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The Role of Sodium Alginate Hydrogel in Maintaining Soil Homeostasis Exposed to Sulcotrione

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Abstract: Herbicides are the most widely used agrochemicals in crop protection, which has led to serious environmental pollution around the world, including soil ecosystems. It is important to look for new solutions that lead to an improvement in soil quality, even if only through the use of hydrogels. The aim of this study was therefore to determine the effect of sodium alginate on the microbiological and biochemical properties of sulcotrione-treated soil. It was found that both the herbicide and the sodium alginate had a significant effect on the soil environment. An amount of 10 g kg^{-1} of sodium alginate was applied to the soil, while sulcotrione was applied to the soil in the following amounts: 0.00 (C), 0.200 (R), 0.999 (5R), and 9.999 mg kg^{-1} (50R). Sulcotrione stimulated the activity of dehydrogenases, catalase, arylsulfatase, and β-glucosidase and inhibited the activities of alkaline phosphatase, acid phosphatase, and urease as well as the proliferation of organotrophic bacteria, actinobacteria, and fungi. This caused an increase in the colony development index (CD) of organotrophic bacteria and fungi and decreased the colony development index value of actinobacteria. It also increased the value of the ecophysiological diversity index (EP) of fungi. The addition of sodium alginate to the soil increased the numbers of organotrophic bacteria, actinobacteria, and fungi as well as the activities of dehydrogenases, catalase, urease, alkaline phosphatase, and arylsulfatase. The hydrogel had different effects on β-glucosidase activity. Acid phosphatase showed a significant decrease in activity after the addition of sodium alginate to the soil. Under the influence of sodium alginate, there was an increase in the index of colony development of actinobacteria and fungi, while there were decreases in organotrophic bacteria and the index of ecophysiological diversity of actinobacteria and fungi. The proliferation of microorganisms and the enzymatic activity of the soil changed over time both in soil enriched with sodium alginate and without its addition. This study may be useful for evaluating the effects of sulcotrione on the microbiological and biochemical properties of soil and the effectiveness of sodium alginate in improving the quality of soil exposed to sulcotrione.

Keywords: sulcotrione; sodium alginate; soil environment; microorganisms; enzymes; soil homeostasis

Citation: Baćmaga, M.; Wyszkowska, J.; Kucharski, J. The Role of Sodium Alginate Hydrogel in Maintaining Soil Homeostasis Exposed to Sulcotrione. *Agriculture* **2024**, *14*, 2081. https://doi.org/10.3390/agriculture14112081

Academic Editor: Vito Armando Laudicina

Received: 24 September 2024
Revised: 4 November 2024
Accepted: 6 November 2024
Published: 19 November 2024

1. Introduction

Food security and environmental sustainability depend largely on the quality and fertility of the soil. However, the excessive and frequent use of herbicides, especially in conventional agriculture, has disrupted the soil environment by altering the activity, number, and diversity of microorganisms and biochemical processes in the soil. This can lead to a deterioration of the physicochemical properties of the soil, which are sometimes irreversible, and contribute to the loss of soil organic matter [1,2].

Soil is one of the most important sites for accumulating herbicides, where these compounds interact with microorganisms. The result of accumulating herbicides is toxic effects on the microbiota or microbial degradation depending on abiotic and biotic factors. Herbicides adhere to soil particles by adsorption and are strongly adsorbed onto soils

with a high clay or organic matter content. This is due to their chemically active and large surface area, which gives chemical compounds a greater sorption capacity [3]. In addition, herbicides are absorbed faster and more easily in dry soils as there is no competition between water and these compounds for binding sites in the soil. Herbicides sorbed by soil particles are more likely to survive in the soil and are more available for plant uptake and chemical and microbial degradation [4].

The toxic effects of herbicides on microorganisms usually occur when they are applied in quantities that are difficult to degrade in the soil, leading to a change in the rates of many microbial processes [5]. Romdhane et al. [6] reported that herbicides that act by inactivating plant enzymes, e.g., acetylacetate synthase or 4-hydroxyphenylpyruvate dioxygenase, exhibit toxicity to microorganisms. Microorganisms in the soil environment are important as they fulfill several key functions, especially for plant production and soil sustainability. Microorganisms can develop defense mechanisms in response to stress to survive in unstable ecosystems [7]. The effects of herbicides on the diversity of microorganisms depend on various factors, e.g., the type and dose of herbicide and environmental conditions [8]. They can remove pollutants from the environment by immobilization (precipitation, biosorption, biostimulation, and complex formation) and mobilization (bioleaching, enzymatic oxidation, enzymatic reduction, biostimulation, and bioaugmentation) [9]. Microorganisms protect themselves from the toxic effects of compounds by forming a hydrophobic or solvent efflux pump that shields the outer cell membrane [10].

In addition to microorganisms, enzymes, which are important from both agricultural and ecological points of view, are very important indicators for assessing soil quality. They are committed in biochemical processes and the nutrient cycle. All biological reactions occur in the soil through catalysis substance-specific enzymes that affect the stabilization of the soil structure and the life processes of microorganisms and are involved in the decomposition of organic material and organic pollutants [3]. There are enzymes in the soil that influence its metabolic processes related to the physical, chemical, microbiological, and biochemical properties of the soil. Enzymes are constantly synthesized, accumulated, inactivated, or degraded [11].

Some herbicides, e.g., dicamba, 2,4-dichlorophenoxyacetic acid (2,4-D), metolachlor, and glyphosate, are resistant to degradation, so they are persistent in the environment and may pose a risk to human health. Therefore, it is desirable to look for new strategies to remove these chemicals from the soil environment, for example, by remediating areas contaminated with herbicides [12]. The main objectives of this process are to remove the pollutants from the soil, increase their transformation into less hazardous metabolites, or immobilize them without impairing soil function. Of all soil remediation methods, the use of sorbents is particularly interesting, as when introduced into contaminated soil, they cause the sorption and immobilization of toxic compounds, reducing their bioretention, infiltration, and bioavailability for organisms [13]. Recently, macromolecular polymers have gained great popularity in improving soil quality due to their affordability, multiple functional groups, pore structure, large specific surface area, and ability to immobilize pollutants [14]. In addition, these substances can conserve water in dry soils, form degradable mulch, control the release of agrochemicals, degrade organic matter, and improve nutrient retention and plant development [15–17].

A common active ingredient in herbicide formulations is sulcotrione ($C_{14}H_{13}ClO_5S$), which belongs to the group of triketone compounds [18], leading to the impairment of carotenoid synthesis, which degrades chlorophyll and inhibits pigment production. This leads to leaf damage and the subsequent death of the plant [19]. Sulcotrione is characterized by the following properties: solubility in water—165 mg dm^{-3}; solubility in organic solvents—2000 mg dm^{-3}; logK_{ow}—3.13; log P—1.7; K_f—1.05 cm^3 g^{-1}; K_{foc}—36.00 cm^3 g^{-1}; vapor pressure—5.00 × 10^{-3}; and soil degradation DT_{50}—from 3.6 to 25.3 days [20]. The structural formula of sulcotrione is shown in Figure 1.

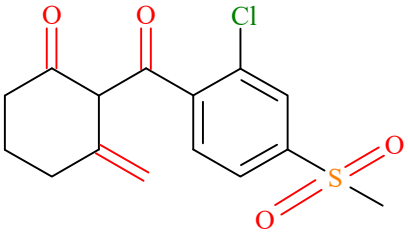


Figure 1. The structural formula of sulcotrione.

Sodium alginate (SA) is a polysaccharide extracted from the cells of the brown algae *Macrocystis pyrifera*, *Laminaria digitata*, *Laminaria cloustoni*, and *Ascophyllum nodosum*, which belong to the *Phaeophyceace* family. It makes up 10% to 50% of the dry weight of the algae cells. It has very good properties, i.e., non-toxicity, biocompatibility, and biodegradability [21]. Sodium alginate has a high water absorption capacity and increases water retention in sandy soils [16,22]. Chemically, sodium alginate is a linear copolymer consisting of monomeric units, which are β-D-mannuronic acid and α-L-guluronic acid residues linked together by β-1,4 and α-1,4 glycosidic bonds. The D-mannuronic acid block is in the $^4C_1$ conformation, while the L-guluronic acid block is in the $^1C_4$ confirmation, regardless of the nearest neighbor unit [23–25]. The very important feature of sodium alginate is its ability to bind multivalent cations (Ca^{2+}, Mg^{2+}, Sr^{2+}, and Ba^{2+}) in blocks of α-L-guluronic acid residues, which results in the formation of hydrogel. Ions can be trapped in the spaces between neighboring mers, leading to the formation of an "egg carton" structure [26]. Sodium alginate leads to the improvement in the soil structure through the formation of colloidal material surrounding soil particles and the modification of pore size distribution, which, in turn, contributes to the stability of soil aggregates, the regulation of water and fertilizer, and herbicide retention. The ability to neutralize the toxic effects of herbicides on soil properties is due to sodium alginate's cross-linked structure and hydrophobic interactions between hydrophobic groups present on its side chains. As a result, these organic pollutants are rapidly absorbed in the soil and they are slowly released into the environment, thereby reducing the adverse effects of herbicides on the soil microbiome and the biochemical processes taking place [27,28]. The structural formula of sodium alginate is shown in Figure 2.

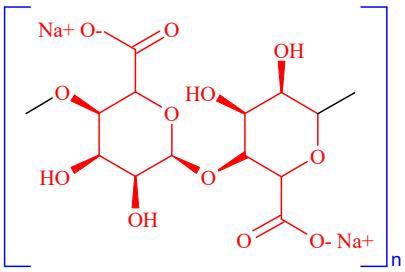


Figure 2. The structural formula of sodium alginate [29].

