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ORIGINAL PAPER

REMEDIATION OF SOIL CONTAMINATED WITH DIESEL OIL*

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Abstract

Petroleum hydrocarbons are the most ubiquitous organic pollutants. In Europe, 45% of polluted habitats are contaminated with these compounds. These observations have prompted a pot experiment aiming to determine the effectiveness of a molecular sieve, alginite, sepiolite and the Ikasorb 1850 sorbent (0 and 15 g kg^{-1} DM soil) in remediating soil contaminated with Verva diesel oil (10 cm^3 DO kg^{-1} DM soil). Soil contamination with diesel oil significantly inhibited the growth and development of maize and modified the chemical composition of this crop. Diesel oil decreased the bioconcentration index of nitrogen, phosphorus, calcium and potassium in maize. The molecular sieve, sepiolite, alginite and sorbent minimized the adverse effects of diesel oil on the aerial parts and roots of maize plants. Diesel oil disrupted the microbial equilibrium in soil, and it decreased the ecophysiological diversity of fungi and organotrophic bacteria. Diesel oil decreased the percentage of *Firmicutes, Actinobacteria, Bacteroidetes* and *Acidobacteria* in the bacterial community, stimulated intracellular and extracellular enzymes, and exerted adverse effects on the physical properties of soil. The evaluated soil remediation agents exerted varied effects on the microbiome of contaminated soil. Sepiolite, alginite and Ikasorb generally enhanced the growth of actinomycetes by increasing the proportion of K-strategists in soil. All remediation agents increased the ecophysiological diversity of organotrophic bacteria, stimulated the activity of dehydrogenases and improved the physicochemical properties of soil. The evaluated compounds intensified the degradation of petroleum hydrocarbons (C_6-C_{12}) and 9 PAHs, and the molecular sieve and alginite also accelerated the biodegradation of mineral oil (C_{12}-C_{35}). The analyzed compounds can be used to reclaim soil polluted with diesel oil, and molecular sieve and alginite are the most effective bioremediation agents.

Keywords: diesel oil, remediation, soil microbiome, hydrocarbon degradation.

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INTRODUCTION

Petroleum hydrocarbons are the most ubiquitous organic pollutants (Liu et al. 2014). According to Masy et al. (2016), 45% of polluted habitats in Europe are contaminated with these compounds. The elimination of oil contaminants from soil with the use of mechanical and chemical methods is very expensive. Bioremediation is one of the most effective methods of soil reclamation. Soil microorganisms which participate in bioremediation, in particular the mineralization of organic pollutants, influence the structure and function of soil as well as the activity of intracellular and extracellular enzymes (Wolińska et al. 2016). Phytoremediation is a bioremediation technique which relies on plants capable of accumulating pollutants and producing large amounts of biomass. The secretions produced by plant roots should be highly resistant to environmental stressors (Liu et al. 2014).

Rhizodegradation is a process by which soil microorganisms and plant roots break down contaminants in the rhizosphere. Rhizofiltration is a form of phytoremediation where pollutants are precipitated or absorbed by roots. The absorbed pollutants are transported with water to plant tissues (Liu et al. 2012, 2014, Moubasher et al. 2015). Pilon-Smits (2005) has identified three processes by which pollutants are transported to plant tissues: phytodegradation, phytoaccumulation and phytovolatilization. Selected root secretions can be utilized by soil-dwelling microorganisms that promote the growth and development of plants to minimize the adverse effects of PAHs of the soil microbiome (Guo et al. 2012, She et al. 2013, Cook, Hesterberg 2013, Liu et al. 2014, Arslan et al. 2015, Moubasher et al. 2015, Chen et al. 2016). According to Arslan et al. (2015) and Chen et al. (2016), plants are a source of nutrients for microorganisms, and microorganisms promote plant growth by degrading and neutralizing soil pollutants.

Petroleum pollutants are eliminated from soil by heterotrophic bacteria. Autochthonous microorganisms adapt to new substrates through mutation and horizontal gene transfer, which enables them to break down petroleum pollutants in contaminated environments (Wolińska et al. 2016). The synergistic interactions between these microorganisms intensify their biological activity and lower their nutritional requirements. These properties can be harnessed to develop commercial strains of soil-purifying bacteria (Margesin et al. 2007, Hibbing et al. 2010).

The success of soil remediation is determined by the choice of plant species which most effectively eliminate diesel oil additives, including anti-corrosion, biocidal, demulsifying, anti-foaming and refining agents, as well as the soil type, physicochemical and chemical properties of soil, soil sorptive capacity, soil pH, autochthonous microorganisms and enzyme activity (Kucharski, Jastrzębska 2005, She et al. 2013, Eibes et al. 2015, Wyszkowska et al. 2015).

The selection of the optimal method of remediating soil contaminated with petroleum hydrocarbons should be dictated not only by economic concerns but also by environmental factors at the local, regional and, above all, the global level. The United States Environmental Protection Agency (US EPA 2017) has recently focused on innovative techniques for the remediation of polluted habitats. These observations have prompted a study aiming to determine the effectiveness of a molecular sieve, alginite, sepiolite and the Ikasorb 1850 sorbent in restoring the equilibrium of soil contaminated with VERVA diesel oil. These remediation agents were evaluated based on their influence on the soil microbiome and the physicochemical and chemical properties of soil, including the activity of selected enzymes that transform carbon, nitrogen, phosphorus and sulfur. The sensitivity of maize plants to diesel oil and the percentage reduction in the content of petroleum hydrocarbons (C_6-C_{12}), mineral oil (C_{12}-C_{35}), volatile hydrocarbons (BETX) and ∑9 PAHs induced by soil-dwelling microorganisms were also determined.

MATERIAL AND METHODS

Soil

The experiment was performed on samples of brown topsoil (Eutric Cambisol) with the granulometric composition of loamy sand (sand fraction – 82%, silt fraction – 17%, clay fraction – 1%). The analyzed soil had the following parameters: C_{org} – 7.7 g kg^{-1}; N_{total} – 0.74 g kg^{-1}; hydrolytic acidity – 8.02 mmol(+) kg^{-1}; exchangeable base cations – 92.3 mmol(+) kg^{-1}; cation-exchange capacity – 100.32 mmol(+) kg^{-1}; base saturation – 92.0%.

Experimental design

A pot experiment was performed in a greenhouse. Polyethylene pots with the volume of 3.0 dm^3 were filled with 2.8 kg of soil thoroughly mixed with $CO(NH_2)_2$, KH_2PO_4, KCl and $MgSO_4 \cdot 7H_2O$. The quantity of the above compounds was determined based on the nutritional requirements of the experimental plant – *PR39H32* maize. The experiment was conducted in 5 replications for each of the two treatments. The first treatment was uncontaminated soil, and the second treatment was soil contaminated with 10 cm^3 of VERVA diesel oil per kg of soil. In each treatment, before filling the pots, soil was combined with the following remediation agents: Activated Molecular Sieve Powder, Sepiolota E-562 (sepiolite), alginite and Ikasorb 1850 sorbent in amounts corresponding to 1.5% of soil weight. Six maize plants were grown in each pot for 60 days. Soil moisture content was monitored throughout the experiment. The aerial parts and roots of maize plants were harvested in stage BBCH 59.

Diesel oil

VERVA diesel oil (type B) was purchased in an Orlen petrol station. The oil had the following characteristics: cetane number – 55, cetane index – 46, density 820-845 g dm^3. A full specification of VERVA diesel oil is available on the manufacturer's website (http://www.orlen.pl/).

