forests

MDPI

Article

Effect of Herbicide Clopyralid and Imazamox on Dehydrogenase Enzyme in Soil of Regenerated Pedunculate Oak Forests

Verica Vasic [1,*], Timea Hajnal-Jafari [2], Simonida Djuric [2], Branislav Kovacevic [1], Srdjan Stojnic [1], Sreten Vasic [1], Vladislava Galovic [1] and Sasa Orlovic [1]

[1] Institute of Lowland Forestry and Environment, University of Novi Sad, Antona Cehova 13, Novi Sad 21000, Serbia; branek@uns.ac.rs (B.K.); srdjan.stojnic@uns.ac.rs (S.S.); sreten.vasic.srecko@gmail.com (S.V.); galovic@uns.ac.rs (V.G.); sasao@uns.ac.rs (S.O.)

[2] Department of Field and Vegetable Crops, Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, Novi Sad 21000, Serbia; mikrobiologija@polj.uns.ac.rs (T.H.-J.); simonida@polj.uns.ac.rs (S.D.)

* Correspondence: vericav@uns.ac.rs; Tel.: +381-62271755

Abstract: Clopyralid and imazamox are successfully used for weed control during the first years of regeneration of pedunculate oak forests. Hence, the question that arises is how these herbicides affect microorganisms, especially the activity of dehydrogenase enzyme, when they reach the soil. Two study sites were selected in regenerated pedunculate oak forests, and the two herbicides were applied in two doses that are used for weed control (clopyralid, 100 g a.i. ha^{-1} and 120 g a.i. ha^{-1}; imazamox, 40 g a.i. ha^{-1} and 48 g a.i. ha^{-1}). The effect of the herbicides was evaluated 7, 14, 21, 30, and 60 days after application. A significant reduction in dehydrogenase activity was found on days 7 and 14 at both sites. However, after 14 days there was a recovery of dehydrogenase activity for all treatments such that the values obtained on day 21 did not differ from the control values. The effect of clopyralid and imazamox on dehydrogenase activity was not dose-dependent. Dehydrogenase activity also depended on soil properties, soil sampling time and climatic conditions during the investigation years. The results show that clopyralid and imazamox can reduce soil dehydrogenase activity, but this effect is transient. This can be attributed to the ability of microorganisms to overcome the stress caused by the herbicide by developing the capability to utilize herbicides as a nutrient source and proliferating in such an environment.

Keywords: dehydrogenase activity; forest soil; herbicides; clopyralid; imazamox

Citation: Vasic, V.; Hajnal-Jafari, T.; Djuric, S.; Kovacevic, B.; Stojnic, S.; Vasic, S.; Galovic, V.; Orlovic, S. Effect of Herbicide Clopyralid and Imazamox on Dehydrogenase Enzyme in Soil of Regenerated Pedunculate Oak Forests. *Forests* **2022**, *13*, 926. https://doi.org/10.3390/f13060926

Academic Editors: Jianping Wu, Jie Zhao and Songze Wan

Received: 17 May 2022
Accepted: 11 June 2022
Published: 13 June 2022

1. Introduction

Regeneration of pedunculate oak stands is a long-lasting and expensive process that requires knowledge and control of numerous factors that can have an adverse effect on the course of regeneration [1]. One of the most serious problems during regeneration of pedunculate oak is eliminating the negative impact of weeds that grow rapidly, even in relatively low light conditions, and can severely interfere or even stop/disable the regeneration of oak. Nowadays, there are several ways to control weeds, however, due to manpower shortages, high labour costs and large areas, forest managers are increasingly turning to herbicide application [2]. Although herbicides are important for weed control during reforestation, there is a question about their fate in the environment and their impact on the biological properties of the soil. During application of herbicides, some amount reaches the soil surface and profile, which may exert certain side effects on the activity of microorganisms in the soil [3]. Some microorganisms can degrade herbicides and use them as a source of biogenic elements and energy to maintain their physiological processes [4,5]. On the other hand, herbicides may alter the number of microorganisms as they change their activity and reproduction [6]. Therefore, there has been considerable interest in the impact of herbicides on microbial and enzyme activity in the soil of regenerated oak forests.

Soil microorganisms and the activity of enzymes found in the soil are considered basic indicators of the pollution status of the soil [7]. The activity of soil enzymes is correlated with soil microorganisms, so the use of enzymes in the study of soils gives us information about the biological characteristics of the soil [8]. Among all enzymes, dehydrogenase enzyme is one of the most important and is used as an indicator of overall soil microbial activity [9]. In addition, dehydrogenase is often used as a parameter for assessing the side effects of pesticides and other pollutions [10,11]. More than 100 different enzymes have been found in the soil that are responsible for various stages of the transformation of organic matter in soil-forming processes. However, dehydrogenase plays a major role during the initial stages of oxidation in soil organic matter as electron or hydrogen donors from substrates to acceptors [12–14]. Dehydrogenases actually do not accumulate extracellularly in the soil [10] but are found in live cells, and they are indicators of soil microbial activity [15]. The main advantage of measuring dehydrogenase activity is that is a quick and relatively simple method for the overall activity of microorganisms [16].

Clopyralid and imazamox are post-emergence herbicides that are successfully used for weed control during the first years of regeneration of pedunculate oak forests [2]. Clopyralid is an auxin-mimic type herbicide, and it is more selective than some other auxin-mimic herbicides, such as picloram, triclopyr, or 2,4-D. Similar to other auxin-mimics, it has no effect on grasses and other monocots, but is intended for the control of many annual and perennial broadleaf weeds [17]. Imidazolinone herbicides, such as imazamox, are applied as a post-emergence herbicide for the control of primarily broadleaf weeds and some grasses [18]. This herbicide inhibits acetolactate synthase enzyme (ALS), which results in disruption of protein synthesis and then DNA synthesis leading to the death of plants [19,20].

The question that arose was how these herbicides affect microbial communities, especially enzyme activity, when they reach the soil. Numerous studies have examined the effects of various herbicides on soil enzyme dehydrogenase [15,21–26]. However, there is almost no data on the impact of clopyralid and imazamox on soil enzymatic activity, especially for forest soil. For that reason, the aim of this study was to examine the effect of clopyralid and imazamox on dehydrogenase enzyme in the soil of regenerated pedunculate oak forests.