Against this background, a study was conducted to evaluate the effectiveness of a hydrogel (sodium alginate) in stabilizing the soil environment exposed to sulcotrione pressure. This evaluation was based on reliable biological indicators, i.e., soil microorganisms and enzymes. Importantly, there have been no studies to date that have evaluated the effectiveness of sodium alginate in rebalancing soils exposed to sulcotrione.

The research hypothesis is that (i) sulcotrione in excessive amounts causes changes in soil microbial populations and biochemical activity and (ii) soil enrichment with sodium alginate neutralizes the negative effects of sulcotrione and improves soil quality.

2. Materials and Methods

2.1. The Procedure for Conducting the Experiment

This study was conducted in a laboratory experiment in six replicates (for each combination and each test date). There was a total of 144 beakers (4 herbicide doses × 2 additions of sodium alginate × 3 soil incubation times × 6 replicates). The scheme for conducting the laboratory experiment is shown in Figure 3.

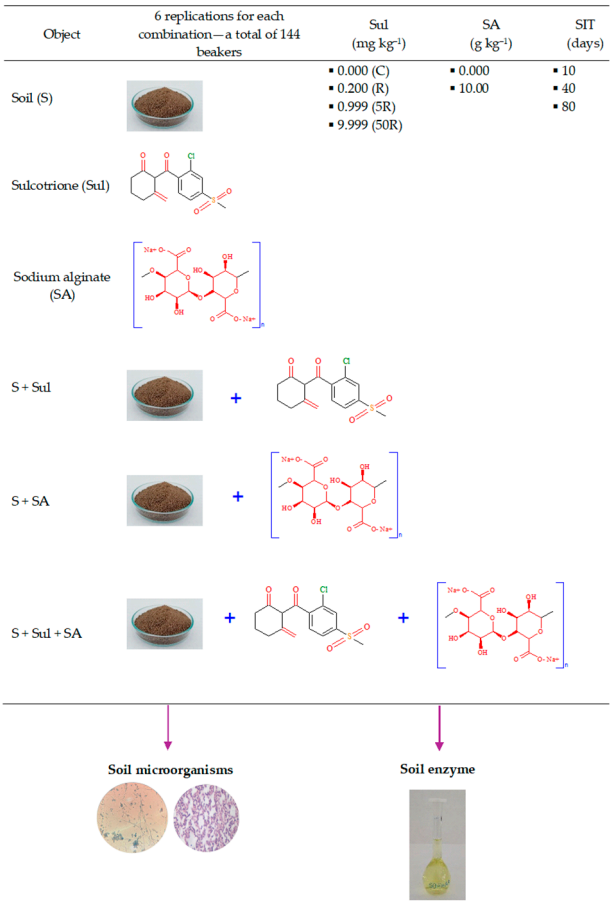


Figure 3. A schematic representation of the laboratory experiment. The "Abbreviations" section includes explanations of the abbreviations used.

The experiment consisted of the following steps:

- First, a separate batch of beakers was prepared for each term.
- An amount of 150 g of air-dried soil (soil sieved through a sieve with a mesh diameter of 2 mm) was weighed into glass beakers (250 cm^3 capacity) for each term.
- The herbicide Sulcogan 300 SC was applied once to the respective sites at doses of 0.000 (C), 0.200 (R), 0.999 (5R), and 9.999 (50R) mg kg^{-1} d. m. of soil, and sodium alginate (SA) at a dose of 10 g kg^{-1} d. m. of soil was applied to neutralize any negative effect of the herbicide on the microbiological and enzymatic properties of the soil.
- The total volume was then homogenized and moistened to 60% capillary water capacity.
- The beakers with soil were covered with perforated foil and incubated at 25 °C for 10, 40, and 80 days. Soil moisture was kept constant throughout the experiment by systematically adding water. For each combination, a bulk sample (total sample weight 900 g) was taken at the respective experimental time points (10, 40, and 80 days), and microbiological and enzymatic analyses were performed. The microbiological analyses were carried out in 4 replicates, while the enzymatic analyses were carried out in 3 replicates for each combination.

2.2. A Description of the Soil Properties

The soil used for this study belonged to Eutric Cambisols [30] with a granulometric composition typical of sandy loam. The soil material was taken from the arable-humus level of Tomaszkowo (53.7161° N, 20.4167° E) in the north-eastern part of Poland from a depth of 0–20 cm. A description of the soil is given in Table 1. Physicochemical analyses of the soil were performed according to the method described in the paper by Wyszkowska et al. [31].

Table 1. Descriptions of the soil properties.

Soil Type	Granulometric Fraction (%)			pH	HAC	EBC	CEC	BS (%)	C_{org}	N_{tot}	C/N
	Sand	Silt	Clay		$mmol^+ kg^{-1}$ d.m. Soil				$g\ kg^{-1}$ d.m. Soil		
sl	66.39	33.58	0.03	5.84	23.62	27.00	50.62	53.32	7.08	1.16	6.10

The "Abbreviations" section includes explanations of the abbreviations used.

2.3. A Description of the Herbicide

The experiment tested the Sulcogan 300 SC preparation, which is used to protect maize against monocotyledonous and dicotyledonous weeds. The manufacturer of the preparation is Nufarm Polska sp. z o. o. (Warsaw, Poland), and the preparation was approved for the Polish market in 2012. It is a systemic preparation in the form of a concentrate for dilution with water. The product is absorbed by weeds mainly through the leaves, but also through the roots. The following weeds are sensitive to this preparation: *Echinochloa crus-galli* (L.) P. Beauv., *Viola arvensis* Murr., *Stellaria media* (L.) Vill., *Chenopodium album* L., *Tripleurospermum inodorum* (L.) Sch. Bip., *Galium aparine* L., *Amaranthus retroflexus* L., and *Thlaspi arvense* L. Moderately sensitive weeds are *Fallopia convolvulus* (L.) Á. Löve and *Polygonum aviculare* L., while the resistant weed is *Solidago virgaurea* L. The recommended dose of the herbicide for a single application is 200 g ha^{-1}. Sulcogan 300 SC contains the substance sulcotrione in the amount of 300 g dm^{-3} of the product.

2.4. Characteristics of the Sodium Alginate

For the neutralization of adverse changes under the influence of the herbicide Sulcogan 300 SC, the hydrogel sodium alginate E401 $(C_6H_7NaO_6)n$) was added to the soil in an amount of 10 g kg^{-1}, produced by Agnex (Bialystok, Poland).

2.5. Microbiological and Enzymatic Analyses of the Soil

Microbiological and enzymatic analyses were carried out on days 10, 40, and 80 of soil incubation.

The microbiological analyses were carried out according to the method described by Wyszkowska et al. [31] and included the determination of the following groups of microorganisms:

- Organotrophic bacteria (Org)—Bunt and Rovira medium, 10^{-5} and 10^{-6} dilutions;
- Actinobacteria (Act)—Küster and Williams medium, 10^{-5} and 10^{-6} dilutions;
- Fungi (Fun)—Martin's medium, 10^{-3} and 10^{-4} dilutions.

The grown colonies of the above groups of microorganisms were counted every day for 10 days, and then their numbers were calculated in units of cfu $\times$ 10^n g^{-1} d.m. (dry matter) of soil.

Enzymatic analyses of the soil were conducted according to the methodology described by Boros et al. [32] and Komorek et al. [33] and included the determination of the activity of the following enzymes:

- Enzymes of the oxidoreductase class: dehydrogenase (Deh) and catalase (Cat). The activity of Deh is expressed in units of µmol TFF kg^{-1} d.m. (dry matter) of soil h^{-1}, and the activity of Cat is expressed in mol O_2 kg^{-1} d.m. (dry matter) of soil h^{-1};
- Hydrolase class enzymes: alkaline phosphatase (Pal), acid phosphatase (Pac), arylsulfatase (Aryl), β-glucosidase (Glu), and urease (Ure). The activities of Pal, Pac, Aryl,

and Glu are expressed in units of mmol PNP kg^{-1} d. m. soil h^{-1}, and the activity of urease is expressed in mmol N-NH_4 kg^{-1} d.m. (dry matter) of soil h^{-1}.

2.6. Calculations of Microbiological and Biochemical Soil Indicators

- Microbiological indicators are calculated based on the number of organotrophic bacteria, actinobacteria, and the fungi/colony development (CD) index [34];
- Ecophysiological (EP) index [35];
- Microorganisms' colony growth (K_s) at specific time intervals (%) [36].

The geometric mean of enzyme activities (GMea) was calculated based on the enzyme activities in the soil [37,38].

The indicators were calculated based on the number of microorganisms and soil enzyme activities:

- Index of sulcotrione effects (IE_{Sul}) [39];
- Index of sodium alginate effects (IE_{SA}) [39].

Formulas and explanations of the listed microbiological and biochemical indicators are given in Supplementary Materials.

2.7. Statistical Analyses

The results obtained were statistically analyzed using the package Statistica 13.3 [40] using a three-factorial ANOVA (factor 1—herbicide dose; factor 2—sodium alginate; factor 3—incubation time in soil) with $p \leq 0.01$. The structural formulation of sulcotrione and sodium alginate was performed using the software ISIS Draw 2.3 [41].

The following statistical analyses were performed:

- For homogeneous groups, significant differences between means were calculated using Tukey's test (HDS);
- Pearson's simple correlation coefficients were calculated for $p \leq 0.01$ between the number of microorganisms and the enzymatic activity in the soil;
- The number of microorganisms and the activity of soil enzymes were presented using a principal component analysis and classification (PCA).

