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The Subsequent Effects of Soil Pollution by Petroleum Products and Its Bioremediation on the Antioxidant Response and Content of Elements in *Vicia faba* Plants

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Abstract: Petroleum-derived substances (PDSs), which are one of the most significant sources of energy, have become key factors that affect soils and, consequently, plants. The aim of this study was to determine the subsequent effects of soil contamination by PDSs such as petrol (P), diesel fuel (DF) and used engine oil (EO), in addition to its bioremediation using a ZB-01 biopreparation, on the activities of antioxidising enzymes as well as on the content of antioxidants in the leaves of *Vicia faba* L. The effects on the growth of the plants and their chemical composition were also determined. The results showed that as many as five years after contamination, engine oil and diesel fuel adversely affected the growth of plants. PDSs caused a significant increase in the activity of peroxidase and an increase in the content of proline. The contamination of the soil with oils (EO and DF) resulted in a decrease in the content of nutrients (Ca, Mg and P) in the plants. DF also decreased the content of K and N while EO decreased the content of Fe. PDSs also increased the content of lead and cadmium, and some resulted in a decrease in the content of zinc, manganese and copper. The ZB-01 biopreparation generally had a beneficial effect on the growth of plants, and contributed to a lowering of the activities of the analysed antioxidative enzymes as well as the content of antioxidants in plants in the soil that had been contaminated with diesel fuel. Furthermore, it most often caused an increase in the nutrient levels in the leaves of plants. The effect of the ZB-01 biopreparation on the content of heavy metals varied and was dependent on the specific contaminant and metal that were analysed.

Keywords: soil pollution; oil derivatives; bioremediation; antioxidant enzymes; antioxidants; broad bean

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1. Introduction

The intensive industrialisation and urbanisation that have occurred in recent years have caused petroleum-derived substances (PDSs) to become one of the most significant factors contaminating the natural environment [1]. These substances adversely affect the physical, chemical and biological properties of soil, which leads to dramatic changes in organic content and disturbs the ratios of carbon to nitrogen and phosphorus. Furthermore, PDSs restrict the air exchange between the soil and the atmosphere, decreases the soil permeability and water-holding capacity and impede ion exchange [2]. PDSs also adversely affect the growth and development in cultivated plants [3,4], cause changes in the content of nutrients [5,6] and modify the content of heavy metals in the plant organs [7].

High concentrations of PDSs in the soil causes oxidative stress in plants, which results in the generation of ROS (reactive oxygen species) [8]. The toxic effect of ROS consists of their reactivity with components in living cells, including lipids, proteins, enzymes, nucleic acids and sugars, which leads to modifications in the structures and functions of

these molecules [9]. The accumulation of ROS in plants that occurs due to environmental stress is the principal cause of the decrease in the productivity of cultivated crops around the world [10]. In order to alleviate the damage caused by ROS plants have developed many protective mechanisms, which are generally called the system of antioxidative protection. This system involves many antioxidants, chemically and actively different, that are effective at different levels of stress [11]. Principally, this activity is divided into enzymatic or non-enzymatic systems. The enzymatic mechanism is based on antioxidative enzymes, e.g., superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), while the non-enzymatic system is comprised of low-molecular-weight antioxidants, e.g., proline, ascorbic acid and glutathione, all of which can neutralise free oxygen radicals [12,13].

In efforts where the goals are the decontamination and bioremediation of toxic substances in soil (such as PDSs), the use of biological methods has become increasingly important. These methods are effective, advantageous in economic terms and safe to the natural environment; they also have remarkably rapid action [14–16]. ZB-01 is an example of a microbial preparation with confirmed usefulness in the decomposition of PDSs [17–21]. ZB-01 is comprised of a mixture of select prokaryotic organisms (*Acinetobacter, Alcaligenes, Burkholderia, Corynebacterium, Comamonas, Moraxella, Pseudomonas, Stenotrophomonas, Ochrobactrum* and *Oligella*). This preparation has already been successfully used to initiate and stimulate the biodegradation of organic contaminants in soil that had been polluted with refinery sludge and oil slurries [19], which led to an approximately four–five-fold decrease in contaminant levels after only 4.5–6 months. ZB-01 has also been used to treat oil-containing wastewater from metal processing plants [20], where it increased the decomposition of organic pollutants by about 20% in only 18 days, compared to decomposition that was based solely on autochthonous microflora. In the case of a highly contaminated, spent-oil-based metalworking fluid, a significant degradation of contaminants after 14 days was achieved using only ZB-01, while autochthonous microflora did not significantly reduce the organic content [21]. The bacteria included in ZB-01 were a component of a mixed biocenosis that was used to biodegrade pollutants in highly loaded and toxic wastewater from the production facilities of the furniture industry [17]. Using ZB-01 for soil pollution (3655 mg kg^{-1}) in the area of a fuel station of a production plant, which was part of the chemical industry, after three corroded and leaking fuel tanks were dismantled resulted in a significant acceleration of the kinetics of organic pollutant removal. The level of contaminants in the soil was almost two-fold lower 40 days after the application of the biopreparation [18]. The above-mentioned applications of the ZB-01 biopreparation argue in favour of testing its potential effectiveness in reducing the adverse effects of PDSs on plant health and thus in restoring the suitability of soils that had been contaminated by PDSs for agricultural production.

The available literature provides little information on the effect of PDSs on the antioxidant response of plants growing in contaminated soil [22–24]. Most of the results on this topic usually concern a short time period following contamination and/or are conducted in laboratory or greenhouse conditions. Conversely, there are no data on its more long-term consequences, especially in field conditions. There is also little information on the effects of bioremediation on these parameters.

The objective of the conducted study was to determine the subsequent effect (within five years after contamination) of the contamination of soils by PDSs, e.g., petrol, diesel fuel and used engine oil, on the activity of selected antioxidising enzymes as well as the content of antioxidants in the leaves of the broad bean (*Vicia faba* L.). The broad bean has already been used as a model plant in studies on the effect of soil contamination with hydrocarbons on the growth and development of plants, and was indicated as being a potential bioindicator of oil pollution [25]. We also determined the effects of PDSs on select morphological features of plants as well as the content of nutrients and heavy metals in plants. The assessment was extended to analyse the effects of bioremediation using ZB-01 preparation on the parameters mentioned above.

These were the hypotheses: 1. Even after five years, PDSs modify the growth of *V. faba* plants as well as the content of nutrients and heavy metals. 2. PDSs in the soil cause stress in plants, which could be manifested as an increase in both the activity of antioxidising enzymes and the content of antioxidants. 3. Bioremediation can reduce the negative impacts of PDSs on plants.

2. Materials and Methods

2.1. Experimental

The field experiment started in the Experimental Station of the University of Agriculture in Kraków (Poland; 50.0815° N, 19.84730° E) in November 2009. A detailed description of the experimental design and the characteristics of the experimental soil are presented in [24]. Briefly, the native soil (loamy sand, pH in H_2O = 7.12, $CaCO_3$ = 0.17%, K_2O = 17.17 mg 100 g^{-1}, P_2O_5 = 16.37 mg 100 g^{-1}, C_{total} = 1.04% and N = 0.09%) was placed in 32 1 m^3 containers. Next, the containers were buried in the ground up to their top edges. The soil in the containers was left untouched throughout the autumn–winter season in order for it to regain its natural biological function. The soil in selected containers was artificially contaminated with a variety of PDSs in June 2010 using petrol (P), used engine oil (EO) and diesel fuel (DF), with 6 g of each PDS per 1 kg of dry soil mass (i.e., the typical petroleum concentrations in medium-contaminated soils), poured uniformly over the surface of the soil in each container. The selected PDSs (characteristics in Table 1) were purchased from a local petrol station. The engine oil had been used in a petrol engine for one year prior to its use in this experiment. The control treatments for the experiment were the same type of containers, containing non-contaminated soil. One week after contamination with PDSs, half of the containers were subjected to bioremediation by first adding a fertiliser and then a biopreparation of ZB-01 that had been specifically produced for this experiment in the Biochemistry Department of the University of Agriculture in Kraków. The ZB-01 biopreparation was sprinkled over the surface of the soil and a 60% sorption humidity of the soil was maintained. The fertiliser compound (Azofoska) applied before the ZB-01 biopreparation consisted of 13.6% N, 2.8% P and 15.8% K at 100 g per container. This same procedure of the application of the fertiliser and the biopreparation was repeated one year later, in the spring of 2011. The experiment was conducted in four replications in accordance with the randomised blocks method. The soil in the containers was left without any intervention for the next three years, in order to permit natural plant succession. No reclamation methods, such as loosening or moistening the soil after the application of the oil products, were used. In the 2013–2014 period, the broad bean was grown in the containers, followed by winter wheat.

