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Article

Biosensor-Based Assessment of Pesticides and Mineral Fertilizers' Influence on Ecotoxicological Parameters of Soils under Soya, Sunflower and Wheat

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Abstract: Pesticides and fertilizers used in agriculture can negatively affect the soil, increasing its toxicity. In this work, a battery of whole-cell bacterial lux-biosensors based on the *E. coli* MG1655 strain with various inducible promoters, as well as the natural luminous *Vibrio aquamarinus* VKPM B-11245 strain, were used to assess the effects of agrochemical soil treatments. The advantages of using biosensors are sensitivity, specificity, low cost of analysis, and the ability to assess the total effect of toxicants on a living cell and the type of their toxic effect. Using the *V. aquamarinus* VKPM B-11245 strain, the synergistic effect of combined soil treatment with pesticides and mineral fertilizers was shown, which led to an increase in the overall (integral) toxicity of soils higher than that of the individual application of substances. Several probable implementation mechanisms of agrochemical toxic effects have been discovered. DNA damage caused by both SOS response induction and alkylation, oxidative stress due to increased superoxide levels, and damage to cellular proteins and membranes are among them. Thus, the usage of biosensors makes it possible to assess the cumulative effect of various toxicants on living organisms without using expensive chemical analyses.

Keywords: whole-cell bacterial lux-biosensors; agricultural soils; fertilizers; pesticides; DNA damage; oxidative stress

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1. Introduction

Modern agricultural practices include an extensive use of fertilizers and pesticides. Despite achievements in organic farming and the use of biological crop protection products, the complete elimination of chemicals in the foreseeable future seems an unlikely scenario. In this regard, the task of agricultural soils condition monitoring is of particular relevance.

Excessive or improper use of pesticides can lead to the accumulation of agrochemical residues, which then enter the ecosystem and food chain directly or indirectly. In turn, this has a negative impact on living beings, including humans [1,2]. Chemical methods (chromatography, mass spectrometry, etc.) are mainly used to detect trace amounts of chemicals in environmental samples [3–5]. The disadvantages of these methods are their relatively high cost, duration of analysis, and equipment requirements. In addition, chemical analysis alone can only provide accurate information about the levels and composition of contaminants but cannot assess their actual toxicity to biological systems.

In this regard, considerable attention has recently been paid to the development of biosensors that can be a good alternative to chemical analysis [6]. Biosensors with various biological parts (whole cells, enzymes, antibodies, DNA and RNA) have emerged as

promising tools for environmental research. In particular, whole-cell biosensors can identify environmental risks associated with pollutants using modeling of microbial interactions with pollutant molecules [7]. These sensors respond to pollutant mixtures and can be used as a net effect sensor, including the response to compounds that cannot yet be identified by chemical analysis.

Whole-cell biosensors are biological reporters that convert chemical signals into a detectable signal using microbial cells as sensors. They typically consist of two parts: biological sensors (microbial cells) that generate signals in the presence of a target chemical (group of substances), and physical devices that convert and detect optical, electrochemical and thermodynamic signals [8,9]. Lux-biosensors containing bacterial luciferase genes under the control of specifically inducible promoters are a type of optical biosensors. Lux biosensors can be divided into two groups—those with inducible and those with constitutive luminescence. Sensors of the first group have low background luminescence, which increases in the presence of the target substance (group of substances, during stress exposure), and the second group (often natural luminous strains) has a high level of luminescence, which decreases in the presence of toxicants in the environment.

Despite a large number of reports on the design of various biosensor types, there are much fewer examples of their actual use for environmental objects' monitoring. Thus, a whole-cell bacterial sensor based on the *Acinetobacter baylyi* ADP1 Tox2 strain with constitutive luminescence was used to determine the toxicity of river water in Bangladesh [10], and contaminated seawater near wastewater outlets in China [11]. In addition, a biosensor based on the same strain, *A. baylyi* ADP1_recA_lux, was used to determine the genotoxicity of groundwater contaminated with a mixture of phenolic compounds [12], soils and groundwater contaminated with petroleum hydrocarbons as a result of an accident in Lanzhou, China [13]. Using the *E. coli* RecA::luxCDABE bioreporter, the genotoxicity of chromium-contaminated soils and seawater contaminated with crude oil after the Jiaozhou Bay spill was studied [14]. Whole-cell bacterial lux-biosensors based on the *E. coli* MG1655 strain with various inducible promoters were used to monitor the genotoxicity of the Don River bottom sediments (Russia) [15], wastewaters of Rostov-on-Don and Munich [16], bottom sediments of the Sea of Azov [17], and soils of various types of land use [18]. The review carried out by Bazhenov [19] systematizes the information on the use of whole-cell bacterial lux biosensors based on various bacterial strains, including their use in ecotoxicological environmental studies.

In addition to determining general genotoxicity in natural samples, the use of biosensors for specific pollutants detection is also described. Using the *A. baylyi* ADPWH_alk strain, which contains the alkM operon, the content of available n-alkanes in soil and groundwater contaminated by an accident in China was determined [13], as well as that in soil from an oil pumping field and chromium slag storage facility in China [20], and in seawater and bottom sediments at the site of an oil reservoir explosion in Dalian, China [21]. Whole-cell biosensors based on *E. coli* were used to determine the concentration of p-nitrophenol in soils near a chemical plant (Nanjing, China) [22] mercury in historically contaminated soils of the Hunan and Guizhou mining areas (China) [23].

Thus, one of the main application areas of bacterial biosensors in environmental studies is the determination of general toxicity/genotoxicity, as well as the presence of specific pollutants. Most often, the objects of study are anthropogenically polluted territories, natural waters (river, sea, underground), and—extremely rarely—agricultural soils. The potential of biotesting using bacterial biosensors can be much wider due to an increase in the range of tested objects and the possibility of using promoters that respond to various groups of pollutants or toxic effects. As a result, we can obtain more complete information about the totality of biological processes occurring in contaminated environmental objects.

In this work, we conducted a study of agricultural soils treated with agrochemicals using a battery of whole-cell bacterial lux-biosensors based on the *E. coli* MG1655 strain with inducible promoters and the natural *Vibrio aquamarinus* VKPM B-11245 strain. The natural strain was used to determine the integral toxicity of soils, and the genetically

engineered strains of E. coli MG1655 were used to study genotoxic, pro-oxidant, protein- and membrane-damaging properties of soils. Using the set of strains made it possible to determine not only the fact of toxicity of soils with various treatments, but also to suggest a number of biological mechanisms through which the toxic effects of agrochemicals are manifested.

