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Original Research

Green Technology as a Way of Cleaning the Environment from Petroleum Substances in South-Eastern Poland

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Abstract

Background: In phytoextraction methods, the problem is the obtained contaminated plant biomass, the selection of the appropriate species, resistant to the type and degree of contamination, as well as the long time needed to completely clean the soil. Goal: when selecting the appropriate method of remediation of soils contaminated with polycyclic aromatic hydrocarbons, not only the effectiveness of the method should be considered, but also the degree of contamination, the location of the site and its current and planned use. **Methods**: Descriptive, laboratory and comparative methods were used. **Results**: Soil contamination with polycyclic aromatic hydrocarbons (PAHs), which can cause mutations and cancer, is of particular concern as it affects not only human health but also vegetation growth and the biological environment. A fast, nature-friendly and cost-effective method is required to remove and minimize the hazardous effects of crude oil. **Conclusions**: Green technology is particularly beneficial, especially the phytoextraction technique, in which plants clean the soil of excess petroleum products, prevent its further movement from the site of contamination and prevent erosion of reclaimed soil. Species such as: *Trifolium repens*, *Trifolium pratense*, *Lotus corniculatus*, *Agrostis stolonifera*, *Festuca rubra* subsp. *trichophylla*, *Arrhenatherum elatius* performed their tasks very well, therefore they can be recommended for use as a factor counteracting environmental degradation.

Keywords: bioremediation; plants; pollution; phytoremediation; soil contaminated with hydrocarbons

1. Introduction

For over a dozen years, all industrialized countries of the world have had the problem of contaminating land with crude oil and its processed products. These pollutants get to the soil mainly as a result of oil extraction and processing in refineries, as well as any failures during fuel storage. Over-exploitation of oil fields, failures during the extraction, storage and transport of raw materials as well as military activities were the main causes of soil contamination with petroleum substances [1–4]. In Poland, the areas of the former bases and post-Soviet military training grounds are heavily contaminated, as well as the former crude oil exploitation areas, land near refineries, gas stations, repair workshops for car-railroad rolling stock, airports [3,5–9]. A large share of soils is in the south-eastern part of the country, contaminated with petroleum substances, after the former extraction of crude oil [1]. Pursuant to the Polish Act of “Environmental Protection Law”, contaminated soils should be remediated [10]. Under this concept, which is understood, among others, actions aimed at the complete removal or reduction of the number of substances causing the risk, but also their monitoring and reduction of soil contamination with these substances. Typically, two main soil remediation strategies are considered. One of them is total or partial purification, combined with a temporary increase in the mobility of petroleum substances in the soil [1]. The second one is based on their immobilization, so that it cannot migrate to other components of the environment and become not available for plants. Currently, many methods of soil remediation are known, including: physical, chemical, thermal and biological. Methods related to extraction, washing or thermal treatment in order to clean the soil from petroleum substances are highly effective, but they usually involve the costly removal of the contaminated soil layer and transport to the place of treatment. Moreover, such methods destroy soil structure and their natural, biological activity, and generate a large amount of waste. On the other hand, methods involving immobilization. In soil xenobiotics only work for a limited time and require constant monitoring. Most friendly for the soil environment and much less expensive are biological methods based on the use of microorganisms (bioremediation) and higher plants (phytoremediation) as remediators. The phytoextraction technique, in which plants clean the soil of excess oil derivatives, and prevent its further displacement, seems to be particularly advantageous from the site of contamination and additionally prevent erosion of the cleaned area. In turn, in phytoextraction methods the problem is the obtained contaminated plant biomass, selection of the appropriate species, resistant to the type and the degree of pollution, as well as the long time needed to completely clean the soils. However, when choosing an appropriate method of re-mediation of soils contaminated with these substances, one should consider not only the effectiveness of the method, but also the degree of contamination, the location of the area as well as its current and planned use [9,11].

When crude oil penetrates deep into the soil, it causes clogging of the spaces through which water and air are transported, and therefore soil clumping occurs and the related deterioration of their physicochemical and biological properties as well as deterioration of production capacity, reduction of soil sorption capacity and its capacity for the exchange of calcium, magnesium and hydrogen, the bioavailability of potassium, magnesium and phosphorus compounds and an excessive increase in the content of carbon compounds [5]. This causes the mass death of animal organisms inhabiting the soil and a rapid increase in nitrogenous organic matter. This, in turn, causes rapid changes in the composition of the soil microflora. The number of aerobic species decreases, nitrifying bacteria are dying, the number of atmospheric nitrogen-fixing bacteria under aerobic conditions decreases, the number of fiber-binding bacteria and fungi decreases, while bacteria that assimilate hydrocarbons and their components develop. Under such conditions, ammonification bacteria and ammonia are formed. In fields contaminated with oil, the emergence of grain and papilionaceous plants is delayed, and the potato plants do not show any emergence. The plants die after 3–4 weeks from delayed emergence. Crude oil with a prominent level of contamination inhibits the growth of crops [4,5,12–14].

Various strategies are used in the remediation of petroleum contaminated sites. The first one consists in the immobilization of these substances in the soil, that is, transforming them into compounds that are insoluble or sparingly soluble in water, thanks to which they become inaccessible to plants and cannot migrate deep into the soil profile [1,8]. Soil remediation can be conducted in the place of contamination (*in situ*) and outside the place of contaminated area (*ex situ*). It depends on the selection of the method and the process it is based on [14]. Natural biodegradation is only about regular monitoring of the level of pollution without human intervention. It involves the use of local microorganisms and natural physicochemical reactions, and it occurs automatically. Remediation of xenobiotics by microorganisms can take place through biodegradation (oxidation and decomposition), assimilation (assimilation) or biotransformation (transformation into non-toxic chemical compounds) [13]. Microorganisms that take part in the degradation of pollutants constitute approx. 10% of their total population, therefore cleaning the environment in this way takes an exceedingly long time [15–20].

The development of the oil extraction industry and economic changes in south-eastern Poland - from the great industrial divergence to contemporary globalization processes, resulted in an increase in the threat to the natural environment in this region [7,9,21–23]. It is therefore important to put in place measures and actions that will help reduce the environmental impact of the oil industry. Therefore, research has been conducted on the creation of a special, ecological technology for remediation and protection of the natural environment. A proposal for the development of these areas was developed with the use of “green technology” and the use of selected plant species for reclamation, which have a significant, beneficial effect on the soil. They are able to eliminate residual aromatic hydrocarbons (PAHs) or significantly reduce their content in the soil [24–26]. Thanks to “Green Technology” it will be possible to prepare the soil for the cultivation of more demanding plant species. As food and energy security is a strategic goal of most countries in the world, this study advocates understanding the concept of soil stability to integrate food and energy security as a new pillar of environmental management, based on an empirical understanding of the relationship between soil and the plants and their interconnections. The aim of the work is to develop “Green Technology” as a way to restore the biological balance of polluted environments on the basis of own research conducted in south-eastern Poland. The alternative hypothesis assumes that “Green Technology” using phytoextraction methods will allow to reduce the content of PAHs in the soil, increase the share of plants in the soil cover and restore food security, contrary to the null hypothesis that such technology and such activities not help to reduce contaminate degraded soils in the region of South-Eastern Poland.

2. Materials and Methods

The research was conducted in the period 2018–2019 on the grounds twelve localities, in the area of crude oil extraction, belonging to two poviats: Krosno and Jasło in South-Eastern Poland. The analysis covered five communes of the Krosno poviat and four communes of the Jasło poviat. The number of examined sites in individual communes was twelve. A profile of up to 120 cm was achieved, and soil samples were collected in accordance with the methodology and analyzed for some physicochemical properties (Tables 1,2,3,4,5,6,7,8) [27,28]. In addition, the research material was a list of plant species located in areas devastated by the oil industry, in the Krosno and Jasło poviats, and a list of photos of reclamation plants that were photographed and described. The key to recognizing herbaceous plants was used in the identification and description of plant species present in this area [29,30]. In addition, a field experiment was conducted in 2018–2019 with red clover as a potential species for the reclamation of soils contaminated by PAHs. The contaminated soil profile was classified according to the World Reference Base for Soil Resources (WRB) [28].

2.1 Chemical and Physicochemical Properties of the Soil

The chemical and physicochemical properties of the soil were determined in the certified laboratory of the Regional Chemical and Agricultural Station in Lublin (Scope of Accreditation No AB 1186) according to the following methods: soil texture – using the Casagrande's method in Prószyński's modification [31]; soil reaction – according to ISO 10390: 2005 [32]; organic carbon content – Corg. – by the method of Tiurin [33]; hydrolytic acidity – Hh – by the Kappen method [33]; content of basic exchangeable cations in 1 M ammonium acetate (Ca^{2+}, K^+, Na^+ - by flame photometry [22], Mg^{2+} content – by ASA method [34]; sorption capacity was calculated, as the sum of the hydrolytic acidity and the sum of basic cations (T = Hh + S) [21]; the content of available magnesium, phosphorus and potassium – according to polish norms [35–37]. The total nitrogen content was determined by the Kjeldahl method [38]. The C/N ratio was also calculated.

2.2 Quantitative Determination of Multicycle Aromatic Carbons

Quantitative determinations of polycyclic aromatic hydrocarbons in the tested samples were conducted on the basis of standard mixtures of these compounds in ampoules containing about 1 mL of solution, which were used to prepare standard solutions. The solutions obtained on the basis of dichloromethane contained approximately 90 μg/mL of each component. Using the solutions prepared in this way, a series of five calibration samples was prepared. For this purpose, the following were introduced into 10 mL volumetric flasks: 0, 100, 400, 600, 800 μg of the stock solution. To perform quantitative determinations, a constant volume (one hundred μg) of an internal standard solution containing acenaphthene d10 and the addition (one hundred μg) of a control standard (d10 phenanthrene) was introduced into the dichloromethane solutions, allowing for the control of the extraction process and determination of the analyte recovery. PAH concentrations in individual standard solutions are summarized in the Table 5. For the standard solutions, chromatograms were recorded with the use of an analytical program. One μg of the standard solution was introduced into the chromatographic column. The area under the peaks of individual compounds from the PAH group was measured, and then the ratios of these areas to the area of the control standard added in a constant amount to the calibration solutions were determined. Based on the calculated surface area ratio the peak of the analyte to the area of the control standard model charts have been developed [39]. Sample graphs prepared for naphthalene, acenaphthylene, fluorene and benzo[k]fluoranthene in the coordinate system:

- X axis - ng of analyte injected into the chromatograph,

- Y axis - ratio of the analyte surface area to the area of the Internal Standard.

In the determination of PAHs, the methodology of Kubacki [40], Steliga and Kluk [15], the PN-ISO 10381-1 [41], PN-ISO 10381-2 [42] and ISO 10381-5 were used [43]. The test samples were collected in sealed containers with a capacity of about 2 kg.

The number of sampling sites in each potentially contaminated zone was proportional to the size of that zone, but keeping a minimum number of six samples. As part of the research, the scheme of systematic sampling with a mesh was used with a regular shape in a randomly selected area from an area located at a certain distance from former crude oil production sites. The samples were taken with the use of a soil stick to the depth of 0.2 m. Such collected samples were transported to the laboratory in the shortest possible time, where the soil was stored in a refrigerator at 4 °C until the preparation of the analytical sample. Five soil samples were collected for the study from the studied localities located near the former drilling shafts. These areas were classified in accordance with the Ordinance of the Minister of the Environment of 2002 (item 1359), as land classified as wasteland (Group B) [44].

2.3 Tests of the Process of PAH Extraction from Soil

Determination of petroleum substances and PAHs in soils was conducted in the accredited laboratories of the Malopolska Provincial Sanitary and Epidemiological Station in Kraków and the Laboratory of Analysis and Physiochemistry of Hydrocarbon Fuels. The 15 PAHs allowed for labeling in environmental samples by the US Environmental Protection Agency were analyzed, excluding hydrocarbons rarely found in soils and the most volatile hydrocarbons. In order to isolate polycyclic aromatic hydrocarbons from the sample matrix used the extraction process with the use of a solvent, assisted by ultrasound. The process involved surrendering five gram of soil sample, placed in the specified organic solvent, the action of ultrasonic waves. It was conducted for 30 min, then the obtained extract was separated from the soil sample by centrifugation in a laboratory centrifuge at 3000 rpm min for a certain period of

Table 1. Soil granulometric composition (%).

