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Biometric indicators of growth and development of *Lolium perenne* and *Trifolium repens* in terms of recultivation of soil contaminated with petroleum

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Abstract: The aim of the research was to determine the factors defining the growth and development of *Lolium perenne* and *Trifolium repens* upon petroleum contamination. The top layer of clay soil contaminated with petroleum products resulting from an oil pipeline failure was collected for the tests. The control was the same type of uncontaminated soil with the addition of, under laboratory conditions, tissue paper. The research was conducted in two stages. The first concerned the germination process and seedlings parameters (Petri dishes). The germination energy (*GE*) and the germination capacity (*GC*) of seeds were determined. The seedling's development was also evaluated based on 'WinRhizo PRO 2009' software. Then, in the second stage, pot tests were carried out, where the growth and development of species in the first year after sowing were temporarily measured. The parameters studied were the number, height, green and dry masses of the plants.

A Gompertz regression model describing seed species germination and number species as time dependent dynamic was applied. The data were analysed statistically using variance analysis (ANOVA) and the PCA (principal component analysis) method. The results of our study indicated that admixture of petroleum into the soil does not seriously affect the development dynamics of *Lolium perenne* seedlings. The diesel oil contamination mostly affects the germination of the *Trifolium repens* by a statistically significant increase of the maximum value of germination and increasing the maximum growth rate.

Keywords: germination dynamic, Gompertz regression model, lawn plants, polycyclic aromatic hydrocarbons (PAH), pot tests, remediation, seedlings parameters

INTRODUCTION

The issue of soil contamination with petroleum substances and the selection of species for phytoremediation is still relevant due to the increasing use of crude oil as an energy raw material [Hussain *et al.* 2019]. Soil contamination resulting from the use of petroleum products is mainly anthropogenic and is highly toxic, persistent and bio-accumulative [Malachowska-Jutsz *et al.* 2012]. The environmental threat with these substances is global [Errington *et al.* 2018; Xie *et al.* 2017; Zhu *et al.* 2019] and is particularly dangerous in the places where deposits are exploited, distributed, transported and at random events (illegal connections to the pipeline) [Chen, Zhong 2019; Smith *et al.* 2015]. The growing number of such events proves the constant and increasing risk of leakages of petroleum products in Poland. In 2011–2018, the number of spills of petroleum substances doubled [KGSP 2020]. The size of the effects of polycyclic aromatic hydrocarbons (PAH) emissions depends on its type, intensity, PAH deposition in soil and the way of using the space, including the type and properties of soils, climatic conditions and microbiological biodiversity of the soil environment [Chaudhary *et al.* 2012]. It is estimated that over 90% of the total contamination with petroleum substances is found in the surface layer of soil. The need to remediate areas contaminated with PAHs is legally determined [Ustawa … 2001] and therefore research is being undertaken on the impact and possibility of restoring the soil environment to a state close to the original one. The effectiveness of these activities should be interdisciplinary because it depends on many abiotic and biotic factors [Gaskin, Bentham 2010; Hewelke *et al.* 2018; Páliz *et al.* 2021]. The response of plants and microorganisms to changes in the soil environment caused by the content of petroleum substances is still insufficiently explained and still arouses the interest of many researchers [Chen, Zhong 2019].

An important element of remediation of soils contaminated with PAHs is the selection of plant species. It should take into account the tolerance of plant species to the amount of pollution and their individual components. Only the recognition of the mechanisms of plant adaptation to such difficult habitat conditions will ensure the effectiveness of their introduction. As a consequence, it will be possible to obtain a permanent turf and initiate the process of decomposition of PAHs by microorganisms [Gałązka, Gałązka 2015]. It seems that a good choice of plant species for remediation are perennial herbaceous species (permanent, turf-forming), especially lawn varieties. These varieties are characterised by rapid growth and development after sowing, and their main root mass is in the top layers of soil, where the concentration of PAHs is the highest. Meanwhile, most research on the remediation of soils contaminated with oil derivatives focuses on the response of annual plants with various fertilisation variants [Wyszkowski *et al.* 2022].

The current state of knowledge in the field of the use of perennial plants for remediation of PAHs indicates that in the initial stage of development, most plant species relatively well adapt to the conditions of contamination in the initial period of development – germination capacity. However, the process of introducing species is not limited to the germination process. Its course is also limited by environmental factors, such as weather conditions [Baskin, Baskin 2014; Hay, Probert 2013; Jensen 2004] and soil humidity [Walker *et al.* 2004], independent of oil contamination. It can be also determined by the parameters of the seed material [Janicka *et al.* 2021]. The importance of further plant development on soils contaminated with PAHs is confirmed by different plant reactions in subsequent development stages in strict pot tests [Małuszyńska, Małuszyński 2009; Wyszkowska *et al.* 2019].

It seems, however, that the studies conducted so far in a given field have been typical of measurements carried out under controlled conditions, i.e. in present doses of petroleum substances. Based on the results of these studies, it is difficult to conclude about the growth and development of plant species under extreme conditions determined by damage to the oil pipeline (uncontrolled leakage of petroleum substances). The aim was to determine the factors determining the growth and development of two plant species commonly used for fast and permanent turfing of difficult areas – *Lolium perenne* and *Trifolium repens* in conditions of soil contamination after leakage of a petroleum product during the initial stages of plant development (germination, emergence and biomass formation in the three months after sowing).