2. Materials and Methods

2.1. Field Experiment

The field experiment was carried out in 2022–2023 in the Rassvet village in the Rostov region, Russia (47°21′40″ N and 39°52′50″ E). The region has a temperate continental climate with an average annual precipitation of 530–550 mm, average monthly temperature from 5 °C to −9 °C in winter, +22–24 °C in summer. Soya (*Glycine max*), sunflower (*Helianthus annuus* L.) (2022) and winter wheat (*Triticum aestivum*) (2023) were grown in four treatments: control (without fertilizers and pesticides), only fertilizers, only pesticides, and combined use of fertilizers and pesticides. The list of soil samples collected during the field experiment is given in Appendix A Table A1.

Ammophos (12:52) was used as a fertilizer on soya and sunflowers for the main treatment (in autumn), and the application dose was P40 (77 kg in physical weight per 1 ha). For wheat, a nitrogen–phosphorus–potassium fertilizer (NPK 16:16:10) was used for the main treatment (in autumn), and the application dose was N40P40K40; in spring, ammonium nitrate (N40) was applied on frozen soil. Details on the applied pesticides are provided in Table A2 (Appendix A).

The area of each plot was 20 × 12 m. Each treatment was carried out in triplicate. Soil samples were taken twice—during the growing season before applying pesticides, and after applying pesticides at the end of the growing season. Soil samples were collected from five different undisturbed locations at the depth of 0–20 cm for each site ("envelope method"). The samples were thoroughly mixed until homogeneous.

2.2. Bacteria Strains

A battery of whole-cell bacterial lux biosensors was used to assess the ecotoxicity of soil samples. The integral (general) toxicity of soil samples was determined using the natural strain of *V. aquamarinus* VKPM B-11245 [24]. The biotest is based on the principle of *V. aquamarinus* VKPM B-11245 bioluminescence "suppression" in the presence of toxic substances. To determine genotoxicity, biosensors with inducible luminescence of *E. coli* MG1655 (pRecA-lux), *E. coli* MG1655 (pDinB-lux) and *E. coli* MG1655 (pAlkA-lux) were used. *PrecA*, *PdinB*, and *PalkA* promoters are induced by DNA damage in the plasmids of these strains. Biosensors *E. coli* MG1655 (pKatG-lux), *E. coli* MG1655 (pSoxS-lux), *E. coli* MG1655 (pOxyR-lux), the promoters of which (*PkatG*, *PsoxS* and *PoxyR*) respond to the presence of pro-oxidant substances [25], were used to detect the substances that induce oxidative stress in cells (hydrogen peroxide (H_2O_2), organic peroxides, superoxide radical ion (O_2^-). *E. coli* strain MG1655 (pGrpE-lux), containing the "heat shock" promoter *PgrpE*, was used as a specific biosensor for toxicants that damage cellular proteins. *E. coli* strain MG1655 (pFabA-lux) was used to determine the toxicity of media containing chemicals and cell-membrane-modifying materials. The strain contains the *fabA* gene promoter, which encodes the ß-hydroxydecanoyl-[acyl-carrier-protein] dehydratase enzyme, a key protein in the synthesis of unsaturated fatty acids (components of cell membranes) [26]. In order to correct artifacts associated with changes in bacterial luciferase activity and not associated with induction, a genetically engineered *E. coli* MG1655 (pXen7) strain with a constitutive promoter was used [25]. All biosensors with inducible luminescence contained hybrid plasmids based on the pBR322 vector, carrying the *Photorhabdus luminescens luxCDABE* gene cassette under the control of the corresponding promoters and a selective marker for ampicillin resistance.

2.3. Assessment of Soil Ecotoxicity Using Bacterial Lux-Biosensors

Soil extracts were prepared according to the protocol described in the article by Sazykina [15]. Cells of bacterial strains were incubated in Luria-Bertani (LB) medium [27] containing 100 μg of ampicillin/mL with constant shaking for 18–20 h at 37 °C. *V. aquamarinus* VKPM B-11245 strain was grown without ampicillin until the early exponential phase at 25 °C. Bacterial strains were immediately used for stress induction tests.

The biosensors' luminescence was measured on a Luminoskan Ascent microplate luminometer (Thermo Fisher Scientific, Waltham, MA, USA) for 120 min in three independent replicates. To carry this out, 20 μL of extracts of the studied soils was added to the wells of a 96-well microplate containing 180 μL of culture. Then, 20 μL of distilled water were added instead of soil extracts into the wells with negative control; 20 μL of a toxicant solution activating the corresponding promoter was added into the wells with the positive control. Numerical values of bioluminescence were expressed in relative luminescence units.

To assess the toxic effect of substances contained in soil extracts, the induction coefficient F_i was calculated, defined as the ratio of luminescence intensity of lux-biosensor suspension containing the test sample (L_c) to luminescence intensity of the control lux-biosensor suspension (L_k): $F_i = L_c/L_k$. Since natural substrates contain substances that can both suppress and stimulate the activity of bacterial luciferase itself, the *E. coli* strain MG1655 (pXen7), in which the lux operon is under the control of a constitutive promoter, was also used.

For this strain, the coefficient of inductive suppression of luminescence (K) was calculated, $K = l_c/l_k$, where l_c is luminescence intensity of the suspension of the strain with a constitutive promoter in the presence of the test compound; l_k represents the luminescence intensity of the lux-strain with a constitutive promoter control suspension. The true values of the induction factor (I) were calculated using the formula $I = F_i/K$, where F_i is the induction coefficient, and K is the luminescence suppression coefficient. The degree of toxicity for genetically engineered *E. coli* strains using I values was assessed as follows: mild toxicity ($I < 2$), moderate toxicity ($2 \leq I \leq 10$), and severe toxicity ($I > 10$) [28].

When determining the integral toxicity of soils using the natural *V. aquamarinus* VKPM B-11245 strain, the toxicity index (T) was calculated as $T = 100\ (I_k - I_c)/I_c$, where I_c and I_k are the luminescence intensity of bacteria in the test and control samples, respectively, at a fixed exposure time of bacteria (30 min) with soil extracts. Soil samples were considered admissibly toxic when $I < 20$, toxic when $20 \leq I < 50$, and highly toxic when $I > 50$ [29]. The experiments were performed in three independent replicates.

2.4. Statistical Processing

Statistical processing of the results was carried out using standard methods of mathematical statistics. Statistical analysis was performed using GraphPad Prism 8.0.2 DEMO (GraphPad Software, Inc., San Diego, CA, USA) using two-way ANOVA and *t*-test ($p \leq 0.05$). For visualization, we used package "seaborn" for Python 3.8.