No	Uptake location	Percentage of fraction diameter (mm Ø)			Composition granulometric
		0.5–2.0	0.5–0.002	<0.002	PTG/USDA*
1	Brzezówka	38.0	56.1	5.9	pyg/SiL
2	Chorkówka	37.2	56.6	6.2	pyg/SiL
3	Folusz	35.8	59.0	5.2	pyg/SiL
4	Harklowa	57.0	34.0	9.0	gl/SL
5	Jedlicze	54.0	36.0	10.0	gl/SL
6	Krościenko	35.5	59.2	5.3	pyg/SiL
7	Równe	37.4	56.4	6.2	pyg/SiL
8	Roztoki	53.0	37.0	10.0	gl/SL
9	Samoklęski	36.4	58.1	5.5	pyg/SiL
10	Tarnowiec	37.2	56.6	6.2	pyg/Silo
11	Węglówka	54.7	34.3	11.0	go/SL
12	Wietrzno	37.5	55.6	6.9	pyg/SiL
	Mean	42.8	49.9	7.3	

* PTG 2008 classification/USDA classification: pyg, clay dust/silt loam; gl, light clay/sandy loam [27]; Source: own study based on the results of the Certified Regional Chemical and Agricultural Station in Lublin.

Table 2. Physicochemical properties of the studied soils.

Uptake location	pH in KCl	Hydrolytic acidity (mmol H^+ kg^{-1})	Humus (g kg^{-1})	Content of Corg (g kg^{-1})	N total (g kg^{-1})	C:N	P_2O_5 (g kg^{-1})	K_2O (g kg^{-1})	Mg (g kg^{-1})
Brzezówka	4.3	**22.9**	2.67	1.36	0.132	10.3	18.9	31.7	4.8
Chorkówka	5.7	14.3	2.35	1.55	0.151	10.3	12.6	20.0	19.5
Folusz	4.8	***22.6***	2.82	1.45	0.143	10.1	13.1	16.6	4.9
Harklowa	6.2	18.8	1.71	1.28	0.173	7.4	24.1	20.2	11.0
Jedlicze	6.1	14.1	1.72	1.29	0.178	7.2	23.0	21.5	9.7
Krościenko	5.7	***22.3***	2.51	1.38	0.158	8.7	13.1	16.6	4.9
Równe	6.4	10.9	2.37	1.45	0.178	8.1	12.0	20.0	19.5
Roztoki	5.9	18.2	1.93	1.24	0.152	8.2	22.2	19.5	10.3
Samoklęski	5.6	22.7	2.87	1.36	0.144	9.4	16.6	25.7	5.1
Tarnowiec	5.4	19.3	2.14	1.24	0.141	8.8	12.5	20.2	19.5
Węglówka	6.1	20.4	1.79	1.35	0.148	9.1	23.1	20.4	10.3
Wietrzno	6.1	19.1	2.59	1.13	0.180	6.3	12.4	20.1	19.7
Mean	5.7	18.8	2.29	1.34	0.157	8.7	17.0	21.0	11.6
LSD$p_{\leq 0.05}$	0.3	1.0	0.1	0.1	ns*	0.5	0.9	ns	0.6

*not significant at $p_{\leq 0.05}$.

Table 3. Sorption properties in dependent of locality.

Locality	Sorption properties							
	Hydrolytic acidity (Hh) (cmol($^{+}$) kg^{-1})	exchangeable calcium (Ca^{2+}) (cmol($^{+}$) kg^{-1})	exchangeable magnesium (Mg^{2+}) (cmol($^{+}$) kg^{-1})	removable sodium (Na $^{+}$) (cmol($^{+}$) kg^{-1})	exchangeable potassium (K^{+}) (cmol($^{+}$) kg^{-1})	sum of exchangeable cations (S) (cmol($^{+}$) kg^{-1})	Soil sorption capacity (T) (cmol($^{+}$) kg^{-1})	Saturation of the sorption complex with basic cations (V) (%)
Brzezówka	2.26	17.55	2.05	0.16	0.78	20.63	21.99	93.49
Chorkówka	2.23	17.78	2.17	0.15	0.76	20.55	21.56	92.11
Folusz	1.68	16.24	1.98	0.14	0.79	20.38	20.98	90.71
Harklowa	1.88	15.79	1.92	0.15	0.80	19.45	21.13	93.14
Jedlicze	1.32	12.67	0.69	0.09	0.67	19.45	21.68	89.67
Krościenko	1.89	12.98	0.78	0.07	0.76	16.89	17.45	89.45
Równe	1.80	17.56	2.06	0.10	0.80	18.35	17.13	90.12
Roztoki	1.74	18.03	2.11	0.16	0.59	17.78	17.18	88.00
Samoklęski	1.67	17.22	1.87	0.15	0.90	15.21	18.45	89.12
Tarnowiec	1.91	16.65	1.69	0.13	1.72	15.38	19.56	88.34
Węglówka	1.56	16.17	1.92	0.13	1.34	15.34	17.16	89.56
Wietrzno	1.67	15.85	1.74	0.14	1.14	16.17	19.12	88.17
Mean	1.80	16.21	1.75	0.13	0.92	17.97	19.45	90.16
LSD$p_{\leq 0.05}$	0.10	0.83	0.09	ns*	ns	0.92	1.00	4.7

*not significant at $p_{\leq 0.05}$.

Table 4. Descriptive statistics of soil sorption properties.

Specification	Hydrolytic acidity (Hh) (cmol($^{+}$) kg^{-1})	Exchangeable calcium (Ca^{2+}) (cmol($^{+}$) kg^{-1})	Exchangeable magnesium (Mg^{2+}) (cmol($^{+}$) kg^{-1})	Removable sodium (Na^{+}) (cmol($^{+}$) kg^{-1})	Exchangeable potassium (K^{+}) (cmol($^{+}$) kg^{-1})	Sum of exchangeable cations (S) (cmol($^{+}$) kg^{-1})	Soil sorption capacity (T) (cmol($^{+}$) kg^{-1})	Saturation of the sorption complex with basic cations (V) (%)
Mean	1.7	16.2	1.7	0.1	0.9	18.0	19.4	90.2
Median	1.7	16.4	1.9	0.1	0.8	18.1	19.3	89.6
Standard deviation	0.2	1.8	0.5	0.0	0.3	2.1	2.0	1.9
Kurtosis	–0.5	0.8	1.8	0.2	2.6	–1.7	–1.9	–0.5
Skewness	–0.5	–1.3	–1.7	–1.1	1.7	–0.1	0.0	0.8
Range	0.6	5.4	1.5	0.1	1.1	5.4	4.9	5.5
Minimum	1.3	12.7	0.7	0.1	0.6	15.2	17.1	88.0
Maximum	1.9	18.0	2.2	0.2	1.7	20.6	22.0	93.5
Variability coefficients (%)	11.1	10.8	28.3	22.2	35.2	11.8	10.0	2.1

Table 5. Content of polycyclic aromatic hydrocarbons in tested soils before the start of the research (μg kg^{-1}).

Locality	Polycyclic aromatic hydrocarbons (PAH)															
	Sum of PAH	naphthalene	Phenanthrene	anthracene	fluoranthene	chrysene	benzo (a) anthracene	benzo (a) pyrene	benzo (a) fluoranthene	benzo (ghi) perylene	fluorene	pyrene	benzo (b) fluoranthene	benzo (bk fluoranthene	dibenzo (ah) anthracene	indenol (1,2,3-cd)pyrene
Brzezówka	238	31	15	0	56	0	1	23	23	43	14	15	4	3	6	4
Chorkówka	312	43	27	14	45	12	12	28	19	22	31	11	2	9	23	14
Folusz	307	31	43	9	38	15	13	31	23	18	29	14	0	7	25	11
Harklowa	256	34	32	11	41	11	9	12	22	15	17	12	0	5	22	13
Jedlicze	234	22	17	14	23	14	10	0	26	23	25	9	3	9	20	19
Krościenko	330	37	35	28	27	19	16	0	29	21	30	28	4	11	22	23
Równe	217	25	27	23	21	17	9	1	21	14	12	25	5	8	9	0
Roztoki	211	19	17	22	18	15	11	0	20	27	9	17	9	10	8	9
Samoklęski	236	21	16	25	19	13	9	0	25	17	10	15	16	12	23	15
Tarnowiec	265	26	24	27	32	18	0	0	16	19	32	21	17	10	14	9
Węglówka	228	18	27	31	30	16	0	0	17	21	30	16	3	11	8	0
Wietrzno	189	21	23	20	25	14	0	0	15	18	21	0	7	13	5	7
Mean	252	27	25	19	31	14	8	8	21	22	22	15	6	9	15	10
Permissible values of PAH*	100	10	10	0	10	10	0	0	10	10	10	10	0	0	10	10
LSD $p \leq 0.05$	13	1	1	1	2	1	ns**	ns	1	1	1	1	ns	ns	1	1

*Permissible values of PAH concentrations (μg kg^{-1}) (soil B), **not significant at $p_{\leq 0.05}$.

Table 6. Descriptive statistics of polycyclic aromatic hydrocarbons (PAH) content in the studied soil before the start of the research (μg kg^{-1}).

Specification	Sum of PAH	naphthalene	Phenanthrene	anthracene	fluoranthene	chrysene	benzo (a) anthracene	benzo (a) pyrene	benzo (a) fluoranthene	benzo (ghi) perylene	fluorene	pyrene	benzo (b) fluoranthene	benzo (bk) fluoranthene	dibenzo (ah) anthracene	indenol (1,2,3-cd)pyrene
Mean	252	27	25	19	31	14	8	8	21	22	22	15	6	9	15	10
Median	237	26	26	21	29	15	9	0	22	20	23	15	4	10	17	10
Standard deviation	44	8	9	9	12	5	6	12	4	8	9	7	6	3	8	7
Kurtosis	–1	0	0	0	0	6	–1	0	–1	6	–2	1	1	0	–2	0
Skewness	1	1	1	–1	1	–2	0	1	0	2	0	0	1	–1	0	0
Range	141	25	28	31	38	19	16	31	14	29	23	28	17	10	20	23
Minimum	189	18	15	0	18	0	0	0	15	14	9	0	0	3	5	0
Maximum	330	43	43	31	56	19	16	31	29	43	32	28	17	13	25	23
Variability of coefficients (%)	17	29	34	49	37	36	76	155	20	36	41	48	96	32	50	68

Table 7. PAH content in soil after completion of field trials (μg kg^{-1}).

Locality	Polycyclic aromatic hydrocarbons (PAH)															
	Sum of PAH	naphtalene	Fenantren	anthracene	fluoranthene	chrysene	benzo (a) anthracene	benzo (a) pyrene	benzo (a) fluoranthene	benzo (ghi) perylene	fluorene	pyrene	benzo (b) fluoranthene	benzo (bk fluoranthene	diabezno (ah) anthracene	indeno (1,2,3-cd) pyrene
Brzezówka	200	28	13	0	49	0	1	20	19	34	11	12	3	2	5	4
Chorkówka	262	38	24	13	40	10	10	23	16	17	24	9	2	7	18	11
Folusz	256	28	37	8	33	13	11	25	19	14	22	11	0	5	19	9
Harklowa	216	30	28	10	36	9	8	10	18	12	13	8	0	4	17	12
Jedlicze	193	20	15	13	20	12	8	0	21	18	19	7	2	7	15	15
Krościenko	275	33	32	26	24	16	14	0	23	16	23	22	3	9	16	18
Równe	181	22	24	20	18	15	7	1	17	11	9	19	4	6	7	0
Roztoki	178	17	16	20	16	13	10	0	18	21	7	14	7	8	6	7
Samoklęski	195	19	14	23	17	12	8	0	20	13	8	12	12	9	18	12
Tarnowiec	220	23	21	25	28	15	0	0	12	15	26	16	13	8	11	7
Węglówka	191	16	24	27	27	14	0	0	13	16	24	12	2	9	6	0
Wietrzno	158	19	21	17	22	12	0	0	12	14	16	0	6	10	4	6
Mean	210	24	23	17	27	12	6	7	17	17	17	12	5	7	12	8
Permissible values of PAH*	100	10	10	0	10	10	0	0	10	10	10	10	0	0	10	10
LSD $p \leq 0.05$	11	1	1	1	1	ns**	ns	ns	1	1	1	1	ns	ns	1	ns

*Permissible values of PAH concentrations (μg kg^{-1}) (soil B), **not significant at $p_{\leq 0.05}$.

Table 8. Descriptive statistics of the PAH content in the soil after the completion of the field experiment (μg kg^{-1}).

