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The Potential for Restoring the Activity of Oxidoreductases and Hydrolases in Soil Contaminated with Petroleum Products Using Perlite and Dolomite

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Abstract: The research focused on assessing the response of oxidoreductases (dehydrogenases and catalase) and hydrolases (urease, acid phosphatase, alkaline phosphatase, arylsulfatase, and *β*-glucosidase) to diesel oil (DO) and gasoline (G) contamination of soils subjected to phytoremediation with *Zea mays*. The activity of enzymes constitutes one of the fundamental mechanisms for the removal of contaminants from soil, which have the potential to contaminate not only the soil but also groundwater and water reservoirs. Additionally, correlations between enzyme activity and the basic physicochemical properties of the soil were determined. The interaction of perlite and dolomite with soil enzymes and the cultivated plant was also tested. The study was carried out in a pot experiment, where soil contaminated with DO or G was artificially treated at doses of 0, 8 cm^3, and 16 cm^3 kg^{-1}. Perlite and dolomite were applied for remediation at doses of 0 and 10 g kg^{-1} of soil. *Zea mays* was found to respond to the tested pollutant with a reduction in biomass. DO affected the growth of this plant more than G. DO reduced the yield of aerial parts by 86% and G by 74%. The negative effects of these pollutants on the growth and development of *Zea mays* were mitigated by both perlite and dolomite. DO exerted greater pressure than G on the activity of oxidoreductases and hydrolases, as well as on the physicochemical properties of the soil. DO enhanced the activity of oxidoreductases and most hydrolases, whereas G inhibited them. The implementation of dolomite intensified the activity of all enzymes, except AcP (acid phosphatase) and Glu (*ß*-glucosidase), in soil contaminated with DO and G, and also improved its physicochemical properties. Perlite induced less significant effects than dolomite on soil enzymes and the physicochemical properties of the soil.

Keywords: adsorption; petrochemical pollutants; soil enzyme activity; phytoremediation

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1. Introduction

Petroleum and petrochemical products are among the most common pollutants worldwide [1,2]. This is due to their use as energy sources [3]. As hydrophobic pollutants [4], these products are classified as hazardous organic pollutants [5]. They disrupt the stability of ecosystems [6,7], leading to the loss of fundamental functions [8,9], and consequently, deteriorate soil quality and fertility [10–12]. Petroleum-derived substances cover the surface of soil aggregates with a thin layer, and hydrocarbons bind to organic matter [13]. These products destroy the colloidal structure of the soil [14]. They disrupt the water, air, and sorption properties of the soil, directly and indirectly affecting the disturbance of biological life in the soil environment at different trophic levels [15,16].

Hence, it is important to pay attention to the actual threats posed by the effects of these pollutants on living organisms [17,18], as well as the uncontrolled spread of petroleum-derived substances in the natural environment [19,20]. Petrochemical products lead to the destabilization of soil health [19,21], contributing to the formation of anaerobic conditions in the soil, leading to the phenomenon known as soil necrosis. In such environments,

anaerobic bacteria capable of transforming petroleum hydrocarbons thrive more intensively [22,23]. This inhibits the ability of plants to absorb water and mineral salts from the soil and leads to a loss of root hair formation capability [16,24]. Additionally, there are also changes in the structure of the soil microbiome [16,25,26], soil microfauna [15,27], and enzymatic activity [25,28,29].

Enzymatic activity is increasingly being utilized not only to assess the fertility and productivity of arable soils [30,31] but also to assess the quality and stability of degraded soil ecosystems [32,33]. Lee et al. [34] and Yang et al. [35] emphasize the importance of oxidoreductases and hydrolases in diagnosing the remediation needs of contaminated soils. Representative enzymes that facilitate the diagnosis of soil quality include dehydrogenases, β-glucosidase, urease, arylsulfatase, and phosphatases [34,36]. As intracellular enzymes, dehydrogenase activity reflects the real-time activity of the soil microbiome [37]. Conversely, extracellular enzymes are released from living or dead cells and form complexes with soil organic matter or humus–clay complexes [38]. They participate in catalyzing the decomposition of organic matter [25,37,39] and constitute approximately 40–60% of the total enzymatic activity of the soil [34,36]. Considering the crucial role of enzymes in biogeochemical cycles and their sensitivity to various stress factors, they are considered to be good and rapid diagnostic indicators for assessing ecosystem responses to environmental changes [40].

In the context of the above considerations, the protection of soil resources is of crucial importance [19,41]. Therefore, research that contributes to the development of a multifaceted strategy aimed at developing innovative remediation methods [3,42–44] is essential. Such methods will help to protect the environment by minimizing the problem of soil contamination with petroleum products [19,45]. Currently, research that is environmentally friendly and characterized by high pollutant degradation efficiency is being promoted [46,47]. These studies focus mainly on bioremediation [43,48,49]. Mekonnen et al. [3] emphasize that between 2020 and 2022, 385 publications dealing with biological soil remediation techniques for hydrocarbon-contaminated soils were published in the Scopus database alone. However, most of the research conducted in the last decade has focused on the degradation of one or two components of petroleum refinery pollutants [50]. There is limited research on the remediation of areas contaminated with petrochemical products, which are mixtures of various simple and complex hydrocarbons.

Among the potential means to improve the health of soil subjected to various contaminants, sorbents such as biochar [46,49,51], halloysite [11,52,53], alginate [11], dolomite [54], sepiolite [52,55], perlite [56], zeolite [49,57,58], kaolinite [49], and vermiculite [49] can be considered. Mineral sorbents, both natural and synthetic, are of particular interest to researchers. The most important of their many advantages, apart from their recyclability [59], is their sorption capacity towards petroleum products, which varies between 0.20 and 0.50 (g petroleum products g^{-1} of sorbent) [60]. Bulk density is also an important parameter that increases their attractiveness, and zeolite is one of the more well-characterized sorbents, with perlite used in its synthesis [61]. The modification of zeolite by perlite is based on expansion [62], whereas dolomite is modified by thermal treatment, defined as calcination. This process induces the transformation of $CaCO_3$ and $MgCO_3$ into CaO and MgO in dolomite, which leads to an increase in the total and short-term alkalinity of this sorbent [63]. In turn, the effectiveness of perlite in soil remediation is supported by its pore diameter, which ranges from 10 µm to 100 µm [64]. According to Rios-Valenciana et al. [65], the application of perlite to soil is a cost-effective strategy for the aerobic biodegradation of organic pollutants. The technology based on the utilization of sorbents can be integrated with phytoremediation, which has recently received increased attention [66,67].

In light of information regarding soil contamination with petroleum products, the authors carried out a study to determine the effect of two different petrochemical substances on *Zea mays* biomass and the activity of soil enzymes from the oxidoreductase and hydrolase classes. Additionally, the role of dolomite and perlite in neutralizing disturbances caused by these products in the soil environment was assessed. The following research hypotheses

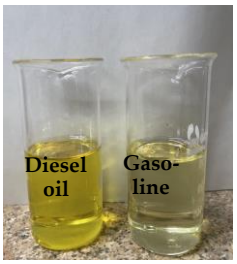
were formulated: (1) petroleum-derived substances induce soil ecological dysfunction, leading to disturbances in the development of *Zea mays* and destabilization of soil enzymatic properties; (2) the degree of soil dysfunction depends on the type of petroleum product; and (3) the implementation of dolomite and perlite reconstitutes soil homeostasis under the pressure of diesel oil and gasoline.

2. Materials and Methods

2.1. Soil, Petroleum Products, and Sorbents

The soil, free of any contaminants, was collected from agricultural fields (0–20 cm) in the vicinity of Olsztyn (Warmian-Masurian Voivodeship, Poland). All soil samples were homogenized and sieved through a 5 mm mesh sieve. Additionally, soil samples designated for grain size analysis, organic carbon content (C_{org}), total nitrogen (N_{total}), physicochemical properties, and soil enzyme activity (Table 1) were sieved through a 2 mm mesh sieve. Table 1 also presents the characteristics of petroleum products, and sorbents used in the study.

Table 1. Characterization of soil, petroleum products, sorbents, and plants.

Parameter	Characteristic
	Eutric Cambisol with a particle size distribution of loamy sand. Content in %: sand—73.46; silt—24.29; clay—2.25. Content per 1 kg d.m. of soil: C_{org}—6.95 g, N_{Total} —1.06 g, HAC—34.56 mmol$^{(+)}$, EBC—44.82 mmol$^{(+)}$, CEC—79.38 mmol$^{(+)}$, BS—56.46%, pH_{KCl} 4.2. Enzyme activity per 1 kg d.m. of Deh—12.863 µmol TFF, Cat—0.161 mol O_2, Ure—0.709 mmol N-NH_4, AcP—2.499 mmol PN, AlP—0.393 mmol PN, Glu—0.260 mmol PN, Aryl—0.098 mmol PN.
	**Diesel oil.** Premium fuel for Diesel engines, purchased from PKN Orlen (Poland). Density: 0.820–0.845 g cm^{-3}, sulfur content—maximum 10 mg kg^{-1}. The detailed characteristics are available on the PKN Orlen website [68]. **Unleaded gasoline 95.** Fuel for gasoline engines of vehicles, purchased from PKN Orlen (Poland). Density: 0.720–0.775 g cm^{-3}, sulfur content—maximum 10 mg kg^{-1}. The detailed characteristics are available on the PKN Orlen website [69]. The diesel oil and gasoline were applied in the experiment at doses of 0, 8, and 16 cm^3 kg^{-1} d.m. of soil.
	**Dolomite.** Ground sedimentary rock with a pH of approximately 9.0, containing Ca—50.1% and Mg—15.8% [54]. **Perlite.** A quartz mineral extracted from volcanic rocks with a pH of approximately 7.0, characterized by an amorphous porous structure. It contains SiO_2—about 73% *w*/*w*, Al_2O_3—about 15% *w*/*w*, Ca—0.36–1.07%, and Mg—0.12–0.42% [56,70]. The dolomite and perlite used in the study were provided by Biovita Sp. z o.o., Tenczynek, Poland. The sorbents were applied in the experiment at doses of 0 and 10 g kg^{-1} d.m. of soil.
	Along with wheat and rice, maize (*Zea mays*) is one of the most important cereals cultivated on Earth [71]. As a C4 plant, maize is very adaptable to different environmental conditions. The global area under maize (for grain) is 197 million hectares and is increasing steadily. According to OECD-FAO [72], global maize production is expected to reach 1.36 billion tons in 2032. In the experiment, hybrid maize of the DS1897B variety (Producer Pioneer, Warsaw, Poland) was grown which can be used for feed and biogas. It is a late-maturing variety [73]. In the study, maize was grown with 4 plants per pot for 60 days. The plants were harvested at growth stage 59 of the BBCH (Biological Bundesanstalt, Bundessortenamt, and Chemical).

