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ORIGINAL PAPER

BIOAUGMENTATION OF SOIL CONTAMINATED WITH DIESEL OIL*

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Abstract

The research objective has been to determine the effectiveness of bioaugmentation of soil polluted with diesel oil VERVA (DO) in restoring the biological balance of the contaminated soil. Another goal was to determine the suitability of bioaugmentation for degradation of petrols (C_6-C_{12}), mineral oils (C_{12}-C_{35}), volatile hydrocarbons (BETX) and ∑9 polycyclic aromatic hydrocarbons (PAHs) present in diesel oil. The research included a pot experiment. Two soils (loamy sand and sandy loam) were tested, either unpolluted or polluted with DO in a dose of 7 cm^3 kg^{-1}. Maize served as a test plant. Bioaugmentation was carried out with the biopreparation BIO ACTIV HGS code 208 (BC) and with a consortium of bacteria (CN) isolated from soil contaiminated with diesel oil. It has been found that the extent of DO toxic effects on plants depended on the textural composition of soil. Diesel oil was more toxic to maize grown on loamy sand than on sandy loam. The pollutant disturbed proportions between fast-growing (strategy r) and slow-growing (strategy K) microorganisms. It had an adverse effect on the ecophysiological diversity of microorganisms. Bioaugmentation of DO-polluted soil changed proportions in the structure of bacterial communities. There was a partial shift of the assemblage of actynomycetes towards strategy r while organotrophic bacteria shifted towards strategy K. Bioaugmentation alleviated the negative influence of DO on the diversity of microorganisms, and on the growth and development of maize. It accelerated degaration of petrols and mineral oils.

Keywords: diesel oil, bioaugmentation, soil microbiome, degradation of hydrocarbons.

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INTRODUCTION

Petroleum hydrocarbons are major pollutants in the natural environment. Their removal has become a great challenge, both ecological and economic one, in the contemporary world (Tyagi et al. 2011, Saleem 2016). In recent years, bioaugmentation has gained importance among numerous other innovative techniques applied for the sake of remediating environmental sites polluted with crude oil products (Dos Santos, Maranho 2018). Bioaugmentation consists of the soil incorporation of selected microbial strains able to decompose pollutants which occur in the environment (Tyagi et al. 2011, Clarkson and Abubakar 2015, Ameen et al. 2016, Chachina et al. 2016). Microorganisms used for bioaugmentation should be highly resistant and adaptable to a contaminated habitat, as well as capable of decomposing pollutants (Yang et al. 2009, Tyagi et al. 2011, Clarkson, Abubakar 2015, Wang et al. 2015). Among the bacteria most frequently employed for bioaugmentation of soils polluted with hydrocarbons, there are: *Pseudomonas, Mycobacterium*, *Paenibacillus, Rhodococcus, Bacillus, Achromobacter, Arthrobacter, Acinetobacter, Chryseobacterium, Stenotrophomonas, Alcaligenes, Gordonia, Nocardia, Corynebacterium, Geobacillus, Klebsiella, Brachybacterium, Sphingobium, Serratia, Micrococcus* (Maddela et al. 2015, Ameen et al. 2016, Chachina et al. 2016). It is essential that microorganisms and biopreparations used for bioaugmentation be safe for people and the natural environment (Tyagi et al. 2011, Saleem 2016).

The rate at which crude oil products are degraded depends on the type of a pollutant and degree of pollution (Wyszkowska et al. 2015), but equally important are the physical and chemical properties of soil, its porosity, structure and permeability (Wolf et al. 2013), temperature, moisture (Laocharoen et al. 2015), plant species (Ogbo 2009, Saleem 2016, Sivaram et al. 2017), as well as a type, species or even strain of microorganisms (Tyagi et al. 2011). It is not uncommon for a soil bioaugmentation treatment to fail because of such numerous and diverse factors (Alrumman et al. 2015, Laocharoen et al. 2015).

According to Chachina et al. (2016), bioaugmentation is effective when soil contamination with petroleum products does not exceed 20 g kg^{-1} DM of soil. Saleem (2016) draws attention to the fact that bioaugmentation can be significantly accelerated by combining it with phytoremediation. Root secretions contain nutrients and essential metabolites, which facilitate colonisation of the rhizosphere by microorganisms, while microorganisms promote the growth and development of plants (Fatima et al. 2016, Dos Santos, Maranho 2018). Also, the activity of enzymatic complexes of metabolic pathways used for degradation of pollutants is stimulated (Lamy et al. 2013).

The above considerations gave rise to the following research, whose aim was to determine the role of the biopreparation BIO ACTIV HGS code 208 and a consortium of autochthonous bacteria in the restoration of the biological equilibrium in soil contaminated with diesel oil Verva. The overriding

goal was to identify to what extent bioaugmentation could help to degrade petrols (C_6-C_{12}), mineral oils (C_{12}-C_{35}), volatile hydrocarbons (BETX) and a sum of 9 PAHs. Our assessment of the role of bioaugmentation was supported by the characteristics of the soil microbiome, activity of intracellular and extracellular enzymes, and the response of maize to the soil contamination with diesel oil.