Remediation agents

The zeolite molecular sieve (AB_xO_{2x} **x** $\mathbf{nH_2O}$, where A denotes Na, K, Li, Ca, Ba, Sr, and B denotes Si and Al in a ratio of 5:1 to 1:1) is hydrated aluminosilicate with sorptive properties and micropores 0.3 nm in diameter. Sepiolite ($Mg_4[Si_6O_{15}(OH)_2]6H_2O$) is hydrated magnesium silicate with sorptive properties. Alginite, a naturally occurring mineral extracted from fossilized algae and weathered tuff, has high water-holding capacity, which enhances the biological activity of soil. The Ikasorb 1850 sorbent is a granulated product with the grain size of 0.3-1 mm. This porous material is extracted from calcined diatomaceous earth, and is characterized by high sorptive capacity.

Microbiological analyses

Microbiological analyses were performed in 4 replications on two dates (experimental days 20 and 60). The counts of organotrophic bacteria (Org), actinomycetes (Act) and fungi (Fun) were determined in soil samples. The analytical procedures were described by BOROWIK et al. (2017). The colony development index (CD) and the ecophysiological diversity index (EP) were calculated based on the method proposed by DE LEIJ et al. (1993). On experimental day 60 (harvest), the taxonomic diversity of prokaryotes in uncontaminated soil and soil contaminated with VERVA diesel oil was determined based on the sequence of the V3-V4 hypervariable region of the 16S rRNA gene. A metagenomic analysis was performed by next-generation sequencing (NGS) with the use of the MiSeq technique (Illumina) (Genomed).

Soil enzyme activity

Soil samples were subjected to biochemical analyses to determine the activity of dehydrogenases (Deh), catalase (Cat), urease (Ure), acid phosphatase (Pac), alkaline phosphatase (Pal), β-glucosidase (Glu) and arylsulphatase (Aryl). The analyses were conducted in 3 replications on two dates (experimental days 20 and 60). The method of enzyme identification and enzyme activity units were described in detail by BOROWIK et al. (2017).

Physicochemical and chemical properties of soil

The analytical procedure described by BOROWIK et al. (2017) was used to determine the granulometric composition, pH_{KCl}, hydrolytic acidity (HAC), exchangeable base cations (EBC), content of organic carbon (C_{org}), total nitro-

gen (N_{total}) and available phosphorus, potassium and magnesium in soil samples. The content of petroleum hydrocarbons (C_6-C_{12}) was determined according to standard PN-EN ISO 22155 (2016), mineral oil (C_{12}-C_{35}) – according to standard PN-EN ISO 16703 (2011), volatile hydrocarbons (BETX) – according to standard PN-EN ISO 22155 (2016), and ∑9 PAHs – according to standard ISO 18287 (2006). The analyses were performed with the use of an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass spectrometer with an EI/CI ion source.

Chemical composition of maize plants

The aerial parts and roots of maize plants were mineralized in concentrated H_2SO_4 with hydrogen peroxide and analyzed to determine their total nitrogen, phosphorus, potassium, magnesium, calcium and sodium content based on the procedure described by Sivitskaya and Wyszkowski (2013).

Statistical analysis

The results were processed statistically in the Statistica 13.0 program (Dell Inc. 2016). The contribution of independent variables to dependent variables was determined by calculating coefficient η^2 in ANOVA. Homogeneous groups were identified in the Tukey's range test at a significance level of $P = 0.01$. The results were also subjected to the principal component analysis (PCA) with the use of multidimensional methods. The influence of diesel oil and remediation agents on the soil microbiome was determined based on microbial counts and enzyme activity levels with the use of the following formula:

$$IF_{DO}\ (IF_R) = (Po\text{-}Co)/Co,$$

where:

IF_{DO} – influence of diesel oil,

IF_R – influence of remediation agents,

Po – microbial counts or enzyme activity in soil contaminated with diesel oil,

Co – microbial counts or enzyme activity in uncontaminated soil.

RESULTS

Yield and chemical composition of maize plants

The analyzed soil remediation agents generally exerted negative effects on the growth and development of maize grown in uncontaminated soil (Figure 1). The yield of aerial plant organs decreased by 27% under the influence of the molecular sieve and sepiolite, and by 17% under the influence of alginite. The Ikasorb 1850 sorbent was the only remediation agent that did not inhibit the development of aerial plant parts. Maize roots responded dif-

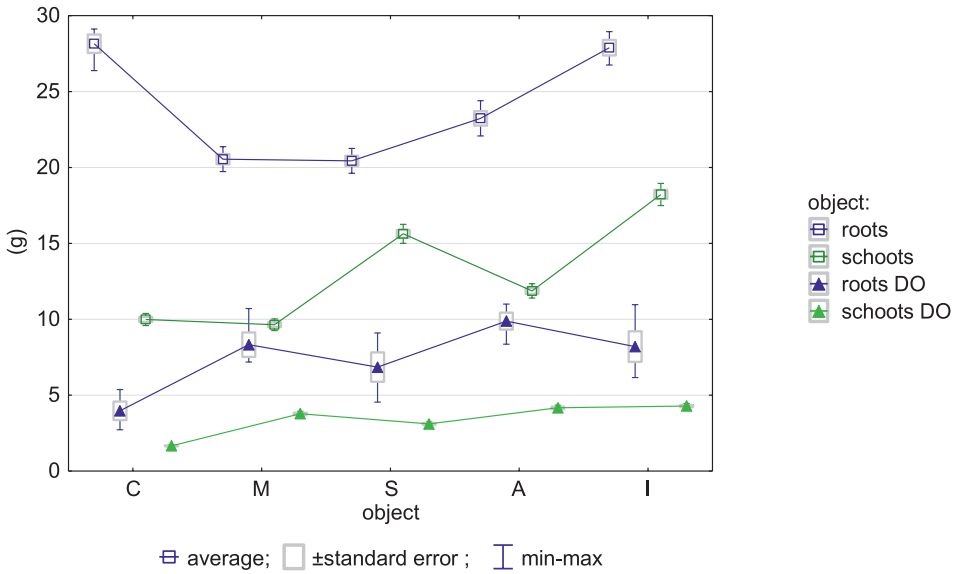

Fig.1. Impact of diesel oil on the yield of maize. The dry mass of aerial parts and roots, g per pot
* explanations are given in Table 1; error bars represent standard error of the mean for $n = 5$

ferently to the applied remediation agents, which increased root mass by 19% (alginite) to 82% (Ikasorb). Soil contamination with diesel oil significantly decreased the yield of aerial parts and roots of maize plants. In treatments where remediation agents were not applied, the yield of aerial plant parts decreased by 86%, and root yield decreased by 83%. The remediation agents minimized the negative effects of diesel oil on aerial plant parts by 72% (sepiolite) to 149% (alginite) and on roots by 87% (sepiolite) to 158% (Ikasorb).

In uncontaminated soil, the molecular sieve increased the concentrations of nitrogen, phosphorus, potassium and sodium and decreased the content of divalent elements in aerial plant parts (Table 1). Sepiolite increased the content of magnesium and sodium and decreased the content of calcium. Alginite increased the concentrations of nitrogen, magnesium and sodium. Ikasorb increased the concentration of sodium and decreased the content of nitrogen, phosphorus, calcium and potassium. Soil contamination with diesel oil increased the content of nitrogen, magnesium, calcium, potassium and sodium in the aerial parts of maize plants. In soil contaminated with diesel oil, the molecular sieve increased the accumulation of potassium and sodium, sepiolite increased the concentrations of magnesium, potassium and sodium, alginite enhanced the accumulation of phosphorus and magnesium, and Ikasorb increased the concentrations of magnesium and sodium relative to contaminated treatments without the addition of remediation agents.