3. Results

3.1. Integral Toxicity Determined Using the V. aquamarinus VKPM B-11245 Strain

The study of integral toxicity of soil samples under soya crops showed an acceptable degree of toxicity for almost all samples, except for the soil treated with both pesticides and fertilizers ($T = 29.05 \pm 4.30$, average toxicity). At the same time, soil under sunflower crops, taken at the end of the growing season, had significant integral toxicity in all treatment options, and the highest with the combined treatment ($T = 71.88 \pm 5.80$) (Figure 1, Table A3 (Appendix A)). In soil under wheat grown after soya, the initial toxicity of all samples was admissible; however, agrochemical treatments in all variants contributed to a significant increase in toxicity—all samples were characterized as highly toxic ($T = 64.29 \pm 4.00$; $T = 59.87 \pm 2.00$; $T = 76.87 \pm 3.00$, Figure 1). Soil under wheat crops after sunflower had admissible initial toxicity (except for the soil with combined treatment, where $T = 35.29 \pm 0.90$). The application of pesticides slightly increased the integral toxicity

of the soil (T = 27.34 ± 2.00). For soil with the combined treatment, a decrease in toxicity was noted during the cultivation of wheat (T = 23.8 ± 3.00). The soil with the application of mineral fertilizers was highly toxic (T = 86.34 ± 6.00).

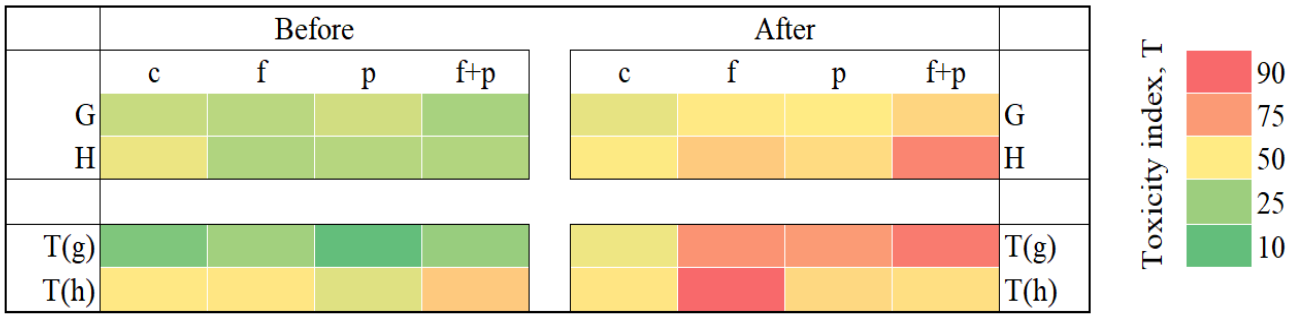

Figure 1. Integral toxicity of agricultural soils under soya crops (G), sunflower (H); wheat grown after soya (T(g)) and after sunflower T(h), determined using the *V. aquamarinus* VKPM B-11245 strain: c—control, f—fertilizers, p—pesticides, f + p—combined treatment. Before—sampling before pesticide application. After—sampling after pesticide application at the end of the growing season.

3.2. Genotoxicity of Agricultural Soils

The response of the *E. coli* MG1655 (pRecA-lux) strain, which detects the presence of DNA-damaging substances, revealed the negative impact of applying pesticides separately and as part of the combined treatment of soil under soya and sunflowers (Figure 2, Table A3). During the subsequent cultivation of wheat after soya, no significant biosensor response was recorded either in the initial sampling or after treatments. At the same time, with the help of this biosensor, it was possible to register the negative effects that persisted after treating the predecessor of wheat (sunflower) with fertilizers and pesticides. Interestingly, at the end of the wheat growing season, genotoxicity could no longer be detected using the *E. coli* MG1655 (pRecA-lux) biosensor.

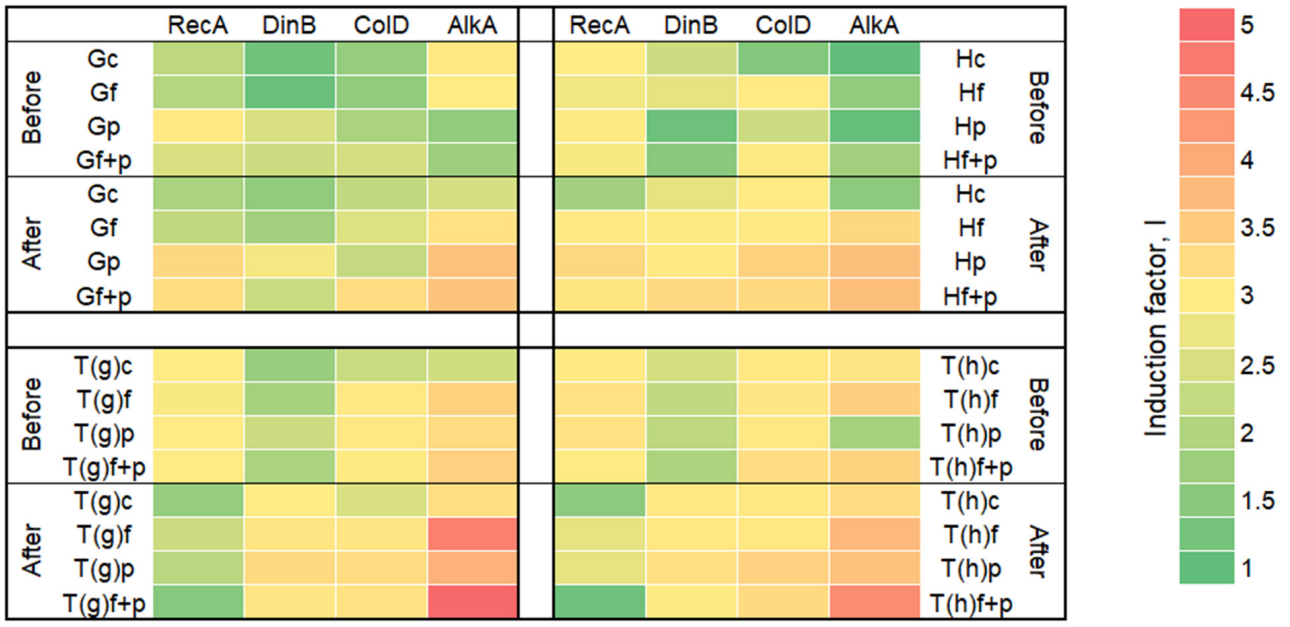

Figure 2. Genotoxicity of agricultural soils under soya crops (G), sunflower (H); wheat grown after soya (T(g)) and after sunflower T(h), determined using *E. coli* MG1655 (pRecA-lux), *E. coli* MG1655 (pDinB-lux), *E. coli* MG1655 (pColD-lux), *E. coli* MG1655 (pAlkA-lux) strains: c—control, f—fertilizers, p—pesticides, f + p—combined treatment. Before—sampling before pesticide application. After—sampling after pesticide application at the end of the growing season.