MATERIAL AND METHODS

Soil

Considering the fact that the soil textural composition has a significant role in the process of degrading petroleum products, two soils with different shares of sand, silt and clay as well as different physicochemical and chemical properties were included in the experiment (Table 1). The soils used in

Table 1

Characteristics of soils used in the experiment

Parametr	Loamy sand (LS)	Sandy loam (SL)
Sand 2000-50 µm (%)	74.30 ± 2.23	70.38 ± 2.11
Silt 50-2 µm (%)	23.69 ± 1.42	27.19 ± 0.82
Clay <2 µm (%)	2.01 ± 0.09	2.43 ± 0.07
pH_{KCl}	6.98 ± 0.17	7.13 ± 0.21
HAC (mmol(+) kg^{-1})	8.00 ± 0.30	5.70 ± 0.34
EBC (mmol(+) kg^{-1})	84.20 ± 4.55	181.80 ± 5.45
CEC (mmol(+) kg^{-1})	92.20 ± 4.84	187.50 ± 5.80
BS (%)	91.32 ± 0.14	96.96 ± 0.09
C_{org} (g kg^{-1})	11.20 ± 0.55	11.50 ± 0.70
N_{tot} (g kg^{-1})	0.89 ± 0.05	1.01 ± 0.06
Content of exchangeable (mg kg^{-1})		
K^+	320.00 ± 19.52	302.00 ± 9.06
Na^+	60.00 ± 2.88	80.00 ± 2.72
Ca^{2+}	600.00 ± 26.40	1238.10 ± 44.57
Mg^{2+}	66.70 ± 3.40	73.80 ± 2.21
Content of available (mg kg^{-1})		
P	164.05 ± 6.89	172.73 ± 5.01
K	53.95 ±2.75	78.85 ± 3.71
Mg	46.00 ± 2.44	38.00 ±2.01

pH_{KCl} – soil reaction, C_{org} – organic carbon content, N_{tot} – total nitrogen content, HAC – hydrolytic acidity, EBC – exchangeable base cations; CEC – cation exchange capacity; BS – base saturation

the experiments were brown eutrophic soils (Eutric Cambisols), which had been sampled from the arable humic horizon (depth of 0 to 20 cm) in a field at the Research Station in Tomaszkowo, which belongs to the University of Warmia and Mazury in Olsztyn, Poland (NE Poland, 53.7167°N, 20.4167°E).

Characteristics of diesel oil

The diesel oil used in the experiments is sold under the brand name Verva – type B (DO). Detailed specification of diesel oil Verva can be found on the manufacturer's website (http://www.orlen.pl/).

Preparation of a consortium of microorganisms

Strains of bacteria acquired from soil polluted with diesel oil Verva were used in the experiment. To this aim, samples of proper brown soil (Eutric Cambisol) weighing 1 kg each were polluted with diesel oil Verva (DO) in a dose of 20 cm^3 kg^{-1} DM of soil, after which they were watered up to 50% capillary water capacity. The polluted soil samples were incubated for 60 days at a temperature of 22°C, while maintaining a constant moisture content. After 60 days since the contamination, bacteria were isolated from the soil by being cultured on the Bunt and Rovira (1955) medium, using the method of serial dillutions. In order to obtain pure cultures, colonies of microorganisms which had developed most intensively in the DO polluted soil were inoculated and then purified through multiple passage. Identification of soil bacteria was achieved on the basis of an analysis of 16S rRNA coding sequences. The following primers were employed: B-all Forward GAG TTT GAT CCT GGC TCA G and B-all Reverse ACG GCT ACC TTA CGA CTT. Sequences of 16S rRNA fragments were compared with the help of BLAST to sequences available in the GenBank of the NCBI. Strains of bacteria used for the further study (Figure 1) were the ones which were idenified at 99-100% score of the sequence match according to the analysis supported by the NCBI dadabase. Prior to the pot expe-

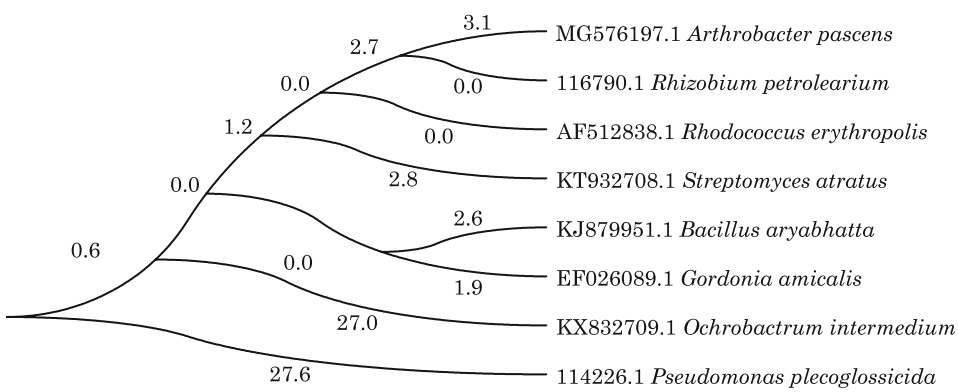

Fig. 1. Phylogenetic tree of bacteria isolated from soil contaminated with diesel oil based on an analysis of the 16S rRNA gene used as a bacterial consortium (CN) in the experiment

riment, 48-hour isolates of bacteria were transferred with the help of 0.85% NaCl to 100 cm^3 of liquid PCA medium and proliferated in an incubator set at a constant temperature of 28°C for 48 h. Each strain was multiplied separately. Afterwards, all cultures were combined into a single sample. 1 cm^3 of the suspension contained $4 \cdot 10^8$ of cells. The composition of the consortium was as follows: *Arthrobacter pascens* – 12%, *Rhizobium petrolearium* – 10%, *Rhodococcus erythropolis* – 13%, *Streptomyces atratus* – 13%, *Bacillus aryabhatta* – 12%, *Gordonia amicalis* – 15%, *Ochrobactrum intermedium* – 12%, *Pseudomonas plecoglossicida* – 13%.

Characteristics of BIO ACTIV HGS code 208

The commercial biopreparation BIO ACTIV HGS code 208 (BC) was used in this study. The preparation is dedicated to biological degradation of hydrocarbons. It contains microorganisms with directed action, bound to mineral carriers (calcium and silicate). The preparation comes in the form of fine powder, with grains size from 20 to 160 µm, density of 1-1.2 g cm^{-3}, and pH 6.5-7.5. The preparation was obtained from the company EKOB-TBA.

