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Risk Assessment of Sulfonylurea Herbicides Based on a Complex Bioindicator

Aurica Breica Borozan ¹, Despina-Maria Bordean ² , Oana Maria Boldura ³ , Sorina Popescu ^{1,*},
Marioara Nicoleta Caraba ⁴ and Camelia Moldovan ²

¹ Faculty of Engineering and Applied Technologies, University of Life Sciences “King Mihai I” from Timisoara, 119 Calea Aradului, 300645 Timisoara, Romania; auricaborozan@usab-tm.ro

² Faculty of Food Engineering, University of Life Sciences “King Mihai I” from Timisoara, 119 Calea Aradului, 300645 Timisoara, Romania; despinabordean@usab-tm.ro (D.-M.B.); cameliamoldovan@usab-tm.ro (C.M.)

³ Faculty of Veterinary Medicine, University of Life Sciences “King Mihai I” from Timisoara, 119 Calea Aradului, 300645 Timisoara, Romania; oanaboldura@usab-tm.ro

⁴ Faculty of Chemistry-Biology-Geography, West University of Timisoara, 4 Vasile Parvan Str., Timisoara 300223, Romania; marioara.filimon@e-uvt.ro

* Correspondence: sorinapopescu@usab-tm.ro

Abstract: The increasing use of herbicides in recent years for improved crop yields requires a risk assessment. To assess their impact on soil, the use of an indicator named the synthetic biological indicator (ISB%) is proposed, which includes a range of biotic and enzymatic parameters derived from previous experiments. Three sulfonylurea herbicides were evaluated, named chlorsulfuron, amidosulfuron, and tifensulfuron. The biotic and enzymatic parameters were monitored using different herbicide doses in field and laboratory experiments. Calculating this indicator for all experimental variants in the field and laboratory showed that the impact of the analyzed herbicides was insignificant, but there were statistically significant differences between the experimental conditions. The registration of an herbicide based on the legislation of different countries requires several toxicity tests of the active substance’s effects against soil microorganisms and some of the soil functions performed by microorganisms, parameters which are also included in the synthetic biological indicator (ISB). This indicator has the capacity to provide important information for sustainable soil management, including a minimum set of parameters, which can provide global information regarding the environment, showing changes in multiple areas of interest, including parameters that can be applied at minimal cost worldwide. In conclusion, we can say that the use of the indicator highlights all the changes caused by various soil chemical treatments because it follows the variation in a large number of parameters, unlike other indicators that follow only one, providing useful information for sustainable farming practices.

Keywords: biological synthetic indicator; biotic and enzymatic parameters; mathematical models



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1. Introduction

The continued increase in the world’s population, which, according to the estimates of the United Nations (UN), will reach 9.7 billion [1] in 2050, requires high amounts of food, water, and energy [2–5]. Therefore, intensive farming is carried out to obtain increased yields by reducing areas with less work, involving intensive soil exploitation with high inputs of chemicals (pesticides) [6,7].

Herbicides represent almost half of all pesticides used. In Romania, farmers use pesticides frequently. The main reasons for this are the lack of a labor force, the aging of the population in rural areas, elimination of or reduction in manual and mechanical tillage, and difficulty controlling weeds and pathogens.

Therefore, overcoming the equilibrium limits and prolonged application of herbicides could produce major changes in the environment, which are concretized by the gradual degradation of the soil and loss of biodiversity [8,9].

The overdose associated with climate change, the removal of protective forest belts, and prolonged drought have even led to the desertification of soils in some parts of Romania.

Soil degradation due to human activities and reduced ability to sustain agricultural production by reducing soil fertility [10] is a major threat to food security, with one of the consequences being increased poverty. All of these are considered serious problems for the environment and humanity worldwide. Under these conditions, sustainable soil management has great importance worldwide.

Therefore, it is necessary to prevent degradation and preserve the soils' optimal functional parameters. This requires knowing the real situation of degraded soils and all the deficiencies that characterize this ecosystem, which is the foundation of all terrestrial ecosystems.

The first step is to develop strategies for an accurate assessment of the real state of the soil and to establish the most appropriate bioindicator management [11,12].

In studies conducted by Ludwig et al. (2017) [13], resilience (the ability to recover soil structural and functional integrity) is mentioned as a measurable bioindicator for soil sustainability. Finding bio-indicators that can optimally express the current condition of agricultural soils is of great interest to researchers from many countries. The aim is to summarize the soil indicators that can be used to meet the goals of sustainable development imposed by the United Nations Organization (UNO) [5].

Although several indicators have been proposed by various authors for soil quality evaluation, such as soil fertility, soil health status, or soil monitoring [5,14–19], a globally accepted and applicable soil fertility assessment methodology is not in place at present [20]. However, many researchers have proposed microorganisms as bioindicators of soil quality and health [21].

Since the 1990s, attention has been drawn to the importance of biological indicators in characterizing the imbalances caused by a chemical. Thus, it was considered that biological and biochemical parameters are sensitive to the smallest changes occurring in the soil due to a degrading agent [22]. Soil enzyme activity is also considered to be sensitive to pollution and has been proposed as a measure of the degree of pollution in degraded soils [23], while the physical and physico-chemical parameters of the soil are not very sensitive and only undergo changes following drastic interventions and change [24]. However, Parr et al. (1992) suggested the importance of finding significant biological/ecological indicators to describe the quality of soil, draw the attention of specialists in cases of early soil degradation, and provide information on the sustainability of agricultural systems [25]. According to Elliott (1997), biological indicators show different aspects of soil quality [26], monitoring soil development and nutrient storage, as well as reflecting biological activity [27].

The use of biological indicators to evaluate pesticide risks became a top priority in April 1997, when the Workshop of the Organization for Economic Cooperation and Development (OECD) held in Copenhagen emphasized that indicators are necessary to complement pesticide risk assessment and registration. OECD is an intergovernmental organization “formed by representatives of 30 industrialized countries in North America, Europe and the Asia and Pacific region, as well as the European Commission”, with the role of co-ordinating and harmonizing policies, discussing issues of mutual concern, and working together to respond to various international problems [28]. The correlation between the indicator and the purpose of the evaluation is of great importance. It is considered that a single parameter, or a small number of parameters, does not provide conclusive results [29,30]. However, a large number of parameters, which require a great deal of work, a long time, and high costs, as well as leading to difficulties in the data collection, processing, and interpretation process, are also not indicated.