MATERIALS AND METHODS

CHARACTERISTIC OF INVESTIGATED SOIL MATERIAL

The soil material used in the research came from the area belonging to the Forest Experimental Station in Rogów, Warsaw University of Life Sciences (Leśny Zakład Doświadczalny SGGW w Rogowie), where the soil was contaminated with petroleum substances. The soil contamination was the result of damage to the hose illegally connected to the fuel pipeline of PERN S.A. of the Płock–Koluszki relation. As a result of a leak (within Rogów PGR, Rogów municipality, geographical coordinates 51.798503, 19.843632), a mixture of oil and gasoline got into the ground environment. For the tests, soil was taken from the top layer of the soil profile (0–30 cm), which was stored in a dump during the rescue operation, and then analysed for PAH content. In the soil from the contaminated area, the sum of C6-C12 hydrocarbons and the sum of C12-C35 hydrocarbons were studied. It was found that the permissible content of hydrocarbons was exceeded by 40% – the sum of C6-C12 hydrocarbons, and the sum of C12-C35 hydrocarbons was exceeded by more than nine time (Tab. 1). The tested substances cause the risk specified in the Rozporządzenie Ministra Środowiska z dnia 1 września 2016 r. w sprawie sposobu prowadzenia oceny zanieczyszczenia powierzchni ziemi [2016] on the method of assessing the pollution of the Earth's surface. The control variant was soil taken from the site above the contamination site in the ravine area (geographical coordinates 51.797902, 19.844337)

Table 1. Summary of the results of laboratory analyses of a soil sample taken from the surface of the area in which it was found that the permissible values of hazardous substances were exceeded

Parameter / Analysed sample	C6-C12, gasoline fraction components	C12-C35, oil fraction components
	mg kg^{-1}·DM	
Analysed land[1)]	69.9	2740
Limit value for the land of group III (Forest area and science services[2)]	50.0	300

[1)] Source: own study based on Annex 1 to the Rozporządzenie Ministra Środowiska z dnia 1 września 2016 r. w sprawie sposobu prowadzenia oceny zanieczyszczenia powierzchni ziemi [2016].
[2)] Source: Intekprojekt [2014].

The studied soil material was initially prepared by removing plants, stones and visible invertebrate parts for further analytical steps. Then, the samples were dried under the room temperature across five weeks in order to obtain an air-stable, dry mass. The dried samples were sieved through a sieve with a diameter of 2 mm. Afterward, the granulometric composition and other physicochemical soil properties were determined using common soil procedures described by Małuszyński and Małuszyńska [2022]: the soil granulometric composition was determined using the Bouyoucos aerometric method modified by Casagrande and Prószyński; pH in 1 mol·dm^{-3} KCl was measured via the potentiometric method using a pH meter model inoLab pH 720

(WTW, Weilheim, Germany) with glass combine electrode; hydrolytic acidity (*Hh*) as well as the sum of alkalinity exchangeable cations (*Sa*) was measured using Kappen's method; *Hh* was measured after extraction of soil with 1 mol·dm^{-3} $(CH_3COO)_2Ca$ using a laboratory shaker WU-4 and determined using an automatic titrator, Jencons Digitrate, to pH 8.2 with 0.1 mol·dm^{-3} NaOH; *Sa* was measured after extraction of soil with 0.1 mol·dm^{-3} HCl using a laboratory shaker WU-4 and determined using an automatic titrator, Jencons Digitrate, to pH 8.2 with 0.1 mol·dm^{-3} NaOH; cation exchange capacity of soil (*CEC*) was calculated by summing the values of *Hh* and *Sa*; organic matter content (OM) was measured by losing mass during anneal in 550°C using the muffle furnace model SNOL 8.2/1100; solid phase density was measured using alcohol method; electrical conductivity (*EC*) was performed with the TDScan4 tester. Results of physicochemical properties of soil samples were presented in Table 2.

Table 2. Physicochemical properties of soil samples

Soil	Percentage share of fraction (mm)			OM (%)	pH in KCl	*Hh*	*Sa*	*CEC*	*EC* (mS·cm^{-1})	*ϱs* (g·cm^{-3})
	2–0.05	0.05–0.002	<0.002			cmol(+)·kg^{-1}				
PCS	43	45	12	7.52	6.29	4.59	17.65	22.24	0.76	2.47
Control (S)	41	45	14	4.90	6.27	5.04	16.30	21.34	1.73	2.54

Explanations: OM = organic matter content, *Hh* = hydrolytic acidity, *Sa* = sum of alkalinity exchangeable cations, CEC = cation exchange capacity, *EC* = electrical conductivity, *ϱ* s = solid phase density, PCS = petroleum-contaminated soil, control – soil (S).

The resulted soil grain size analysis enables to classify both tested soil samples as a loam mechanical group. The solid phase density (*ϱs*), both in the soil in the control area (2.47 g·cm^{-3}) and in the polluted area (2.54 g·cm^{-3}), indicated the values typical for mineral soils. Both tested soils were characterised by a slightly acidic reaction, a similar value of the sorption capacity, with a significant predominance of alkaline ions in the sorption complex over acid ions. The soil from the control area was characterised by a lower value of electrical conductivity and at the same time contained more organic matter than the soil from the polluted area.

