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EFFECTS OF CHEMICALS USED IN CULTIVATION OF RAPESEED ON THE MICROORGANISMS AND THEIR ACTIVITY IN SOIL

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1. INTRODUCTION

Currently, intensive crop protection treatment consisting in application of various groups of pesticides, particularly fungicides, insecticides, and herbicides, is very widely used both in Poland and worldwide. Protection of agricultural crops based on agrochemical treatments improves crop productivity and quality. Noteworthy, plant protection agents are not yield-promoting factors; yet, they protect crops and ensure cost-effectiveness of other inputs (Golinowska 2009). Therefore, only appropriate use of chemical formulations has a stabilising effect on plant yield and ensures high yields of good-quality crops (Łozowicka and Bułatowicz 2009).

Plant protection products, commonly referred to as pesticides, are synthetic or natural substances comprising numerous chemically diverse groups of compounds applied for crop protection against e.g. pests, diseases, and weeds (Piwowar 2012). Currently, the market offers a very wide range of chemical formulations. However, the choice of an appropriate agent depends primarily on the type of cultivated crops, the condition and degree of weed infestation, severity of disease, or degree of pest infestations.

Winter rapeseed (*Brassica napus* L.) is one of the basic and most important oilseed crops grown both in Poland and in many European countries. In the recent years, the area of cultivation of this plant in the crop structure has increased markedly. This is mainly associated with the increasing demand for its seeds, which are a valuable raw material for not only the food and feed industry but also the fuel industry.

Winter rapeseed has high agrotechnology and fertilisation requirements; it also needs advanced, intensive plant protection (Mrówczyński *et al.* 2006). Pesticide-based protection of rapeseed not only comprises pest, disease, and weed control but also aims at prevention of plant lodging. Furthermore, due to uneven ripening, high susceptibility of pods to shattering, and excessive seed loss, agrochemicals are applied also during preparation of plantations to harvest in order to reduce seed loss. Hence, throughout the vegetation season, the plant is subjected to a variety of crop-protection treatments based on different groups of pesticides.

Besides the economic benefits ensured by good production results, intensive use of chemicals in the modern agriculture may pose a risk to consumers' health and lead to a number of adverse changes in the natural environment (Łozowicka and Bułatowicz 2009). Active substances contained in chemical agents are not indifferent to the soil environment either. This is related to the fact that these compounds are generally characterised by high toxicity, ability to accumulate, and persistence in the environment (Beyer and Biziuk 2007). Excessive accumulation of pesticides in soil may lead to disturbances in the abundance, composition, and activity of soil microflora; additionally, it may exert a significant effect

on processes carried out by microbes (Przybulewska and Nowak 2004ab). The degree and direction of these changes is strongly dependent on the type and dose of the preparation, the type and chemical structure of the active substance, and the physico-chemical properties of soil (Niewiadomska *et al.* 2009, Jastrzębska 2010). Introduction of chemical agents into soil, particularly those with the capacity for long-term persistence, may result in deterioration of the physico-chemical and biological soil properties. This, in turn, may lead to impairment of soil fertility and a decline in yield quality and quantity (Siwek *et al.* 2008). Therefore, constant monitoring of the effects of chemical agents on the parameters of the soil environment is indispensable (Wyszkowska and Kucharski 2004). Microbiological indicators are particularly useful in the analysis of the soil environment and assessment of not only the ecological condition of soils but also soil biological activity, fertility, and fecundity (Quemada and Menacho 2001). Determination of the abundance of soil microorganisms and enzymatic activity facilitates comprehensive identification of changes occurring in contaminated soil and estimation of the capability of contaminant biodegradation (Baćmaga *et al.* 2007).

Contamination of soil with chemical preparations is frequently caused by irrational and inadequate application thereof as well as by lack of basic knowledge of the toxicity and threats posed by improper use of such chemicals. Therefore, in order to maintain proper balance in the function of agroecosystems and ensure adequate levels of agricultural production, assessment of changes caused by chemical plant protection agents in the soil environment is essential. Moreover, monitoring of the response of biological soil indicators to application of agrochemicals is important from the point of view of environmental protection and human health and for appropriate application of these substances.

