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Original Research

The Effect of Petroleum-Derived Substances on the Growth and Chemical Composition of *Vicia faba* L.

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Abstract

Petroleum and petroleum-derived products can contribute to the inhibition of growth and development of plants. Soil contamination by these compounds either hampers or makes difficult the intake of water and mineral salts from the substrate, and also breaks a number of metabolic processes. As a result of the deficiency of chlorophyll and nutrients, the affected plants grow deformed roots, shoots, leaves, and flowers, and show numerous chloroses and necroses.

The objective of our research was to determine the effects of petrol, used engine oil, and diesel oil on select morphological parameters of *Vicia faba* L., and on the content of select nutrients and heavy metals in the leaves of plants. The effect of the bioremediation process initiated by biopreparation ZB-1 on the above-mentioned parameters was also determined. The analyzed plants were obtained from the Experimental Station of the University of Agriculture in Cracow, located in Mydlniki near Cracow. In June 2010, the soil placed in special containers was contaminated with petroleum-derived substances at a quantity of 6,000 mg per 1 kg of dry mass, by pouring it on the soil. Half of the objects were subjected to bioremediation. The evaluation of the morphology of plants and the structural elements of yield was made at the stage of technological maturity of broad bean seeds. The evaluation of heavy metals content was conducted using flame absorption spectrometry. The carbon, nitrogen, and sulphur content was determined in a Variomax CNS analyzer. The results of the experiment showed that diesel oil had the most adverse effects on the morphological parameters of plants. Engine oil caused a decrease in the nitrogen, carbon, and sulfur contents in the leaves of plants, but on the other hand this substance, like other petroleum-derived compounds, increased the content of most heavy metals. The effect of biopreparation was diverse and depended on the type of substance and on the analyzed parameter.

The conducted experiment showed that petroleum-derived substances continued to adversely affect the growth of plants even three years after soil contamination. Additionally, it increased the accumulation of harmful substances in the organs of plants which could, in turn, seriously impact the economy and human health. However, the intensity of the impact depends on the type of derivative. Bioremediation supported by the use of microorganisms is an advantageous solution that permits the improving of the growth parameters of plants, as well as offsetting the harmful effects of petroleum-derived products upon the majority of the analyzed elements.

Keywords: petroleum-derived substances, macronutrients, heavy metals, bioremediation

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Introduction

At present, petroleum and its derivatives are used primarily to generate energy, and to produce fuels and greases, as well as many other products such as medicines, pigments, cosmetics, fertilizers, and explosives. The intensive development of urbanization and mechanisation is associated with increases in the contamination of the environment by petroleum and petroleum-derived products. It is a matter of grave concern, primarily because of the acute toxicity of these compounds, the rate of their spread, and their specific complexity. The components of petroleum, chiefly hydrocarbons with long carbon chains, do not undergo degradation, are extremely durable and stable, and thus remain long in the environment and are very difficult to remove [1, 2].

The contamination of soil by petroleum-derived substances results in far-reaching physical, chemical, and biological changes that are most clearly visible in the health status of plants [3-5]. Very often the contaminants contribute to alterations in the content of macro- and microelements in plant organs [6, 7], and also modify the content of heavy metals in them [8], but published studies on this topic are scarce.

Heavy metals (trace elements) play a significant role in metabolic processes in all living organisms. Some of them are essential for the normal growth and development of plants (copper, cobalt, nickel, zinc, chromium, and manganese) because they take part in many enzymatic reactions. However, among trace elements there are heavy metals such as cadmium, lead, and mercury, which may be toxic to cultivated plants even in low concentrations [9-12]. They are responsible for upsetting water management, photosynthesis, and other biochemical processes, and for damaging the root systems in plants [13]. Many of them have carcinogenic and mutagenic effects [14]. Cadmium, lead, and zinc are particularly dangerous as they can cause the degradation of the DNA and RNA structures, and disturb the synthesis of these compounds. The effects involve the impairment of the growth and reproduction of plants. Furthermore, they are often capable of bioaccumulation, and can remain in the environment for a long period and enter into subsequent levels of the trophic chain with ingested food [15-17]. In animals as well as in humans, heavy metals primarily cause changes in protein synthesis and disturb ATP production, which can result in dire pathological effects, including neoplasms [18].

They may also cause stress reactions in plants, which depend on the developmental stage of a plant, its genotype, and on the kind and concentration of the given heavy metal [19-21]. The uptake of heavy metals by plants most often occurs via the root system, but also via leaf blades. The most easily taken up from the soil are metals that occur in the form of free ions, whereas metals occurring in the form of complexes can be mobilised by active substances released by roots and then taken up by plants [22].

Bioremediation – using living organisms to remove harmful xenobiotics from soils – is one of the most advantageous methods for cleaning up petroleum-derived substances. It presents an alternative to conventional clean-up methods, being also much cheaper and simpler because it enables the decontamination of soil at the same place where it was contaminated. The ability to decompose petroleum-derived products is shown, e.g., by the bacteria of *Pseudomonas*, *Arthrobacter*, *Acinetobacter*, *Nocardia*, *Corynebacterium*, *Geobacillus*, and *Mycobacterium* genera, and by fungi of the *Candida* genus. Five months after the contamination incident, bioremediation using these microorganisms may result in the degradation of petroleum-derived hydrocarbons by 50%, and also in reducing the aromatic compounds content in the soil by nearly 65% [2].