Experimental protocol

In order to compare the effectiveness of the microbial consortium composed of bacteria isolated from diesel oil contaminated soil and the preparation BIO ACTIV HGS code 208 in the degradation of hydrocarbons, a plant growing pot experiment was conducted in a greenhouse. Plants were grown in polyethylene pots filled with 3.0 dm^3 of soil each. The experimental variables were: (1) type of soil: loamy sand (LS) and sandy loam (SL); (2) degree of soil contamination with diesel oil Verva: 0 and 7 cm^3 kg^{-1}; (3) type of the inoculum stimulating the natural soil microbiota: a consortium of microorganisms (CN) composed of microorganisms isolated from soil contaminated with diesel oil, and the preparation BIO ACTIV HGS code 208 (BC). Before placing soil in pots, LS and SL batches weighing 2.5 kg were mixed thoroughly with $CO(NH_2)_2$, KH_2PO_4, KCl and $MgSO_4 \cdot 7H_2O$. Identical amounts of fertilisers, such as (per kg^{-1} DM of soil) 112 mg of nitrogen, 39 mg of phosphorus, 112 mg of potassium and 15 of magnesium, were added to each pot. The pots were divided into three groups: one was inoculated with microorganisms (CN), by adding a dose of the microbial consortium (CN) equal 10 cm^3 kg^{-1} d.m. of soil to each pot; the second group was treated with the biopreparation BIO ACTIV HGS code 208 in a dose of 0.6 g kg^{-1} DM of soil and 10 cm^3 of the liquid PCA medium, and the third one, which received 10 cm^3 of the liquid PCA medium, served as the control. Once the soil batches were placed in the pots, the soil moisture level was raised to 60% of capillary water capacity, after which maize PR39H32 was sown. The experiment was conducted with 5 replicates. Maize plants (6 per pot) were grown for 60 days, during which the soil moisture content was monitored. The aerial parts and roots of maize were harvested at the BBCH 59 stage.

Methodology of microbiological assays

On the maize harvest day, loamy sand (LS) and sandy loam (SL) soil samples were submitted to the determination, by the serial dilution method with 4 replications, of the counts of organotrophic bacteria (Org), actinomycetes (Act) and fungi (Fun), according to the protocol presented by Borowik et al. (2017a). The counts of bacteria, actinomycetes and fungi served to calculate the colony development index (CD) and ecophysiological diversity coefficient (EP), as instructed by De Leij et al. (1993).

Methodology of the biochemical assays

Parallel to the microbiological assays, soil samples were submitted to determinations of the activity of dehydrogenases (Deh), catalase (Cat), urease (Ure), acid phosphatase (Pac), alkaline phosphatase (Pal), β-glucosidase (Glu) and arylsulfatase (Aryl). These determinations were replicated thrice. The detailed protocol for determination of the above enzymes and activity units can be found in the paper by Borowik et al. (2017*a*).

Methodology of the determination of physicochemical and chemical soil properties

Soil samples were analysed to determine the grain-size composition, pH_{KCl}, hydrolytic acidity (HAC) and exchangeable base cations (EBC), content of organic carbon (C_{org}), total nitrogen (N_{tot}), assimilable phosphorus, potassium and magnesium, all according to the protocol presented by Borowik et al. (2017*a*). Further determinations included the soil content of petrols (C_6-C_{12}) and volatile aromoatic hydrocarbons (BETX) compliant to EN ISO 22155 (2016), mineral oil (C_{12}-C_{35}) in line with EN ISO 16703 (2011) and $\sum$9 PAHs according to ISO 18287 (2006). All these determinations were made on a gas chromatographer Agilent 7890A.

Statistical analysis

The experimental results were processed statistically with the support of Statistica 13.0 (Dell Inc. 2016). The following were calculated: the coefficient η^2 – with the analysis of variance ANOVA method, homogeneous groups – applying the Tukey's test at $P = 0.01$, PCA (principal component analysis) – using multidimensional and exploratory analyses. In addition, the factors corresponding to the impact of diesel oil as well as bioaugmentation on the soil microbiome were derived from the formulas presented by Borowik and Wyszkowska (2018).

RESULTS AND DISSCUSSION

Effect of diesel oil on maize yields

Maize responded negatively to the soil contamination with diesel oil (Figure 2). Under the influence of this pollutant, the yield of aerial parts

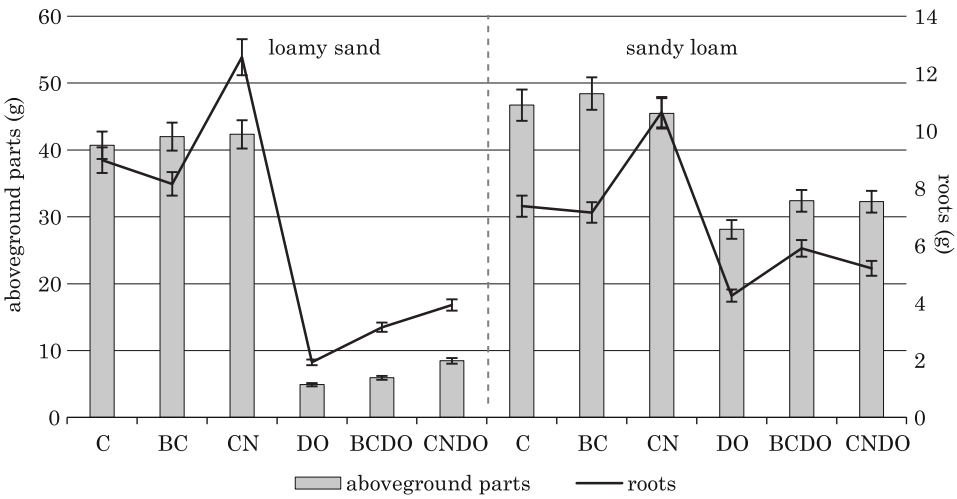

Fig. 2. The impact of diesel oil and bioaugmentation on the yield of maize (g DM): per pot C – control, DO – diesel oil, BC – BIO ACTIV HGS 208, CN – bacterial consortium. Error bars represent standard error of the mean for n=5.

