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The resistance of *Lolium perenne* L. × *hybridum*, *Poa pratensis*, *Festuca rubra*, *F. arundinacea*, *Phleum pratense* and *Dactylis glomerata* to soil pollution by diesel oil and petroleum

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Abstract: Resistance of common European grasses to diesel oil and petroleum pollution is not well-known. Therefore, this study aimed at determining the level of resistance of selected grasses to pollution by diesel and petroleum using the pot experiment. The achieved results were compared with those determined for grasses grown on the non-polluted soil. Soil pollution with the tested products was found to significantly decrease the yield of all grasses, with the decrease being lower upon soil pollution with petroleum than with diesel oil. The most resistant to the pollution with diesel oil and petroleum were *Phleum pratense* L., *Lolium perenne* L. and *Lolium* × *hybridum* Hausskn. The degradation of particular groups of polycyclic aromatic hydrocarbons (PAHs) depended on their chemical properties, on the type of pollutant and grass species. The greatest degradation was determined in the case of BTEX, C_6–C_{12} benzines as well as 2- and 3-ring hydrocarbons, whereas the lowest in the case of 5-and 6-ring hydrocarbons and C_{12}–C_{25} oils. The most useful species in the remediation of soils polluted with diesel oil and petroleum turned out to be: *Lolium perenne* L., *Lolium* × *hybridum* Hausskn and *Phleum pratense* L., whereas the least useful appeared to be: *Festuca rubra*, *Dactylis glomerata* L. and *Poa pratensis* L.

Keywords: grasses species; phytoremediation; contamination; Poaceae; liquid fuel

Environmental pollution is a global issue of the XXIst century. The number of potentially polluted areas in the EU is successively increasing. In 2014, their total number was estimated at 2.5 million (Van Liedekerke et al. 2014); whereas in 2018, it increased to 2.8 million sites (Payá-Pérez and Rodríguez-Eugenio 2018). About 19% of the registered potentially polluted habitats in Europe require precautionary measures or risk reduction (Payá-Pérez and Rodríguez-Eugenio 2018). The most frequent pollutants include petroleum-based substances (Masy et al. 2016, Telesiński et al. 2018). Among the 28 Member States of the European Union, as many as 24 refer in their legal acts to the problem of soil environment pollution. In general, all regulations aim at preventing adverse changes in the soil and reclaiming polluted soils and underground waters (Payá-Pérez and Rodríguez-Eugenio 2018). The removal of pollution outcomes with these substances is a long-lasting process which requires complicated technologies (Kuppusamy et al. 2017). However, not only economic but also social aspects should be taken into consideration while choosing the remediation method. Benzo(a)pirene (BaP) is the strongest carcinogenic agent to the living organisms, including human. Also, a long-term exposure to BTEX group compounds, i.e., benzene, toluene, ethylbenzene or xylene, induces severe diseases in human (Santiago et al. 2014). For this reason, monitoring of pollutants in the natural environment is a must. Hence, technologies should be developed which would enable highly effective remediation of the natural environment at relatively low costs and which would be acceptable

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by the society, i.e., would not pose any threat to the environment nor to human health (Khan et al. 2018). It is also important to follow guidelines for the sustainable development in contaminated land management (CLM), which is perceived today as a highly important issue in many countries of the world (Reinikainen et al. 2016). Considering the extent of contamination, special conditions occurring in a given place and the effect which this contamination has on the natural environment, the *in situ* techniques often prove better than the *ex situ* ones (Kuppusamy et al. 2017). Phytoremediation is one of the most environmentally-friendly *in situ* biological methods for soil remediation which leaves soil structure intact and minimizes the potential migration of contaminants (Saleem 2016). However, considering that it is a time-consuming method, a special attention should be paid while choosing plant species for the elimination of potential contamination. Literature data (Hall et al. 2011, Fatima et al. 2018) indicate that the phytoremediation of soils polluted with petroleum-based products may be aided by plants representing both the Fabaceae and the Poaceae family. The usability of grasses in this process is associated with their well-developed root system, which results in a high rhizospheric effect being extremely important in the degradation of organic contaminants. Nonetheless, the available literature lacks reports from studies that would simultaneously compare the resistance of a few grass species to the effects of petroleum-based products and to the remediation of soils polluted with these products. This has prompted us to undertake a study aiming at determining the resistance of selected grasses to soil contamination with petroleum-based products and to evaluate the effectiveness of phytoremediation of soil polluted with BP Diesel with the active technology and with 98 BP lead-free petroleum with the active technology. Finally, it helped indicate the most effective grass species in removing hydrocarbons from these products.