2.2. Experimental Design

The basis of the research consisted of a three-factorial pot experiment conducted in a split plot design, with four replications. The vegetative pot experiment was conducted in the greenhouse of the University of Warmia and Mazury in Olsztyn, Poland (NE, Poland, 53.759° N, 20.454° E). The first-order factor was the type of petroleum product: control, diesel oil, and unleaded gasoline 95; the second-order factor was the dose of the contaminating product, in $cm^3\ kg^{-1}$ d.m. of soil: 0, 8, and 16; and the third-order factor was the type of sorbent: control, dolomite, and perlite. The first step in setting up the experiment was to mix aqueous solutions of $CO(NH_2)_2$, KH_2PO_4, KCl, $MgSO_4 \times 7H_2O$ with soil (3.4 kg pot^{-1}), followed by the addition of the respective petroleum products and sorbents in the designated pots. Subsequently, the soil material was then placed in pots with dimensions of 14.5 cm (ϕ base diameter) × 19.5 cm (ϕ top diameter) × 16.5 cm (height) and moistened to 60% of the maximum water holding capacity by watering the plants 3–4 times a day with demineralized water. In the end, there were 72 pots in the experiment. A total of 250 kg of soil was used. Fertilization with N, P, K, and Mg was constant throughout the experiment (uncontaminated and contaminated treatments, with and without sorbent application), with the following amounts per kg of soil: N—225 mg, P—50 mg, K—150 mg, and Mg—20 mg. The aqueous solutions were added to the soil once on the day the experiment was set up to cover the nutrient requirements of the maize. This fertilization was the same throughout the experiment. The next step involved sowing maize into the pots. The duration of the pot experiment was 60 days. On the day of maize harvest, the Chlorophyll Meter (Spectrum Technologies, Inc., KONICA MINOLTA, Inc., Chiyoda, Japan) was used to determine the leaf greenness index (SPAD). Subsequently, the plants were harvested, and aerial parts and roots were carefully separated, rinsed with distilled water, and dried in a Binder D-78532 dryer (Binder GmbH, Tuttlingen, Germany) at 60 °C. Soil samples were also collected on the day of plant harvest for further laboratory analysis.

2.3. Methodology of Soil Property Determinations

The activity of selected enzymes from the oxidoreductase and hydrolase classes was determined in the soil samples, both before the experiment setup and after its completion (Table 2). The concentration of the released product in the case of Deh, Ure, AcP, AlP, Aryl, and Glu was determined using a Perkin-Elmer Lambda 25 spectrophotometer (Waltham, MA, USA). In the air-dried soil, the content of total organic carbon and nitrogen was determined using a macroanalyzer Vario MaxCube CN (Hanau, Germany), soil pH in 1 mol KCl dm^{-3}. An aqueous 0.5 M calcium acetate solution was used to determine hydrolytic acidity (HAC), and an aqueous 0.1 M hydrochloric acid solution was used to determine the sum of exchangeable base cations (EBC). The filtrates were titrated in the presence of phenolphthalein with an aqueous 0.1 M sodium hydroxide solution. The content of exchangeable cations (CEC) was calculated by summing the results of the HAC and EBC determinations, and then base cations saturation in soil (BS) was calculated according to the formula BS = EBC/CEC × 100%. A detailed description of these methods is given in our previous study [74]. All determinations were performed in 4 replications.

Table 2. Methods of determination of soil enzymes activity.

Enzyme Name	Enzyme Abbreviation	Enzyme Number—International Union of Biochemistry	Circulation of Elements	Substrate	Product	Unit in kg d.m. of Soil per Hour	References
Dehydrogenases							
Dehydrogenases	Deh	EC 1.1	C-cycle	2,3,5-triphenyl tetrazolium chloride	triphenyl fomazan (TFF)	μmol	[75]

Table 2. *Cont.*

Enzyme Name	Enzyme Abbreviation	Enzyme Number—International Union of Biochemistry	Circulation of Elements	Substrate	Product	Unit in kg d.m. of Soil per Hour	References
Catalase	Cat	EC 1.11.1.6	C-cycle	H_2O_2—aqueus solution	O_2	mol	[76]
Hydrolases							
Urease	Ure	EC 3.5.1.5	N-cycle	Urea—aqueous solution	N-NH_4	mmol	[77]
ß-glucosidase	Glu	EC 3.2.1.21	C-cycle	4-nitrophenyl-ß-D-glucopyranoside	4-nitro-phenol (PN)	mmol	[77]
Acid phosphatase	AcP	EC 3.1.3.2	P-cycle	Disodium 4-nitrophenyl phosphate hexahydrate	4-nitro-phenol (PN)	mmol	[77]
Alkaline phospha-tase	AlP	EC 3.1.3.1	P-cycle	Disodium 4-nitrophenyl phosphate hexahydrate	4-nitro-phenol (PN)	mmol	[77]
Aryosulphatase	Aryl	EC 3.1.6.1	S-cycle	Potassium-4-nitrophenyl-sulfate	4-nitro-phenol (PN)	mmol	[77]

2.4. Statistical Analyses

The statistical analysis of the data was conducted using Statistica 13.3 software [78]. Significant differences between treatments were determined using three-way ANOVA analysis ($p = 0.05$) with Tukey's HSD test. Four repetitions were used for statistical calculations. Additionally, the degree of dependence between the variables was assessed. Pearson's correlation analysis ($p < 0.05$) was performed separately for soil contaminated with diesel oil and unleaded gasoline. The methodology and formulas for defining the influence factor (IF) of petroleum products and sorbents are thoroughly described in our previous research. Two soil quality indices were used to assess soil health: BA_1 and BA_2. The BA_1 index considers the activity of seven enzymes investigated in this study and has been extensively described in our prior research [79,80]. Additionally, a modification of index BA_1 was proposed by incorporating the $\%C_{org}$ content ($BA_2 = BA_1 \times \%C_{org}$). To highlight the interrelationships between biochemical, physicochemical properties, and productivity of soils exposed to diesel oil and gasoline, the authors proposed a diagram illustrating the relationships between total biomass of *Zea mays* aerial parts and roots, the activity of oxidoreductases and hydrolases, soil organic matter, CEC, and soil quality index (BA_2). Plots representing plant biomass, SPAD, BA_1 and BA_2 indices, as well as the effect indices of petroleum products and sorbents, were generated using Microsoft Office 365 software [81] and R v1.2.5033 software (Boston, MA, USA) [82] with R v3.6.2 addition [83] and gplots library [84], Principal Component Analysis (PCA) in Statistica 13.3 software [78], and variable loadings in the determination of dependent variables using InteractiVenn software [85].

3. Results

3.1. The Response of Zea mays to DO and G

In pursuit of one of the stated objectives of the study, the response of *Zea mays* was verified on the basis of the dry weight of the above-ground parts of maize grown on soil not contaminated with petroleum products. It was relatively constant, ranging from 69.544 to 74.073 g d.m. pot^{-1} (Figure 1a, Table S1). The differences between these values were not statistically significant. However, the biomass yield obtained from soil contaminated with DO and G was significantly lower. Under the influence of DO at a quantity of 16 g kg^{-1} d.m. of soil, the maize yield decreased by 7.1-fold.

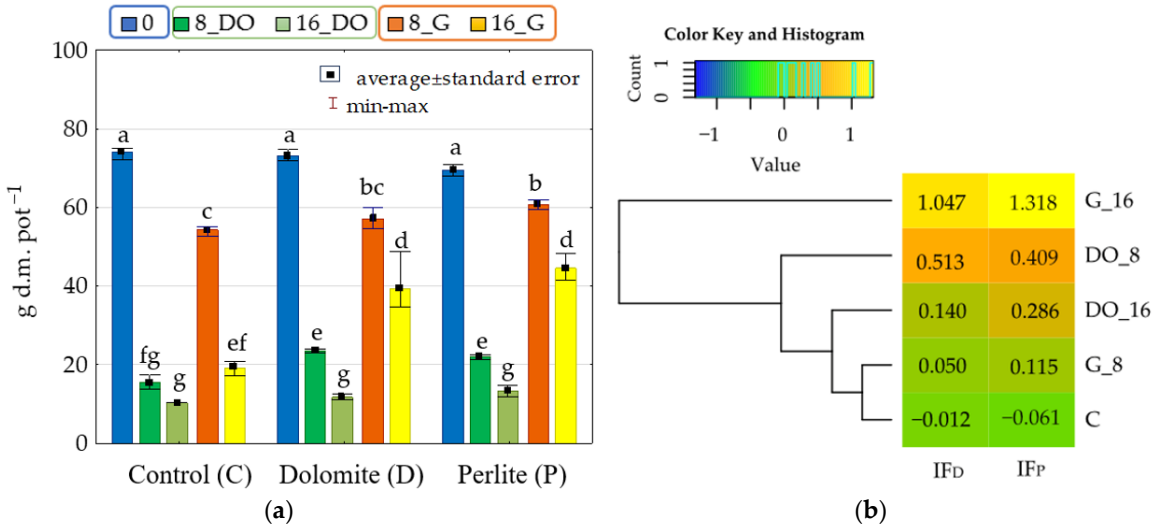


Figure 1. The yield of aerial parts (Ya) of *Zea mays* (**a**) and the impact factor of dolomite (IF_D) and perlite (IF_P) on Ya (**b**). Diesel oil (DO) and gasoline (G) doses, in cm^3 kg^{-1} d.m. of soil: 0 (C), 8, 16. The same letters on the bar graph (a–g) denote homogeneous groups, $p < 0.050$, N = 4.