In order to limit the deleterious effects of petroleum-derived substances on plants, and particularly of their most dangerous components (polycyclic aromatic hydrocarbons, or PAHs), various techniques of supported bioremediation (biostimulation, supplementation with compost, bentonite, calcium oxide, nutrients, organic matter, municipal waste) are recommended. These measures can influence the yield of plants and their chemical composition [7, 23, 24]. Among the above-mentioned methods, the use of purposefully selected microorganisms decomposing chemical compounds provides beneficial effects in medium- and low-PAH-contaminated soils [25]. The available academic literature lacks information on the effect of microbiological biopreparations used for accelerating the decomposition of petroleum-derived products in contaminated soils, on growth and chemical composition of plants cultivated on these soils.

The objective of the conducted research was to determine the effects of petroleum-derived substances such as: petrol, used engine oil, and diesel oil, after three years from the date of contamination, on the select morphological parameters of the broad bean, and on the content of selected macronutrients and heavy metals in the leaves of plants growing on contaminated soils. The effect of the bioremediation process initiated by biopreparation ZB-01 on the above-mentioned parameters was also determined.

Experimental Procedures

The analyzed plants were obtained from the Experimental Station of the University of Agriculture in Cracow, located in Mydlniki near Cracow. In November 2009, indigenous soil (loamy-sand, pH=6.45 in 1 mol/dm^3 KCl solution and 7.12 in water, organic carbon content = 1.04%) was placed in special containers of 1 m^3 volume, retaining the natural arrangement of layers. The containers were sunk in the ground so that their upper edge was at the same level as the surface of the soil. All containers had a pipe leading to the surface, enabling the excess water to be pumped out, and three plastic tubes for suitable aeration of the soil, the latter being necessary for the correct course of bioremediation. The soil in the containers was left for eight months without any intervention in order to regain its natural biological functions. In June 2010, the soil surface was artificially contaminated with petrol, used engine oil, and diesel oil in a quantity of 6,000 mg of petroleum-derived substance per 1 kg of dry mass, by pouring it on the soil.

Table 1. Effects of petroleum-derived substances on select morphological parameters of plants.

Treatments	Sum of shoots length [cm]	Mass of shoots [g]	Root length [cm]	Mass of root [g]	Number of leaves [pcs.]
EO	188.2 ab	69.1 a	17.7 ab	26.1 ab	154.8 a
EOr	203.5 abc	71.9 abc	16.7 ab	25.0 a	157.4 a
DO	161.8 a	70.7 ab	14.5 a	27.8 ab	134.4 a
DOr	218.2 abc	92.2 bcd	17.0 ab	29.8 ab	163.1 a
P	263.0 c	97.9 d	19.1 b	33.7 b	259.4 c
Pr	223.3 abc	99.9 d	17.2 ab	28.4 ab	187.8 ab
C	253.6 bc	102.9 d	19.7 ab	32.9 ab	223.9 bc
Cr	190.4 ab	92.7 cd	18.8 b	33.3 ab	163.9 a

EO – soil contaminated with engine oil, DO – soil contaminated with diesel oil, P – soil contaminated with petrol, C – control soil, r – with bioremediation. Values marked by different letters are statistically different (α=0.05).

After one week, half of the number of containers were subjected to the bioremediation process by adding biopreparation ZB-01, which was specially produced for this experiment and contained select prokaryotic organisms, mainly bacteria from the following genera: *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Oligella*, *Alcaligenes*, *Methylobacterium*, *Bacillus*, *Stenotrophomonas*, *Corynebacterium*, and *Morganella*. The treatment was performed by sprinkling, maintaining 60% sorption humidity of the soil. The non-contaminated soil was placed in identical containers and constituted the control treatment. The experiment was set in four repetitions in line with the randomized blocks method. In three subsequent years, the soil in the containers was left without any intervention to enable natural plant succession.

After 24 months from the date of pollution, content of petroleum-derived substances in soil contaminated with engine oil was more than 10 times higher than in the control soil. In soil contaminated with diesel oil this value was more than twice higher, while soil contaminated with petrol was similar to the control soil. The biopreparation applied resulted in a decrease to almost double in the content of TPH in soil contaminated with engine oil and with diesel oil. In the case of soil contaminated with petrol and control soil TPH content was similar.

The seeds of the Windsor White variety of broad bean were sown in the containers at the beginning of April 2013, after earlier preparation of the soil (i.e. loosening and fertilizing). Pre-sowing soil fertilization with NPK fertilizer ('polifoska') was applied, providing 2.88 g N, 3.77 g P, 7.16 g K, and 1.30 g S per container.

