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Effects of Polycyclic Aromatic Hydrocarbons on Germination and Initial Growth of Selected Lawn Grass Species in Soil Polluted with PAHs

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ABSTRACT

Grasses are often used to recultivate areas contaminated during shale gas extraction. This is due to the fact that they adapt very well to unfavorable soil conditions such as: high pH, salinity, water deficit or the presence of harmful substances. Additionally, the grass root system releases enzymes into the soil that increase the activity of microorganisms and bacteria that decompose polycyclic aromatic hydrocarbons (PAH), which are the main component of drilling waste. In turn, assessment of initial growth and development (germination tests) is a cheap and quick method to assess the sensitivity of the tested plants to pollutants. Young plants are more susceptible to harmful substances. The study aimed to determine the effect of drilling waste, containing polycyclic aromatic hydrocarbons (PAHs) on the initial growth and development of selected grass species, with a specific focus on germination energy and capacity, as young plants are more sensitive to harmful substances compared to older plants. Among the tested species, *Lolium perenne* and *Festuca rubra* showed the highest energy and germination ability, while *Poa pratensis* showed the lowest. The experiment showed that of the tested grass species, *Lolium perenne* and *Festuca rubra* were the least sensitive to the effects of PAHs, with the smallest reductions in root length and seedling height observed in these species. Additionally, the highest concentration of PAHs was found in soil seeded with *Lolium perenne*, while the lowest was found in soil seeded with *Poa pratensis*.

Keywords: drilling waste, polycyclic aromatic hydrocarbon, soils, grasses, germination energy and capacity, initial growth.

INTRODUCTION

The Scientific Committee on Food of the European Union stated that polycyclic aromatic hydrocarbons (PAHs) have mutagenic and carcinogenic properties that have a tendency to accumulate in soil and plants for long periods of time and recommended the monitoring of 15 PAHs (Directive 2004/107/E). Also, the US Environmental Protection Agency recognized these 15 PAHs as mutagenic and carcinogenic, which is confirmed by also numerous scientific studies (Abdel-Shafy and Mansour 2016; Beriro et al., 2016, Mojiri et al. 2019, Ossai et. al., 2020, Patel et al., 2020, Lasota et al. 2021). PAH are the pollutants that have a tendency to accumulate in soil and plants for long periods of time (Antizar-Ladislao and al., 2006, Gabov et al. 2010, Aranda and Martinez-Pagán 2012, Gennadiev and Tsibart, 2013,Abdel-Shafy and Mansour 2016).Therefore, biological methods are commonly used for removing such pollutants from the soil, which, unlike chemical methods, do not produce secondary pollutants. However, their application in phytoremediation is still in the developmental phase.

Currently, phytoremediation is considered a cheap and environmentally friendly method for the recultivation of large areas contaminated with organic chemical substances such as PAHs (Rajakarunaet al., 2006; Ma et al.2010, Gałązka and Gałązka 2015; Cristaldi et al., 2017). Plants can exude various compounds, such as organic acids and sugars, into the rhizosphere, which provide nutrients and energy to soil microorganisms. These microorganisms can degrade PAHs and other organic pollutants through processes such as mineralization, oxidation, hydroxylation, and esterification. In addition, plants may uptake and store PAHs in their tissues, limiting their availability in the soil and reducing potential exposure risks. Overall, the use of plants for PAH remediation has shown promising results and may be a viable option for sustainable and environmentally friendly cleanup of contaminated sites (Lalande et al., 2003; Huang et al. 2004; Parrish et al., 2010; Gałązka and Gałązka 2015). Phytoremediation is a technology that relies on the catabolic potential of microorganisms associated with plant roots, aided by enzymes secreted in the root zone (rhizosphere) (Joner et al. 2001; Parrish et al., 2010).

Using grasses for PAH remediation offers several benefits beyond just the removal of contaminants. As mentioned, plants can improve the physical and chemical properties of contaminated soil, which can enhance soil structure (Afegbua and Batty, 2018). Moreover, "greening" the contaminated area contributes to protecting the soil against wind erosion, surface water runoff, strengthening the soil with roots and improving the aesthetic value of the area (Harris et al 1996). To achieve the maximum reduction of PAHs in a contaminated area, various factors should be taken into account, such as: optimal selection of plants and the costs generated by fertilization or frequent mowing of plants (Smith et al. 2006, Conte et al., 2016).