2. Materials and Methods

2.1. Study Sites

Two regeneration oak sites areas located at Public Enterprise Vojvodinasume, SG Sremska Mitrovica were chosen. One experiment was established at Varadin (44°56′43.74″ N, 19°15′48.97″ E) and a second at Vinicna (44°56′43.74″ N, 19°15′48.97″ E). Both prepared sites were seeded with acorns in spring 2015 using a seed sower and 500 kg of acorns per hectare. In this way, for both regenerated forest areas, the pedunculate oak seedlings were 1 year old in spring 2016, 2 years old in spring 2017 and 3 years old in spring 2018. When the acorns were sown, there were no weeds on the investigated sites. Study sites were not irrigated or fertilized either before or during the experiment. The application of herbicides was carried out when the oak plants had fully developed leaves and the weeds were in a phase of intensive growth. The major weed species in the regenerated forest areas were *Ambrosia artemisiifolia* L., *Cirsium arvense* (L.) Scop., *Solanum nigrum* L., *Sonchus arvensis* L., *Geum urbanum* L., *Polygonum lapathifolium* L., *Datura stramonium* L., *Galium aparine* L., *Ranunculus ficaria* L. and *Erigeron canadensis* L. Air temperature (Figure 1) was measured continuously by an EMS33 (EMS Brno, Brno-Královo Pole, Czechia) sensor located at a height of 2 m and precipitation (Figure 1) was recorded at 1 m above the ground using a Raingauge SR03 (Fiedler, Ceske Budejovice, Czech Republic).

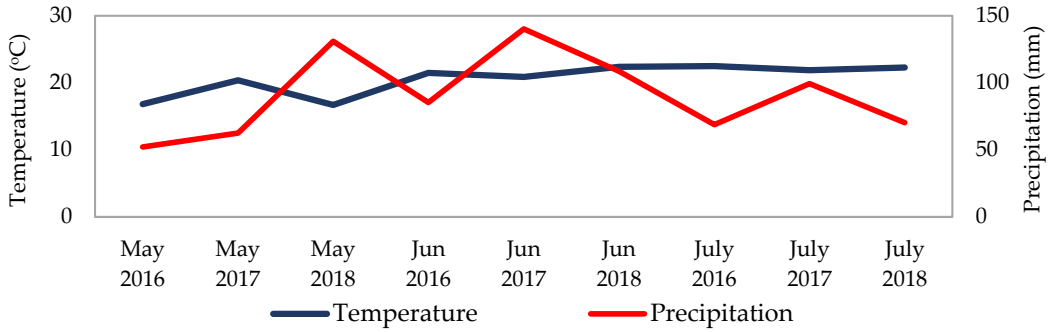

Figure 1. Average temperature and precipitation over the period May to July in the years of investigation for both sites.

2.2. Soil Analyses and Soil Sampling

Experimental plots were established at two sites where the physical and chemical properties of the soil differed. Analysis of the physical properties (soil texture and type) was performed by using the international B-pipette method of sample preparation in sodium pyrophosphate [27]. The study soil at Varadin is classified as loamy with a textural composition of 37.56% sand, 29.55% silt and 62.44% clay, and the soil at Vinicna is sandy-loamy with a textural composition of 57.68% sand, 32.28% silt and 42.32% clay. In order to obtain data on the chemical properties of the soils, the following parameters were monitored: soil pH was determined using a pH meter (pH/Ion/Cond 750, WTW, Weilheim, Germany), and humus content and carbonate content were measured volumetrically using a Scheibler calcimeter. The content of N, P and C was determined according to Hadzic et al. [28]. The soil at Varadin had a pH of 6.61 and 6.21% organic matter, whereas the soil at Vinicna had a pH of 5.85 and 5.61% organic matter. $CaCO_3$ concentrations were 3.84% at Varadin and 2.40% at Vinicna. All other characteristics of the soils (content of N, P and C) are presented in Table 1 for a depth ranging from 0 to 20 cm. At each experimental site, volumetric water content (m^{-3}) and soil temperature (°C) were measured at a depth of 20 cm automatically using ML3 ThetaProbe Soil Moisture Sensors (Delta-T Devices, Ltd., Burwell, United Kingdom). In total, three sensors per site were evenly deployed at the soil surface and connected to central data logger units.

Table 1. Soil physical and chemical properties at each study site.

Site	Textural Class	Sand (%)	Silt (%)	Clay (%)	pH	$CaCO_3$ (%)	P_2O_5 (mg kg^{-1})	K_2O (mg kg^{-1})	N (%)	Humus (%)
Varadin	Loamy soil	37.56	29.55	62.44	6.61	3.84	13.10	10.60	0.20	6.21
Vinicna	Sandy-loamy soil	57.68	32.28	42.32	5.85	2.40	9.50	7.60	0.17	5.61

Soil samples for dehydrogenase enzyme analysis were collected in each year 7, 14, 21, 30 and 60 days after the application of herbicides. From each replicate subplot, four soil samples were taken from a depth of 0–20 cm with a steel soil tube drill. The four samples were taken in different parts of each subplot and then mixed to make one composite sample. Soil samples were passed through a 2 mm sieve to remove stones and plant material, placed in sterile plastic bags and put on ice packs. The samples were transported to the laboratory and stored in a refrigerator at 4 °C and within 48 h, laboratory analysis was performed.

2.3. Experimental Design and Herbicide Treatments

At both sites, the experiments had a completely randomized block design with four replications and the following treatments: control, two doses of clopyralid (100 g a i. ha^{-1} and 120 g a.i. ha^{-1}) and two doses of imazamox (40 g a.i. ha^{-1} and 48 g a.i. ha^{-1}). The examined doses are commonly used in practice, depending on the degree of weeds, development stage and type of weeds in the regenerated area. When annual weeds are present and in smaller numbers, a lower dose of clopyralid (100 g a.i. ha^{-1}) or imazamox (40 g a.i. ha^{-1}) is usually used, and if perennial weeds are present in greater numbers, a higher dose of clopyralid (120 g a.i. ha^{-1}) or imazamox (48 g a.i. ha^{-1}) is used. The experiments were performed using a commercial product under the trade name Lontrel 100 (Dow AgroSciences) that contains 100 g a.i. L^{-1} clopyralid and Pulsar 40 (BASF) that contains 40 g a.i. L^{-1} imazamox. For herbicide application, spraying was performed with an air-pressurized hand-sprayer and a boom fitted with one Hypro polyjet nozzle, calibrated to deliver 300 L ha^{-1} of water at 250 kPa pressure. The application of herbicides was performed in conditions without wind and precipitation and when the air temperature did not exceed 20 °C. Each spring (26 May 2016, 17 May 2017 and 22 May 2018), the application of herbicides was always performed on the same experimental plots and seedlings.

