bases was grouped in the same quadrant with enzymatic activities in unpolluted soil, while appeared in opposite quadrants in soil under bioaugmention.

4. Discussion

4.1. Soil physicochemical properties

Initial parameters of the soil are extremely important in defining the eventual success of remediation after oil spill. Among the main limiting factors are: soil texture, pH, temperature, water holding capacity and nutrient content (Das and Chandran, 2010). To support cell growth and sustain biodegradation, microorganisms require optimal temperature and pH, water, oxygen and inorganic nutrients.

In the oil-polluted soils there was a continuous decrease in the soil pH induced by the decrease of the mobility of exchangeable bases, and increase in hydrolytic acidity. The increase of the sum of exchangeable bases after a year of experiment can be attributed to the gradual dissolution of carbonates. Later on the gradual desalination of carbonates and recovery of initial soil acidity due to TPH biodegradation was observed.

The PCA showed that hydrolytic acidity was the highest positive loading on PC2. Soil acidity is an important factor limiting soil fertility, and oil contamination may intensify and speed up acidification process (Wyszkowski and Sivitskaya, 2015). Bioremediation decreased hydrolytic acidity and contributed to the rise in the base saturation. The restored soil balance had positive effect on the TPH biodegradation rate.

Both biostimulation and bioaugmentation had a favourable effect on soil hydrolytic acidity, pH and the sum of exchangeable bases, although the effect of bioaugmentation was much stronger than the effect of biostimulation. By the end of the experiment, there was no significant

difference in acid-base ratio in different treatment types.

The PCA showed a strong effect of crude oil on the content of organic carbon. Similarly, Wang et al. (2010) demonstrated a strong positive correlation between TPH and TOC in the oil contaminated soil. Our results showed that while total organic carbon was significantly higher ($p < 0.05$) in contaminated soils, introduction of oil significantly reduced ($p < 0.05$) available potassium, phosphorous and nitrate nitrogen concentrations.

In soils under natural attenuation and biostimulation, the nitrate nitrogen content decreased by 80–85%, which can be explained as a consequence of microbial utilization and alterations in microbial community composition (John et al., 2011; Urakawa et al., 2012; Marchand et al., 2017). The disappearance of available phosphorous and potassium in polluted soil over time can be attributed to microbial metabolism, immobilization in biomass, immobilization on soil colloids, and washing out (Margesin and Schinner, 2001). The strong positive correlations of the available nutrient content with the hydrocarbon concentration, basal respiration, and the enzymatic activities in the polluted soil indicate the importance of nutrients for biodegradation processes.

4.2. TPH biodegradation

Removal of the crude oil from soil is the combined result of chemical oxidation, photo-oxidation, evaporation, and microbial oxidation (mineralization). Depending on the time of contamination and environmental factors, one or more of these processes may play key role in oil degradation, but in general, mineralization is the most important in decontamination. All biological methods of rehabilitation of contaminated sites are based on this process. Among a variety of remediation methods, bioremediation has been recognized as a cost-effective clean-up technology (Sarkar et al., 2005; Liu et al., 2011; Wu et al., 2016).

Natural attenuation, including evaporation, photo-oxidation, and natural biodegradation, removed 13.4, 28.3, 67.6 and 91.2% of hydrocarbons in 3 month, 1 year, 3 and 9 years, respectively. The degradation rate decreased after the third year, presumably because most of available hydrocarbons were removed and only recalcitrant compounds with higher molecular weight were present (Gomez and Sartaj, 2013; Riveroll-Larios et al., 2015). It is also possible that degradation of higher molecular weight hydrocarbons may produce toxic intermediates that can inhibit oil-degrading microorganisms (Frankenberger, 1992).

Biodegradation of crude oil by natural populations of microorganisms is well known, as many bacteria and fungi can mineralize hydrocarbons (Marchand et al., 2017). Microorganisms are able to degrade petroleum hydrocarbon pollutants both in aerobic and anaerobic conditions (Mbadinga et al., 2011). Anaerobic degradation is very common and occurs under methanogenic, nitrate-, iron-, manganese- and sulfate-reducing conditions (Varjani and Upasani, 2017). Nevertheless, oil biodegradation in soil occurs mainly in the surface layer under aerobic conditions. During aerobic oxidation microorganisms use hydrocarbons as both energy and carbon sources and can fully degrade them with CO_2 as an end-product.