2. LITERATURE REVIEW

2.1. Characteristics, consumption, and legal regulations for application of plant protection products in Poland

Currently, increased agricultural production is associated with the use of a variety of agrochemicals, primarily plant protection agents and fertilisers, which guarantee high quantity and quality of yields (Łozowicka and Bułatowicz 2009).

In accordance with the Plant Protection Act of December 18, 2003 (Journal of Laws No. 11, item 94 and 96), plant protection products are biologically active substances or formulations containing one or more bioactive compounds. These substances are designed for the control of unwanted plant and animal organisms, decreasing the prevalence and development of many diseases, and for desiccation, defoliation, and regulation of plant growth (Kosikowska and Biziuk 2009).

In the international nomenclature, plant protection products are also called pesticides (Latin: *pestist* – pest, plague and *caedere* – kill). Colloquially, they are often referred to as biocides and agrochemicals.

The first recorded case of pesticide application was the use of tobacco infusion for aphid control in 1763. In turn, the first synthetic organic pesticide, potassium dinitroorthocresolate, was introduced in 1892. Another important step was the discovery of the DDT preparation. It was synthesized as early as in 1874, but its insecticidal properties were only detected in 1939 by Müller. DDT was first used in 1943 to combat the typhus epidemic in Naples and for control of malaria-transmitting mosquitoes in India (Kowalik 2012). The discovery of the insecticidal properties of the DDT formulation and application thereof during World War II was the beginning of the modern development of the synthesis and use of pesticides (Sikorska and Wędzisz 2009).

Currently, plant protection products constitute a rich chemically diverse group of compounds. In their composition, pesticides contain appropriately selected mixtures comprising one or several active substances. Additionally, they may contain additives such as surfactants with emulsifying properties, wetting and foaming compounds and buffering agents, as well as synergists and fillers, whose function is to facilitate distribution and penetration of the formulation (Turos-Biernacka and Walcerz 1990). The chemical structure of an active substance is one of the most important factors determining the biological activity and degradability of the pesticide. Currently, pesticide formulations are available on the market in the solid (powders, granules) and liquid form (concentrated aqueous solutions).

Given their high diversity, there are many ways of classification of pesticides. The most common classification is based on the application, mode of action, chemical structure, and toxicity of the compounds (Brzeziński and Sęńczuk 2002). According to the mode of action, the agents are divided into (Kowalik, 2012) insecticides (insect control), herbicides (weed control), fungicides (fungus control), molluscicides (slug control), nematicides (nematode control), acaricides (mite control), bactericides (bacteria control), rodenticides (rodent control), defoliants (leaf-removing substances), desiccants (substances inducing plant apex withering), attractants (substances attracting insects, rodents, and other pests), and repellents (substances repelling pests.). Another common classification of pesticides based on their chemical structure distinguishes inorganic (arsenic and fluorine insecticides) and organic (chloroorganic and phosphoroorganic compounds, carbamates, phenoxyacetic acid derivatives, and triazine derivatives) pesticides. In turn, according to their toxicity, pesticides in Poland have been divided into 5 classes (I-V). Assignment to a toxicity group is determined on the basis of the LD_{50} (Grosicka-Maciąg 2011).

Chemical plant protection agents are used in agriculture primarily to control a variety of pests and plant diseases and to control and destroy weeds. This is

confirmed by the data from the statistical yearbook, which clearly indicate that herbicides (61.2%), fungicides (23.1%), and insecticides (5.7%) were the dominant pesticides used in Poland in 2011.