Some studies have found that certain plants can transform PAHs into less toxic and more easily degradable compounds through through root exudates (Banks et al., 1999, Kolb and Harms, 2000; Liste and Alexander 2000a, b; Kucerová et al., 2001; Bisht et al. 2015). Grasses, particularly *Lolium perenne* and *Festuca arundinacea* have been used in numerous studies for the biodegradation of PAHs in soil due to their tolerance to PAH contamination (Allard et al. 2000; Sverdrup et al. 2003; Kirk et al. 2005; Liste and Prutz, 2006). The use of perennial ryegrass for the biodegradation of PAHs has been successful according to various studies (Günther et al. 1996; Binet et al. 2000, 2001; Fang et al. 2001). However, researchers have noted that the biodegradation process of PAHs by grasses is dependent on several factors, such as exposure time to contamination, the physicochemical properties of the soil, and the microbial activity. Therefore, further research is necessary to explore the potential of this species for PAH removal from soil.

The results of studies by Aprill and Sims (1990), Günther et al. (1996) and Liste and Alexander (2000b) showed that the biodegradation of PAHs occurs in the rhizosphere through secreted enzymes and is dependent on the development of the grass root system. Meanwhile, Patowary et al. (2016) and Alagić et al. (2016) showed that the reduction of PAHs in soil can occur through the accumulation of pollutants in the intercellular tissue of the plant. Other researchers have pointed out that water-soluble PAH damage root cell membranes, resulting in increased permeability that promotes the uptake of PAH by roots (Sivaram et al.,2018, Molina and Segura, 2021). On the other hand, Fang et al. (2001) and Kirk et al. (2005) showed that the rate of PAH degradation in planted and unplanted soil was similar.

The research will allow us to understand the basic processes and mechanisms of reducing complex PAH mixtures in soil sown by selected grass species, and their ability to phytoremediate. This will, in turn, help to identify new methods for the remediation of PAH-contaminated soils.

MATERIAL AND METHODS

The drilling waste used in the experiment came from a waste disposal site located in Luchów, near Biłgoraj in Poland. The drilling waste was mainly composed of sand fraction (2.0–0.05 mm) which accounted for 74%±0.52 of the total mass, followed by 20%±0.43 dust fraction (0.05–0.002 mm) and a 6%±0.32 of clay fraction smaller than 0.002 mm). The drilling waste had a strongly alkaline pH (8.0–9.3) and a high electrical conductivity value (EC>40dS/m) (Fig. 1).

The research covered four old, registered old Polish lawn species: *Festuca arundinacea* variety Tarmena, *Festuca rubra* variety Areta, *Lolium perenne* variety Gazon and *Poa pratensis* variety Alicja,which are currently included in the Polish National List of Agricultural Plant Varieties (2022).The list of varieties, their origin, and registration dates to the Polish National List of Agricultural Plant Varietie are provided in Table 1. Species selected for laboratory testing were those that, according to many researchers (Rutkowska and Pawluśkiewicz, 1996; Golińska, 2009) are usually used to sowing soil in difficult terrain,

Fig. 1. The granulometric composition of the drilling waste used in the experiment

which are characterized by high tolerance for the presence of PAH in the soil (Sverdrup et al., 2003; Liste and Prutz, 2006; Smith et al., 2006) and the ability to accumulate PAH (Khashij et al., 2018; Borowik et al., 2019; Wyszkowska et al., 2019; Gawryluk et al., 2022).