produced by maize grown on loamy sand decreased by 88% while the root yield fell by 79% relative to the control. The loss in yields of maize cultivated on sandy loam was 40 % (aerial parts) and 42% (roots). Similar results have been obtained by other researchers (Ogbo 2009, Wyszkowski, Ziółkowska 2013, Basumatary et al. 2012). In soils not polluted with diesel oil, neither BIO ACTIV HGS code 208 (BC) nor the consortium of bacteria (CN) changed significantly the aerial yield of maize. Differences between the individual treatments were small, ranging from -3% to 4%. The root biomass was positively affected by the CN consortium, which raised the root yield by 40% in the trial on sandy loam and by 44% in the variant on loamy sand. In contrast, the development of roots produced by maize grown on either of the soils was not stimulated by the preparation BC. The soil biaaugmentation contributed to reducing the scale of the negative impact of diesel oil on the development of maize. The preparation BC reduced the adverse impact of DO on the maize aerial yield by 3% and on the root yield by 14% with respect to the maize plants grown on loamy sand. The effect of this preparation on maize cultivated on sandy loam consisted of reducing the negative impact of contamination on yield by 9% (aerial parts) and 22% (roots). In turn, the consortium of bacteria used in the experiment limited the destructive influence of diesel oil on maize grown on loamy sand by 9%

(aerial parts) and by 22% (roots), while its influence on maize grown on sandy loam resulted in the maize yields higher by 9% (aerial parts) and 13% (roots).

The negative response of maize to DO contamination was stronger in plants grown on loamy sand rather than on sandy loam, which was due to these two types of soil being different in terms of their richness in nutrients as well as the sorption complex capacity (Table 1). According to ADAMS et al. (2015) and CURY et al. (2015), the abundance of nutrients in soil is one of the factors that determine the success of bioremediation.

Effect of diesel oil on soil microorganisms

Soil contamination with diesel oil had the strongest impact on the counts of soil microorganisms (Figure 3). This independent variable decided in 49%

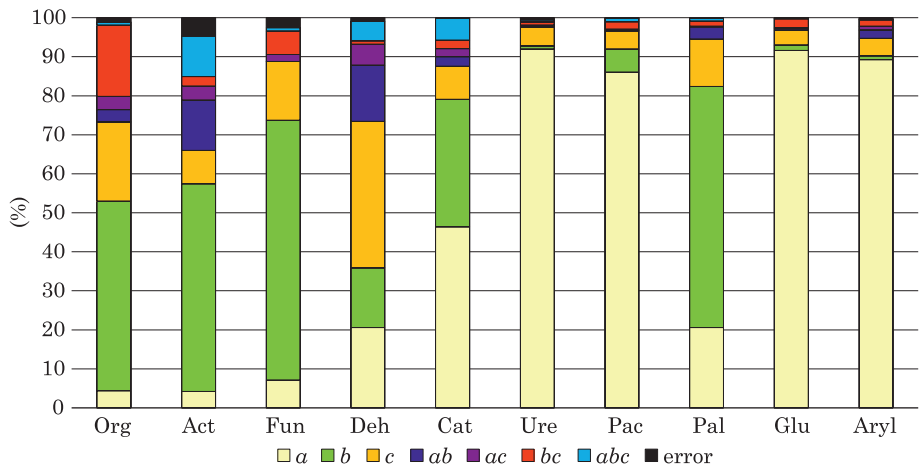

Fig. 3. The share of independent variables in the evolution of the counts of microorganisms and soil enzymatic activity (η^2): *a* – kind of soil, *b* – contamination with diesel oil (DO), *c* – bioaugmentation, Org – organotrophic bacteria, Act – actinomycetes, Fun – fungi, Deh – dehydrogenases, Cat – catalase, Ure – urease, Pac – acid phosphatase, Pal – alkaline phosphatase, Aryl – arylsulphatase, Glu – β-glucosidase

about the number of organotrophic bacteria and in as much as 66% about the number of fungi. Bioagumentation had a much weaker effect. The number of bacteria depended on this factor in 20%, and the number of fungi – in 15%. The type of soil had the smallest contribution to the shaping of the counts of microorganisms (from 4% to 7%).

The prinicpal component analysis proved that the abundance of microorganisms was negatively correlated with the first principal component (Figure 4, Table 2) and dependend significantly on both the soil contamination with diesel oil and the applied bioaugmentation. This conclusion is supported by the positive factors of the impact of DO (IF_{DO}) on counts of microorganisms (Figure 5). On average, irrespective of their type, microorganisms

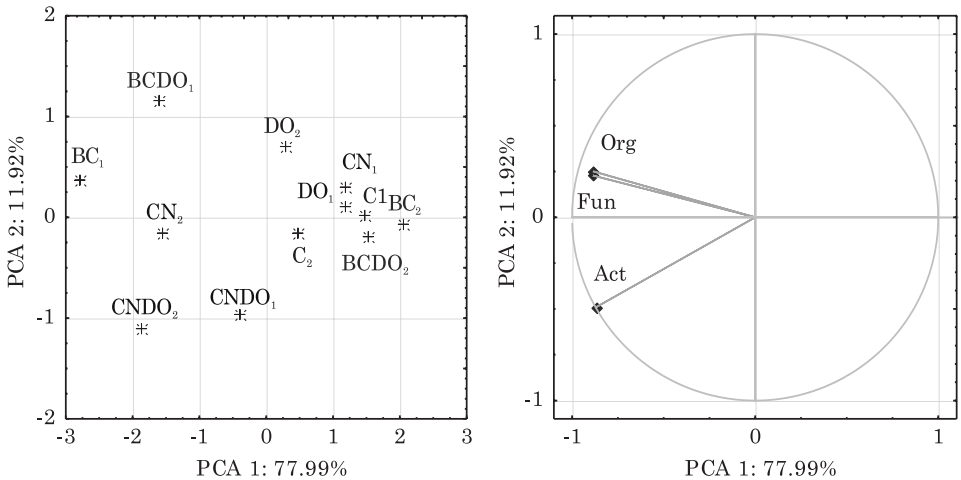

Fig. 4. The influence of the factors on the counts of soil microorganisms presented by the principal component analysis: 1 – loamy sand, 2 – sandy loam, C – control, DO – diesel oil, BC – BIO ACTIV HGS 208, CN – bacterial consortium, Org – organotrophic bacteria, Act – actinomycetes; Fun – fungi