MATERIAL AND METHODS

Description of soil. The experiment was performed on samples of agricultural topsoils typical for the Olsztyn Lake District, being part of the Mazurski Lake District in north-eastern Poland. The exact growing experiment was carried out using Eutric Cambisols collected at the Didactic and Experimental Station of the University of Warmia and Mazury in Olsztyn (north east Poland, 53.7167°N, 20.4167°E). Samples of topsoil were collected from a depth of 20 cm. In terms of the granulometric composition, it was loamy sand, the main characteristics of which were presented in Table 1 which were determined according to the procedures provided in work by Borowik et al. (2017a,b).

Studied grasses. Assuming that the appropriate choice of plants, based on their durability and stability in the natural environment, is a prerequisite for successful remediation of soil polluted with petroleum-based products, this research focused attention on plants having a well-developed bundle root system. Thus, 7 grass species were used in our experiment. They included 3 species of lawn grasses: *Lolium perenne* L. (Lp) cv. Bajka, *Poa pratensis* L. (Pr) cv. Sójka, and *Festuca rubra* (Fr) cv. Dark, as well as 4 species of fodder grasses: *Lolium* × *hybridum* Hausskn (Lh) cv. Gala, *Festuca arundinacea* (Fa) cv. Rahela, *Phleum pratense* L. (Pp) cv. Kaba and *Dactylis glomerata* L. (Dg) cv. Bepro.

Description of petroleum-based products. Two commonly used petroleum-based products were tested: BP Diesel with active technology (DO) and 98 BP lead-free petroleum with active technology (P). Their detailed characteristics are provided at the following website: https://www.bp.com/.

Description of the experiment. The effectiveness of grasses in accelerating the degradation of polycyclic aromatic hydrocarbons (PAHs) contained in DO and P, and the most appropriate grass species for phytoremediation were determined in a specially designed pot experiment performed under controlled

Table 1. Characteristics of soil used in the experiment

Soil subgroup	pH	Content in 1 kg DM											
		granulometric fractions (g)			total forms (g)		content of available (mg)			content of exchangeable (mg)			
		sand	silt	clay	N	C	P	K	Mg	K	Ca	Na	Mg
Loamy sand	6.7 ± 0.3	749 ± 57	229 ± 12	22 ± 1.6	0.6 ± 0.04	9.3 ± 0.7	94 ± 8.3	141 ± 11	42 ± 3.9	156 ± 13	624 ± 54	40 ± 2.7	60 ± 4.0

± standard deviation of the mean for n = 4; DM – dry matter

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conditions in a greenhouse. The soil characterized earlier was transported to the greenhouse and sifted through a screen with a mesh size of 5 mm. The experiment was established in 10 dm^3 Kick-Brauckman pots and conducted in 4 replications, each in 3 series: (1) non-polluted soil (loamy sand); (2) soil exposed to DO and (3) soil polluted with P. The petroleum-based products were used in a dose of 7 cm^3/kg soil dry matter (DM). The tested grass was sown in the pots in the amount of 22 seeds per pot. Regardless of the experimental series, before the soil had been polluted with the petroleum-based products, all grasses were once fertilized with (in mg/kg soil DM): N – 80 [$CO(NH_2)_2$]; P – 20 [KH_2PO_4]; K – 40 [KH_2PO_4 + KCl]; and Mg – 10 [$MgSO_4 \cdot 7 H_2O$]. Throughout the experimental period (105 days), the humidity was maintained at the level of 50% maximum water content using distilled water. The grasses were cut 3 times. On the day of harvest, plant yield was determined and soil samples were collected for further analyses.