3. Results

3.1. The Responses of Microorganisms to the Herbicide and Sodium Alginate

The number of actinobacteria and fungi was significantly dependent on all tested factors, with a *p*-value of <0.001, while the proliferation of organotrophic bacteria was significantly affected by the dose of herbicide—DSul ($p < 0.001$)—and the addition of hydrogel—SA ($p < 0.001$) (Table 2). It was found that sulcotrione (Sul) applied to the soil at a dose of R (0.200 mg kg^{-1} d. m. soil) to 50R (9.999 mg kg^{-1} d. m. soil) inhibited the proliferation of organotrophic bacteria (Org) and fungi (Fun). The number of organotrophic bacteria decreased on average from 6.78% to 21.99% compared to the control soil, while the number of fungi decreased from 7.04% to 28.51%. The number of actinobacteria (Act) after the application of herbicides at doses of 5R (0.999 mg kg^{-1}) and 50R (9.999 mg kg^{-1}) decreased by 32.52% and 4.87%, respectively. Considering the incubation period of the soil, the average numbers of organotrophic bacteria, actinobacteria, and fungi were the highest on day 40 (0.656×10^7 cfu, 0.563×10^7 cfu, and 0.321×10^5 cfu, respectively). The negative effect of the herbicide on microbial growth is confirmed by the sulcotrione effect index (IE_{Sul}) on microorganisms (Figure 4a). The greatest changes in the proliferation of organotrophic bacteria, actinobacteria, and fungi were caused by the 50R dose. The average IE_{Sul} index values were −0.457, −0.447, and −0.246, respectively. Excluding the herbicide dose, the IE_{Sul} index values of organotrophic bacteria and fungi were the lowest on day 10 (−0.408 and −0.020, respectively), while that of actinobacteria was the lowest on day 40 (−0.535).

The introduction of sodium alginate to soil has a positive effect on the proliferation of the tested groups of microorganisms (Table 2). It was observed that their numbers increased

significantly compared to the objects without the addition of hydrogel. The organotrophic bacteria increased, on average, by 4.44-fold (C) to 5.48-fold (5R), the actinobacteria by 4.71-fold (R) to 6.37-fold (5R), and the fungi by 5.05-fold (C—control soil) to 7.05-fold (50R). The highest numbers of organotrophic bacteria, actinobacteria, and fungi were observed on day 10 in the plots supplemented with sodium alginate (their average numbers were 3.363×10^7 cfu, 3.478×10^7 cfu, and 1.931×10^5 cfu, respectively). The hydrogel neutralized the adverse effects of sulcotrione on the soil microbiota (Figure 4b), resulting in an increase in the numbers of organotrophic bacteria, actinobacteria, and fungi compared to the soil without hydrogel addition, as shown by the index of sodium alginate effect (IF_{SA}). The IE_{SA} index of organotrophic bacteria was the highest in the soil with the 5R dose (IE_{SA} average of 4.983), and that of actinobacteria and fungi was the highest with the 50R dose (IE_{SA} averages of 7.888 and 8.646, respectively). The highest average IE_{SA} values for organotrophic bacteria, actinobacteria, and fungi were recorded on day 10 (IE_{SA} values of 4.437, 7.035, and 9.475, respectively).

Table 2. Microbial numbers in soil with herbicide and sodium alginate (cfu 10^n g^{-1} d. m. soil).

Object	Org $\times 10^7$			Act $\times 10^7$			Fun $\times 10^5$		
	Soil Incubation Time (Days)								
	10	40	80	10	40	80	10	40	80
Soil without the addition of sodium alginate (S)									
C	0.936 ^{k}	0.898 ^{l}	0.726 ^{m}	0.450 ^{l}	0.941 ^{h}	0.612 ^{j}	0.271 ^{j}	0.327 ^{h}	0.133 ^{o}
R	0.595 ^{o}	0.728 ^{m}	0.674 ^{n}	0.760 ^{i}	0.534 ^{k}	0.413 ^{m}	0.300 ^{hi}	0.418 ^{g}	0.226 ^{k}
5R	0.579 ^{q}	0.506 ^{r}	0.581 ^{p}	0.372 ^{n}	0.454 ^{l}	0.301 ^{op}	0.141 ^{n}	0.334 ^{h}	0.228 ^{k}
50R	0.489 ^{r}	0.492 ^{s}	0.572 ^{q}	0.324 ^{o}	0.321 ^{o}	0.272 ^{p}	0.128 ^{p}	0.206 ^{l}	0.156 ^{m}
Average	0.650 ^{D}	0.656 ^{D}	0.638 ^{D}	0.477 ^{E}	0.563 ^{D}	0.400 ^{F}	0.210 ^{E}	0.321 ^{D}	0.186 ^{F}
Soil with the addition of sodium alginate (SSA)									
C	3.679 ^{b}	3.429 ^{d}	4.267 ^{a}	3.601 ^{b}	2.829 ^{d}	3.782 ^{a}	2.020 ^{b}	1.775 ^{c}	1.706 ^{c}
R	3.432 ^{d}	3.031 ^{f}	3.578 ^{c}	3.582 ^{c}	3.111 ^{d}	2.558 ^{f}	2.328 ^{a}	2.007 ^{b}	1.237 ^{e}
5R	3.392 ^{e}	3.022 ^{f}	2.715 ^{h}	3.564 ^{c}	2.868 ^{d}	2.536 ^{f}	1.748 ^{c}	2.015 ^{b}	1.123 ^{e}
50R	2.949 ^{g}	2.438 ^{j}	2.631 ^{i}	3.163 ^{d}	2.783 ^{e}	2.192 ^{g}	1.629 ^{d}	1.685 ^{d}	1.015 ^{f}
Average	3.363 ^{A}	2.980 ^{C}	3.298 ^{B}	3.478 ^{A}	2.898 ^{B}	2.767 ^{C}	1.931 ^{A}	1.871 ^{B}	1.270 ^{C}
p-value									
DSul	<0.001			<0.001			<0.001		
SA	<0.001			<0.001			<0.001		
DSul × SA	0.025			<0.001			<0.001		
DSul × SIT	<0.001			<0.001			<0.001		
SA × SIT	0.159			<0.001			<0.001		
DSul × SA × SIT	0.016			<0.001			<0.001		
	0.006			<0.001			<0.001		

The "Abbreviations" section includes explanations of the abbreviations used. Homogeneous groups marked with letters (a–s) were calculated separately for each group of microorganisms depending on the herbicide dose and sodium alginate addition. The average soil incubation times marked with capital letters (A–F) were calculated separately for each group of microorganisms.

The application of sulcotrione to the soil has a significant effect on the colony development index (CD) of the microorganisms (Figure 5). On average, the CD value of organotrophic bacteria was between 47.830 (50R) and 55.091 (5R), that of actinobacteria was between 24.342 (R) and 26.552 (5R), and that of fungi was between 34.127 (C) and 42.041 (R). Considering the average values for the incubation period of the soil, the highest CD values for organotrophic bacteria and actinobacteria were seen on day 10 (58.505 and 26.202, respectively), and that for fungi was seen on day 40 (38.992). Sodium alginate led to the occurrence of CD effects of actinobacteria and fungi compared to the effects without the use of hydrogel. The CD values of actinomycetes ranged from 27.671 (5R) to 30.789 (C),

while those of fungi ranged from 47.672 (C) to 49.750 (50R). On the other hand, the CD value of organotrophic bacteria decreased under the influence of sodium alginate compared to the object without its addition. The CD values of this group of microorganisms ranged from 37.076 (5R) to 41.868 (C). In soils with hydrogel, regardless of the herbicide dose, the highest CD values of organotrophic bacteria and actinobacteria were seen on day 80 (CD averages of 40.084 and 29.647, respectively), and that of fungi was seen on day 10 (CD average of 51.009).

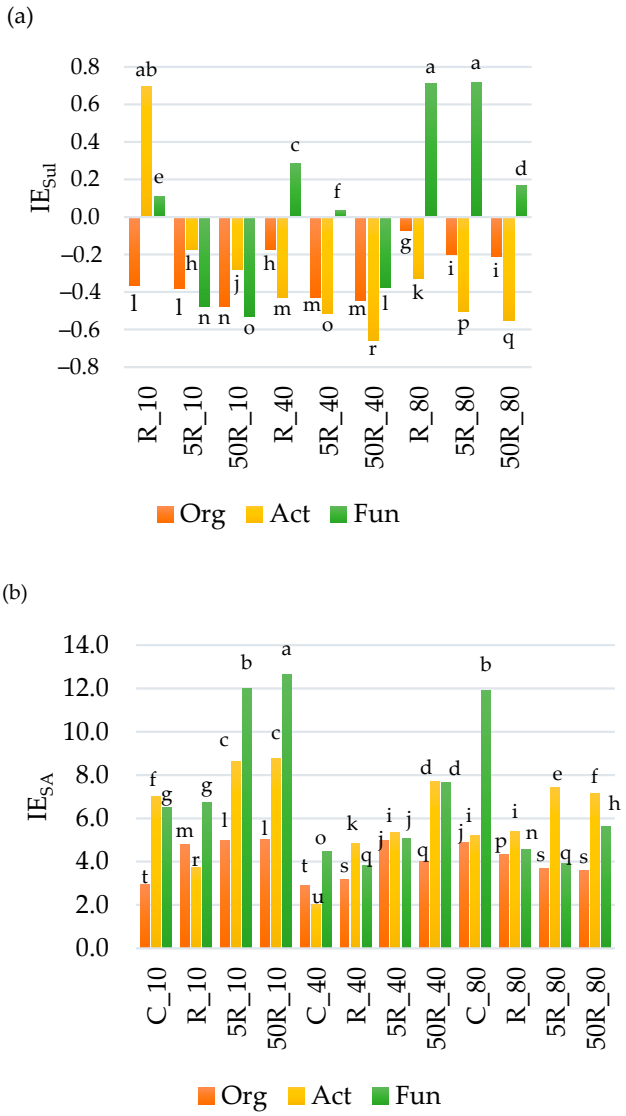


Figure 4. An index of the effects of (**a**) sulcotrione (IE_{Sul}) and (**b**) sodium alginate (IE_{SA}) on microbial proliferation in soil. The "Abbreviations" section includes explanations of the abbreviations used. Homogeneous groups are denoted by letters (a–u).