The evaluation of the morphology of plants, and the structural elements of yield (plant height, root length, mass of shoots, number and mass of leaves per plant, number and mass of pods per plant, number and mass of seed per pod) was made at the stage of technological maturity of broad bean seeds.

In order to determine the heavy metal concentrations in the leaves of plants, plant material was cleaned of any patches of deposited aphid honeydew and other surface contaminants, washed in tap water, next in distilled water. It was then dried at 105ºC. A portion of 0.25 g dried plant material was digested with 5 ml of HNO_3 at 110ºC and then diluted to 10 ml with deionized water. Next, the metal content (Cd, Pb, Zn, Ni, Cu, and Mn) was measured using flame absorption spectrometry (Thermo Scientific iCE 3500) [17, 26]. Carbon, nitrogen, and sulphur contents were determined in a Variomax CNS analyzer.

The obtained results were then subjected to analysis by STATISTICA 10.0 software. The significance of differences between the means were tested by two-factor variance analysis, and the means were differentiated by Fisher's LSD test at α=0.05.

Results and Discussion

Among all oil-derived substances used in the experiment, diesel oil had the most adverse impact on the morphological parameters of broad bean plants, contributing to significant decreases in the lengths of roots and shoots, number and mass of leaves, number of pods, and the mass of shoots (Tables 1 and 2). Compared with the control treatment, engine oil caused a decrease in the number of leaves developed by plants and decreased mass of shoots by more than 30%. Petrol did not exert significant effects on any of the analyzed morphological features, probably due to the fact that petrol contains a considerable proportion of volatile compounds that can rapidly evaporate from the soil, thus it could be significant that the experiment was conducted three years after initial soil contamination. In the case of soil contaminated by diesel oil, the biopreparation applied caused an increase in the number of pods and seeds developed by plants, and also an increase in the mass of pods. In the remaining cases, the biopreparation had no significant effect on the analyzed parameters, and only in the case of soil contaminated with petrol did it cause a significant decrease in the number of leaves and the mass of seeds, as well as – in the case of the control treatment – a decrease in the number of leaves developed by plants.

Table 2. Effects of petroleum-derived substances on select morphological parameters of plants, continued.

Treatments	Mass of leaves [g]	Number of pods [pcs.]	Mass of pods [g]	Number of seeds [pcs.]	Mass of seeds [g]
EO	56.9 [ab]	4.8 [ab]	89.0 [ab]	15.1 [ab]	31.8 [a]
EOr	52.8 [a]	4.8 [ab]	104.2 [ab]	15.5 [ab]	35.9 [a]
DO	54.1 [a]	3.7 [a]	77.0 [a]	11.8 [a]	31.8 [a]
DOr	66.5 [ab]	5.8 [b]	132.8 [b]	18.3 [bc]	40.2 [a]
P	87.0 [c]	6.8 [b]	132.2 [b]	22.2 [c]	61.2 [b]
Pr	67.1 [abc]	5.7 [ab]	129.4 [b]	17.2 [abc]	34.0 [a]
C	73.7 [bc]	5.8 [b]	113.8 [ab]	17.9 [abc]	45.5 [ab]
Cr	70.5 [abc]	5.3 [ab]	109.6 [ab]	15.6 [ab]	38.9 [a]

EO – soil contaminated with engine oil, DO – soil contaminated with diesel oil, P – soil contaminated with petrol, C – control soil, r – with bioremediation. Values marked by different letters are statistically different (α=0.05).

In recent years, a number of diverse studies have been conducted on the effects of petroleum-derived compounds on plants. Wyszkowski et al. [27] showed that diesel oil at a rate of 5.07 g/kg of soil caused substantial losses in the yield of aboveground parts of the yellow lupine by nearly 80%, and in the yield of root by nearly 40%. Similar regularities were observed in this study where this substance most negatively affected the studied morphological features of plants, resulting in a reduced aerial parts yield (shoots, leaves, pods, and seeds by around 30%) compared to the control treatment. Małachowska-Jutsz and Miksch [28] found that in rye, red clover, and white mustard the contamination of soil with engine oil provoked toxic effects, and these were greater when a higher rate of xenobiotics was applied. Małuszyński and Małuszyńska [29] demonstrated that used engine oil significantly reduced plant growth in spring vetch, orchard grass, and white mustard plants. Similar regularities were also presented in studies by Liste and Felgentreu [3], and Okonokhua et al. [30]. The presence of engine oil in soil results in the growth of *Amaranthus hybridus* L. almost halving, and a reduction of the leaf surface to one-third [31]. The plants grown in our experiment on the soil contaminated with engine oil also had a lower number of leaves and a lower mass of shoots than those in the control experiment. Similar results were obtained in studies on the effect of crude oil on the growth of plants. Osuagwu et al. [32] showed that the contamination of soil by crude oil at the rate of 5 ml/kg of soil results in the reduction of the numbers of leaves and tubers developed by *Dioscorea bulbifera* L., a reduction in their surface and mass, inhibiting the growth of plants by c. 10 %, as well as limiting the production of chlorophyll. Similar regularities were noted by Njoku et al. [33], who studied the impact of petroleum-derived compounds on select morphological features of *Glycine max* L. Lopes and Piedade [34] – proving that petroleum-derived substances contribute to an increase in mortality and a decrease in the biomass in *Eichhornia crassipes* (Mart.). The adverse impact of the petroleum-derived products on the morphological features of plants follows the disturbances in the uptake of water and nutrients. Even more, the petroleum-derived compounds can block the transport of many substances in plant cells, which can later be reflected in the limited growth of vegetative and generative organs [32].