In order to confirm the activity of ZB-01, the content of total petroleum hydrocarbons (TPHs) in the investigated soils was monitored once a month for a period of 24 months after contamination (i.e., in the years 2010–2012). At the beginning of the experiment, the soil that had been contaminated with EO had more than a ten-fold-higher TPH content (36,372 mg kg^{-1}), the soil that had been contaminated with DF had an almost four-fold-higher TPH content (13,406 mg kg^{-1}) and the soil that had been contaminated with P had a TPH content (2675 mg kg^{-1}) that was similar to that of the control (3527 mg kg^{-1}). Inoculation with ZB-01 biocenosis caused the degradation of petroleum derivatives that were present in the soil that had been contaminated with DF and EO with 82.3% and 75.4% efficiency, respectively, after 24 months [26].

2.2. Heavy Metal Concentrations in the PDS Samples

In order to determine the possible causes of the changes in the elemental content of the plant samples, the PDSs that were used in the experiment were analysed for their contents of heavy metals. The PDS samples were analysed using ICP-OES emission spectrometry, according to BOSMAL/I-7-43/06, to determine their content of heavy metals such as cadmium, copper, manganese, nickel, lead and zinc. The diesel fuel sample was tested directly (i.e., without the preparation), the petrol sample was diluted with an organic

solvent and the engine oil sample was digested in an oxidising environment in a closed microwave system. The analyses were performed in the BOSMAL laboratory (Bielsko-Biała, Poland).

Table 1. Characteristics of the PDSs that were used in the experiment (content in %) according to the material safety data sheets and according to Polish legal regulations.

Ingredient Name	Petrol	Diesel Fuel	Engine Oil
Gasoline	≥50		
Toluene	5–30		
Benzene	0.1		
Methyl t-amyl ether (TAME)	≤15		
2-ethoxy-2-methylpropane (ETBE)	≤15		
Tert-butyl methyl ether	≤15		
Propan 2-ol	≤10		
Izobutyl alcohol	<10		
Tert-butyl alcohol	≤7		
Ethanol	≤5		
Methanol	<3		
Olefin hydrocarbons	<18 [1]		
Aromatic hydrocarbons	<35 [1]		
Polycyclic aromatic hydrocarbons (PAHs)		<8 [1]	
Diesel fuel		≥90	
Heavy paraffin distillates, treated with hydrogen (crude oil); base oil—unspecified 01-2119484627-25			~43
Heavy distillates from hydrocracking; base oil—unspecified 01-2119486951-26			~35
Highly refined mineral oil			~4.5
Zinc salts of mixed esters of O,O-bis (sec-Bu I 1,3-dimethylbutyl) phosphorodition acid 01-2119657973-23-0000			0.54–1.0
Benzenesulfonic acid, methyl mono-C20-26-branched alkyl derivatives, calcium salts			0.54–1.05
Benzenesulfonic acid, methyl mono-C20-24-branched alkyl derivatives, calcium salts			0.11–0.54

[1] According to Polish law for the quality requirements for liquid fuels.

2.3. Petroleum-Derived Hydrocarbons and Element Concentrations in the Soil Samples

In order to monitor the changes in the content of PDSs in the experimental soil at five years after contamination (i.e., in 2015), the soil was analysed for petrol (C_6–C_{12} hydrocarbons in acc. with the standard PN-ISO 22155: 2013) and mineral oil (C_{12}–C_{36} hydrocarbons in acc. with the standard PN-EN ISO 16703: 2011). These analyses were performed in the WESSLING Polska laboratory in Kraków.

The soil concentrations of heavy metals (Cd, Mn, Zn, Fe, Pb and Ni) and macronutrients (K, Mg, Ca, P and S) in 2015 were estimated in air-dried soil samples that had been sieved through a 2 mm sieve according to the method that was used by Zheljazkov et al. [27] and Wójcik et al. [28]. We used concentrated extractants of HNO_3 (65%) for the acid-extracted elements and 0.01 M $CaCl_2$ for the potentially bioavailable elements, except Ca. The levels of the metals were measured in the filtered extracts using inductively coupled plasma atomic emission spectroscopy (Spectro Analytical Instruments, Kleve, Germany).

2.4. Plants

In the beginning of April 2015 (five years after the soil contamination), broad bean ('Bartek' variety) seeds were sown in the soil in the containers: 30 seeds per container (in accordance with the sowing standard) after the soil had been prepared (loosening, fertilising). A pre-sowing fertilisation with 'Polifoska® 8' fertiliser (8% N, 24% P_2O_5, 24% K_2O and 9% SO_3) was applied, which supplied the soil with 2.88 g of N, 3.77 g of

P, 7.16 g of K and 1.30 g of S per container. 'Polifoska® 8' fertiliser was used due to the requirements of the broad bean for macronutrients, and was also based on the results of a soil analysis that had been performed in 2014, which showed a slight decrease in the available content of potassium and phosphorus but no changes in the content of nitrogen. The detailed results of this soil analysis can be found in [6]. After the emergence of the seedlings, 25 plants were left in each container.

Plant growth was assessed at the processing maturity (milk-ripe stage) of the broad bean seeds. Six plants were randomly collected from each container and the following elements were determined: the height of the plants, the number and mass of the shoots in addition to the mass of the leaves, pods and seeds. The rest of plants were left to grow to full maturity in order to assess the effect of the investigated factors on the damage to the seeds that was caused by *Bruchus rufimanus* Boh. weevils (data not presented in this manuscript).

2.5. Biochemical Parameters of the Plants

In order to determine the antioxidant enzymes in the plant samples, ten randomly selected plants were taken from each container during the flowering stage, which is the highest metabolic point during the life cycle of plants. It is recommended that ecophysiological (stress) parameters be estimated in this plant stage [29]. Crushed plant parts were homogenised in a 100 mM phosphate buffer (pH of 6.8) for POD and a 50 mM sodium-potassium phosphate buffer for CAT, and centrifuged at 12,000× *g* for 20 min; then, the supernatant was used to determine the enzyme activity levels. The entire procedure was conducted at 4 °C. The CAT activity was estimated as described in the method of Aebi [30], and was expressed as μmol of consumed H_2O_2 min^{-1} mg $protein^{-1}$. SOD was analysed according to Beauchamp and Fridovich [31]. One unit of SOD was defined as the amount of enzyme activity that was able to inhibit 50% of the photoreduction of nitroblue tetrazolium (NBT) to blue formazan. The total protein was determined using the Bradford (1976) method [32]. The non-protein thiols were analysed according to Mass et al. [33]. The content of proline was measured according to the method described by Bates et al. [34]. All of the above-mentioned methods have been described in detail previously [35,36].

2.6. Element Concentration in the Plant Samples

The plant material that was used to measure the element concentration was the leaves of six randomly selected plants from each container at the stage of processing maturity (milk-ripe). The older tissues of plant foliar parts are generally more efficient in retaining elements than younger leaves or stems [37]. Therefore, we decided not to measure their content in the flowering stage (as was the case for the biochemical parameters) but in the older stage of plants. In order to determine the concentrations of macroelements (Ca, Mg, K, N, S, C and P) and trace elements (Fe, Zn, Ni, Cu, Mn, Cd and Pb) in the plant samples, the plant material was washed and dried at 105 °C. Ground, dry weight subsamples of 0.25 g were wet digested in concentrated HNO_3 at a maximum temperature of 120 °C [38]. The concentrations of the trace elements and macroelements (Ca, Mg and K) were measured using flame absorption spectrometry (Thermo Scientific iCE 3500) and the P content was measured using inductively coupled plasma emission spectroscopy (ICP, Spectro Analytical Instruments, Germany) [35,36]. The reference material was certified reference material CTA-OTL-1, Oriental Tobacco Leaves, with the same quantities of samples used to check the quality of the analytical procedures. The content of carbon, nitrogen and sulfur was were determined in a Variomax CNS analyser.