Sterile sand was used as a substrate for grass growth, with more than 90% of the granulometric composition being clayey sand fraction. The sand was sterilized by heating it at 180°C for 60 minutes. Then, sterile sand was mixed with drilling waste in proportions of 10%, 15%, 20% and 30% of drilling waste based on the mass of the substrate. This way created mixtures: P-5, P-10, P-15, P-20 and P-30 were placed in pots with a capacity of 11.8 liters. The control sample (P-0) contained only sterile sand, without any impurities. When sowing the tested grass species, the actual germination capacity of the seeds was taken into account so that 100 seeds germinated in each pot. During the research period (6 weeks from the sowing date), the laboratory had optimal conditions for grass growth: 12-hour lighting (4000 Lux) and air temperature ranging from 24 to 25°C. In all pots, the substrate humidity was maintained at a constant level of 80% of the field water capacity (ppw).The assessment of energy and germination capacity of seeds was carried out according to the method used in recommendations ISTA (2015).

In this study, the growth rate of seedlings was assessed by measuring root length and seedling height on the 60th daya fter the experiment was established (Fig. 2). According to Pawluśkiewicz (2000), the sensitivity of species and varieties to stress factors is manifested already in the earliest phases of their growth and development, research by Dziadczyk (2002) also showed that stress tolerance is a continuous feature, inherited in a multigene manner (whole number of genes are regulated by more than one stress factor). The experiment included three replicates of each variety, with five representative plants measured for each replicate. The PAH content in soil samples was determined using high-performance liquid chromatography using the Acella UHPLC System (Thermo Scientific, USA). Soil samples (15 g) were carefully weighed and then subjected to solid-liquid extraction using a solvent (30 ml of acetone) and ultrasound. The samples were shaken in an ultrasonic bath for 30 minutes. After this time, the extract was poured off and the sample was poured with a new portion of solvent. The process was repeated three times and the extracts obtained as a result of this process were combined. The extracts obtained after centrifugation were cleaned using membrane filters (Restek 17 mm 0.45 um PTFE). After purification, the extracts were concentrated using SPE columns filled with silica gel (Phenomenex Strata PAH).

Chromatographic separation was performed on an ACQUITY UPLC BEH C18 column (2.1×50 mm, 1.7 µm) in a reversed phase system using the gradient method using water and acetonitrile. Detection of individual aromatic hydrocarbons was carried out using a DAD detector at a

Table 1. List of the studied grass species and varieties, their origins, and dates of registration in the polish national list of agricultural plant varieties

Species	Variety	The origin	Date of entryinto the register
Festucaarundinacea	Tarmena	PL	24.02.2004
Festuca rubra	Areta	PL	13.01.1993
Loliumperenne	Gazon	PL	31.12.1966
Poapratensis	Alicja	PL	31.12.1966

Fig. 2. Grass seedlings on the 60th day of the experiment

wavelength of 254 nm. The PAH Calibration Mix Supelco standard, purchased from Sigma Aldrich, was used to identify and quantify individual hydrocarbons. The chromatographic system was calibrated and the analytical method was validated using calibration solutions prepared from the standard solution. The calibration curve consisted of five prepared standard solutions ranging from 0 to 200 ug/ml. The limit of detection (LOD) and limit of quantification (LOQ) of the tested aromatic hydrocarbons were determined (Table 2).

STATISTICAL ANALYSIS

The experiments were conducted in three replicates, with the average values being reported and a coefficient of variation of 10%. Data processing and statistical analyses were carried out using STATISTICA 11.0 and Sigma-Plot 12.5. The significance of differences between replicates was determined at a significance level of $p<0.05$. The error bars used in the figures represent the standard deviation.

RESULTS AND DISCUSSION

Our research findings indicate that drilling waste contain high level of low molecular weight PAHs such as naphthalene, acenaphthylene, and Fluorene, as well as high molecular weight PAHs such as benzo(a)anthracene, pyrene, fluoranthene and benzo(b)fluoranthene (Table 3).