BIOMETRIC PLANT MEASUREMENTS

The studies were carried out under controlled laboratory conditions, in Petri dishes with a diameter of 10 cm, filled with a certain substrate i.e. paper (P), soil (S), and petroleum-contaminated soil (PCS). The soil substrate was the topsoil with the granulometric composition of loam. The seeds of *Lolium perenne* L. (variety 'Nira') and *Trifolium repens* L. (variety 'Grass Huia') were sown in the first decade of May 2021 in the quantity of 50 per 1 Petri dish, in 5 rows of 10 seeds per row. Five replicates were used for each research treatments (P, S, PCS). The seeds germinated in the light, at a temperature of approximately 25°C, under the optimal moisture of the substrate and closed Petri dish. The vigour (first counting, previously called the energy of germination – *GE*) and the germination capacity (*GC*) – last counting of seeds were determined according to the methodology of the research subject [Dorywalski *et al.* 1964; Małuszyńska *et al.* 2015]:

$$GE(GC) = \frac{N_{sn}}{N_c} 100\% \tag{1}$$

where: N_{sn} = the number of the seeds with the radicle not shorter than the caryopsis, N_c = the number of the seeds placed on the Petri dish.

The energy of germination (*GE*) and the germination capacity (*GC*) of *L. perenne* were determined after 5 and 14 days respectively, and for *T. repens* after 3 and 7 days after sowing. The seedlings development was determined on the basis of the following parameters: the length of seedlings (*LSe*), the projected area of the leaves (*PAL*), length of the roots (*LRo*), surface area of roots (*SARo*), the projected area of the roots (*PARo*) and average root diameter (*AvgD*). These features were determined on five randomly chosen 8-day-old seedlings of the tested species taken from each dish (in total 5 seedlings × 5 repetitions × 3 treatments). The "WinRhizo PRO 2009" scanned image technique was used for the analysis of plant materials.

Pot tests were carried out in the period from the second decade of June to mid-September 2021 in the vegetation hall of the Warsaw University of Life Sciences. Pots with an area of 86.55 cm^2 and a height of 12 cm were filled with the same amount of soil (700 g) taken from the site of contamination resulting from damage of the pipeline (PCS) and above the site of contamination – control soil (S). For both analysed plants, eight pots were prepared for uncontaminated soil (S) and soil contaminated with petroleum substances (PCS), a total of 32 pots. The soil surfaces were levelled, and seeds were sown. The sowing amount (pcs. per pot) was determined taking into account: the sowing standard for pure sowing (kg·ha^{-1}), the area of the pot and the weight of a thousand seeds. For *L. perenne* 16 and for *T. repens* 20 seeds were sown for each pot. During the research period, the number of shoots was systematically counted every 7–10 days and their shoot length was measured on five selected plants from the pot. In the final stage of the pot experiments, the plants were cut at the base of the shoot. Immediately after cutting, the shoots were weighed in grams (green mass). Then the shoots were left to dry, and their air-dry mass (in grams) was determined (dry mass).

The distribution of temperatures and precipitation during the pot tests (Tab. 3) favoured the growth and development of the studied species and did not require additional soil irrigation.

Table 3. Weather conditions during the pot tests according to the meteorological station of the Warsaw University of Life Sciences

Period	Parameter							
	Air temperature (°C)				Precipitation (mm)			
Month	VI	VII	VIII	IX	VI	VII	VIII	IX
Decade 1	18.8	21.8	18.1	15.1	3.1	90.5	96.7	1.8
Decade 2	20.6	23.1	19.5	14.9	37.5	79.5	55.4	30.2
Decade 3	22.9	22.3	15.3	12.3	32.7	31.1	51.0	10.0
Average / Sum	**20.8**	**22.4**	**17.6**	**14.1**	**73.3**	**201.1**	**203.1**	**42.0**

Source: own study.

STATISTICAL PROCEDURES

A Gompertz regression model describing seed species germination (and the number of plant shoots – N) as a time-dependent dynamic was applied [Yin *et al.* 2003]. This flexible three-parametric equation enables mathematical quantification of the impact of diesel oil contents in the soil on the germination dynamic (Eq. 2) or number of plant shoots increase (Eq. 3) with time (t) by studying changes of the maximum growth rate (MGR) at the time of inflection (tm and tn) and analysing the relative growth rate (RGR) by establishing the curvature parameter (k and kn).

$$GR = w_{\max}\, e^{-e^{-k(t-tm)}} \tag{2}$$

$$N = n_{\max}\, e^{-e^{-kn(t-tn)}} \tag{3}$$

Equation (2) predicts inflection time tm, when the maximum growth rate MGR is equal to $w_{\max}/e$. The $w_{\max}$ parameter indicates the maximum number of the germinated seeds (upper asymptote) and e is base of the natural logarithm. Respectively the maximum rate of plants number increasing (MNS) with time (tn) is equal to $n_{\max}/e$ (Eq. 3). Consequently the $n_{\max}$ parameter indicates the maximum number of plants.

The data of the Petri dishes and pot tests were analysed statistically using variance analysis (ANOVA). For this reason, the *GE*, *GC*, green mass and dry mass parameters were illustrate using box-plot with the p-value indications. The WinRhizo results (*LSe, PAL, LRo, SARo, PARo, AvgD*) were evaluated by establishing homogenous groups. Both experiments were presented using PCA (principal component analysis) biplots [R Core Team 2021] for determination of the main factors influencing the germination and pots developments of the investigated species.