Upon accession to the European Union, Poland accepted the obligation to adapt the provisions of the plant protection products to the requirements of EU Directives. The strict European Union regulations imposing new provisions concerning plant protection products resulted in a decline in the number of registered and available agrochemicals on the Polish market. According to Matyjaszczyk (2007), between May 1, 2004 and December 31, 2006, the number of withdrawn plant protection products was substantially higher than the number of newly registered agents. The author reports that during that period the number of authorized plant protection products available on the market was 84, while 155 agents were withdrawn. The largest decrease in the number of registered formulations was recorded in the group of herbicides (by 60). 562 plant protection products in 1994 and ca. 880 formulations in 2011 were accepted for use in Poland (Matyjaszczyk, 2011).

In Poland, the principles concerning introduction, trading, and application of plant protection products as well as control systems were initially regulated by the Act of 1995 on protection of crop plants and by the Act of 18 December 2003 on the protection of plants. Currently, the Act of 8 March 2013 (Journal of Laws, item 455) is in force and regulates admission of plant protection products to trading and agricultural use. The Act implements the provisions of the Directive of the European Parliament and the Council 2009/128/EC of 24 November 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. It is also an implementation of the provisions of Regulation (EC) No. 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

According to the data from the Central Statistical Office, the domestic production of pesticides in 2011 was by 1% higher than in 2010 and amounted to 29 thousand tonnes. Additionally, approximately 59 thousand tonnes of bulk plant protection products were sold for agricultural use (by 13.8% more than in 2010). In turn, the amount of purchased active substances contained in plant protection products was by 12.0% higher than in 2010. The sales structure was clearly dominated by herbicides (ca. 36 thousand tonnes in bulk product), and their total proportion in the trading of plant protection products was 61.2% (Statistical Yearbook of the CSO GUS, 2012).

In Poland, particularly before accession to the European Union, the use of pesticides remained at a relatively low level and never exceeded 2 kg of an active ingredient per hectare of arable land and orchards. However, after the accession to the EU member states, the level of chemical plant protection and use of agro-

chemicals have increased (Matyjaszczyk 2011, Falger and Jaworski 2011). The surface area of land treated with these formulations has increased as well (Zalewski 2007). Over the last few years, elevated rates of trading chemical plant protection products have been reported as well.

2.2. Rapeseed and its importance in modern agriculture

Rapeseed (*Brassica napus* ssp. *oleifera* Metzg.) is one of the basic and most important oil-protein plants cultivated both in Poland and in many European countries. In Poland, rapeseed cultivation was introduced in the 18th century (Muśnicki, 1999), as confirmed by the first statistical data concerning areas of rapeseed cultivation from 1811 (Pietruszyński 1949). Yet, it should be emphasised that cultivation of rapeseed as an industrial crop was developed in Poland only in the 60 s of the 20th century (Brzóska 2006).

Rapeseed is cultivated primarily for seeds, which are characterised by high contents of oil and protein. Depending on the variety, the seeds of the plant contain from 43% to 49% of oil and over 20% of crude protein. The high content of fat renders rapeseed a valuable raw material for production of edible oil, which has an important role in human nutrition. In turn, seeds devoid of oil are mainly used as animal feed (Rudko 2011). Moreover, oil extracted from rapeseed is used not only as food and feed, but also in the cosmetic and chemical industries (Dudek *et al.* 2011). Additionally, it can be used in energetic industry as a biocomponent for production of diesel fuel, i.e. biodiesel (Tys *et al.* 2003).

In recent years, or more precisely after Poland's accession to the European Union, there has been a demand for a systematic increase in rapeseed cultivation areas. European Union Directive No. 2003/30/EC imposes wider use of renewable energy in the national economy and obliges member states, including Poland, to increase the share of biocomponents in the fuel market structure (Klimek and Sajdak 2007). The growth of the demand for rapeseed, caused mainly by the necessity to produce biofuels (biodiesel), has stimulated interest in cultivation of this plant and in increasing the area of rapeseed cultivation in the crop structure. According to the data presented by the CSO Statistical Yearbook, the area of rapeseed and agrimony cultivation in Poland in 2010 was as much as 946 thousand ha, which was followed by a decline to 830 thousand ha and 720.3 thousand ha in 2011 and 2012, respectively. This decrease was largely caused by adverse atmospheric conditions. Yet, according to the estimates of the CSO, the cultivation area of rapeseed and agrimony (winter and spring varieties in total) increased again in 2013 and reached 846.6 thousand ha, which was by 17.5% higher than in the previous year (Statistical Yearbook of the CSO GUS, 2011, 2012, 2013).