2.7. Statistical Analysis

The obtained results were analysed and checked for normality (Shapiro–Wilk test with Lilliefors correction) and equality of variance (Levene's test). The significance of any differences between the means were tested by one-factor variance analysis (STATISTICA 13.1 software), and the means were differentiated with Fisher's LSD test (plant growth and

biochemical parameters of the plants) or Tukey's multiple range test (element concentrations in the soil and plant samples) at $p < 0.05$. CANOCO 4.5 was used to perform principal component analysis (PCA) [39]. The PCA assessed the similarities and relationships between the biochemical parameters and content of elements in the plants. The data were subjected to logarithmic transformation Y = log (Y + 1).

3. Results

3.1. Heavy Metal Concentrations in the PDS Samples

The concentrations of the tested heavy metals (Cd, Cu, Mn, Ni, Pb and Zn) in the diesel fuel, and Cd, Mn, Ni and Pb in the petrol were below the method's limit of quantification (Table 2). The used engine oil contained the highest amounts of the analysed heavy metals (especially zinc).

Table 2. The content of heavy metals in the PDSs that were used in the experiment (mg kg^{-1}) as means (±SD).

Ingredient Name	Petrol	Diesel Fuel	Engine Oil
Cd	<0.1 [1]	<0.1	0.23 (±0.01)
Cu	0.13 (±0.03)	<0.1	11.30 (±0.70)
Mn	<0.1	<0.1	1.90 (±0.70)
Ni	<0.1	<0.1	3.10 (±0.70)
Pb	<0.1	<0.1	3.90 (±0.10)
Zn	0.61 (±0.08)	<0.1	865.00 (±21.00)

[1] Values < 0.1 mean that the result was below the limit of detection of the method.

3.2. Petroleum-Derived Hydrocarbons and Element Concentrations in the Soil Samples

At five years after the soil contamination, the content of C_6–C_{12} hydrocarbons was lower than 0.8 mg kg^{-1} in all of the analysed soils (Table 3). However, the content of the C_{12}–C_{36} hydrocarbons (particularly in the soils that had been contaminated with EO and DF) was still significantly higher than in the control soil. In the soil that had been contaminated with P, this value only slightly exceeded that in the non-contaminated soil. The applied ZB-01 biopreparation resulted in a marked drop in the content of mineral oil hydrocarbons in all of the contaminated soils. The content of the C_{12}–C_{36} hydrocarbons in the soils that had been contaminated with PDSs and then subjected to bioremediation was approx. 15% lower in the case of EO and nearly 50% lower in the case of DF compared to the soils in which no ZB-01 was used.

Table 3. The content of hydrocarbons in the soil five years after contamination (mg kg^{-1}).

Treatment	Gasoline Range Hydrocarbons (C_6–C_{12})	Mineral Oil Hydrocarbons (C_{12}–C_{36})
EO 0R [1]	<0.8	600
EO R	<0.8	520
DF 0R	<0.8	370
DF R	<0.8	190
P 0R	<0.8	9.5
P R	<0.8	<6
C 0R	<0.8	<6
C R	<0.8	<6

[1] EO—soil that had been contaminated with engine oil; DF—soil that had been contaminated with diesel fuel; P—soil that had been contaminated with petrol; C—control soil; 0R—without bioremediation; and R—with bioremediation.

An analysis of the macronutrients in the experimental soil that had been extracted with HNO_3 (acid-extracted elements) showed lower contents of potassium, magnesium, phosphorus and iron in the soils that had been polluted with EO or DF compared to the control (unpolluted soil) (Figure 1). Contamination with P resulted in a decrease

in the content of magnesium and iron, while there were no significant changes in the content of calcium between the treatments. The application of ZB-01 increased the content of magnesium in the soil that had been contaminated with P, as well as the levels of magnesium and potassium in the control soil.

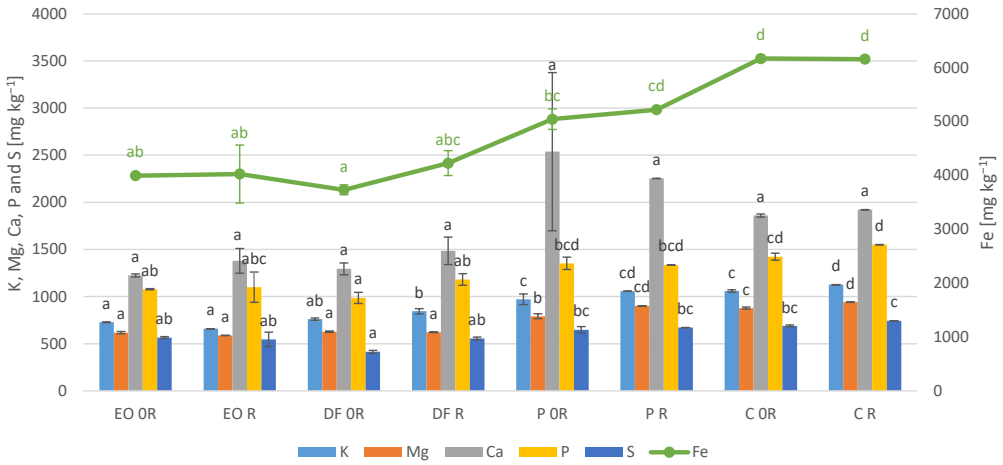

Figure 1. The effect of PDSs and bioremediation on the content of potassium, magnesium, calcium, phosphorus, sulfur and iron in the experimental soil (acid-extracted elements). The means (±SE) for individual elements that are marked with the same letters did not differ significantly according to Tukey's test at $p < 0.05$. The symbols are the same as those in Table 3.

An analysis of the potentially bioavailable elements showed no significant differences except for potassium, whose highest content was found in the soil that had been contaminated with DF and then subjected to bioremediation (Figure 2). Because the concentration of iron was below the quantification limit of the method, it is not presented in Figure 2.

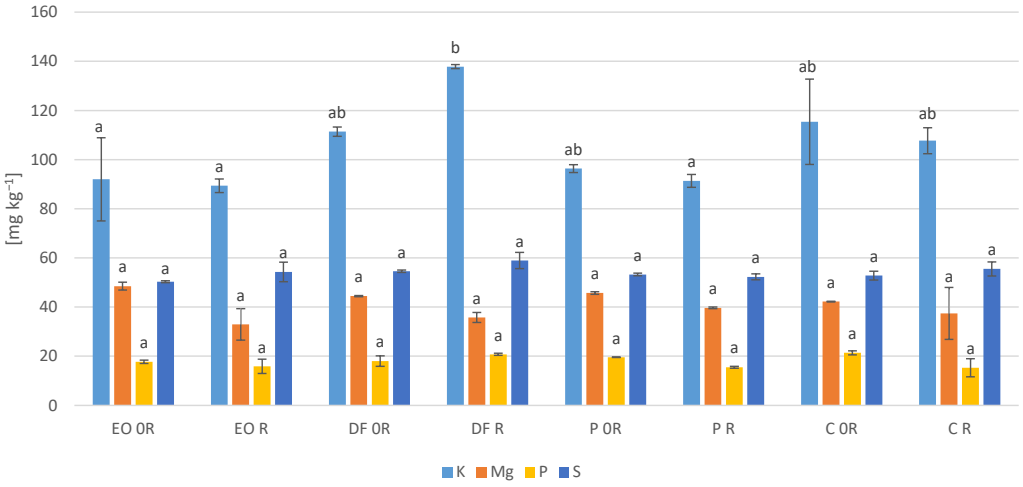

Figure 2. The effect of PDSs and bioremediation on the content of potassium, magnesium, phosphorus and sulfur in the experimental soil (potentially bioavailable elements). The means (±SE) for individual elements that are marked with the same letters did not differ significantly according to Tukey's test at $p < 0.05$. The symbols are the same as those in Table 3.