2.4. Dehydrogenase Enzyme Analysis

Dehydrogenase activity (DHA) was determined by a spectrophotometric method according to Lenhard [29], modified by Thalmann [30]. The method is based on the ability of the dehydrogenase enzyme to transfer hydrogen from the substrate to colourless 2,3,5-triphenyltetrazolium chloride (TTC), reducing and converting it to red coloured triphenyl formazan (TPF). Dehydrogenase activity is determined based on measurements of the intensity of the red colour of TPF at 546 nm. The reaction mixture containing 10 g air dry soil and 10 mL 0.5% TTC in Tris buffer was mixed and incubated at 30 °C in thermostat for 24 h. At the same time, a prepared control sample that contained only the TTC solution in Tris buffer was also incubated. After incubation, 40 mL of extraction solution was added, all samples were shaken and incubated for another 2 h at room temperature and filtered. The colour intensity of the samples was read at a wavelength of 546 nm. Dehydrogenase activity is expressed as μg TPF g^{-1} soil.

2.5. Statistical Analysis

In order to analyse the effect of the applied clopyralid and imazamox on dehydrogenase enzyme activity in soils of reforested oak forests, we used repeated measures analysis of variance (ANOVA) that allows us to prove statistically significant differences in the studied variants. In cases where a statistically significant difference between variants was identified, the differences were tested with Fisher's LSD test. All statistical analyses were performed with a level of confidence of $p = 0.05$. Data analysis was conducted using Statistical software (Statistica 12.0, Hamburg, Germany). The experimental results of the study for dehydrogenase activities were subjected to repeated measures variance analysis performed using an ANOVA to test the effect of various doses of herbicides, depending on the date and place of sampling across of years of study.

3. Results

The repeated measures analysis of variance (ANOVA) showed us that the application of clopyralid and imazamox affected dehydrogenase activity in the soil. There were no statistically significant differences between the investigated doses of each herbicide, but there were significant differences when it came to the time of soil sampling, the site, as well as the year of investigation.

3.1. Dehydrogenase Activity after Application of Herbicides at Various Doses

Results of this experiment indicated that 7 days after application of both doses of clopyralid and imazamox, dehydrogenase activity in the soil decreased significantly. At 14 days, this trend was maintained and there were no significant changes in dehydrogenase enzyme activity. However, for all treatments, the dehydrogenase activity significantly increased at 21 days and ranged from 182.69 to 203.25 µg TPF g^{-1} soil. The obtained values did not differ significantly from the control values (199.54 µg TPF g^{-1} soil) except for the higher dose of imazamox (48 g a.i. ha^{-1}) where recovery of dehydrogenase activity was somewhat slower (182.69 µg TPF g^{-1} soil) but was not statistically significant. Generally, dehydrogenase activity increased after 14 days so that from day 21 onwards, the activity of dehydrogenase significantly increased in both control and treated soils. The inhibitory effect of clopyralid and imazamox on the activity of dehydrogenase was not dose-dependent. These data show that less than 21 days are necessary for the recovery of dehydrogenase activity in forest soils treated with common doses of clopyralid and imazamox used in field. Mean values for the measured enzymatic activity 7, 14, 21, 30 and 60 days after application of herbicides are presented in Figure 2.

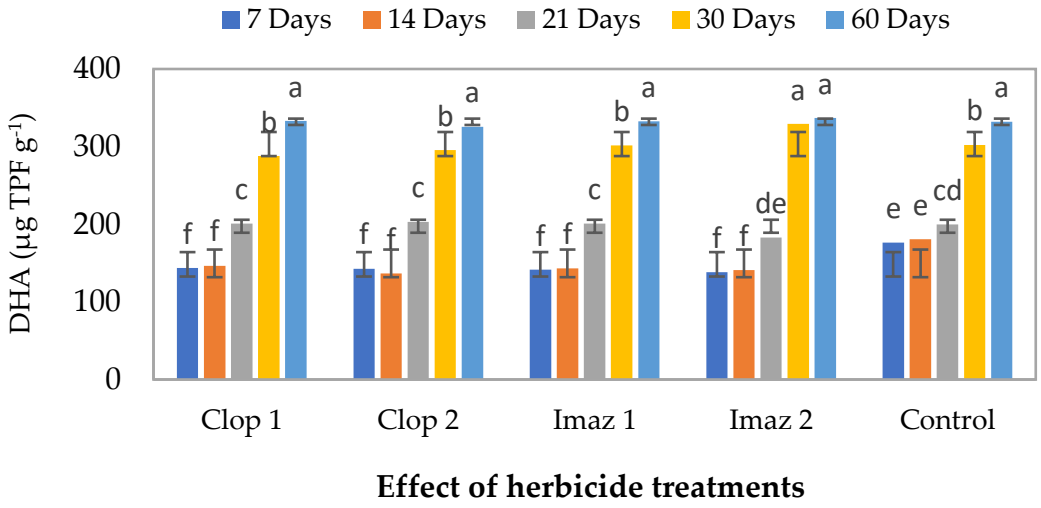

Figure 2. Dehydrogenase activity (DHA) in soil after application of herbicides at various doses. Different lower-case letters above the bars denote significant differences ($p < 0.05$) in dehydrogenase activity. Clop 1, clopyralid at 100 g a.i. ha^{-1}; Clop 2, clopyralid at 120 g a.i. ha^{-1}; Imaz 1, imazamox at 40 g a.i. ha^{-1}; Imaz 2, imazamox at 48 g a.i. ha^{-1}.