The conditions prevailing in our country are suitable for cultivation of two forms of the plant: winter and spring varieties. However, due to its higher yields, winter rapeseed is cultivated more commonly. The spring form is cultivated on a lesser scale, mainly in the case of freezing of winter rapeseed (Adomas and Murawa 2005). In 2011, winter rapeseed accounted for as much as 93.6% of all rapeseed cultivation, whereas the spring variety constituted only 6.4% (Rosiak 2012a).

In the recent years, rapeseed production in Poland has become the fastest developing branch of plant production (Klugmann-Radziemska *et al.* 2010). Currently, Poland is one of the biggest producers, processors, and exporters of rapeseed in Europe. Since 2007, Poland has ranked third after Germany and France in rapeseed production. It should be emphasised that Poland is also an important producer of rapeseed oil and rapeseed meal in the European Union (Rosiak 2012b).

2.3. Chemical protection of winter rapeseed plantations

The main objective in rapeseed cultivation is to obtain a stable and the greatest possible yield of seeds with high technological value for oil industry. This goal can be mainly achieved through the use of appropriate agrotechnology based on basic yield-enhancement and yield-protection procedures. In the agrotechnology of the species, not only rotation, tillage, or seeding techniques but also proper fertilisation and plant protection are essential (Adamiak and Adamiak 2010, Wójtowicz and Jajor 2010, Jaskulski and Jaskulska 2012). Moreover, growers of this crop plant should be focused on obtaining the highest seed yield through maximisation of yield per unit area and minimisation of harvest and storage losses (Bartkowiak-Broda *et al.* 2005).

Winter rapeseed represents crops that require adequate, high-level, and intensive protection against weeds, diseases, and pests throughout the vegetation season. The investigations conducted by Łozowicka and Bułatowicz (2009) demonstrate that winter rapeseed plantations receive more intensive chemical protection treatment than cereals. During the vegetation season, plantations of this species in south-eastern Poland are subjected to 7 protection procedures based on pesticides, whereas wheat receives 4 and oats merely 1.5 treatments. According to Mrówczyński *et al.* (2006), appropriate protection is particularly important for this plant species, as it reduces the risk of yield losses. Furthermore, Muśnicki *et al.* (1995) emphasise that abandonment of rapeseed protection not only leads to reduction of the amount of seeds but also affects their development and chemical composition.

One of the fundamental factors influencing the profitability of winter rapeseed production is protection against weeds (Mrówczyński *et al.* 2006), especially given the fact that already in the initial developmental stage the plant is exposed to strong competition from weeds (Ciesielska *et al.* 2009). Particularly disadvanta-