RESULTS AND DISCUSSION

PETRI DISHES EXPERIMENT

The tested plant species differed in the germination rate and the response to the content of oil in the soil (Fig. 1). *Lolium perenne* germinated the fastest and most intensively on blotting paper (P) – on average 20 pcs a day up to 96 h after placing them on the substrate (Fig. 1a). In hour 144 the 91% of germination seeds were obtained. On the soil (S), the germination intensity of seeds was also high (on average 13 pcs a day), but the number of germinated seeds as on tissue paper (P) was obtained by 192 h after sowing. On soil contaminated with oil derivatives (PCS), *L. perenne* seeds initially germinated (up to 72 h after sowing) much slower than on tissue paper (P), but similarly to uncontaminated soil (S). In the following hours, the seed germination process was not as fast as on the control media (P, S). In the period between 72 and 144 h, an average of 30 pcs per day germinated. As a result, the number of germinated seeds after 192 h on PCS was 41 pcs (82%).

The germination course of *Trifolium repens* was different than that of *L. perenne* (Fig. 1b). The seeds of this species germinated most quickly in soil contaminated with petroleum substances (PCS). After 48 h, it germinated 2.5 times more than on the control media (P, S). In the following days, the number of germinated seeds (PCS substrate) dynamically increased (on average 10 pcs a day) and after 96 h on average 76.8% of germinated seeds was obtained. The seed germination dynamics on control media in the period between 24 and 96 h was significantly lower than on contaminated soil (PCS), on average by 32% on unpolluted soil and on average by 66% on filter paper. In the following days, the number of germinated seeds on PCS did not increase significantly, while on the control media (P and S) the number of germinated seeds gradually increased, though not as intensely as during the first four days.

The applied Gompertz function (Eq. 2) described the course of seed germination of plant species very well, despite certain limitations [Yin *et al.* 2003] related to the lower and upper asymptotes (e.g. $w_{\max}$ with t going to infinity). The compliance of the measurement results with the mathematical function is on average over 99% for *L. perenne* and 82% for *T. repens* (Tab. 4).

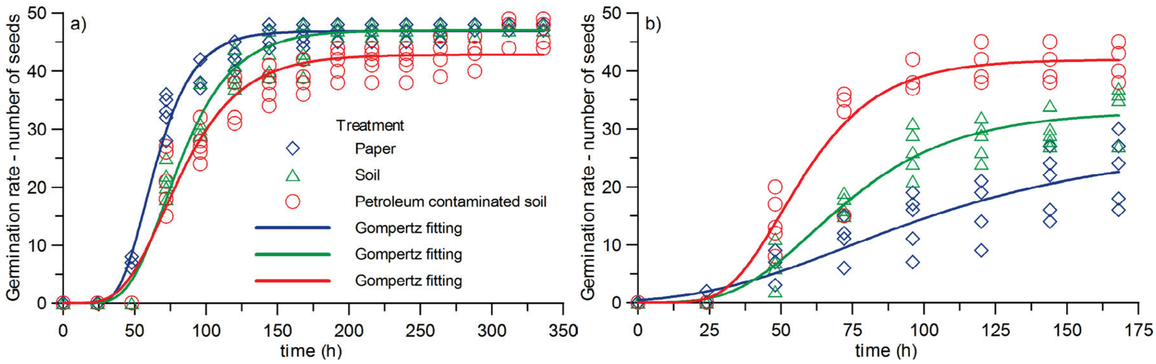

Fig. 1. Measured and fitted number of germinated seeds for: a) *Lolium perenne*, b) *Trifolium repens*; source: own study

Table 4. Parameters of the Gompertz function – experiment on Petri dishes

Parameter	Paper (P)			Soil (S)			Petroleum contaminated soil (PCS)		
	mean	min.	max.	mean	min.	max.	mean	min.	max.
				Lolium perenne					
w_{max}	46.82	45.47	47.72	46.97	45.76	47.74	42.81	39.67	45.19
k	0.05	0.05	0.08	0.04	0.03	0.06	0.03	0.03	0.05
tm	57.65	56.60	61.43	71.13	66.63	73.90	70.42	64.64	73.81
MGR	17.22	16.73	17.56	17.28	16.83	17.56	15.75	14.59	16.62
R^2	0.99	0.99	1.00	0.99	0.98	1.00	0.99	0.96	0.99
				Trifolium repens					
w_{max}	26.46	19.68	39.55	32.98	27.13	37.73	41.94	38.05	45.24
k	0.02	0.01	0.03	0.04	0.02	0.06	0.05	0.04	0.11
tm	73.66	57.63	85.35	61.02	58.14	67.29	50.00	46.03	67.14
MGR	9.73	7.24	14.55	12.13	9.98	13.01	15.43	14.00	16.64
R^2	0.82	0.97	1.00	0.96	0.96	0.99	0.96	0.98	1.00

Explanations: w_{max} = maximum number of seeds, MGR = maximum growth rate of the seeds, tm = inflection point (h), k = parameters of Equation (2), R^2 = coefficient of determination (–).
Source: own study.

The most important parameter differentiating the germination curves was the *tm* parameter, meaning the achievement of the maximum growth rate (*MGR*) at the curve's inflection point. *Lolium perenne* on the paper (P) was characterised by the lowest time *tm* of the inflection point of the curve, which indicates the absence of stress factors during the germination period. In the case of the other two substrates (S and PCS), the values of *tm* times were similar and indicated a delay in reaching the maximum growth of germinated seeds by about 13 h. The w_{max} value for the PCS substrate was slightly less than the measured germination values, confirming the possibility of underestimating the growth curve with values striving for the germination calculation times (*GC*). The characteristics of the germination curve of *T. repens* showed that the PCS substrate influence both the germination rate (*tm* and *MGR*) and the maximum number of germinated seeds (w_{max}). The highest values of these parameters were demonstrated in relation to the control substrates (P, S). Comparing the germination curves of the two analysed plant species (*L. perenne and T. repens*) on the soil contaminated with petroleum product (PCS), it can be concluded that they had a similar course, which was reflected in particular in the similar values of the *MGR* and w_{max} parameters.