Our own studies indicate that diesel oil contributes to a greater increase in soil density than engine oil (the bulk density of the soil contaminated by diesel oil amounted to 1.25 g/cm^3, and 1.17 g/cm^3 with engine oil), which can explain the greater harm exerted on morphological features of plants studied in our experiment. The excessive density may cause the blocking of soil pores through which water and air are transported, leading to the clumping of the soil and therefore to changes in their physical, chemical, and biological properties, and – consequently – to the reduction in its production potential [32].

Little is known about the impact of various techniques of supporting the natural bioremediation of soils contaminated by petroleum-derived products on the growth of plants cultivated in these soils. For example, adding compost to soil contaminated with engine oil can contribute to the better growth and development of [7, 35, 36]. Ogboghodo et al. [37] studied the possibility of applying poultry manure to improve the properties of soils contaminated by petroleum-derived compounds, and to increase the yield of maize. The authors found that in the sample where the highest rate of xenobiotic and the highest rate of fertilizer were applied, the properties of the soil changed significantly: the plants were more than seven times taller, and the dry mass yield was nearly twice greater than in the sample without the addition of poultry manure. The microbiological biopreparation used in the experiment presented in this paper also mostly had a beneficial effect on the analyzed morphological parameters of plants.

Nitrogen, carbon, and sulphur are the most important organogenic compounds that form the principal mass of plants. These elements are principal factors for the normal growth and development of plants, their yield production, and the quality of obtained yield [38].

All the oil-derived substances applied in the experiment contributed to a significant drop in the nitrogen content in broad bean leaves (Fig. 1). The most detrimental effect to the analyzed parameter was exerted by engine oil, which caused a decrease in the content of this macrocomponent by nearly 15% compared with the control treatment. In the case of soils contaminated with engine oil and petrol, the biopreparation applied led to a significant increase in the content of nitrogen in leaves of plants, whereas in the control treatment it led to a decrease of nearly 50%. Wyszkowski and Ziółkowska [36] demonstrated in their studies that diesel oil and petrol contributed to the decrease in nitrogen content in the leaves of spring rape and oats, which coincides with the results of this study. The authors also emphasized that the intensity of the effects exerted by petroleum-derived compounds depends much on their type, amount applied, as well as on the species of the tested plant. On the basis of another experiment, Wyszkowski and Wyszkowska [39] found that diesel oil applied at a rate of 9 g/kg of dry mass of soil caused the nitrogen content to almost double its rate of decrease in the aboveground parts of oat plants. Wyszkowski et al. [27] also found that a rate of diesel oil more than 6 g/kg of dry mass of soil results in nearly triple the reduction in the nitrogen content in the aboveground parts of yellow lupine. Because of the presence of hydrocarbons, soil contaminated with petroleum-derived products shows a disturbed proportion between nitrogen and carbon. It contributes to the inhibition of many reactions involving nitrogen (in both mineral and organic forms) in the soil, as well as to the decrease in the intensity of the ammonification and nitrification processes [40]. These phenomena could explain the lower content of this element in experimental plants. As the production process of ZB-01 biopreparation applied in the experiment uses small quantities of petroleum-derived products, the aforementioned mechanism can partly explain the observed drop in nitrogen content in plants grown on control soil, which was subject to the supported bioremediation.

Engine oil and petrol caused a significant drop in the carbon content of broad bean leaves (429.4 g/kg and 432.2 g/kg, respectively), whereas diesel oil caused the carbon content to increase to 451.8 g/kg (Fig. 2). The application of the bioremediation process most often resulted in decreasing the content of this macronutrient, which was best seen in the case of applying it on the control soil where the carbon content dropped by nearly 40%. Petroleum-derived substances contribute to the increase in carbon content in soil, which is linked to their structure (they contain aliphatic hydrocarbons, cycloalkanes, olefins, and arenes) [41, 42]. It should, nevertheless, be remembered that plants obtain most of their required carbon from air, and the intensity of that process depends also on the content of other macrocomponents in their organs. Plants growing on soil contaminated with petroleum-derived substances show a variable accumulation of nutrients [7, 36, 39], resulting in diverse carbon content in different parts of the experiment.