The usefulness of the two main bioremediation technologies, bioaugmentation and biostimulation harnesses the metabolic ability of the microorganisms. Biostimulation boosts the degrading capacity of the indigenous microbial community by adding nutrients to avoid metabolic limitations, while bioaugmentation introduces the exogenous degrading microorganisms into the soil (Wu et al., 2016).

Many bioaugmentation studies have indicated that the use of associations of degrading microorganisms (bacteria from genera *Achromobacter, Alcaligenes, Bacillus*, *Flavobacterium, Mycobacterium, Pseudomonas, Rhodococcus, Sphingobium*, and fungi *Absidia, Achremonium, Aspergillus, Mucor, Penicillium and Verticillium*) significantly enhanced crude oil biodegradation (Mrozik and Piotrowska-Seget, 2010). Due to complex chemical nature of petroleum hydrocarbons, microbial consortia are more effective in removing hydrocarbons than individual strains.

On the other hand, it was repeatedly demonstrated that stimulating the indigenous soil microbiota by nutrients was sufficient to achieve successful bioremediation (Margesin and Schinner, 1997; Tyagi et al., 2011). The advantage of biostimulation lies in the use of the already present, native microorganisms that are well-matched to specific soil environment, compared to the transient allochthonous organisms that do not have a place in the existing community (Bento et al., 2005).

Although bioaugmentation strategy have been reported as a powerful tool to enhance the removal of crude oil in contaminated soil, our data have found a comparable effect of simpler nutrient addition. Oil degradation activities reached their maximum during the first 3 months of experiment, resulting in decontamination of 26.5% and 28.8% in soils under biostimulation and bioaugmentation, respectively. Similar biodegradation rates obtained under conditions of bioaugmentation and biostimulation point out the high activity of indigenous microorganisms.

During the first year, additions of both microbial inoculate and nutrients showed continued improvement in the level of TPH degradation compared to biodegradation observed under natural attenuation. In the consecutive years, however, TPH concentration and biodegradation rates remained similar in all three treatment types.

The hydrocarbon content remained nearly unchanging during the last years of experiment. It was shown that hydrocarbons cannot be completely removed by biological decontamination, even after a prolonged exposure (Juhasz and Naidu, 2000; Haritash and Kaushik, 2009). The remaining compounds consist of hydrocarbons that are structurally less available for biodegradation due to their recalcitrance and very limited bioavailability (Stroud et al., 2007). Other reasons for the diminishing degradation rate may include the accumulation of inhibiting metabolites, and the lack of microbial growth factors (Margesin and Schinner, 1997; Juhasz and Naidu, 2000).

Native oil-degrading soil microorganisms are enriched by the presence of contaminant, but they may be constrained in their degradation capability by limiting factors such as insufficient nutrients (Silva-Castro et al., 2015). High degree of response to nutrient addition indicates that the metabolic activity is closely related to these soil characteristic. Our results demonstrated that the addition of mineral nitrogen, phosphorus and potassium increased the biodegradation rate compared to unaided, natural process. It should be noted that bioaugmentation only worked more effectively than the indigenous microorganisms at the initial stages of decontamination. With increasing incubation time no difference between remediation strategies were detectable.

Our data suggest that the considerable part of decontamination could be attributed to activity of indigenous microorganisms, and biostimulation is a better technology for bioremediation of the studied soil. These results are in line with previous findings. For example, Margesin and Schinner (1997) demonstrated that in alpine soils, introduction of a diesel-oil-degrading inoculum increased biodegradation rates only for a short time. Indeed, the addition of allochthonous microorganisms gives the best results when the contaminant has a toxic effect on the indigenous microorganisms. It can accelerate the initial phase of biodegradation but it was repeatedly demonstrated that stimulating the indigenous soil microbiota by nutrients was also sufficient (Tyagi et al., 2011; Wu et al., 2016).

4.3. Basal respiration

Quantitative characters of soil biological activity are often used as relevant parameters during microbial oil decontamination (Maila and Cloete, 2005; Riffaldi et al., 2006; Wolińska et al., 2016). It is usually assumed that, even though CO_2 production is not a direct measure of the oil carbon biotransformation, changes in the microbial activity indirectly reflect microbial breakdown of oil (Oh et al., 2002).