Using the *E. coli* MG1655 (pDinB-lux) strain (detects genotoxicants that trigger SOS response in the cell), a weak toxic effect of the combined soil treatment under sunflower crops (Figure 2, Table A3) of all variants of agrochemical soil treatments under wheat crops after soya was revealed, and that of introducing fertilizers and pesticides separately into the soil under wheat after sunflower.

The genotoxic effects of fertilizers and pesticides applied jointly to soya crops were noted using the *E. coli* MG1655 (pColD-lux) biosensor (I = 1.81 ± 0.27) (Figure 2, Table A3). For almost all treatments in soil under sunflower, a negative effect was found due to the presence of genotoxicants, which persisted during the subsequent cultivation of wheat, increasing towards the end of the growing season. Genotoxicity due to the presence of alkylating agents was detected using the *E. coli* MG1655 (pAlkA-lux) strain in all soil treatments for soya, sunflower and wheat after both predecessors (Figure 2, Table A3).

3.3. Pro-oxidant Properties of Agricultural Soils

Using biosensor strains that detect the presence of substances generating oxidative stress (*E. coli* MG 1655 (pKatG-lux), *E. coli* MG 1655 (pOxyR-lux), *E. coli* MG 1655 (pSoxS-lux)), no significant pro-oxidant effect of all variants of soil treatments under soya was found, except for one sample (*E. coli* MG 1655 (pOxyR-lux) sensor, soil with pesticides, I = 1.64 ± 0.03) (Figure 3, Table A3). In soil under sunflower, the response of the *E. coli* MG 1655 (pKatG-lux) sensor to pesticide treatment (I = 1.57 ± 0.04), and that of the *E. coli* MG 1655 (pSoxS-lux) sensor to a combined treatment (I = 1.59 ± 0.04) were registered. There was a much stronger oxidative stress, caused by the presence of substances that generate superoxide radicals in soil under wheat crops after both predecessors—the response of the *E. coli* MG 1655 (pSoxS-lux) sensor was recorded in all variants of agrochemical treatments. In addition, weak toxicity due to peroxides (*E. coli* MG 1655 (pOxyR-lux) sensor) was observed when fertilizers and pesticides were applied separately to soil under wheat after sunflower.

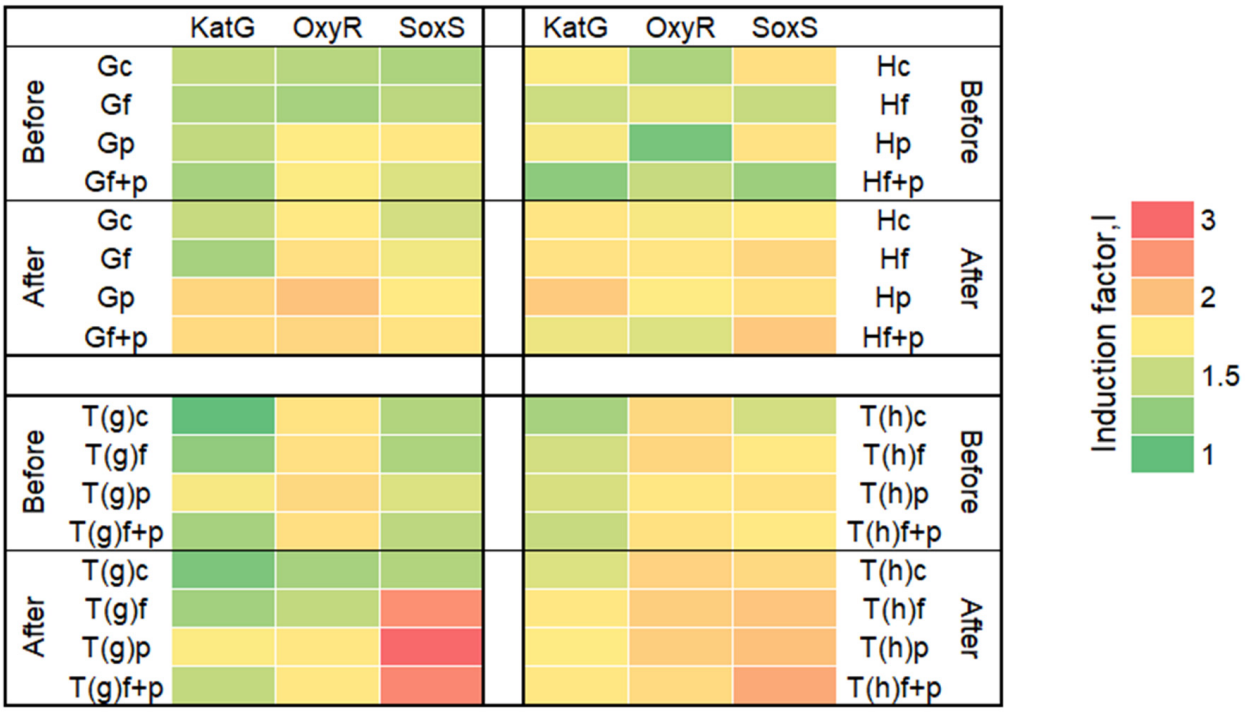

Figure 3. Pro-oxidant properties of agricultural soils under soya crops (G), sunflower (H); wheat grown after soya (T(g)) and after sunflower T(h), determined using *E. coli* MG 1655 (pKatG-lux), *E. coli* MG 1655 (pOxyR-lux), *E. coli* MG 1655 (pSoxS-lux) strains: c—control, f—fertilizers, p—pesticides, f + p—combined treatment. Before—sampling before pesticide application. After—sampling after pesticide application at the end of the growing season.

3.4. Protein- and Membrane-Damaging Properties of Soils

All variants of soil treatments under soya and sunflower did not lead to an increase in the protein-damaging properties of the studied soils extracts, detected using the sensor *E. coli* MG1655 (pGrpE-lux) (Figure 4, Table A3). However, when growing wheat, the emergence of soil toxic properties due to protein damage was recorded in all variants of agrochemical treatments (except for soil after sunflower with mineral fertilizers application). Weak toxicity due to the presence of cell-membrane-damaging substances was demonstrated using the *E. coli* MG1655 (pFabA-lux) strain when pesticides were applied (separately and together with fertilizers) to soya crops (Figure 4, Table A3). All variants of soil treatments under sunflower led to an increase in the membrane-damaging properties of soils (from weak to medium levels). Regarding further wheat growth after soya, the toxic effect was recorded at the end of the growing season with the combined treatment, and when grown after sunflower, it was found in the initial soil samples in all treatment options. By the end of the wheat growing season, membrane-damaging properties were recorded only in the soil with the pesticides' addition.