The rate of microbial multiplication varied, as shown by the growth of microbial colonies (K_s) at specific time intervals (Figure 6). In the soil treated with sulcotrione, the organotrophic bacteria and fungi multiplied most rapidly during the first 6 days. For organotrophic bacteria, the average colony growth rate ranged from 97.00% (50R) to 99.39% (C), and that for the fungi ranged from 90.34% (50R) to 94.69% (5R). The actinobacteria, on the other hand, multiplied most rapidly in 3 to 8 days. During this time, the growth rates of the colonies were between 86.54% (C) and 89.86% (R).

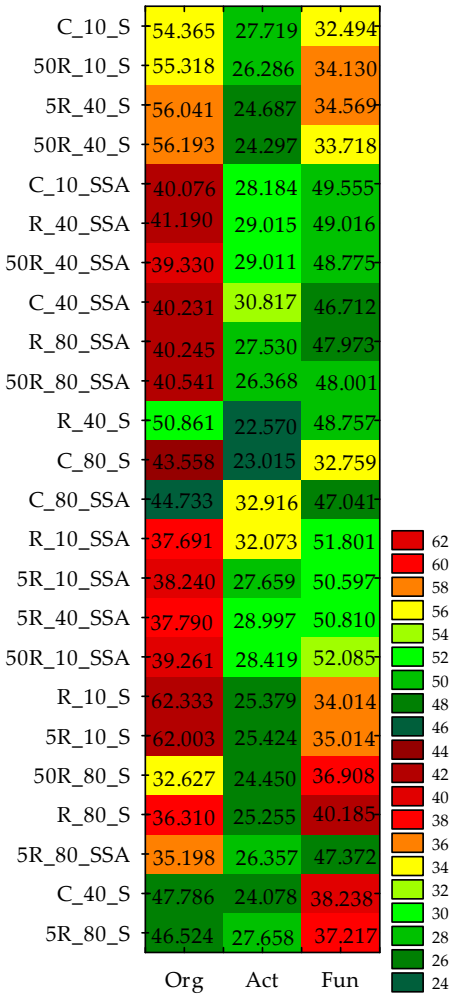


Figure 5. Colony development (CD) index of microorganisms in soil with sulcotrione and sodium alginate. "Abbreviations" section includes explanations of abbreviations used.

The introduction of sodium alginate into the soil also led to the fastest colony growth of organotrophic bacteria and fungi in the first 4 days of incubation. During this time, 89.73% (5R) to 94.28% (R) of the organotrophic bacterial colonies and 99.00% (R) to 100.00% (50R) of the fungal colonies proliferated. Actinobacteria in soils with herbicide doses of C to 5R proliferated most intensively from day 3 to 8 (88.33% to 90.90%). In soils with a dose of 50R, actinobacteria proliferated most rapidly in the first 8 days of incubation (96.12% of colonies grew in this period).

The ecophysiological diversity index (EP) of the microorganisms (Figure 7) in the sulcotrione-treated soils varied and was dose-dependent. The EP of organotrophic bacteria and actinobacteria did not change significantly under the influence of the herbicide, as the EP was at a similar level as in the control soil. Only the EP value of the fungi increased significantly in the soil with R and 5R doses ranging from 0.591 to 0.643 and 0.636, respectively. The highest EP value of organotrophic bacteria was recorded on day 80 (average EP value 0.857), and those of actinobacteria and fungi were recorded on day 40 (average EP values of 0.819 and 0.670, respectively). The application of sodium alginate to the soil helped reduce the EP values of actinobacteria and fungi. The average EP value of actinobacteria was between 0.754 (50R) and 0.780 (R), while that of fungi was between 0.205 (R) and 0.313 (C). The EP value of the fungi increased in the soil with herbicide doses of R and 5R ranging from 0.743 to 0.784 and from 0.730 to 0.782, respectively. At sites C and 50R, the EP value of fungi decreased from 0.772 to 0.734 and from 0.763 to 0.758, respectively. After soil enrichment with sodium alginate, the highest EP values for organotrophic bacteria and

actinobacteria were recorded on day 40 (average EP values of 0.777 and 0.796, respectively), and that for fungi was recorded on day 10 (average EP value of 0.270) irrespective of the herbicide dose.

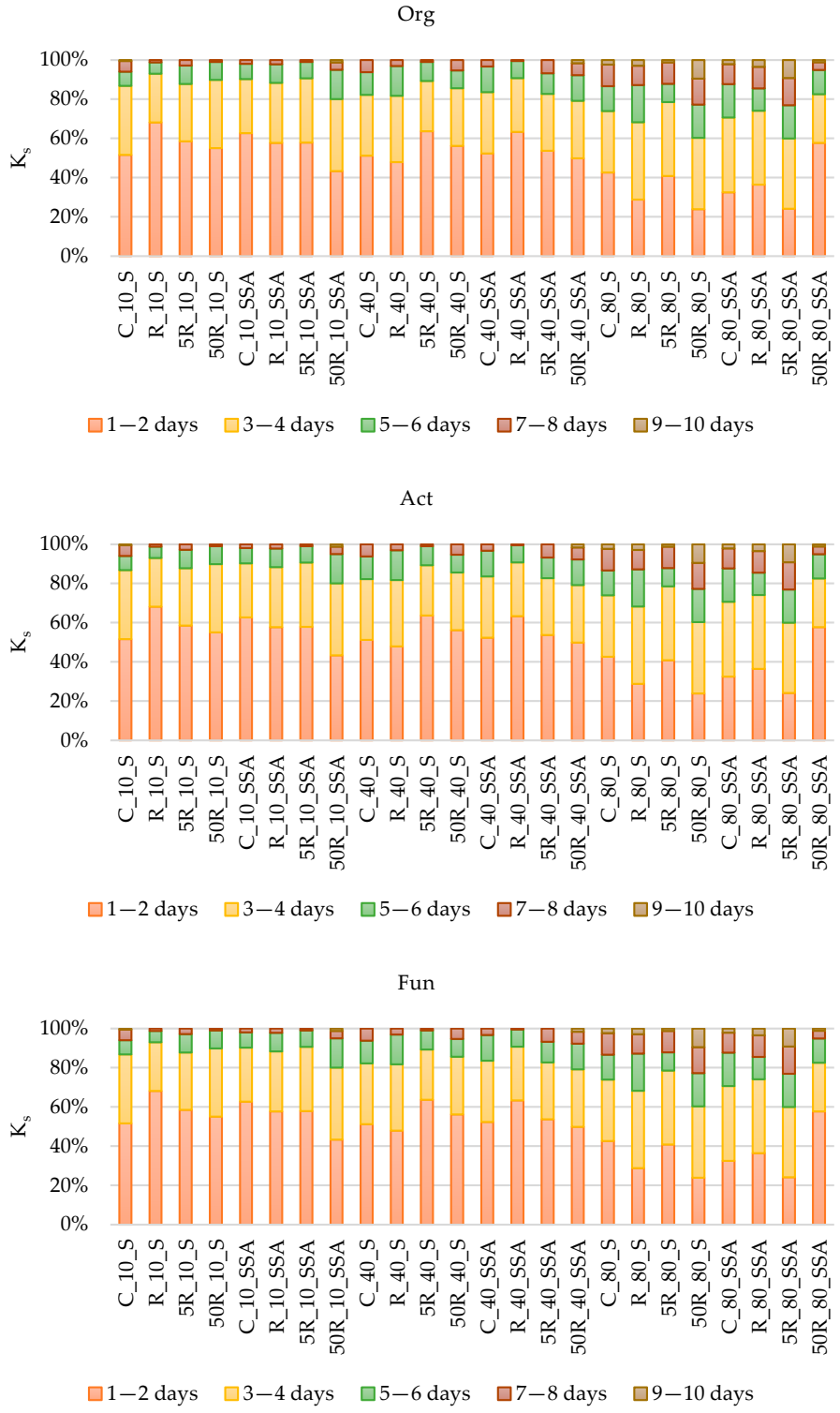


Figure 6. Microorganism colony growth (K_s) at specific time intervals (%). The "Abbreviations" section includes explanations of the abbreviations used.