The evaluation of bio-indicators is quite difficult, as the soil is a living, complex ecosystem, and one or more randomized tests cannot be used to monitor the soil characteristics that are important for agricultural practice [31,32]. Therefore, it is necessary to develop a bio-indicator that summarizes several biotic or vital and enzymatic parameters to accurately monitor the soil changes, determine the subsequent anthropogenic interventions,

support improvements in the applied technologies, preserve the natural heritage, and protect the environment.

The following questions need to be answered: Which is the best one? Is there a simple or complex worldwide indicator that is applicable to all countries? Is there an indicator that provides a comprehensive assessment of soil degradation?

In this situation, our main objective was to present research comparing biological concepts and indicators targeting herbicide-treated soils (Supplementary Material, Table S1) and observe whether sulfonylurea remains a risk to the environment 30 days after field application and 7 days after homogenizing herbicides with chernozemic cambic soil under laboratory conditions, because, as mentioned by Poporisch et al. (2020), the information related to the accelerated degradation of sulfonylurea herbicides is quite limited [33].

This paper would like to draw the attention of specialists to a synthetic bioindicator relevant in the context of using any chemical substance which may reveal an influence on the soil.

For demonstration, sulfonylurea herbicides have been evaluated, which are very popular around the world because of their low toxicity, reduced volatilization, high selectivity, and herbicide activity at a low concentration (20–200g/ha) (above 100–1000 times that of traditional herbicides), in addition to weed control in agricultural crops [34].

Despite all the advantages of sulfonylureas, information regarding the negative aspects of herbicides requires particular attention, namely the risk of bioconcentration and phytotoxicity referred to in numerous literature sources [35–38]. Some studies have found that at pH 5.0, the maximum bioconcentration factor of *Chlorella chlorsulfuron* is 53 [39].

Herbicide residues have been reported in cereals, legumes [40], vegetables, and fruits [41], and it was observed that they could delay blooming, resulting in reduced yield [42]. Sulfonylurea herbicides can also infiltrate groundwater.

The target for sulfonylurea is the enzyme acetolactate synthase [43], which only occurs in microorganisms and plants (which justifies the use of biological indicators) but is missing in humans and animals [44].

Knowledge of the impact of sulfonylurea herbicides in soil and water is important for agricultural practices and the development of environmental protection strategies [45].

Therefore, we propose a synthetic biological indicator (ISB), defined by all biotic and enzymatic processes that are described mathematically by the following partial indicators: The Vital Activity Potential Indicator (VAPI), which includes the biotic parameters respiratory potential, cellulosic activity, nitrification, and has been presented in six research papers [46–51]; the Enzymatic Activity Potential Indicator (EAPI), which contains the enzymatic parameters catalase, urease, saccharase, phosphatase, presented in three research papers [52–54].

As herbicides fall under the broad term of pesticides, serving as a subcategory of pesticides, the recommended criteria related to this (scientifically robust, user-friendly; linking hazard and exposure data with herbicides' use data; providing meaningful information, etc.) are met. Compared to other complex indicators, ISB can be used worldwide and not only at a regional level [28].

2. Materials and Methods

2.1. The Methodology Used to Calculate Biological Indicators

The synthetic biological indicator (ISB%) [32,54,55] considered two parameters, namely the vital activity potential indicator (VAPI%) and the enzyme activity potential indicator (EAPI%), underlining the level of alteration in the balance of soil life produced by the studied herbicides and the ecological implications of those changes.

$$ISB\% = \frac{VAPI\% + EAPI\%}{2} \quad (1)$$

The vital activity potential indicator (VAPI %) [32,54,55] quantified the biotic soil activity, including respiratory, celulosic, and nitrification potential.

$$\text{VAPI \%} = \sum_{k=1}^3 \frac{(R, \text{Cel}, \text{nbN})}{3} \quad (2)$$

where R = breathing, Cel = cellulose, and nbN = most likely number of autotrophic nitrifying bacteria.

According to studies conducted by Stefanic and Sandoiu (2011) [32], this formula may include other terms if other analyses have been carried out (for example, microbial soil biomass).

The enzyme potential indicator (EAPI%) [32,54,55] expressed the enzymatic activity of the soil, including catalase, saccharase, urease, and phosphatase.

$$\text{EAPI \%} = \sum_{k=1}^4 \frac{(C, Z, U, F)}{4} \quad (3)$$

where C = catalase, Z = saccharase, U = urease, and F = total phosphatase.

The quantitative indicators created by Ștefanic (1994) [56] could be applied to both temperate and polar soil areas [55,57,58], allowing for soil quality control in terms of biological processes, the accumulation of nutrients, and soil type under certain ecological conditions. Specialty references mention that these achievements are originally in the soil sciences [23,59].

2.2. The Experimental Design

Previous experiments were used to calculate these indicators. To provide an overview of this, the experimental design is outlined below.

The following sulfonylurea herbicides were used for the calculation of these indicators:

- Chlorsulfuron (2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl]-benzene sulfonamide) (Glean 75 DF), produced by Nemours, USA (A1);
- Amidosulfuron 4,6-dimethoxypyrimidin-2-yl-1-(N-methyl-N-sulfonylurea) (Grody 75 DF), produced by AgrEvo, Germany (A2);
- Tifensulfuron carboxylate de methyl-2 (methoxy-4-methyl-6-triazine-1-methoxy-4-methyl-6-triazine-1,3,5-yl-2) amino-carbonyl amynosulfuronyl-3 thiophene), (Harmony 75DF), produced by Nemours, USA (A3).

For studies, chernozem cambic soil was selected, located in the southwestern part of Romania 45°47'07" N, 21°12'38" E, Timisoara, Romania.

The chemical treatment of the soil was carried out in the spring, during May. Before the application of herbicide treatments and soil sampling, the plots were not biologically or chemically fertilized, and they were kept in the "black field" until the samples were collected.