The obtained value of the germination rate (*GE*) of *L. perenne* indicates (Fig. 2a) that there is only a significant statistical difference between the PCS medium (71.2%) and the P medium (86.0%). However, this value was much lower (by 10%) than the average of all measurements (P, S, PCS). It should be emphasised that the variance resulting from the observation on this medium (PCS) was much higher than on the other media, which may be due to the different individual tolerance of *L. perenne* to very high soil contamination with oil derivatives. On the other hand, the germination rate (*GE*) of *T. repens* seeds (Fig. 2b) was significantly higher on contaminated soil (PCS) than on other substrates and was on average 61% (median 70%). As for *L. perenne,* some individual differences were observed, but in the statistical analysis these values were treated as an error. The mean

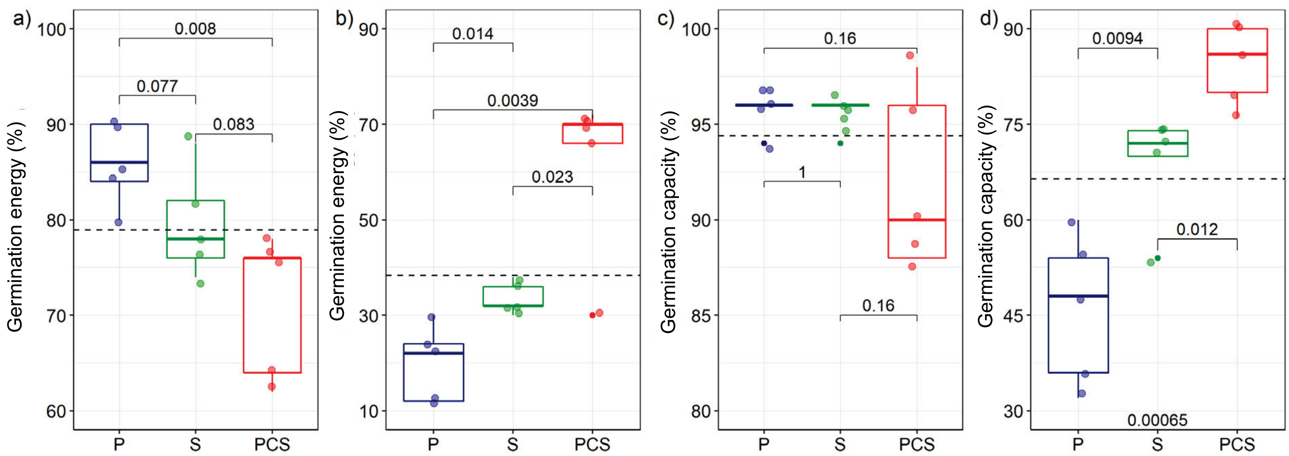

Fig. 2. Box-plots for germination parameters and for of investigated species with *p*-value: a) the germination energy (%) – *Lolium perenne*, b) the germination energy (%) – *Trifolium repens*, c) the germination capacity (%) – *Lolium perenne*, d) the germination capacity (%) – *Trifolium repens*; the scattered point indicates the repetition of measured value characterising certain treatments (P = paper, S = soil, PCS = petroleum contaminated soils); dotted line indicates overall average of the measured values; source: own study

GE value of *T. repens* seeds was 1.6 times higher than the mean value (Fig. 2b) of all measurements (P, S, PCS). Significant differences between the *GE* value obtained on contaminated soil (PCS) and the control variants (P, S) indicated that the emission of gases released from this medium (with closed Petri dishes) stimulated germination of seeds of this species. This kind of phenomenon (regulation of seed dormancy and germination under the influence of endogenous factors) was observed in the studies by Gniazdowska *et al.* [2013].

The germination capacity (*GC*) of *L. perenne* was high (average 94.4%) and did not differ statistically significantly on the tested media (Fig. 2c). This indicated a potentially large tolerance of this species to soil contamination with petroleum substances [Wyszkowska *et al.* 2019] during this period of development. Nevertheless, a large difference (large variance in observation) in the response to a stress factor may have a significant impact on the growth and development of this species in the later stages of development [Janicka *et al.* 2021]. The germination capacity (*GC*) of *T. repens* seeds (Fig. 2d) was highest on the PCS medium (such as *GE*) and averaged to 84.4%. This value was 1.8 times higher than on filter paper (P) and 1.2 times higher than on unpolluted soil (S). Such a high viability of *T. repens* seeds on soil contaminated with diesel oil was not demonstrated in the studies by Pawluśkiewicz *et al.* [2020]. However, the concentrations of diesel oil (DO) used in these studies were much lower (2.5 and 5 g DO on 1 kg of dry mass of soil).