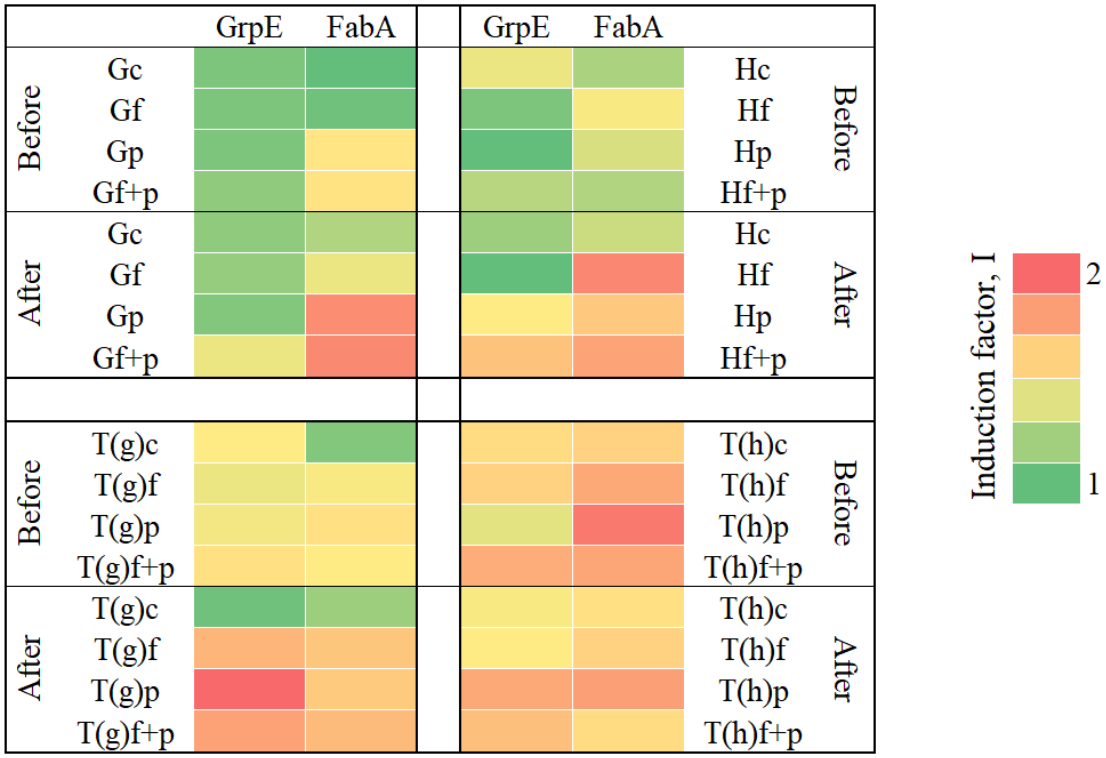

Figure 4. Protein- and membrane-damaging properties of agricultural soils under soya crops (G), sunflower (H); wheat grown after soya (T(g)) and after sunflower T(h), determined using *E. coli* MG1655 (pGrpE-lux) and *E. coli* MG1655 (pFabA-lux) strains: c—control, f—fertilizers, p—pesticides, f + p—combined treatment. Before—sampling before pesticide application. After—sampling after pesticide application at the end of the growing season.

4. Discussion

Soil is a complex matrix in which living components, chemical and physical factors interact. When studying the influence of agrochemical treatments on agricultural soils' ecotoxicity, we are dealing not only with the toxicity of individual chemicals introduced into the soil, but also with the result of complex interactions of chemicals, microorganisms, plant root exudates, and physical and chemical soil parameters. A number of studies have shown that, indeed, the toxicity or environmental risk of pollutants in soil is influenced not only by their total amount or availability, but also by physicochemical soil properties, pH value, environmental properties, and the ionic strength of contaminated areas [30].

In this work, a synergistic negative effect of pesticides and fertilizers when applied to plant crops was discovered using the natural bioluminescent strain *V. aquamarinus* VKPM B-11245, the operating principle of which is based on suppressing the strain luminescence in the presence of a sum of toxicants. Similar results were shown by Yang et al. [31], who used biosensors to find both synergistic and antagonistic effects of the combination of heavy metals and pesticides on soil cytotoxicity. The bioavailability of pollutants, not just their direct concentration in environmental samples, is also important. This was shown by Sazykin et al. [32] when studying the ecotoxicity of river Don bottom sediments using bacterial lux biosensors, as well as by Azhogina et al. [18], who found a close connection between the response of biosensors and the concentration of bioavailable PAHs.

Integral toxicity. In this work, we also observed an indirect effect of agrochemical treatments on the response of the *V. aquamarinus* VKPM B-11245 strain, which was especially pronounced in the soil under wheat crops. At the same dosages of agrochemicals, the integral toxicity of soil after growing soya and after growing sunflowers changed significantly. The highest toxicity was found in the soil under wheat after sunflower with mineral fertilizers' application. At the same time, a response of comparable strength from other biosensors for this sample was not found. This may indicate other mechanisms of toxicity that are not detected by the biosensors used. In addition, a negative effect can be realized not through the direct action of a chemical, but through its influence on plants and soil microbiome.

Genotoxicity. Using several sensors that respond to the presence of DNA-damaging substances, the genotoxic properties of soil with pesticides application (both separately and in combination with mineral fertilizers) were discovered. The biosensors set was formed in such a way as to cover the main mechanisms of genotoxic effects (DNA damage that blocks replication and induces an SOS response, as well as DNA alkylation, which does not always stop the replication fork and does not cause SOS response induction). The strongest reaction to agrochemical treatments was shown by the *E. coli* MG1655 (pAlkA-lux) strain, which detects alkylating agents. This corresponds to the evidence that a number of pesticides have alkylating properties and can react with nucleophilic regions of DNA, causing genotoxicity [33]. The *E. coli* MG1655 (pRecA-lux) strain response to the application of pesticides separately and as a part of combined application to soil under soya and sunflower was also recorded. The genotoxic effects of pesticide residues in contaminated soil were also shown by Zeyad et al. [34]. Using prokaryotic tests (in particular, *E. coli* K-12 mutants with a DNA repair defect), it was found that survival of *polA*, *lexA* and *recA* mutants was 39%, 47% and 55% when treated with hexane extract of contaminated soil. The same was previously shown for soils irrigated with wastewater from pesticide production plants—the survival of E. coli K-12 mutants with DNA repair defect decreased when exposed to soil extracts [35].