Specification	Polycyclic aromatic hydrocarbons (PAH)															
	Sum of PAH	naphtalene	Fenantren	anthracene	fluoranthene	chrysene	benzo (a) anthracene	benzo (a) pyrene	benzo (a) fluoranthene	benzo (ghi) perylene	fluorene	pyrene	benzo (b) fluoranthene	benzo (bk fluoranthene	diabezno (ah) anthracene	indeno (1,2,3-cd) pyrene
Mean	210	24	23	17	27	12	6	7	17	17	17	12	5	7	12	8
Median	198	23	23	18	26	13	8	0	18	15	18	12	3	7	13	8
Stand. Dev.	37	7	7	8	10	4	5	10	3	6	7	6	4	2	6	6
Kurtosis	–1	0	0	0	0	6	–1	0	1	6	–2	1	1	0	–2	0
Skewness	1	1	1	–1	1	–2	0	1	0	2	0	0	1	–1	0	0
Range	117	22	24	27	33	16	14	25	11	22	19	22	13	7	15	18
Minimum	158	16	13	0	16	0	0	0	12	11	7	0	0	2	4	0
Maximum	275	38	37	27	49	16	14	25	23	34	26	22	13	10	19	18
V* (%)	18	29	33	49	37	36	76	156	20	36	42	48	96	31	50	67

* variability coefficient.

time. The separated extract was purified on SPE columns in a vacuum system. Compounds adsorbed on the column were eluted to 2 mL vials with an appropriately selected solvent. The extract was concentrated under a stream of nitrogen and an internal standard solution was added in the last step (Syringe Standard) and directed to quantification. In the first stage, the extraction process was evaluated on synthetic samples - 5 g of roasted sand with the addition of four hundred μg of the standard mixture PAHs and two hundred μg of internal standard containing deuterated compounds of acenaphthene, chrysene, 1,4-dichlorobenzene, naphthalene, perylene and phenanthrene. Extraction was performed in two cycles of 30 min, each time a new portion of solvent, a which 2-propanol was selected. The centrifugation time was set to 10 min. An SPE column was used for purification with octadecyl stationary phase, conditioned 3 mL 2-propanol and 3 mL of a mixture of propanol and water. The compounds to be labeled were eluted from the column with two five hundred μg portions of dichloromethane. The extract was concentrated to a volume of approximately one hundred μL under a nitrogen stream. In order to control the extraction process, the addition of two hundred μg of the control standard was introduced into the test sample before extraction [15,45,46].

2.4 Sampling of Plants and Determining their Dry Weight

Plants from the test objects were harvested using a 75 cm^{-2} quadrant, and the dried above-ground plant biomass obtained from the quadrant was extrapolated to one m^2. The plants were dried at 60 °C in an oven (SIM 500 model, Memmert, Schwabach, Germany) for 48 hours until constant weight was obtained. The dry weight of the samples was then determined and ground to a fine powder using an ultra centrifugal mill (model ZM1000, Retsch, Haan, Germany). Then 100 mg of dried plant sample for microwave digestion (MLS-ETHOS plus, MLS GmbH, Dorsten, Germany) was weighed according to Krachler *et al*. [47]. Before digestion, the samples were mixed with two hundred μL of ultrapure water and 1.9 mL of nitric acid and allowed to react overnight before adding six hundred μL of 4.9% hydrofluoric acid. After digestion, the samples were transferred to centrifuge tubes, and the volume was made up to 10 mL [40].

2.5 Research Conditions

The Jasło-Krosno Basin and the adjacent foothills are the oldest region of crude oil extraction in Europe and were clearly subject to consumption and, consequently, a decrease in their content [48–50]. The oil trail in the vicinity of Jasło – Krosno – Iwonicz – Saroj (Poland), to which the surveyed localities belong, is shown in Fig. 1.

2.5.1 Climatic Conditions

Climatic region in the Jasło poviat is located (49°73’67”N; 21°48’08” E, height above sea level 280-350 m) and Krosno (49°41’19”N; 21°46’14”E; height above sea level: 278–340 m), was characterized by a level of climate, with an average temperature drop of 0.5 °C per 100 m altitude and a rainfall increase of approx. 60 mm at 100 m in height. Average rainfall is 750–800 mm in the western part, and 800–850 mm in the eastern part. South-west winds prevail here. The climate of the Krosno and Jasło poviats is a transitional climate between the oceanic and continental climate. The average annual temperatures range between 6.0–8.5 °C. The growing season here lasts up to 230 days [48,51]. The area of poviats Jasło and Krosno belongs to the Subcarpathian agro-climatic district, constituting a transition zone between mountains and sub-mountain valleys [9,48].

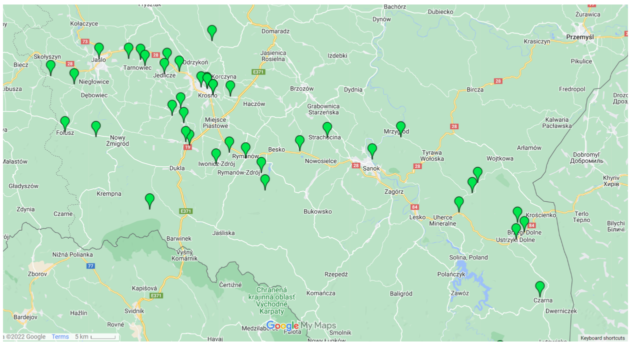

Fig. 1. The oil trail Jaslo-Krosno. Source: The oil trail Jasło-Krosno – Google map (https://www.google.com/maps/d/viewer?mid=1otBYNjAI19hxCf9LKssL5azbv_A&ie=UTF8&hl=en&msa=0&ll=49.494890999999974).

2.5.2 Soil Conditions

The soils of the Krosno and Jasło poviats are characterized by typological variability related to the geological structure, land morphology, water conditions, the nature of vegetation and human activity. The most common here are acidic brown soils, less often leached, made of clay and dust [28], medium-deep and deep. In mountainous regions, the soils are more homogeneous, shallow, skeletal, acidic, brown and podzolic. Soils of classes IIIb, IV and V dominate here. The main factors of soil degradation are erosive phenomena, soil acidification, soil contamination with chemical substances and exploitation of raw materials such as crude oil. Pollution has a point character (emission of industrial plants and landfills) and linear (along communication routes with significant traffic). Most of the soils are clean class II and III in terms of heavy metal content [52].

The soils of the Krosno Basin are diversified due to the varied terrain relief and diverse rock material. Due to their location, the soils of the Basin can be divided into: mountain, mountain (valley) and valley soil. Mountain soils are deluvial soils and are formed by erosion. Intra-mountain and valley soils are formed by influx of sediments. Peat soils and black earth developed on low peat bogs [23]. In

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the Jasło poviat, acidic and very acidic soils, with exceedingly high liming requirements, constitute the advantage. The abundance of nutrients in soils in the poviat is low. The soils show a low and extremely low content of available phosphorus. The content of heavy metals and sulphate sulfur in the soils of the Jasło poviat does not exceed the applicable standards [4,51,53].

2.6 The Field Experiment with Red Clover

A field experiment was conducted using the randomized block method in four repetitions, on eighteen m^2 plots for harvesting, in the Jasło and Krosno poviats, in all the investigated localities in the fields of individual farmers. In the experiment, the same fertilization with phosphorus and potassium was used in the amount of: 43.8 P + 124.6 K kg ha^{-1}, in the form of triple phosphate superphosphate and 60% potassium salt, and one diploid Milena variety. Red clover seeds in the amount of 12 kg ha^{-1} were sown in the year preceding the research, in spring barley (Atico variety - 90 kg ha^{-1}) in the third decade of April (2018). In the sowing year, additional nitrogen fertilization was applied in the amount of 60 N kg ha^{-1}. The experiment was located on the soils of the complex from wheat-rye to good wheat, graded in class IIIb-IVa, the characteristics of which are presented in Tables 1,2,3,4,5,6. During the sowing years, spring barley was harvested in the first ten days of August, and stubble was harvested in the third decade of September. In the year of full use (2019), three cuts of the green mass of clover were harvested, each in the budding phase. During the harvest of plants, samples of green fodder weighing 0.5 kg were collected in order to determine the dry matter content.

2.7 Statistical Analyses

Statistical analyzes were based on two-way analysis of variance models in one-year data experiments and multiple T-Tukey tests, with an assumed significance level of α = 0.05. An analysis of variance with the main effects of the studied factors and their interactions was used. The detailed analysis focused on the main effects and two-way interactions. The study also analyzed the simple correlation of the obtained research results. The significance of the sources of variation was evaluated with the Fischer-Snedecor F test, and the significance of differences between the compared means was assessed using Tukey's confidence intervals. The obtained values were used to determine the optimal conditions for the correct and most expected soil quality indicators, as well as to apply support in the face of changing edaphic conditions and to eliminate the negative effects of the weather. The significance level was used. $p < 0.05$, considering that there were uncontrolled changes in the experimental field, such as slope, growth rate of replicates, as well as soil pH, etc. In addition, descriptive statistics of the studied traits and simple Pearson correlation were performed using the IBM program SPSS Statistics 26 [54–57].

3. Results

3.1 Characteristic of Soils

In the vicinity of Jasło there are brown and podzolic soils, silty-clay soils, medium and medium-deep muds, podzols, clay and brown soils. Brown-acid soils, formed on substrates rich in phosphorus, potassium, calcium and magnesium compounds, as well as brown-leached soils, occur in the Krosno poviat [27]. In the studied localities, the soil grain composition varied depending on the bedrock on which they were formed, the topography and anthropogenic factors (Table 1, Ref. [27]).

The following markings for soil samples were adopted:

(1) soil from an area located 500 m from an active oil and gas mine in Brzezówka, Tarnowiec commune, Jasło poviat,

(2) soil from an area located 300 m from the oldest in Poland and in the world, active Crude Oil Mine in Chorkówka, commune Chorkówka, Krosno poviat,

(3) soil from an area located 300 m from the active Crude Oil and Natural Gas Mine in Folusz, commune Dębowiec, Jasielski poviat,

(4) soil from the area located 400 m from the Oil Mine in Harklowa, gm. Skołyszyn, on the cross-border oil route running through the territory of Poland and Ukraine, Jasło poviat,

(5) soil from the area located 400 m from the Crude Oil Mine in Jedlicze, commune Jedlicze, Krosno poviat, located on the cross-border oil route running through the territory of Poland and Ukraine,

(6) soil from the area in the immediate vicinity of the crude oil well in Krościenko Wyżne, Jasło poviat,

(7) soil from an area located 400 m from the Bóbrka-Równe Crude Oil and Natural Gas Mine in Równe, commune Dukla, Krosno poviat,

(8) soil from the area located 500 m from the Crude Oil and Natural Gas Mine in Roztoki, Jasło poviat,

(9) soil from the area located 500 km from the Crude Oil Mine in Samoklęski, Osiek Jasielski commune, Jasło poviat,

(10) soil from the area located 400 m from the Crude Oil Mine in Tarnowiec, Tarnowiec commune, Jasło poviat,

(11) soil from the area located 500 m from the Crude Oil and Natural Gas Mine in Węglówka, commune Korczyna, Krosno poviat,

(12) soil from the area located 600 m from the old Crude Oil Mine in Wietrzno, Dukla commune, Krosno poviat [27,28].

These were fallow soils on acidic and alkaline soils and on clay formations. Most of the studied soils were located on fallow soils created from dust deposits in the Haplic Luvisol [27,28], classified as a defective wheat complex (class IIIa and IIIb). The soil was classified to the subgroup of clay dust. Only in four localities (Harklowa, Jedlicze, Roztoki and Węglówka) the soil granulometric composition corresponded to that of light clay. The leading fraction have the dust fraction (49.9%), the sand fraction constituted on average 42.8%, and the clay fraction –7.3% (Table 1). Such share of individual fractions corresponds average to the composition of clay dust. The granulometric composition allows the studied soils to be classified as mineral, medium soils [27]. This soil is classified as a medium, dusty soil. The tested soils were acidic (4.3) to neutral (6.4 pH). The hydrolytic acidity was quite varied and ranged from 10.9 to 22.9 mmol H·kg^{-1}. The sum of the exchangeable bases showed a decreasing tendency, from 64.7 mmol H·kg^{-1} to 61.1 mmol H·kg^{-1}. The humus content in the studied soils was high and ranged from 1.92 to 2.87 g kg^{-1} (Tables 2,3).

Considering the content of assimilable forms of phosphorus (13.1–23.1 mg·100 g^{-1} of soil), potassium (16.6–31.7 mg·100 g$^{-1)}$ and magnesium (4.8–19.5 mg·100g^{-1}), the studied soils should be classified as medium- and high-phosphorus-rich, medium-high-potassium-rich and low- to high-magnesium-rich soils. Agronomically this soils are classified as a medium, dusty soil (Table 2). The tested soils were characterized reaction from highly acidic (4.3) to slightly acidic (6.4 pH). The hydrolytic acidity was quite varied and ranged from 10.9 to 22.9 mmol H·kg^{-1}. The sum of the exchangeable bases showed a decreasing tendency. The humus content in the studied soils was high and ranged from 1.92 to 2.87 g kg^{-1}. The content of total nitrogen in the soil did not significantly depend on the study location. The C:N ratio in the soil was the lowest in Wietrzno, the highest, and in the villages of: Brzezówka and Horkówka, while it turned out to be homogeneous in this respect in the villages of: Brzezówka, Chorkówka and Folusz; Harklow and Jedlicze; Równe and Roztoki; Samoklęski, Tarnowiec and Węglówka (Table 2).