The plots were delimited in the experimental fields of the Plant Breeding Department of the University of Agricultural Sciences and Veterinary Medicine of Banat, "King Mihai I of Romania" in Timisoara. The experiments were placed into a Latin rectangle with two replicates, with each plot being 18 m².

For each herbicide, the concentrations were set according to the approved dose of agricultural practice in quantum of 1 × (d1), 2 × (d2), and 5 × (d3). In parallel with the treated variants, a nonherbicide variant (control) was also used (A0d0) (Table 1).

Table 1. Experimental scheme in field and laboratory.

Sulfonylurea Herbicide	Experimental Variant
Control (A0)	A0 d0
Chlorsulfuron (A1)	A1 d1 (20 g/ha)
	A1 d2 (40 g/ha)
	A1 d3 (100 g/ha)
Amidosulfuron (A2)	A2 d1 (60 g/ha)
	A2 d2 (120g/ha)
	A2 d3 (300 g/ha)
Tifensulfuron (A3)	A3 d1 (60 g/ha)
	A3 d2 (120g/ha)
	A3 d3 (300 g/ha)

Legend: d0—dose 0 (untreated); d1—normal dose; d2—two-fold normal dose; d3—five-fold normal dose; A0—control, without herbicides; A1—chlorsulfuron variant, A2—amidosulfuron variant, A3—tifensulfuron variant.

One month after field treatments, separate soil samples were taken for all 10 variants (9 chemically treated and 1 untreated). The soil samples collected from the untreated plots were used as a control for the field experiment and biological materials for the laboratory experiments.

Under experimental laboratory conditions, the soil samples collected from the untreated variant, arranged diagonally from certain points, were mixed in an average sample, which was processed in the laboratory. This was divided into 10 equal samples: 1 used as a control and the other 9 treated with the 3 herbicides (according to the experimental design above).

In lab models, soil samples remained in contact with the herbicide for seven days. For each of the 20 soil samples from field and lab tests, the stabilization of biotic and enzymatic processes at the typical level of a natural soil sample was performed [54,55]. Then, vital and enzymatic processes were assessed to form the basis for the determination of the synthetic biological indicator (ISB%).

2.3. Methods for Evaluating Biotic and Enzymatic Parameters

To provide an overview of the disturbance caused by the three herbicides and evaluate the synthetic indicator, the following biotic and enzymatic parameters were used:

- Cellulosic activity was determined according to Vostrov and Petrova (1961) [60], with some amendments: the cotton cloth was replaced with cotton mixed with 50% polyester fiber cloth, spinning on cotton yarn, and flaming the edge of the cloth patch [56,57].
- The most likely number of autotrophic nitrifying bacteria was determined on liquid mineral media supplemented by a color indicator in accordance with the Saratchandra (1979) method [61].
- Respiratory potential was assessed according to Dommergue (1960) [62] using Ștefanic's modifications (1988, 1994) [56,63]. The respirometer obtained by Ștefanic (1988) was used, maintaining the oxygen concentration in the respirometer [63].
- Catalase potential was determined by the method described by Ștefanic et al. (1984) [64].
- Soil urease potential was determined using the method described in Ștefanic, 2000, 2006 [54,55].
- Saccharase potential was determined according to Ștefanic and Irimescu-Orzan (2000) [65].

It was considered that simultaneously with the saccharase activity, a totally spontaneous phosphatase activity develops, determined by the accumulated phosphatases and the phosphorus resources originating from the soil.

The determination of phosphatase potential was based on a method developed by Ștefanic et al. (1965) [66], with the modifications of Ștefanic and Irimescu (1998) [67]. The method was based on the presence of phosphorous compounds in the soil, as well as

the accumulation of phosphatase enzymes in the soil. To assess phosphatase activities, glucose was added to the reaction mixture to combine with the phosphate ions that were enzymatically released or already present in the soil at the time of analysis. Consequently, the unbound glucose excess was determined, but the free phosphate ions released into the soil by various biotic and enzymatic activities were not determined.

2.4. Statistical Analysis

The statistical evaluation of the experimental data was carried out using MVSP 3.1 and PAST 2.14 software [68].

The mathematical models (principal component analysis, kernel density, and generalized linear model) were selected to identify the differences, as well as the correlations in the data for both laboratory and field experiments.

Principal component analysis (PCA) maximizes the variance explained by each successive axis [68,69].

The estimation of the density probability based on known points was determined according to kernel density. The use of kernel density shows the details of the probability density function using all sample points' locations and suggested multimodality [70]. The density estimate provided a rapid overview of the shape of the data distribution [71].

The generalized linear model was adopted because it “permits the response variable y to have an error distribution other than a normal distribution” [72]. The Iterative Reweighted Least Squares (IRLS) algorithm was used to estimate the maximum probability. For normal distributions, the dispersion parameter φ (used only for the inference) was estimated using Pearson's chi-square. G was the difference in D between the full model and an additional Generalized Linear Model (GLM) analysis where only the intercept was installed. G was approximately chi-squared with one degree of freedom, providing significance for the slope [73].

3. Results

Results regarding the effect of sulfonylurea herbicides on the synthetic biological indicator after 30 days of field treatment and 7 days of laboratory models are shown in Figures 1–4.

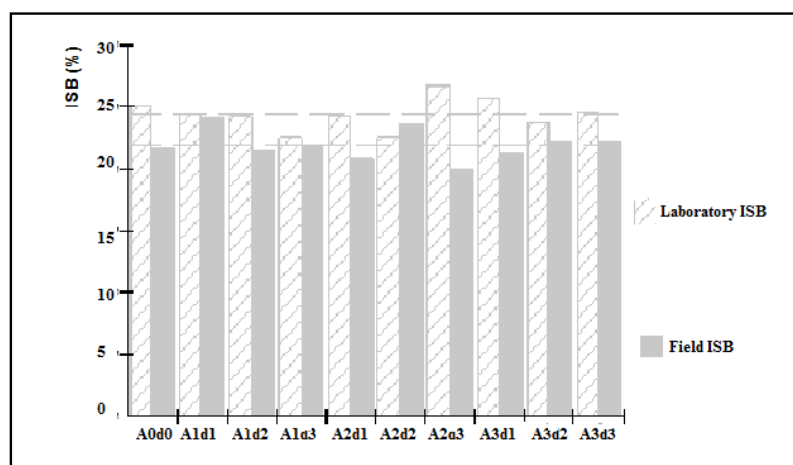


Figure 1. Synthetic biological indicator (ISB %) assessment under field and laboratory conditions. Legend: A0—control; A1—chlorsulfuron; A2—amidosulfuron; A3—tifensulfuron; d0—0 dose (untreated); d1—normal dose; d2—two-fold normal dose; d3—five-fold normal dose.