The analyzed remediation agents also modified the chemical composition of maize roots (Table 2). The roots of maize plants grown in uncontaminated

Table 1

The content of elements in aerial parts of maize (mg kg^{-1} DM)

Remediation substance	DO dose (cm^3 kg^{-1} of soil)	N	P	Mg	Ca	K	Na
Control - C	0	15.60^{ef}	2.09^{bc}	3.30^{d}	7.15^{c}	20.25^{d}	0.14^{c}
	10	30.70^{a}	2.09^{bc}	4.50^{b}	10.73^{a}	26.56^{c}	0.20^{c}
Molecular sieve - M	0	18.00^{d}	2.49^{a}	1.50^{f}	4.58^{d}	34.86^{a}	4.58^{a}
	10	19.80^{cd}	2.09^{bc}	2.50^{e}	6.29^{c}	37.18^{a}	4.44^{a}
Sepiolite - S	0	15.60^{ef}	2.09^{bc}	3.80^{c}	6.65^{c}	19.92^{d}	0.45^{b}
	10	30.40^{a}	2.09^{bc}	5.80^{a}	10.80^{a}	29.88^{b}	0.33^{bc}
Alginite - A	0	30.70^{a}	2.09^{bc}	3.70^{cd}	6.94^{c}	20.92^{d}	0.17^{c}
	10	21.60^{bc}	2.31^{ab}	4.80^{b}	8.87^{b}	25.23^{c}	0.17^{c}
Ikasorb - I	0	14.00^{f}	1.92^{c}	3.30^{d}	6.29^{c}	18.59^{d}	0.15^{c}
	10	23.50^{b}	2.09^{bc}	4.80^{b}	9.22^{b}	27.22^{bc}	0.34^{bc}

DO – diesel oil;
The same letters show homogeneous groups separately for the content of elements in aerial parts of maize. Different letters show significant differences separately for the content of elements in aerial parts of maize (Tukey test, $P = 0.01$).

Table 2

The content of elements in the roots of maize (mg kg^{-1} DM)

Remediation substance	DO dose (cm^3 kg^{-1} of soil)	N	P	Mg	Ca	K	Na
Control - C	0	10.90^{d}	1.31^{cd}	3.20^{cd}	5.58^{c}	8.96^{c}	2.68^{c}
	10	26.30^{a}	1.57^{ab}	3.68^{c}	15.52^{a}	13.28^{ab}	2.46^{c}
Molecular sieve - M	0	11.50^{d}	1.70^{a}	1.93^{e}	2.07^{de}	14.28^{ab}	7.96^{b}
	10	14.90^{c}	1.57^{ab}	1.75^{e}	2.29^{d}	14.94^{a}	10.42^{a}
Sepiolite - S	0	6.30^{f}	1.13^{d}	5.13^{a}	6.44^{c}	6.64^{d}	2.39^{c}
	10	20.70^{b}	1.61^{ab}	5.61^{a}	9.37^{b}	13.28^{ab}	2.89^{c}
Alginite - A	0	10.50^{d}	1.44^{bc}	4.40^{b}	8.51^{b}	8.63^{c}	2.25^{cd}
	10	13.90^{c}	1.48^{bc}	4.34^{b}	8.94^{b}	12.95^{b}	2.46^{c}
Ikasorb - I	0	7.50^{e}	1.13^{d}	2.71^{d}	1.00^{e}	7.64^{cd}	1.69^{d}
	10	15.00^{c}	1.13^{d}	4.28^{b}	6.65^{c}	13.28^{ab}	2.53^{c}

DO – diesel oil;
The same letters show homogeneous groups separately for the content of elements in roots of maize. Different letters show significant differences separately for the content of elements in roots of maize (Tukey test, $P = 0.01$).

soil treated with the molecular sieve were characterized by a higher content of nitrogen, phosphorus, potassium and sodium, and lower concentrations of magnesium and calcium. Sepiolite increased the accumulation of magnesium and calcium, and decreased the concentrations of nitrogen, phosphorus, potassium and sodium. Alginite increased the content of phosphorus, magnesium and calcium, and decreased the accumulation of sodium. Ikasorb decreased the content of all the analyzed elements. In treatments without the addition of any remediation agent, diesel oil contributed to an increase in the concentrations of all elements, excluding sodium, in maize roots. Such unequivocal relationships were not observed in contaminated soil subjected to remediation.

The bioconcentration index of the evaluated elements, calculated as the ratio of their concentrations in aerial plant parts and roots, was determined at 1.17-2.92 for nitrogen, 1.33-1.85 for phosphorus, 0.74-1.43 for magnesium, 0.69-2.75 for calcium, 1.95-3.00 for potassium, and 0.05-0.58 for sodium (Figure 2). Soil contamination decreased the bioconcentration index of nitro-

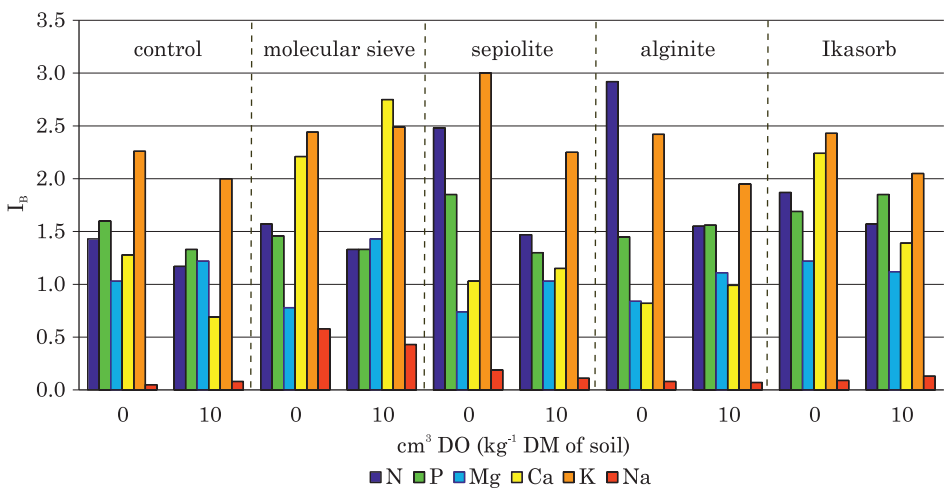

Fig. 2. Index of bioconcentration (I_B) of elements in maize (ratio of content in aerial parts to content in roots)

gen, phosphorus, calcium and potassium, and the molecular sieve increased the bioconcentration index of nitrogen, magnesium, calcium, potassium and sodium in maize plants grown in contaminated soil. Sepiolite increased the bioconcentration index of nitrogen, calcium, potassium and sodium. Alginite increased the bioconcentration index of nitrogen, phosphorus and calcium. Ikasorb increased the bioconcentration index of nitrogen, phosphorus, calcium and sodium. The tested bioremediation agents minimized the adverse changes induced by diesel oil in soil.