The degree of saturation of the sorption complex with bases was not high and ranged from 63.5–64.2% (Table 3) [29]. The highest soil hydrolytic acidity was found in the Brzezówka and Chorkówka, and the lowest in Jedlicze. The soils in the villages of Harklowa, Krościenko, Równe, Tarnowiec and Folusz, Samoklęski, Wietrzno and Węglówka turned out to be homogeneous in terms of this feature. The highest content of exchangeable calcium was in the soil from Roztoki, while the lowest in Jedlicze. The value of this feature turned out to be homogeneous in the following towns: Brzezówka, Równe, Chorkówka and Samoklęski; Folusz, Harklowa, Tarnowiec, Węglówka, Jedlicze and Krościenko. Less calcium and exchangeable magnesium mean a lower degree of saturation of the soil with alkaline cations. The average of exchangeable potassium content was 0.92 cmol (+)·kg^{-1} and was not significantly dependent on the locality of field research. The content of exchangeable sodium was on average 0.13 cmol (+)·kg^{-1}, but it was not significantly dependent on the study location. The sum of exchangeable cations (S) turned out to be the highest in Brzezówka, but in Chorkówka and Folusz it was homogeneous in this respect. The lowest sum of exchangeable cations was found in Samoklęski, and homogeneous results were obtained for soils in: Samoklęski, Tarnowiec, Węglówka and Wietrzno. Soil sorption capacity (T) was the highest in the village of Brzezówka, but the value of this feature turned out to be homogeneous, in this respect, in the villages of Chorkówka, Harklowa and Jedlicze. The lowest sorption capacity was found in the soil in Równe, but homogeneous, in terms of the value of this feature, the soil in Roztoki, Krościenko and Węglówka. The highest saturation of the soil sorption complex with alkaline cations (V) was in Brzezówka, but due to this feature, homogeneous results were also obtained in the following villages: Chorkówka, Folusz, Harklowa, Jedlicze, Krościenko and Równe. The lowest saturation of the soil sorption complex with alkaline cations was in Roztoki village. However, this value turned out to be homogeneous with soils in the villages of Samoklęski, Tarnowiec, Węglówka, Wietrzno and Krościenko (Table 3).

The values of descriptive statistics for soil sorption properties are presented in Table 4. In the case of hydrolytic acidity as well as sum of exchangeable cations (S), soil sorption capacity (T) and saturation of the sorption complex with basic cations (V), the kurtosis obtained a negative value, which means that a greater number of extreme results were observed in the data set (far from the average). The remaining soil quality measures obtained a positive kurtosis value, which means that there was a significant concentration of results around the mean, and the kurtosis value was above "0". The skewness coefficient above 0 indicates a right-hand asymmetry of the distribution. Skewness coefficients for Hydrolytic acidity, exchangeable calcium (Ca^{2+}), exchangeable magnesium (Mg^{2+}), removable sodium (Na^{+}) and sum of exchangeable cations (S) were negative, which indicates a left-hand asymmetry of the distribution (otherwise called negative skew distribution). The skewness coefficient, when it takes a value close to "0", proves the lack of asymmetry of the results. The most variable feature of the soil sorption properties was exchangeable potassium (K^{+}), and the most stable – saturation of the sorption complex with basic cation (Table 4).

3.2 Oil Pollution of Soil

In the studied soils, the content of polycyclic aromatic hydrocarbons was monitored (Tables 5,6). All analyzed soil samples showed the presence of polycyclic aromatic hydrocarbons. Table 5 shows the concentration of PAH (μg kg^{-1} dry weight) in soil samples collected from twelve towns in south-eastern Poland at different distances from the source of pollution. 15 PAH compounds were found. The highest concentrations of total PAHs (330 μg kg^{-1}) were found in the soil from Krościenko, located remarkably close to the oil wells. The PAH content in soil samples, however, decreased with the distance from the source of contamination.

PAH loads from soil samples were low in areas away from oil wells and other sources of land pollution, such as Równe (217 μg kg^{-1}) and Roztoki (211 μg kg^{-1}). In Wietrzno, an extremely low concentration of PAH (189 μg kg^{-1}) was found. The sum of 15 PAHs presents in these soil samples turned out to be 2.5 times higher than the limit values specified in the ordinance of the Minister of the Environment for soils from group B [Journal of Laws 02.165.1359, 2002]. This may suggest the need for another, redetermination of PAH concentration, but in a much larger area adjacent to the former oil well. High concentrations, up to 307 μg kg^{-1} PAH in total, were also found in Chorkówka and Folusz. However, the PAH load in soil samples decreased rapidly with the distance from the source of contamination in both locations. This suggests the need for another, redetermination of PAH concentration, but in a much larger area adjacent to the former or currently operating oil wells. The following hydrocarbons had the highest share in total PAHs: fluoranthene (31; 12%), naphthalene (27; 11%), phenanthrene (25; 10%), and the lowest: pyrene (6; 2%) (Fig. 2).

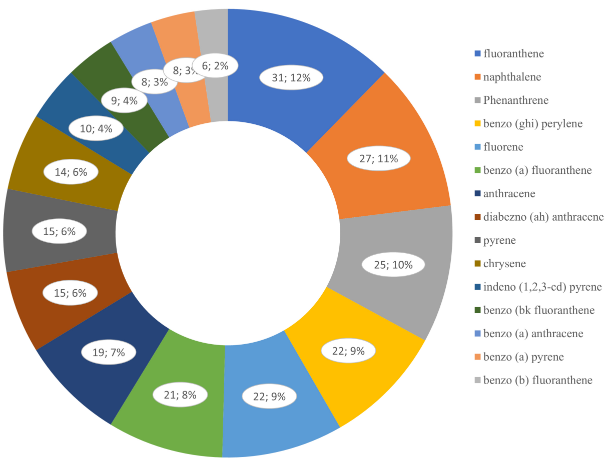

Fig. 2. The share of individual aromatic hydrocarbons in the total PAH content in soil, before the start of the research, in 2019.

The content of the following aromatic hydrocarbons (benzo (a) anthracene, benzo (a) pyrene, benzo (b) fluoranthene, benzo (bk-fluoranthene) did not differ significantly between localities. The content of the remaining aromatic hydrocarbons significantly depended on the research location (Table 5). The highest content of naphthalene (56 μg kg^{-1}) and benzo (ghi) perylene (43 μg kg^{-1}) was found in Brzezówka, naphthalene (43 μg kg^{-1}) and fluorene (31 μg kg^{-1}) were the highest in Chorkówka. Among the aromatic hydrocarbons in Folusz: phenanthrene (43 μg kg^{-1}), benzo (a) pyrene (31 μg kg^{-1}) and diabezno (ah) anthracene (25 μg kg^{-1}). In Krościenko, the closest to the oil wells, the highest concentration of chrysene (19 μg kg^{-1}), benzo (a) anthracene (19 μg kg^{-1}), benzo (a) fluoranthene (29 μg kg^{-1}), pyrene (28 μg kg^{-1}) and indeno (2,3-cd) pyrene (23 μg kg^{-1}) were determined. In Tarnowiec, the most numerous of all localities were fluorene (32) and benzo (b) fluoranthene (17 μg kg^{-1}), and in Wietrzno – diabezno (ah) anthracene (13 μg kg^{-1}). The content of individual polycyclic aromatic hydrocarbons was from 0.0 (in the case of indeno (1,2,3-cd) pyrene) to 9-times higher (in the case of benzo (bk) fluoranthene), than the permissible values of concentration of PAH. The smallest pollution with polycyclic aromatic hydrocarbons was found in Wietrzno and was on average 1.9 times higher, and the highest was in Krościenko – 3.3 times higher than the PAH pollution standard for class B soils.

Descriptive statistics on PAHs are presented in Table 6. Among the analyzed aromatic hydrocarbons, benzo (a) pyrene showed the greatest variability, while the sum of PAHs was the most stable. Kurtosis, as a relative measure of the concentration and flattening of a distribution, defines the distribution and concentration of values (collectives) close to the mean. The higher the kurtosis value, the more the community is concentrated around the mean value. Its low value gives the opposite effect, i.e., a greater dispersion of values, poor concentration and, consequently, a flattening of the abundance curve. For the normal distribution and the kurtosis value is assumed to be three, for values greater than three the distribution is slenderer, and for the smaller values it is more flattened. Skewness is a measure of the asymmetry of the observed results. The skewness coefficient above 'zero' indicates a right-hand asymmetry of the distribution, and the results below 'zero' indicate a left-hand asymmetry of the distribution. The most variable aromatic hydrogen was benzo (a) pyrene (V = 155%), and the most stable – benzo (a) fluoranthene (V = 20%).

After the end of the field experiment, the sum of PAHs in the soil decreased by an average of 42 μg kg^{-1}, compared to the initial state, which was on average over 19.0% of its initial content; this value depended on the locality and individual PAHs. The reduction in the amount of PAHs in soil ranged from 18 to 22%, depending on the locality. The greatest decrease in the value of this feature was recorded in Jedlicze, and the smallest in the village of Harklowa. The smallest decrease in the value was observed for anthracene (by 11.7%), and the greatest decrease in the content of hydrocarbons in the soil was observed for diabetic (ah) anthracene (by 29.7%). In the case of chrysene, benzo (a) anthracene, benzo (a) pyrene, benzo (b), fluoranthenobenzo (bkfluoranthene and indeno (1,2,3-cd) pyrene, no significant differences in PAH values were found between the localities found (Table 7).

The descriptive characteristics of PAHs concerning the results obtained after the completion of the field experiment are presented in Table 8. The sum of PAHs turned out to be the most stable of the assessed traits (V = 18%), while the most variable trait was benzo (a) pyrene (V = 156%). The obtained positive kurtosis indicated that there were more outliers in the data than in the normal distribution, while negative kurtosis indicated that there were fewer positive outliers in the data than in the normal distribution. Pearson's skewness coefficient describes to what extent the standard deviation of a given feature represents the difference between the arithmetic mean and the mode. The asymmetry coefficient turned out to be better than the third central moment and made it possible to compare the values for different distributions. Positive values of this coefficient indicate positive asymmetry, and negative values - negative asymmetry, and in the case of "0" values of the asymmetry coefficient we had a symmetrical distribution, indicating a situation in which the numbers of individual variants of a given feature were evenly distributed around the appropriate value. measure of central tendency (Table 8).

3.3 Green Technology as a Way of Cleaning the Environment from Petroleum Substances in Degraded Positions

The research material was reclamation plants, both legumes (*Fabaceae*) and grasses (*Poaceae*), which grow in areas damaged by industry in the Krosno and Jasło counties. Their frequency of appearance is indicated in Table 9. These data were used to assess the state of the environment in the Jasło and Krosno poviats. In addition, the possibilities of better use of the existing seed base of plants were identified, including the determination of the possibilities of increasing the level of use of the natural production potential of the region, thanks to environmental and other activities.

The most common species in the study area were red clover and white clover, each constituting 100% each of the species composition of the assessed sites (Fig. 3). Significant differences in soil cover were found between red clover and white clover and other species, and between Red fescue and French ryegrass. The differences between the French ryegrass and the Horn trefoil and between the French ryegrass and the Horn trefoil turned out to be homogeneous in terms of this feature (Fig. 3). Also, on the basis of the number of sites in which individual species occur, it can be concluded that the species of the *Fabaceae* family, especially Red clover (*T. pratense*) and White clover (*T. repens*) and were most frequently found in the studied areas (Table 9, Figs. 3,4d,e). In the conducted research, the horn trefoil covered 45% of the tested soil surface (Figs. 3,4b).

French ryegrass (*A. elatius*), also called European ryegrass, Haughty ryegrass, European pinworm – is a species of plant from the *Poaceae* family (Fig. 4c). Its share in soil cover averaged 48% (Fig. 3), i.e., was half less than that of legumes, which may result from its soil requirements and tolerance of this species to PAHs pollution.

In seven of the twelve studied sites, the presence of red fescue (*F. rubra* subsp. *trichophylla*) was found, which accounted for 60% of the total species composition (Figs. 3,4f, Table 9).

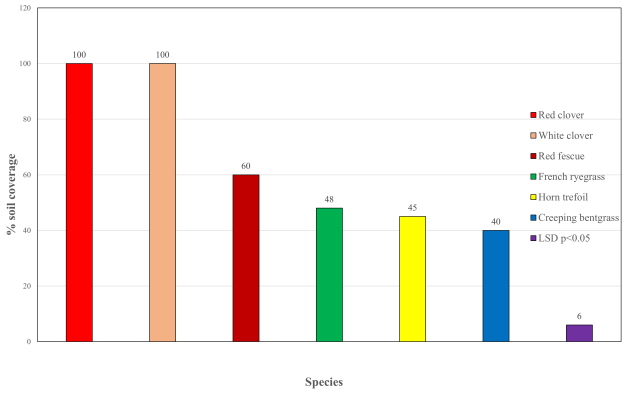

Fig. 3. The degree of soil coverage with phytomelioration plants in percent.