geous is the presence of self-seeded cereals, cleavers, couch grass, and odourless grass in winter rapeseed plantations (Praczyk 2005). Besides, weed infestation of rapeseed plantations leads to seed loss as well as development of diseases and pest infestations (Mrówczyński 2003). In general, yield losses caused by abandonment of weed control reach even 40% in comparison with plantations receiving full chemical treatment (Tys *et al.* 2003). A method for reduction of excessive weed infestation is application of e.g. preventive measures such as selection of an appropriate locality of cultivation, sowing at an optimum time, careful use of agrotechnical treatments, and use of dressed seeds free of weed seeds (Mrówczyński *et al.* 2006). Yet, it should be emphasised that the use of appropriate agrotechnical treatments does not fully eliminate weeds, but only facilitates protection of plantations. Currently, the most effective way to control the competitive effects of weeds is to use a chemical method based on herbicides (Ciesielska *et al.* 2009). Selection of the type and dose of chemicals corresponding to the condition and degree of weed infestation of rapeseed plantations as well as application of treatment at a proper time ensure effective protection and achievement of the most desirable results (Franek and Rola 2002, Mrówczyński *et al.* 2006). According to Franek and Rola (2002), elimination of weed infestation of rapeseed is more efficient when the agents applied combine two biologically active substances, as these usually exhibit a broader spectrum of weed-killing activity. In turn, Rola *et al.* (2004) argue that lack of diversity and rotation in the use of herbicides may lead to weed compensation and development of resistance. Products applied to the soil in rapeseed plantations are usually amide derivatives such as dimethachlor, metazachlor, propachlor, napropamide, propyzamide, and dinitroaniline derivatives (trifluralin). In turn, after the emergence of dicotyledonous weed seedlings, formulations containing such active substances as chlopyralid, metazachlor, propachlor, and propyzamide are applied (Praczyk and Bączkowska 2008, Rudko 2011). Graminicides are used to eliminate infestations of winter rapeseed plantations with monocotyledonous weed species (Badowski and Kucharski 2010).

Another very important factor ensuring high rapeseed yields is adequate pathogen control. Throughout the vegetation season, the plants may be infected by a variety of pathogenic fungal species, which reduce not only the quantity but also the quality of yields (Korbas *et al.* 2010). It has been estimated that the average losses caused by pathogens range from 15 to 20%, but they may be higher at a more severe infection of the field (Wałkowski *et al.* 2006) and reach even 50-60% (Gwiazdowski and Korbas 2006). One of the most severe diseases infecting rapeseed is stem canker. In Poland, losses caused by the disease are estimated at even do 60% (Gwiazdowski and Korbas 2006). Additionally, rapeseed plantations are relatively often infected by such diseases as seedling canker, pod spot, noble rot, downy mildew, powdery mildew, and clubroot (Gwiazdowski and Korbas

2006). Factors contributing to the occurrence and severity of fungal diseases primarily include high relative humidity and temperature (Korbas *et al.* 2010). The use of appropriate agricultural treatments as well as cultivation of varieties with greater resistance helps reduce the occurrence of rapeseed diseases. However, when the prevalence of pathogens is too high, fungicidal treatments need to be employed (Jajor *et al.* 2008, Jajor *et al.* 2010, Jajor *et al.* 2012). The use of fungicides in rapeseed protection is quite popular due to the efficiency of their action and the possibility of obtaining high yields (Korbas 2002). Chemical protection involves spraying rapeseed in the flowering phase with triazole and imidazole fungicides (Pruszyński 2009). The most commonly used fungicides are triazole formulations (Korbas *et al.* 2010) containing tebuconazole and metconazole, as besides the fungicidal effect, these substances exhibit growth-regulating properties (Cieślicki and Toboła 2007).

Winter rapeseed plantations are also severely damaged by pest insect infestations (Tys *et al.* 2003). Yield losses caused by the presence of many pest species are estimated to be in the range between 10 and 50% and, in some cases, they may lead to total destruction of the plantation (Mrówczyński *et al.* 2007). The most serious pests causing the most detrimental damage to rapeseed plantations include the turnip sawfly, rape stem weevil, cabbage seedstalk, *Meligethes aeneus*, brassica pod midge, and cabbage aphid (Mrów-czyński *et al.* 2006). Currently, the chemical method is the primary and most effective method used for rapeseed pest control. However, it should be remembered that the insecticidal chemical treatment should be applied in an environmentally safe manner and in accordance with the instructions placed on the formulation label. In pest control procedures, an alternating use of insecticides from different chemical groups is recommended. Compliance with these recommendations can reduce the degree of pest resistance, which may otherwise develop if only one formulation is applied. Moreover, while choosing this type of plantation protection not only monitoring and thresholds for economic harm should be taken into account but also a suitable plant protection product should be chosen (Mrówczyński *et al.* 2006).