Table 2. The limit of detection (LOD) and limit of quantification (LOQ) of the tested aromatic hydrocarbons

PAHs	Compound	LOD [ng/g]	LOQ [ng/g]
Low molecular weight PAHs	Naphthalene	0.22	0.71
	Acenaphthylene	0.42	1.28
	Fluorene	0.56	1.81
	Phenanthrene	0.04	0.14
	Anthracene	0.02	0.07
High molecular weight PAHs	Benzo(a)anthracene	0.05	0.16
	Chryzene	0.04	0.14
	Benzo(b)fluoranthene	0.04	0.14
	Benzo(k)fluoranthene	0.06	0.21
	Benzo(a)pyrene	0.10	0.33
	Dibenz(a,h)anthracene	0.21	0.65
	Benzo((ghi)perylene	0.20	0.64
	Fluoranthene	0.14	0.46
	Pyrene	0.16	0.49

Table 3. Content of PAH in control soil and drilling waste (µg/kg)

PAHs	Compound	P-0	100% drilling waste
Low molecular weight PAHs	Naphthalene	2.23	742.66
	Acenaphthylene	3.49	703.83
	Fluorene	0.06	94.50
	Phenanthrene	0.25	73.68
	Anthracene	4.79	35.56
High molecular weight PAHs	Benzo(a)anthracene	0.33	506.73
	Chryzene	0.16	54.42
	Benzo(b)fluoranthene	0.12	117.00
	Benzo(k)fluoranthene	0.82	97.57
	Benzo(a)pyrene	1.02	65.82
	Dibenz(a,h)anthracene	0.86	83.34
	Benzo((ghi)perylene	0.15	39.99
	Fluoranthene	1.01	133.80
	Pyrene	0.79	407.44

Note: P-0-control soil.

The germination energy of seeds of the studied grass species decreased with the increase in the cuttings content in the substrate (Fig. 3 and 4). The findings of Soleimani et al. (2010),Wei et al. (2014) and Zhu et al. (2019) confirms that germination energy declines with an increase in PAH concentration in the soil, but Henner et al. (1999) contradict these findings, stating that adding 5% drill cuttings encourage germination rate. Also, Allard et al. (2000) in their research did not observe a negative effect of PAH addition to the soil on the germination of *Lolium perenne*. However, the current study showed that addition 5% of drill cuttings had a negative impact on the seed germination and energy of *Poa pratensis*, while *Lolium perenne* seeds were less affected and maintained similar germination energy and capacity level seven with higher doses of drilling waste (15% and 20% respectively). PAH phytotoxicity can inhibit germination at the early stage of growth and limiting biomass production. The inhibitory effect of PAHs on plant germination and growth is well-documented in the literature (Ouvrard et al., 2014; Wei et al., 2014; Yun et al., 2019; Reddy et al., 2020).

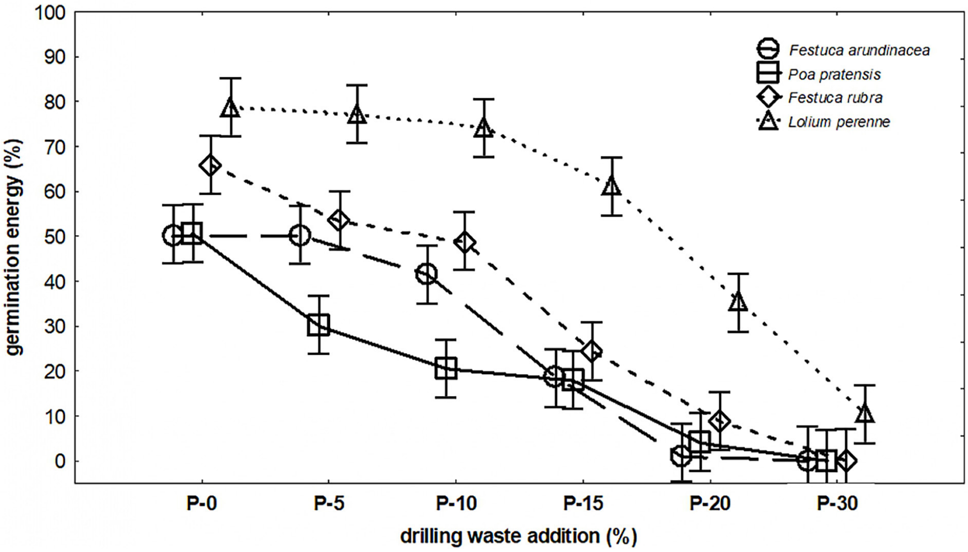

Fig. 3. Influence of drilling waste concentration on germination energy (%) of the tested grass species. The bars at the top and bottom represent the mean± SE. Bars at the same level indicate no significant difference (p ≤ 0.05 by Tukey's test)