Microbial diversity

Diesel oil significantly influenced microbial counts. The counts of organotrophic bacteria were influenced by diesel oil in 32%, by date of analysis – in 18%, and by remediation agents – in just 8%. Fungal counts were influenced by diesel oil in 53%, by date of analysis – in 18%, and by remediation agents – in 8% (Figure 3). Diesel oil induced the least significant changes in the size

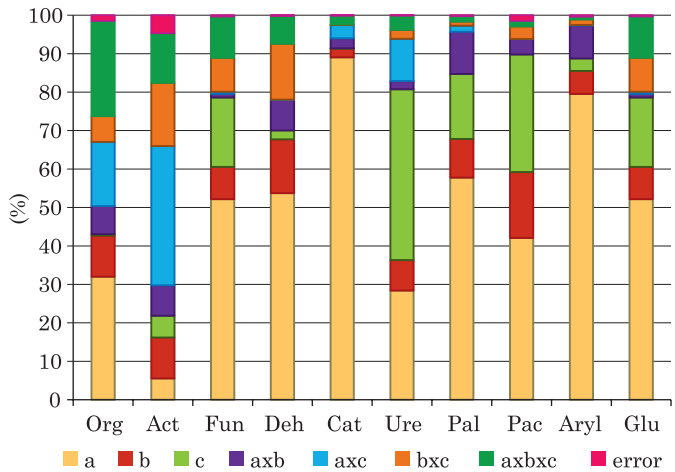

Fig. 3. The share of independent variables in the evolution of the number of microorganisms and soil enzyme activity (η^2): a – dieslel oil, b – remediation substance, c – date of analysis, Org – organotrophic bacteria, Act – actinomycetes, Fun – fungi, Deh – dehydrogenases, Cat – catalase, Pac – acid phosphatase, Pal – alkaline phosphatase, Aryl – arylsulphatase, Glu – β-glucosidase

actinomycetes populations. Actinomycete counts were influenced by diesel oil in just 6%, by remediation agents – in 11%, and by date of analysis – in 6%.

The counts of actinomycetes and organotrophic bacteria were positively correlated with the first principal component, and fungal counts were positively correlated with the second principal component (Figure 4). Both components accounted for 91.76% of the variation in original variables, and they were robust indicators of microbial proliferation under the influence of diesel oil and remediation agents.

Diesel oil had a particularly significant influence on microbial counts in the first 20 days of the experiment. On day 20, the counts of organotrophic bacteria increased 18-fold, the counts of actinomycetes increased 68-fold, and fungal counts increased 20-fold. On day 60, diesel oil increased only fungal counts, but decreased the counts of organotrophic bacteria (by 31%) and actinomycetes (by 57%). The above changes were determined based on the calculated values of IF_{DO} and IF_{R} (Figure 5).

The applied remediation agents exerted different effects on microbial proliferation. In uncontaminated soil, the molecular sieve (M) increased the counts of organotrophic bacteria and actinomycetes on day 20, but induced a

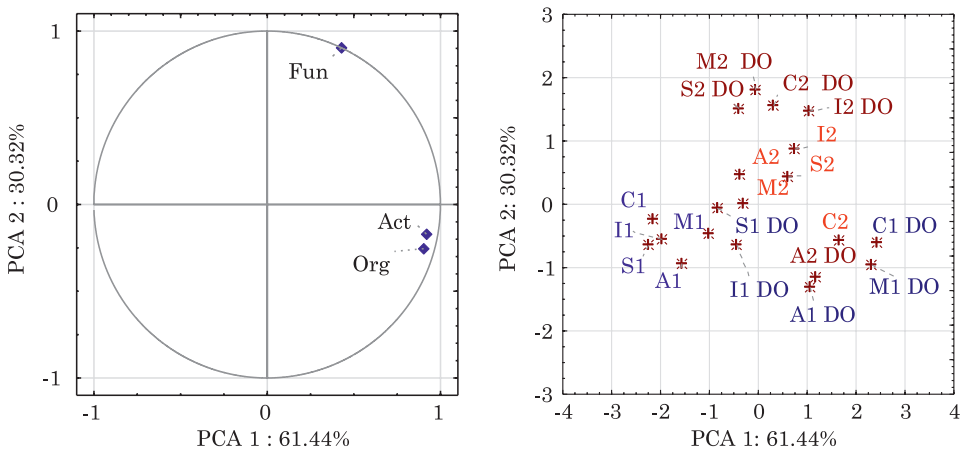

Fig. 4. The influence of the factors studied on the counts of soil microorganisms presented by the principal component analysis: C – control; DO – diesel oil; M, S, A, I – remediation substances (see Table 1), 1 – 20th day, 2 – 60th day, Org – organotrophic bacteria, Act – actinomycetes, Fun – fungi

	Org	Act	Fun
DO_1	18.40	67.56	19.73
DO_2	-0.31	-0.57	0.87
M_1	0.10	3.19	0.08
M_2	-0.39	-0.55	-0.02
M_1 DO	0.07	-0.06	-0.16
M_2 DO	-0.48	0.13	0.02
S_1	-0.74	-0.43	-0.38
S_2	-0.51	-0.17	0.31
S_1 DO	-0.72	-0.60	-0.25
S_2 DO	6.53	5.62	-0.07
A_1	-0.51	0.78	-0.45
A_2	-0.67	-0.44	0.18
A_1 DO	-0.24	-0.19	-0.52
A_2 DO	15.56	17.34	-0.65
I_1	-0.58	-0.35	-0.20
I_2	-0.43	-0.23	0.56
I_1 DO	-0.59	-0.50	-0.44
I_2 DO	0.37	0.25	0.04

(-∞; -0.1>
(-0.1;0.2>
(0.2;0.5>
(0.5;1.0>
(1.0;2.0>
(2.0;4.0>
(4.0;8.0>
(8.0;12>
(12; ∞)

Fig. 5. The index of the influence of diesel oil (IF_{DO}) and remediation substances (IF_R) on the counts of soil microorganisms: DO – diesel oil, M, S, A, I – remediation substance (see Table 1), 1 – 20 day, 2 – 60 day, Org – organotrophic bacteria, Act – actinomycetes, Fun – fungi

significant decrease in the counts of organotrophic bacteria (by 39%) and actinomycetes (by 55%) on day 60. The tested agent had a negligent influence on fungal counts in uncontaminated soil on both dates of analysis. In contrast, sepiolite (S) decreased the counts of organotrophic bacteria by 71% on day 20 and by 51% on day 60, decreased actinomycetes counts by 43% and 17%, respectively, and decreased fungal counts by 38% on day 20 in uncontaminated soil. Similarly to sepiolite, alginite (A) and Ikasorb (I) induced a significant decrease in the counts of organotrophic bacteria and actinomycetes in uncontaminated soil, and exerted varied effects on fungal counts. The remediation agents had a varied influence on microbial counts in soil contaminated with diesel oil. On day 60, sepiolite, alginite and Ikasorb significantly increased the counts of organotrophic bacteria and actinomycetes, and alginite decreased fungal counts.

The colony development index (CD) of organotrophic bacteria and actinomycetes isolated from uncontaminated soil increased significantly over time (Figure 6), whereas fungal CD values remained fairly stable throughout the experiment. The CD values of organotrophic bacteria increased also in contaminated soil. The CD values of actinomycetes and fungi decreased on day 60 relative to day 20. The CD values of organotrophic bacteria isolated from uncontaminated soil treated with remediation agents were higher on day 60 than on day 20. Similar results were noted in contaminated and remediated soil, excluding treatments with the addition of the molecular sieve. The CD values of organotrophic bacteria and actinomycetes isolated from contaminated soil on day 60 were lower than the values noted in uncontaminated soil. In contrast, the CD values of fungi were higher in isolates from contaminated that uncontaminated soil. The ecophysiological diversity index (EP) of organotrophic bacteria and fungi decreased in soil contaminated with diesel oil (Figure 7). The EP values of organotrophic bacteria tended to increase under the influence of all the remediation agents in both uncontaminated and contaminated soil. The EP values of actinomycetes increased only in uncontaminated soil. The EP values of fungi isolated from remediated soil were similar under the influence of all tested agents, whereas the EP values of fungi isolated from contaminated soil decreased not only in response to diesel oil, but also under the influence of the molecular sieve and sepiolite.