Methodology of chemical analysis of soil. Both before and after the experiment, the soil sifted through a screen with a mesh size of 2 mm was determined for the content of volatile aromatic hydrocarbons (BETX) according to EN ISO 22155 (2016), mineral oils (C_{12}–C_{35}) according to EN ISO 16703 (2011) and benzines (C_6–C_{12}) according to EN ISO 22155 (2016). In addition, the content of the following 9 PAHs: naphthalene [2 rings]; anthracene [3 rings]; chrysene [4 rings]; benzo(a)anthracene [4 rings]; dibenz(ah)anthracene [5 rings]; benzo(a)pyrene [5 rings]; benzo(b)fluoranthene [5 rings]; benzo(k) fluoranthene [5 rings]; and benzo(ghi)perylene [6 rings], were determined according to the ISO 18287 (2006). These determinations were carried using an Agilent 7890A gas chromatograph (Wilmington, USA) coupled with an Agilent 5975C mass spectrometer (Wilmington, USA) equipped in an EI/CI ion source, in an accredited laboratory Weeseling (Krakow, Poland). The accuracy of all PAHs determination was 0.005 mg/kg. Values greater than the median were attributed to 1 point and less to 0 points.

Data collection. Formulas developed by Orwin and Wardle (2004) were used to calculate the resistance of grass species to petroleum-based products (RS) and their adaptation to the polluted soil (RL):

$$RS = 1 - \frac{2|D|}{(|C| + |D|)}$$

Where: RS – resistance index of grass species to pollution; D – difference between grass yield obtained at non-polluted soil (C) and grass yield obtained at polluted soil.

In turn, the adaptation index of grass species to pollution was computed using the following formula:

$$RL = \frac{2|D_1|}{(|D_1| + |D_3|)} - 1$$

Where: RL – adaptation index of grass species to pollution; D_1 – difference between grass yield from swath I obtained at non-polluted soil and grass yield obtained at polluted soil; D_3 – difference between grass yield from swath III obtained at non-polluted soil and grass yield obtained at polluted soil.

Evaluation of the usability of particular grass species was based on the response of the tested grass species to pollution with DO and P, and the extent of hydrocarbons degradation. In total, 9 different parameters were evaluated in the case of each grass species: resistance index (RS), the percentage of degraded C_6–C_{12}, C_{12}–C_{35}, 2, 3, 4, 5 and 6-ring hydrocarbons, as well as BTEX. If the value of the tested parameter was higher or equal to the median, it was ascribed the value of 1; if the value of the tested parameter was lower than the median, it was ascribed the value of 0. The usability index of grass species was computed from the following formula:

$$UI = \frac{m}{\sum a}$$

Where: UI – usability index of grass species to phytoremediation; m – number of evaluated parameters ≥ median; Σa – total number of analysed parameters.

The usability of individual grass species for phytoremediation was determined based on their productivity level and PAHs degradation degree and expressed in a scale from 0 to 1, where 1 denoted full usability and 0 complete uselessness.

Statistical analysis. The results were subjected to a statistical analysis using the Statistica 13.0 package (Dell Inc. 2016). Homogenous groups were calculated using the Tukey's distribution test at $P = 0.01$ and the principal component analysis (PCA) was conducted.

RESULTS AND DISCUSSION

Grass yield. This study conducted with plants from the Poaceae family proves the response of plants to the pollutants above to be a species-specific trait. Among the 7 analysed grass species, the highest yield (Figure 1) was produced by tall fescue (Fa); cultivar ryegrass (Lh); and perennial ryegrass (Lp), whereas the lowest one by bluegrass (Pr), red fescue (Fr), and timothy-grass (Pp). Soil pollution with diesel oil and petroleum contributed to a significant decrease in the crop yield of all grass species, with the decrease being smaller after soil pol-

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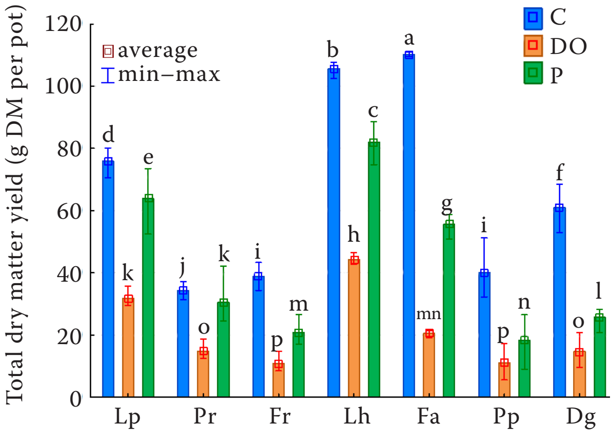