Henner et al. (1999) reported that naphthalene, the most volatile and water-soluble PAH compound among those tested, can reduce germination energy. The results of our study support these findings, as we observed a reduction in germination energy and capacity in *Poa pratensis* seeds when drilling waste with PAHs was added. On the other hand, *Lolium perenne* showed greater tolerance to the presence of PAHs in drilling waste, as there were no significant differences in germination energy and capacity even at higher doses of drilling waste. Our results also agree with the previous study by Smith et al. (2006), which found that *Lolium perenne* was less affected by the addition of PAHs than other species. These findings highlight the importance of considering the species-specific responses to PAHs in assessing the phytotoxicity of drilling waste. Rodriguez-Campos et al. (2018) observed that the presence of PAHs in the soil had a significant impact on increasing the biomass of *Panicum maximum*. Similarly, Kaur et al. (2017) showed that exposure to crude oil and its derivatives significantly decreased the germination rate and seedling growth of in many different plant species. These findings suggest that PAHs have a broad inhibitory effect on plant growth and development, and different species may show varying degrees of sensitivity to their presence. Therefore, it is important to assess the species-specific responses to PAHs in evaluating their phytotoxicity and developing effective management strategies for contaminated soils.

The study found that adding drill cuttings to substrate reduced the root length and seedling height ($P < 0.001$). *Festuca arundinacea* and *Lolium perenne* exhibited significantly the higher seedlings and root length, while *Poa pratensis* showed significantly slower growth rates and root elongation ($P < 0,001$). The studies have shown the strongest decrease in root length in *Festuca rubra* and in seedling height in *Festuca arundinacea* (Fig. 5 and 6). According to Kujawska et al. (2020), even a small amount (5%) of drilling waste harms most grass species. The authors suggest that this could be attributed not just to the substantial levels of PAHs in the waste, but also to its elevated salinity.

The above results are confirmed by the research of Reed and Glick (2005) and Gawryluk et al. (2022), which show that PAH has a toxic effect on plants, which is manifested by shorter roots and lower above-ground plant mass than in the absence of soil contamination. However, Ghavidel et al. (2018) showed that the reduction in root length and shoot height of *Lolium perenne* is caused by anthracene. Other researchers have also shown that PAHs cause a reduction in the chlorophyll content in leaves, which is caused by mechanical damage to cell membranes, decreased ability of plants to retain water and absorb nutrients, disrupting the metabolism of cell organelles is disrupted, which largely results in inhibition of growth plants (Maila and Cloete, 2002; Oguntimehin et al., 2010, Wei et al., 2014).

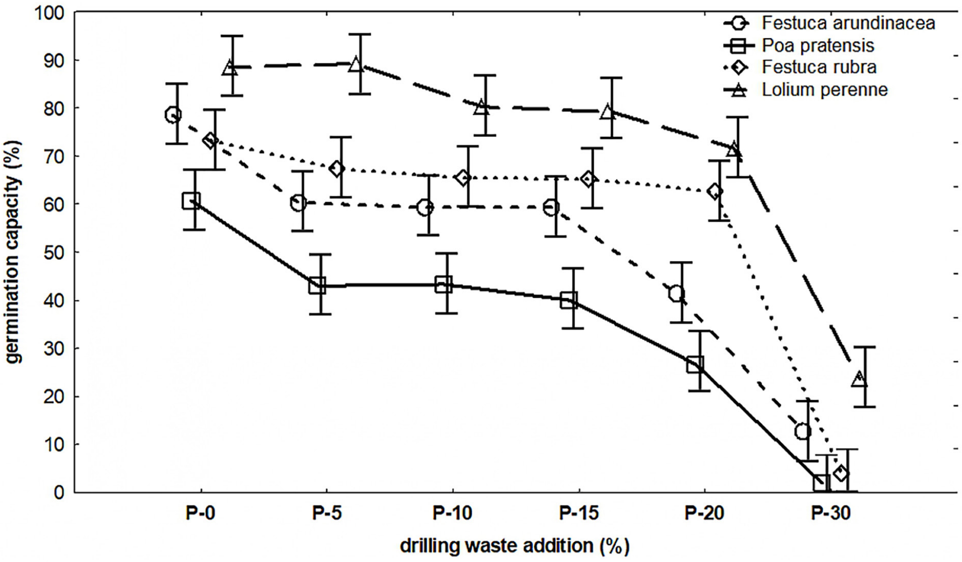

Fig. 4. Influence of drilling waste concentration on germination capacity (%) of the tested grass species. The bars at the top and bottom represent the mean ± SE. Bars at the same level indicate no significant difference ($p \leq 0.05$ by Tukey's test)