When studying genotoxicity of total petroleum hydrocarbons in contaminated soils and groundwater using the biosensor strain *Acinetobacter baylyi* ADPWH_recA, a high level of genotoxicity was found in soil and groundwater samples with lower concentrations of TPH (4338.0 mg/kg and 1.4 mg/L mitomycin C equivalent). This may indicate a significant influence of geochemical variables and alkanes availability on ecological risks of oil pollution [13]. We believe that this assumption is also valid for agricultural soils contaminated with agrochemicals. In addition, introduced substances can increase soil genotoxicity due to reaction of soil organisms and plants. Using the *E. coli* MG1655 (pRecA-lux) strain, it was possible to register residual genotoxicity during further cultivation of wheat after sunflower. This can be explained by a longer growing season of sunflower and the need to apply more pesticides and use desiccants. Soya harvest is completed early, and, apparently, soil has enough time to recover before the wheat growing season.

The ability of whole-cell bacterial biosensors with the recA promoter to detect changes in genotoxicity has been well demonstrated in oil-contaminated seawater. Using the *E. coli* RecA::*luxCDAB* biosensor strain, Jiang et al. [14] found a spatial and temporal variation in genotoxicity of seawater contaminated with crude oil, most likely due to crude

oil degradation process. In earlier work, using another biosensor strain, *Acinetobacter* ADPWH_recA, it was also possible to record a decrease in alkane content and genotoxicity to the detection threshold [21].

However, as the results of the present study show, it is important to use a combination of biosensor strains. This is clearly seen in the example of soil under wheat, where at the end of the cultivation period the *E. coli* MG1655 (pRecA-lux) strain no longer detects genotoxicity, but other sensors, especially *E. coli* MG1655 (pAlkA-lux), reacting to the presence of alkylating agents, indicate the presence of genotoxic properties of the soil. Apparently, over time, partial degradation of pesticides in soil occurs under the influence of temperature, humidity, pH, etc., forming partial decomposition products that are more or less toxic than the original compounds.

They may no longer cause structural disturbances of the bacterial genome, including single- and double-strand DNA breaks, which leads to a decrease in the induction of the biosensor strain *E. coli* MG1655 (pRecA-lux), which responds to the expression of the SOS response.

Oxidative stress. Using biosensor strains that detect oxidative stress, it was found that agrochemical treatments (in all variants) increase oxidative stress caused by the presence of superoxide anion in the medium. It is known that pesticides can act as potent inducers of oxidative stress, which results from an imbalance between reactive oxygen species (ROS) and antioxidant mechanisms [36]. In a recent study, Sazykin et al. [37] showed that glyphosate pesticide caused oxidative stress due to increased levels of superoxide and peroxide (determined using *E. coli* MG 1655 (pSoxS-lux) and *E. coli* MG 1655 (pKatG-lux) strains), and increased the level of mutagenesis in *E. coli*. Similar results were obtained in the work of Yang et al. [31], where, using the biosensor strain *Acinetobacter* ADPWH_recA, it was shown that combined exposure of soil to Ag(I), Cr(VI) and four pesticides (dichlorvos, parathion, omethoate, monocrotophos) aggravated oxidative damage associated with ROS compared with individual pollutants. For other organisms (mammals, plants), there is ample evidence of the ability of pesticides to induce oxidative stress and generate ROS, especially when interacting with heavy metals [38–41]. Oxidative stress in the cell triggers a cascade of protective reactions, including the SOS response, accompanied by DNA damage. In this work, an increase in the genotoxicity of treated soils was detected using lux biosensors, which suggests a mechanism of toxic action of agrochemicals due to oxidative DNA damage. Thus, the data on oxidative stress in soils treated with agrochemicals, obtained using bacterial lux biosensors, are consistent with the results observed for other objects. We believe that this tool can be used to quickly and inexpensively assess oxidative stress caused by various environmental pollutants.

Damage to proteins and membranes. Soil under wheat after both predecessors had protein-damaging properties. The response of the *E. coli* MG1655 (pGrpE-lux) sensor in these soils looks quite natural, given that in these samples, with the help of other strains, both an increased level of oxidative stress and genotoxicity due to DNA alkylation were observed. Interestingly, in this work, we did not observe a coordinated response of sensors to protein damage and membrane damage. Using the *E. coli* MG1655 (pFabA-lux) strain, toxic effects of both pesticides and fertilizers on cell membranes in the soil under soya and sunflowers were detected, while the *E. coli* MG1655 (pGrpE-lux) strain did not show any negative effects here.

Membrane damage was recorded both with individual and combined application of chemicals, while, for example, with the combined application of Ag(I), Cr(VI) and four pesticides in the work of Yang [31], membrane damage was not observed in bioreporter cells in response to mixtures of heavy metals and pesticides. Summarizing the responses of various biosensors, it can be assumed that the application of agrochemicals to the soil increases oxidative stress caused by superoxide level, which leads to triggering an SOS response, accompanied by damage to DNA, cell membranes and proteins.

5. Conclusions

A battery of whole-cell bacterial lux-biosensors based on the *E. coli* MG1655 strain with inducible promoters can be used to monitor the ecotoxicity of natural environments, in particular that of agricultural soils. Using biosensors, it is possible to assess the total impact of pollutants on living organisms and biological mechanisms that mediate toxicity. It has been shown that the application of pesticides and fertilizers to plant crops increases the overall soil toxicity, which may be due to a damaging effect on DNA, proteins and membranes, as well as an increase in the level of superoxide anion radical.

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Abbreviations

soya crops (G), sunflower (H), wheat grown after soya (T(g)) and after sunflower T(h), c—control, f—fertilizers, p—pesticides, f + p—combined treatment. Before—sampling before pesticide application. After—sampling after pesticide application at the end of the growing season.

Appendix A

Table A1. Soil samples collected during a field experiment.

№	Designation	Crop	Agrochemical Treatment	Sampling Time	Forecrop
			Sampling before pesticide application		
1	Gc	soya	control	14.06.2022	–
2	Gf	soya	fertilizers	14.06.2022	–
3	Gp	soya	pesticides	14.06.2022	–
4	Gf + p	soya	fertilizers + pesticides	14.06.2022	–
5	Hc	sunflower	control	14.06.2022	–
6	Hf	sunflower	fertilizers	14.06.2022	–
7	Hp	sunflower	pesticides	14.06.2022	–
8	Hf + p	sunflower	fertilizers + pesticides	14.06.2022	–
9	T(g)c	winter wheat	control	15.05.2023	soya
10	T(g)f	winter wheat	fertilizers	15.05.2023	soya
11	T(g)p	winter wheat	pesticides	15.05.2023	soya
12	T(g)f + p	winter wheat	fertilizers + pesticides	15.05.2023	soya
13	T(h)c	winter wheat	control	15.05.2023	sunflower
14	T(h)f	winter wheat	fertilizers	15.05.2023	sunflower
15	T(h)p	winter wheat	pesticides	15.05.2023	sunflower
16	T(h)f + p	winter wheat	fertilizers + pesticides	15.05.2023	sunflower