The analysis of heavy metals in the soils (acid-extracted elements) showed that the soil contaminated with EO had high levels of zinc, lead and nickel (Figure 3). The soil that had been contaminated with DF had a higher content of manganese, lead, nickel and cadmium, whereas the soil that had been contaminated with P had higher levels of manganese, zinc, lead, cadmium and nickel. The ZB-01 biopreparation resulted in a significant decrease

in the content of manganese, lead and nickel, and a slightly higher level of zinc in the DF-treated soil.

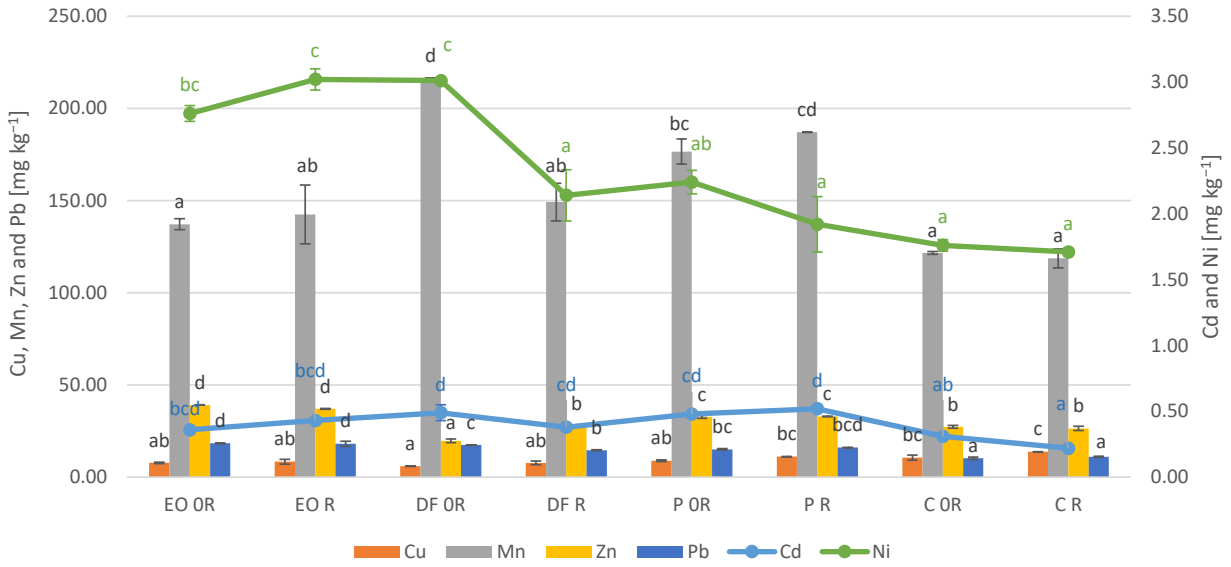

Figure 3. The effect of PDSs and bioremediation on the content of heavy metals in the experimental soils (acid-extracted elements). The means (±SE) for individual elements that are marked with the same letters did not differ significantly according to Tukey's test at $p < 0.05$. The symbols are the same as those in Table 3.

The analysis of the potentially bioavailable heavy metals in the experimental soils showed that the content of copper and, in most cases, nickel, lead and cadmium were below the method's quantification limit (Figure 4). Therefore, the content of copper is not presented in Figure 4. The content of manganese and zinc in the EO-contaminated soil was significantly higher than in the control soil. A higher content of manganese was recorded in the soil that had been contaminated with P, while a slightly higher content of cadmium was recorded in the soil that had been contaminated with DF. The addition of ZB-01 resulted in a significant decrease in manganese in the EO-treated soil and cadmium in the DF-treated soil.

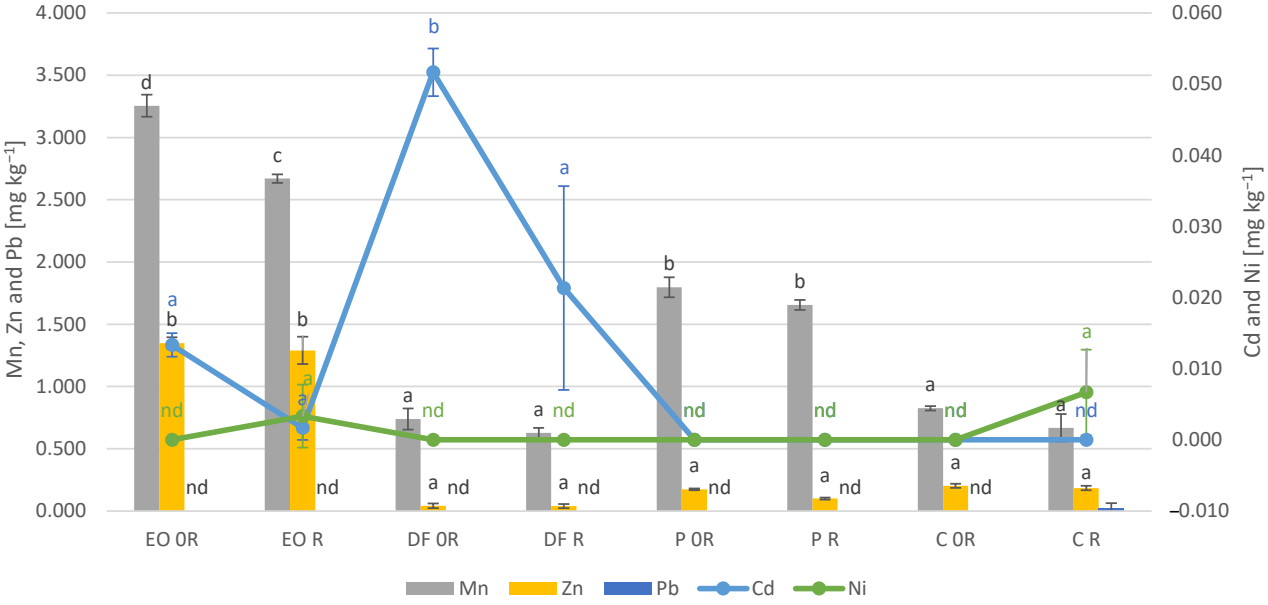

Figure 4. The effect of PDSs and bioremediation on the content of heavy metals in the experimental soils (potentially bioavailable elements). The means (±SE) for individual elements that are marked with the same letters did not differ significantly according to Tukey's test at $p < 0.05$. The symbols are the same as those in Table 3.

3.3. Plant Growth

The DF had the most detrimental effect on the studied morphological traits of the broad bean, which resulted in a significant decrease in the number of shoots as well as in the shoot and pod masses—by approx. 30% (Table 4). EO also reduced the pod mass compared to the control; however, similar to P, it did not affect the other morphological features of plants. Generally, the applied ZB-01 preparation beneficially affected the analysed features and, in the case of the soil that had been contaminated with DF, resulted in an almost two-fold increase in the mass of shoots and a nearly three-fold increase in the mass of pods. Furthermore, the plants that were growing on the control soil and on the P-contaminated soil were significantly taller after the application of the preparation than those to which no preparation was applied.

Table 4. The effect of PDSs and bioremediation on the growth of *Vicia faba* L.

Treatment	Height of Plant (cm)	Number of Shoots per Plant	Mass of Shoots (g per Plant)	Mass of Leaves (g per Plant)	Mass of Pods (g per Plant)	Mass of Seeds (g per Plant)
EO 0R	63.55 (±11.4) ab [1]	2.08 (±0.9) ab	54.78 (±14.0) bc	39.20 (±13.4) ab	45.84 (±21.3) b	11.18 (±2.3) ab
EO R	64.92 (±6.4) ab	2.25 (±0.8) ab	51.62 (±9.1) b	38.84 (±8.3) a	61.12 (±12.7) bc	10.83 (±4.5) ab
DF 0R	51.73 (±7.1) a	1.83 (±0.7) a	36.17 (±12.3) a	28.41 (±8.2) a	19.78 (±6.8) a	4.86 (±2.6) a
DF R	78.59 (±5.0) ab	2.00 (±0.4) ab	66.44 (±13.2) bc	48.86 (±14.1) ab	57.42 (±19.6) bc	12.72 (±5.7) ab
P 0R	55.56 (±5.3) a	2.50 (±1.0) ab	62.84 (±14.3) bc	42.95 (±13.0) ab	53.69 (±21.1) bc	15.82 (±8.2) ab
P R	84.98 (±10.0) b	2.08 (±0.7) ab	79.01(±15.1) c	53.06 (±14.2) ab	53.61 (±24.3) bc	14.98 (±4.6) ab
C 0R	50.92 (±8.6) a	2.42 (±0.8) b	53.16 (±6.1) b	42.68 (±10.1) ab	63.25 (±23.5) c	17.02 (±8.4) ab
C R	94.66 (±6.4) b	1.92 (±0.7) ab	88.36 (±18.3) d	64.92 (±12.1) b	64.29 (±22.2) c	17.14 (±5.1) b

[1] The means (±SE) in the columns that are marked with the same letters did not differ significantly according to the LSD test at $p < 0.05$. The symbols are the same as those in Table 3.