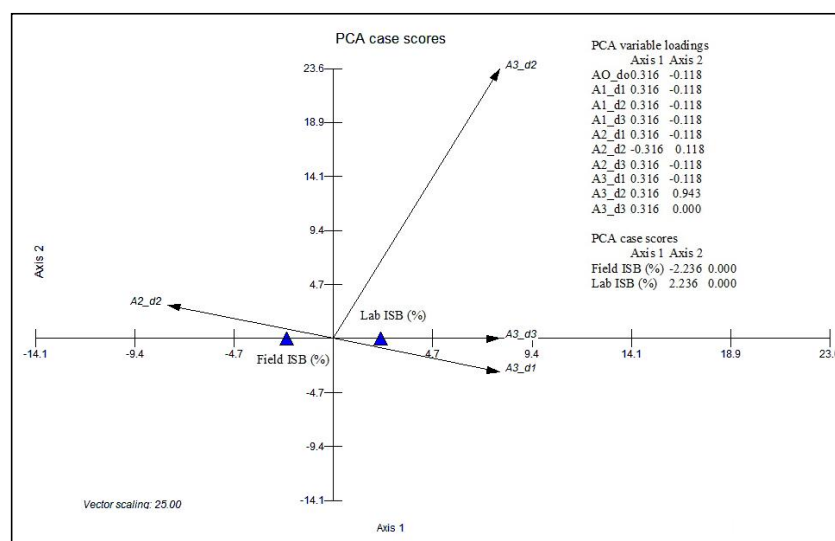


Figure 2. PCA applied to the two datasets (field and laboratory). Legend: A0—control variant; A1—chlorsulfuron; A2—amidosulfuron; A3—tifensulfuron; d0—dose 0 (untreated); d1—normal dose; d2—two-fold normal dose; d3—five-fold normal dose. The blue triangles are the graphical point symbols.

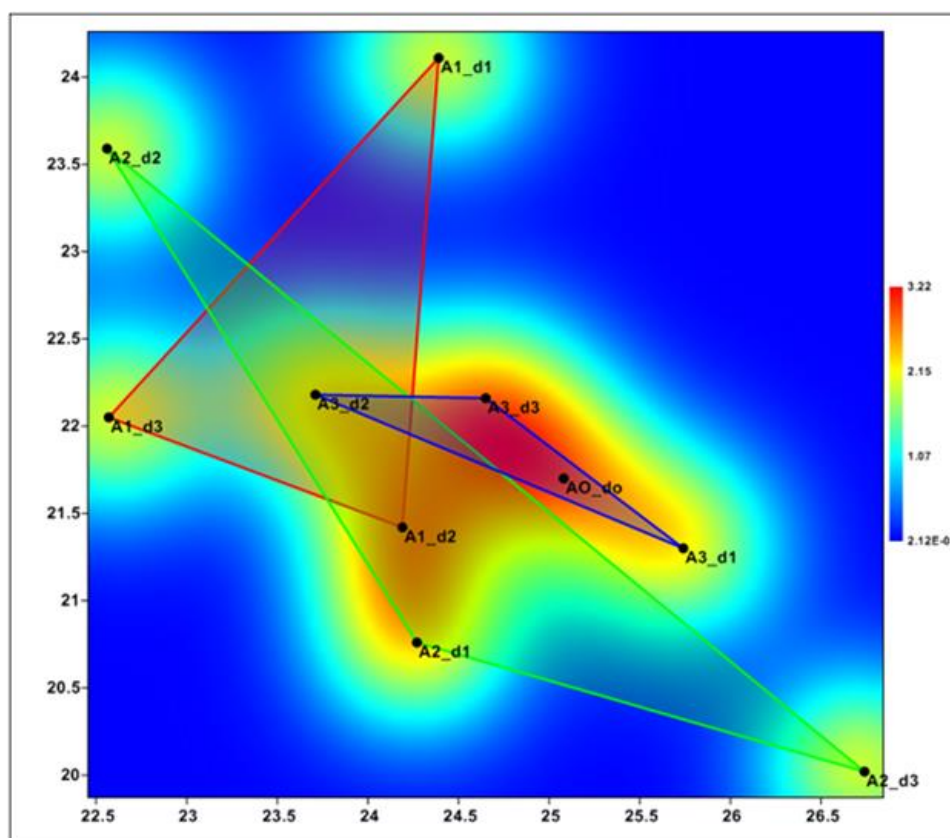


Figure 3. Map of experimental determinations based on Kernel density and calculated -ISB for field and laboratory conditions. Legend: A0—control variant; A1—chlorsulfuron; A2—amidosulfuron; A3—tifensulfuron; d0—dose 0 (untreated); d1—normal dose; d2—two-fold normal dose; d3—five-fold normal dose.