Table A1. *Cont.*

№	Designation	Crop	Agrochemical Treatment	Sampling Time	Forecrop
			Sampling after pesticide application		
17	Gc	soya	control	07.07.2022	–
18	Gf	soya	fertilizers	07.07.2022	–
19	Gp	soya	pesticides	07.07.2022	–
20	Gf + p	soya	fertilizers + pesticides	07.07.2022	–
21	Hc	sunflower	control	22.09.2022	–
22	Hf	sunflower	fertilizers	22.09.2022	–
23	Hp	sunflower	pesticides	22.09.2022	–
24	Hf + p	sunflower	fertilizers + pesticides	22.09.2022	–
25	T(g)c	winter wheat	control	04.07.2023	soya
26	T(g)f	winter wheat	fertilizers	04.07.2023	soya
27	T(g)p	winter wheat	pesticides	04.07.2023	soya
28	T(g)f + p	winter wheat	fertilizers + pesticides	04.07.2023	soya
29	T(h)c	winter wheat	control	04.07.2023	sunflower
30	T(h)f	winter wheat	fertilizers	04.07.2023	sunflower
31	T(h)p	winter wheat	pesticides	04.07.2023	sunflower
32	T(h)f + p	winter wheat	fertilizers + pesticides	04.07.2023	sunflower

Table A2. Chemical plant protection products used in this study.

Plant-Protecting Agent	Trade Name	Composition	Application	Dose (L ha^{-1})	Treatment Method	Crop
Herbicides	Gardo Gold	312.5 g L^{-1} c-metolachlor 187.5 g L^{-1} terbutylazine	SE	4.0	application to the soil before sowing	soya
				3.0		sunflower
	Benito	300 g L^{-1} bentazone	CC	2.0	during vegetation	soya
	Reglon Super	150 g L^{-1} diquat	WS	2.0	before harvesting (desiccant)	sunflower
Fungicides	Maxim	25 g L^{-1} fludioxonil	SC	5.0	pre-sowing seed treatment (protectant)	sunflower
	Optimo	200 g L^{-1} pyraclostrobin	EC	1.0	during growing season	sunflower
	Ceriax Plus	66.6 g L^{-1} pyraclostrobin + 41.6 g L^{-1} fluxapyroxad + 41.6 g L^{-1} epoxiconazole	EC	0.4	during growing season	winter wheat
Insecticides	Cruiser	350 g L^{-1} thiamethoxam	SC	0.5	pre-sowing seed treatment (seed dresser)	sunflower
	Ampligo	50 g L^{-1} lambda-cyhalothrin; 100 g L^{-1} chlorantraniliprole	MS	0.2	during growing season	sunflower
	Fascord	100 g L^{-1} alpha-cypermethrin	EC	0.15	during growing season	winter wheat

Notes: SE is suspension emulsion, CC are colloidal concentrates, WS is water solution, SC are suspension concentrates, EC are emulsion concentrates, MS is microencapsulated suspension.

Table A3. Variation in toxicity of the studied soils under different experimental conditions.