After the application of dolomite, the diminishment was 6.2 fold, and for perlite, it was 5.2-fold. The negative effect of G on maize was less substantial than that of DO. The highest dose of this product (16 g kg^{-1} of soil) decreased the aerial parts biomass by 3.8 fold, while after the application of dolomite, it was diminished by 1.9 fold, and for perlite, by 1.6-fold. The low values of the dolomite (D) and perlite (P) influence indices on the aerial parts biomass in the control treatments (Figure 1b) indicate that these sorbents when added to soil not degraded by petroleum products, did not exert a significant influence on the physiological processes of maize. However, relatively high values, especially in the DO_8 and G_16 treatments, suggest that the implementation of D and P partially mitigates the negative effects of DO and G on the plant.

Diesel oil (DO) and gasoline (G) affected the chlorophyll index (SPAD) of *Zea mays* leaves (Figure 2a). Their effects were opposite. The application of DO at a rate of 16 g kg^{-1} soil resulted in a decrease in SPAD, whereas G applied at the same dose contributed to a significant increase. In contrast to DO and G, neither dolomite nor perlite modified the intensity of the green color of *Zea mays* leaves, as evidenced by the low values of the influence index (IF_D and IF_P) on the magnitude of SPAD (Figure 2b).

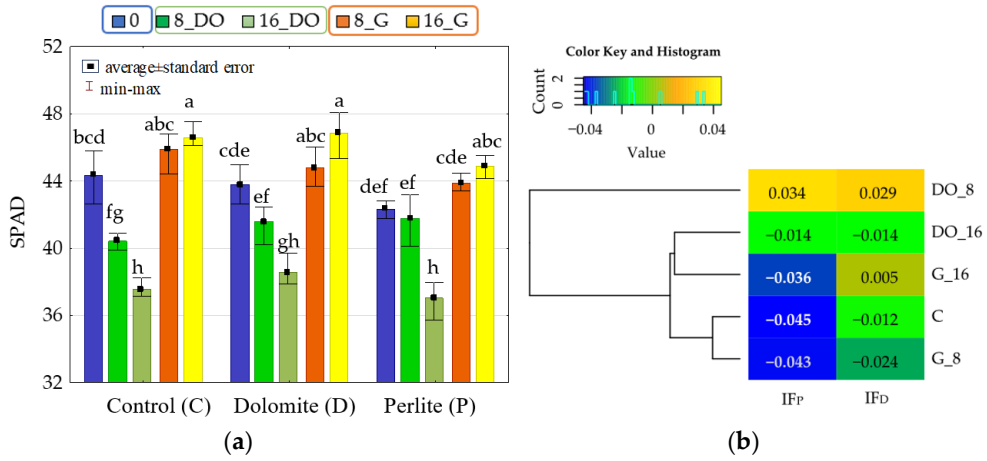
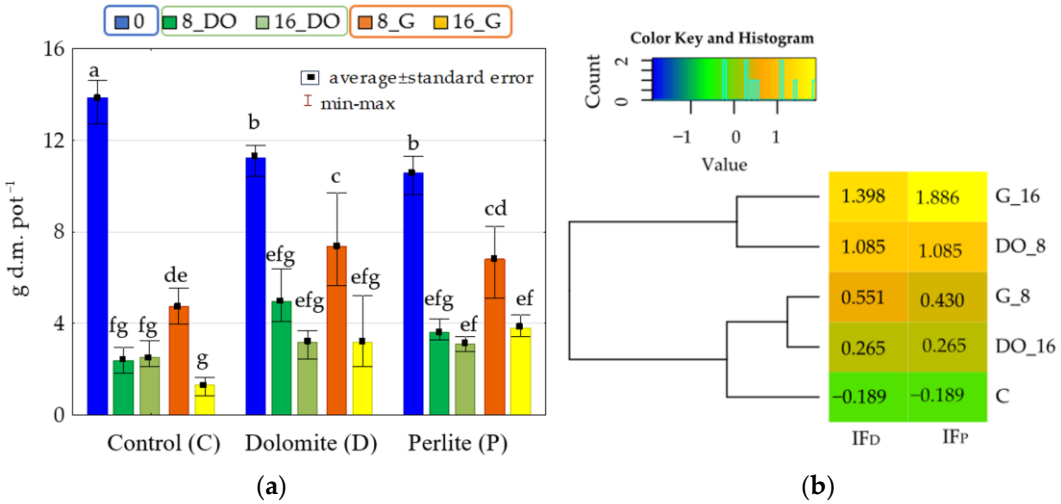


Figure 2. Greenness index (SPAD) of *Zea mays* (**a**) and the influence index of dolomite (IF_D) and perlite (IF_P) on SPAD (**b**). Explanations of abbreviations are provided in Figure 1. The same letters on the bar graph (a–h) denote homogeneous groups, $p < 0.050$, N for each error bar = 4.

Roots play a fundamental role in the response of the whole plant to environmental stress. In the presented study, both DO and G caused the underdevelopment of *Zea mays* roots (Figure 3a). The IF_D and IF_P indices (Figure 3b) demonstrate that both dolomite and perlite reduced the negative effects of DO and G on root growth and development. However, *Zea mays* roots grown in the control soil, unaffected by DO and P, showed reduced development under the influence of the sorbents tested.



(**a**) (**b**)

Figure 3. Root yield (Yr) of *Zea mays* (**a**) and the influence index of dolomite (IF_D) and perlite (IF_P) on Yr (**b**). Explanations of abbreviations are provided in Figure 1. The same letters on the bar graph (a–g) denote homogeneous groups, $p < 0.050$, N for each error bar = 4.

3.2. Soil Enzymes Response to DO and G

One of the main research objectives of the experiment was to determine the effects of DO and G on soil enzyme activity (Tables 3 and S1). The response of individual enzymes was related to the type of oil product pressure. Although both products, DO and G, destabilized the enzymatic properties of the soil, their direction of influence was opposite.

Diesel oil acted as a stimulator of dehydrogenases, catalase, alkaline phosphatase, *β*-glucosidase, and arylsulfatase, while inhibiting acid phosphatase. Conversely, gasoline served as an inhibitor for all enzymes. Therefore, the influence factors of DO (IF_{DO}) on the activity of individual enzymes, except for acid phosphatase, were positive, whereas those of G (IF_G) were negative (Figure 4). Generally, higher positive or negative values of these indices were induced by the implementation of both tested products at a dose of 16 cm^3 compared to 8 cm^3 kg^{-1} of soil.

Table 3. Soil enzyme activity per kg DM of soil in 1 h.

Dose DO/G, cm^3 kg^{-1} of d.m. Soil	Diesel Oil (DO)			Gasoline (G)		
	Sorbent (S)					
	Control (C)	Dolomite (D)	Perlite (P)	Control (C)	Dolomite (D)	Perlite (P)
	Dehydrogenases (Deh), μM TFF					
0	13.798 ± 0.071 ^{hi}	21.920 ± 0.327 ^{d}	16.287 ± 0.384 ^{f}	13.798 ± 0.071 ^{hi}	21.920 ± 0.327 ^{d}	16.287 ± 0.384 ^{f}
8	14.253 ± 0.199 ^{h}	26.514 ± 0.455 ^{b}	20.056 ± 0.313 ^{e}	11.522 ± 0.199 ^{k}	13.541 ± 0.000 ^{i}	12.404 ± 0.398 ^{j}
16	15.476 ± 0.398 ^{g}	27.396 ± 0.313 ^{a}	24.323 ± 0.085 ^{c}	1.195 ± 0.114 ^{n}	10.384 ± 0.825 ^{l}	5.576 ± 0.000 ^{m}
	Catalase (Cat), M O_2					
0	0.167 ± 0.004 ^{h}	0.237 ± 0.004 ^{f}	0.215 ± 0.009 ^{g}	0.167 ± 0.004 ^{h}	0.237 ± 0.004 ^{f}	0.215 ± 0.009 ^{g}
8	0.329 ± 0.002 ^{e}	0.531 ± 0.002 ^{b}	0.443 ± 0.009 ^{d}	0.083 ± 0.009 ^{jk}	0.114 ± 0.004 ^{i}	0.096 ± 0.004 ^{j}
16	0.447 ± 0.013 ^{d}	0.574 ± 0.009 ^{a}	0.478 ± 0.003 ^{c}	0.066 ± 0.009 ^{l}	0.096 ± 0.004 ^{j}	0.079 ± 0.004 ^{kl}