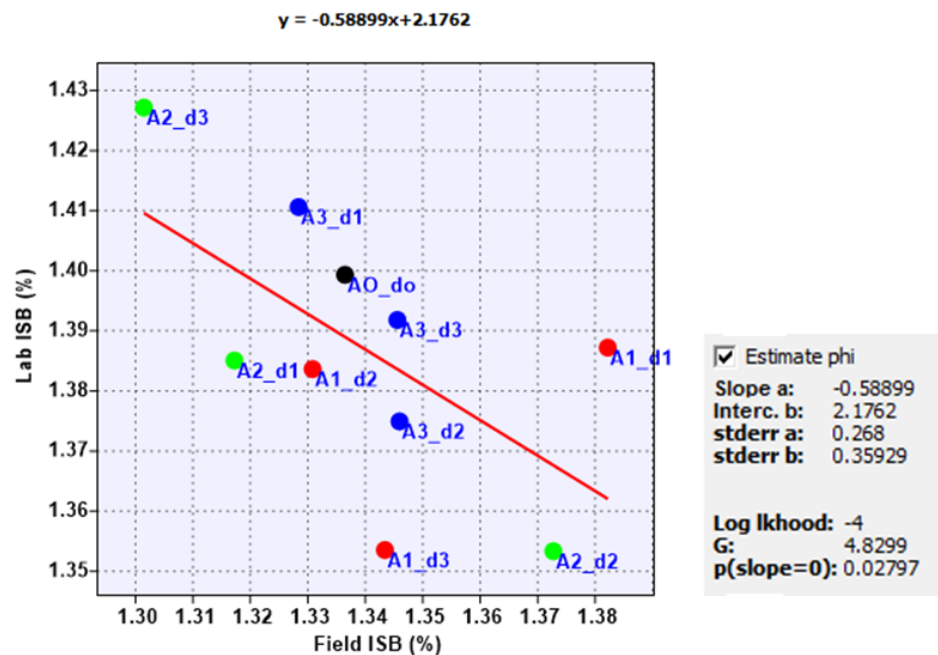


Figure 4. The generalized linear model of variations between data corresponding to field and laboratory conditions. Legend: Dots: black = control variant; red = A1; green = A2; blue = A3. A0—control; A1—chlorsulfuron; A2—amidosulfuron; A3—tifensulfuron; d0—0 dose (untreated); d1—normal dose; d2—two-fold normal dose; d3—five-fold normal dose.

The values obtained in the two experimental conditions indicate some fluctuations in ISB, in ascending or descending order, at the dose approved for agricultural practice, or at increased doses ($2 \times$ normal dose or $5 \times$ normal dose) compared to the control variant (Figure 1).

The information from Figure 1 is complemented by Table 2, which illustrates the statistically significant differences between laboratory and field treatments.

Table 2. The behavior of herbicides in the field versus in the laboratory.

Cell No.	Experimental Variants	Duncan's Test: (in the Field and in the Laboratory) Approximate Probabilities for Post Hoc Tests					
		A2 Field 21.457	A3 Field 21.880	A1 Laboratory 23.717	A2 Laboratory 24.523	A3 Laboratory 24.700	A1 Field 22.527
1	A2 field		0.722903	0.096941	0.033091 *	0.027127 *	0.399843
2	A3 field	0.722903		0.159192	0.056674	0.047112 *	0.589363
3	A1 laboratory	0.096941	0.159192		0.502221	0.438097	0.327539
4	A2 laboratory	0.033091 *	0.056674	0.502221		0.882173	0.128514
5	A3 laboratory	0.027127 *	0.047112*	0.438097	0.882173		0.109028
6	A1 field	0.399843	0.589363	0.327539	0.128514	0.109028	

Legend: A1 = chlorsulfuron, A2 = amidosulfuron, A3 = tifensulfuron. Marked boxes (*) indicate significant differences as compared to the reference group (Duncan's test, $p < 0.05$).

According to these data (Figure 1), under field conditions, there is a slight increase in the ISB compared to the control variant (A0d0) in descending order $A1d1 > A2d2$, which generally indicates a slight stimulation of the biotic and enzymatic activities occurring after applying the treatments with the three sulfonylurea herbicides at the doses mentioned in the experimental scheme presented in the Material and Methods section (Table 1).

In laboratory models, there is a slight stimulation of the ISB compared to the control variant (A0d0) in the following order: $A2d3 > A3d1$.

Regarding the inhibitory effect, in reference to the control, the herbicidal doses exhibited a low inhibitory effect. This effect had the following ascending path: $A2d2, A1d3,$

and A3d2. In general, under laboratory conditions, an inhibiting effect is observed with all herbicides at doses above normal.

Under field conditions, in reference to the control, a low negative influence of the tested herbicides was included in the following decreasing scheme: A2d3 > A2d1. However, in the latter case, the difference is insignificant.

When a comparison was made between the two experimental conditions, higher ISB values (%) were recorded in laboratory models, both in the herbicide variants and the control variant. Extreme values, either due to the mild inhibitory effect or the mild stimulation, were highlighted in both contexts. If we refer to controlled conditions, the highest ISB value was observed in A2d3, and the smallest values were in A2d2, A1d3, and A3d2.

Under field conditions, in the amidosulfuron-treated plots (A2d3 and A2d1), ISB had lower values; the extreme opposite was shown by variant A2d2.

After 30 days in the field and 7 days in the laboratory, the slight influence of sulfonylurea herbicides was always obvious.

A comparative analysis of the data shows statistically significant differences between the two experimental conditions, the field, and the laboratory. There are no statistically significant differences between treatments with the three sulfonylurea herbicides in the field or in the laboratory (Table 2).

It is noted that statistically significant differences were observed between variant A2 under field conditions and variants A2 and A3 under laboratory conditions. Statistically significant differences were also found between the A3 variants in the field and the A3 variants in the laboratory models.

Several homogeneous groups were identified, formed by experimental variants with no statistically significant differences. For example, group 1 was represented by the experimental variants A1 (treatments in the field and laboratory) and A3 (treatment in the field), but also group 2, which includes the experimental variants A1 (treatments in the field and laboratory) and A2 (treatment in the laboratory). Variant A3 (laboratory) is not statistically significantly different from variants A2 (treatment in the laboratory) and A1 (treatments in the field and laboratory), and A2 is not statistically significantly different from A3 (treatment in the field) and from A1 (treatments in the field and laboratory).

The ISB values were investigated using statistical and mathematical models (PCA, kernel density, and generalized linear models) to identify the differences and correlations between laboratory and field results. The PCA routine found the eigenvalues and eigenvectors of the correlation matrix, providing the measure of the variance reported for the corresponding eigenvectors (components), displayed together with the percentages of variance accounted for by each of these components [74].

The analysis of the main components of the logarithmized ISB values used the correlation matrix.

The maximum variation represented on the principal component axis PC1 is 80.678%, and that represented on PC2 is 19.322%, at a Joliffe cut-off of 0.7 (Figure 2).

The scatter plot of these data, projected onto the principal components presented in Figure 2, and used as a visual aid in grouping close points [74], might be associated with kernel density to identify the optimal version and to create a map that is helpful for its categorization. The resulting map is shown in Figure 3.