3.2. Dehydrogenase Activity after Herbicide Application Depending on the Time of Soil Sampling

There were significant differences in dehydrogenase activity after herbicide application depending on the time of soil sampling. During the experiment, as the incubation period increased, there was recovery of dehydrogenase activity in both soils (Figure 3). The lowest values for dehydrogenase activity were recorded at day 7 (148.39 µg TPF g^{-1} soil) and day 14 (149.62 µg TPF g^{-1} soil) for all treatment with herbicides and controls. After 14 days, the dehydrogenase activity slowly started to increase and at day 21 dehydrogenase activity was significantly higher with a value of 197.43 µg TPF g^{-1} soil. Furthermore, dehydrogenase activity progressively grew over time to significantly higher values of 303.33 µg TPF g^{-1} soil and 332.01 µg TPF g^{-1} soil at day 30 and 60, respectively. From the data, one can clearly observe that significant differences in dehydrogenase activity existed and depended on the time of soil sampling after herbicide application.

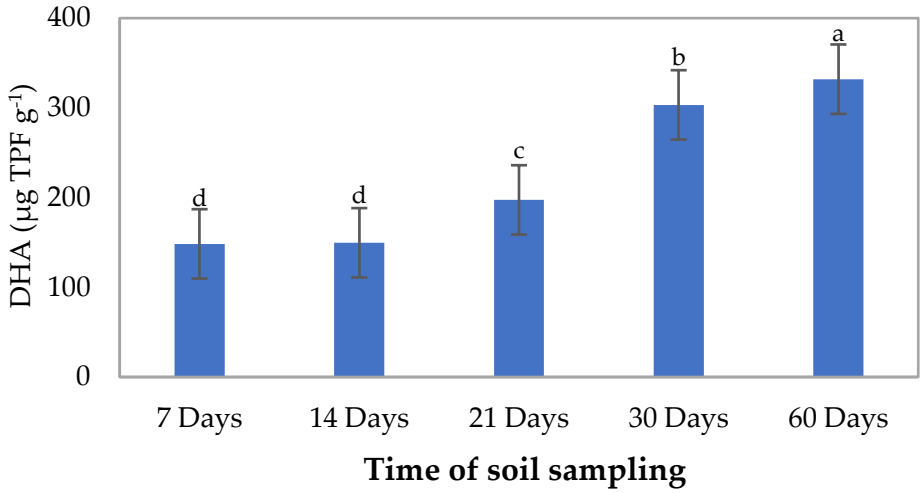

Figure 3. Dehydrogenase activity (DHA) in soil after application of herbicides depending on the time of sampling. Different lower-case letters above the bars denote significant differences ($p < 0.05$) in dehydrogenase activity.

3.3. Dehydrogenase Activity after Herbicide Application for Different Soil Types

Statistically significant differences in dehydrogenase activity were proven between the studied sites by repeated measures analysis of variance. Significantly higher dehydrogenase activity was measured in Varadin forest soil than Vinicna soil for all samples (Figure 4). Measured mean values for Varadin soil ranged from 253.73 to 280.64 µg TPF g^{-1} soil, whereas values for Vinicna soil values ranged between 186.76 and 195.76 µg TPF g^{-1} soil. Analysis of forest soils showed that differences in physical and chemical properties existed. At Varadin, the soil pH was 6.61, which certainly contributed to higher dehydrogenase enzyme activity in comparison with Vinicna soil, where the pH value was 5.85. In addition, organic matter content was higher in Varadin soil (6.21%) than Vinicna soil (5.61%) as well as the content of $CaCO_3$ (Varadin soil, 3.84%; Vinicna soil, 2.40%), which affected the dehydrogenase enzyme activity. The content of N, P and K were also higher in Varadin soil as well. Textural analysis of soils showed that the soil at the site Varadin belonged to the class of loamy soils, whereas the soil at the Vinicna site was classified as sandy-loamy soil. The more favourable soil texture at the Varadin site also contributed to the higher activity of dehydrogenase enzyme.

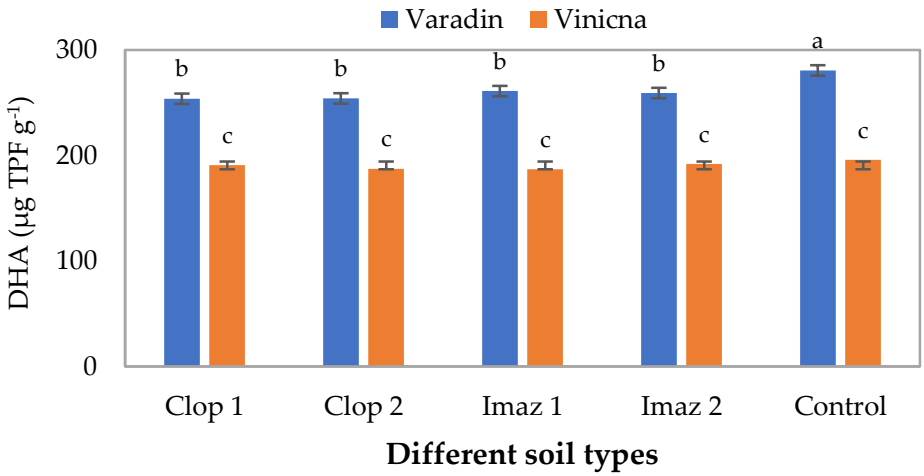

Figure 4. Dehydrogenase activity (DHA) after herbicide application for different soil types. Different lower-case letters above the bars denote significant differences ($p < 0.05$) in dehydrogenase activity. Clop 1, clopyralid at 100 g a.i. ha^{-1}; Clop 2, clopyralid at 120 g a.i. ha^{-1}; Imaz 1: imazamox at 40 g a.i. ha^{-1}; Imaz 2: Imazamox at 48 g a.i. ha^{-1}.