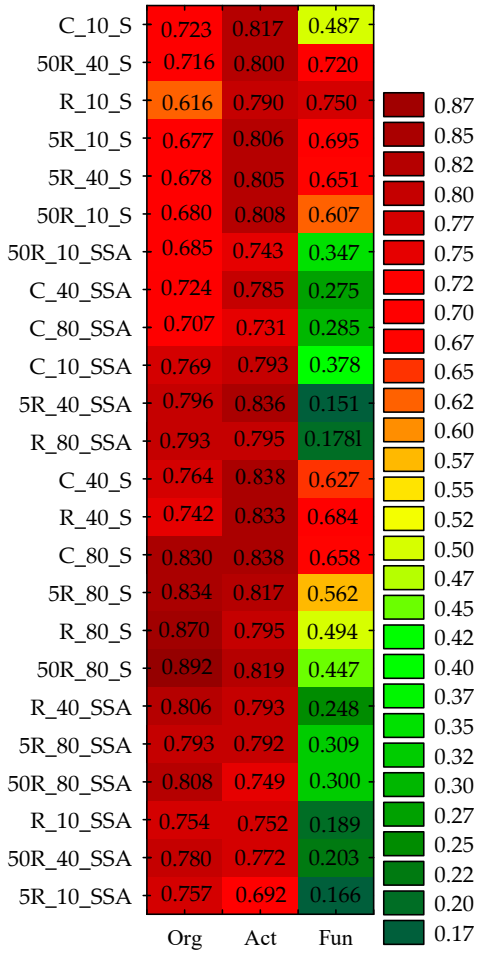


Figure 7. The ecophysiological diversity (EP) index of microorganisms in soil with sulcotrione and sodium alginate. The "Abbreviations" section includes explanations of the abbreviations used.

3.2. The Response of the Enzyme to the Herbicide and Sodium Alginate

The activity of all soil enzymes was significantly dependent on the sulcotrione dose ($p < 0.001$), the addition of sodium alginate ($p < 0.001$), and the soil incubation time—SIT ($p < 0.001$) (Table S1). Sulcotrione at a dose of R to 50R stimulated the activities of dehydrogenases (Deh), arylsulfatase, and β-glucosidase (Glu). The activities of these enzymes increased on average compared to the control soil in the following ranges: dehydrogenases—from 9.70% to 27.58%; arylsulfatase—from 10.83% to 31.11%; and β-glucosidase—from 19.14% to 28.12%. The activity of catalase (Cat) increased due to the herbicide when the soil dose was applied at R and 5R (there were 4.20% and 15.69% increases in activity, respectively). The herbicide had an inhibitory effect on the activities of alkaline phosphatase (Pal), acid phosphatase (Pac), and urease (Ure). Doses of 5 R and 50 R reduced the activity of alkaline phosphatase by averages of 21.30% and 24.70%, that of acid phosphatase by 3.81% and 7.06%, and that of urease by 5.92% and 46.35%. The highest activities of dehydrogenases, catalase, arylsulfatase, β-glucosidase, and urease were recorded on day 10 (the average activities were 12.000 µmol TFF, 0.159 mol O_2, 0.044 mmol PNP, 0.114 mmol PNP, and 0.224 mmol N-NH_4), those of alkaline phosphatase and arylsulfatase were recorded on day 80 (the average activity was 0.262 mmol PNP), and that of acid phosphatase was recorded on day 10 (the average activity was 1.355 mmol PNP). The differential effects of the herbicide on soil enzymes are confirmed by the IE_{Sul} index of the effects of sulcotrione on the soil biochemical properties (Figure 8). Based on the IE_{Sul}, it was found that the herbicide had an activating effect on dehydrogenases (the IE_{Sul} value ranged from 0.099 to 0.282 on average), arylsulfatase (the IE_{Sul} value ranged from 1.475 to 3.296 on average), and β-glucosidase (the IE_{Sul} value ranged from 0.185 to 0.290) at a dose of R to 50R. Cata-

lase activity was also stimulated by sulcotrione at doses of R and 50R (the average IE_{Sul} values were 0.172 and 0.034, respectively). The incorporation of 5R and 50R doses into the soil inhibited alkaline phosphatase activity (the average IE_{Sul} values were –0.208 and –0.240, respectively) and acid phosphatase activity (average IE_{Sul} values were –0.033 and –0.064, respectively). The IE_{Sul} index values of dehydrogenases, alkaline phosphatase, and *β*-glucosidase were the highest on day 10, those of catalase and urease were the highest on day 40, and that of arylsulfatase on day 80.

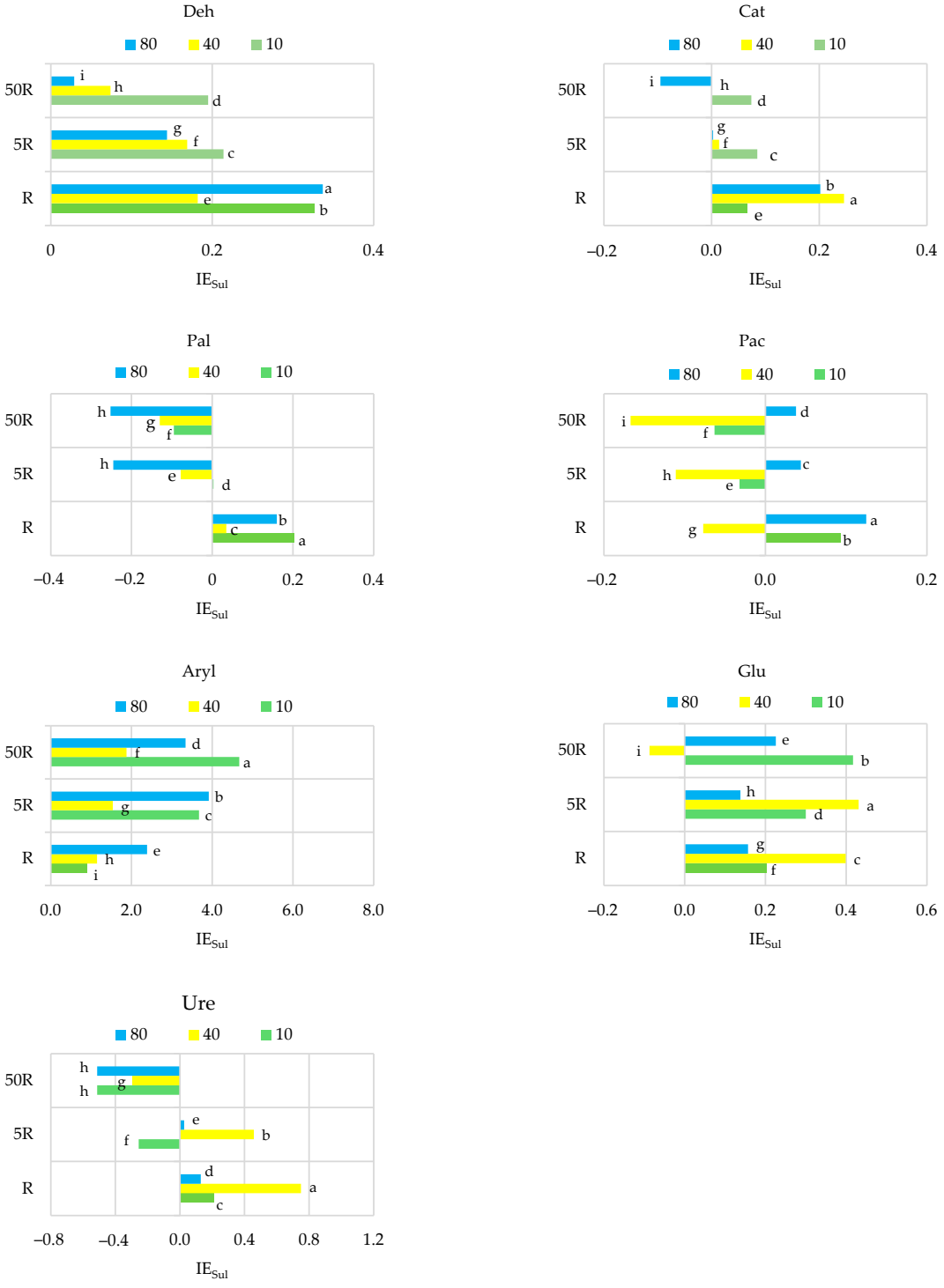


Figure 8. The index of the effects of sulcotrione (IE_{Sul}) on the activity of soil enzymes. The "Abbreviations" section includes explanations of the abbreviations used. Homogeneous groups, denoted by letters (a–i), were calculated for individual enzymes.

The enrichment of the soil with sodium alginate (Table S1) had a positive effect on the activities of dehydrogenases, catalase, alkaline phosphatase, and arylsulfatase. On average, the activities of these enzymes increased from 2.73-fold to 4.57-fold for dehydrogenases, from 2.21-fold to 2.71-fold for catalase, from 1.59-fold to 2.48-fold for alkaline phosphatase, and from 3.05-fold to 17.89-fold for arylsulfatase compared to the soil without hydrogel addition. Sodium alginate had an inhibitory effect on acid phosphatase, as its activity decreased from 3.27-fold to 3.62-fold. The activity of β-glucosidase increased under the influence of sodium alginate in the soil containing the herbicide at doses C and R (increases of 1.20-fold and 1.08-fold, respectively), while doses of 5R and 50R inhibited its activity (the average activity decreased by 1.02-fold and 1.01-fold, respectively). The addition of hydrogel to the soil in objects C, R, and 5R had an inactivating effect on urease activity (1.01-fold, 1.45-fold, and 1.11-fold decreases in activity, respectively). It was observed that the activities of dehydrogenases, catalase, arylsulfatase, β-glucosidase, and urease were the highest in the sodium alginate-supplemented objects on day 10, while the activities of alkaline phosphatase and acid phosphatase were the highest on day 40. The significant effect of the hydrogel on the enzymatic activity of the soil was confirmed by the sodium alginate impact index (IE_{SA}) (Figure 9). The hydrogel stimulated the activities of dehydrogenases (the average IE_{SA} value ranged from 1.679 to 3.597), catalase (the average IE_{SA} value ranged from 1.207 to 1.430), alkaline phosphatase (the average IE_{SA} value ranged from 2.355 to 3.766), arylsulfatase (the average IE_{SA} value ranged from 4.237 to 19.778), and urease (the average IE_{SA} value ranged from 3.568 to 10.805). Sodium alginate had an inhibitory effect on acid phosphatase activity as the IE_{SA} index took on negative values ranging from –0.378 (5R) to –0.199 (C) for all objects. The IE_{SA} index values of β-glucosidase in C, R, and 50R soil were 0.203, 0.083, and 0.039, respectively. In sodium alginate-enriched soil, the IE_{SA} values of acid phosphatase, dehydrogenases, and β-glucosidase were the highest on day 10, that of alkaline phosphatase on day 40, while those of catalase and arylsulfatase were the highest on day 80.