Cultivation of winter rapeseed requires advanced skills and experience from growers not only during the cultivation itself but also while preparing the plantation to harvest (Tys *et al.* 2003). Application of appropriate procedures in the final phase of rapeseed maturation is particularly important, since the plant is among the group of plants posing a number of difficulties before and during harvesting (Tys *et al.* 2003). The difficulties arising during harvest are primarily associated with the uneven ripening of rapeseed and relatively high susceptibility to pod shattering and seed loss (Markowski *et al.* 2003), which on average may reach 10-15% or, in more adverse conditions, 50% of the biological yield (Bączkiewicz *et al.* 2001). Therefore, proper preparation of the plantation for harvesting involves supporting the process of natural plant maturation, protection of pods against ex-

cessive shattering, and equalisation of canopy maturation by accelerated chemical desiccation of weeds and rapeseed plants (Wałkowski *et al.* 2007).

Acceleration and equalisation of canopy maturation as well as reduction of rapeseed losses can be obtained by application of the desiccation treatment based on various chemical preparations called desiccants (Markowski *et al.* 2003, Choszcz *et al.* 2005). The action of desiccants mainly involves chemical dehydration of green plant parts through inhibition of respiration and chlorophyll degradation. This leads to reduction in the moisture content and acceleration of plant desiccation. However, the condition of achieving the desirable effects does not only comprise an appropriate choice of chemical agents, but also proper timing of application thereof, given the fact that desiccation preparations should be applied in rapeseed plantations only at full physiological maturity and development, i.e. after reaching harvest maturity. Furthermore, precise determination of the time of spraying eliminates the possibility of yield reduction, as premature use of desiccants may result in lowered seed quality and a decrease in their quantity even to 7 dt ha $^{-1}$ (Tys 2007).

Chemical formulations designed for pre-harvest desiccation of winter rapeseed are most often contact herbicides containing such active substances as glyphosate and diquat. Formulations composed of these substances are also used to eliminate weed infestation of crop fields. Desiccation of winter rapeseed plantations can be achieved with the use of such chemical formulations as Roundup 360 SL (Jaskulski and Jaskulska 2012, Pits *et al.* 2008), Reglone 200 SL, and Avans Premium 360 SL (Choszcz *et al.* 2005).

The preparations Roundup 360 SL and Avans Premium 360 SL contain an active substance called glyphosate. After entering the plant, the substance primarily causes inhibition of the enzyme 5-enolpyruvylshikimate- 3-phos-phate synthetase (EPSP), thereby impeding formation of aromatic amino acids (phenylalanine, tyrosine, and tryptophan). Additionally, glyphosate causes disturbances in the stomatal movement and reduction of the respiration process, which impairs the photosynthetic process in plants and results in dehydration of plant tissues (Pieniążek *et al.* 2003). In turn, ions of diquat, the active substance in the Reglone 200 SL preparation, cause oxidative stress in plants and inhibition of photosynthesis. The mechanism of action of bipyridyl herbicides, represented by the Reglone formulation, involves capturing electrons from the photosynthetic electron transport chain in PS1, resulting in generation of reactive oxygen species (ROS) in cells (Popova *et al.* 2003). The activity of these two active substances is used for preparation of winter rapeseed plantations for harvesting, since they induce rapid and uniform drying out of plants in the field. In their investigations of the impact of the Avans Premium 360 SL and Reglone 200 SL preparations on harvest seed loss, Choszcz *et al.* (2005) found that application of these chemical agents increased the yield. Furthermore, the authors showed that the average seed losses during combine-

harvester-aided swathing were over 16% lower after application of the Reglone 200 SL preparation.