The highest level of basal respiration was found one week after the start of the experiment, when hydrocarbon concentrations were at their highest, but declined after three months, when TPH decreased by 26–28%. After the first year, both the level of hydrocarbon contamination and BR decreased even further in our experiment. Other studies also reported the same effect: initial BR stimulation of the indigenous microorganisms faded with time in oil-contaminated soils (Allard and Neilson, 1997). At first, soil respiration rate grows rapidly because the most labile hydrocarbons stimulate high microbial activity (Lee et al., 2008). However, easily available compounds are quickly used up and BR drops in response. This decline may be also associated with the depletion of the nutrients added at the beginning of decontamination or initially present in the soil, thus disrupting the optimum conditions for microbial growth (Riffaldi et al., 2006).

The addition of nutrients (biostimulation) increased the initial basal respiration by factor of 5, indicating that NPK accelerated the oil-degrading activity of the indigenous microbiota. The application of both nutrients and biopreparation (bioaugmentation) increased biodegradation efficiency even further: BR grew 7 times. Natural attenuation showed the least changes in basal respiration. These results suggest that nutrient availability was an important factor limiting oil biodegradation in soil, in agreement with a commonly held view (Kim et al., 2005).

The stimulating effect of bioaugmentation on BR declined with time in the same way as the effect of biostimulation. This phenomen may be explained by reduction of inoculum concentration in the soil after 3 month (Masy et al., 2016). We found that one year after the start of the experiment, BR was 1.3 times higher in the samples exposed to biostimulation compared to the samples with added degrading microorganisms. Other studies also report higher efficiency of biostimulation compared to other treatment types (Balba et al., 1998).

Respirometric measurements are used repeatedly as a monitoring instrument for the decontamination process of oil-contaminated soil (Margesin et al., 2000). However, the respiration rates reflect the non-specific degradation of added amendments as reported by Jørgensen et al. (2000). These researchers also concluded that the respiration rates are not directly correlated with oil degradation. Since respiration does not represent an actual biodegradation, soil enzyme activity could be more useful parameter to interpret the intensity of microbial metabolism in soil (Lee et al., 2008).

4.4. Soil enzymatic activity

Correlations between the soil biological parameters and the levels of hydrocarbon residues could help in elucidating microbial contributions to oil elimination from soil (Margesin et al., 2000). The PCA showed a strong effect of crude oil on metabolic activity of soil microorganisms (PC1). The TPH concentration was mainly responsible for changing enzymatic activity; this variable was the one with the highest negative loadings on PC1for all treatments.

The biodegradation mechanism of petroleum hydrocarbons involve metabolic reactions catalyzed by a variety of enzymes. Enzymes playing important role are oxygenases, reductases, hydroxylases and dehydrogenases (Mbadinga et al., 2011; Varjani and Upasani, 2017). In the initial step, biodegradation mechanisms require the presence of dioxygenase and monooxygenase to catalyze oxidation reactions. Other principle agent in the degradation of soil petroleum hydrocarbon pollutants is dehydrogenase. This enzyme transports electrons and hydrogen through a chain of intermediate electron carriers to a final electron acceptor (oxygen) (Tate, 2002). Alcohol dehydrogenase oxidizes both short- and long-chain alkanes to the aldehyde while aldehyde dehydrogenase oxidizes aldehyde to the acid.

Soil dehydrogenase activity measured in this study was significantly higher in control than in contaminated soil under all treatments. Low DA reflects toxic effect of oil on the process of dehydrogenation of organic matter. Dehydrogenase activity is often strongly affected by introduction of oil; both positive and negative correlations with oil concentration were reported under various conditions (Bento et al., 2005; Lapinskiene et al., 2006; Wyszkowska and Wyszkowski 2010; Riveroll-Larios et al., 2015). In our experiment, dehydrogenase activity fell abruptly for the first few months after contamination and recovered slowly and incompletely in the following nine years.

A steady initial decline in DA was seen in contaminated soils under both natural attenuation and biostimulation. By contrast, we found that bioaugmentation stimulated DA during the first week of the experiment. However, shortly thereafter, it also began to decline, so that the decrease was similar in all three conditions by 3 month. After two years, the negative effect of oil contamination was partially mitigated, but DA was still lower in contaminated soil than in control regardless of the remediation strategy, even after 9 years of experiment. Dehydrogenase activity reflects the microbial biomass and degradation activity of organic matter. Our results, together with previous studies by Lapinskiene et al. (2006) and Gianfreda et al. (2005) indicate high susceptibility of dehydrogenase to the presence of crude oil in the soil.