Table 3. *Cont.*

Dose DO/G, cm^3 kg^{-1} of d.m. Soil	Diesel Oil (DO)			Gasoline (G)		
	Sorbent (S)					
	Control (C)	Dolomite (D)	Perlite (P)	Control (C)	Dolomite (D)	Perlite (P)
	Urease (Ure), mM N-NH_4					
0	0.741 ± 0.741 ^{e}	2.802 ± 0.026 ^{a}	0.758 ± 0.013 ^{e}	0.741 ± 0.041 ^{e}	2.802 ± 0.026 ^{a}	0.758 ± 0.013 ^{e}
8	0.754 ± 0.030 ^{e}	2.031 ± 0.026 ^{b}	0.514 ± 0.000 ^{f}	0.180 ± 0.026 ^{g}	0.951 ± 0.026 ^{d}	0.180 ± 0.026 ^{g}
16	0.797 ± 0.045 ^{e}	1.877 ± 0.026 ^{c}	0.540 ± 0.026 ^{f}	0.129 ± 0.026 ^{g}	0.745 ± 0.028 ^{e}	0.129 ± 0.023 ^{g}
	Acid phosphatase (AcP), mM PNP					
0	2.569 ± 0.011 ^{a}	2.555 ± 0.013 ^{a}	2.014 ± 0.013 ^{fg}	2.569 ± 0.011 ^{a}	2.555 ± 0.013 ^{a}	2.014 ± 0.013 ^{fg}
8	2.114 ± 0.028 ^{de}	2.073 ± 0.051 ^{def}	2.054 ± 0.010 ^{ef}	2.360 ± 0.019 ^{b}	2.341 ± 0.049 ^{b}	1.957 ± 0.019 ^{gh}
16	2.106 ± 0.011 ^{de}	2.190 ± 0.010 ^{c}	2.119 ± 0.013 ^{d}	1.550 ± 0.017 ^{j}	1.718 ± 0.059 ^{i}	1.909 ± 0.054 ^{h}
	Alkaline phosphatase (AlP), mM PNP					
0	0.406 ± 0.008 ^{h}	0.901 ± 0.002 ^{f}	0.659 ± 0.006 ^{g}	0.406 ± 0.008 ^{h}	0.901 ± 0.002 ^{f}	0.659 ± 0.006 ^{g}
8	0.959 ± 0.030 ^{e}	1.483 ± 0.013 ^{b}	0.885 ± 0.011 ^{f}	0.282 ± 0.002 ^{j}	0.650 ± 0.016 ^{g}	0.352 ± 0.002 ^{i}
16	1.448 ± 0.025 ^{c}	2.040 ± 0.011 ^{a}	1.312 ± 0.003 ^{d}	0.243 ± 0.006 ^{k}	0.645 ± 0.027 ^{g}	0.355 ± 0.024 ^{i}
	β-glucosidase (Glu), mM PNP					
0	0.267 ± 0.003 ^{fg}	0.278 ± 0.002 ^{cde}	0.280 ± 0.002 ^{bcd}	0.267 ± 0.003 ^{fg}	0.278 ± 0.002 ^{cde}	0.280 ± 0.002 ^{bcd}
8	0.278 ± 0.001 ^{cde}	0.285 ± 0.005 ^{b}	0.285 ± 0.001 ^{b}	0.263 ± 0.001 ^{g}	0.274 ± 0.006 ^{de}	0.272 ± 0.004 ^{ef}
16	0.282 ± 0.001 ^{bc}	0.292 ± 0.001 ^{a}	0.295 ± 0.002 ^{a}	0.254 ± 0.002 ^{i}	0.256 ± 0.003 ^{hi}	0.262 ± 0.003 ^{gh}
	Arylsulfatase (Aryl), mM PNS					
0	0.106 ± 0.005 ^{g}	0.215 ± 0.002 ^{c}	0.119 ± 0.002 ^{g}	0.106 ± 0.005 ^{g}	0.215 ± 0.002 ^{c}	0.119 ± 0.002 ^{g}
8	0.148 ± 0.012 ^{f}	0.239 ± 0.002 ^{b}	0.150 ± 0.005 ^{f}	0.106 ± 0.005 ^{g}	0.191 ± 0.012 ^{d}	0.116 ± 0.002 ^{g}
16	0.155 ± 0.007 ^{f}	0.336 ± 0.002 ^{a}	0.169 ± 0.010 ^{e}	0.063 ± 0.003 ^{h}	0.193 ± 0.002 ^{d}	0.106 ± 0.010 ^{g}

The same letters (a–n) within one enzyme indicate a homogeneous group, $p < 0.050$, N for each standard deviation = 4, and N for each property tested = 72.

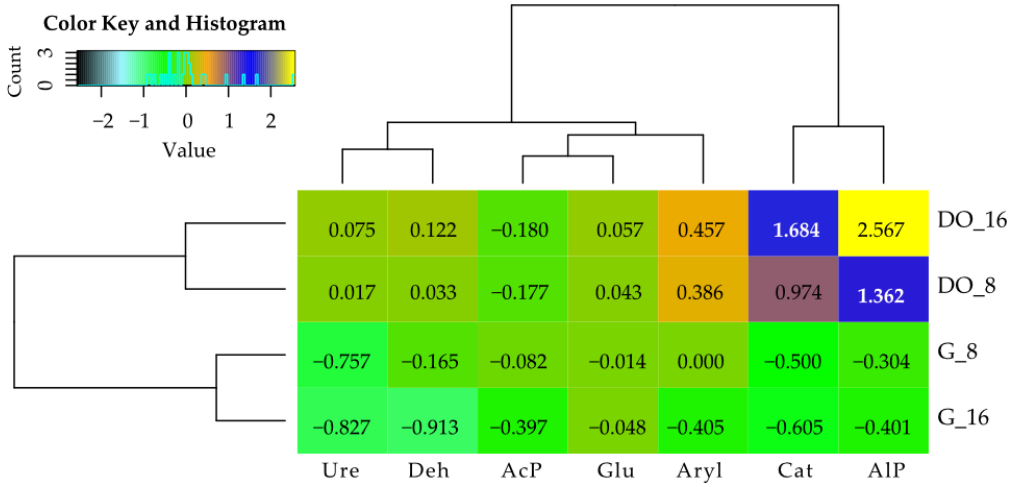


Figure 4. Influence indices of diesel oil (IF_{DO}) and gasoline (IF_G) on soil enzyme activity. Explanations of abbreviations are provided in Figure 1 and Table 2.

The implementation of dolomite to the control soil (C) increased the activity of all enzymes except AcP and Glu (Figure 5a). It most significantly stimulated Ure (IF 2.780), AlP (IF 1.222), and Aryl (IF 1.023). It did not only affect the activity of AcP and Glu. It also increased the activity of all enzymes, except AcP and Glu, in soil contaminated with diesel oil and gasoline. A particularly high intensification of activity was observed in the G_16 soil, where the IF_D index ranged from 0.467 (Cat) to 7.69 (Deh) and 4.80 (Ure).

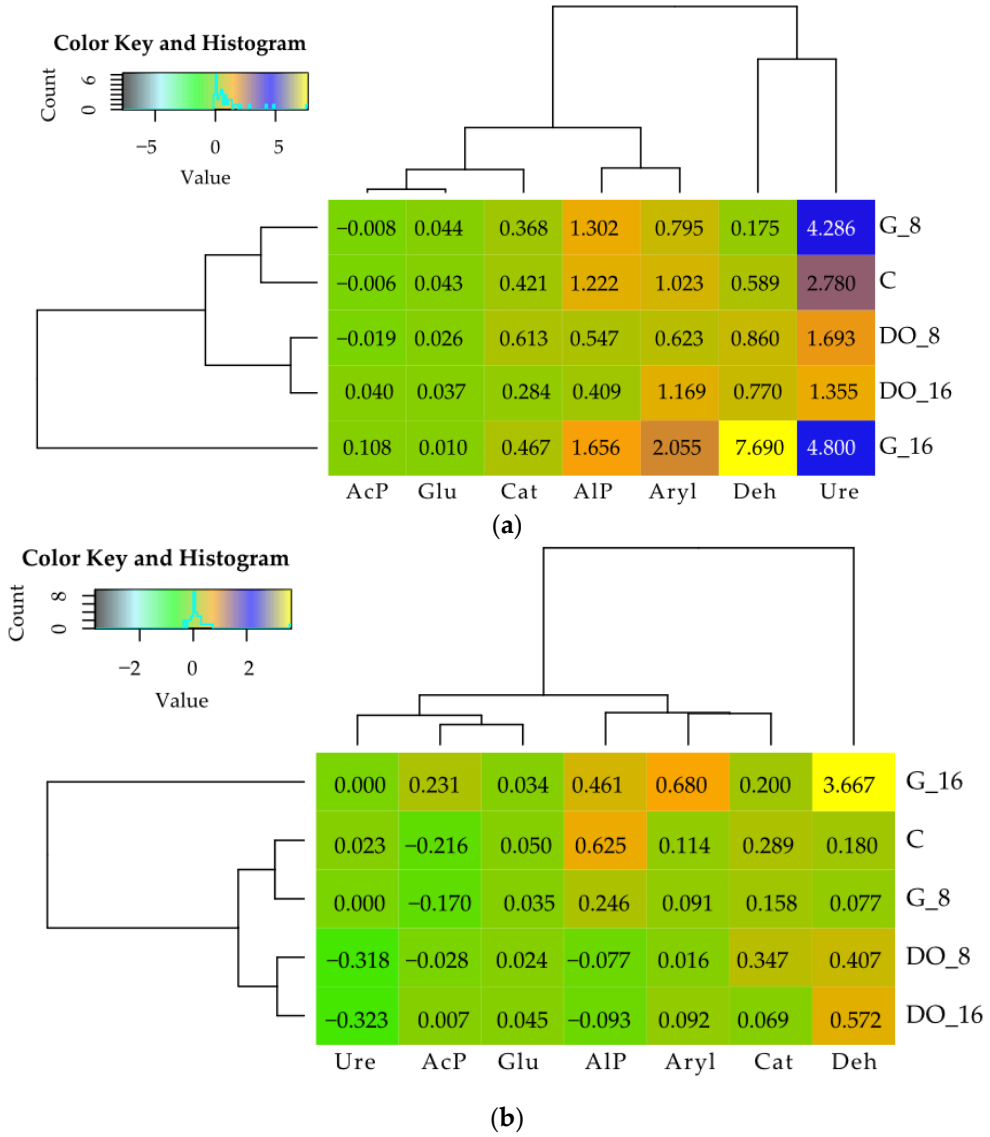


Figure 5. Influence index: (**a**) dolomite (IF_D) and (**b**) perlite (IF_P) on soil enzyme activity. Explanations of abbreviations are provided in Figure 1 and Table 2.