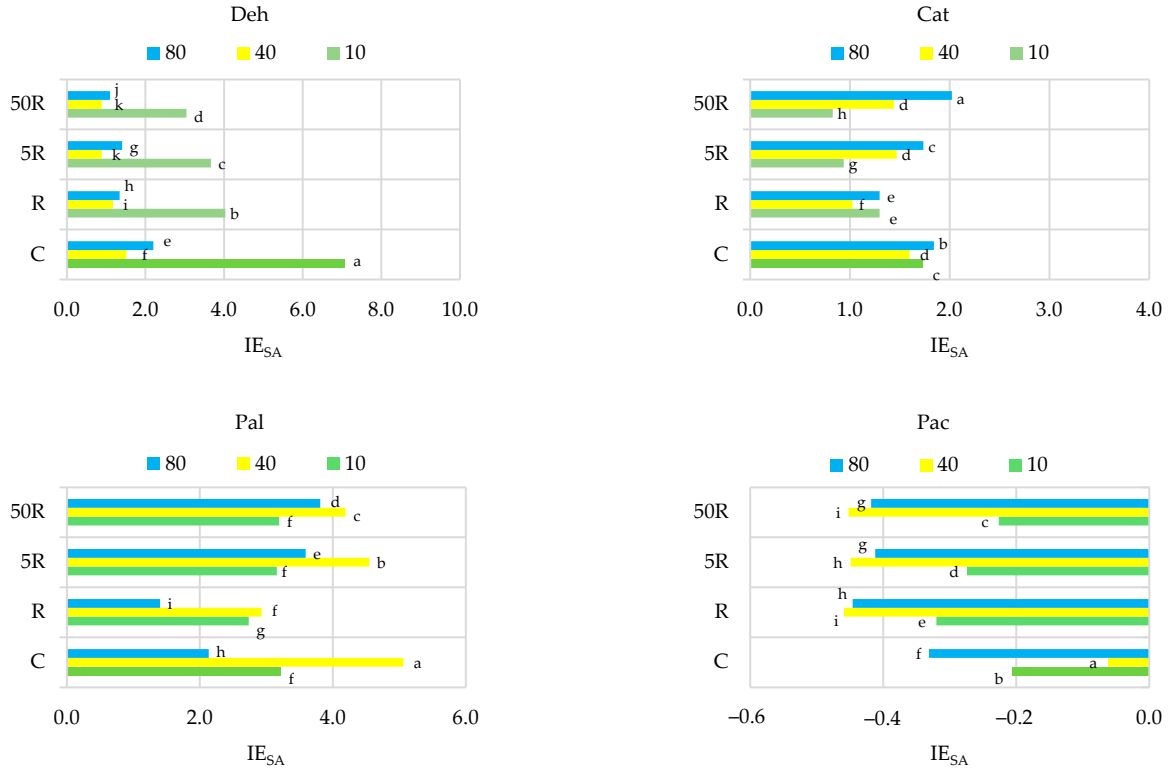


Figure 9. *Cont.*

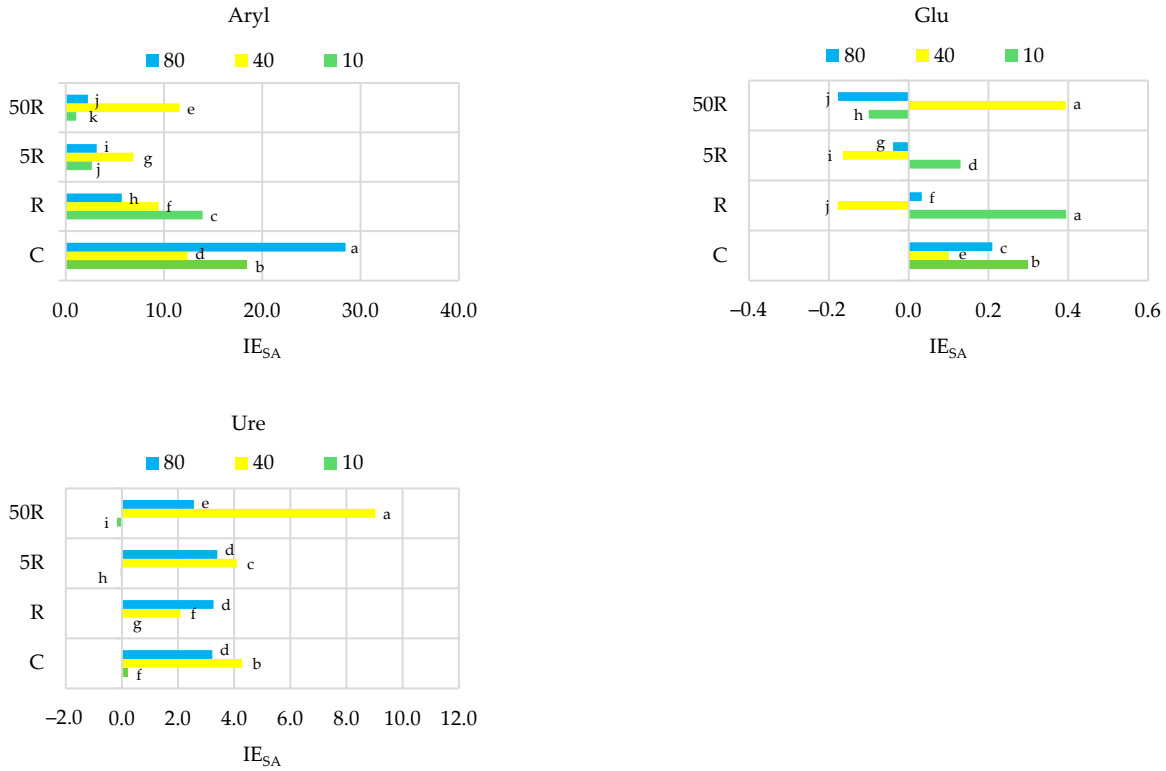


Figure 9. The index of the effects of sodium alginate (IE_{SA}) on the activity of soil enzymes. The "Abbreviations" section includes explanations of the abbreviations used. Homogeneous groups, denoted by letters (a–k), were calculated for individual enzymes.

The geometric mean enzyme activity (GMea) (Figure 10) provides valuable information about soil quality. In the soil without hydrogel addition, the GMea index value was the highest on day 10 in the treatment with dose C (GMea = 0.175) and on days 40 and 80 in the treatment with dose R (the GMea values were 0.279 and 0.313, respectively). The addition of sodium alginate to the soil contributed to the increase in the GMea index compared to the soil without this addition, being between 1.92-fold (dose 50R, 80 days) and 5.21-fold (dose C, 10 days).

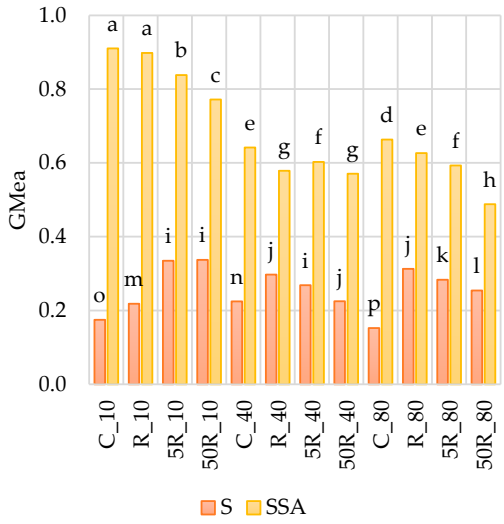
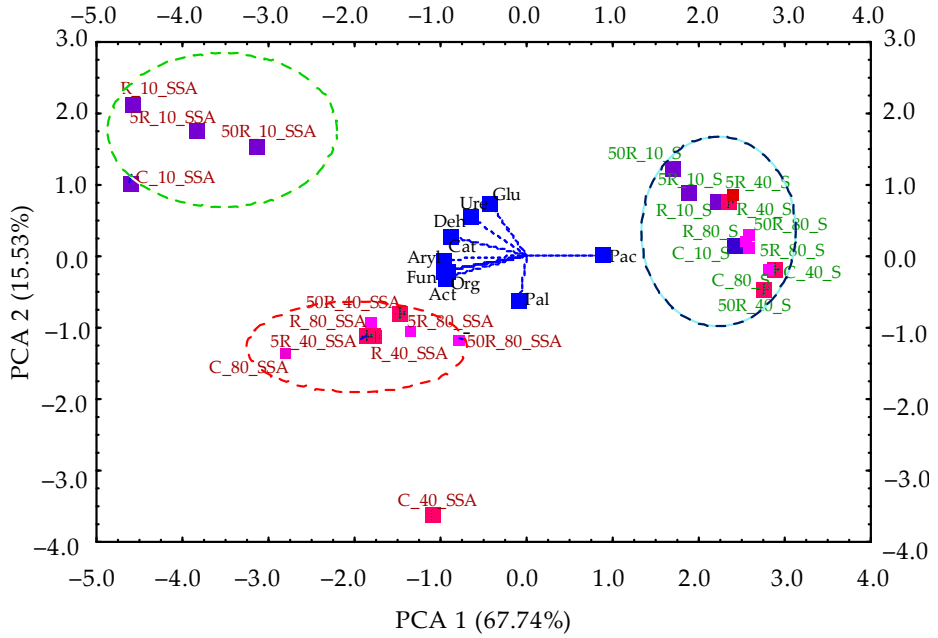


Figure 10. Effects of sulcotrione and sodium alginate on the geometric mean enzyme activity (GMea). The "Abbreviations" section includes explanations of the abbreviations used. Homogeneous groups are denoted by letters (a–p). The background colors of the table are explained in the table as values from −1 to 1.