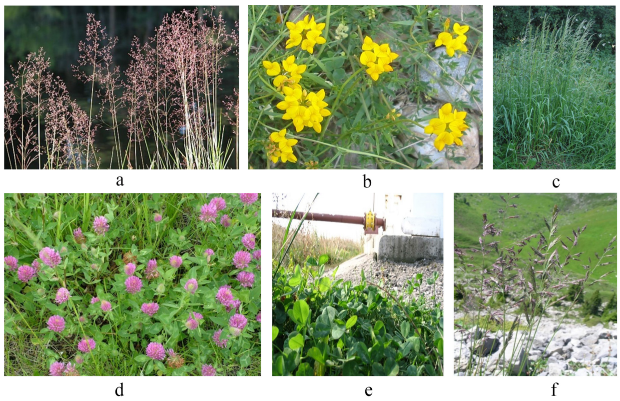

Fig. 4. The studied species of grasses and legumes. (a) creeping bentgrass (*A. stolonifera* L.), Harklowa, Skołyszyn commune, Jasło poviat. (b) Horn trefoil (*L. corniculatus* L.), Krościenko Wyżne commune, Krosno poviat. (c) French ryegrass (*A. elatius* L.), Węglówka, Korczyna commune, Krosno poviat. (d) Red clover (*T. pratense* L.), Samoklęski, Osiek Jasielski commune, Jasło poviat. (e) White clover (*T. repens* L.), Folusz, Dębowiec commune, Jasło poviat. (f) Red fescue (*F. rubra* L.), Harklowa, commune Skołoszyn; Source: own.

Creeping bentgrass (*A. stolonifera* L., f. *Poaceae*), as a native species, characteristic of floodplain grasslands (Fig. 4a), covered the soil with the least amount of vegetation, only in 40% (Table 9, Fig. 3).

3.4 The Fresh Mass of the Aboveground Parts of the Red Clover

The highest fresh and dry matter yield of red clover was obtained in the first harvest date, and the lowest in the last one. The total fresh weight (from three cuts) was 56.1 t·ha^{-1} and dry mass – 11.3 t·ha^{-1}. The highest yield of both fresh and dry matter of red clover was obtained in Jedlicze, on a slightly acidic soil, rich in available phosphorus, potassium and magnesium, and an average PAH content. The lowest fresh and dry matter yield of this species

Table 9. Occurrence of the studied species of white clover (*T. repens* L.), red clover (*T. pratense* L.), horn trefoil (*L. corniculatus* L.), French ryegrass (A. elatius [L.] P. Beauv.), of red fescue (*F. rubra* subsp. *trichophylla* L.) and creeping broom (*A. stolonifera* L.), at sites in individual localities, communes and counties (number of sites in each locality n = 12).

Locality	Community	Count
	White clover (*T. repens* L.)	
Harklowa, Folusz, Samoklęski, Roztoki, Brzezówka, Tarnowiec	Skołyszyn, Dębowiec, Osiek Jasielski, Tarnowiec	Jasło
Węglówka, Jedlicze, Krościenko, Równe, Wietrzno, Chorkówka	Korczyna, Jedlicze, Krościenko Wyżne, Dukla, Chorkówka	Krosno
	Red clover (*Trifolium pratense* L.)	
Harklowa, Folusz, Samoklęski, Roztoki, Brzezówka, Tarnowiec	Skołyszyn, Dębowiec, Osiek Jasielski, Tarnowiec	Jasło
Węglówka, Jedlicze, Krościenko, Równe, Wietrzno, Chorkówka	Korczyna, Jedlicze, Krościenko Wyżne, Dukla, Chorkówka	Krosno
	Horn trefoil (*L. corniculatus L.*)	
Folusz, Samoklęski, Roztoki, Osiek Jasielski, Tarnowiec	Dębowiec, Osiek Jasielski, Tarnowiec	Jasło
Jedlicze, Krościenko Wyżne, Równe, Jedlicze, Dukla	Jedlicze, Krościenko Wyżne, Dukla,	Krosno
	French ryegrass (*A. elatius* [L.] P. Beauv. Ex. Presl & C. Presl)	
Harklowa, Samoklęski, Brzezówka	Skołyszyn, Osiek Jasielski, Tarnowiec	Jasło
Węglówka, Krościenko, Chorkówka	Korczyna, Krościenko Wyżne, Chorkówka	Krosno
	Red fescue (*F. rubra subsp. Trichophylla* L.)	
Harklowa, Brzezówka, Tarnowiec	Skołoszyn, Tarnowiec	Jasło
Węglówka, Jedlicze, Wietrzno, Chorkówka	Korczyna, Jedlicze, Dukla, Chorkówka	Krosno
	Number of examined natural habitats of creeping bentgrass (A. stolonifera L.) in individual localities, communes and counties	
Harklowa, Folusz,	Skołyszyn, Dębowiec,	Jasło
Jedlicze, Równe, Wietrzno	Jedlicze, Dukla	Krosno

Source: own.

was obtained in the village of Chorkówka, on acidic soil, low abundance in available phosphorus and characterized by a high PAH content (Fig. 5).

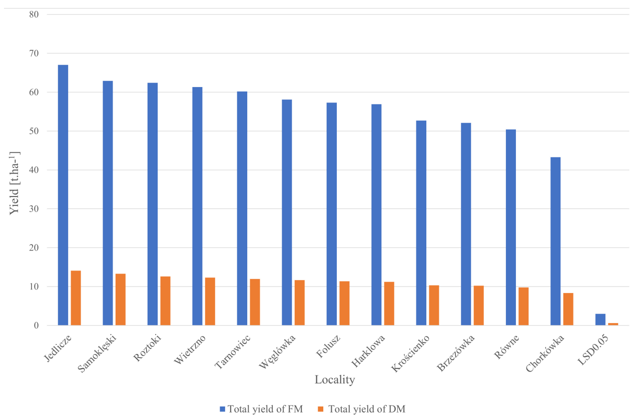

Fig. 5. Ranking of localities in terms of the yield of fresh and dry matter of red clover.

The yield of fresh and dry matter of red clover differed significantly in the dates of harvest. The highest value of this feature was obtained in the first term, and the lowest – in the last date of harvest, which results from the rate of physiological development of plants. Significantly, the highest yield was obtained in Jedlicze, and the lowest in Chorchówek, at all harvest times (Table 10). Homogeneous dry matter yield of red clover was obtained in the following villages: Roztoki, Samoklęski, Wietrzno and Tarnowiec; Węglówka, Folusz, Harklowa; Krościenko, Równe and Brzezówka (Table 10).

Table 11 presents the descriptive statistics of the fresh and dry weight of the above-ground parts of red clover collected in three cuts.

The first cut was characterized by the smallest variation in the dry weight of the above-ground parts, and the last cut for the red clover had the largest variation. However, the dispersion of the research results turned out to be the largest in the first harvest date. Most of the observed results were close to the left mean value. In other words, in our set of observations, there were more scores that were below or equal to the mean. Kurtosis informs how large the "spread" of the obtained results is, whether they are concentrated around the mean. A significant concentration of results around the mean occurs when the kurtosis value is above 0. We can then say that a significant part of the results/observations is similar to each other, and observations significantly different from the mean are few. If there is a weak concentration of the results around the mean (the kurtosis, then takes a value below 0) we can say that there is a sizable proportion of the results that are far away from the mean. Such a situation took place in the third harvest date (Table 11).

Table 10. The fresh and dry matter of the aboveground parts of the red clover (*T. pratense*) ($t \cdot ha^{-1}$).

No.	Location	Fresh mass				Dry matter			
		first cut	second cut	third cut	Mean	first cut	second cut	third cut	Mean
1	Brzezówka	20.7	17.3	14.1	17.4	4.3	3.6	3.0	3.6
2	Chorkówka	15.6	14.3	13.4	14.4	3.3	3.0	2.8	3.0
3	Folusz	21.1	20.5	15.7	19.1	4.3	4.1	3.2	3.9
4	Harklowa	20.6	19.4	16.9	19.0	4.1	3.9	3.4	3.8
5	Jedlicze	24.5	22.7	19.8	22.3	4.9	4.5	3.9	4.4
6	Krościenko	21.7	18.7	12.3	17.6	4.4	3.8	2.5	3.5
7	Równe	21.4	16.7	12.3	16.8	4.2	3.3	2.4	3.3
8	Roztoki	23.6	21.7	17.1	20.8	4.6	4.3	3.4	4.1
9	Samoklęski	24.1	21.0	17.8	21.0	4.7	4.1	3.5	4.1
10	Tarnowiec	23.4	20.3	16.5	20.1	4.6	4.0	3.2	3.9
11	Węglówka	22.8	19.5	15.8	19.4	4.4	3.8	3.1	3.8
12	Wietrzno	24.0	20.5	16.8	20.4	4.6	3.9	3.2	3.9
LSD$p_{\leq 0.05}$			ns*		4.0		ns		1.2
Mean		21.5	19.1	15.5	18.7	4.3	3.8	3.1	3.7
LSD$p_{\leq 0.05}$			1.0				0.3		

*not significant at $p_{\leq 0.05}$.

Table 11. Descriptive statistics of fresh and dry matter of red clover ($t \cdot ha^{-1}$).

Specification	First cut	Second cut	Third cut	Total fresh matter	First cut	Second cut	Third cut	Total dry matter
Mean	22.0	193.8	157.1	570.5	4.4	3.9	3.1	11.4
Median	22.3	199.0	161.5	577.0	4.4	3.9	3.2	11.5
Standard deviation	2.4	23.4	22.9	65.2	0.4	0.4	0.4	1.1
Kurtosis	0.4	0.7	–0.5	0.4	4.5	0.6	0.3	0.6
Skewness	–0.2	–0.9	–0.1	–0.6	–1.8	0.6	–0.1	0.4
Range	8.9	84.0	75.0	237.0	1.6	1.5	1.5	4.2
Minimum	156.0	143.0	123.0	433.0	3.3	3.0	2.4	9.1
Maximum	245.0	227.0	198.0	670.0	4.9	4.5	3.9	13.3
CV*	11.1	12.1	14.5	11.4	9.3	10.6	13.5	9.8

* Coefficient of variation (%).

3.5 Dependence of Dry Matter Content of Red Clover on Abiotic Factors

3.5.1 Dependence of Dry Weight of Red Clover on Physicochemical Properties of Soil

The largest negative relationship between the yield of dry matter of red clover (dependent variable y) and independent variables (x) was demonstrated with the content of organic carbon in the soil; with the content of humus in the soil; C:N ratio, of the abundance of calcium and magnesium in the soil, sum of exchangeable cations (S) and saturation of the sorption complex with basic cations (V). On the other hand, a significant positive relationship was found between the dry matter yield of *T. pratense* and the abundance of phosphorus in the soil and with the hydrolytic acidity of the soil. The remaining simple correlations between the independent variables are internal interrelationships (Table 12).

3.5.2 Dependence of Clover Dry Matter on the Content of PAHs

The relationships between the yield of *T. pratense* dry matter and the content of PAHs in the soil are presented in Table 13. The largest, negative relationship between the dry matter yield and naphthalene was (r = –0.68), and the other significant relationships were as follows: sum of PAHs (r = –0.43), phenanthrene (r = –0.38), fluoranthene (r = –0.45), benzo (a) pyrene (r = –0.44), fluorene (r= –0.20), pyrene (r = –0.32), benzo (b) fluoranthene (r = –0.33), benzo (bk) fluoranthene (r = –0.21) and indeno (1,2,3-cd) pyrene (r = –0.22). The remaining correlations concern internal intercorrelations.

3.6 Dependence of Physicochemical Properties of the Studied Soils on Petroleum Contamination

Correlations measure the degree of relationship between numerical or qualitative variables that can be logically ordered. The correlation coefficients shown in Fig. 6 can take values from –1 to +1. Positive correlation means that as the value of one feature increases, the value of the other increases, and the correlation coefficient of one means the strongest positive correlation. On the other hand, we can interpret the negative correlation in such a way that as the value of one feature increases, the value of the other decreases. A correlation coefficient of –1 indicates the strongest negative correlation. The value of the coefficient equal to 0 means that the variables are not related to each other in any way.