Perlite exerted a significantly reduced effect on soil enzyme activity compared to dolomite (Figure 5b). In the control treatment (C), it stimulated the activity of AlP (IF 0.625), Cat (IF 0.289), Deh (IF 0.180), and Aryl (IF 0.114), whereas it inhibited the activity of AcP (IF −0.216). This sorbent had a marginal effect on enzyme activity in soil contaminated with gasoline at a dose of 8 cm^3 kg^{-1} of soil. The highest IF value for this substance was observed for AlP (0.246) and Cat (0.158), while the lowest was observed for AcP (−0.170). Increased efficacy of perlite was observed in soil exposed to gasoline applied at a dose of 16 cm^3 kg^{-1} of soil (G_16). Perlite stimulated the activity of all enzymes except for Ure and Glu. It stimulated the activity of Deh, Aryl, and AlP the most, with IF values for these enzymes of 3.667, 0.680, and 0.461, respectively. The sorbent tested was less effective in influencing the enzymes in soil contaminated with diesel oil. In the most heavily contaminated soil (DO_16), it led to a notable increase in the IF value for Deh (0.572) and a decrease for Ure (−0.323). A similar trend was also observed in the soil of treatment DO_8, although with an additional stimulation of Cat (IF 0.347) in this soil.

3.3. Physicochemical Properties of Soil Subjected to Pressure from DO and G

An important research step in assessing the condition of soils exposed to DO and G pressures was to track changes in soil physicochemical properties (Tables 4 and S1). Diesel oil (DO) exerted greater pressure than gasoline (G) on the physicochemical properties of the soil (Table 4). In the soil not modified by sorbents, DO increased the content of C_{org} from 7.10 g to 10.19 g kg^{-1} of soil, N_{total} from 1.12 to 1.27 g, C:N ratio from 6.34 to 7.99, pH value from 4.30 to 4.90 and decreased HAC from 35.25 mmol(+) kg^{-1} of soil to 27.87 mmol(+) kg^{-1} of soil. It did not change the values of EBC and CEC but caused an increase in BS from 54.17% to 64.4%. A similar direction of DO effects was observed in the soil with the addition of perlite, while the implementation of dolomite contributed to an increase in soil pH, EBC, CEC, and BS in all treatments, and decreased HAC. The above changes were more strongly determined by the application of dolomite than by DO.

Table 4. Soil physicochemical properties after the completion of plant vegetation.

Dose DO/G, cm^3 kg^{-1} of d.m Soil	Diesel Oil (DO)			Gasoline (G)		
	Sorbent (S)					
	Control (C)	Dolomite (D)	Perlite (P)	Control (C)	Dolomite (D)	Perlite (P)
	Total Organic Carbon (C_{org}) in g kg^{-1}					
0	7.100 ± 0.110 ^{gh}	8.685 ± 0.325 ^{de}	7.310 ± 0.030 ^{g}	7.100 ± 0.110 ^{gh}	8.685 ± 0.025 ^{de}	7.310 ± 0.030 ^{g}
8	8.850 ± 0.240 ^{d}	9.885 ± 0.105 ^{c}	8.515 ± 0.205 ^{e}	7.110 ± 0.090 ^{gh}	8.495 ± 0.025 ^{e}	7.115 ± 0.065 ^{gh}
16	10.190 ± 0.000 ^{b}	11.560 ± 0.040 ^{a}	9.605 ± 0.035 ^{c}	6.745 ± 0.015 ^{i}	7.800 ± 0.050 ^{f}	6.820 ± 0.050 ^{i}
	Total Nitrogen (N_{total}) in g kg^{-1}					
0	1.120 ± 0.020 ^{def}	1.110 ± 0.010 ^{ef}	1.105 ± 0.005 ^{ef}	1.120 ± 0.020 ^{def}	1.110 ± 0.020 ^{ef}	1.105 ± 0.015 ^{ef}
8	1.215 ± 0.035 ^{b}	1.155 ± 0.015 ^{cd}	1.200 ± 0.010 ^{b}	1.155 ± 0.025 ^{cd}	1.120 ± 0.010 ^{def}	1.055 ± 0.015 ^{g}
16	1.275 ± 0.015 ^{a}	1.185 ± 0.005 ^{bc}	1.280 ± 0.020 ^{a}	1.265 ± 0.020 ^{b}	1.135 ± 0.015 ^{de}	1.090 ± 0.010 ^{fg}
	C:N					
0	6.339	7.824	6.615	6.339	7.824	6.615
8	7.284	8.558	7.096	6.156	7.585	6.744
16	7.992	9.755	7.504	5.332	6.872	6.257
	pH_{KCl}					
0	4.300 ± 0.000 ^{i}	6.450 ± 0.050 ^{d}	4.300 ± 0.000 ^{i}	4.300 ± 0.000 ^{i}	6.450 ± 0.050 ^{d}	4.300 ±0.000 ^{i}
8	4.550 ± 0.005 ^{h}	6.550 ± 0.050 ^{b}	4.600 ± 0.000 ^{g}	4.300 ± 0.000 ^{i}	6.600 ± 0.000 ^{a}	4.300 ± 0.000 ^{i}
16	4.900 ± 0.000 ^{e}	6.500 ± 0.000 ^{c}	4.800 ± 0.005 ^{f}	4.300 ± 0.000 ^{i}	6.600 ± 0.000 ^{a}	4.300 ± 0.000 ^{i}
	Hydrolytic Acidity (HAC) in mmol(+) kg^{-1} soil					
0	35.250 ± 0.750 ^{a}	11.625 ± 0.375 ^{d}	35.625 ± 0.375 ^{a}	35.250 ± 0.750 ^{a}	11.625 ± 0.375 ^{d}	35.625 ± 0.375 ^{a}
8	33.000 ± 3.000 ^{b}	11.250 ± 0.000 ^{d}	28.500 ± 0.750 ^{c}	35.250 ± 0.000 ^{a}	10.500 ± 0.000 ^{d}	35.625 ± 0.375.^{a}
16	27.875 ± 0.573 ^{c}	12.000 ± 0.000 ^{d}	28.875 ± 0.375 ^{c}	35.625 ± 0.375 ^{a}	10.875 ± 0.375 ^{d}	36.000 ± 0.000 ^{a}
	Total Exchangeable Base Cations (EBC) in mmol(+) kg^{-1} soil					
0	44.075 ± 3.075 ^{de}	214.225 ± 7.175 ^{bc}	45.100 ± 0.000 ^{de}	44.075 ± 3.075 ^{de}	214.225 ± 7.175 ^{bc}	45.100 ± 0.000 ^{de}
8	47.150 ± 2.050 ^{de}	238.825 ± 7.175 ^{a}	44.075 ± 1.025 ^{de}	43.563 ± 0.512 ^{e}	211.150 ± 10.250 ^{c}	49.200 ± 0.000 ^{de}
16	54.325 ± 3.075 ^{d}	235.750 ± 4.100 ^{a}	43.050 ± 2.050 ^{e}	43.050 ± 0.000 ^{e}	222.425 ± 11.275 ^{b}	46.125 ± 1.025 ^{de}
	Total Cation Exchange Capacity of Soil (CEC) in mmol(+) kg^{-1} soil					
0	79.325 ± 3.825 ^{de}	225.850 ± 7.550 ^{bc}	80.725 ± 0.375 ^{de}	79.325 ± 3.825 ^{de}	225.850 ± 7.550 ^{bc}	80.725 ± 0.375 ^{de}
8	80.150 ± 2.656 ^{de}	250.075 ± 7.175 ^{a}	72.575 ± 1.025 ^{e}	78.813 ± 0.512 ^{de}	221.650 ± 10.250 ^{c}	84.825 ± 0.375 ^{d}
16	82.200 ± 2.735 ^{de}	247.750 ± 4.100 ^{a}	71.925 ± 2.425 ^{e}	78.675 ± 0.375 ^{de}	233.300 ± 10.900 ^{b}	82.125 ± 1.025 ^{de}
	Base Cations Saturation Ratio in Soil (BS) in %					
0	54.170 ± 1.171 ^{f}	92.539 ± 0.006 ^{a}	54.507 ± 0.253 ^{f}	54.170 ± 1.171 ^{f}	92.539 ± 0.006 ^{a}	54.507 ± 0.253 ^{f}
8	57.424 ± 2.813 ^{cd}	93.170 ± 0.126 ^{a}	59.244 ± 0.541 ^{c}	53.924 ± 0.284 ^{f}	92.933 ± 0.214 ^{a}	56.588 ± 0.250 ^{de}
16	64.445 ± 1.579 ^{b}	92.835 ± 0.078 ^{a}	58.376 ± 0.813 ^{cd}	53.385 ± 0.254 ^{f}	93.002 ± 0.370 ^{a}	54.790 ± 0.534 ^{ef}

The same letters (a–i) within each property indicate a homogeneous group, $p < 0.050$, N for each standard deviation = 4, and N for each property tested = 72.