The taxonomic diversity of prokaryotes was analyzed based on the sequence of the V3-V4 hypervariable region of the 16S rRNA gene to determine the composition of bacterial communities in uncontaminated soil and soil contaminated with diesel oil (Figure 8). *Proteobacteria* was the dominant phylum in both contaminated and uncontaminated soil. Diesel oil decreased the percentage of *Firmicutes, Actinobacteria, Bacteroidetes* and *Acidobacteria* in comparison with uncontaminated soil. Bacterial genera were identified to describe changes in the taxonomic composition of prokaryotes.Bacteria of the genera *Janthinobacterium* and *Arthrobacter* were the most isolated from both contaminated and uncontaminated soil. In the soil contamineted with DO were dominated bacterial genera *Burkholderia*, *Sphingomonas*, *Caulo-*

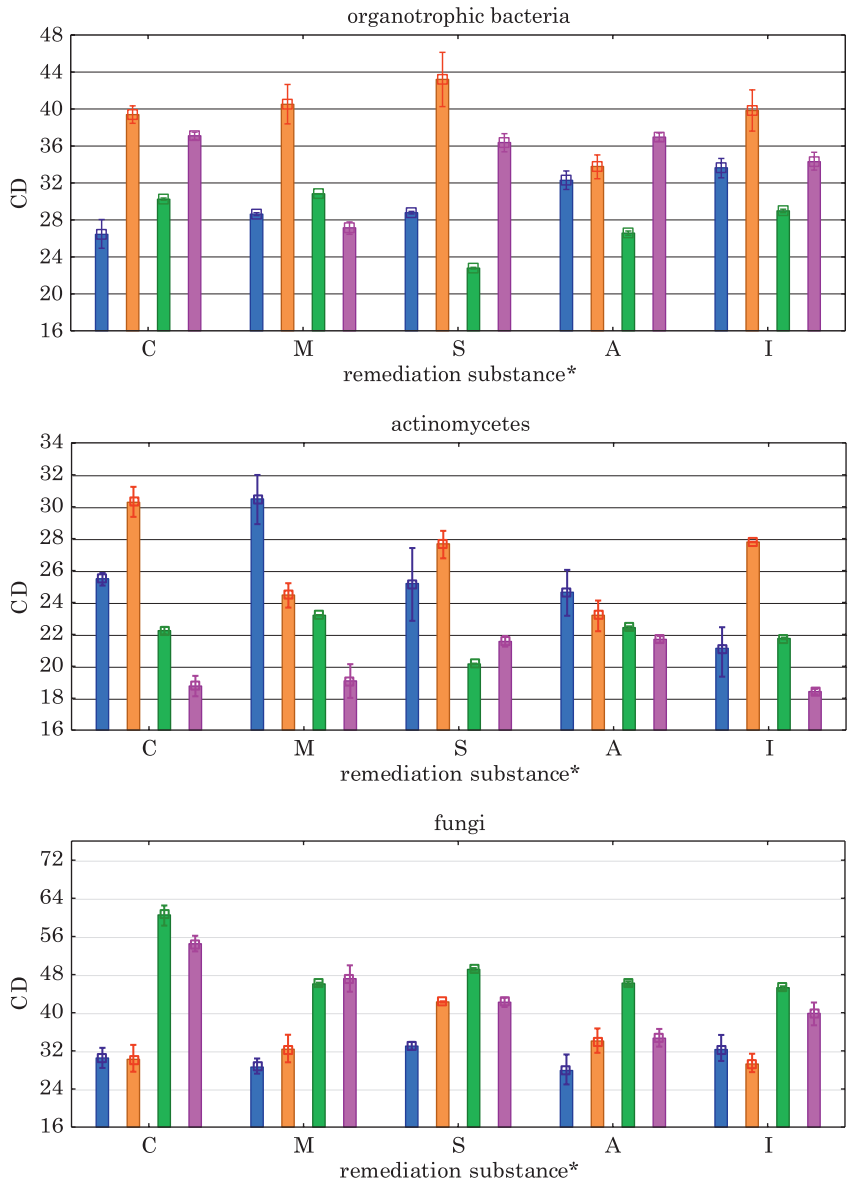

Fig. 6. Colony development index (CD) of organotrophic bacteria, actinomycetes and fungi in soil: C – control, DO – diesel oil, 1 – 20th day, 2 – 60th day.
Error bars represent standard error of the mean for n = 4; * explanations are given in Table 1

bacter, *Streptomyces* and *Pseudomonas*, but in the not contaminated were *Janthinobacterium*, *Bacillus*, *Arthrobacter*, *Kaistobacter*, *Oxalobacter*, *Pedobacter* and *Paenibacillus*.