3.4. Dehydrogenase Activity after Herbicide Application in the Years of Investigation

Dehydrogenase enzyme activity after herbicide application was different depending on the year of investigation (Figure 5). In 2016, both soils had the lowest dehydrogenase activity values for all treatments and control in relation to 2017 and 2018. In contrast, in 2017 and 2018 dehydrogenase activity values in all treatments were significantly higher that the higher values recorded in 2017. Figure 1 shows that during all years of the experiment, air temperature and thus soil temperature gradually increased from month to month, and monthly soil temperatures (Figure 6a) were more or less uniform over all three years except May 2017, when the measured temperatures were somewhat higher than in 2016 and 2018. When it comes to precipitation (Figure 1), it was the lowest in 2016 during all months of soil sampling, so the soil water content in those months was also the lowest (Figure 6b). In 2017 and 2018, the amount of precipitation varied depending on the month, but soil water content in all months was significantly higher than 2016. The analysed data suggest that the dehydrogenase activity was more strongly influenced by soil moisture content than the temperature in both regenerated forest soils. The lowest amount of precipitation in 2016 affected the measured dehydrogenase activity values, which were lower for all treatments and the control in relation to the measured values for 2017 and 2018. More precipitation and thus higher water content in soil contributed to dehydrogenase activity values that were higher in 2017 than 2018.

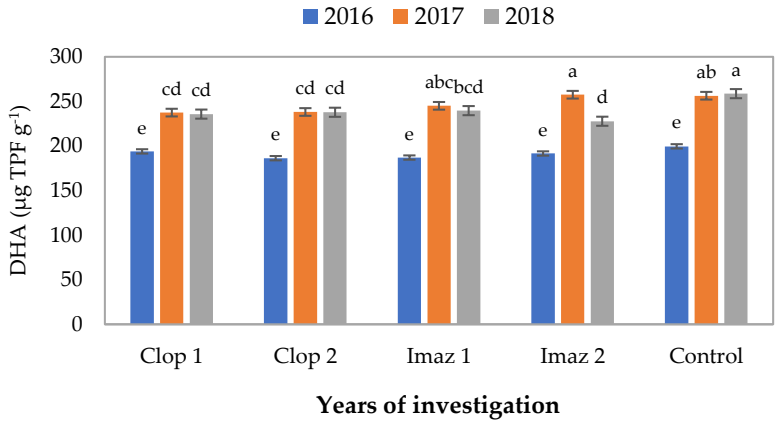

Figure 5. Dehydrogenase activity (DHA) after herbicide application across of investigation years. Different lower-case letters above the bars denote significant differences ($p < 0.05$) in dehydrogenase activity Clop 1, clopyralid at 100 g a.i. ha^{-1}; Clop 2, clopyralid at 120 g a.i. ha^{-1}; Imaz 1, imazamox at 40 g a.i. ha^{-1}; Imaz 2, imazamox at 48 g a.i. ha^{-1}.

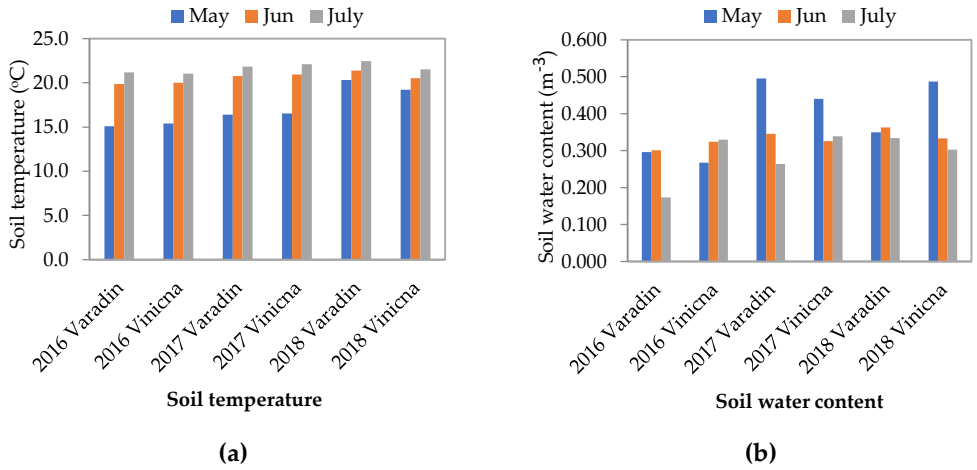

Figure 6. The monthly mean values for (**a**) soil temperature and (**b**) soil water content for the period May to July in the years of investigation.

4. Discussion

The results of our study and previous studies [15,25,26,31,32] indicate that herbicides were responsible for some distortion of dehydrogenase activity in soil. The analysed data revealed that clopyralid and imazamox applied in recommended doses led to a significant reduction in dehydrogenase activity at day 7 and 14 after application. However, the inhibitory effect was transient and after that, dehydrogenase activity values started to increase for all treatments such that the obtained values on day 21 did not differ from the controls. After 30 and 60 days, dehydrogenase activity was significantly increased for all treatments, though there were no significant differences between the treatments and the control. In addition, no statistically significant differences were found between the investigated doses of herbicides. The impact of herbicides on dehydrogenase activity has been widely investigated with researchers mostly reporting a temporary inhibitory effect [26,32,33]. Kaur et al. [34] proved that after application of imidazoline (imazamox and imazethapyr), dehydrogenase activity in soil decreased and that after a certain time, dehydrogenase activity increased. Even though the interpretation and understanding of enzymatic responses after the application of pesticides are very difficult because there are numerous direct and/or indirect interactions of pesticides with soil enzymes [35], some authors [36] reported that herbicide effects on soil enzymes depend on their mechanism of action. In a review paper, Riah et al. [36] reported that herbicides can show negative and neutral effects on soil enzymes based on the mechanism of action. Imazamox is an herbicide with the fastest degradation in soil compared to other imidazolinones [37]. As well as imazamox, under normal field conditions, clopyralid is dissipated rapidly [38]. The degradation of clopyralid and imazamox in soil is mainly performed by microbial processes, but their fate depends on many other factors, such as environmental conditions, physical and chemical properties of the soil, and the properties of the herbicides. Herbicides can affect microorganisms, reducing their number, biochemical activity and/or changing the structure of the soil microbial community.