Table 2

Correlations of counts of microorganisms and enzymatic activity with PCA 1 and PCA 2

Microbial counts/enzymatic activity	PCA1	PCA2
Organotrophic bacteria	-0.889	0.252
Actinomycetes	-0.871	-0.491
Fungi	-0.890	0.229
Dehydrogenases	-0.629	0.639
Catalase	-0.795	0.433
Urease	-0.974	-0.038
Acid phosphatase	0.829	0.505
Alkaline phosphatase	0.275	0.933
Arylsulphatase	-0.985	-0.041
β-glucosidase	-0.982	0.006

were more numerous in sandy loam than in loamy sand. The preparation BIO ACTIV HGS code 208 had a positive influence on the count of organotrophic bacteria in loamy sand unpolluted with DO, and on actinomycetes and fungi in sandy loam. Contrary to BC, the consortium of bacteria added to soil stimulated the reproduction of all microorganisms in both unpolluted soils. Values of the impact factor of bioaugmentation (IF_B) determined for the BC preparation and CN bacterial consortium in loamy sand polluted with DO were positive, whereas BC was not found to have affected actinomycetes in sandy loam. This preparation increased the counts of organotrophic bacteria

index	object*	Org	Act	Fun
IF_{DO}	DO_1	1.62	0.07	0.58
	DO_2	1.34	1.31	1.65
IF_B	BC_1	0.58	0.06	0.01
	BC_1 DO	1.42	1.04	0.34
	BC_2	0.25	0.24	0.44
	BC_2 DO	1.02	0.00	0.29
	CN_1	0.95	0.39	0.27
	CN_1 DO	0.30	0.35	0.90
	CN_2	1.68	0.12	0.40
	CN_2 DO	0.40	0.25	0.50

(0.0;0.1>
(0.1;0.2>
(0.2;0.5>
(0.5;1.0 >
(1.0;2.0)

Fig. 5. The index of the impact of diesel oil (IF_{DO}) and bioaugmentation (IF_B) on the counts of soil microorganisms. * Explanations are given in Fig. 4

and fungi. The consortium of bacteria (CN) had a positive impact on all the analysed microorganisms in sandy loam.

The contamination of loamy sand with DO depressed the values of the colony development (CD) index for organotrophic bacteria and actinomycetes but raised the CD value for fungi (Figure 6). Diesel oil pollution of sandy loam was found to have caused the same effect as in loamy sand only on actinomycetes, whose CD was lowered. The value of the CD index of organotrophic bacteria in sandy loam soil increased due to the soil pollution with DO, while that of fungi decreased. In loamy sand, bioaugmentation of uncontaminated soils decreased the values of the CD index for actinomycetes and fungi when either BC or CN was applied, and the CD index for organotrophic bacteria was also depressed after the application of BC. In sandy loam, both BC and CN raised the CD index values for organotrophic bacteria. Bioaugmentation with the preparation BC and consortium CN increased the value of the colony development index for actinomycetes in both types of soil polluted with DO. This proves that the structure of these microorganisms shifted towards strategy r ones at the expense of strategy K representatives, a development associated with the occurrence of intermediate organic compounds characterised by various degrees of assimilability during the decomposition of diesel oil (Gianfreda 2015). In this study, bioaugmentation decreased the CD index for organotrophic bacteria (in both soils). Changes in the colony development index for fungi in soils contaminated with DO and submitted to bioaugmentation were not unequivocal. In loamy sand, the preparation BC decreased this index, whereas the bacterial consortium CN did not change its value significantly. In sandy loam, the relationships were exactly opposite (Figure 6).

In response to DO pollution, the ecophysiological (EP) index for fungi decreased in both types of soil, for actinomycetes – only in loamy sand, and for organotrophic bacteria – only in sandy loam (Figure 7). Bioaugmentation