3.4. Biochemical Parameters of the Plants

PDSs generally caused an increase in the activity of POD and SOD as well as a decrease in the activity of CAT (Figure 5) and in the content of the non-protein thiol groups. PDSs also caused an increase in the content of proline in the leaves of the broad bean, with the strongest effect of as much as a five-fold increase in the case of DF contamination. Assisting the bioremediation process using the ZB-01 preparation in the DF and P treatments in addition to the control resulted in a decrease in the activity of the majority of the studied antioxidative enzymes as well as in a decrease in the content of proline. The level of this antioxidant was remarkably low in the leaves of the broad bean plants that had been exposed to DF (three-fold drop). In the EO contamination, however, a reverse relationship was found, i.e., a higher level of proline was found after the use of ZB-01, which was similar to the activity of POD.

3.5. Element Concentration in the Plant Samples

Plants that were grown in soil that had been contaminated by DF were characterised by having a lower content of nitrogen in the leaves than that of the control plants (Table 5). PDSs generally had no effect on the content of carbon in the plants except for the DF contamination, where the content of carbon decreased. EO contamination led to an increase in the content of sulfur in the leaves of the broad bean by more than 1.5 g kg^{-1}, while the presence of the P contamination resulted in a decrease in the content of sulfur by 0.64 g kg^{-1}. When the ZB-01 biopreparation was applied to the DF-contaminated plants, it resulted in an increase in the content of nitrogen and carbon in the leaves to a level that was similar to that in the control plants, while in the P-contaminated plants it resulted in a decrease in their content. In the EO-contaminated plants, the application of the ZB-01 biopreparation resulted in a decreased content of sulfur in the leaves, so that it was slightly closer to the level that was found in the control plants.

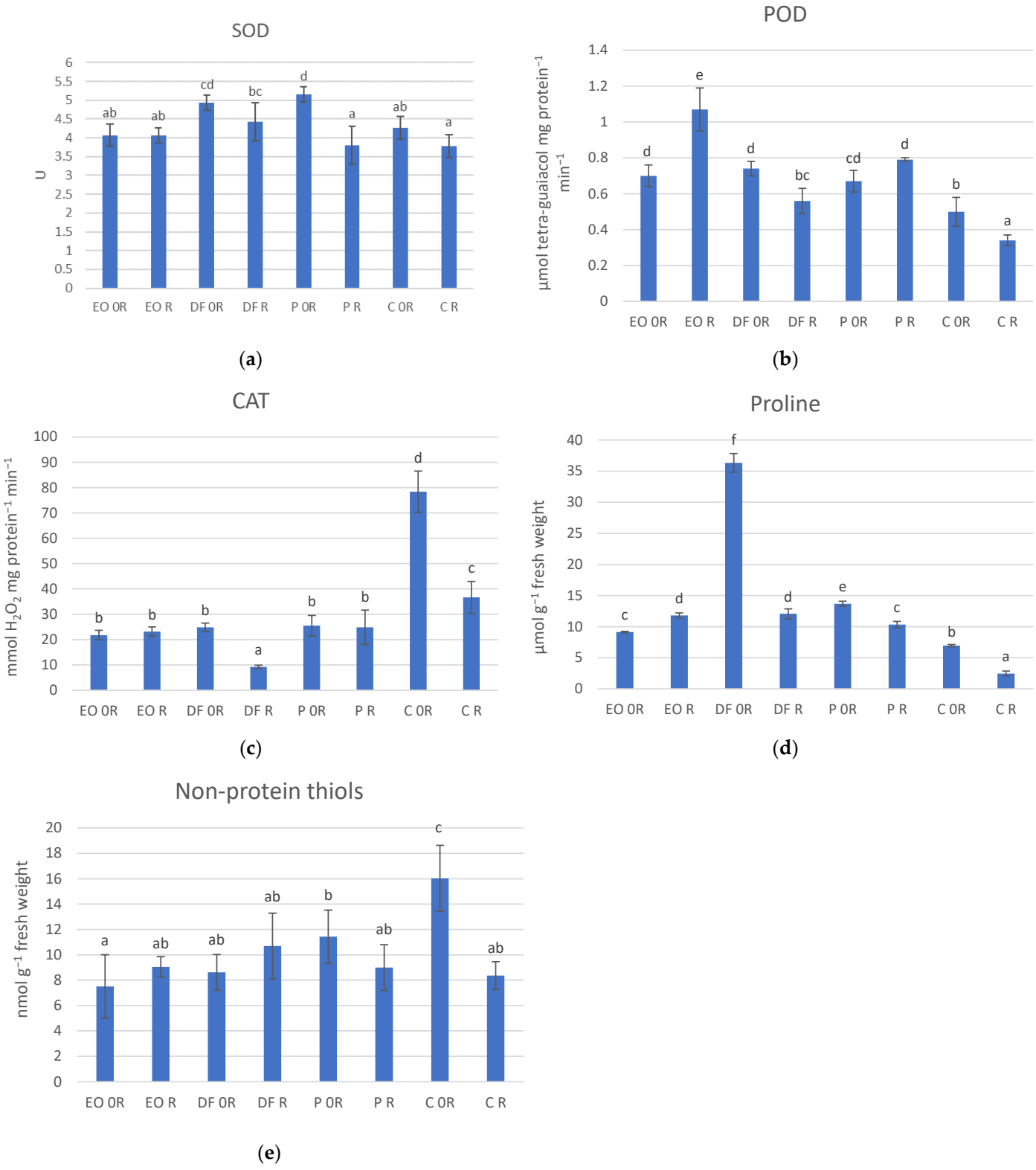

Figure 5. The effect of PDSs and bioremediation on the activity of antioxidant enzymes (superoxide dismutase (**a**), peroxidase (**b**), catalase (**c**)) and the content of antioxidants (proline (**d**), non-protein thiols (**e**)) in broad bean leaves. The means (±SE) for individual parameters that are marked with the same letters did not differ significantly according to the LSD test at $p < 0.05$. The symbols are the same as those in Table 3.

Generally, during our experiment, EO and DF contamination caused a decrease in the content of calcium, magnesium, phosphorous and potassium in *V. faba* leaves (Table 6). P

contamination did not affect the content of magnesium and calcium, but it resulted in a decrease in the content of potassium and phosphorus in the leaves.

Table 5. The effect of PDSs and bioremediation on the content of nitrogen, carbon and sulfur in the leaves of *Vicia faba* L. (g kg^{-1}).

Treatment	N	C	S
EO 0R	48.71 (±2.89) c [1]	418.48 (±1.89) c	3.95 (±0.02) e
EO R	47.21 (±1.27) c	419.28 (±0.08) c	2.91 (±0.01) d
DF 0R	38.02 (±7.20) a	411.53 (±6.92) ab	2.17 (±0.04) bc
DF R	49.02 (±0.45) c	418.16 (±0.26) c	2.29 (±0.01) c
P 0R	45.29 (±2.50) bc	415.15 (±0.71) bc	1.77 (±0.01) ab
P R	38.52 (±0.41) a	407.53 (±1.27) a	1.38 (±0.08) a
C 0R	44.56 (±1.41) bc	419.55 (±2.62) c	2.41 (±0.02) c
C R	42.32 (±4.90) ab	415.00 (±0.48) bc	2.22 (±0.01) c

[1] The means (±SE) in the columns that are marked with the same letters did not differ significantly according to the LSD test at $p < 0.05$. The symbols are the same as those in Table 3.