Additionally, formulations enhancing pod resistance are used in winter rapeseed plantations to prevent excessive seed shedding. One of such preparations is the chemical agent Spodnam 555 SC containing 555 g dm^{-3} of a bioactive substance called di-1-P-menthene. Spodnam 555 SC is a chemical agent from the group of local-systemic regulators of plant growth and development. After application thereof onto rapeseed plants, the preparation forms a waterproof and permanent "protective" layer on pods. Therefore, the formulation is recommended for use in a vegetation season characterised by adverse weather conditions. Investigations carried out by Szot and Tys (1991) showed that application of the Spodnam preparation in winter rapeseed plantations increased shatter-resistance of pods in the range from 10 to 45%. Similarly, the research conducted by Rudko (1995) confirmed the effectiveness of this agent in reduction of seed shedding in the plant.

2.4. Transformation of pesticides in soil

Intensive protective treatment of crop fields based on various groups of such as pesticides, fungicides, insecticides, and herbicides is still an important and predominant element of the modern agricultural practice (Fliesbach and Mader 2004). However, due to intensive and long-term application of chemical preparations to crop plantations, there is a possibility of dispersal thereof outside the original area of use. Therefore, agrochemicals used in agricultural practice are one of the primary factors of contamination of the natural environment and soil in particular (Błaszak and Nowak 2006).

After entering the substrate, active substances contained in pesticides can undergo various transformations leading to degradation under the impact of many environmental factors (Andreu and Picó 2004, Lewandowska 2008). According to Adamczewski and Banaszak (2000), the mechanism of pesticide action after penetration of soil involves the following processes:

- adsorption by soil particles,
- washing off by rainfall,
- oxidation,
- disintegration under UV light,
- chemical decomposition,
- soil erosion,
- decomposition by microorganisms,
- uptake by plants and animals.

Depletion of pesticides from soil is mainly related to the decomposition processes, which lead to gradual degradation of active substances, first to metabolites and next to simple substances (Sadowski and Sekutowski 2008). However, it should

be borne in mind that intermediate metabolites may also exhibit toxicity, which is sometimes substantially higher than that of end products (Jastrzębska 2010). In soil, or more precisely in the soil sorption complex, there may appear immobilisation of both residues of native substances and those formed by decomposition of metabolites (Lewandowska 2008).

Decomposition of pesticides, including plant protection agents, may take place in the soil under the effect of various physico-chemical and biological processes. However, Gregorczyk and Swarcewicz (2012) emphasise the dominant role of the microbial factor in decomposition of biocides in the soil complex. Degradation of organic compounds in the environment by microorganisms and enzymes is called biodegradation. This process may lead to complete degradation of these substances to carbon dioxide, water, and organic salts, i.e. compounds that occur naturally in the environment (Jeanot 1994).

According to Wrzosek *et al.* (2009), besides hydrolysis and photolysis, soil microbial biodegradation is one of the major processes leading to decomposition of the compounds. However, Reid *et al.* (2000) claim that contact between the contaminant and the microorganism is indispensable for the biodegradation process to occur; moreover, the substance should be biodegradable and organisms must have the capability of biodegradation thereof.

Biodegradation of plant protection products by microorganisms is the result of their metabolic processes. Enzymes secreted by soil microbes catalyse the degradation of compounds contained in plant protection agents (Zemleduch and Tomaszewska 2007). Microorganisms induce biotransformation of pesticides and xenobiotics in soil through changes in the structure of their components, thereby contributing to a decline in their toxicity (Gianfreda and Rao 2004). Furthermore, besides chemical and microbial degradation, an important role in the depletion of the pesticide active substance is played by migration into the soil profile (Sadowski and Sekutowski 2008), sorption, and uptake by crop plants (Kucharski and Domaradzki 2009, Wrzosek *et al.* 2009).

The rate of degradation of pesticides in soil depends on many factors including (Kucharski and Sadowski 2006, Arias-Estevez *et al.* 2008, Siwek *et al.* 2008):

- the chemical composition and structure of the active substance,
- the type of the pesticide,
- the dose applied,
- the type and properties of soil (organic matter content, pH, temperature,
- humidity),
- climatic conditions (temperature, precipitation),
- the intensity of ultraviolet radiation.