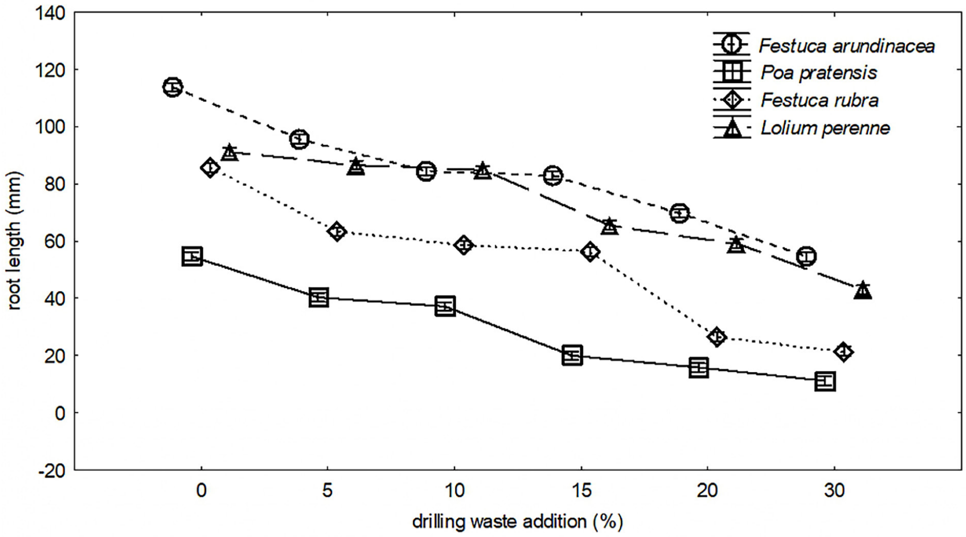

Fig. 5. Impact of different drilling waste addition (%) on the root length (mm) of tested grass species in 60th day from the sowing date. The bars above and below the mean represent the standard error (SE). Bars at the same level indicate no significant difference according to Tukey's multiple range test ($P \leq 0.05$)

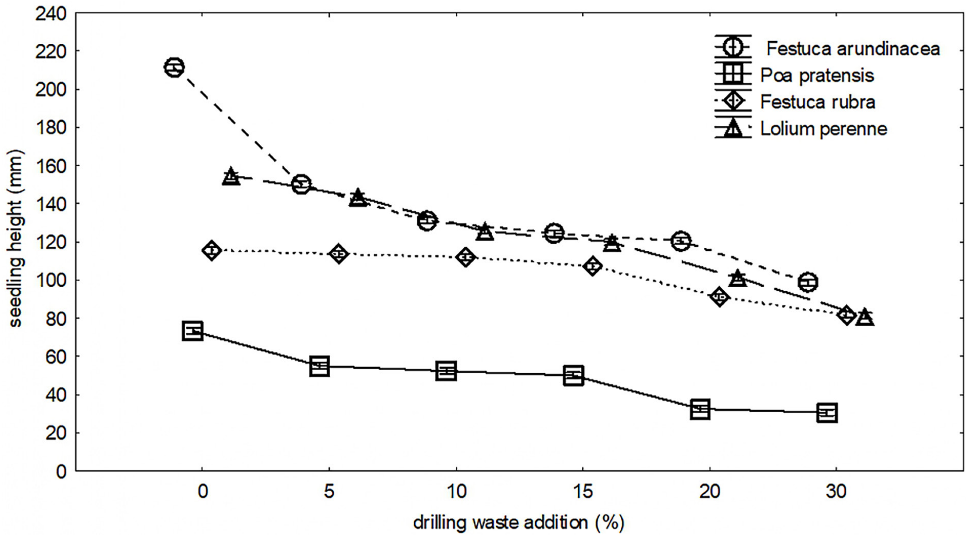

Fig. 6. Impact of different drilling waste addition (%) on seedling height (mm) of tested grass species in 60th day from the sowing date. The bars above and below the mean represent the standard error (SE). Bars at the same level indicate no significant difference according to Tukey's multiple range test ($P \leq 0.05$)