Figure 1. Total dry matter yield of grass species in all cuts. The same letter shows homogeneous groups. Different letters show significant differences between objects (two-way ANOVA, Tukey's test, $P < 0.01$). Error bars represent a range (min–max), $n = 4$. Lp – *Lolium perenne* L.; Pr – *Poa pratensis* L.; Fr – *Festuca rubra*; Lh – *Lolium* × *hybridum*; Fa – *Festuca arundinacea*; Pp – *Phleum pratense* L.; Dg – *Dactylis glomerata* L.

lution with petroleum than with diesel oil. Upon soil exposure to petroleum, the reduction in crop yield ranged from 11% (bluegrass) to 58% (cocksfoot), and upon soil exposure to diesel oil – from 57% (bluegrass) to 81% (tall fescue). Many authors (Wyszkowski and Ziółkowska 2011, Fatima et al. 2018) have emphasized that soil pollution with either petroleum-based products or PAHs alone impairs the growth and development of plants. A research conducted by Wyszkowski and Ziółkowska (2011) indicated that the stronger toxic effect of diesel oil than of petroleum on plants is not a rule but is rather associated with the affiliation of plants to particular families.

Considering their sensitivity to the effect of petroleum, the analysed grass species could be ordered as follows: cocksfoot grass > timothy-grass > tall fescue > red fescue > cultivar ryegrass > perennial ryegrass > bluegrass, whereas considering their response to soil pollution with diesel oil they could be ordered as follows: tall fescue > cocksfoot grass > timothy-grass = red fescue > perennial ryegrass = cultivar ryegrass = bluegrass. The above-presented orders of grass species are consistent with their resistance (RS) to the pollution with petroleum and diesel oil (Figure 2a). In the case of petroleum, the highest values of the resistance index, being higher than the median, were determined for smooth-stalked meadowgrass (0.796), perennial ryegrass (0.727) and cultivar ryegrass (0.651). The same grass species were also the most resistant to the pollution with diesel oil, but their resistance to this pollutant was significantly lower than to petroleum and ranged from 0.276 to 0.265.

It is worth emphasizing that the resistance of particular grass species to the tested pollutants was not always concomitant with their adaptation to the polluted soil (Figure 2b). Values of the RL in-