Gasoline induced minor changes in the values of the studied parameters (Table 4). These changes were limited to a decrease in the content of C_{org} and an increase in the

accumulation of N_{total} under the influence of a dose of 16 cm^3 kg^{-1} soil. This naturally led to a reduction in the C:N ratio in the soil. The application of perlite did not have a significant effect on the soil properties, whereas the application of dolomite, similar to the series of experiments with DO, reduced the acidification of the soil and improved its sorption capacity.

3.4. The Interrelationships between Biochemical, Physicochemical Properties, and Soil Fertility Exposed to the Effects of DO and P

The implementation of petroleum products resulted in significant changes in the values of the soil biochemical quality indicators (Figure 6). DO led to a greater increase in the values of BA_1 and BA_2 with higher concentrations in the soil, while G acted inversely to DO. It significantly decreased the magnitude of these indicators, the higher the soil contamination. Both sorbents (D and P) significantly increased the values of the BA indicators in soils destabilized by DO and G, as well as in stable soils unaffected by the influence of petroleum products.

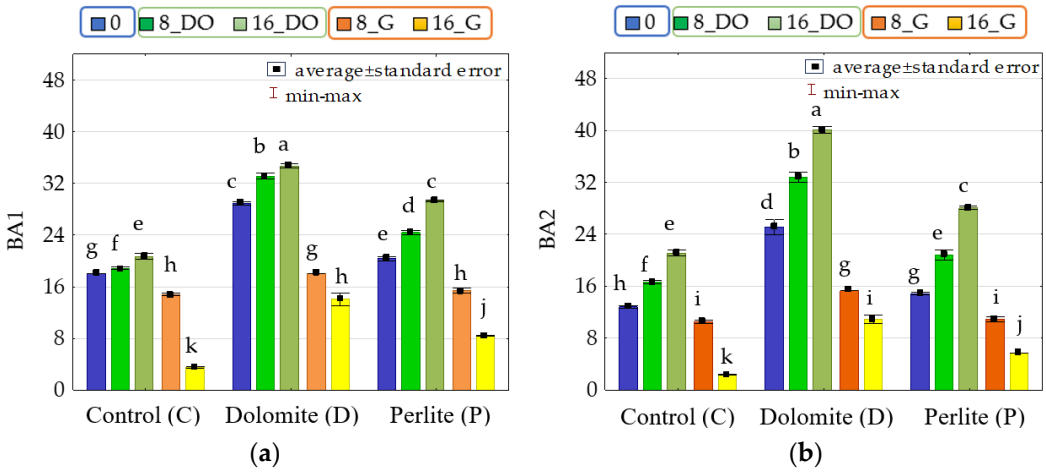


Figure 6. Soil biochemical quality indices BA_1 (**a**) and BA_2 (**b**). Explanations of abbreviations are provided in Figure 1. The same letters on the bar graph (a–k) denote homogeneous groups, $p < 0.050$, N for each error bar = 4.

Among the three variables: type of petrochemical product (Cont. A), dose of petrochemical product (Dose B), and type of sorbent (Sorbent C), Cont. A predominantly influenced the activity of Cat, Glu, AlP, Deh, SPAD index, C_{org} content, and BA indices, while it had the least effect on the aerial parts and root biomass of *Zea mays*, soil pH, HAC, EBC, SEC, BS, AcP, and Ure activity (Figure 7). Dose B had the greatest effect on the *Zea mays* aerial parts and root biomass, AcP activity, and soil N_{total}. The third factor investigated, the type of sorbent (Sorbent C), was most significant in determining soil pH, HAC, EBC, CEC, BS, Aryl, and Ure activity.

The aerial parts biomass of maize grown on soil degraded by DO was significantly positively correlated (Table 5) with root biomass (0.973), the SPAD chlorophyll index (0.802), and AcP activity (0.640), and negatively correlated with Deh (−0.343), Cat (−0.872), AlP (−0.740), Aryl (−0.372), and Glu (−0.685) activity, soil C_{org} content (−0.767), and soil N_{total} content (−0.832), as well as the BA_2 index (−0.501). However, there was no significant correlation between aerial parts biomass and soil pH, HAC, EBC, CEC, BS, the BA_1 index, or Ure activity. The activities of all enzymes, except for AcP, were positively correlated with each other and with soil pH, C_{org} content, EBC, CEC, BS, and BA indices, and negatively correlated with HAC.

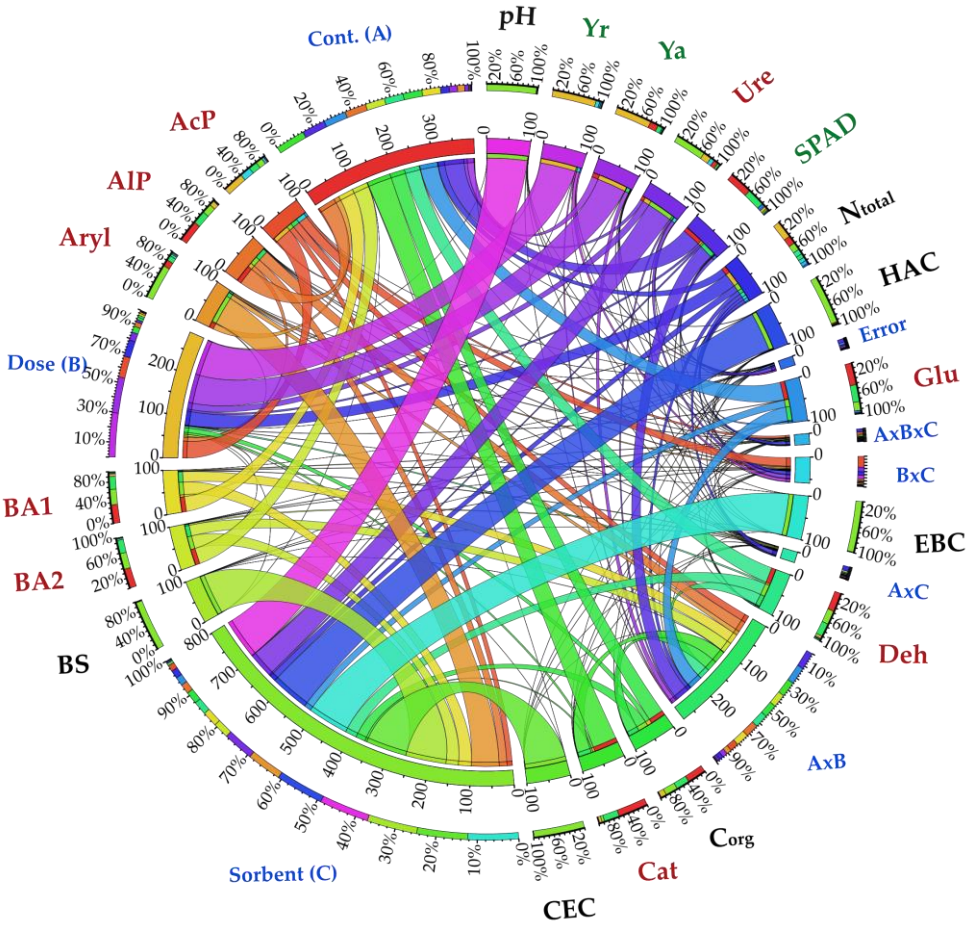


Figure 7. Loadings of independent variables in explaining dependent variables, in %. Explanations of abbreviations are provided in Figure 1 and Tables 2 and 4.

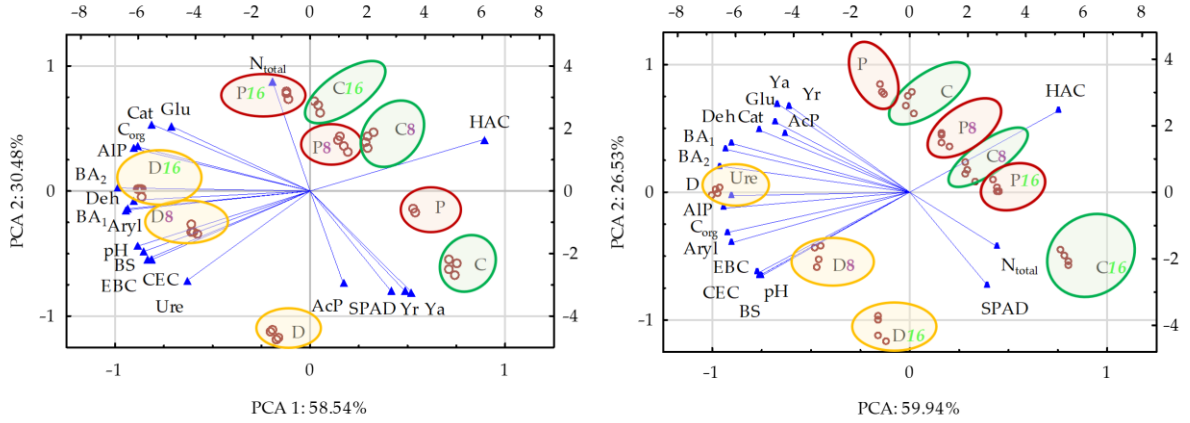
Similarly, the aerial parts biomass of maize cultivated on soil degraded by G was significantly positively correlated with root biomass (0.907). However, in contrast to maize cultivated on soil contaminated with DO, it was negatively correlated with the SPAD chlorophyll index (−0.708), and positively correlated with the soil C_{org} content (0.394), as well as with the activity of all enzymes and both soil quality indices. In this series of experiments, there was a positive correlation between the activity of all enzymes and soil pH, and, similarly to the DO series, a positive correlation with the same parameters and a negative correlation with HAC.