Our research showed that the content of individual PAH groups depended on the physicochemical properties of these compounds (molecular weight, number of aromatic rings) and the grass species (Table 4).The addition of drilling waste to soilmixtures (P-5, P-10, P-15, P-20, P-30) increased the amount of PAHs in the samples. Laboratory tests revealed that the content of PAHs in soil sown with different grass species was significantly different.

The conducted research showed that among PAHs (with low molecular weight), the lowest concentrations of Phenanthrene and Anthracene were found in soil mixtures planted with *Lolium perenne*. These results suggest that the presence of *Lolium perenne* had a significant impact on the degradation of PAHs in the soil. These findings are consistent with the study by Binet et al. (2000b), which indicates that the roots of *Lolium perenne* accelerated the dispersion of 3-6 ring PAHs, with 3-ring PAHs such as Phenanthrene and Anthracenehaving a higher dispersion than high molecular weight PAHs. This is not consistent with the results of Fang et al. (2001), who showed that the

Table 4. Content of PAH in substrate mixtures (µg/kg) regardless of the species (s) and drilling waste addition (A)

Low molecular weight PAHs					
Species (S)	Naphthalene	Acenaphthylene	Fluorene	Phenanthrene	Anthracene
Festucaarundinacea	104.10a	123.63a	22.08c	12.76bc	7.13a
Festuca rubra	103.79a	112.77b	28.91b	12.26c	7.06a
Loliumperenne	96.49b	107.50c	43.49a	10.99d	6.11b
Poapratensis	88.76c	108.05c	20.60d	13.04a	6.97a
Drilling waste addition (A)					
P-5	24.13e	19.90e	10.75e	4.33e	3.17d
P-10	65.93d	69.18d	19.98d	8.56d	5.53c
P-15	83.95c	81.86c	23.31c	9.97c	5.36c
P-20	117.17b	165.42b	33.31b	16.40b	8.34b
P-30	200.23a	228.58a	56.50a	22.05a	11.64a
SEM	5.605	7.048	1.849	0.590	0.289
P - value					
Species (S)	<0.001	<0.001	<0.001	<0.001	<0.001
Addition (A)	<0.001	<0.001	<0.001	<0.001	<0.001
Interaction S x A	<0.001	<0.001	<0.001	<0.001	<0.001

High molecular weight PAHs									
Species (S)	Fluoranthene	Pyrene	Benzo(a) anthracene	Chryzene	Benzo(b) fluoranthene	Benzo(k) fluoranthene	Benzo(a) pyrene	Benzo((ghi) perylene	Dibenz(a,h) anthracene
Festucaarundinacea	34.54b	79.31a	16.64b	12.12b	22.56b	25.61c	18.50b	14.47a	81.46a
Festuca rubra	41.02a	58.71d	16.26c	9.66d	22.51b	26.79b	19.95a	13.05c	67.55d
Loliumperenne	32.32c	66.82c	18.92a	13.35a	22.81a	28.24a	17.99c	9.68d	72.35b
Poapratensis	30.35d	72.11b	16.14c	11.10c	21.92c	23.89d	16.45d	13.35b	71.79c
Drilling waste addition (%) (A)									
P-5	16.47e	24.33e	6.98e	2.66e	8.23e	7.21e	5.18e	5.23e	30.18e
P-10	24.73d	46.09d	10.84d	7.50d	16.69d	17.05d	11.48d	9.95d	66.66d
P-15	25.30c	66.15c	13.92c	10.89c	19.26c	21.84c	13.53c	12.24c	63.63c
P-20	41.66b	89.50b	24.56b	15.97b	26.95b	35.54b	27.98b	13.81b	94.20b
P-30	64.61a	120.11a	28.65a	20.77a	41.12a	49.02a	32.94a	21.97a	111.77a
SEM	1.986	3.298	0.802	0.633	1.053	1.384	1.002	0.592	3.153
P - value									
Species (S)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Addition (A)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Interaction S x A	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: a-e – designation of homogeneous species groups at the significance level α =0.05.