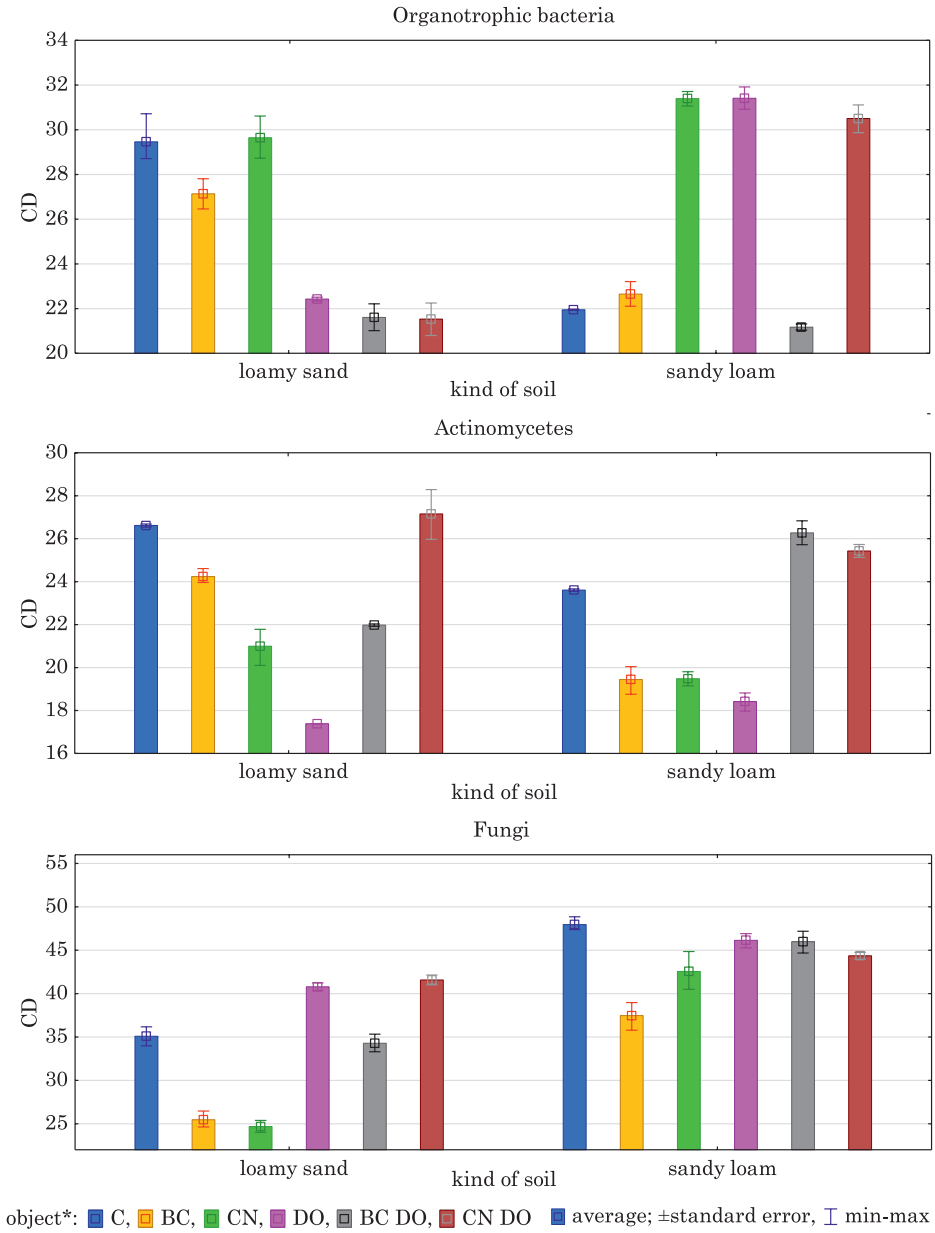

Fig. 6. Colony development index (CD) of organotrophic bacteria, actinomycetes and fungi in soil. * Explanations are given in Fig. 4. Error bars represent standard error of the mean for n=4.

(BC and CN) of soils polluted with DO raised the EP values for organotrophic bacteria and fungi in sandy loam, as well as for actinomycetes in loamy sand. Borowik et al. (2017b) and Cury et al. (2015) determined that while the

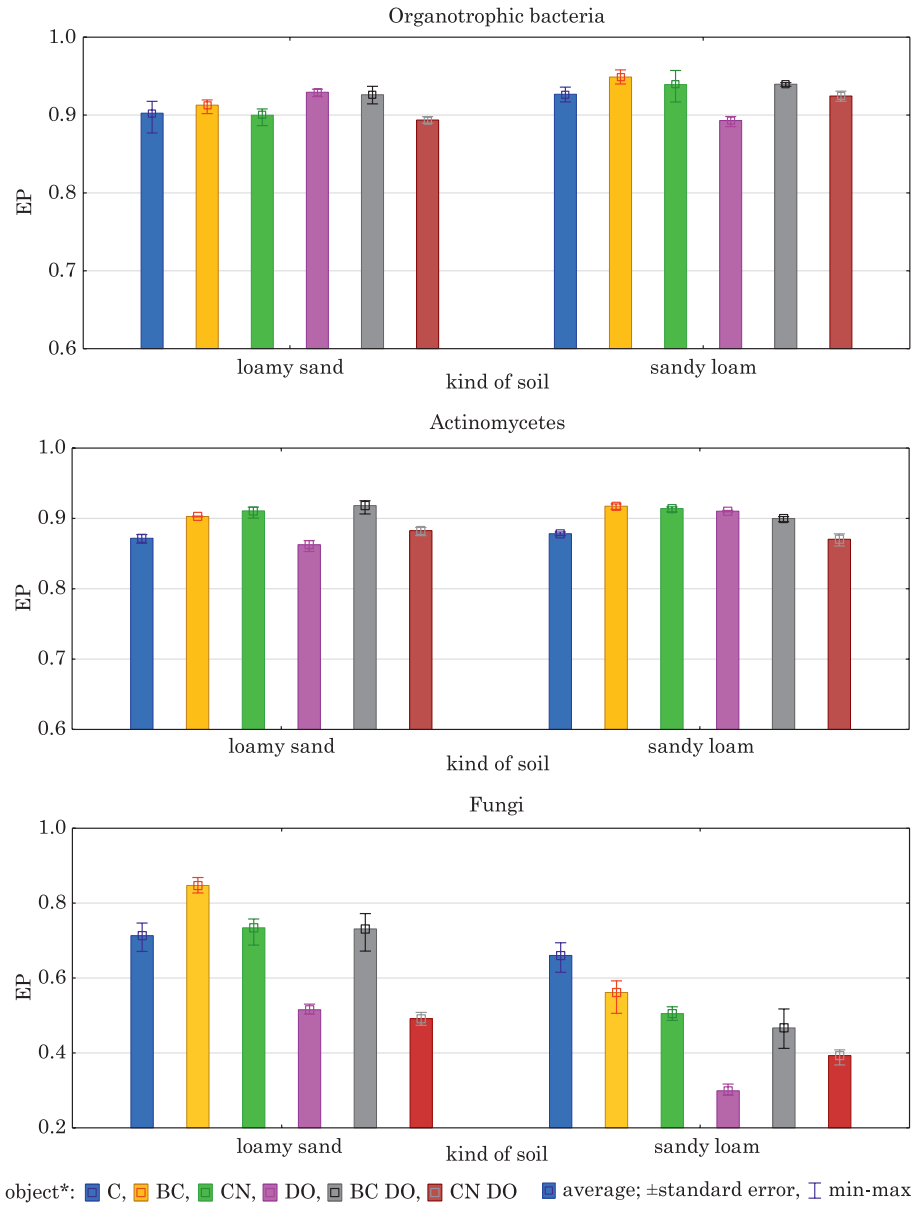

Fig. 7. Ecophysiological index of diversity (EP) of organotrophic bacteria, actinomycetes and fungi in soil. * Explanations are given in Fig. 4.
Error bars represent standard error of the mean for n=4