Among all oil-derived substances applied in the experiment, only engine oil contributed to a significant decrease in sulphur content in plant leaves (Fig. 3). The remaining

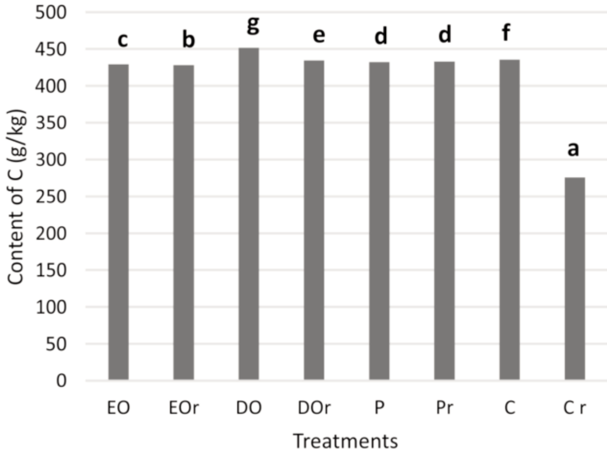

Fig. 2. The effect of petroleum-derived substances on content of carbon in broad bean leaves (g/kg). Symbols as in Fig. 1. Values marked by different letters are statistically different (α=0.05).

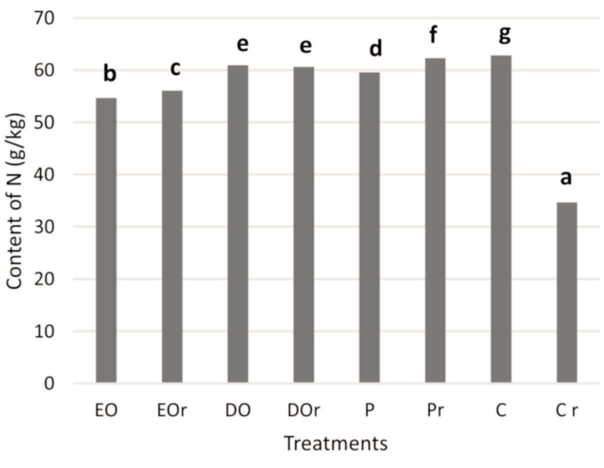

Fig. 1. The effect of petroleum-derived substances on content of nitrogen in broad bean leaves (g/kg). Symbols as in Table 1. Values marked by different letters are statistically different (α=0.05).

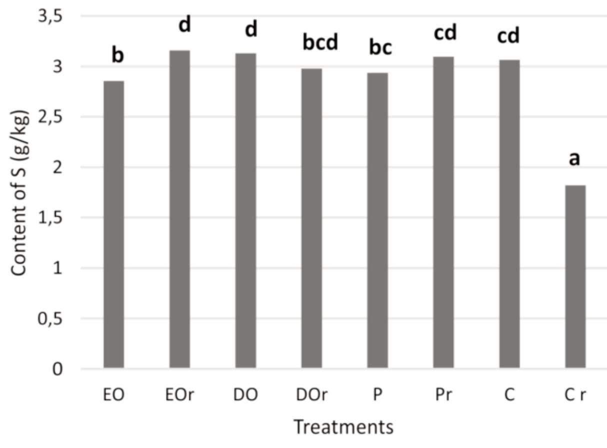

Fig. 3. The effect of petroleum-derived substances on content of sulphur in broad bean leaves (g/kg). Symbols as in Table 1. Values marked by different letters are statistically different (α=0.05).

compounds did not significantly affect this parameter. A similar situation occurred in studies carried out by Moubasher et al. [43], where they found that, most often, petroleum-derived hydrocarbons did not result in significant changes in the content of this component in the roots and shoots of *Bassia scoparia* (L.). The biopreparation applied in this experiment resulted in a significant increase in sulphur content in the leaves of plants growing on soil contaminated with engine oil. Sulphur is chiefly bound by hydrocarbons with a high molecular weight, and thus the degradation of the polycyclic aromatic hydrocarbons resulting from the introduction of select microorganisms causes an increase in the bioavailability of this nutrient to plants. The microorganism also influences a number of processes associated with the metabolism of biogenic elements, like sulphur [44], which may also explain why the biopreparation caused the rate of decrease to almost double in the content of sulphur in the control treatment.

Dimitrov and Mitov [45] found that the content of nutrients in plants growing on soils contaminated with petroleum-derived substances depends much on the species of plant. Among seven species of plants tested by these authors, only three of them showed increases in the content of macroelements in their organs.

Cadmium is an element relatively easily taken up by plants because it occurs in soil chiefly as a Cd^{2+} ion and can be easily transported to all plant organs. However, even at low concentrations it causes disturbances in the process of photosynthesis, transformations of nitrogen compounds and in the DNA structure, as well as in the permeability of cell membranes [46]. Diesel oil and petrol contributed to a significant decrease in cadmium content in leaves of the plants growing on contaminated soil, whereas engine oil did not significantly affect this parameter (Fig. 4). Furthermore, it was noted that the use of biopreparation only led to a significant increase in cadmium content (by more than 25%) in the treatment with petrol contamination.