degradation of PAH in plots seeded with grass and in unplanted plots was at the same level. On the other hand, Günther et al. (1996) obtained reverse results suggesting that the degradation of PAH was higher in soil samples planted with *Lolium perenne*. This is consistent with the results of Cheema et al. (2009), who showed higher biological activity and significant degradation of PAHs in soils planted with plants (*Festuca arundinacea*) compared to control soils without plants. However, Binet et al. (2000a) and Ghavidel et al. (2018) demonstrated the high effectiveness of *Lolium perenne* in the phytoremediation of anthracene at over 81% reduction compared to the initial concentration. Such a high removal rate of anthracene suggests that *Lolium perenne* can be considered one of the best grasses for the phytoremediation of soils contaminated with PAHs, bearing in mind that anthracene is used as a model compound for PAHs (Kanaly and Harayama 2000). Among the high molecular weight polycyclic aromatic hydrocarbons (WWA), significantly lower concentrations of Dibenz(a,h)anthracene, Pyrene, Benzo(a)anthracene, and Chryzene were found in soil mixtures seeded with *Festuca rubra*. Meanwhile, significantly lower concentrations of Fluoranthene, Benzo(k)fluoranthene, Benzo(b) fluoranthene, Benzo(a)pyrene, and Benzo(a)anthracene were found in soil sowing with Poa pratensis. This contradicts the findings of Cheema et al. (2009), which suggest that the degradation of Fluoranthene and Pyrene in soil seeded with *Festuca arundinacea* occurred faster than in unseeded soil. Also research Binet et al. (2000a) showed that lividomycin was able to accelerate the dispersion of a series of PAHs, including 5 and 6-ring PAHs such as dibenzo(a,h)anthracene and benzo(g,h,i)perylene, which are known for their low solubility and bioavailability. As indicated by the research of Kirk et al. (2005), such different results may be due to varying numbers of microorganisms and bacteria stimulated by root exudates, which are a specific feature of particular plants. However, according to Ghavidel et al. (2018), the effectiveness of phytoremediation depends on the growth and development of the plant. The faster the growth and development of the plant, the higher the degree of PAH degradation that can be achieved. The research showed that, regardless of the species, with the increase in the content of drilling waste in the substrate, the content of all tested PAHs increased in direct proportion to the applied dose. The exception was soil contaminated with drilling waste, in the proportion of 10% and 15% cuttings in relation to the sand mass, where the Anthracene content, regardless of the doses used, was at a similar level (differences not significant). The analysis of the results did not show any significant interactions between Species x Drilling waste addition.

CONCLUSIONS

The study found that assessing the germination energy and capacity of tested grasses does not predict their growth in PAH-contaminated soil. The germination energy of seeds decreased with the increase in drill cuttings in the substrate. *Lolium perenne* and *Festuca rubra* had the highest germination energy and ability among the tested species, while *Poa pratensis* had the lowest. Even with the addition of 5% drill cuttings to the substrate, *Poa pratensis* had significantly lower germination energy and capacity compared to other tested species. However, the germination capacity of *Lolium perenne* was similar to that in the control sample (P-0) even with a 15% dose of cuttings (no significant differences). Moreover, the experiment showed that *Lolium perenne* had the smallest reduction in root length and *Festuca rubra* had the smallest reduction in seedling height. The soil sown with *Poa pratensis* had the lowest PAH content, while the soil sown with *Lolium perenne* had the highest. The study suggests that *Festuca arundinacea* and *Lolium perenne*can be used for land reclamation in post-drilling areas (significantly the higher seedlings and root length), but close monitoring of waste impact on soil properties is needed since the experiment was conducted only for 60 days in laboratory conditions.

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