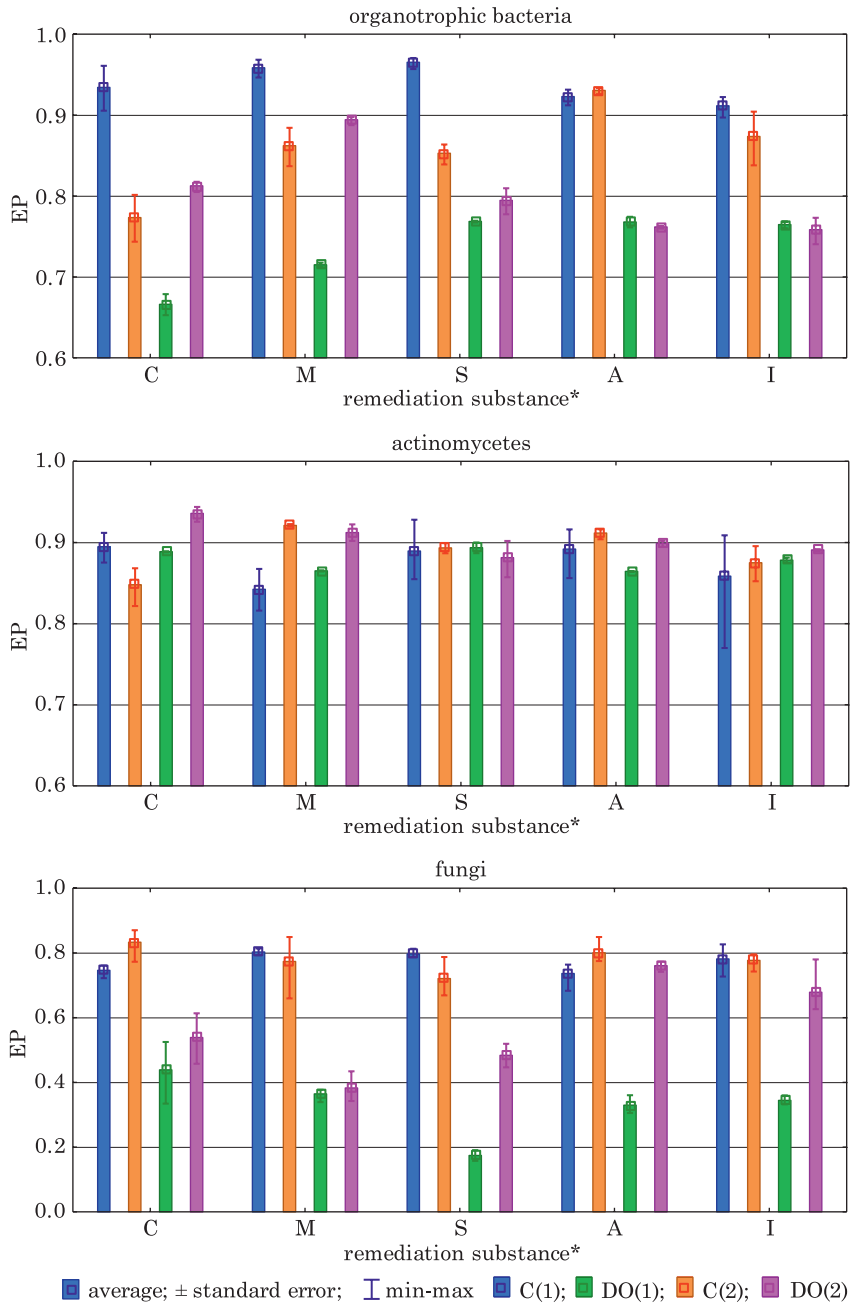

Fig. 7. Ecophysiological index of diversity (EP) of organotrophic bacteria, actinomycetes and fungi in soil: C – control, DO – diesel oil, 1 – 20th day; 2 – 60th day.
Error bars represent standard error of the mean for n = 4; * explanations are given in Table 1

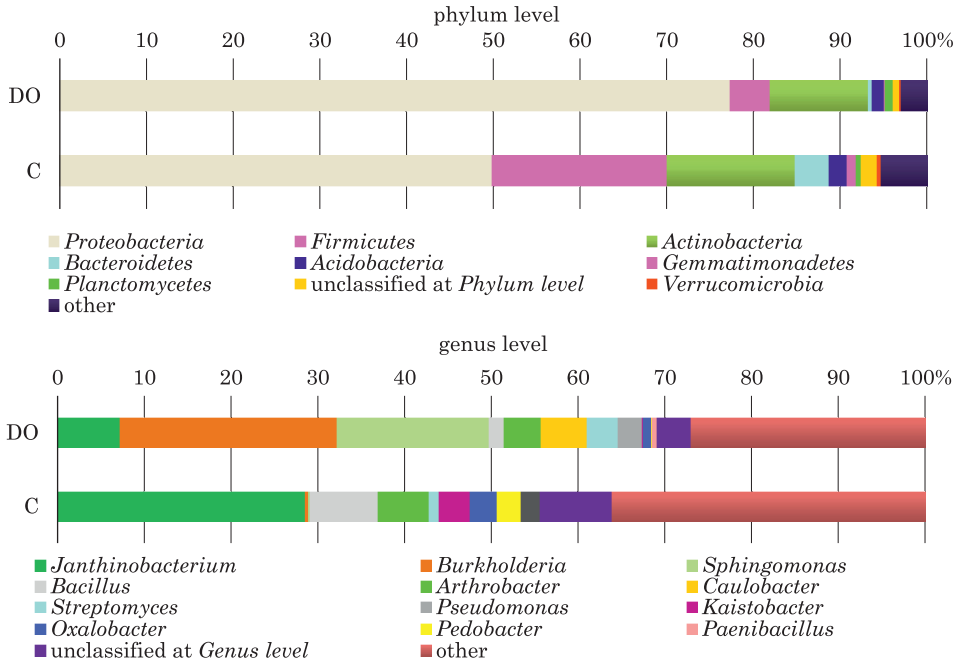

Fig. 8. Composition of bacterial biocenosis after the end of the experiment, C – soil uncontaminated with diesel fuel, DO – soil contaminated with diesel oil

Enzyme activity

In the group of the analyzed independent variables, the activity of soil enzymes was most influenced by diesel oil (28%-89%), and it was least affected by the applied remediation agents (2.3%-14%) – Figure 9. The date of analysis influenced enzyme activity in 2.3% to 31%. The activity of dehydrogenases, catalase, alkaline phosphatase, acid phosphatase, arylsulphatase and ß-glucosidase was adequately described by the first principal component (Figure 9). The above enzymes were bound by significant positive correlations. The coordinates on the PC1 axis ranged from -0.77 (dehydrogenases) to -0.95 (arylsulphatase). Urease was the only enzyme whose response to the analyzed factors was described by the second principal component. The value of urease on the PC2 axis was 0.89.

The activity of all soil enzymes increased under the influence of diesel oil (Figures 9, 10). Diesel oil induced the greatest increase in dehydrogenases activity and the smallest increase in the activity of acid phosphatase (Figure 10). Selected enzymes were stimulated by both diesel oil and remediation agents. On day 60, dehydrogenases activity increased most notably in response to the molecular sieve (M), sepiolite (S), alginite (A) and sorbent (I). In contaminated soil, catalase was stimulated only by alginite. Urease activity was most effectively enhanced by the molecular sieve in both contaminated and uncontaminated soil. The activity of acid phosphatase was not influenced or

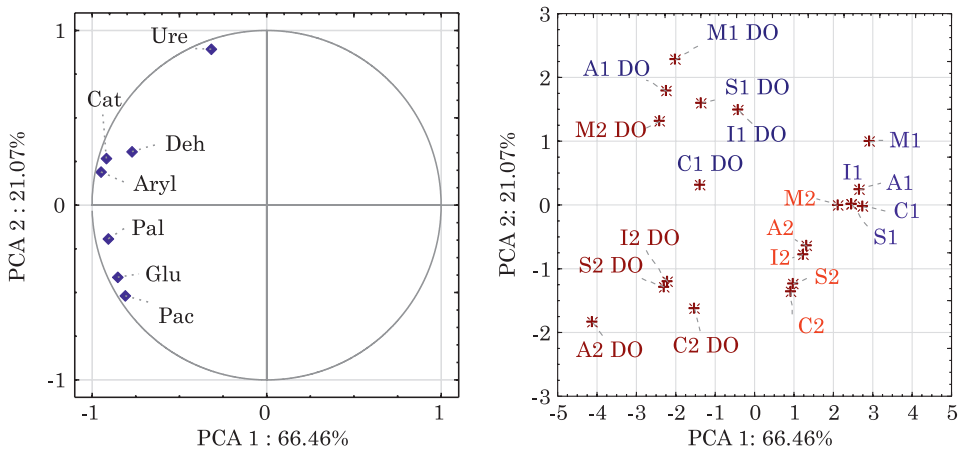

Fig. 9. The influence of the factors studied on the soil enzymes activity presented by the principal component analysis: C – control, DO – diesel oil, M, S, A, I – remediation substances (see Table 1), 1 – 20th day, 2 – 60th day, Deh – dehydrogenases, Cat – catalase, Pac – acid phosphatase, Pal – alkaline phosphatase, Aryl – arylsulphatase; Glu – β-glucosidase