The above dependencies are also confirmed by the results presented in Figure 8, which shows the data analyzed using PCA. The reliability of the data is underlined by the high degree of determination attributed to the first two principal components. It was 89.02% for soils polluted with diesel oil and 86.47% for soils polluted with gasoline.

Table 5. Simple correlation coefficients in soil contaminated with diesel oil and gasoline.

		Ya	Yr	SPAD	Deh	Cat	Ure	AcP	AlP	Aryl	Glu	C_{org}	N_{total}	pH	HAC	EBC	CEC	BS	BA_1	BA_2	
Ya	Diesel oil	1.000	0.907 *	−0.708 *	0.879 *	0.774 *	0.521 *	0.809 *	0.492 *	0.351 *	0.791 *	0.394 *	−0.688	0.063	−0.077	0.068	0.067	0.091	0.856 *	0.776 *	Gasoline
Yr		0.973 *	1.000	−0.673 *	0.777 *	0.824 *	0.541 *	0.758 *	0.467 *	0.246	0.677 *	0.338 *	−0.473	0.032	−0.048	0.032	0.029	0.050	0.770 *	0.702 *	
SPAD		0.802 *	0.781 *	1.000	−0.585 *	−0.660 *	−0.301	−0.384 *	−0.355 *	−0.068	−0.736 *	−0.148	0.527	0.163	−0.153	0.157	0.158	0.137	−0.558 *	−0.492 *	
Deh		−0.343 *	−0.310	−0.276	1.000	0.851 *	0.783 *	0.746 *	0.779 *	0.662 *	0.794 *	0.701 *	−0.560	0.413 *	−0.429 *	0.429 *	0.428 *	0.441 *	0.996 *	0.968 *	
Cat		−0.872 *	−0.825 *	−0.689 *	0.717 *	1.000	0.788 *	0.573 *	0.757 *	0.435 *	0.735 *	0.510 *	−0.331	0.227	−0.242	0.240	0.240	0.246	0.863 *	0.837 *	
Ure		0.239	0.224	0.274	0.558 *	0.088	1.000	0.557 *	0.877 *	0.761 *	0.528 *	0.824 *	−0.186	0.655 *	−0.668 *	0.671 *	0.671 *	0.674 *	0.835 *	0.900 *	
AcP		0.640 *	0.693 *	0.532 *	−0.141	−0.555 *	0.430 *	1.000	0.367 *	0.373 *	0.566 *	0.477 *	−0.374	0.181	−0.202	0.180	0.176	0.194	0.754 *	0.724 *	
AlP		−0.740 *	−0.695 *	−0.689 *	0.718 *	0.899 *	0.331 *	−0.367 *	1.000	0.876 *	0.585 *	0.875 *	−0.333	0.771 *	−0.776 *	0.783 *	0.783 *	0.786 *	0.811 *	0.863 *	
Aryl		−0.372 *	−0.340 *	−0.274	0.835 *	0.674 *	0.689 *	−0.014	0.844 *	1.000	0.409 *	0.933 *	−0.370	0.933 *	−0.935 *	0.942 *	0.942 *	0.946 *	0.695 *	0.768 *	
Glu		−0.685 *	−0.691 *	−0.687 *	0.750 *	0.822 *	0.052	−0.591 *	0.760 *	0.576 *	1.000	0.499 *	−0.606	0.154	−0.160	0.157	0.156	0.182	0.777 *	0.744 *	
C_{org}		−0.767 *	−0.720 *	−0.703 *	0.671 *	0.883 *	0.334 *	−0.299	0.982 *	0.819 *	0.718 *	1.000	−0.236	0.896 *	−0.903 *	0.898 *	0.897 *	0.908 *	0.741 *	0.831 *	
N_{total}		−0.832 *	−0.803 *	−0.846 *	0.067	0.602 *	−0.455 *	−0.459 *	0.476 *	0.013	0.513 *	0.542 *	1.000	−0.084	0.079	−0.095	−0.098	−0.122	−0.522 *	−0.452 *	
pH		−0.123	−0.104	−0.021	0.792 *	0.477 *	0.914 *	0.178	0.650 *	0.885 *	0.364 *	0.642 *	−0.181	1.000	−0.999 *	0.997 *	0.996 *	0.998 *	0.461 *	0.570 *	
HAC		0.152	0.139	0.026	−0.810 *	−0.509 *	−0.892 *	−0.160	−0.648 *	−0.876 *	−0.391 *	−0.643 *	0.149	−0.989 *	1.000	−0.997 *	−0.996 *	−0.998 *	−0.477 *	−0.585 *	
EBC		−0.016	0.006	0.094	0.752 *	0.390 *	0.923 *	0.212	0.578 *	0.870 *	0.265	0.553 *	−0.331	0.980 *	−0.960 *	1.000	1.000 *	0.998 *	0.476 *	0.585 *	
CEC		0.002	0.024	0.109	0.740 *	0.373 *	0.922 *	0.217	0.565 *	0.865 *	0.248	0.538 *	−0.352	0.974 *	−0.949 *	0.999 *	1.000	0.998 *	0.476 *	0.584 *	
BS		−0.078	−0.065	0.032	0.752 *	0.434 *	0.928 *	0.199	0.618 *	0.873 *	0.309	0.608 *	−0.243	0.994 *	−0.987 *	0.987 *	0.981 *	1.000	0.488 *	0.596 *	
BA_1		−0.319	−0.287	−0.253	0.991 *	0.698 *	0.649 *	−0.072	0.744 *	0.884 *	0.704 *	0.705 *	0.034	0.862 *	−0.874 *	0.823 *	0.812 *	0.828 *	1.000	0.985 *	
BA_2		−0.501 *	−0.451 *	−0.435 *	0.938 *	0.813 *	0.566 *	−0.151	0.896 *	0.944 *	0.747 *	0.869 *	0.202	0.834 *	−0.837 *	0.786 *	0.775 *	0.800 *	0.957 *	1.000	

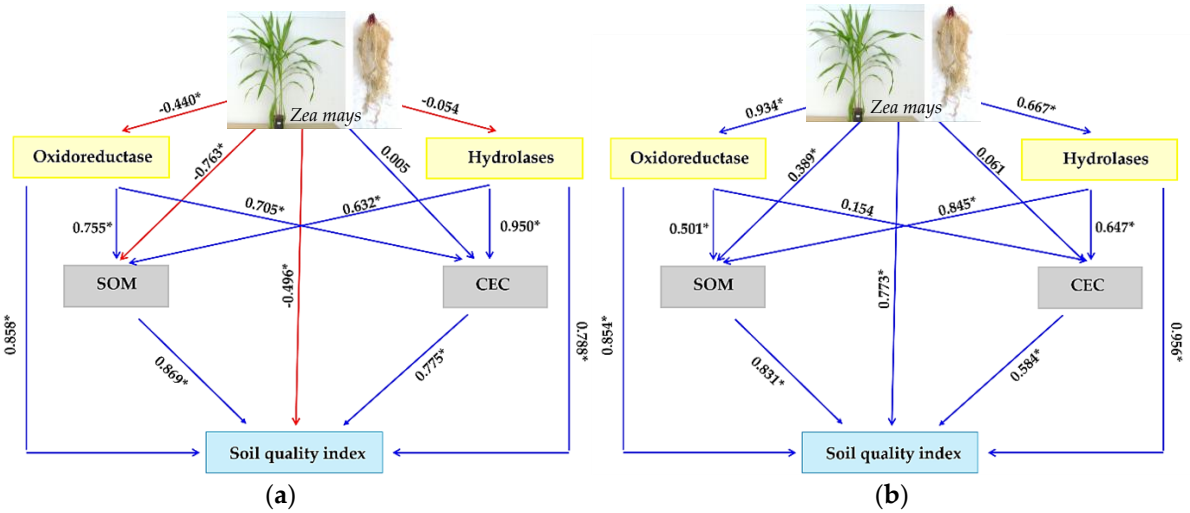
Explanations of abbreviations are provided in Figure 1 and Tables 2 and 4. *—homogeneous groups, $p < 0.050$, N = 36. —Diesel oil, —Gasoline, red color—statistically significant, black color—statistically insignificant.



(**a**) (**b**)

Figure 8. PCA for crop yield and soil properties contaminated with (**a**) diesel oil, (**b**) gasoline. Explanations of abbreviations are provided in Figure 1 and Tables 2 and 4.

Among the enzymes analyzed, dehydrogenases and catalase belong to the class of oxidoreductases, while the remaining enzymes are part of hydrolases. Figure 9 illustrates the correlations between these enzyme classes and *Zea mays* and SOM (soil organic matter) along with CEC (cation exchange capacity). Regardless of individual enzymes, both classes were significantly positively correlated with the biochemical soil quality indicator, independent of the influence of DO and G. They were positively correlated with *Zea mays* biomass cultivated on soil affected by G, and negatively correlated on soil affected by DO, with a statistically significant correlation occurring between oxidoreductases and *Zea mays* biomass in the case of the latter pollutant. Both enzyme classes were significantly positively correlated with soil SOM and CEC. In both experimental series, with DO and G, there was also a positive correlation between SOM and CEC with the biochemical soil quality indicator and the activity of hydrolases with SOM. Additionally, a positive correlation between CEC and oxidoreductase activity was observed exclusively in soil treated with DO.