Sulfonylurea herbicides A2 and A3 exhibit similar behavior in both laboratory models and in natural conditions. With good behavior in natural conditions, sulfonylurea A2 and A1 have the largest area of application (Figure 3).

Kernel density was chosen because it makes a smooth map of point density in 2D. The density estimate was based on Gaussian kernel functions, with radius parameter r (in our case, the chosen radius was $r = 0.409$), with the scaling providing an estimation of the number of points per area, not a probability density [68].

Figure 3 shows that A2 and A3 exhibit similar behavior in both laboratory and field conditions; the largest area is represented by A2, but A1 also behaves well in the field.

The general linear model

The function on which ISB values can be estimated at doses other than those studied is the following:

$$y = -0.58899x + 2.1762 \quad (4)$$

where x = ISB resulting in different doses under laboratory conditions (logarithmized value); y = ISB resulting at different dosages under field conditions (logarithmized value). The graphical representation is shown in Figure 4.

For the normal distributions, the dispersion parameter φ (used only for the inference) is estimated using Pearson's chi-square. The use of normal distribution and the function identity link is equivalent to ordinary least squares linear regression: $G: 4.8299$; P (slope = 0): 0.02797.

The generalized linear model was used to emphasize the importance of ISB and the possibility of using some diagrams to anticipate some effects in the field based on the results observed in the laboratory.

This model allows for the estimation of ISB values for other herbicide doses, as it could be used to obtain a linear model with a continuous response variable based on the normal distribution using the identity link function.

The generalized approach to the linear model is particularly useful and attractive because it provides a general theoretical framework that can be used to estimate ISB for different doses to those used for these three experiments.

4. Discussions

4.1. Analysis of the Impact of Herbicides on the Synthetic Biological Indicator in the Documentation

Based on the results obtained (Figure 1), herbicide fluctuations were observed, sometimes towards stimulation and sometimes towards inhibition of the analyzed synthetic indicator. These effects were observed even at normal dosages (for example, variants A2d1, A1d1, and A3d1) under both field and laboratory conditions.

Rose et al. (2018) show that the use of herbicides at recommended doses may have inconsistent, transient, or negligible effects on enzymatic activities, mineralization processes, and mineral nitrogen content, which are included in this synthetic biological indicator [75].

Some authors note that the stimulating or inhibiting effect of herbicides on soil microbiota can depend on the type and toxicity of herbicide molecules, the concentration, the method of application, the bioavailability and persistence of the herbicide, and the microbial or enzymatic processes' interactions with the herbicide substance [76], particularly under laboratory conditions where the soil sample is smaller and well homogenized with the herbicide solution, and microorganisms and enzymes can better interact with herbicide molecules.

In accordance with the above, there have also been cases where herbicides tested at higher rates than those recommended for agricultural practice have influenced the ISB. This is the situation for chlorsulfuron at a triple dose (A1d3) and amidosulfuron at a double dose (A2d2) when applied under laboratory conditions. The effect of amidosulfuron at a normal dose and a triple dose was similar under both field and laboratory conditions. These differences between field and laboratory treatments were very well illustrated by the Duncan test (Table 2), which demonstrates that there are statistically significant differences between treatments with amidosulfuron (A2) in the field and in the laboratory when using treatments with this herbicide and thifensulfuron (laboratory models), respectively. The same differences were reported between thifensulfuron (A3) treatments under both test conditions. It can be seen that the inhibitory effects occur at double or even triple doses of the herbicide. Some studies indicate that before starting the degradation process, nontarget soil microorganisms have to tolerate the oxidative stress caused by the herbicide and must also withstand the pressure of the herbicide interfering with the host metabolism [77].

Microbial species have different degradation capabilities. For example, some authors have noted that some microbial species degrade certain sulfonylurea herbicides very rapidly (e.g., *Bacillus cereus*), whereas others, such as *Pseudomonas fluorescens*, have lower

degradability. In this case, the process can take many weeks, with the growth of the bacteria being affected by the herbicide's presence [78]. This demonstrates the importance of the microbial consortium in the degradation and removal of toxic substances from the soil.

Furthermore, herbicides behave differently in the soil because their degradation depends on the structure and other characteristics that cause them to persist in the environment. In addition, environmental, climatic, and environmental factors (physical, chemical, and biological properties) play an important role [79].

In the literature, it is also emphasized that the effect of herbicides is reduced by adsorption on soil colloids and the stabilization of soil microbiota [80], as well as the selection of highly biodegradable species as herbicides during the degradation process and their conversion into accessible sources of nutrients and energy [81].

As some authors note, sulfonylurea herbicides can be degraded in a very short period of time (7 days) at a relatively large proportion (more than 90%), and subsequent metabolites can be used as carbon and energy sources [82], which allows for the estimation of biotic and enzyme processes in the soil. It should be noted that microorganisms must have the ability to adapt to and metabolize the herbicide [83]. The same authors note that experimental environments may affect biological indicators, which was also found in this study. Special caution should be taken in case of adsorption of the herbicide on soil particles, as phytotoxicity phenomena [35], infiltration into groundwater [84], and accumulation in the environment [85,86] may occur.

In addition, the pH of the tested soil meets the conditions for the degradation of sulfonylurea herbicides. Sarmah et al., 1998 [87], cited by Zhang et al., 2022 [88], specify that sulfonylurea behaves well and does not cause problems under acid conditions at pH 4–7.

Although biological indicators for soil quality are often ignored, the most efficient pathway for herbicide degradation is considered to be the microbial pathway [89], especially under conditions of alkaline pH.

As can be seen in these experiments (Table 2), the area of influence was higher for herbicide A2 (amidosulfuron) than other herbicides when the results were compared with the literature data.

Some studies have shown that, in some situations, amidosulfuron has a phytotoxic and inhibitory effect on test organisms in the aquatic environment [90]. This herbicide was also detected at a depth of 0–10 cm, and herbicidal activity was detected at a proportion of 30% at 0–30 cm depth after two years of field experimentation [91]. In addition, the bioavailability differences at concentration intervals of lower than 1 mg/kg ranged from 0.5 to 1.7 mg/kg.