	Deh	Cat	Ure	Pac	Pal	Glu	Aryl
DO_1	6.80	0.93	1.36	0.41	0.58	1.95	0.94
DO_2	3.35	0.20	2.90	0.28	1.15	0.31	0.41
M_1	0.31	0.06	1.01	-0.18	-0.04	-0.13	-0.02
M_2	9.50	-0.03	0.67	-0.26	-0.14	-0.55	-0.17
M_1 DO	0.33	0.06	0.79	-0.14	0.35	-0.18	0.28
M_2 DO	11.20	0.13	0.76	-0.28	-0.04	-0.32	0.40
S_1	-0.31	0.17	0.13	0.05	-0.13	0.12	0.08
S_2	10.49	-0.17	0.44	0.05	0.11	-0.15	-0.08
S_1 DO	-0.26	0.03	0.61	-0.07	0.66	-0.35	0.10
S_2 DO	4.54	0.23	-0.59	-0.11	0.05	0.07	0.10
A_1	0.04	0.04	0.18	-0.08	-0.17	0.18	0.18
A_2	7.94	-0.17	1.24	-0.12	0.03	-0.19	0.01
A_1 DO	0.24	0.08	0.54	-0.05	1.04	-0.38	0.28
A_2 DO	4.63	0.30	-0.50	0.01	0.59	0.26	0.38
I_1	0.15	-0.16	0.39	0.01	-0.17	0.47	0.28
I_2	12.07	-0.20	1.09	-0.04	0.13	-0.27	-0.06
I_1 DO	-0.54	0.02	0.35	-0.15	0.10	-0.43	0.07
I_2 DO	9.21	0.08	-0.52	-0.07	-0.19	0.14	-0.09

(-∞;-0.1>
(-0.1;0.2>
(0.2;0.5>
(0.5;1.0>
(1.0;2.0>
(2.0;4.0>
(4.0;8.0>
(8.0;12>
(12; ∞)

Fig. 10. The index of the influence of diesel oil (IF_{DO}) and remediation substances (IF_R) on the soil enzymes activity: DO – diesel oil, M, S, A, I – remediation substances (see Table 1), 1 – 20 day, 2 – 60 day, Deh – dehydrogenases, Cat – catalase, Pac – acid phosphatase, Pal – alkaline phosphatase, Aryl – arylsulphatase; Glu – β-glucosidase

was inhibited by the tested remediation agents, whereas alkaline phosphatase was stimulated by alginite in contaminated soil. The activity of β-glucosidase was inhibited by the molecular sieve in both soil treatments, and the remaining remediation agents had a mostly inhibitory effect on this enzyme. Arylsulphatase was stimulated by the molecular sieve and alginite in contaminated soil.

Physicochemical and chemical properties of soil

Soil contamination with diesel oil increased hydrolytic acidity and decreased exchangeable base cations, sorptive capacity and base saturation (Table 3).

Table 3

Physical and chemical properties of the soil

Remediation substance	DO dose (cm^3 kg^{-1} of soil)	pH_{KCl}	HAC	EBC	CEC	BS (%)
			mmol(+) kg^{-1} DM of soil			
Control - C	0	6.63^{b}	8.13^{c}	93.33^{d}	101.46^{de}	91.99^{c}
	10	6.70^{b}	12.50^{ab}	47.33^{f}	59.83^{f}	79.11^{h}
Molecular sieve - M	0	7.20^{a}	8.13^{c}	138.00^{a}	146.13^{a}	94.44^{a}
	10	7.10^{ab}	12.19^{ab}	118.67^{b}	130.86^{b}	90.68^{d}
Sepiolite - S	0	6.87^{ab}	8.75^{c}	131.33^{a}	140.08^{a}	93.75^{b}
	10	6.80^{ab}	11.88^{b}	102.67^{c}	114.55^{c}	89.63^{e}
Alginite - A	0	6.80^{ab}	8.13^{c}	122.00^{b}	130.13^{b}	93.75^{b}
	10	6.80^{ab}	12.81^{a}	104.67^{c}	117.48^{c}	89.10^{f}
Ikasorb - I	0	6.90^{ab}	6.88^{d}	102.67^{c}	109.55^{cd}	93.72^{b}
	10	6.70^{b}	12.81^{a}	84.00^{e}	96.81^{e}	86.77^{g}

DO – diesel oil; HAC – hydrolitic acidity; EBC – exchangeable base cations; CEC – cation exchange capacity; BS – base saturation;
The same letters show homogeneous groups separately for the physical and chemical properties of the soil. Different letters show significant differences separately for the physical and chemical properties of the soil (Tukey test, $P = 0.01$).

All the remediation agents significantly increased exchangeable base cations, sorptive capacity and base saturation of both contaminated and uncontaminated soil. Diesel oil increased the concentrations of available phosphorus and magnesium and exchangeable magnesium and potassium (Table 4). In contaminated soil, the molecular sieve increased the content of available potassium and exchangeable calcium, potassium and sodium. Sepiolite increased the concentrations of exchangeable calcium, magnesium and sodium and available magnesium. Alginite increased the accumulation of available magnesium and exchangeable calcium and magnesium. Ikasorb increased the content of exchangeable calcium in soil.

Hydrocarbons were rapidly degraded in soil (Figure 11). The concentra-

Table 4

The content of elements in the soil

Remediation substance	DO dose (cm^3 kg^{-1} of soil)	Available forms			Removable cations			
		P	K	Mg	Ca	Mg	K	Na
		(mg kg^{-1} DM of soil)			($mmol^+$ kg^{-1} DM of soil)			
Control - C	0	16.09^{a}	8.72^{f}	5.30^{e}	40.51^{e}	5.29^{d}	2.15^{e}	1.74^{d}
	10	15.13^{ab}	25.32^{c}	6.30^{cd}	40.51^{e}	7.25^{c}	6.29^{b}	1.74^{d}
Molecular sieve - M	0	10.68^{c}	83.00^{a}	3.60^{f}	68.91^{ab}	4.11^{e}	16.98^{a}	22.62^{b}
	10	9.24^{e}	75.36^{b}	2.80^{g}	72.48^{a}	3.45^{e}	17.49^{a}	25.40^{a}
Sepiolite - S	0	15.78^{a}	12.87^{efg}	8.70^{a}	52.28^{d}	12.71^{a}	3.63^{d}	4.35^{c}
	10	14.56^{ab}	16.19^{d}	8.80^{a}	53.46^{d}	13.03^{a}	6.04^{bc}	2.61^{d}
Alginite - A	0	14.13^{b}	10.79^{ef}	7.60^{b}	60.59^{c}	9.28^{b}	2.92^{de}	1.74^{d}
	10	15.04^{ab}	15.36^{d}	6.80^{e}	64.75^{bc}	8.86^{b}	5.32^{bc}	1.7^{d}
Ikasorb - I	0	13.82^{b}	10.38^{f}	6.20^{cd}	52.87^{d}	6.85^{c}	2.92^{de}	1.74^{d}
	10	14.74^{ab}	14.94^{de}	5.90^{de}	52.28^{d}	6.46^{c}	5.22^{c}	1.74^{d}

DO – diesel oil;
The same letters show homogeneous groups separately for the content of elements in soil. Different letters show significant differences separately for the content of elements in soil (Tukey test, $P = 0.01$).