proliferation of microorganisms was stimulated under the influence of petroleum products, the diversity of microorganisms deteriorated.

Generally, soil pollution with crude oil products stimulates multiplica-

tion of microorganisms and enhances the activity of soil enzymes (Kucharski, Jastrzębska 2006, Wyszkowska et al. 2015, Borowik et al. 2017a, Ramadass et al 2017). Organic compounds found in diesel oil are a source of carbon and a donor of electrons for some autochthonous microorganisms (Gianfreda 2015). Hence, the soil amendement with an additional pool of microorganisms able to decompose hydrocarbons (Mrozik and Piotrowska-Seget 2010) acclerates remediation of soil polluted with DO (Adams et al. 2015, Cury et al. 2015).

Effect of diesel oil on soil enzymes

Regardless their origin, soil enzymes are responsible for soil metabolism, associated with the activation of nutrients as well as the degradation of pollutants (Kucharski, Jastrzębska 2005, Szulc et al. 2014, Gianfreda 2015, Borowik et al. 2017a). The activity of soil enzymes, unlike the counts of microorganisms, was more strongly determined by the type of soil than by the other factors (Figure 3). The eta-square for the activity of individual enzymes depending on: the type of soil ranged from 21% (dehydrogenases) to 92% (urease and β-glucosidase); soil contamination with DO – from 0.8% (urease) to 62% (alkaline phosphatase); and bioaugmentation – from 4% (β-glucosidase and arylphosphatase) to 38% (dehydrogenases).

The analysis of principal components (Table 2, Figure 8) indicates that the activity of catalase, urease, acid phosphatase, arylsulfatase and β-glucosidase is described well by the first principal component, while the activity of alkaline phosphatase is described well by the second principal component. The activity of dehydrogenases is nearly equally described by

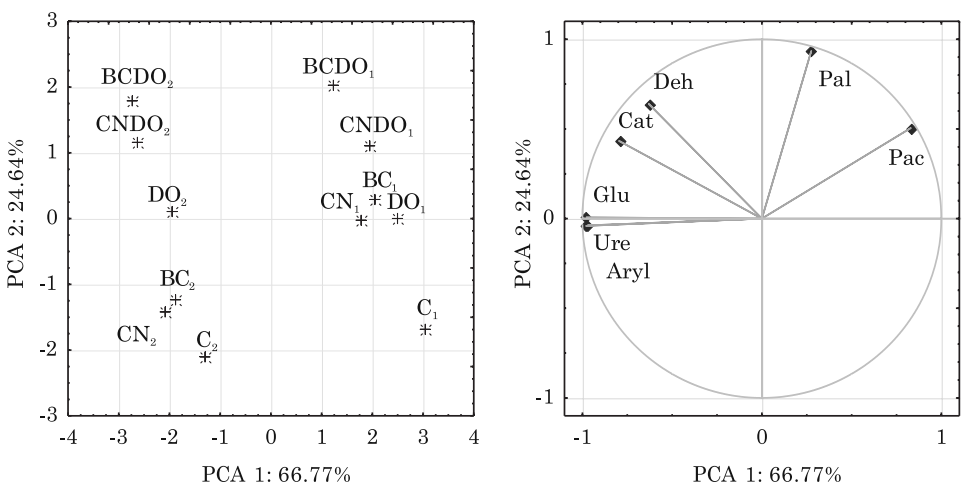

Fig. 8. The influence of the factors on the soil enzymatic activity presented by the principal component analysis: 1 – loamy sand, 2 – sandy loam, C – control, DO – diesel oil, BC – bioactive, CN – bacterial consortium, Deh – dehydrogenases, Cat – catalase, Ure – urease, Pac – acid phosphatase, Pal – alkaline phosphatase, Aryl – arylsulphatase, Glu – β-glucosidase.

PCA1 and PCA2. Irrespective of the soil contamination with DO and applied bioaugmentation, significant differences appeared in the activity of enzymes between the two types of soil (Figure 8).

The values of the IF_{DO} suggests that in response to the action of DO in loamy sand the activity of all enzymes, except β-glucosidase (Figure 9),

index	object	Deh	Cat	Ure	Pac	Pal	Glu	Aryl
IF_{DO}	DO_1	0.16	0.50	0.65	0.35	0.21	0.37	0.19
	DO_2	0.33	0.11	0.23	0.62	0.43	0.05	0.19
IF_B	BC_1	0.87	0.09	1.97	0.43	0.11	0.22	0.50
	BC_1 DO	0.48	0.43	0.95	0.00	0.20	0.12	0.16
	BC_2	0.09	0.12	0.45	0.37	0.15	0.03	0.04
	BC_2 DO	0.30	0.15	0.09	0.11	0.17	0.09	0.03
	CN_1	0.75	0.17	1.23	0.27	0.09	0.44	0.55
	CN_1 DO	0.54	0.05	0.49	0.08	0.04	0.01	0.08
	CN_2	0.22	0.05	0.03	0.49	0.02	0.20	0.20
	CN_2 DO	0.12	0.28	0.02	0.18	0.06	0.08	0.05