Table 6. The effect of PDSs and bioremediation on the content of magnesium, calcium, potassium and phosphorus in the leaves of *Vicia faba* L. (g kg^{-1}).

Treatment	Mg	Ca	K	P
EO 0R	1.88 (±0.11) a [1]	11.31 (±0.47) b	12.02 (±0.66) bc	8.01 (±0.20) a
EO R	1.82 (±0.02) a	12.36 (±0.01) b	14.07 (±0.44) d	8.82 (±0.39) a
DF 0R	1.94 (±0.06) a	8.12 (±0.26) a	10.61 (±0.28) a	8.52 (±0.17) a
DF R	2.54 (±0.09) b	12.64 (±0.25) b	18.77 (±0.35) e	11.67 (±0.31) b
P 0R	2.46 (±0.08) b	15.47 (±0.16) c	10.76 (±0.34) ab	7.83 (±0.30) a
P R	2.62 (±0.25) b	19.30 (±0.20) d	11.95 (±0.71) bc	8.25 (±0.71) a
C 0R	2.55 (±0.03) b	15.61 (±1.30) c	12.88 (±1.30) cd	11.98 (±1.74) b
C R	2.88 (±0.05) c	17.96 (±0.02) d	10.93 (±0.02) ab	8.11 (±0.56) a

[1] The means (±SE) in the columns that are marked with the same letters did not differ significantly according to the LSD test at $p < 0.05$. The symbols are the same as those in Table 3.

In all of the treatments, the ZB-01 biopreparation generally caused an increase in the content of the studied nutrients in the leaves of the plants. The exception was the content of potassium and phosphorus in the control plants (a decrease after the application of the ZB-01 biopreparation).

All of the PDSs resulted in a significantly higher content of cadmium and lead in the leaves of the broad bean (Table 7). The highest level of Pb (6.81 mg kg^{-1}) was recorded for the EO contamination. Furthermore, the P contamination caused an increase in zinc and copper. The plants that were grown in soil that had been contaminated with EO, however, had a lower content of iron, manganese and copper in the leaves, and those that were grown in the DF contamination had a lower content of zinc and copper than the control plants. There was no significant effect of PDSs on the content of nickel in the broad bean.

The ZB-01 biopreparation caused an approx. 20% decrease in the content of manganese in the leaves of the plants that were grown in PDS-contaminated soils and a decrease in the content of lead to a level that was similar to that in the control plants in the case of the EO treatment. In the DF treatment, this resulted in an increase in the content of some of the heavy metals, e.g., zinc (by 57.7 mg kg^{-1}), copper (by 1.8 mg kg^{-1}), lead (by 2.5 mg kg^{-1}) and cadmium (by 0.2 mg kg^{-1}).

PCA was performed in order to determine the relationship between the analysed parameters in the plants. Figure 6 presents the first two components, which represent a combined 96.8% variance. The strongest correlations with the first axis appeared in POD activity, the content of cadmium and the content of C_{12}–C_{36} hydrocarbons. The activity of SOD was negatively correlated with the second axis. The analysis showed a significant effect of bioremediation on the studied parameters in the samples as well as on the morphological features of the plants, in addition to the effect of a specific contaminant on the activity of POD and proline.

Table 7. The effect of PDSs and bioremediation on the content of the selected microelements and heavy metals in the leaves of *Vicia faba* L. (mg kg^{-1}).

Treatment	Fe	Zn	Pb	Mn	Cd	Cu	Ni
EO 0R	321.65 (±4.0) a [1]	174.10 (±7.0) bc	6.81 (±0.1) cd	85.68 (±2.3) b	1.60 (±0.1) d	8.16 (±0.1) a	1.33 (±0.1) a
EO R	385.84 (±12.8) cd	208.56 (±5.2) d	4.14 (±2.5) ab	70.30 (±7.2) a	1.75 (±0.2) d	15.10 (±0.5) d	1.41 (±0.1) a
DF 0R	394.09 (±10.9) cd	123.27 (±3.0) a	5.73 (±0.2) bc	113.54 (±2.9) cd	1.14 (±0.2) b	12.10 (±0.4) b	1.27 (±0.1) a
DF R	409.08 (±9.3) d	181.01 (±3.1) c	8.26 (±0.7) d	90.21 (±3.6) b	1.38 (±0.1) c	13.92 (±0.4) c	1.43 (±0.2) a
P 0R	353.97 (±21.9) b	180.68 (±6.0) c	6.04 (±0.5) c	125.69 (±2.7) d	1.74 (±0.1) d	14.98 (±0.7) d	1.47 (±0.2) a
P R	450.19 (±23.0) e	178.57 (±17.5) c	4.04 (±0.1) ab	103.49 (±12.7) c	1.36 (±0.1) c	16.61 (±1.0) e	1.47 (±0.1) a
C 0R	368.00 (±17.2) bc	161.69 (±14.5) b	3.71 (±0.6) a	118.34 (±13.4) d	0.85 (±0.1) a	13.95 (±0.2) c	1.25 (±0.1) a
C R	439.13 (±14.4) e	243.63 (±3.7) e	4.11 (±0.7) ab	179.29 (±4.7) e	0.88 (±0.1) a	12.41 (±0.1) b	1.45 (±0.1) a

[1] The means (±SE) in the columns that are marked with the same letters did not differ significantly according to the LSD test at $p < 0.05$. The symbols are the same as those in Table 3.

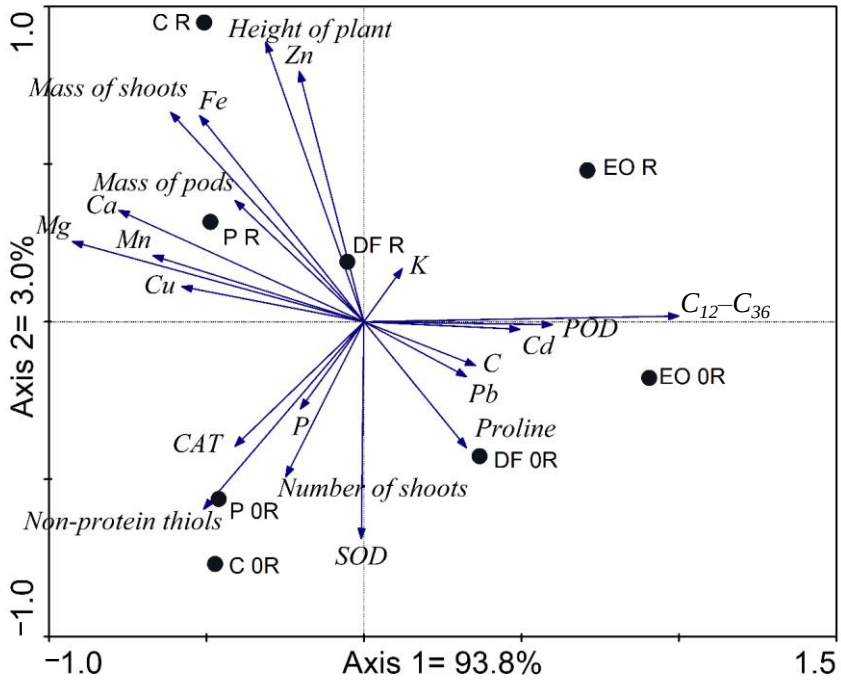

Figure 6. Principal component analysis that was performed on the broad bean biochemical parameters and content of elements in the plants that were grown in PDS-contaminated soils. The symbols are the same as those in Table 3.

4. Discussion

4.1. Petroleum-Derived Hydrocarbons and Heavy Metal Concentrations in Soil Samples

Five years after the soil contamination (when the presented experiment was performed), the content of C_{12}–C_{36} hydrocarbons (particularly in the soils that had been contaminated with EO and DF) was still much higher in all of the analysed soils than in the control soil and the maximum permissible contents, which indicates the persistence of these compounds in the natural environment. According to Polish norms [40], the maximum permissible contents of the tested hydrocarbons for a soil type such as the experimental soils (subgroup II-2) are as follows: 1 mg kg^{-1} for C_6–C_{12} hydrocarbons and 50 mg kg^{-1} for C_{12}–C_{36} hydrocarbons. PDSs can remain in the soil for as many as 12 years following contamination, and their rate of decomposition not only depends on their concentration, but also on the structure of the compounds of which they are composed and on the physicochemical properties of the soil that was contaminated [41]. In its composition, petrol contains considerable amounts of volatile substances which are able to quickly evaporate from the soil; furthermore, it is characterised by a greater susceptibility to natural biodegradation [42]. Hence, the content of petroleum-derived hydrocarbons in the soil that had been contaminated with petrol was only slightly higher than in the control

soil. The applied ZB-01 biopreparation resulted in a marked drop in the content of mineral oil hydrocarbons in all of the contaminated soils (Table 3).