The analysis of the condition of seedlings growing on soil contaminated with petroleum product in comparison to control variants shows that this pollution causes morphological reactions in the tested plant species (Tab. 5). The length of the aerial parts of *L. perenne* (*LSe*) and their surface area (*PAL*) under soil contamination conditions (PCS) were significantly shorter than on tissue paper (by 14.7 and 26.0%, respectively) and on uncontaminated soil (by 35.5 and 45.0%, respectively). Root elongation is a response to soil contamination with petroleum substances, which can more than above-ground biomass, determine the adaptability to difficult environmental conditions. On contaminated soil (PCS), the roots were on average 29.2% longer than under control conditions (P, S). Compared to the paper (P), they also had (on average 2 times) larger areas (*SAR*, *PAR*) and diameters (*AvgD*). These parameters were also greater on contaminated soil (PCS) than on uncontaminated soil (S), with an average of 20% for the area parameters and 40% for the diameter, however, no statistically significant differences between these values were found.

The response to soil contamination with petroleum product of the *T. repens* was slightly different from that of *L. perenne*. The height of seedlings (*LSe*) was, as in *L. perenne,* also much lower on PCS than on uncontaminated soil (S), but the values of the embryonic root parameters (*SARo*, *PARo*) on soil contaminated with oil derivatives (PCS) were similar to blotting paper (P) and significantly lower than on uncontaminated soil (S). The exception was the root length (*LRo*), which was even 55% smaller on PCS substrate compering to paper (P). The root diameter (*AvgD*), was twice as large as on the blotting paper (P). The length, diameter and root area (*SARo*, *PARo*) of *T. repens* seedlings in the soil contaminated with petroleum product (PCS) were lower than in non-contaminated soil, on average by 17, 33 and 40%, respectively.

POT EXPERIMENTS

The research showed significant differences in the number of plants (n_{max}) growing on the soil with the petroleum product (PCS) and on the control soil (S), both for *L. perenne* and *T. repens* (Fig. 3, Tab. 6). The value of n_{max} for *L. perenne* was 2.1 times and *T. repens* 1.6 times lower on PCS than on uncontaminated soil. The shape parameters *kn* of the curve for *L. perenne* were similar in the analysed substrates (S and PCS). The curves significantly differed in the values of the *tn* and *MNS* parameters. For *T. repens*, the *kn* parameters were also similar on the tested substrates, and the values of *tn* and *MNS* slightly differed, which was important for the time of reaching the n_{max} value – close to approx. 48 d. Regardless of the course of the Gompertz curve showing the growth rate of the studied species, the number of *L. perenne* plants was much greater than that of *T. repens,* both in the substrate contaminated with petroleum product (PCS) and under control conditions (S). This may result from the different intensity of branching of these species, but also from the fact that *T. repens* seed material is characterised by a larger number of fresh non-germinating seeds [Janicka *et al.* 2021]. The stimulation of the embryo activity (observed in plate tests) was not so strongly manifesting due to the fact that research was conducted in the vegetation hall with non-limiting, of the germination process weather conditions, repres-

Table 5. Biometric parameters of seedlings of tested plant species depending on treatment

Parameter	*Lolium perenne*			*Trifolium repens*		
	paper (P)	soil (S)	petroleum contaminated soil (PCS)	paper (P)	soil (S)	petroleum contaminated soil (PCS)
LSe	5.44a	7.21b	4.65c	2.07ab	2.18a	1.87b
PAL	0.42a	0.56b	0.31c	0.16a	0.18a	0.15a
LRo	3.95a	4.04a	5.16b	1.98a	1.08b	0.89c
SARo	0.24a	0.44b	0.54b	0.14a	0.20b	0.12a
PARo	0.08a	0.14b	0.17b	0.04a	0.06b	0.04a
AvgD	0.04a	0.05ab	0.07b	0.04a	0.12c	0.08b

Explanations: *LSe* = length of seeds (cm), *PAL* = projected area of leaf (cm^2), *LRo* = length of root (cm), *SARo* = surface area of root (cm^2), *PARo* = projected area of root (cm^2), *AvgD* = average diameter of root (cm).
Source: own study.

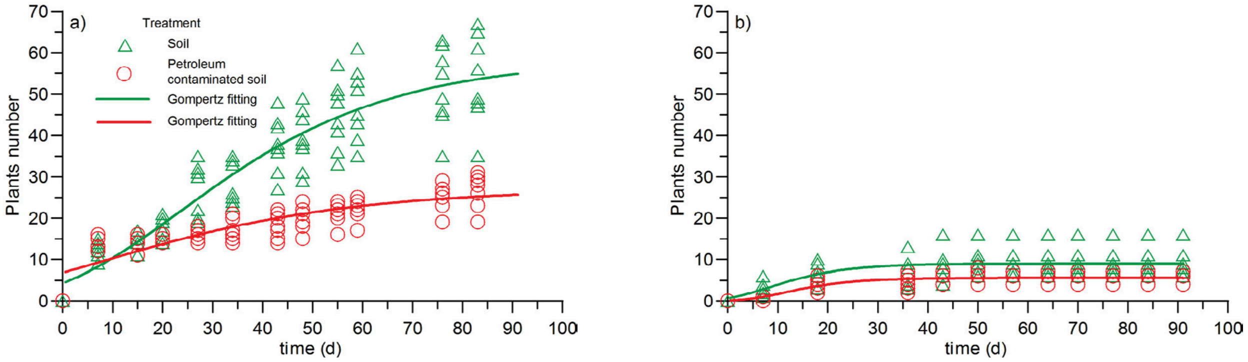

Fig. 3. Measured and fitted plant shoots for: a) *Lolium perenne*, b) *Trifolium repens*; source: own study