Table 12. Pearson's simple correlation coefficients between the yield of dry matter of red clover and the physicochemical and sorption properties.

	y	*x1*	*x2*	*x3*	*x4*	*x5*	*x6*	*x7*	*x8*	*x9*	*x10*	*x11*	*x12*	*x13*	*x14*	*x15*	*x16*
y	1.00																
x1	0.17	1.00															
x2	–0.26*	–0.57	1.00														
x3	–0.74**	–0.23	0.29*	1.00													
x4	0.17	0.80**	–0.37**	–0.29*	1.00												
x5	–0.52**	–0.71**	0.45**	0.74**	–0.85**	1.00											
x6	0.44**	0.19	–0.70**	–0.29*	0.06	–0.21*	1.00										
x7	0.01	–0.48**	0.22*	–0.05	–0.38**	0.28*	0.25*	1.00									
x8	–0.23*	0.49**	–0.22*	–0.13	0.42**	–0.37**	–0.39**	–0.25*	1.00								
x9	–0.20*	–0.63**	0.43**	–0.19	–0.63**	0.36**	0.06	0.23*	–0.69**	1.00							
x10	–0.34*	–0.22*	0.25*	0.21	–0.42**	0.41**	–0.15	0.33**	0.28*	–0.05	1.00						
x11	–0.37**	–0.18	0.19	0.22*	–0.35**	0.37**	–0.07	0.23*	0.25*	–0.01	0.95**	1.00					
x12	–0.02	–0.36**	0.13	–0.10	–0.45**	0.28*	0.20	0.43**	0.01	0.28*	0.77**	0.81**	1.00				
x13	0.17	0.01	–0.08	–0.35**	–0.22*	–0.04	–0.22*	–0.05	0.42**	0.18	0.08	0.07	0.04	1.00			
x14	–0.44**	–0.35**	0.02	0.48**	0.02	0.28*	0.09	0.12	–0.14	–0.24*	0.06	0.14	0.15	–0.66**	1.00		
x15	–0.12	–0.48**	0.03	0.15	–0.11	0.21	0.09	0.34**	–0.08	0.02	–0.09	–0.02	0.28*	–0.18	0.71**	1.00	
x16	–0.58**	–0.37**	0.02	0.49**	–0.16	0.41**	0.19	0.40**	–0.21*	0.02	0.15	0.27*	0.27*	–0.36**	0.75**	0.66**	1.00

y – yield of dry matter of red clover (t ha^{-1} DM); x1, pH in KCl; x2, humus (g kg^{-1}); x3, content of Corg (g kg^{-1}); x4, N total (g kg^{-1}); x5, C:N; x6, P_2O_5 (g kg^{-1}); x7, K_2O (g kg^{-1}); x8, Mg (g kg^{-1}); x9, Hydrolytic acidity (mmol H^+ kg^{-1}; x10, exchangeable calcium (Ca^{2+}); x11, exchangeable magnesium (Mg^{2+}); x12, removable sodium (Na^+); x13, exchangeable potassium (K +); x14, sum of exchangeable cations (S); x15, Soil sorption capacity (T); x16, Saturation of the sorption complex with basic cations (V); **significant at $p_{\leq 0.01}$, *significant at $p_{\leq 0.05}$.

Table 13. Pearson's simple correlation coefficients between the yield of dry matter of red clover and the content of PAHs and individual polycyclic aromatic hydrocarbons.

	y	*x1*	*x2*	*x3*	*x4*	*x5*	*x6*	*x7*	*x8*	*x9*	*x10*	*x11*	*x12*	*x13*	*x14*	*x15*	*x16*
y	1.00																
x1	–0.43**	1.00															
x2	–0.68**	0.82**	1.00														
x3	–0.38**	0.65**	0.49**	1.00													
x4	0.05	–0.10	–0.41**	0.02	1.00												
x5	–0.45**	0.40**	0.65**	0.18	–0.72**	1.00											
x6	0.06	0.16	–0.20	0.42**	0.81**	–0.71**	1.00										
x7	–0.15	0.59**	0.47**	0.41**	–0.06	–0.18	0.28*	1.00									
x8	–0.44**	0.52**	0.67**	0.39**	–0.76**	0.80**	–0.52**	0.22*	1.00								
x9	–0.12	0.44**	0.29*	0.10	–0.19	–0.05	–0.07	0.70**	0.06	1.00							
x10	0.01	–0.07	0.07	–0.49**	–0.53**	0.53**	–0.76**	–0.24*	0.30*	0.12	1.00						
x11	–0.20*	0.62**	0.38**	0.54**	0.17	0.25*	0.37**	–0.01	0.20*	–0.14	–0.17	1.00					
x12	–0.32*	0.39**	0.17	0.23*	0.39**	–0.14	0.35**	0.32*	–0.19	0.38**	–0.03	0.02	1.00				
x13	–0.33*	–0.28*	–0.44**	–0.51**	0.50**	–0.44	0.22*	–0.33*	–0.53**	–0.22*	–0.08	–0.22*	0.17	1.00			
x14	–0.21*	–0.16	–0.43**	–0.10	0.81**	–0.74**	0.69**	–0.04	–0.64**	–0.22*	–0.47**	0.19	–0.09	0.49**	1.00		
x15	–0.01	0.75**	0.50**	0.47**	–0.11	0.08	0.20*	0.71**	0.35**	0.57**	–0.39**	0.33*	0.09	–0.17	–0.05	1.00	
x16	–0.22*	0.58**	0.42**	0.14	–0.02	–0.11	0.20*	0.66**	–0.00	0.66**	–0.13	0.25*	0.03	–0.02	0.17	0.76**	1.00

y, yield of dry matter of red clover (t ha^{-1} dm); x1, sum of PAHs; x2, naphthalene; x3, phenanthrene; x4, anthracene; x5, fluoranthene; x6, chrysene; x7, benzo (a) anthracene; x8, benzo (a) pyrene; x9, benzo (a) fluoranthene; x10, benzo (ghi) perylene; x11, fluorene; x12, pyrene; x13, benzo (b) fluoranthene; x14, benzo (bk) fluoranthene; x15, diabezno (ah) anthracene; x16, indeno (1,2,3-cd) pyrene; **significant at $p_{\leq 0.01}$, *significant at $p_{\leq 0.05}$.

Fig. 6 shows the dependence of the physicochemical properties of soil on its contamination with polycyclic aromatic hydrocarbons. Most of the dependencies are in the range from 0.80 to –0.70. The greatest number of correlations between the examined features was found in the range from 0.5 to –0.5, and the least number of correlations close to 1.0 or –1.0 and also close to "0".

The acidity of the soil (pH in KCl) turned out to be most dependent on PAH impurities, on the content of fluoranthene (x21), benzo (a) pyrene (x24) and benzo (ghi) perylene, respectively (r = –0.65, –0.5, 0.63, 0.66). The content of humus, total nitrogen, available phosphorus (x7), and also the available Mg (x8), hydrolytic acidity (x9) and the sum of exchangeable cations (S) (x14) were not significantly associated with any of the aromatic hydrocarbons. The carbon to nitrogen ratio turned out to be positively related to the sum of PAHs (x17) and naphthalene soil contamination (x18). Exchangeable calcium (Ca^{2+}) (x10) and exchangeable magnesium (Mg^{2+}) (x11) were negative corelated to the benzo (a) fluoranthene content. Exchangeable sodium (Na^{+}) and exchangeable potassium (K^{+}) (x13) were turned out to be negatively correlated with the content of chrysene (x22) and benzo (a) anthracene (x23) in soil. Whereas of soil sorption capacity (T) (x15) and saturation of the sorption complex with basic cations (V) (x16) turned out to be correlated with fluoranthene (x21) and benzo (a) fluoranthene (x25) (Fig. 6).

4. Discussion

4.1 Soil Diversity

The studied soils of the Krosno Basin are diversified due to the varied topography and various rock material. Contamination of the soils of the Krosno and Jasło poviats with crude oil causes their degradation and transformation into rainfall and glial soils [23,27,28]. This is the reason for the creation of anaerobic (anaerobic) conditions and, consequently, the inhibition of gas exchange between the atmosphere and soil air [9,22]. The soil becomes saturated with gaseous hydrocarbons (methane, ethane, propane, butane). Rainwater does not freely penetrate the soil horizons, it stagnates. Excessive moisture and anaerobic environment intensify the processes of reduction of trivalent iron to divalent iron, which is manifested in soil lubrication. Petroleum kills small invertebrates, thus destroying the biological life of the soil. Only anaerobic bacteria live in such soil: hydrogen sulfide, hydrogen, methane and iron reducing bacteria (*Alteromonas*) [58]. Khatisashvili *et al*. [59] found that oil pipelines in Georgia pose a significant risk of soil contamination with petroleum hydrocarbons, which requires the development of a special ecological remediation and environmental protection technology. They were developed a strategy for the joint use of specially selected plants and microorganisms for phytoremediation of soils contaminated with petroleum hydrocarbons [60].

In the conducted research, plant species characterized by high resistance to PAHs were selected. According to Ziarati *et al*. [4] these species are also characterized by high activity of enzymes involved in the metabolism of hydrocarbons. It was also shown that thanks to the use of selected plant species, almost 20% of hydrocarbons were effectively removed from the soil, but despite this, the soil still contained a heavy fraction of PAH. Khatisashvili *et al*. [59] achieved over 25% reduction in PAHs in the soil under the influence of the technology used by him. The function of these plants can also be reduced to providing these areas with appropriate aesthetic and scenic values, protection against industrial pollution by creating special filters (belts, zones) of tall and low vegetation, initiating and stimulating the development of grass and legumes in degraded areas [1,58,61–64].

PAHs represent a group of environmental pollutants that are recognized as priorities in Europe as well as in all international organizations related to maintaining a clean environment and the ocean. In conducted study were identificated of fifteen basic compounds from the group of polycyclic aromatic hydrocarbons. Their highest concentration was found in the town of Krościenko, located in the immediate vicinity of the oil well (now a district of Krosno). The sum of PAHs presents in this soils samples was exceeding 3.3 times the limit values specified in the ordinance of the Minister of the Environment for soils from group B. This suggests the need to redefine the concentration of PAHs, but in a larger area adjacent to the mine in Krościenko. The current extraction is small and does not constitute a significant share in the region or the country [65,66]. Based on the details of WHO [67], where their estimated of background levels for soils without point sources or influence from traffic are less than 50–100 $\mu g \cdot kg^{-1}$ can bee conclude that those samples in an average degree were contaminated. The conducted research revealed significant relationships between the physicochemical features and soil contamination with polycyclic aromatic hydrocarbons. Most of these were negative, which confirms the negative impact of PAHs on soil quality and, consequently, soil water quality and plant condition. Only some soil characteristics, such as the content of humus, total nitrogen, available phosphorus, and also the available Mg, hydrolytic acidity and the sum of exchangeable cations (S) were not significantly associated with soil contamination with PAHs. These dependencies are confirmed by Ziarati *et al*. [4] and Khatisashvili *et al*. [59]. There is little research on this subject, so there is a need for research to monitor the state of soil contamination with PAHs, especially in vulnerable zones, and their impact on the state of the environment.