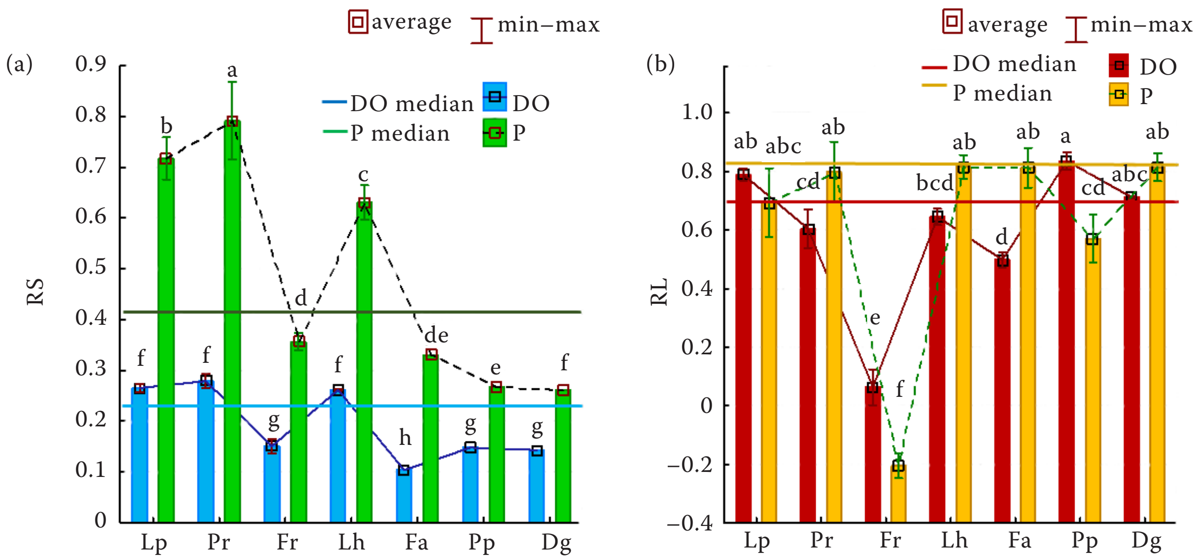

Figure 2. (a) Resistance index (RS) and (b) adaptation index (RL) of grass species to BP diesel (DO) and 98 BP lead-free petroleum (P) pollution. The same letter shows homogeneous groups. Different letters show significant differences between objects (two-way ANOVA, Tukey's test, $P < 0.01$). Error bars represent a range (min–max), $n = 4$. Lp – *Lolium perenne* L.; Pr – *Poa pratensis* L.; Fr – *Festuca rubra*; Lh – *Lolium* × *hybridum*; Fa – *Festuca arundinacea*; Pp – *Phleum pratense* L.; Dg – *Dactylis glomerata* L.

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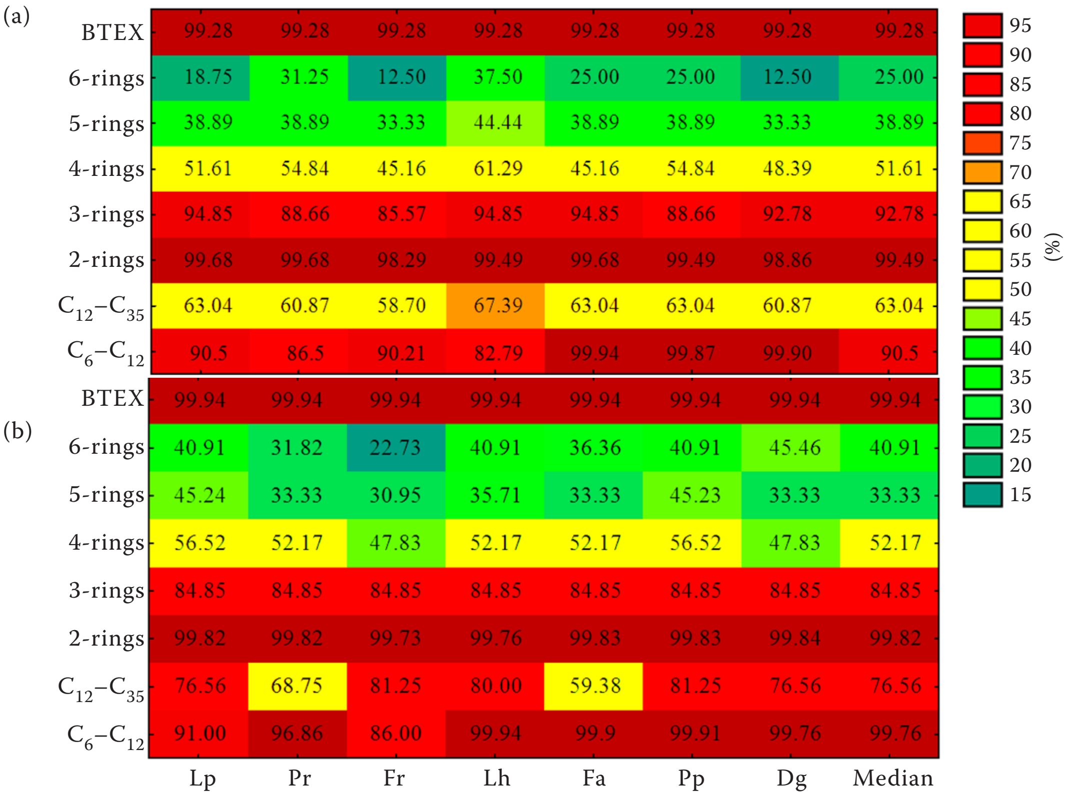