There have been only a limited number of studies conducted to date on the effects of oil-derived compounds on

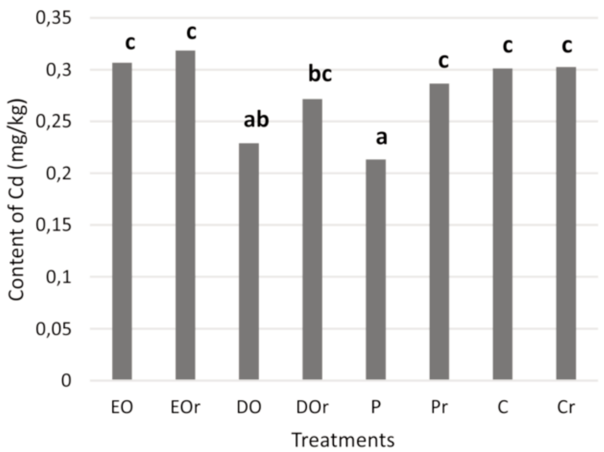

Fig. 4. The effect of petroleum-derived substances on content of cadmium in broad bean leaves (mg/kg). Symbols as in Table 1. Values marked by different letters are statistically different (α=0.05).

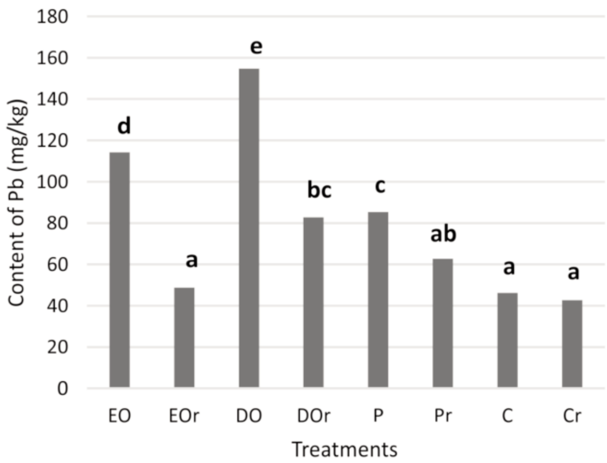

Fig. 5. The effect of petroleum-derived substances on content of lead in broad bean leaves (mg/kg). Symbols as in Table 1. Values marked by different letters are statistically different (α=0.05).

the contents of heavy metals in plants. Nwaichi et al. [47] showed that petroleum-derived hydrocarbons reduce the cadmium content in the leaves of *Vernonia amygdelina* and *Talinum triangular*, which coincide with the results of our experiment. Additionally, it was found that the uptake of heavy metals by plants increased in line with their higher amounts in the soil on which the plants grow [48]. Wyszkowski and Sivitskaya [49] found that heating oil caused a significant decrease in the cadmium content in soil. This phenomenon can provide explanation for the lower accumulation of this element in the leaves of plants used in the presented experiment.

Lead occurs naturally in plants but its function in plant metabolism has not been identified. In excessive amounts it adversely affects the basic life processes of plants. This element weakens growth and development, impairs germination, and disturbs the processes of photosynthesis and chlorophyll production, as well as cell division, nitrogen metabolism, and water management in plants [50]. All the petroleum-derived substances used in the experiment contributed to a significant increase in lead content in leaves (Fig. 5). The strongest impact on the analyzed parameter was diesel oil, which caused the lead content to nearly triple its rate of increase compared with the control treatment, at 154.7 mg/kg. The bioremediation applied had a beneficial effect on the analyzed parameter, causing a reduction in the content of the analyzed metal in plant leaves. The statistically significant differences were noted in treatments where soils were contaminated with engine oil, diesel oil, and petrol. The increase in the lead content in the leaves of *Vernonia amygdelina*, *Talinum triangulare*, *Manihot esculenta*, and *Xanthotosoma sagittifolium* (resulting from petroleum-derived hydrocarbons) was also found by other authors [47]. Benka-Coker and Ekundayo [51], Wyszkowski and Sivitskaya [49], Ujowundu et al. [8], and Okonokhua et al. [30] found that the contamination of soil by oil-derived substances contributed to an increased lead content in the soil, which may explain the increase in the content of this metal in the leaves of plants growing on soils contaminated with engine oil, diesel oil, and petrol in the experiments described in the presented study.

Zinc plays an essential role in metabolic processes in plants, and also takes part in gene transcription. However, the excessive amounts of this element may result in toxic effects, causing a reduction in yield, damage to the root system, and disturbing the process of photosynthesis. Zinc is taken up by plants in quantities proportional to its content in soil, but both soil properties and the selection of species significantly affect its accumulation in plants [52]. Engine oil caused a significant increase in the content of zinc in plant leaves; the concentrations of the elements in this treatment amounted to 144.9 mg/kg (Fig. 6). Petrol, however, contributed to a significant reduction in zinc content by nearly 20 mg/kg compared with the control treatment. In most cases, the applied biopreparation had no effect on the content of this metal, and only caused a significant reduction in the zinc content in leaves in the case of soil contaminated with engine oil. Similar regularities were noted by Nwaichi et al. [47], who found that petroleum-derived substances caused an increase in the zinc content in the leaves of *Vernonia amygdelina*, *Talinum triangulare*, *Manihot esculenta*, and *Xanthotosoma sagittifolium*. Benka-Coker and Ekundayo [51] also found that petroleum-derived substances contribute to an increase in the zinc content in soil, which is then reflected in the elevated content of this element in plants growing on soil contaminated with engine oil.