Some authors found a positive correlation between dehydrogenase activity and CO_2 production in contaminated soil (Riveroll-Larios et al., 2015), while others have not (Silva-Castro et al., 2015, 2016). In these studies DA and CO_2 production had an inverse relationship or the correlation was not significant ($p > 0.10$). Our results suggest the inactive state of a part of the native soil microbiota.

The metabolism of hydrocarbons in microorganisms produces a huge amount of H_2O_2 as biproduct inside the cells which leads to cell damage. Catalase is highly efficient H_2O_2 metabolizing enzyme. It protects cells from damage by reactive oxygen species and can be found in all aerobic microorganisms (Stepniewska et al., 2009). In our study catalase activity (as well as DA) was strongly inhibited by oil contamination. A short-term initial increase in catalase activity under conditions of bioaugmentation may be caused by the increased microbial biodegradation of the easily available hydrocarbons. The induction of peroxide degrading enzyme genes (katG) encoding catalase-peroxidase has been reported from PAH degrading bacteria (Wang et al., 2000).

It is known that fertilization increases CA (Margesin and Schinner, 1997), and, indeed, in our experiment treatment with nutrients had a positive effect. Still, in the presence of oil catalase activity never reached the control level. These results agree well with previous studies (Achuba and Peretiemo-Clarke, 2008; Wolińska et al., 2016). The enzyme induction can be blocked by accumulation of toxic products and competition at the active site of enzymes (Bouchez et al., 1995). The decreased enzymatic activities lead to a lower rate of autotrophic and heterotrophic processes.

Urease activity of polluted soil fluctuated during the experiment, with a marked initial increase, followed by a significant decrease after 3 month of experiment. After one year it grew again, reached the control level and remained unchanged in the following years. Such fast recovery indicates that urease is a weak parameter for testing hydrocarbon degradation in soil. Similarly, Wyszkowska and Wyszkowski (2010) demonstrated that petroleum pollution disturbed the biochemical balance by strongly inhibiting the activity of soil dehydrogenases, while the least change occurred in the activity of soil ureases.

Bioremediation increased the activity of urease, with the greatest effect seen under conditions of bioaugmentation, although very strong and positive initial effect on the activity of urease was also produced by addition of nutrients. This result agrees well with the study by Lee et al. (2008), who found that nitrogen supplements significantly enhanced the initial activity of urease.

Based on the response of microbial parameters, we distinguish two periods during experiment: fast initial disturbance and slow recovery. During the first year we found an increase in respiratory activity, which later approached the control level. Activities of soil dehydrogenase and catalase suffered an initial fast decrease and over the next years moved closer to the control, but did not complete the recovery. Urease activity fluctuated wildly during the first year, but stabilized around control

value in the second period.

Here, again, changes in the concentration of readily degradable hydrocarbons between the two identified periods may play a role. Recently contaminated soil contains easily biodegradable compounds like n-alkanes (< n-C_{21}) and low molecular weight aromatic compounds. Later the residual hydrocarbons are composed mostly of more recalcitrant and resistant to biodegradation compounds (Liu et al., 2011; Riveroll-Larios et al., 2015). When the concentration of easily utilizable substrates decreases below a critical level, microorganisms shift towards production of enzymes to regenerate nutrients from organic matter, and vice-versa, when the hydrolysis products are sufficient to meet microbial demands, enzymatic production becomes suppressed (Chróst, 1991). Conversely, synthesis of constitutive enzymes is not affected by nutrient availability.

Enzyme activities play an important role in nutrient cycling, and can be sensitive indicators of environmental pollution. Between the three examined enzymes, dehydrogenase demonstrated the best sensitivity to TPH (also reported by Kaczyńska et al., 2015) and has a close response to composition of hydrocarbon fractions (Sarkar et al., 2005; Silva-Castro et al., 2015). We believe that dehydrogenase activity is one of the most useful microbial parameters for testing bioremediation methods and evaluating the factors affecting oil bioremediation.

5. Conclusions

Various aspects of soil biological activity proved to be powerful tools for the assessment of long-term changes in oil-contaminated podzolic soil. By the ninth year of the experiment a 90% decontamination level was reached, but microbial communities continued to suffer beyond the time scope of our study. Dehydrogenase activity, in particular, was the most sensitive biological indicator, suitable for use under all remediation strategies. We found that a considerable part of decontamination could be attributed to degradation activities of indigenous microorganisms. Thus, we conclude that biostimulation is a better bioremediation strategy for examined podzolic soil.

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