An analysis of the acid-extracted heavy metals in the soils indicated that the soil that had been contaminated with PDSs had high levels of Zn, Pb, Cd, Mg and Ni (Figure 3). However, it should be emphasised that none of the recorded contents exceeded the permissible content of risk-causing substances in soils according to the Polish norms [40]. The maximum permissible contents of the tested heavy metals for the experimental soils (subgroup II-2) were as follows: Zn—500 mg kg^{-1}; Cd—3 mg kg^{-1}; Cu—150 mg kg^{-1}; Ni—150 mg kg^{-1}; and Pb—250 mg kg^{-1}.

4.2. Plant Growth

In the presented experiment, DF and, to a lesser extent, EO were the most detrimental to broad bean plants (Table 4). As has been shown by many authors [43–45], PDSs adversely affect the morphological features of plants, including plants from the Fabaceae family [46]. However, the above-mentioned studies were conducted immediately after PDS contamination, and the subsequent effects of these pollutants were not examined. In our investigations of the broad bean, it was indicated that even after five years there was a marked limitation of growth, which shows its high sensitivity. Małachowska-Jutsz and Miksch [47] emphasised that the hydrocarbons contained in PDSs accumulate in plant roots, and therefore their negative effects can be long-lasting. They have an adverse effect on the transpiration and respiration processes in plants in addition to the transportation of nutrients through cell membranes [48], and they result in a decrease in the content of the nutrients that are available to plants [49]. Additionally, these compounds contribute to an increase in soil density, which results in clogging soil pores and, as a result, leads to changes in the physical, chemical and biological properties of the soil, which then disturb the water and nutrient uptake of plants [44].

Generally, the applied ZB-01 preparation had a beneficial effect on the growth of the broad bean (Table 4). In the available references, there are only limited data concerning the effect of using various techniques of supported bioremediation on the growth of plants; applying compost [50] or bird droppings [51] to the contaminated soil resulted in improved growth and development of plants.

4.3. Biochemical Parameters of the Plants

In the presented experiment, PDSs caused an increase in the activity of POD and SOD (except for EO) and a decrease in the activity of CAT (Figure 5). Marti et al. [52] found that soil contamination with refinery sludges containing petroleum-derived hydrocarbons resulted in an increased activity of SOD and POD in the leaves of alfalfa (*Medicago sativa* L.). According to the authors, the induction of the activity of SOD in plants that were grown in the presence of this sludge could be favoured by higher concentrations of zinc, iron and manganese in the leaves [52]. In our study, this might be associated with the increased content of Zn (in the petrol-contaminated soil) as well as Cd and Pb (in all of the contaminated soils) (Table 7). The increases in POD activity in the plants following the presence of heavy metals (which were also present in PDSs—Table 2) in the soil has also been observed by many authors, and therefore this activity has been used as a non-specific biomarker of environmental pollution [12,35,36,52,53]. Increases in POD activity protect plants from the toxic effects of H_2O_2. This protection might be the result of a strategy that is adopted by plants to survive under stress [54]. Lead contamination of soil results in an increase in POD activity in the leaves of *Vicia faba*, which is markedly different than CAT, whose activity decreases [55]. A decrease in the activity of CAT was also found in the aboveground parts of *Mirabilis jalapa*, which was caused by spent engine oil contamination [24], as well as in the leaves of cowpea and maize seedlings that had been contaminated with kerosene, diesel, engine oil and petrol [23], which is in agreement with our results.

Soil contamination with heavy metals resulted in a decreased content of the non-protein thiol groups in the leaves of *Cardaminopsis arenosa*, *Plantago lanceolata* and *Philadel-*

phus coronarius [12,36]. In the experiment presented here, five years after the soil contamination, PDSs caused a decrease in the content of the non-protein thiol groups in the leaves of broad bean plants. However, other authors have observed that soil contamination with cadmium [56], lead, copper and nickel [35] might lead to an increase in the content of the –SH groups in the leaves of plants. Sulfur-containing molecules are present in many plant cells and perform a number of diverse functions, which is why they can be regulated independently; this might also explain the existing discrepancies [57]. The content of proline in plants often increases in response to the presence of heavy metals [12,35,36,58]. In the presented experiment, PDSs also caused an increase in proline in the leaves of the broad bean, especially in the case of the DF contamination. As was described by Pilipović et al. [22] and Merkl et al. [59], an increased accumulation of free proline in plants that have been exposed to crude oil contamination might be the result of a disturbed water regime. Water availability could be critical, since oil-impregnated soil does not pass water homogeneously. The results of the soil moisture analysis that were performed a year earlier (i.e., in 2014) showed its highest level in the DF-treated soil [6].

Assisting the bioremediation process using the ZB-01 preparation in the DF treatment resulted in a decrease in the activity of SOD, POD and CAT, as well as in a decrease in the content of proline. The level of this antioxidant was three-fold lower in the leaves of the broad bean plants that had been exposed to DF and then bioremediated than in the non-bioremediated plants. Therefore, this might indirectly indicate that by contributing to the decomposition of the hydrocarbons that are dangerous to plants (as was confirmed by a soil analysis of DF contamination), ZB-01 can reduce the level of stress in plants. In the EO-contaminated plants, however, a reverse relationship was found, i.e., a slightly higher level of proline after ZB-01 was applied, which was similar to the activity of POD. This phenomenon requires more research.

4.4. Element Concentration in the Plant Samples

DF contamination caused a decrease in the content of nitrogen in broad bean leaves (Table 5). Other research has confirmed that the contamination of soil with PDSs can cause a decrease in the content of N in plants [60]. In soil that has been contaminated with PDSs, the nitrogen/carbon ratio is disturbed, which restricts many nitrogen reactions in the soil and decreases the intensity of the ammonification and nitrification processes [61]. In the conducted experiment, PDSs generally had no effect on the content of carbon in the plants, except for the DF contamination where the content of carbon decreased. Because PDSs contain aliphatic hydrocarbons, cycloalkanes, olefins and arenes, they can cause an increase in the content of carbon in soil [62]. However, for plants, the principal source of carbon is atmospheric air. A lower content of carbon in the DF-treated plants could have been the result of disturbances in photosynthesis, which was also confirmed by their limited growth. Moubasher et al. [63] found that petroleum-derived hydrocarbons most often failed to cause changes in the sulfur content in the roots and shoots of *Bassia scoparia* (L.). In our experiment, EO contamination led to an increase in the content of sulfur in the leaves of the broad bean by approximately 60%, while the presence of P contamination resulted in a decrease in the content of sulfur by 26%. Sulfur is mainly attached to high-molecular-weight polycyclic aromatic hydrocarbons (PAHs), which are present in EO. The degradation of PAHs might have caused an increase in the availability and uptake of sulfur by the plants under the EO treatment. However, an analysis of the sulfur content in the soil that had been treated with $CaCl_2$ did not exhibit any differences from the control soil (Figure 2).

Generally, during our experiment, EO and DF contamination caused a decrease in the content of calcium, magnesium, phosphorous and potassium in *V. faba* leaves (Table 6). This was reflected in the content of these elements in the soil (Figure 1). P contamination did not affect the content of magnesium and calcium, but it did result in a decrease in the content of potassium and phosphorus in the leaves. This corresponds with the results of other authors for oat and spring rape [64]. Higher concentrations of potassium in the roots

and phosphorus in the shoots and roots were also found in alfalfa plants that were growing in soil that had been contaminated with refinery sludge [65].