4.2 Advantages and Disadvantages of Phytoremediation Technique

Phytoremediation, also called "green remediation" or "green technology", the soil cleaning process is conducted

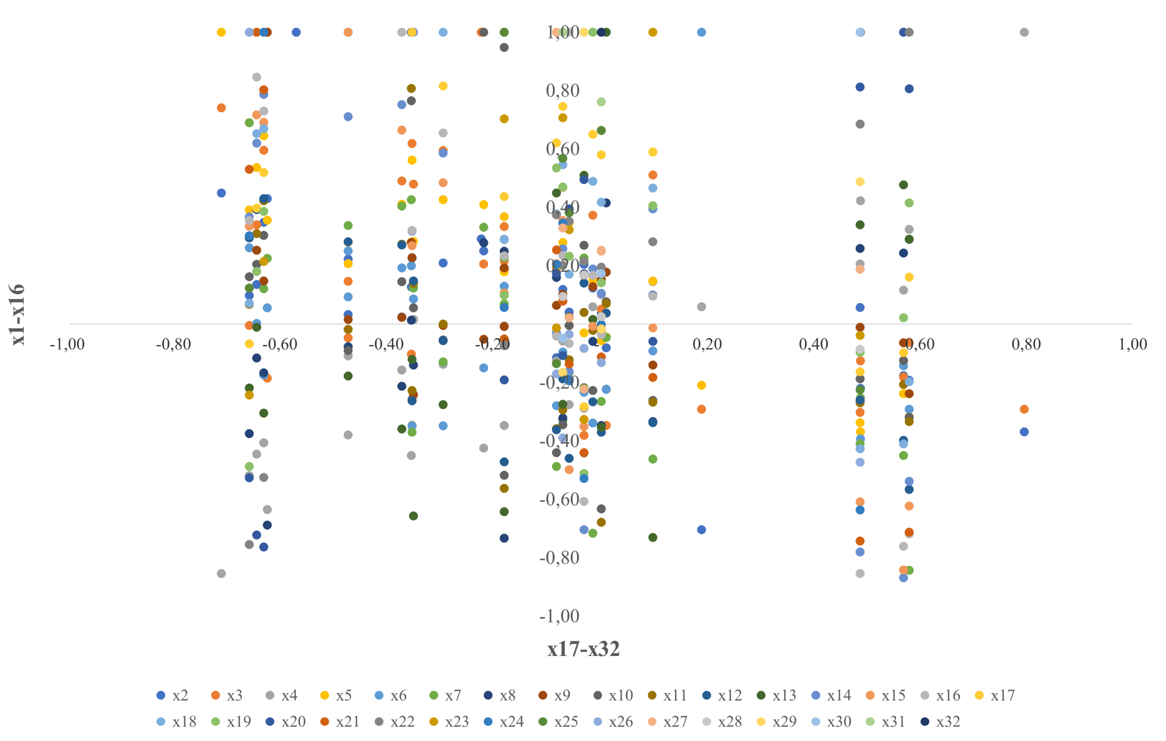

Fig. 6. Pearson's simple correlation coefficients between soil variables. x1 - pH in KCl; x2 - humus (g kg^{-1}; x3 content of Corg (g kg^{-1}); x4 -N total (g kg^{-1}); x5 - C:N; x6 - P_2O_5 (g kg^{-1}); x7 - K_2O (g kg^{-1}); x8 - Mg (g kg^{-1}); x9 - Hydrolytic acidity (mmol H^+ kg^{-1}; x10 - exchangeable calcium (Ca^{2+}); x11 - exchangeable magnesium (Mg^{2+}); x12 - removable sodium (Na^+); x13 - exchangeable potassium (K +); x14 - sum of exchangeable cations (S); x15 - Soil sorption capacity (T); x16 - Saturation of the sorption complex with basic cations (V); x17 – sum of PAHs; x18 – naphthalene, x19 – phenanthrene; x20 – anthracene, x21 – fluoranthene, x22 – chrysene, x23 – benzo (a) anthracene, x24 – benzo (a) pyrene, x25 – benzo (a) fluoranthene, x26 – benzo (ghi) perylene, x27 – fluorene, x28 – pyrene, x29 – benzo (b) fluoranthene, x30 – benzo (bk) fluoranthene, x31 – diabezno (ah) anthracene, x32 – indeno (1,2,3-cd) pyrene.

by green plants, but also because phytoremediation techniques are environmentally friendly [58]. Phytoremediation methods can be used as separate technologies, but also as a supplement to traditional methods of soil treatment and reclamation of degraded areas [64]. Contrary to traditional methods, no additional extracting compounds are used in the phytoremediation process, which means a lower amount of secondary pollution and a reduction of landfilled waste [58,68,69].

In the conducted research, high soil coverage *T. repens, T. pratense, L. corniculatus* on soils contaminated with petroleum substances was high (100%, 100% and 48%, respectively) and thus the soil could be enriched with nitrogen, which improved its structure, as a result of which these species left a good forecrop for successive plants. In the opinion of Sui *et al.* [58] these plants play an influential role in increasing the fertility and efficiency of the soil, thanks to their large root mass. Moreover, *Rhizobium leguminosarum* biotype *Trifolii*, which live with the root system of both clover species, can bind from 40–700 kg N·ha^{-1} annually [70].

L. corniculatus L. is a species commonly known and found in the wild throughout Poland. In the conducted research, it covered 45% of the soil surface. It is resistant to biting and trampling. It is also frost-resistant, because without snow cover it can withstand air temperatures down to – 25 °C. It works well in poorer soils, but requires more moisture in the substrate [58,70]. The legume's species tolerated the most unfavorable environmental conditions at most of the research sites. They were the most resistant to harmful substances in the soil. Therefore, it can be concluded that due to the presence of the tested species, the process of colonization of the soil by nitrifying bacteria and other bacteria may take place, which positively affects the improvement of soil quality. In order to make them a resilient and reliable tool to meet the new challenges in the field of climate change, to close the yield gap and to recover the soil that society has lost due to contamination with oil derivatives.

European ryegrass (*A. elatius*, *Poaceae* family) is a phytophilous species and, as it turns out, it quickly grows on the surface covered with other plants, which seriously limits their development possibilities, besides, it quickly grows on any soil [8]. This species is characteristic of the ryegrass meadows of the *A. elatioris* association. Its share in soil cover averaged 48%. This species starts vegetation early (in the air temperature of 3–4 °C), it may be damaged by frosts; in mild winters, the plants remain green, in harsh, snowless winters, they can freeze. On the other hand, in dry summers, after flowering, the above-ground parts of plants may die early. Hence, this species gave significantly lower soil cover than both species of clover [8,11].

F. rubra subsp. *Trichophylla*, family *Poaceae,* accounted for 60% of the total species composition of the studied sites in the area of the former and active crude oil extraction areas. A characteristic feature of this species is the high tillering capacity, i.e., the release of numerous blades from the so-called tillering nodes in the lower parts of the shoot, thanks to which *F. rubra* maintains well in meadow or pasture sward. *F. rubra* subsp. *trichophylla* is also characterized by low soil requirements, therefore it tolerates well the unfavorable climatic and soil conditions of the studied region and contributes to filling gaps in the turf after drying out of other grass species. Moreover, it is characterized by a high self-regeneration ability of sward, which is important in areas degraded by the refining industry [4,8]. According to Różański [21,23], it is suitable for the management of PAH contaminated areas, as oil residues do not inhibit the growth of above-ground parts of F. rubra.

Creeping bentgrass (*A. stolonifera* L., family *Poaceae*), as a native species, is found it occurs throughout the country. It is a perennial tuft grass, blooming from June to August, inhabiting medium-fertile soils, mainly wet meadows and pastures, and is considered a species characteristic of floodplain grasslands. Therefore, its share in the soil cover was only 40%. A. stolonifera turned out to be the most demanding of the species studied, hence its occurrence was clearly lower [4,8,21]. This species is not very resistant to the lack of water [11,59].

Ziarati *et al*. [4] believe that the factor limiting phytoremediation is the possible negative impact of PAHs on the biology of plants used for soil treatment. This research has shown that the effect of PAHs on plant genes deserves serious attention to prevent a decline in species diversity in nature.

Each of these techniques of "phytoremediation" is based on the use of one of the four types of plant physiological responses to the presence of a pollutant in the environment: accumulation, hyperaccumulation, indication and exclusion. An important criterion for assessing phytoremediation methods is the type of remediated matrix and the type of contamination [71], where plants serve as a tool for carrying, removing, degrading and stabilizing pollutants in soil, sediment and water [63]. Phytoremediation, especially phytoextraction, phytostabilization and Phyto transformation are methods of *in situ* decomposition of hydrocarbons using plants capable of functioning on contaminated soil [61]. Biological treatment of the environment is much cheaper and safer compared to physicochemical methods and gives incredibly reliable results of soil contamination decomposition, especially with multi-ring aromatic hydrocarbons. Ziarati *et al*. [4] obtained the best results in the bioremediation process using monocotyledonous plants and inoculants of mycorrhizal fungi isolated from contaminated soil. The symbiosis of mycorrhizal fungi with monocotyledons resulted in the removal about 40% of WWA, compared to the samples in which the plants were not inoculated with mycorrhizal fungi [31]. Phytoremediation plays an integral role in reducing risk on future.

The dependence of plant vegetation on the climate is also a serious limitation in the application of phytoremediation techniques. The effectiveness of soil treatment may decrease due to damage to plants during the growing season, e.g., due to extreme weather conditions, e.g., drought or flooding. The plant species that have adapted to life in extreme conditions, in the soil saturated with crude oil or petroleum products are the naphthophytes. Anaerobic conditions in the soil inhibit the growth of bacteria that degrade petroleum hydrocarbons [21–23].

According to Kubińska [72], phytoremediation can be used as an independent treatment, as well as a complementary element to other strategies. Due to the dense root system, both legumes and grasses additionally protect degraded soils against erosion, which is a natural process caused by weather conditions, but is intensified by anthropogenic activities [63]. The reduction of surface erosion processes reduces the risk of pollutants entering the water and atmosphere [68,69]. The use of phytoremediation techniques does not require the use of professional equipment or specialized personnel. Its advantage is economic profitability [64]. The costs of the treatment of soil loaded with petroleum substances, both at the level of planning and subsequent practical implementation, are many times lower than the financial expenditure necessary to conduct treatment with classical physicochemical methods [71]. It is estimated that cleaning one hectare of the surface layer of soil (up to approx. 50 cm) would cost around forty thousand. dollars: the cost is spread over 30 years of the process. For comparison, the cost of using physicochemical techniques can reach up to $2 million [2,15,73].

The European Union Commission has published Regulation 2020/1255 of September 7, 2020, amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons (PAHs) in traditionally smoked meats and its products and in traditionally smoked fish and its products, as well as on the establishment of a maximum level for PAH in powdered food of plant origin used in the preparation of beverages. As a result, the provisions on the maximum permissible levels of polycyclic aromatic hydrocarbons (PAHs) were amended and certain exemptions were allowed for some Member States, including Poland [26]. This allows you to adapt to the applicable standards.

Despite its many advantages, phytoremediation also has a number of limitations. The main disadvantage is the long duration of the entire process, which, depending on the degree of contamination and the plant species used, may take from several to several years before the concentration of heavy metals or petroleum substances accumulated in the soil reaches an acceptable level [4,73]. The speed of their removal is influenced by the type of soil and its reaction, the right amount of nutrients and water, the length of the growing season, the type of plant, and the chosen method. The longest phytoremediation process is conducted using continuous phytoextraction. Most of the plants used are annual or biennial herbaceous plants, which are characterized by a slight increase in biomass during the growing season [68]. The limited photoprotection of petroleum substances and heavy metals is also a problem in the use of phytoextraction. Natural and synthetic chelating compounds, such as EDTA, seem to be the solution, but improper use of this type of substance may lead to contamination of neighboring areas and waters [4]. Therefore, an important aspect when conducting assisted phytoextraction is the constant monitoring of the treated area. Moreover, plant biomass additionally contaminated with heavy metals is a highly toxic waste, even after thermal management [64,74]. A significant limitation of all phytoremediation techniques is the depth of root penetration, solubility and availability of contaminants [63,74]. A similar problem is the movement of heavy metals from the soil to another environmental matrix, namely to the plants growing there or to the atmosphere. Vegetation contaminated with heavy metals and petroleum derivatives is not only a threat resulting from direct contact, but also the possibility of including the removed pollutants into the food chain [1,2,4,63]. Phytoulating is considered the riskiest method, because the pollutants removed during this process penetrate the atmosphere and, despite the less toxic form, still pose a serious threat to the environment [68,69,75]. When using a given phytoremediation technique, the introduction of unsuitable invasive plants should be avoided, as alien species may disturb the local biological diversity [63,68,75]. Therefore, the functions of areas treated with phytoextraction should be limited to a minimum, it is unacceptable, for example, to use such an area as a pasture [2,71].

4.3 Factors Determining the Effectiveness of Bioremediation Processes

The way a species acts on pollutants depends on the type of pollutant, the type, family and class of plants, and on abiotic factors. Some species have the ability to accumulate xenobiotics because they incorporate them into their own cells, while others are able to incorporate them into their metabolic pathway [4]. Thanks to special substances secreted by the roots, plants can immobilize harmful substances and limit their migration into the soil profile [1,2,68]. The dependence of plant growth on the climate and their seasonality also constitutes a serious limitation in the use of phytoremediation techniques. The effectiveness of the soil treatment process may decrease as a result of damage to plants during the growing season, e.g., as a result of extreme weather conditions or the appearance of pests or diseases.

The efficiency of bioremediation of soils from crude oil and its derivatives depends on the rate of decomposition of these pollutants by soil microorganisms, which are influenced by such factors as: chemical structure, concentration of hydrocarbons and their toxicity in relation to the microflora, microbiological potential of soil (biomass concentration, population diversity, enzyme activity), physicochemical environmental parameters (including reaction, air and soil temperature, organic matter content, humidity) and the availability of hydrocarbons for microbial cells [24,45,59–61,76,77].

The bioavailability of petroleum hydrocarbons for microbial cells depends on various physical, chemical and microbiological factors that affect both the transport of these compounds and the migration of microorganisms in the soil. Poorly soluble aliphatic and aromatic hydrocarbons with four or more rings are easily adsorbed on soil particles [24]. Due to the well-developed and negatively charged surface, soil particles, organic matter and clay, may also bind microorganisms with hydrophobic cell sheaths [78].