Obtained data showed that the level of dehydrogenase activity differed between the investigated sites. This difference is likely a consequence of the various physical and chemical properties of the investigated sites, primarily soil type, organic matter content and pH. Hence, each type of soil has its inherent level of enzyme activity [39]. Significantly higher dehydrogenase activity was observed in Varadin soil because of higher organic matter content, which supports increased microbial activity and biomass, and consequently, the activity of dehydrogenase [40]. In addition, the pH of the soil at Varadin was 6.61, which also contributed to higher dehydrogenase enzyme activity than Vinicna soil, where the pH was 6.05. A study performed by Brzezinska et al. [41] reported that the best pH conditions for dehydrogenase activity ranged between 6.6 and 7.2, though Wolinska and Stepniewska [40] demonstrated that dehydrogenase activity can also be high at pH values between 5.5 and 5.73. In the literature, there are many data about the effect of various soil factors on enzyme activities [42–45] that can be positive or negative. Furthermore, the content of N, P and K were higher in Varadin soil than Vinicna soil. Stark et al. [46] stated that the presence or absence of N in soil has a strong effect on the microbial community structure, but the effects of K and P are in question. Blonska [42] determined that there was no correlation between dehydrogenase activity and the content of K and P in soil. As a far as $CaCO_3$ content is concerned, the study of [43] indicated that enzyme dehydrogenase is highly associated with microbial biomass. Microbial biomass has an effect on the decomposition of organic matter, which contributes to the formation of $CaCO_3$. Finally, one other reason for higher enzymatic activity in Varadin soil is that it contained more clay particles than Vinicna soil, which contained more sand particles. Clay minerals enhance potential enzyme activity in soil [47,48]. Chodak and Niklinska [49] also reported that soils that contain more clay particles maintain larger microbial communities, and thus, promote enzyme activity.

The significantly higher dehydrogenase activity measured in 2017 and 2018 was due to better soil conditions for microbial activity, especially soil moisture, which favours the active proliferation of microbes. In the period May–July, favourable temperature conditions prevailed for the development of microorganisms during all three years. However, precipitation was significantly lower in 2016 than 2017 and 2018, which certainly reduced microbial activity, and thus, dehydrogenase activity. Besides temperature, which influences dehydrogenase activity [50–52], soil water availability strongly effects soil microbial activity, and consequently, its enzymatic activity [53,54].

5. Conclusions

Our results showed that clopyralid applied at doses of 100 g a.i. ha^{-1} and 120 g a.i. ha^{-1} and imazamox applied at doses 40 g a.i. ha^{-1} and 48 g a.i. ha^{-1} significantly reduced dehydrogenase activity in both regenerated forest soils. However, after 14 days there was a recovery in dehydrogenase activity in all treatments, and on day 21, the obtained values did not differ from the control values. The inhibitory effect of clopyralid and imazamox on dehydrogenase activity was not a dose-dependent. Aside from the effect of the herbicides, dehydrogenase enzyme activity also depended on the incubation period, forest soil type and climatic conditions during the years of investigation. This indicates that any study on the effects of herbicides on dehydrogenase enzyme activity must take into account all soil aspects, not just the effect of herbicides. It has been shown in the present study, that when clopyralid and imazamox are applied at recommended doses, there is no long-term adverse effects on dehydrogenase activity in soil. This can be attributed to the ability of microbial populations to overcome herbicide stress and develop the capability to utilize them as a food source to proliferate in such an environment. Generally, it can be concluded that when clopyralid and imazamox are used for weed control during the regeneration of oak forests [55,56], the changes they generate in the dehydrogenase activity of soil are transitory.

Author Contributions: All authors designed the study, analysed, and interpreted the results; V.V., S.D. and T.H.-J. performed the laboratory analyses; V.V., S.V. and S.S. performed the field experiment and took soil samples; V.V., B.K. and V.G. performed statistical analysis and interpreted the results; V.V. and S.O. edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No: 451-03-68/2022-14/200197).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

References

1. Bobiec, A.; Reif, A.; Ollerer, K. Seeing the oakscape beyond the forest: A landscape approach to the oak regeneration in Europe. *Landscape Ecol.* **2018**, *33*, 513–528. [CrossRef]
2. Vasic, V.; Konstantinovic, B.; Orlovic, S. Application of post-emergence herbicides in the regeneration of pedunculate oak (*Quercus robur* L.) forest. *Forestry* **2014**, *87*, 407–415. [CrossRef]
3. Lupwayi, N.Z.; Harker, K.N.; Clayton, G.W.; Turkington, T.K.; Rice, W.A.; O'Donovan, J.T. Soil microbial biomass and diversity after herbicide application. *Can. J. Plant Sci.* **2004**, *84*, 677–685. [CrossRef]
4. Kizildag, N.; Sagliker, H.; Cenkseven, S.; Cengiz Darici, H.; Kocak, B. Efects of imazamox on soil carbon and nitrogen mineralization under Mediterranean climate. *Turk. J. Agric. For.* **2014**, *38*, 334–339. [CrossRef]
5. Vasic, V.; Djuric, S.; Hajnal-Jafari, T.; Orlovic, S.; Vasic, S.; Poljakovic Pajnik, L.; Galovic, V. The microbiological response of forest soils after application of nicosulfuron, imazamox and cycloxydim. *Int. J. Environ. Sci. Technol.* **2019**, *16*, 2305–2312. [CrossRef]
6. Rhine, E.D.; Fuhrmann, J.J.; Radosevich, M. Microbial community responses to atrazine exposure and nutrient availability: Linking degradation capacity to community structure. *Microb. Ecol.* **2003**, *46*, 145–160. [CrossRef]