(**a**) (**b**)

Figure 9. The influence of plants, soil enzymes, SOM, and CEC on soil quality contaminated with diesel oil (**a**) and gasoline (**b**). Blue arrows represent positive effects, while red arrows indicate negative effects. Numbers next to the arrows represent the magnitude of the dependency effect. *—statistically significant.

4. Discussion

4.1. Plant and Enzyme Response to Petrochemical Products

The effectiveness of phytoremediation processes in soils contaminated with petroleum products depends on the selection of appropriate plants [86–89]. These should be fast-growing plants with high adaptability to challenging environmental conditions and a well-developed root system [88,90]. Examples of such plants include *Sorghum bicolor* [86], *Iris lacteal* [91], *Festuca arundinacea* [92,93], and *Lathyrus sativus* [94]. Plants obtained from degraded areas can be used for energy purposes, as their combustion heat and energy values remain unchanged [74,95]. In our own research, *Zea mays* was utilized, which meets all the aforementioned criteria. This is evidenced by the biomass yield of *Zea mays* obtained in the experiment, which ranged from 69,544 to 74,073 g pot^{-1} in uncontaminated objects. Comparing the two environments contaminated with petroleum products, we found that *Zea mays* adapted better in the G-contaminated soil than in the DO-contaminated soil.

The influence of pollutants on plants depends on the chemical composition of petroleum products [96,97] as well as on soil properties [98,99]. Particularly long-lasting effects occur in soils characterized by low organic carbon content and low biological activity, as the biodegradation rate of hydrocarbons is slow under such conditions [36]. In our study, the negative effect of diesel oil (DO) applied to the soil at a rate of 16 cm^3 kg^{-1} on *Zea mays* biomass was almost twice as strong as that of gasoline (G). This was probably due to greater adsorption of oil residues on the soil mineral surfaces, resulting in a change in the redox potential of the soil [100]. Soil colloids covered with hydrophobic films lose their water-holding capacity and decrease their conductivity [16,24]. As a result, the upper parts of the contaminated soil dry out, while the lower parts become excessively moist, leading to the predominance of anaerobic processes [98]. The negative impact of petroleum products on the environment depends on the density of the product [101]. According to Korshunova [99], the effect of heavy oil fractions on plants is long-lasting compared to light fractions, which are more rapidly decomposed by microorganisms and rapidly migrate out of the soil. This observation agrees well with our results, since the density of diesel oil, which destabilized plant growth and development more, ranged from 0.820 to 0.845 g cm^{-3}, while that of unleaded gasoline ranged from 0.720 to 0.775 g cm^{-3}.

According to Tripathi [102], the negative effect of petroleum products results not only from their direct effect on the root system and the reduction of soil oxygen content but also from the decreased availability of nutrients for plants. An important factor limiting nutrient availability to plants is the increase in organic carbon content, leading to an increase in the C:N ratio [92,98,103]. Similarly, in our own study, a significant increase in soil organic carbon content and consequently an expansion of the C:N ratio were observed in soil contaminated with diesel oil (DO). There was also a significant increase in soil pH and sorption capacity. In contrast, the effect of gasoline on these parameters was minimal. These changes are consistent with our earlier studies [21,104].

The destabilization of water–air conditions under the influence of petroleum-derived products and changes in soil properties, such as pH value, C_{org} content, total nitrogen (N_{total}), and sorption capacity, indirectly affect not only the plant growth but also the activity of soil enzymes, which are considered reliable indicators of soil health and accurately reflect the ecological state of the soil [34]. According to Moradi et al. [105], the implementation of any soil remediation strategy should be preceded by the determination of enzyme activity since they are the driving force behind biochemical transformations.

In our studies on the bioindication of soil pollution by DO and G, we used enzymes of the oxidoreductase and hydrolase classes. Enzymes of the first class are considered primary bioindicators, while those of the second class are considered auxiliary [35]. We found that these enzymes were significantly positively correlated with SOM and CEC of the soil, as well as with the type and degree of contamination by DO and G. We observed a stimulation of oxidoreductase activity and most hydrolase enzymes by DO, while an inhibition of the investigated oxidoreductases and hydrolases by G was noted. Differences in enzyme response to contamination by both petroleum-derived products are likely to be due to the

nature of the hydrocarbons they contain. Some microorganisms use these hydrocarbons as a source of carbon and energy [29,34,105], leading to increased proliferation [106] and expansion of the enzyme pool [107]. However, certain fractions of petroleum-derived products may contaminate microbial cells, perforate their cell membrane, and thus exert a toxic effect on them. Moreover, they may limit enzyme production and reduce substrate availability for enzymes [34]. The increased Corg content in the soil and consequently higher C content in microbial biomass result in enhanced enzyme activity, which explains the intensified enzyme activity in soil contaminated with DO. Generally, diesel oil is considered less harmful than gasoline.

4.2. The Role of Dolomite and Perlite in Mitigating the Effect of Petroleum-Derived Products on Plants and Enzyme Activity

Considering the fact that soil contamination with petroleum and its products poses a significant threat to the environment [9,47,102], often leading to the formation of "technological deserts" [98], there is an urgent need to improve methods for cleaning up areas affected by these products [98,108,109]. One such method is to combine phytoremediation with the simultaneous application of soil adsorbents to support bioremediation processes [110]. In our own research, dolomite and perlite were used to assist *Zea mays* in detoxifying soils contaminated with DO and G. Dolomite is a carbonate adsorbent, while perlite is a silica-based adsorbent [54,56,59]. Both adsorbents possess properties such as sorption capacity and buffering capacity. They regulate soil water–air properties [110], and dolomite can additionally improve sorption capacity and restore soil morphology and physical and chemical properties disrupted by petrochemical contamination, mainly through processes such as sorption, precipitation, and dissolution. Sorption relies on two mechanisms. The first is determined by capillary phenomena leading to the filling of both pores and capillaries of appropriate diameter and surface energy [111]. One parameter of perlite that favors capillary formation is its low bulk density, not greater than 0.25 kg dm^{-3} [62]. The second mechanism is sorption, which leads not only to the formation of a uniform oil layer around the grains but also to clusters of irregular structure. It is determined by both the morphology of the sorbent surface, including the presence of hydroxyl groups, and the molecular weight of the petrochemicals [59,111]. The formation of stable macroaggregates with dolomite is the result of H^+ neutralization and suppression of H^+ dispersion and K^+ and Na^+ cations in the soil. Macroaggregates are formed by two types of bridges: Ca^{2+} and Mg^{2+} binding and salt bridges ($CaCO_3$, $MgCO_3$). Al_2O_3, Fe_2O_3, and SiO_2 also play an important role in this process [112]. This explains the preferential adsorption of higher molecular weight petroleum products [113]. These factors indicate that in our studies both sorbents mitigated but did not completely eliminate the negative effects of the tested petrochemical products on the growth and development of maize. This is confirmed by the plant impact indices for dolomite and perlite. In the soil under pressure with 16 cm^3 DO, the IF_D was 0.140 and in the soil with P, it was 0.286. On the other hand, in the soil contaminated with G, IF_D = 1.047 and IF_P = 1.318. Thus, the adsorption of petrochemical products on mineral sorbents involves their penetration into large pores and adhesion to the external surface of the adsorbent [59]. Adsorption can be partially disrupted by the formation of a coating around the adsorbent by soil-soluble organic matter [13].

Dolomite, due to its alkalinity and solubility in soil water, increased soil pH, EBC, CEC, BS, and decreased HAC in all experimental plots, both contaminated and control. These factors, among others, contributed to the higher enzymatic activity of the soil treated with dolomite compared to perlite. On the other hand, perlite did not alter the physicochemical properties to the same extent as dolomite. Nevertheless, both sorbents played a positive role in restoring soil quality, as evidenced by the magnitude of the biochemical soil quality indicator, which reflects their overall enzymatic activity. The higher the enzymatic activity of the soil, the greater the potential for the decomposition of petrochemical products [12,87,105].

Conclusively, it can be stated that the combined use of physical methods (application of adsorbents) and biological methods (phytoremediation) can be effective in remediating

areas contaminated with petroleum products. These methods are environmentally friendly and, moreover, economically justified.

5. Conclusions

In soil contaminated with diesel oil (DO) or gasoline (G), unfavorable changes occur that reduce the biomass of cultivated *Zea mays*. Diesel oil disturbs the development of this plant more than gasoline. DO applied at 16 g kg^{-1} soil reduced maize yield by 7.1 fold, and G by 3.8 fold. DO exerts a greater pressure than G on the physicochemical properties of the soil and on the activity of enzymes belonging to the classes of oxidoreductases and hydrolases. In particular, DO stimulates oxidoreductases and most hydrolases, whereas G inhibits their activity. Application of dolomite and perlite to soil contaminated with petroleum products reduces the degree of negative effect of these pollutants on the growth and development of *Zea mays*. Dolomite also intensifies the activity of all enzymes, except for AcP and Glu, in soil contaminated with DO and G, and improves the physicochemical properties of the soil. However, perlite induces a lesser effect on soil enzymes and physico-chemical properties. Dolomite is more suitable than perlite for the remediation of soils contaminated with petroleum products and for the stabilization of their biochemical and physico-chemical properties. The activities of all enzymes, except AcP, were positively correlated with each other and with soil pH, Corg, EBC, CEC, BS, and BA indices, and negatively correlated with HAC.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14093591/s1, Table S1. The level of significance (*p*-value) of the factors studied, determined by means of three-factor ANOVA (N = 72).

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