Table 6. The Gompertz function parameters – pot experiment

Parameter	Soil (S)			Petroleum contaminated soil (PCS)		
	mean	min.	max.	mean	min.	max.
			Lolium perenne			
n_{max}	58.42	37.11	57.91	27.09	15.62	34.14
kn	0.04	0.03	0.05	0.03	0.03	0.44
tn	23.40	14.64	35.22	9.22	3.30	21.39
MNS	21.49	13.65	29.47	9.97	5.75	12.56
R^2	0.86	0.94	0.99	0.74	0.73	0.89
			Trifolium repens			
n_{max}	8.93	6.33	16.03	5.51	4.08	7.00
kn	0.12	0.07	0.33	0.13	0.06	0.77
tn	8.31	4.84	19.96	11.40	7.76	15.74
MNS	3.28	2.33	5.90	2.03	1.47	2.57
R^2	0.49	0.39	0.99	0.71	0.91	1.00

Explanations: n_{max} = maximum number of plants, *MNS* = maximum growth rate of plants, *tn* = inflection point (d), *kn* = curvature parameter (d), R^2 = coefficient of determination (–).
Source: own study.

ented by stable temperature and optimal soil moisture content [Hay, Probert, 2013; Walker *et al.* 2004].

The variance analysis (p-value < 0.05) for the tested plant species showed that the values of their biomass (green mass, dry mass) were significantly different depending on the substrate used (Fig. 4). The value of green mass here was on average by approx. 2.5 times, and the dry mass for *L. perenne* and *T. repens*, respectively from 3.0 to 3.5 times lower than in the control (S). Similar results were indicated in the studies of the influence of diesel contamination soil on the biomass of *L. perenne*

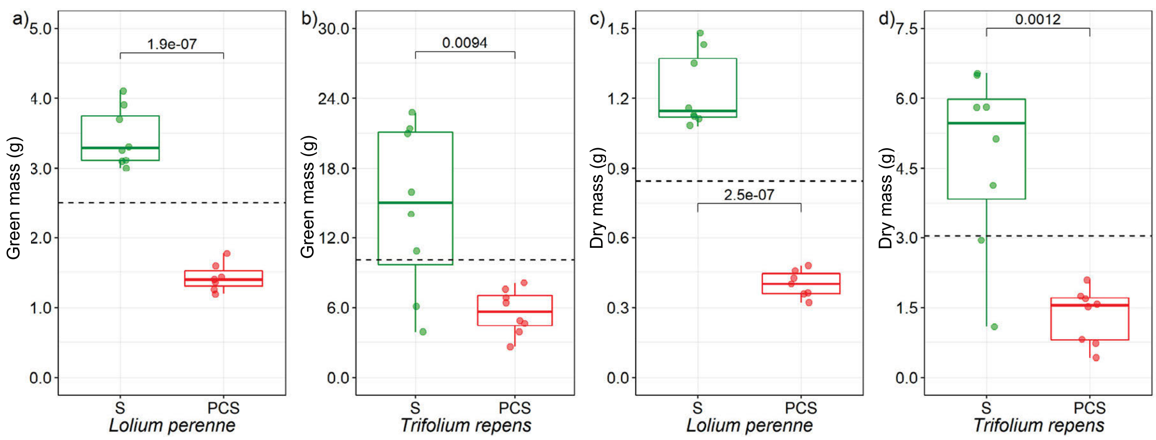

Fig. 4. Box-plots for the green and dry mass of investigated species with p-value: a) green mass – *Lolium perenne*, b) green mass – *Trifolium repens*, c) dry mass – *Lolium perenne*, d) dry mass – *Trifolium repens*; the scattered point indicates the repetition of measured value characterising certain treatments (S = soil, PCS = petroleum contaminated soils). Dotted line indicates overall average of the measured values. Source: own study

[Kechavarzi *et al.* 2007]. The desiccation coefficient (the ratio of green to dry plant mass) of the tested species on uncontaminated soil (S) was approx. 2 times higher than on contaminated soil PCS.

MULTIVARIATE ANALYSIS OF EXPERIMENTAL DATA

The use of PCA analysis showed that in the dish test it is possible to isolate six homogenous clusters (three for each plant) directly resulting from the different type of substrate (Fig. 5a). The presented data in the form of a biplot also take into account the mutual relations between the variables explaining the studied plant species samples. In this case, four groups of interdependent explanatory variables can be distinguished. The first one includes parameters describing the root surface. The second was dominated by the parameters of above-ground plant parts (*LSe*, *PAL*). The third interdependent group of explanatory variables was related to the parameters defining the germination curve, including the *GE* and *GC* parameters. In the fourth group, the parameters of the Gompertz function growth curve were inversely correlated, i.e. *tm* and *k*. It was shown that the initial development of *L. perenne* on soil contaminated with petroleum product (*Lp*_PCS) was mainly related to the size of the seedling root area (*SARo*, *PARo*) and their length (*LRo*). This group was also characterised by a low value of the root diameter (Avg*D*), much smaller than the diameter of *T. repens* roots on uncontaminated soil (S). A characteristic feature of the *T. repens* cluster on the soil contaminated with petroleum product (*Tr*_PCS) was the high value of the Gompertz *k* growth curve shape index with a simultaneous low time (*tm*) of its maximum growth rate (*MGR*). It can also be concluded that *T. repens* on soil contaminated with oil derivatives (PCS) was characterised by a lower value of the *AvgD* parameter compared to the *Tr*_S treatment.