The same authors consider that the area of influence of the herbicide amidosulfuron can be determined to a certain proportion by the experience factors. Cambic-chernozem has a slightly acidic pH, with values ranging from 4.40 to 6.80 in surface horizons and pH that promotes the accelerated degradation of sulfonylureas and reduction in ecotoxic effects. In addition, Sondhia (2008) showed that sulfonylurea herbicides are poorly adsorbed by soil when the pH is increased [92].

Yousef et al. (2016) showed that the rate of herbicide dissipation is fundamental when predicting the consequence of introducing the herbicide to the soil [93].

According to the mathematical model of the three herbicides, chlorsulfuron (A1) behaves well under field conditions. The values of ISB obtained when using treatments with this herbicide are close to the control value, an increase over the control seen in A1d1.

This indicates that the herbicide is metabolized in the first centimeters of the soil, where the soil microbiota are concentrated, as its quantitative reduction along the soil profile is an indication of the persistence of herbicides in the soil. This finding is also underlined by Sarmah et al. (1998) [87].

In our field experiments, ISB values were lower in all experimental variants than in laboratory experimental designs when environmental factors are controlled, and the herbicide–soil–microorganism relationship is much closer.

Under field conditions, lower (A2d1 and A2d3) or higher (A2d2) values of ISB were recorded in the amidosulfuron-treated variants, showing the wide variation in amidosulfuron's influence.

However, there have also been situations where no differences have been observed between the behavior of ISB at normal and higher herbicide levels.

This outcome is supported by other authors: there is likely to be no imbalance in the activity of the soil microbiota at the doses of sulfonylurea used in agricultural practice when compared to the high levels tested by the research team [94].

Comparing the three herbicides, A1 is the most effective on the soil. The information provided by Sofo et al. (2012) is in favor of the post-treatment results [95] since it shows that biological indicators return to normal values 30 days after treatment. A2 and A3 herbicides showed similar behavior under laboratory conditions (Figure 1).

This indicator was successfully used in the analysis of herbicide behavior in soil over a 2-year period by some Romanian researchers [32].

The synthetic biological indicator has also been used to evaluate soils treated with triazine herbicides (a group of substances widely used in Romania, including in the 1990s), proving that overdoses and producer noncompliance can cause many problems to the soil ecosystem [32]. The diversity of responses reveals the role of biodiversity in soil function and expresses the fertility of soil treated with herbicides through mineralization activities and biosynthesis, resulting in the evolution of organic matter in the soil and, implicitly, the increase in the fertility potential of the soil [57,67]. In this work, 30 days after the application of the herbicides in the field and 7 days after application in the laboratory, only a slight, insignificant influence of sulfonylurea herbicides was observed “in vitro and in vivo” as shown by the mathematical models used for the interpretations in this work. The rapid dissipation of sulfonylureas is also supported by Maznah et al. (2020), who show that their half-life can be between 6 and 8 days [96], suggesting that the accumulation of residues and the impact of sulfonylureas at the tested doses are low. According to other studies, the half-life can still vary between 3 and 24 days at an acidic pH, while the halving process is longer in the case of an alkaline pH [97,98].

However, Wu et al. (2022) have shown that chlorsulfuron derivatives degrade rapidly at an alkaline pH [99].

4.2. Synthetic Biological Indicator as Distinct from Other Indicators

It is possible to argue, as other authors have, that the quality of soil depends on the level of fertility, which is the sum of all its physical, chemical, and biological properties [100]; however, of these three properties, the biological factor determines the fertility of the soil. Can we respond to these introductory questions with yes or no?

There are pros and cons to each indicator, but we believe that using the proposed indicator includes a minimum set of biological indicators, which are inexpensive, widely applicable, and capture changes in multiple settings at the same time, in comparison with individual settings.

We can also mention the advantages of such an indicator, which show the intrinsic value of each underlying parameter and the contribution the indicator makes using these parameters.

In addition, the synthetic biological indicator (ISB%) obtained using these parameters provides important information for sustainable soil management with an economic and social impact.

The selection of biotic and enzymatic indicators was not carried out randomly but in accordance with their role in soil quality and their sensitivity to chemical treatments. It should be specified that the indicator includes the soil enzymatic activities involved in cycles C, N, and P, which are essential for obtaining each crop. For example, Sun et al. (2018) showed that these enzyme activities are affected after each harvest [101], showing the importance of including these activities in a more complex indicator.

Several authors have demonstrated that urease is a highly sensitive enzyme and a useful indicator to evaluate soil pollution [102]; it is present in many bacteria and fungi and is positively correlated with soil organic matter content, soil biological activity, and the high availability of nitrogen and other nutrients. The catalase activity is influenced by the degree of soil aeration and favors the processes of mineralization and humification of organic matter [32].

Nitrification is one of the biotic processes included in this synthetic indicator since, together with nitrogen fixation and denitrification, it is one of the most important circuits in the earth's ecosystem. Nitrification provides information about the level of the nitrogen source, nitrogen losses, and environmental pollution by leaching, which leads to ground-water contamination. Through the emission of nitrogen gas, nitrification contributes to the greenhouse effect and global climate change [103] and is considered an indicator for soil health assessments [104]. It also provides information on how the nitrogen fertilization of agricultural land should be managed.

Free nitrogen-fixing bacteria (*Azotobacter* spp.) are part of the rhizobacteria that stimulate the production of phytohormones, improve plant health, intervene through different mechanisms in the removal of stressors and soil recovery, and serve as biofertilizers, playing a role in the metabolism of pesticides and heavy metals [105].

In most countries, the registration of a herbicide requires several toxicity tests of the active substance against soil microorganisms, some soil functions performed by microorganisms, and some microbial processes involved in the performance of significant soil functions.

In this sense, the microbial processes of interest are respiration and nitrification processes, which are also included in the ISB [106,107].

Respiratory activity measures global microbial activity [108] and, together with sucrose and cellulose [109], plays a major role in the global carbon cycle and agricultural management [110,111]. According to different researchers, cellulose is the inexhaustible carbon source of the biosphere [112–114], with the decomposition degree of organic matter in the soil being determined by the C:N ratio.

This indicator cannot be considered perfect, but we can highlight the disadvantages of individual parameters mentioned by other authors in order to understand better what the indicator offers.