(0.0;0.1>
(0.1;0.2>
(0.2;0.5>
(0.5;1.0 >
(1.0;2.0)

Fig. 9. The index of the impact of diesel oil (IF_{DO}) and bioaugmentation (IF_B) on the soil enzymatic activity. * Explanations are given in Fig. 8

increased by 16% (dehydrogenases) up to 65% (urease), while in sandy loam it rose from 11% (catalase) up to 62% (acid phosphatase). A similar influence on petroleum products on soil enzymes has been determined by Kucharski and Jastrzębska (2006) and Wyszkowska et al. (2015). In loamy sand unpolluted with DO, the preparation BC did not modify the activity of catalase, while the activity of all other enzymes was increased within the range of 11% (alkaline phosphatase) up to 197% (urease). In sandy loam unpolluted with DO, the preparation BC stimulated the activity of catalase (by 12%), alkaline phosphatase (by 15%), acid phosphatase (by 37%) and urease (by 45%). The consortium of bacteria (CN) in loamy sand unpolluted with DO stimulated the activity of all enzymes except alkaline phosphatase, while in sandy loam it raised the activity of β-glucosidase and arylsulfatase (by 20%), dehydrogenases (by 22%) and acid phosphatase (by 49%).

Application of the preparation BC to loamy sand polluted with DO led to a higher activity of all enzymes within the range of 12% (β-glucosidase) up to 95% (urease), except acid phosphatase. This preparation was less effective in sandy loam. It only enhanced the activity of dehydrogenases (by 30%), catalase (by 15%), acid phosphatase (by 11%) and alkaline phosphatase (by 17%). A smaller pool of enzymes was activated by the applied bacterial consortium (CN) in soils contaminated with DO. In loamy sand, it increased the activity of dehydroegnases (by 54%) and urease (by 49%), while in sandy loam it stimulated the activity of dehydrogenases (by 12%), catalase (by 28%) and acid phosphatase (by 18%) – Figure 9.

The role of bioaugmentation in the removal of PAHs from soil contaminated with diesel oil

Degradation of ∑9 PAHs after 60 days since the soil contamination with DO was high and ranged from 95% to 97% (Table 3). It did not depend on

Table 3

Percentage of removed hydrocarbons from soil contaminated with diesel oil (DO)

Object	Kind of soil	Petrols (C_6-C_{12})	Mineral oil (C_{12}-C_{35})	BTEX	∑9 PAHs
Control	LS	93.60^b	87.30^b	97.88^a	96.01^a
	SL	75.13^c	36.51^d	97.88^a	94.65^a
Bio Activ HGS kod 208 (BC)	LS	99.46^a	92.38^a	97.88^a	97.03^a
	SL	77.83^c	39.68^d	97.88^a	96.73^a
Bacterial consortium (CN)	LS	99.95^a	91.11^a	97.88^a	97.32^a
	SL	76.89^c	46.03^c	97.88^a	97.03^a

LS – loamy sand, SL – sandy loam, BTEX – volatile hydrocarbons, PAHs – polycyclic aromatic hydrocarbons; The same letters in columns show homogeneous groups.

either the type of soil or the applied bioaugmentation treatment. An even higher level of degradation was achieved for BTEX (98%). However, the rate of decomposition of petrols varied, reaching 94% in loamy sand and 75% in sandy loam. Bioaugmentation of loamy sand with the preparation BC or the consortium of bacteria CN increased the degradation of petrols up to 99%. However, no such effect was observed in sandy loam. Both the preparation BC and the consortium CN significantly accelerated the degradation of mineral oils in loamy sand, while the consortium CN had the same effect in sandy loam. Intensive decomposition of PAHs and BTEX was achievable owing to the slight degree of soil pollution with diesel oil tested in our experiment, the fact also noticed by Adams et al. (2015), while the positive effect of bioaugmentation on the degradation of the remaining pollutants can be attributed to the microbiological oxidation-reduction processes. Ameen et al. (2016) and Cury et al. (2015) highlighted the usefulness of bioaugmentation in the removal of hydrocarbons contained in petroleum products from the environment. In turn, Fatima et al. (2016) and Dos Santos and Maranho (2018) have demonstrated that phytoremediation, which stimulated the growth of microorganisms in the rhizosphere, thereby stimulating the activity of enzymes, might also enhance the degradation of hydrocarbons. What the cited findings suggest is that bioaugmentation should be applied together with phytoremediation (Saleem 2016).

CONCLUSIONS

1. The textural composition of soil determines the extent of toxic effects of DO on plants. Diesel oil was more toxic to maize grown on loamy sand than on sandy loam. In both soils, bioaugmentation with the preparation BIO ACTIV HGS code 208 (BC) or with a consortium of bacteria (CN) alleviated the inhibitory impact of DO exerted on the growth and development of maize.

2. Positive values of IF_{DO} prove that diesel oil stimulated the proliferation of microorganisms in both tested soils, but a more intensive growth in their counts occurred in sandy loam than in loamy sand. At the same time, diesel oil caused disturbances in the proportions of fast-growing (strategy r) and slow-growing (strategy K) microoorganisms.

3. Bioaugmentation of soil polluted with DO carried out by applying the preparation BC or consortium of bacteria CN changed proportions in the structure of bacterial communities. Among actinomycetes, there was a shift in favour of strategy r organisms, while among organotrophic bacteria the shift was towards strategy K. Diesel oil had a negative effect on the ecophysiological diversity of microorganisms, and bioaugmentation with the preparation BC or bacterial consortium CN alleviated these distrubances.

4. Soils contaminated with diesel oil were characterised by higher enzymatic acivity than unpolluted soils, and bioaugmentation with the preparation BC or the bacterial consortium CN stimulated selectively the activity of soil enzymes.

5. Bioaugmentation is an effective treatment in degradation of petrols and mineral oils in soils contaminated with diesel oil. However, its effectiveness depends on the textural composition of soil.

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