In the pot tests (Fig. 5b) it was found that the distinguishing element of the *T. repens* group growing on the control soil (S) was the biomass value (group 1), both green and dry. A characteristic feature of the *L. perenne* group in the control (*Lp*_S group) was the rapid growth (height after seven days – $H_{\min}$) and development of the plants ($n_{\max}$, *kn*, *MNS*). The results of the PCA analysis also indicated that the formation of the *L. perenne* group on soil contaminated with oil derivatives (group 2 *Lp*_PCS) was associated only with a low biomass value. The fourth group that was separated was *T. repens* growing on contaminated soil (PCS). However, this was a heterogenous group as there were two cases with *T. repens* growing on the control. This group was distinguished by the parameter of the shape of the plant appearance rate curve (*kn*). It should be emphasised, however, that the discussed group, despite the dynamic increase in the number of plants in the first days (*kn*), was characterised by very low values of the maximum number of plants ($n_{\max}$) and their maximum height ($H_{\max}$).

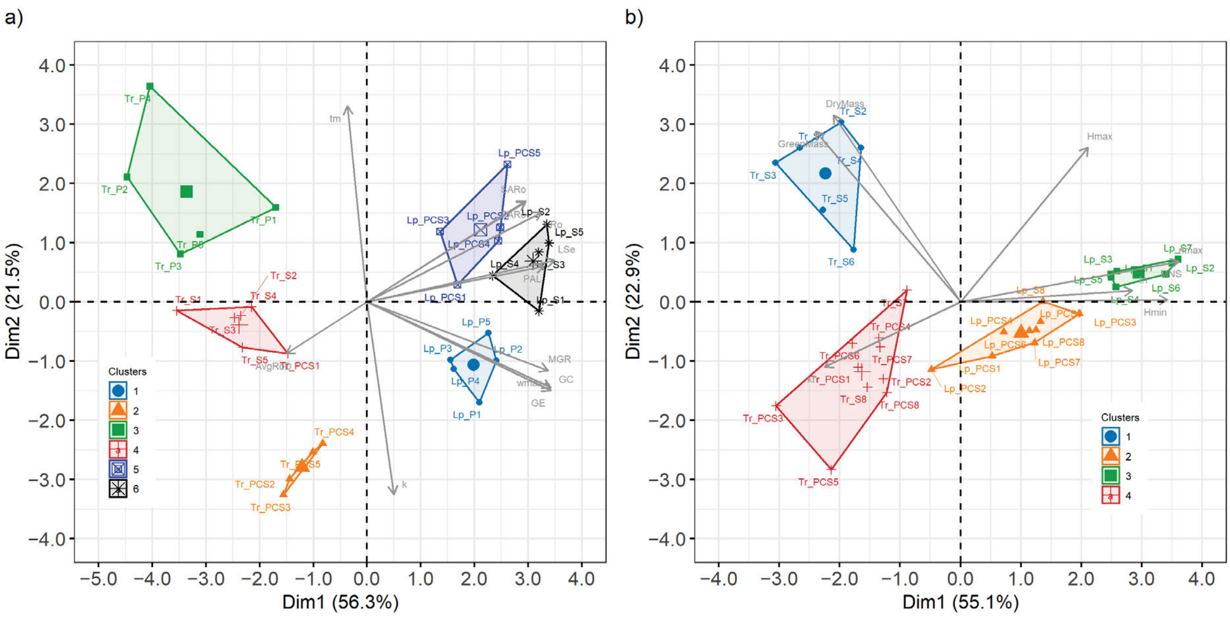

Fig. 5. Principal component analysis biplots indicating distinguished groups of species and descriptive variables for: a) Petri dish experiment, b) pots experiment; source: own study

CONCLUSIONS

The tested plant species reacted differently to the excess oil content in the soil during the period of initial growth and development after sowing. The factor determining the possibility of soil surface turfing by *Lolium perenne* on soil contaminated with petroleum product was the time necessary to start the processes of embryo activation in the seed material – the *tm* parameter of the Gompertz growth curve. This time probably allows the plants to react to the stress factor and change the morphology of the roots (elongation, increase in the diameter and surface of the embryonic root), and thus adapt to the contamination of the substrate. In the subsequent stages of development, one main factor influencing the condition of plants was not found, and the main effect of soil contamination was significantly lower biomass of the aboveground parts. The decisive factors for the initial development of *Trifolium repens* on the soil contaminated with petroleum product was the very fast germination rate – the Gompertz curve shape parameter *k* and the small diameter of the germinal root. The germination rate of seeds of this species in the closed Petri dishes does not fully reflect the tolerance range for the stress factor under study. In the later development stages, the surface coverage was determined by poor emergence (n_{max} parameter of the Gompertz curve).

Among the studied plant species, *Lolium perenne* can be used for turfing area contaminated with petroleum substances as a result of an emergency spill. However, it is recommended to increase the seed sowing rate.

The Gompertz growth curve reflects the measured data well and can be used especially to describe the course of seed germination. The values of the curve parameters were significantly positively correlated with the germination energy, germination capacity, and for *L. perenne* also with the above-ground parameters of seedlings. The values of the parameters characterising the development of *L. perenne* roots were negatively correlated with the estimators of the growth curve (w_{max} and *MGR*), and the values of the root area were statistically significantly related to the value of the *tm* parameter.

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