When the ZB-01 biopreparation was applied to the DF-contaminated plants, it resulted in an increase in the content of nitrogen and carbon in the leaves to a level that was similar to that in the control plants, while in the P-contaminated plants it resulted in a decrease in the content of N and C by 15% and 2%, respectively (Table 5). In the EO-contaminated plants, the application of the ZB-01 biopreparation resulted in a decrease in the content of sulfur in the leaves, making it was much closer to the level that was found in the control plants. In almost all of the treatments, the ZB-01 biopreparation caused an increase in the content of Ca, K and P in the leaves of the plants (Table 6). In the available references, there is very little information on this topic. This kind of bioremediation can reduce the differences in the content of some macronutrients between wheat plants that are growing in PDS-contaminated soils and those that are growing in control soils [6], which corresponds to the results of the present experiment.

Five years after the PDS contamination, all of the PDSs continued to maintain a significantly higher content of cadmium and lead in the leaves of the broad bean (Table 7). The highest level of Pb was recorded for the EO contamination. Furthermore, P contamination caused an increase in zinc and copper. The source of these last two elements could have been the P contaminant, which was confirmed by an analysis of heavy metals in the studied PDSs (Table 2). Moreover, the analysis of Pb in the EO-contaminated plants showed a much higher level than in the case of the other two PDSs. The observed effect in plants in terms of Pb was confirmed by the Pb levels in the soil that had been contaminated with EO (Figure 3), which had the highest level among all of the soils that were studied. The zinc content in soil that had been contaminated with P was also higher than in the control soil. The soils that had been contaminated with PDSs were also characterised by a higher cadmium content than the control soil, which explains the increased content of this element in the broad bean leaves. Nwachi et al. [66] observed that PDSs caused an increase in the content of lead and zinc in the leaves of *Vernonia amygdalina*, *Talinum triangulare*, *Manihot esculenta* and *Xanthotosoma sagittifolium*. The plants that were growing in the soil that had been contaminated with EO, however, had a lower content of iron, manganese and copper in the leaves, and those that were growing in the DF contamination conditions had a lower content of zinc and copper (Table 7). These results are mostly in line with those concerning the levels of these elements in soils (Figures 1 and 3). There was no significant effect on the nickel content in the broad bean plants. The content of acid-extracted Ni was only significantly higher in the soil that had been contaminated with EO than in the control soil (Figure 1); however, the potentially biodegradable fraction of this element was below the detection level in most cases (Figure 2). The effect of PDSs on the content of heavy metals varies and depends on the kind of component being analysed, the doses and the applied contaminant, as well as the analysed part of plant [60]. Undoubtedly, the length of time after the PDS contamination is also important.

The ZB-01 biopreparation caused a decrease in the content of manganese in the PDS-contaminated plants by approximately 20% and a decrease in the content of lead to a level that was similar to that in the control plants in the case of the EO treatment. In the DF treatment, however, it resulted in an increase in the content of some heavy metals, e.g., zinc (by 47%), copper (by 15%), lead (by 44%) and cadmium (by 21%) (Table 7). This indicates the varied effects of the ZB-01 biopreparation, depending on the kind of analysed metal as well as on the kind of contaminating substance. The decrease in the content of some heavy metals after the application of the ZB-01 biopreparation might have resulted from the ability of micro-organisms to utilise harmful substances in their growth and development [67]. The analysis of the effect of ZB-01 on the levels of heavy metals in the soil only partly confirms this statement—the ZB-01 biopreparation contributed to a significant decrease in the level of acid-extracted manganese, lead and nickel in the soil that had been contaminated with DF. In regard to the potentially bioavailable fraction, such a beneficial effect was demonstrated for manganese for the EO contamination and

cadmium for the DF contamination. The increase in the content of some heavy metals in DF treatment after using the ZB-01 biopreparation could suggest a limitation of its use on agricultural land. A comparison of the Pb and Cd concentrations in broad bean leaves from the DF R treatment with the relevant regulations [68,69] showed that Cd was below the maximum permissible concentrations in foodstuffs, while Pb exceeded this level (for the comparison, we assumed that the fresh mass of broad beans = 7.16 × dry mass, based on the measurement). However, it is broad bean seeds, not leaves, that are eaten. Still, the increased content of lead indicates that the content of heavy metals in the plants that were grown in diesel-fuel-contaminated soils and then subjected to microbiological remediation should be monitored.

In conclusion, the observed changes in the content of nutrients in *V. faba* plants might lead to a deterioration in the quality of herbivore food, and this may have consequences for further links in the trophic chain, i.e., for predators, parasitoids and human consumers. An increase in the content of heavy metals in plants might cause them to lose their value for consumption and fodder purposes. This indicates a need to monitor their content when we introduce plants into areas that have been exposed to PDS pollution. The obtained results also indicate that the broad bean is very sensitive to the presence of PDSs in the soil, which was indicated even five years after contamination by changes in growth, the content of nutrients and heavy metals, the activity of antioxidant enzymes and the content of antioxidants. This indicates its usefulness in bioindication (e.g., via POD activity or content of proline), but also that it should not be considered to be introduced into areas that have been exposed to PDSs during their restoration.

Moreover, it is worth emphasising that we observed positive effects of the applied ZB-01 biopreparation on the condition of broad bean plants, namely in an improvement of morphological parameters, an increase in the content of some macronutrients and a decrease in the activity of antioxidant enzymes and the content of proline in the case of DF treatment. This suggests its usefulness in restoring the suitability of soils that have been contaminated with petroleum-derived substances for agricultural production. ZB-01 contains bacterial strains that naturally occur in the environment, which are specialised in the decomposition of organic pollutants. The bacterial population decreases as the petroleum products decompose, thereby allowing typical microflora to develop in the soil [19]. For a period of 24 months after contamination the content of TPHs was monitored once a month, not just in the soils that had been contaminated with PDSs but also in the control soil (both with and without ZB-01) [26]. The addition of ZB-01 did not significantly disturb the content of TPHs in the non-contaminated soils at any time during the analysis, which could also indirectly indicate the absence of a negative effect from the circulation of ZB-01 in the soil. The production cost of the biopreparation was not high, and therefore it could be used on a large scale. This rich, balanced biocenosis was created by isolating micro-organisms from many samples of contaminated soil and water from a variety of locations in Poland; therefore, it could be used in this experiment without a long-lasting and tedious (as well as cost-prohibitive) process of making a specialised preparation using autochthonous micro-organisms that can be obtained from a specific soil batch intended for purification [70]. At the same time, its effectiveness could be continuously improved by adding such autochthonous micro-organisms in a situation in which the pollution is untypical or difficult to biodegrade.

It should also be emphasised that in our experiment the aim of the biopreparation was to initiate the process of spontaneous soil cleaning, and therefore it was applied only twice, i.e., one week and one year after soil contamination. Despite this, the effect of its action in the form of the clearly improved condition of plants was visible even after five years.

5. Conclusions

- Even five years after contamination with PDSs, engine oil and diesel fuel adversely affected the morphological features of broad bean plants. This confirms the susceptibility of the analysed plant to this kind of contamination, and also indicates the durability

of the xenobiotics in the soil environment, which was confirmed by chemical analysis of the soil;

- The applied contaminants significantly affected the activity of antioxidative enzymes and the content of antioxidants, which resulted in a significant increase in the activity of POD and a higher content of proline content in the plant leaves;
- The contamination of the soil with spent engine oil and diesel fuel caused a decrease in the content of calcium, magnesium and phosphorus in the plants. Diesel fuel also decreased the content of potassium and nitrogen while engine oil decreased the content of iron. Petrol had no significant effect on the content of the majority of the analysed nutrient components in the leaves of the broad bean. The introduction of the biopreparation into non-contaminated soil decreased the content of potassium and phosphorus as much as contamination with petroleum products;
- PDSs significantly modified the content of heavy metals in the plants. In particular they increased the content of lead and cadmium, while some of them caused a decrease in the content of zinc (diesel fuel), manganese (engine oil) and copper (engine oil and diesel fuel);
- The applied ZB-01 biopreparation had a generally beneficial effect on the analysed morphological features of the plants and contributed to lowering the activity of the analysed antioxidative enzymes, as well as to a decrease in the antioxidants in the plants from the soil that had been contaminated with the diesel fuel. Furthermore, ZB-01 most often caused an increase in the nutrients in the leaves of the plants. However, its effect on the content of heavy metals varied depending on the contaminant and the analysed metal.

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