3.3. The Relationship Between the Number of Microorganisms and the Activity of the Soil Enzymes

Using Pearson's simple correlation coefficients (Table 3), it was found that the addition of hydrogel correlated significantly positively with the numbers of organotrophic bacteria, actinobacteria, and fungi and the activities of dehydrogenases, catalase, arylsulfatase, and urease, while it correlated negatively with the activity of acid phosphatase. The incubation time of the soil correlated significantly negatively with the activities of β-glucosidase and urease; the numbers of organotrophic bacteria, actinobacteria, and fungi; and the activities of dehydrogenases and catalase with the activity of acid phosphatase. The numbers of organotrophic bacteria, actinobacteria, and fungi correlated significantly with the activities of dehydrogenases, catalase, arylsulfatase, and urease.

The principal component analysis (PCA) showed (Figure 11) that PCA1 (explained 67.74% of the variance) positively correlated with the activity of acid phosphatase and PCA2 (explained 15.53% of the variance) correlated with the activities of dehydrogenases, acid phosphatase, β-glucosidase, and urease. The analyzed variables formed four homogeneous groups. The first group consisted of the variables Glu, Ure, Deh, and Cat; the second group consisted of the variables Aryl, Fun, Org, and Act; the third group consisted of the variable Pal; and the fourth group consisted of the variable Pac.



Variable	Org	Act	Fun	Deh	Cat	Pal	Pac	Aryl	Glu	Ure
PCA 1	−0.949	−0.975	−0.955	−0.889	−0.974	−0.088	0.891	−0.916	−0.435	−0.665
PCA 2	−0.290	−0.195	−0.201	0.302	−0.053	−0.606	0.027	−0.208	0.740	0.581

Figure 11. Responses of soil microorganisms and enzymes to sulcotrione and sodium alginate (principal component analysis—PCA). The "Abbreviations" section includes explanations of the abbreviations used. Vectors are marked in blue (Org, Act, Fun, Deh, Cat, Pal, Pac, Aryl, Glu, Ure), cases in green (soil without the addition of sodium alginate—S) and maroon (soil with the addition of sodium alginate—SSA).

Table 3. Simple Pearson's correlation coefficients between the numbers of microorganisms and the activities of soil enzymes ($p \leq 0.01$, $n = 24$).

Variable	DSul	SA	SIT	Org	Act	Fun	Deh	Cat	Pal	Pac	Aryl	Glu	Ure
DSul	1.000												
SA	−0.000	1.000											
SIT	−0.000	0.000	1.000										
Org	−0.125	0.977 *	−0.004	1.000									
Act	−0.078	0.976 *	−0.090	0.986 *	1.000								
Fun	−0.088	0.953 *	−0.158	0.953 *	0.978 *	1.000							
Deh	−0.150	0.698 *	−0.382	0.745 *	0.791 *	0.779 *	1.000						
Cat	−0.106	0.919 *	−0.303	0.925 *	0.945 *	0.941 *	0.876 *	1.000					
Pal	−0.280	0.210	−0.024	0.232	0.176	0.220	0.018	0.171	1.000				
Pac	−0.154	−0.897 *	0.037	−0.865 *	−0.888 *	−0.847 *	−0.682 *	−0.848 *	0.226	1.000			
Aryl	−0.036	0.914 *	0.050	0.949 *	0.933 *	0.879 *	0.699 *	0.872 *	0.067	−0.886 *	1.000		
Glu	0.114	0.150	−0.628 *	0.193	0.263	0.280	0.581 *	0.413 *	−0.169	−0.256	0.262	1.000	
Ure	0.078	0.439 *	−0.510 *	0.457 *	0.542 *	0.520 *	0.834 *	0.603 *	−0.091	−0.473 *	0.387	0.650 *	1.000

Legend:
0.80 ÷ 1.00
0.60 ÷ 0.79
0.40 ÷ 0.59
0.20 ÷ 0.39
0.00 ÷ 0.19
−0.19 ÷ −0.01
−0.39 ÷ −0.20
−0.59 ÷ −0.40
−0.79 ÷ −0.60
−1.00 ÷ −0.80

The "Abbreviations" section includes explanations of the abbreviations used. *—significant at $p \leq 0.001$.

4. Discussion

4.1. The Responses of Microorganisms to the Herbicide and Sodium Alginate

Chen et al. [42], de Mesquita et al. [43], and Araujo et al. [44] reported that soil microorganisms play a special role in soil ecosystems as they are responsible for nutrient cycling and energy flow and are also bioindicators. In the study by Bezuglova et al. [45], a reduction in the number of bacteria was found after the application of sulfonylurea herbicides, and Kepler et al. [46] did not find any significant responses of microorganisms to the applied glyphosate.

Sunulahpašić et al. [8] found a detrimental effect of nicosulfuron on the numbers of total bacteria, ammonifying bacteria, actinobacteria, and fungi. However, the greatest changes occurred in the fungal population, whose numbers decreased from 38.00% to 60.00% compared to the control. The inhibition of the growth of bacterial, actinobacterial, and fungal populations under the influence of pendimethalin, oxyfluorfen, and propaquizafop was observed by Adhikary et al. [47]. Du et al. [48] also tested mesotrione at concentrations of 1.0 and 5.0 mg kg^{-1} and observed a reduction in the numbers of bacteria, actinomycetes, and fungi. Our studies also confirm the toxic effect of sulcotrione on the population of soil microorganisms. The highest dose of sulcotrione 50R (9.999 mg kg^{-1}) decreased the number of organotrophic bacteria by 21.21–47.76%, actinobacteria by 28.00–65.89%, and fungi by 37.00–52.77% compared to the control soil. This could be due to interspecific competition for the ecological niche of the microorganisms, which were more resistant to high doses of the herbicide, as well as to the toxicity and persistence of sulcotrione in the soil [49]. Microorganisms can degrade herbicides and then use them as a source of biogenic elements for physiological processes in their cells. However, before these compounds are degraded, they are usually toxic to the microbiota, especially after their direct application when the content of these compounds is the highest. Significant changes in number, activity, and microorganism diversity can then be observed [50]. Our studies have shown that sulcotrione (Sul) can disrupt the structure and diversity of soil microorganisms. This herbicide increased the colony development index (CD) of organotrophic bacteria (Org) and fungi (Fun) and decreased it in actinobacteria (Act), indicating changes in the ratio between r-strategists and K-strategists [51]. However, the diversity of organotrophic bacteria and actinobacteria was not affected by sulcotrione, as the ecophysiological diversity index (EP) remained at a similar level as in the control soil. In the fungi, however, sulcotrione led to an increase in the EP value. In their study, He et al. [52] investigated the effects of the sole and combined use of glyphosate and diquat on the structures of bacterial and fungal communities. These herbicides increased the number of Acidobacteria and decreased the number of Proteobacteria, with the mixture of these herbicides having a smaller effect than the individual preparations. These authors also noted an increase in the number of fungi of the genus *Talaromyces* and *Culvuralia* after the application of the herbicide mixture and a decrease in the case of a single compound. Kepler et al. [46] and Lupwayi et al. [53] reported that herbicides have little or no effect on the soil microbial community structure. The continuous use of herbicides allows microorganisms to adapt to changing environmental conditions, and these chemicals can be utilized as an additional source of nutrients for their growth. Omidvar et al. [54] found no significant differences in the diversity and structure of microbial communities in soils treated with Round Herbicide, BioWeed, and Slasher.

The negative effects of chemical pollutants on the soil microbiota can be mitigated by sorbents increasingly using natural polymers. They can be considered renewable sources due to their abundant occurrence in nature, suitable structural composition, non-toxicity, and biocompatibility [55,56]. One such natural polymer is sodium alginate (SA), which we used in our study to mitigate the potential adverse effects of sulcotrione. In this study, we found a positive effect of sodium alginate on soil microorganisms, as evidenced by significant increases in the numbers of organotrophic bacteria (Org), actinobacteria (Act), and fungi (Fun) compared to soil without the addition of the hydrogel. After the application of this preparation to the soil, there was an increase in all microorganisms analyzed. Such a

correlation could be due to the presence of hydroxyl and carboxyl groups in this hydrogel, which have the potential to adsorb pollutants from the environment. In addition, sodium alginate has high mechanical and thermal stability due to its binding to carbon compounds, microorganisms, and other polymers [26,57,58]. Furthermore, it has enormous potential for water retention [59], which helps to maintain adequate soil moisture necessary for the development of the soil microbiome, which is the main producer of enzymes. Despite this positive effect of sodium alginate on the proliferation of microorganisms, different effects were observed on the colony development index (CD) and the ecophysiological diversity index (EP) of the microorganisms. Under the influence of the hydrogel, the CD value of organotrophic bacteria decreased, while the CD values of actinobacteria and fungi increased. Sodium alginate showed no clear effect on the EP value of the organotrophic bacteria, and only fungi reacted with a reduction in this index.