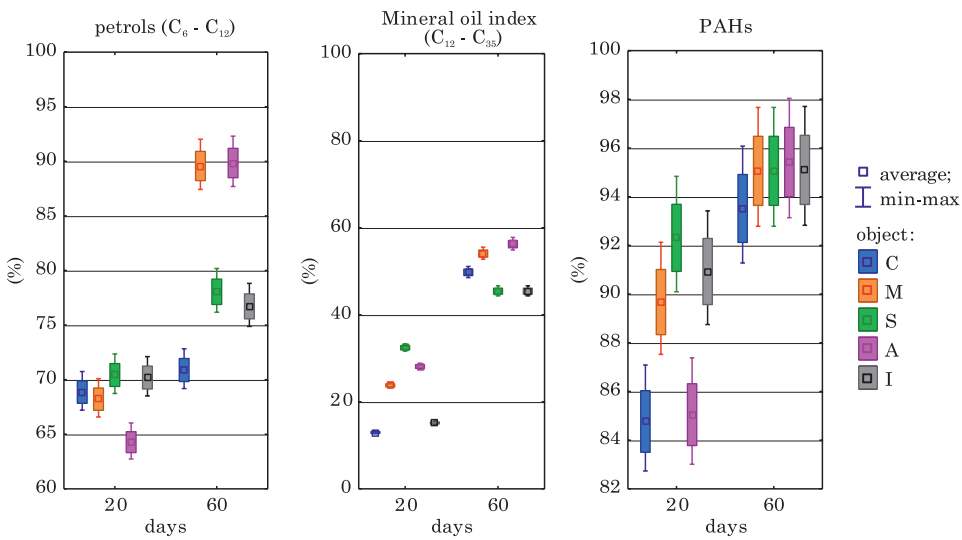

Fig. 11. Percentage of removed hydrocarbons from soil contaminated with diesel oil (DO): C – control without remedition substance, M – molecular sieve, S – sepiolite, A – alginite, I – Ikasorb; Error bars represent standard error of the mean for $n = 3$

tions of BETX are not presented in Figure 11 because 98.5% of volatile hydrocarbons were degraded already on day 20. On day 60, ∑9 PAHs were degraded in 96%, mineral oil (C_{12}-C_{35}) – in 51%, and petroleum hydrocarbons

(C_6-C_{12}) – in 73%. Volatile hydrocarbons were broken down most rapidly, and only 1.5% of BETX was detected in soil on day 20. Mineral oil (C_{12}-C_{35}) was degraded at the slowest rate, and its accumulation in soil remained high at 87% on day 20. All remediation agents accelerated the degradation of petroleum hydrocarbons (C_6-C_{12}) and ∑ 9 PAHs. The biodegradation of mineral oil (C_{12}-C_{35}) was intensified under the influence of the molecular sieve and alginite.

DISSCUSSION

Environmental pollution caused by hydrocarbons poses a significant threat to various biocenoses regardless of the source of contamination (Labud et al. 2007, Yang et al. 2016). Rapid and effective methods of soil remediation are needed to counteract the negative consequences of hydrocarbon pollution (Hernández-Espriú et al. 2013, Zadaka-Amir et al. 2013, Chen et al. 2016, Kuppusamy et al. 2017). In this study, diesel oil significantly inhibited the growth and development of maize plants. Diesel oil exerts both direct (Kucharski, Jastrzębska 2006, Labud et al. 2007) and indirect effects on plants by modifying the physicochemical properties of soil (Polyak et al. 2018). In the present study, diesel oil increased soil acidity and decreased the sorptive capacity of soil. The above inhibited the growth and development of the aerial parts and roots of maize plants, it increased the concentrations of nitrogen, magnesium, calcium, potassium and sodium in both organs, and decreased the bioconcentration index of nitrogen, phosphorus, calcium and potassium in maize plants.

Diesel oil disrupted the microbiological equilibrium in soil and induced changes in microbial diversity. The evaluated pollutant decreased the ecophysiological diversity of organotrophic bacteria and fungi and lowered the percentage of *Firmicutes, Actinobacteria, Bacteroidetes* and *Acidobacteria* in the structure of soil-dwelling bacteria. The above modifications resulted from microbial adaptation to a changing environment (Nwankwegu et al. 2016).

Diesel oil stimulated the activity of intercellular and extracellular enzymes. This is a desirable outcome because enzymatic stimulation intensifies the biodegradation of hydrocarbons. On day 20, BTEX hydrocabons were eliminated in 98.5%, and on day 60, ∑9PAHs were degraded in 96%. Mineral oil (C_{12}-C_{35}) was bio-transformed at the slowest rate. According to Nwankwegu et al. (2016) and Silva-Castro et al. (2015), the biodegradation rate of diesel oil is correlated with microbial biostimulation. Every factor that stimulates microbial activity contributes to soil sanitation.

The analyzed remediation agents minimized the negative effects of diesel oil on aerial plant parts by 72% (sepiolite) to 149% (alginite) and on roots by 87% (sepiolite) to 158% (Ikasorb). The bioconcentration index of nitrogen, phosphorus, calcium and potassium in maize decreased under exposure to

diesel oil, but increased in response to remediation agents, in particular the molecular sieve. Remediation agents neutralized the adverse changes in the chemical composition of maize plants induced by diesel oil. The analyzed compounds improved the physicochemical and chemical properties of soil by decreasing its hydrolytic acidity, increasing its exchange capacity and, in some cases, exchangeable base cations. These results can be attributed to the chemical composition (Wu et al. 2016, Júlio et al. 2018) and considerable sorptive capacity of the evaluated compounds (Lehmann et al. 2011, De Lima et al. 2017). The tested remediation agents exerted varied effects on the soil microbiome. Sepiolite, alginite and Ikasorb generally enhanced the growth of actinomycetes by increasing the proportion of K-strategists in soil. The ecophysiological diversity of organotrophic bacteria increased and the ecophysiological diversity of fungi remained unchanged under the influence of all remediation agents. According to Sun et al. (2013), sepiolite promotes the proliferation of bacteria and actinomycetes.

All of the analyzed remediation agents increased the activity of dehydrogenases, the most sensitive indicators of soil microbial activity. The evaluated compounds inhibited the activity of acid phosphatase, and exerted varied effects on urease, alkaline phosphatase, β-glucosidase and arylsulphatase. The tested remediation agents intensified the degradation of petroleum hydrocarbons (C_6-C_{12}) and $\sum$9 PAHs, and the molecular sieve and alginite also accelerated the biodegradation of mineral oil (C_{12}-C_{35}). According to Silva-Castro et al. (2015), the degradation of diesel oil can also be intensified through biostimulation and bioaugumentation.

CONCLUSIONS

1. Soil contamination with diesel oil significantly inhibited the growth and development of maize and modified the chemical composition of this crop. Diesel oil decreased the bioconcentration index of nitrogen, phosphorus, calcium and potassium in maize. The molecular sieve, sepiolite, alginite and sorbent minimized the adverse effects of diesel oil on the aboveground parts and roots of maize plants.

2. Diesel oil disrupted the microbial equilibrium in soil, and it decreased the ecophysiological diversity of fungi and organotrophic bacteria. Diesel oil decreased the percentage of *Firmicutes, Actinobacteria, Bacteroidetes* and *Acidobacteria* in the bacterial community, stimulated intracellular and extracellular enzymes, and exerted adverse effects on the physical properties of soil.

3. The evaluated soil remediation agents exerted varied effects on the microbiome of contaminated soil. Sepiolite, alginite and Ikasorb generally enhanced the growth of actinomycetes by increasing the proportion of K-strategists in soil. All the remediation agents increased the ecophysiological di-

versity of organotrophic bacteria, stimulated the activity of dehydrogenases and improved the physicochemical properties of soil.

4. All the remediation agents intensified the degradation of petroleum hydrocarbons (C_6-C_{12}) and ∑9 PAHs, and the molecular sieve and alginite also accelerated the biodegradation of mineral oil (C_{12}-C_{35}).

5. The effectiveness of the molecular sieve, sepiolite, alginite and sorbent as soil remediation agents was evaluated in analyses of the microbiological, biochemical, physicochemical and chemical properties of soil and in analyses of plant responses and changes in the chemical composition of maize plants under the influence of diesel oil. It can be concluded that all of the tested compounds minimize the negative consequences of soil contamination with diesel oil and that the molecular sieve and alginite are the most effective bioremediation agents.

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