7. Bacmaga, M.; Borowik, A.; Kucharski, J.; Tomkiel, M.; Wyszkowska, J. Microbial and enzymatic activity of soil contaminated with a mixture of diflufenican + mesosulfuron-methyl + iodosulfuron-methyl-sodium. *Environ. Sci. Pollut. Res.* **2015**, *22*, 643–656. [CrossRef]
8. Das, S.K.; Varma, A. Role of Enzymes in Maintaining Soil Health. In *Soil Enzymology*; Soil Biology; Shukla, G., Varma, A., Eds.; Springer: Berlin/Heidelberg, Germany, 2011; pp. 25–42.
9. Salazar, S.; Sanchez, L.E.; Alvarez, A.; Valvedre, A.; Galindo, P.; Igual, J.M.; Peixa, A.; Santa-Regina, I. Correlation among soil enzyme activities under different forest system management practices. *Ecol. Eng.* **2011**, *37*, 1123–1131. [CrossRef]
10. Kumar, S.; Chaudhuri, S.; Maiti, S.K. Soil Dehydrogenase Enzyme Activity in Natural and Mine Soil—A Review. *Middle East J. Sci. Res.* **2013**, *13*, 898–906.
11. Hanajik, P.; Gafrikova, J.; Zvarik, M. Dehydrogenase activity in topsoil at windthrow plots in Tatra National Park. *Cent. Eur. For. J.* **2017**, *63*, 91–96. [CrossRef]
12. Zhang, C.; Liu, G.; Xue, S.; Wang, G. Soil bacterial community dynamics reflect changes in plant community and soil properties during the secondary succession of abandoned farmland in the Loess Plateau. *Soil Biol. Biochem.* **2016**, *97*, 40–49. [CrossRef]
13. Quilchano, C.; Maranon, T. Dehydrogenase activity in Mediterranean forest Soils. *Biol. Fert. Soils* **2002**, *35*, 102–107. [CrossRef]
14. Małachowska-Jutsz, A.; Matyja, K. Discussion on methods of soil dehydrogenase determination. *Int. J. Environ. Sci. Technol.* **2019**, *16*, 7777–7790. [CrossRef]
15. Sebiomo, A.; Ogundero, V.W.; Bankole, S.A. Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. *Afr. J. Biotechnol.* **2011**, *10*(5), 770–778.
16. Jarvan, M.; Edesi, L.; Adamson, A.; Vosa, T. Soil microbial communities and dehydrogenase activity depending on farming systems. *Plant Soil Environ.* **2014**, *60*, 459–463. [CrossRef]
17. Mithila, J.; Hall, J.C.; Johnson, W.G.; Kelley, K.B.; Riechers, D.E. Evolution of resistance to auxinic herbicides: Historical Perspectives, Mechanisms of Resistance, and Implications for Broadleaf Weed Management in Agronomic Crops. *Weed Sci.* **2011**, *59*, 445–457. [CrossRef]
18. Arora, A.; Sondhia, S. Persistence of imazethapyr residues in soybean and soil. *Indian J. Weed Sci.* **2013**, *45*, 226–227.
19. Cobucci, T.; Prates, H.T.; Falcao, C.L.M.; Rezende, M.M.V. Effect of imazamox, fomesafen, and acifluorfen soil residue on rotational crops. *Weed Sci.* **1998**, *46*, 258–263. [CrossRef]
20. Tan, S.; Evans, R.R.; Dahmer, M.L.; Singh, B.K.; Shaner, D.L. Imidazolinone-tolerant crops: History, current status and future. *Pest Manag. Sci.* **2005**, *61*, 246–257. [CrossRef]
21. Wyszkowska, J.; Kucharski, J. Biochemical and Physicochemical Properties of Soil Contaminated with Herbicide Triflurotox 250 EC. *Pol. J. Environ. Stud.* **2004**, *13*, 223–231.
22. Bacmaga, M.; Boros, E.; Kucharski, J.; Wyszkowska, J. Enzymatic activity in soil contaminated with the Aurora 40 WG herbicide. *Environ. Prot. Eng.* **2012**, *38*, 91–102.
23. Rasool, N.; Reshi, Z.A.; Shah, M.A. Effect of Butachlor (G) on soil enzyme activity. *Eur. J. Soil Biol.* **2014**, *61*, 94–100. [CrossRef]
24. Das, S.K.; Mukherjee, I.; Das, S.K. Metsulfuron-methyl Herbicide on Dehydrogenase and Acid Phosphatase Enzyme Activity on Three Different Soils. *Int. J. Bio-Resour. Stress Manag.* **2017**, *8*, 236–241. [CrossRef]
25. Medo, J.; Hricakova, N.; Makova, J.; Medova, J.; Omelka, R.; Javorekova, S. Effects of sulfonylurea herbicides chlorsulfuron and sulfosulfuron on enzymatic activities and microbial communities in two agricultural soils. *Environ. Sci. Pollut. Res.* **2020**, *27*, 41265–41278. [CrossRef]
26. Filimon, M.N.; Roman, D.L.; Bordean, D.M.; Isvoran, A. Impact of the Herbicide Oxyfluorfen on the Activities of Some Enzymes Found in Soil and on the Populations of Soil Microorganisms. *Agronomy* **2021**, *11*, 1702. [CrossRef]
27. Bošnjak, Đ.; Hadžić, V.; Babović, D.; Kostić, N.; Burlica, Č.; Đorović, M.; Pejković, M.; Mihajlović, T.D.; Stojanović, S.; Vasić, G.; et al. *Metode Istraživanja I Određivanja Svojstava Zemljišta*; Jugoslovensko Društvo Za Proučavanje Zemljišta Komisija Za Fiziku Zemljišta: Novi Sad, Serbia, 1997; p. 278.
28. Hadžić, V.; Belić, M.; Nešić, L. *Praktikum Iz Pedologije*; Poljoprivredni Fakultet, Departman Za Ratarstvo I Povrtarstvo: Novi Sad, Serbia, 2004; p. 80.
29. Lenhard, G. The dehydrogenase activity in soil as a measure of activity of soil microorganisms. *Z. Pflanzenernaehr. Dueng. Bodenkd.* **1956**, *73*, 1–11. [CrossRef]
30. Thalmann, A. Zur Methodikde Bestimmung der dehydrogenase aktivitat im Boden mittels Triphenytetrazoliumchlorid (TTC). *Landwirsch. Forsch.* **1968**, *21*, 249–257.
31. Kucharski, J.; Wyszkawska, J. Biological properties of soil contaminated with the herbicide Apyros 75 WG. *J. Elem.* **2008**, *13*, 357–371.
32. Cycon, M.; Wojcik, M.; Borymski, S.; Piotrowska-Seget, Z. Short-term effects of the herbicide napropamide on the activity and structure of the soil microbial community assessed by the multi-approach analysis. *Appl. Soil Ecol.* **2013**, *66*, 8–18. [CrossRef]
33. Moreno, J.L.; Aliaga, A.; Navarro, S.; Hernandez, T.; Garcıa, C. Effects of atrazine on microbial activity in semiarid soil. *Appl. Soil Ecol.* **2007**, *35*, 8–18. [CrossRef]
34. Kaur, P.; Kaur, P.; Kaur, N.; Jain, D.; Singh, K.; Bhullar, M.S. Dissipation and phytotoxicity of imazethapyr and imazamox in soils amended with β-cyclodextrin-chitosan biocomposite. *Sci. Total Environ.* **2020**, *735*, 139566. [CrossRef] [PubMed]
35. Gianfreda, L.; Rao, M.A. Interactions Between Xenobiotics and Microbial and Enzymatic Soil Activity. *Crit. Rev. Environ. Sci. Technol.* **2008**, *38*, 269–310. [CrossRef]