Crop	Sampling Time	Abbreviation	Response of Lux-Biosensor Strains								
			***V. aquamarinus* VKPM B-11245**	***E. coli* MG1655 (pRecA-lux)**	***E. coli* MG1655 (pDinB-lux)**	***E. coli* MG1655 (pColD-lux)**	***E. coli* MG1655 (pAlkA-lux)**	***E. coli* MG1655 (pKatG-lux)**	***E. coli* MG1655 (pOxyR-lux)**	***E. coli* MG1655 (pSoxS-lux)**	***E. coli* MG1655 (pGrpE-lux)**	***E. coli* MG1655 (pFabA-lux)**
Soya	before	Gc	12.60 ± 1.20	1.24 ± 0.04	1.05 ± 0.03	1.14 ± 0.05	1.5 ± 0.18	1.16 ± 0.01	1.14 ± 0.08	1.12 ± 0.01	1.09 ± 0.03	1.05 ± 0.04
		Gf	11.50 ± 0.90	1.22 ± 0.02	1.03 ± 0.03	1.13 ± 0.20	1.41 ± 0.12	1.13 ± 0.04	1.11 ± 0.04	1.15 ± 0.07	1.09 ± 0.05	1.07 ± 0.02
		Gp	13.45 ± 2.00	1.48 ± 0.06	1.31 ± 0.02	1.19 ± 0.03	1.13 ± 0.04	1.16 ± 0.03	1.28 ± 0.05	1.32 ± 0.06	1.09 ± 0.04	1.32 ± 0.02
		Gf + p	10.05 ± 0.40	1.31 ± 0.07	1.28 ± 0.12	1.3 ± 0.10	1.16 ± 0.04	1.11 ± 0.08	1.27 ± 0.11	1.21 ± 0.09	1.12 ± 0.08	1.33 ± 0.10
	after	Gc	15.11 ± 1.10	1.19 ± 0.04	1.13 ± 0.02	1.25 ± 0.05	1.3 ± 0.02	1.17 ± 0.10	1.3 ± 0.03	1.19 ± 0.13	1.12 ± 0.05	1.17 ± 0.08
		Gf	18.40 * ± 2.50	1.25 ± 0.06	1.17 ± 0.08	1.32 ± 0.20	1.64 * ± 0.13	1.11 ± 0.04	1.38 ± 0.04	1.25 ± 0.06	1.13 ± 0.01	1.26 ± 0.11
		Gp	17.24 * ± 1.20	1.95 * ± 0.11	1.38 ± 0.07	1.26 ± 0.06	2.53 * ± 0.06	1.47 ± 0.13	1.64 * ± 0.03	1.29 ± 0.06	1.1 ± 0.05	1.76 * ± 0.19
		Gf + p	29.05 * ± 4.30	1.76 * ± 0.19	1.27 ± 0.09	1.81 * ± 0.27	2.47 * ± 0.46	1.43 ± 0.12	1.48 ± 0.12	1.35 ± 0.10	1.26 ± 0.13	1.78 * ± 0.18
Sunflower	before	Hc	15.6 ± 2.00	1.43 ± 0.05	1.28 ± 0.11	1.09 ± 0.04	1.01 ± 0.03	1.27 ± 0.05	1.12 ± 0.09	1.39 ± 0.12	1.26 ± 0.03	1.16 ± 0.01
		Hf	10.7 ± 1.50	1.37 ± 0.12	1.34 ± 0.05	1.45 ± 0.16	1.13 ± 0.04	1.18 ± 0.13	1.23 ± 0.03	1.17 ± 0.06	1.09 ± 0.07	1.28 ± 0.09
		Hp	11.2 ± 2.30	1.4 ± 0.07	1.04 ± 0.01	1.28 ± 0.06	1.02 ± 0.03	1.26 ± 0.06	1.02 ± 0.02	1.36 ± 0.12	1.05 ± 0.02	1.23 ± 0.02
		Hf + p	10.9 ± 0.80	1.39 ± 0.12	1.11 ± 0.09	1.4 ± 0.05	1.17 ± 0.05	1.06 ± 0.12	1.17 ± 0.03	1.09 ± 0.04	1.18 ± 0.06	1.17 ± 0.03
	after	Hc	17.05 ± 2.00	1.17 ± 0.01	1.34 ± 0.04	1.44 ± 0.1	1.12 ± 0.1	1.33 ± 0.02	1.26 ± 0.02	1.29 ± 0.04	1.14 ± 0.02	1.21 ± 0.08
		Hf	35.1 *1 ± 5.00	1.47 ± 0.04	1.40 ± 0.07	1.48 ± 0.09	1.94 * ± 0.31	1.36 ± 0.03	1.33 ± 0.04	1.47 ± 0.04	1.05 ± 0.06	1.79 * ± 0.06
		Hp	25.88 * ± 4.10	1.96 * ± 0.03	1.48 ± 0.03	2.11 * ± 0.07	2.59 * ± 0.07	1.57 * ± 0.04	1.28 ± 0.02	1.37 ± 0.04	1.29 ± 0.07	1.47 ± 0.07
		Hf + p	71.88 * ± 5.80	1.55 * ± 0.06	1.91 * ± 0.02	1.87 * ± 0.11	2.60 * ± 0.14	1.24 ± 0.07	1.21 ± 0.02	1.59 * ± 0.04	1.5 ± 0.04	1.65 * ± 0.06
Wheat grown after soya	before	T(g)c	6.72 ± 0.60	1.41 ± 0.02	1.15 ± 0.02	1.27 ± 0.08	1.29 ± 0.1	0.98 ± 0.01	1.35 ± 0.02	1.13 ± 0.06	1.29 ± 0.07	1.1 ± 0.05
		T(g)f	9.54 ± 0.07	1.39 ± 0.04	1.18 ± 0.18	1.49 ± 0.16	2.1 * ± 0.46	1.07 ± 0.03	1.38 ± 0.03	1.12 ± 0.15	1.26 ± 0.04	1.28 ± 0.05
		T(g)p	4.36 ± 0.04	1.41 ± 0.10	1.28 ± 0.04	1.52 * ± 0.03	1.85 * ± 0.42	1.26 ± 0.08	1.45 ± 0.04	1.21 ± 0.03	1.27 ± 0.09	1.34 ± 0.06
		T(g)f + p	8.76 ± 0.11	1.41 ± 0.06	1.19 ± 0.07	1.4 ± 0.12	2.15 * ± 0.13	1.11 ± 0.04	1.39 ± 0.05	1.15 ± 0.05	1.34 ± 0.05	1.29 ± 0.05
	after	T(g)c	15.8 * ± 1.00	1.14 ± 0.06	1.43 ± 0.06	1.31 ± 0.03	1.73 * ± 0.57	1.03 ± 0.06	1.11 ± 0.04	1.13 ± 0.06	1.07 ± 0.04	1.14 ± 0.02
		T(g)f	64.29 * ± 4.00	1.28 ± 0.05	1.58 * ± 0.06	1.58 * ± 0.07	4.28 * ± 0.13	1.1 ± 0.04	1.16 ± 0.06	2.11 * ± 0.10	1.56 * ± 0.12	1.48 ± 0.08
		T(g)p	59.87 * ± 2.00	1.23 ± 0.05	1.88 * ± 0.06	1.81 * ± 0.13	2.93 * ± 0.66	1.27 ± 0.04	1.32 ± 0.07	2.46 * ± 0.09	1.94 * ± 0.08	1.45 ± 0.18
		T(g)f + p	76.87 * ± 3.00	1.10 ± 0.03	1.58 * ± 0.08	1.64 * ± 0.11	4.88 * ± 0.92	1.16 ± 0.05	1.31 ± 0.11	2.19 * ± 0.13	1.66 * ± 0.09	1.53 * ± 0.15
Wheat grown after sunflower	before	T(h)c	19.24 * ± 0.10	1.48 ± 0.05	1.3 ± 0.05	1.49 ± 0.06	1.58 * ± 0.17	1.11 ± 0.05	1.45 ± 0.04	1.19 ± 0.04	1.37 ± 0.09	1.42 ± 0.06
		T(h)f	20.23 * ± 0.30	1.62 * ± 0.07	1.25 ± 0.06	1.54 * ± 0.12	2.17 * ± 0.19	1.19 ± 0.06	1.47 ± 0.05	1.3 ± 0.08	1.42 ± 0.10	1.63 * ± 0.06
		T(h)p	14.55 * ± 0.10	1.62 * ± 0.07	1.24 ± 0.06	1.52 * ± 0.16	1.18 ± 0.06	1.2 ± 0.11	1.32 ± 0.06	1.37 ± 0.04	1.25 ± 0.11	1.86 * ± 0.08
		T(h)f + p	35.29 * ± 0.90	1.45 ± 0.07	1.2 ± 0.09	1.78 * ± 0.14	2.05 * ± 0.16	1.17 ± 0.09	1.37 ± 0.07	1.3 ± 0.05	1.6 * ± 0.07	1.64 * ± 0.14
	after	T(h)c	20.65 * ± 2.00	1.12 ± 0.03	1.49 ± 0.08	1.5 ± 0.10	1.8 * ± 0.27	1.21 ± 0.07	1.51 * ± 0.03	1.44 ± 0.09	1.28 ± 0.08	1.34 ± 0.18
		T(h)f	86.34 * ± 6.00	1.34 ± 0.08	1.53 * ± 0.09	1.52 * ± 0.09	2.75 * ± 0.23	1.31 ± 0.06	1.54 * ± 0.09	1.63 * ± 0.11	1.29 ± 0.05	1.42 ± 0.05
		T(h)p	27.34 * ± 2.00	1.34 ± 0.03	1.71 * ± 0.02	2.08 * ± 0.12	2.5 * ± 0.57	1.28 ± 0.02	1.54 * ± 0.04	1.66 * ± 0.04	1.63 * ± 0.02	1.67 * ± 0.03
		T(h)f + p	23.8 * ± 3.00	1.04 ± 0.06	1.40 ± 0.05	1.88 * ± 0.14	3.95 * ± 0.20	1.32 ± 0.07	1.43 ± 0.10	1.88 * ± 0.09	1.51 * ± 0.06	1.37 ± 0.07

* Differences compared to the control samples are statistically significant. The values were expressed as mean ± SD. Student's *t*-test was used to compare these values. Values of p lower than 0.05 were considered significant. Each experiment was performed in triplicate and repeated on three different occasions.

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