Figure 3. The number of degraded hydrocarbons in soil polluted with (a) BP diesel and (b) 98 BP lead-free petroleum. 2–6 rings, C_6–C_{12}, and C_{12}–C_{35} hydrocarbons. Lp – *Lolium perenne* L.; Pr – *Poa pratensis* L.; Fr – *Festuca rubra*; Lh – *Lolium* × *hybridum*; Fa – *Festuca arundinacea*; Pp – *Phleum pratense* L.; Dg – *Dactylis glomerata* L.

dex prove cultivar ryegrass (0.812), tall fescue (0.809) and cocksfoot grass (0.809) to have the highest ability to adapt to soil pollution with petroleum, whereas timothy-grass (0.833), perennial ryegrass (0.790) and cocksfoot grass (0.712) to soil pollution with diesel oil.

Various responses of plants to the pollution with diesel oil and petroleum were previously described in the literature (Kucharski and Jastrzębska 2006, Wyszkowska et al. 2006), including some opposites to our results, like greater inhibition of plant growth and development after pollution with petroleum than with diesel oil (Wyszkowski and Ziółkowska 2011). Differences in plants responses to the pollution with petroleum-based products are due to their species-specific traits (Hall et al. 2011), as was confirmed in our study with grasses from the Poaceae family.

Degradation of PAHs. The extent of degradation of particular groups of PAHs in the soil polluted with diesel oil (Figure 3a) and petroleum (Figure 3b) depended mainly on their chemical structure, on the type of pollutant (DO, P), and finally on grass species. Regardless of grass species, the percentage of elimination of particular groups of PAH is well reflected by the value of median; in the soil polluted with DO it ranged from 99% for BTEX and 2-ring hydrocarbons to 25% for 6-ring hydrocarbons, whereas in the soil polluted with petroleum it ranged from 99% for BTEX and 2-ring hydrocarbons to 33% for 5-ring hydrocarbons. Red fescue and cocksfoot grass turned out to be the least effective in PAHs degradation in DO-polluted soil whereas in petroleum-polluted soil it was red fescue. In turn, the most effective in degrading PAHs in the soil polluted with DO was cultivar ryegrass and in the soil polluted with petroleum it was timothy-grass. Differences in the PAHs degradation in soil grown with various grass species may be explained by the fact that phytoremediation involves a synergistic interaction of plants and microorganisms associated with them (Hall et al. 2011, Fatima et al. 2018).

Evaluation of the usability of the tested grass species for soil remediation. The evaluation of the usability of individual grass species for the remediation of soils polluted with petroleum-based products should take account of not only their productivity but, most of all, their capability to stimulate degradation of these pollutants by microorganisms. The latter is associated with the quantity and quality of chemical compounds secreted by the root system to the soil (Hall et al. 2011,

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Table 2. The assessment of the usefulness of grass species in the remediation of soil contaminated with diesel oil (DO) and petroleum (P)

Grass species	DO	P	Average
Lolium perenne L.	0.889	0.889	0.889
Poa pratensis L.	0.667	0.667	0.667
Festuca rubra L.	0.111	0.333	0.222
Lolium × hybridum Hausskn	0.889	0.889	0.889
Festuca arundinacea Schreber	0.778	0.778	0.778
Phleum pratense L.	0.889	0.889	0.889
Dactylis glomerata L.	0.333	0.778	0.556
Median	0.778	0.778	0.778

Khan et al. 2018) that stimulate the activity of rhizospheric microorganisms, as well as with the activities of endophytes (Fatima et al. 2018) and enzymes (Bielińska et al. 2018). These two elements are taken into account in the applied score evaluation, which covers both the yield of the examined grass species and the degree of PAHs degradation as a result of their culture (Table 2).

The adopted concept of evaluation allowed concluding that the most useful for the remediation of soils polluted with diesel oil and petroleum were: perennial ryegrass, cultivar ryegrass and timothy-grass. Their usability index was high and reached 0.889. The least useful in phytoremediation turned out to be: red fescue, orchard grass and bluegrass; their usability indices ranged from 0.222 (red fescue) to 0.667 (bluegrass) on average.

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