Small quantities of nickel are indispensable to the normal growth and development of plants. At higher concentrations it can, however, disturb seed germination, water management, and photosynthesis, and can also limit the development of root system, shoots, and leaves, as well as provoke stress reactions in plants [53]. Among all oil-derived substances used in the experiment, it was only diesel oil that caused an increase in the nickel content of broad bean leaves (Fig. 7). In the treatment where plants are grown on soils contaminated by diesel oil, the nickel content in the leaves of plants was almost three times higher

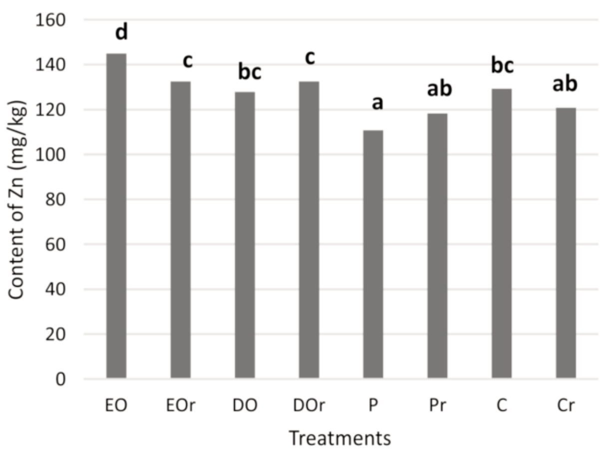

Fig. 6. The effect of petroleum-derived substances on content of zinc in broad bean leaves (mg/kg). Symbols as in Table 1. Values marked by different letters are statistically different (α=0.05).

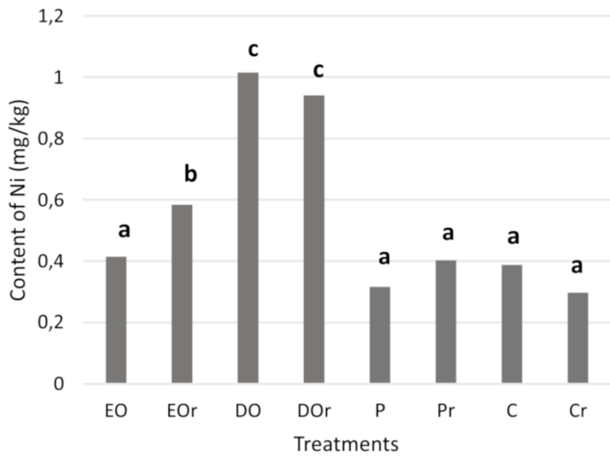

Fig. 7. The effect of petroleum-derived substances on content of nickel in broad bean leaves (mg/kg). Symbols as in Table 1. Values marked by different letters are statistically different (α=0.05).

compared with the control treatment. The remaining oil-derived substances used did not affect the content of the studied metal. The biopreparation used caused an increase in the nickel content in leaves of plants growing on soil contaminated with engine oil, whereas in other treatments it did not affect the analyzed parameter. Nakata et al. [54] showed that petroleum coke contributed to increased nickel content in the shoots of *Triticum aestivum*, as well as in the shoots and roots of *Deschampsia caespitosa*, similarly to the effects noted in the case of engine oil in our experiment. Nickel is more available to plants in soils with low pH, and among all petroleum-derived substances used in the experiment, the addition of engine oil has the greatest lowering effect on the pH of soil. This fact was confirmed by analyses (pH in KCl in soil contaminated with engine oil amounted to 4.95, and in the control soil 6.12) and can partly explain the obtained results.

Copper, at low concentrations, is – similar to nickel – needed for the normal development of plants. However, excessive amounts of this component damage cell membranes and the root system, and decrease chlorophyll production. Furthermore, copper participates in the formation of hydroxyl radicals, which are toxic to living cells [55]. All the oil-derived substances used in the experiment caused a significant increase of copper content in plant leaves (Fig. 8). This effect was most visible in the case of engine oil contamination, which caused the content of this component to increase more than threefold compared with the control treatment. Diesel oil and petrol contamination led to an increase in copper content by ca. 15 mg/kg. In the control soil, the biopreparation used contributed to an increase in the content of the analyzed metal by more than 7 mg/kg. Okonokhua et al. [30] also found that diesel oil causes an increase in the copper content in soil. Furthermore, similar to the case of nickel, the uptake of copper was easier for plants growing on soils with lower pH, and all petroleum-derived substances reduced the pH values of soil by c. 15% compared with the control experiment.