Microorganisms that degrade petroleum hydrocarbons produce surfactants [24,79]. The proper course of the biodegradation process, in the opinion El-Said [79] can lead to an almost 100% reduction of pollutants in just a few weeks. At present, removing light pollutants from soils, such as diesel fuel, gasoline, kerosene, is not an issue. The problem appears in the case of bioremediation of heavy soils from pure oil [2,13]. The bioavailability of petroleum hydrocarbons for microbial cells depends on various physical, chemical and microbiological factors that affect both the transport of these compounds and the migration of microorganisms in the soil. Poorly soluble aliphatic and aromatic hydrocarbons with four or more rings are easily adsorbed on soil particles [80,81]. Chemotaxis plays a key role in directing bacteria towards petroleum hydrocarbons and other petroleum pollutants that have been sorbed on soil particles [24]. Due to the well-developed and negatively charged surface, soil particles, organic matter and clay, may also bind microorganisms with hydrophobic cell sheaths [78].

4.4 Oil Pollution of the Natural Environment

Extraction, transport, industrial processing as well as the use of crude oil and petroleum products are associated with increasing pollution of the natural environment. Crude oil drilling, production and transport, including floods, are a serious and particularly dangerous factor that massively distributes petroleum pollutants in nature [50,59,82]. These substances are introduced into the soil layers either as a result of deliberate inadequate discharge by petrochemical plants or unintentionally as a result of tank, well, pipeline failure. Crude oil initially covers the plants and topsoil. Its migration into the soil depends on the physical, chemical and biological properties of the soil, the type of soil-covering phytocoenoses and the intensity of atmospheric precipitation. Polycyclic aromatic hydrocarbons form different structures in which the benzene rings assume different mutual positions. Some PAH particles have characteristic regions called the K region (outer edge of the phenanthrene ring) and the M region (opposing atoms of the anthracene structure) [78,79,82].

Petroleum pollutants accumulate in humus particles and in the cavities and capillaries of the soil of A0, A1, B and R levels chloro- and fluor derivatives. Crude oil also moves along the soil formations crossing the dense glial layer. If, on the other hand, the G level contains coarse sand and stones, then it becomes permeable to all hydrocarbons [2]. According to Sęk *et al.* [82], petroleum pollutants also have a significant impact on the geological and engineering properties of soil. They cause modification of grain size and porosity, increase of soil compressibility and reduction of plasticity, which in turn causes shrinkage or swelling and, as a result, a reduction in the strength parameters of the soil [82,83].

Oil pollution is one of the most important environmental problems affecting both terrestrial and aquatic environments. Currently, about 80% of the land contains petroleum products, i.e., hydrocarbons, and these products are used in the oil and chemical industry as a source of energy [2]. Oil forms a film on the soil surface and traps carbon dioxide produced by soil organisms. It also reduces soil porosity by sticking soil particles, and disturbs its water, air and thermal properties. As a result, the food chain is contaminated with petroleum substances. The number of losses depends on the amount and type of the spilled oil [2]. Crude oil components, especially long-chain hydrocarbons, are very stable and not biodegradable; so, they stay in the environment for a long time and are difficult to remove.

Phytoremediation (phytoextraction, phytostabilization, phytotransformation) is a method of *in situ* decomposition of hydrocarbons using plants capable of growing on contaminated soil. Based on the own research on the methods of soil purification from petroleum substances, it can be suggested that biological methods, compared to physicochemical methods, are cheaper, safer and give better results of decomposition of contaminated soils, especially polycyclic aromatic hydrocarbons. This is confirmed by the other research results [2,4,12,59].

4.5 The Yielding Potential of Red Clover under Abiotic Stressful Conditions

For centuries, red clover has played a key role as a supplier of reactive nitrogen to farming systems and as a valuable animal feed. Today, it is valued not only for its good nutritional properties for ruminants, but for reducing the need for nitrogen fertilizers and for the properties reducing hydrocarbons in the environment. The yielding potential of this species was compared by Gaudin *et al.* [84]. Diversification of the crop sequence using perennial legumes is encouraged as part of the solution to improve the resistance of the field crop system to multiple environmental stresses and to improve the sustainable use of nitrogen resources. The influence of cover crops is related to the ability of the soil to function, this enables the improvement of the water and nutrient supply for agro-ecological functions. These ground cover plants affect the nitrogen cycle, reduce soil erosion, water run-off and soil loss during heavy rainfall, increase organic matter and soil fertility, inhibit weed growth, improve soil structure and water retention, provide suitable habitats for beneficial predatory insects and function as non-hosts for nematodes and other pests in rotation [84,85]. Clavin *et al.* [85] at a rainfall level of 743–1066 mm, during the growing season, they obtained as many as four cuts of red clover with a total yield of 14.8–15.0 tha^{-1} dry matter. Liatukas and Bukauskaitë [86] on Endocalcari-Endohypogleyic Cambisols, at pH = 7.2–7.3, obtained a higher yielding potential of red clover within the limits of 16.6–17.8 tha^{-1}. In the conditions of central-eastern Poland, Przybylska *et al.* [87], with lower rainfall below 240 mm during the growing season, obtained a dry matter yield of clover, within the range of 9.52–12.40 tha^{-1}.

Particularly good survival of this species (100%) in the conditions of soils contaminated with polycyclic aromatic hydrocarbons, as well as a high yield under the stressful conditions of this experiment, should be due to the genetic characteristics and the content of polyphenol oxidase (PPO) in of the leaves of red clover. It is an important economic trait of this species that improves livestock production, while reducing the environmental impact of this industry. While PPO is not needed for the normal growth and development of red clover plants, it protects the plants from pathogens, resulting in a cleaner environment. According to Boeckx *et al.* [88] and of Przybylska *et al.* [87] red clover possesses a large family of PPO genes, including one allelic gene that regulates most of the PPO activity of both leaves and nodules in healthy tissue. PPO mediates the oxidation of phenols and diphenols to quinones, which are readily reactive with nucleophilic binding sites. Such binding sites can be found on proteins, resulting in the formation of protein-bound phenols [88]. Knowledge on this subject is of immense importance for the use of PPO in lipid protection. The cultivar, genotype, age of leaves, and abiotic and biotic stresses alter the PPO activity of the leaves of *T. pratense*. Understanding what controls PPO gene expression and its enzymatic activity will allow use this species in phytoremediation as well as in seed production to be adapted to the current needs.

In the conducted research, the dry matter yield of red clover turned out to be significantly negatively related to the sum of PAHs and the majority of polycyclic aromatic hydrocarbons as well as to selected physicochemical properties of the soil, such as: the content of humus and organic carbon in the soil, C:N ratio, soil abundance in calcium and magnesium, the sum of exchangeable cations (S) and the saturation of the sorption complex with basic cations (V), and positively with the abundance of available phosphorus in the soil and with the hydrolytic acidity of the soil. These are innovative results that will allow to adapt this species to the phytoremediation of soils contaminated with polycyclic aromatic hydrocarbons.

4.6 Soil Contamination

The CIEP 2020 report [1] indicates that soil contamination in Poland is usually of a permanent nature and its rapid cleaning should not be expected. Areas where soil contamination has been found should be excluded from food production, which protects the environment and humans against the displacement of contaminants. Restoration and/or cultivation of industrial plants as part of the so-called phytoremediation and non-food agriculture should lead to the gradual cleansing of brownfields.

Bioremediation of crude oil from soil, consisting in the use of living organisms (bacteria, fungi and plants) for the degradation of harmful substances present in the environment in the region of South-Eastern Poland, turned out to be an ecological solution. However, the effectiveness of this method, according to Ahmad *et al*. [2] depends on the concentration of hydrocarbons, soil properties and pollutant composition.

PAHs, as compounds occurring in nature in various forms and structures, are environmentally incredibly important due to their strong genotoxic, mutagenic and carcinogenic properties [39,61]. Polycyclic hydrocarbons are the most resistant and toxic group of soil pollutants present in crude oil. PAHs become trapped in the pores of the soil after they penetrate into the soil and are retained by the soil matrix. Therefore, their removal from the soil is difficult [2,89]. Bioremediation is the most appropriate method of removing PAHs from soil as microbes and plant roots have easy access to these tiny pores.

The method of dealing with contaminated soils depends on the current and planned use, the type of contamination and the available technology. Cleaning up polluted sites to enable their re-use without harming future users requires the implementation of environmentally friendly technologies that are economically attractive at the same time. Phytoremediation may prove to be an effective method of soil cleaning at lower pollutant concentrations. This method is one of the biological methods of soil cleaning. It uses the ability of some plant species to collect pollutants from the soil and accumulate in their tissues in an amount up to a hundred times greater than that found in the tissues of other plants. Phytoremediation is considered to be the cheapest method of soil purification and the most environmentally friendly. It can be used in the form of phytoextraction, in which plants absorbing hydrocarbons or heavy metals are used to transport these unfavorable residues from the soil and their bioaccumulation in the above-ground parts, the pollutants are therefore carried away with the yield of plants [4,75,89].

However, further efforts are needed to incorporate phytoremediation processes, including phytoremediation and photooxidation, to forecast pollution and the fate of petroleum pollutants, not only on a field scale, but also on a macro scale. Environmental legislation has significantly reduced the frequency of new point source pollution problems; however, the rehabilitation of many older sources of pollution remains a current challenge.

In 2021, the CAP Commission updated the Soil Protection Strategy to address soil degradation comprehensively and contribute to achieving land degradation neutrality by 2030. The new Action Plan for the Elimination of Water, Air and Soil Pollution deals in particular with soil contamination and remediation of contaminated of places. On industrial emissions, an integrated prevention approach is foreseen emissions to air, water and soil and for their control, for waste management, for energy efficiency [90].

5. Conclusions

The neutralization of harmful pollutants can be performed through the use of a variety of physical and chemical methods; however, full recreation of the natural environment conditions is achieved only by using the achievements of biological and biotechnological technologies. The biological treatment methods are cheaper, easier to use and more effective, and the reclaimed soils exhibit properties similar to non-polluted soils. Due to the random selection of soil sampling areas, it is necessary to consider the need to conduct further, more precise research and develop an appropriate systematic sampling scheme. As a result, a detailed map of organic compounds contamination of the area of Krosno and Jasło poviats should be created.

The physicochemical parameters of the environment determined the effectiveness of *in situ* bioremediation of soils contaminated with petroleum products. The degree of contamination of the soils had a significant impact on the growth and yield of crops.

“Green technology” is particularly beneficial, especially the phytoextraction technique, in which plants clean the soil of excess petroleum products, prevent its further movement from the site of contamination and prevent erosion of reclaimed soil. In the years 2019–2021, the content of PAHs in the soil decreased by about 19%. Perennial legumes plants and grasses belong to plants that grow well on the ground of soils damaged by the oil industry, because they tolerate unfavorable soil conditions exceptionally well.

The development and yield of dry matter of red clover turned out to be significantly dependent on abiotic factors. This characteristic was negatively related to the content of organic carbon in the soil; with the C:N ratio, the abundance of calcium and magnesium in the soil, sum of exchangeable cations (S), saturation of the sorption complex with basic cations (V), sum of PAHs, naphthalene, phenanthrene, fluoranthene benzo (a) pyrene, benzo (b) fluoranthene, benzo (bk) fluoranthene and indenol (1,2,3-cd) pyrene.

Plant species adapted to the type of soil in South-Eastern Poland, characterized by high resistance to petroleum hydrocarbons, were selected. The species resistant to unfavorable conditions of the soil environment in the area of the former and present petroleum industry included: white clover (*T. repens* L.), red clover (*T. pratense* L.), horn trefoil (*L. cornicultus* L.), creeping bentgrass (*A. stolonifera* L.), running red fescue (*F. rubra* subsp. *trichophylla*), French ryegrass (*A. elatius* L.). These species performed their tasks very well, therefore they can be recommended for use as a factor counteracting the degradation of the natural environment.

The vegetation cover ensures a positive course of plant succession and the intensive development of bioecological processes, and the plants analyzed in the research prepare post-exploitation areas unsuitable for agricultural use. Sowing reclamation vegetation in areas degraded by the oil industry should initiate turf processes, and the emerging surface root zone can successfully protect such soil against erosion. "Green technology" can be used in regions contaminated with PAHs to regain food potential and open up the possibility of cleaning up endangered areas.

Abbreviations

A. elatius, *Arrhenatherum elatius*; *A. stolonifera*, *Agrostis stolonifera*; *F. rubra*, *Festuca rubra*; *L. corniculatus*, *Lotus corniculatus*; PAHs, Polycyclic Aromatic Hydrocarbons; *T. pratense*, *Trifolium pratense*; *T. repens*, *Trifolium repens*.

Author Contributions

BS, VV, BK-M and DS designed the research study. BS, BK-M and PP performed the research. PB, MM, AKF and PP provided help and advice on methodology. AKF analyzed the data. BS, BK-M, MM, PB, DS and VV wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

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Conflict of Interest

The authors declare no conflict of interest.

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