Each parameter has a level of importance in measuring the quality of the soil but has individual drawbacks.

The biological indicators frequently used to evaluate disturbances in agricultural soils include microbial community structure, microbial biomass, enzymatic activity, microbial metabolites, respiration, and molecular markers. The choice was based on the fact that these indicators correspond to a wide range of agricultural soil functions. The most widely used indicator is microbial biomass [115], which cannot be quantified by direct methods and is difficult to estimate in the soil [83,116].

Molecular markers are also used in the study of microorganisms involved in the degradation of herbicides in soil. However, their disadvantages are mainly related to the difficulty of isolating and purifying the microbial material and the reduced possibilities of verifying the results [83,116].

Respiration is another indicator recommended for assessing the toxicity of a pesticide, but it is also sensitive to environmental factors, such as the ecophysiological indicators mentioned above.

Consequently, microorganisms may be considered an indicator of changes in soil health and provide early information on improvements in soil quality and warnings of soil degradation [117], but they must be corroborated with enzymatic analyzes, especially in the case of chemical treatments that usually produce a selection of resistant bacterial strains [118]. Furthermore, enzymes are catalysts for all chemical processes of interest in the soil and the biotic processes considered in the determination of the synthetic biological indicator's components.

The indicator includes the enzymes related to the most important elements of the biogeochemical circuit, namely phosphatase, catalase, sucrase, and urease. The most commonly evaluated individual enzyme indicators include dehydrogenase [119], urease, phosphatase, beta-glucosidase, catalase, sucrase, fluorescein diacetate hydrolysis [120,121], and phospholipid fatty acid profile [122].

Fluorescein diacetate hydrolysis activity is determined by a simple technique, but it is not possible to accurately specify which enzymes are involved in the degradation of compounds [83,116]. The relations between the degree of pesticide degradation and the overall vital activity of the soil, i.e., biomass, is also monitored, and this applies only to certain substances.

In addition, there are no validated and accepted methods worldwide. However, the overall parameters can show symptoms on the basis of which the degradation process can be prevented, or the soil may be helped to return to a state of “climax” or equilibrium, depending on environmental factors and other above-ground ecosystems.

“Nature as a whole is well created and recovers, if man respects the uniqueness of each ecosystem and only intervenes when necessary”. This means that assessment methods must be adapted to the specificities of each region.

However, the proposed indicator includes parameters that are common and can be applied at minimal cost in many regions of the world.

Many studies on biodegradation focus on selected microbial strains [123,124], but if a microbial consortium is involved, the chance of eliminating the herbicide and the environmental risks is high [77]. This can highlight many of the changes that occur in the soil ecosystem because of chemical treatments. As a result, the indicator used in testing sulfonylurea herbicides comprises enzymes and vital processes underlying a community of microorganisms in the soil. The advantage is that herbicides will be degraded in a shorter time, as mentioned by other bibliographic sources [125] because enzymes common to several microorganisms will participate in these degradation processes [126].

Lehmann et al. (2020) state that these indicators can be used in combination in the form of complex equations [127].

Some combinations of indicators include the following:

- The biological fertility index, which includes respiration and enzyme activities [128]. The soil biological fertility index, in addition to microbial biomass carbon, also includes basal respiration, cumulative effects, metabolic coefficient, and organic matter [129].
- The soil microbial index is composed of microbiota, microbial biomass carbon, microbial biomass nitrogen, mycorrhizal infection, soil respiration, dehydrogenase, phosphatase, and nitrogen mineralization capacity [130].
- The enzyme activity number, which includes several enzymes (amylase, catalase, dehydrogenase, alkaline phosphatase, and protease) [131].

Which indicator is better?

By referring to physical and chemical indicators, studies show that the response of the soil to anthropogenic actions is faster and more obvious when biological indicators are used. Some data from the specialized literature show that intensive soil management has a high negative impact on the quality of biological indicators [132].

5. Conclusions

The imbalances caused by the application of the sulfonylurea herbicides and quantified by the synthetic biological indicator under field conditions and laboratory conditions are considered negligible relative to the control.

Thirty days after treatment in the field, mild stimulating effects were maintained in the A1d1 and A2d2 variants and under laboratory conditions in the A2d3 and A3d1 variants. Additionally, slight changes in the indicator were observed in the variants treated with amidosulfuron (A2d1 and A2d3) and with all three herbicides in variants A2d2, A1d3, and A3d2 under laboratory conditions.

The indicator showed upward and downward fluctuations at both normal and higher doses. Statistical and mathematical methods provided information on the differentiation and correlation of the outcomes obtained under the two experimental conditions.

The Duncan test revealed statistically significant differences between the two experimental variants (field and laboratory).

Thus, determinations based on kernel density showed that the herbicide chlorsulfuron (A1) behaves well under field conditions, while the herbicides amidosulfuron (A2) and thifensulfuron (A3) showed similar behavior under the two test conditions. Based on the mathematical model, the herbicide amidosulfuron is the most influential of the three studied herbicides. Information about amidosulfuron is limited. It had the greatest area of influence, which is why the study will continue to follow its disappearance from the soil until the time of rebalancing of microbial processes in the soil is rebalanced.

A comparison of the synthetic biologic indicator with other indicators that are commonly used in the analysis of herbicide-treated soils revealed the limitations of individual indicators and the potential benefits of an indicator composed of a minimum set of parameters.

The indicator can provide more answers and solutions for the rational exploitation of the soil and forecasts related to the type and doses of herbicides.

In addition, each region and each soil needs personalized parameters according to the characteristics of the region, such as the human body, but we consider that this indicator includes parameters that are common to most areas.

In order to preserve and restore the fertility of the soil, it is necessary for specialists in the field, dedicated researchers, and especially farmers to receive solid information about the health of the soil through such indicators.

With the help of this complex indicator and the proposed mathematical models, the study can be extended to other pesticides, taking into account various exposure routes. We also intend to develop new mathematical models to create maps that can combine the trends shown by the simple indicators, which usually reflect the risks at a national level, in order to identify the risk trends at a larger scale.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agriculture13050924/s1>, Table S1: Other biological indicators used to quantify the impact of herbicides on soil.

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