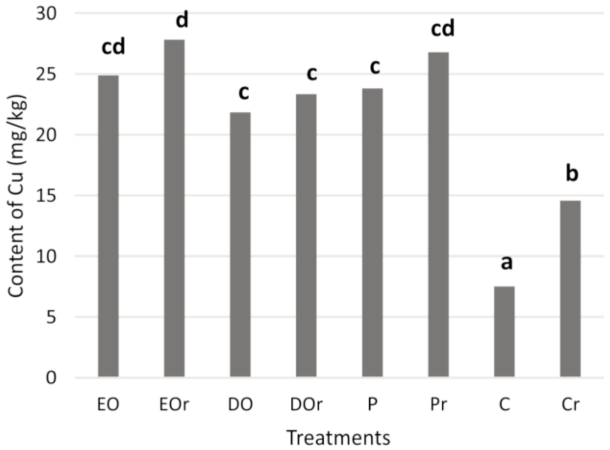

Fig. 8. The effect of petroleum-derived substances on content of copper in broad bean leaves (mg/kg). Symbols as in Table 1. Values marked by different letters are statistically different (α=0.05).

Manganese is an element that takes part in the formation of proteins and photosynthetic enzymes, as well as in metabolic processes. When occurring in excess quantities, it contributes to a decrease in the mass of plants, and disturbs the process of photosynthesis and other biochemical processes, causing the plants to suffer oxidation stress [56]. As with copper, all the oil-derived substances used in the experiment caused the amount of manganese in broad bean leaves to increase (Fig. 9). The bioremediation process initiated by the use of biopreparation ZB-01 resulted in a significant increase in the content of this component in the treatments with soils contaminated with diesel oil and petrol, as well as in the control treatment. It only caused a significant drop in the content of manganese in broad bean leaves in the case of soil contaminated with engine oil. Nwaichi et al. [47] also found that petroleum-derived substances caused an increase in the manganese content in leaves of *Xanthotosoma sagittifolium* and *Manihot esculenta*.

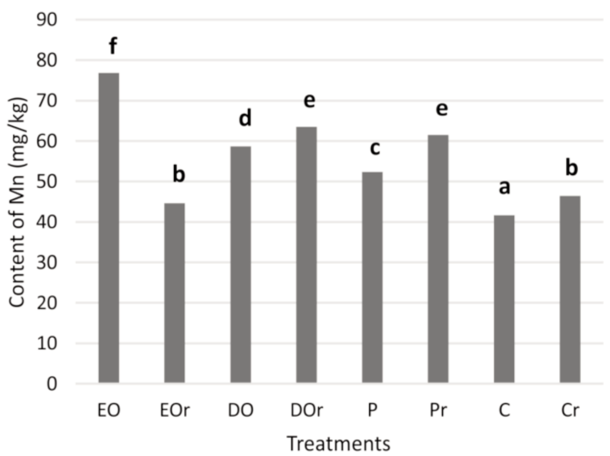

Fig. 9. The effect of petroleum-derived substances on content of manganese in broad bean leaves (mg/kg). Symbols as in Table 1. Values marked by different letters are statistically different (α=0.05).

In the studies reported by Wyszkowski and Sivitskaya [49], it was found that engine oil contamination at the rate of 5 g/per kg of soil resulted in a remarkable increase of manganese content in the soil, which can contribute to the enhanced absorption of this element by plants.

Nanekar et al. [57] proved that adding extra microorganisms (bioaugmentation) to the soil contaminated with petroleum-derived compounds decreased the amount of total petroleum hydrocarbons (TPH) in the soil, and also resulted in lowering the lead, cadmium, and zinc content while increasing manganese content. Most often, these results coincide with the outcome of our experiment. They are the consequences of the ability of microorganisms to adapt to adverse conditions, and to use harmful substances for their growth and development, therefore accelerating the rate of their decomposition. Similar regularities also have been indicated by other authors [58].

It should nevertheless be noted that the uptake of heavy metals by plants depends on many factors, e.g. type of soil, type of metal and its concentration, the form in which the metal occurs in the environment, and the species of tested plant [59, 60].

Conclusions

1. The contamination of soil by diesel oil, even three years after its application, adversely affected the majority of morphological parameters of broad bean plants. After the same period of time, petrol did not affect the growth of plants, whereas engine oil caused a drop in the mass of shoots and the lowering of the number of leaves grown by the plants.
2. Engine oil in soil caused a decrease in the content of all studied macronutrients in broad bean leaves, whereas petrol contributed to a decrease in the nitrogen and carbon contents, while diesel oil resulted in a decrease in nitrogen content but led to an increase in carbon content.
3. All oil-derived substances applied in the experiment caused increases in the content of lead, copper, and manganese in leaves of plants. Furthermore, petrol led to a decrease in cadmium and zinc content. Engine oil contributed to a decrease in cadmium content, but resulted in an increase in nickel content.
4. The process of bioremediation applied in the experiment beneficially affected the studied morphological parameters of broad bean plants most often in the case of soil contaminated with diesel oil. Furthermore, it contributed to either eliminating or reducing differences in the content of nitrogen, carbon, sulphur, cadmium, lead, and zinc between the plants growing on contaminated soil and those on the control soil. The manganese content in plants after applying biopreparation changed depending on the type of contaminant (a decrease in the case of engine oil, and increases in the cases of diesel oil and petrol), whereas the use of biopreparation did not affect the content of copper and nickel (except in the treatment with soil contaminated with engine oil) in the plants growing on contaminated soils.

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