4.2. The Responses of Enzymes to the Herbicide and Sodium Alginate

In this study, we analyzed disturbances in the activities of soil enzymes that responded with varying degrees of sensitivity to increasing doses of sulcotrione. Soil enzymes, which are involved in biochemical processes and nutrient cycling in soil, influence soil microecology [42,60]. As Filimon et al. [61] pointed out, enzyme activity may depend on the type of herbicide, its dose, the interval between applications, the soil organic matter content, and the soil type. In our study, the enzymes found to be most resistant to sulcotrione were dehydrogenases, catalase, arylsulfatase, and β-glucosidase, whose activities increased with an increasing herbicide dose. Dehydrogenases are intracellular enzymes found only in living cells and are therefore an indicator of oxidative metabolism in soil [11]. These enzymes can react differently to soil contamination with herbicides, i.e., there can be an increase, a decrease, or no reaction. An example of the negative effect of herbicides on these enzymes is the study by Siddagangamma et al. [62], who tested two herbicides: pendimethalin at a dose of 0.34 kg ha^{-1} and oxadiargyl at a dose of 0.04 kg ha^{-1} applied to clay soil. In turn, Pertile et al. [63] found that the activity of dehydrogenases increased in up to 15 days of soil incubation under the influence of the herbicides imaceptyril and flumioxazin and then decreased to the level of the control soil. Another enzyme that plays an important role in protecting microbial cells from oxidative stress caused by toxic hydrogen peroxide is catalase [64]. In our study, this enzyme was stimulated by sulcotrione. Athia et al. [65] investigated the effect of three herbicides, namely tribenuron-methyl, diflufenican + isoproturon, and clodinafop-propargyl, and found a significant inhibition of catalase activity after the application of contaminating amounts. Arylsulfatase, in turn, is an enzyme that controls the uptake of organic sulfur and thus the sulfur cycle in the soil, which is very important for plant growth and development [42]. The study by Medo et al. [66] showed that sulfonylurea herbicides (chlorsulfuron at a dose of 26 g ha^{-1} and sulfosulfuron at a dose of 25 g ha^{-1}) inhibited the activity of arylsulfatase, especially after up to 28 days of soil incubation. However, greater changes occurred after the application of chlorosulfate to the soil than with sulfosulfate. The activity of β-glucosidase was increased by sulcotrione in the present study. Li et al. [67] also observed a stimulating effect of the herbicide pyroxsulam on the activity of β-glucosidase during the 56-day experiment. For example, Du et al. [68] analyzed the effect of different amounts of mesotrione (0.1, 1.0 and 5.0 mg kg^{-1}) on the activities of the enzymes and showed an inactivating effect of 5.0 mg kg^{-1} mesotrione on the activity of β-glucosidase. The increased activity of these enzymes could be due to the presence of a large number of living microbial cells in the soil, which have adapted to unfavorable environmental conditions and use the herbicide as a source of carbon and energy. An enzyme that plays an important role in the soil environment is urease, which is involved in the transformation of organic nitrogen in the soil and thanks to which the nitrogen cycle in the soil is regulated [69,70]. Our studies have shown an inhibitory effect of sulcotrione on urease, which was also confirmed in our previous study [51]. Kumari et al. [69] found an increase in urease activity in response to pendimethalin up to day 60 of the experiment, while atrazine inhibited the activity of this enzyme mainly at the

beginning of the experiment on days 0 and 15. In these studies, sulcotrione also had an inhibitory effect on alkaline phosphatase and acid phosphatase activity. These are enzymes actively involved in the catalytic processes of hydrolysis of organic phosphates released into the soil [71,72]. Filimon et al. [61] also observed a reduction in the activity of phosphatases under laboratory and field conditions after the application of the recommended dose (2.0 g kg^{-1}), while their activity returned to equilibrium after 21 days. The inhibition of phosphatase and urease activity could have the result of disturbances in the number and structure of microorganisms that affected the biochemical activity of the soil. In addition, sulcotrione could affect soil enzymes by lysing cells and changing the permeability of cell membranes, which influences the secretion of enzymes into the soil environment [73].

In the conducted studies, sodium alginate increased the activities of dehydrogenases, catalase, alkaline phosphatase, arylsulfatases, β-glucosidase, and urease compared to soils that were were not supplemented with this hydrogel. Sodium alginate owes its positive effect on the activity of these soil enzymes to its high contents of carboxyl and hydroxyl groups, which have an affinity for different cations and can form chelates with other ions present in the soil [74]. Due to its pore structure and cross-linking, it has a high absorption capacity that can immobilize or remove chemical pollutants in the soil [75]. The use of sodium alginate also allows for the controlled release of agrochemicals into the environment, which can limit their negative impact on the soil ecosystem [14,76]. However, in our studies, it was found that the analyzed polymer can inhibit acid phosphatase activity and enhance the harmful effect of sulcotrione. This enzymatic reaction may be the effect of the immobilization of microorganisms producing acid phosphatase, which is an extracellular enzyme, making it less stable on soil colloids [77,78].

5. Conclusions

The microbiological and biochemical properties of the soil were significantly altered by the dose of sulcotrione, the addition of sodium alginate to the soil, and the incubation time of the soil. Considering the average values of the terms, sulcotrione stimulated at a dose of 0.200 mg kg^{-1} d.m. of soil to 9.999 mg kg^{-1} d.m. of soil stimulated the activities of dehydrogenases, catalase, arylsulfatase, and β-glucosidase, while it decreased the activities of alkaline phosphatase, acid phosphatase, and urease as well as the numbers of organotrophic bacteria, actinobacteria, and fungi compared to the control soil. The herbicide, which was applied at a dose of 0.200 mg kg^{-1} d.m. of soil to 9.999 mg kg^{-1} d.m. of soil, increased the colony development index (CD) value of fungi, while at a dose of 0.200 mg kg^{-1} d.m. of soil to 0.999 mg kg^{-1} d.m. of soil, the colony development index of organotrophic bacteria was increased. This preparation did not change the value of the ecophysiological diversity index (EP) of organotrophic bacteria and actinobacteria but increased that of fungi. The enrichment of the soil with sodium alginate contributed to the increase in the number of analyzed microorganism groups and the activities of dehydrogenases, catalase, alkaline phosphatase, arylsulfatase, and urease compared to the soil without the addition of hydrogel. However, acid phosphatase reacted negatively to sodium alginate as its activity decreased. β-glucosidase showed a different response to this polymer. In addition, this substance decreased the colony development index (CD) value of organotrophic bacteria and increased those of actinobacteria and fungi. Under the influence of sodium alginate, reductions in the ecophysiological diversity (EP) index values of actinobacteria and fungi was also observed. Both in soil without and with the addition of sodium alginate, the r-strategies dominated among organotrophic bacteria and fungi, and the K-strategies dominated among actinobacteria. Sodium alginate improves soil quality by stimulating the growth of microorganisms and the activity of most soil enzymes. Studies have shown that sodium alginate has the potential to be a promising sorbent for remediating soils contaminated with organic compounds, i.e., herbicides. However, further research should be conducted to determine the doses of this polymer that are effective in restoring soil homeostasis.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14112081/s1, Table S1: Enzyme activity in soil treated with sulcotrione and sodium alginate (kg^{-1} d.m. soil h^{-1}).

Author Contributions: Conceptualization, M.B., J.W. and J.K.; methodology, M.B., J.W. and J.K.; conducted the experiments, M.B.; formal analysis, M.B.; statistical analyses, M.B; visualization, M.B.; validation, J.W.; investigation, M.B., J.W. and J.K.; writing—original draft preparation, M.B.; writing—review and editing, M.B., J.W. and J.K.; supervision, J.K. All authors contributed significantly to the discussion of the results and the preparation of this manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of Warmia and Mazury in Olsztyn, Faculty of Agriculture and Forestry, Department of Soil Science and Microbiology (grant No. 30.610.006-110) and was financially supported by the Minister of Education and Science under the program "The Regional Initiative of Excellence Program".

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors upon request.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

sl—sandy loam; Sul—sulcotrione; SA—sodium alginate; DSul—dose of sulcotrione; addition; SIT—soil incubation time; DSul × SA, DSul × SIT, SA × SIT, and DSul × SA × SIT—interactions of factors studied; C—control soil; R—herbicide dose recommended by manufacturer; 5R—herbicide dose 5-fold higher than recommended by manufacturer; 50R—herbicide dose 50-fold higher than recommended by manufacturer; 10—10 days of soil incubation; 40—40 days of soil incubation; 80—80 days of soil incubation; pH—soil reaction; HAC—hydrolytic acidity; S—soil without addition of sodium alginate; SSA—soil with addition of sodium alginate; EBC—sum of exchangeable bases; CEC—sorption capacity; BS—base saturation; C_{org}—organic carbon content; N_{tot}—total nitrogen content; C/N—ratio of organic carbon content to total nitrogen content; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi; CD—colony development index; EP—ecophysiological diversity index; K_s— microorganisms' colony growth at specific time intervals; Deh—dehydrogenases; Cat—catalase; Pal—alkaline phosphatase; Pac—acid phosphatase; Aryl—arylsulfatase; Glu—β-glucosidase; Ure—urease; GMea—geometric mean of enzyme activities; IE_{Sul}—index of sulcotrione effects of sulcotrione; IE_{SA}—index of sodium alginate effects.

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