36. Riah, W.; Laval, K.; Laroche-Ajzenberg, E.; Mougin, C.; Latour, X.; Trinsoutrot-Gattin, I. Effects of pesticides on soil enzymes: A review. *Environ. Chem. Lett.* **2014**, *12*, 257–273. [CrossRef]
37. Bundt, A.C.; Avila, L.A.; Pivetta, A.; Agostinetto, D.; Dick, D.P.; Burauel, P. Imidazolinone degradation in soil in response to application history. *Planta Daninha* **2015**, *33*, 341–349. [CrossRef]
38. Ahmad, R.; James, T.K.; Rahman, A.; Holland, P.T. Dissipation of the herbicide Clopyralid in an allophanic soil: Laboratory and field studies. *J. Environ. Sci. Health B.* **2003**, *38*, 683–695. [CrossRef] [PubMed]
39. Pitrowska-Dlugosz, A.; Kobierski, M.; Dlugosz, J. Enzymatic activity and physicochemical properties of soil profiles of luvisols. *Materials* **2021**, *14*, 6364. [CrossRef] [PubMed]
40. Wolinska, A.; Stepniewska, Z. Dehydrogenase Activity in the Soil Environment. In *Dehydrogenases*; Canuto, R.A., Ed.; InTechOpen: London, UK, 2012; pp. 183–210.
41. Brzezinska, M.; Stępniewski, W.; Stępniewska, Z.; Przywara, G. Effect of oxygen deficiency on soil dehydrogenase activity in a pot experiment with triticale CV. Jago Vegetation. *Int. Agrophys.* **2001**, *15*, 145–149.
42. Blonska, E. Enzyme activity in forest peat soils. *Folia For. Pol. A* **2010**, *52*, 20–25.
43. Zhang, N.; He, X.; Gao, Y.; Li, Y.; Wang, H.; Ma, D.; Zhang, R.; Yang, S. Pedogenic carbonate and soil dhydrogenase activity in response to soil organic matter in artemisia ordosica community. *Pedosphere* **2010**, *20*, 229–235. [CrossRef]
44. Moeskops, B.; Buchan, D.; Sleutel, S.; Herawaty, L.; Husen, E.; Saraswati, R.; Setyorini, D.; De Neve, S. Soil microbial communities and activities under intensive organic and conventional vegetable farming in West Java, Indonesia. *Appl. Soil Ecol.* **2010**, *45*, 112–120. [CrossRef]
45. Levyk, V.; Maryskevych, O.; Brzezinska, M.; Włodarczyk, T. Dehydrogenase activity of technogenic soils of former sulphur mines (Yvaoriv and Nemyriv, Ukraine). *Int. Agrophys.* **2007**, *21*, 255–260.
46. Stark, C.; Condron, L.M.; Stewart, A.; Di, H.J.; O'Callaghan, M. Influence of organic and mineral amendments on microbial soil properties and processes. *Appl. Soil Ecol.* **2007**, *35*, 79–93. [CrossRef]
47. Allison, S.D. Soil minerals and humic acids alter enzyme stability: Implications for ecosystem processes. *Biogeochemistry* **2006**, *81*, 361–373. [CrossRef]
48. Ling, N.; Sun, Y.; Ma, J.; Guo, J.; Zhu, P.; Peng, C.; Yu, G.; Rau, W.; Guo, S.; Shen, Q. Response of the bacterial diversity and soil enzyme activity in particle-size fraction of Mollisol after different fertilization in a long-term experiment. *Biol. Fert. Soils* **2014**, *50*, 901–911. [CrossRef]
49. Chodak, M.; Niklinska, M. Effect of texture and tree species on microbial properties of mine soils. *Appl. Soil Ecol.* **2010**, *46*, 268–275. [CrossRef]
50. Brzezinska, M.; Stepniewska, Z.; Stepniewski, W. Soil oxygen status and dehydrogenase activity. *Soil Biol. Biochem.* **1998**, *30*, 1783–1790. [CrossRef]
51. Trasar-Cepeda, C.; Gil-Sotres, F.; Leiros, M. Thermodynamic parameters of enzymes in grassland soils from Galicia, NW Spain. *Soil Biol. Biochem.* **2007**, *39*, 311–319. [CrossRef]
52. Cirilli, F.; Bellincontro, A.; De Santis, D.; Botondi, R.; Colao, M.; Muleo, R.; Mencarelli, F. Temperature and water loss affect ADH activity and gene expression in grape berry during postharvest dehydration. *Food Chem.* **2012**, *132*, 447–454. [CrossRef]
53. Geisseler, D.; Horwath, W.; Scow, K. Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. *Pedobiologia* **2011**, *54*, 71–78. [CrossRef]
54. Uhlirova, E.; Elhottova, D.; Triska, J.; Santruckova, H. Physiology and microbial community structure in soil at extreme water content. *Folia Microbiol.* **2005**, *50*, 161–166. [CrossRef]
55. Radjevic, V.; Pap, P.; Vasic, V. Management of the common oak forests in Ravni Srem: Yesterday, today, tomorrow. *Topola/Poplar* **2020**, *206*, 41–52.
56. Pap, P.; Drekic, M.; Poljakovic Pajnik, L.; Vasic, V.; Markovic, M.; Zlatkovic, M.; Stojanovic, D. Monitoring and forecasting of harmful organisms in forests and plantations in Vojvodina, Serbia in 2018. *Topola/Poplar* **2018**, *201/202*, 251–274.