Undoubtedly, the most important factor determining degradation of pesticides is the chemical structure of the active substance. In ionic compounds, anionic bonds are considerably more susceptible to degradation, whereas aromatic bonds

exhibit greater stability than aliphatic bonds (Wrzosek *et al.* 2009). An important role is also played by the composition of the pesticide itself. In her analysis of the Roundup chemical formulation containing the active substance glyphosate carried out in controlled conditions, Skoczko (2013) showed that the half-life of the formulation is generally from 45 to 60 days. In special conditions, the period may even extend up to 360 days. However, in the natural environment conditions, the Roundup formulation may persist in soil of from 1.5 to 2 years. In turn, its active ingredient, i.e. glyphosate, is degraded in the environment within five days (Baylis 2000). The higher toxicity of preparations than the toxicity of the active substance contained therein (glyphosate) is caused by the presence of additional surfactant and carrier substances in the formulations (Skoczko 2013).

The rate of pesticide decomposition in soil also depends on the quantity of pesticides applied per crop plant. According to Swarcewicz and Gregorczyk (2012), application of a mixture of agents exerts an effect on the rate of degradation of the active substance in herbicides. In their examinations performed in controlled conditions and aimed at determination of the interaction between linuron (phenylurea herbicide) applied in a liquid mixture with other plant protection products (fungicide and insecticide), the authors found that linuron in a mixture was degraded at a substantially lower rate than the herbicide alone. This was also confirmed by other investigations of Swarcewicz and Gregorczyk (2012), in which a mixture of pendimethalin, thiamethoxam, and mancozeb significantly inhibited the rate of degradation of the herbicide.

The dose of plant protection agents applied can also determine the length of degradation thereof in soil. Włodarczyk *et al.* (2007) demonstrated that increased doses of an oxyacetamide herbicide (flufenacet) in field conditions extend half-life of the agent. This was also confirmed by laboratory analyses performed by Gupta and Gajbhiye (2002). In contrast, the results obtained by Rouchaud *et al.* (2001) did not confirm this relationship, as they showed that an increased dose of an oxyacetamide derivative in field conditions did not affect the stability of the herbicide in the soil.

The dynamics and direction of transformations of active substances in soil can also be related to the soil type and properties. Soil variability, e.g. in terms of the organic carbon content, granulometric composition, and pH, may exert an effect on both degradation of herbicides and their residues in soil and plants (Sadowski *et al.* 2012). According to Kucharski and Sadowski (2009) and Sadowski *et al.* (2012), the course and rate of degradation of the herbicide active substance is determined by soil texture and the content of organic matter. In soil with the highest content of carbon and clay fraction in its structure, the rate of degradation of active substances of herbicides is the highest. Elevated levels of soil moisture also enhance the rate of degradation of these compounds (Kucharski and Sadowski

2006). Soil pH also has an impact on the degradation and sorption of pesticides (Sheng *et al.* 2005). Low soil pH generally increases the persistence of pesticides in soil (Biziuk 2001).

The process of sorption of pesticides in soil determines the transport, bioavailability, and degradation of these chemicals. It is assumed that the process of pesticide sorption is significantly influenced by the type and content of organic matter and clay minerals as well as moisture and soil pH (Oleszczuk 2007, Muszyński 2011). It should be noted, however, that sorption limits the availability of contaminants to microorganisms, hence the lower rate of degradation thereof. Additionally, the rate of physicochemical degradation is reduced and the rate of translocation of contaminants in soil is considerably slower (Oleszczuk 2007).

2.5. The use of microbial indicators in the analysis of the soil environment

Protection of agricultural crops based on treatment with various groups of pesticides is still a basic and dominant protection method employed in agricultural practice with the aim of producing efficient yields. However, intense, often incompetent, and long-term use of a variety of chemicals in agriculture may lead to excessive accumulation thereof and environmental pollution (Błaszak and Nowak 2006). The common use of pesticides characterised by high toxicity, ability to accumulate, and persistence in the environment can cause adverse ecological effects (Beyer and Biziuk 2007).

Regardless of the method of application on crop fields, pesticides ultimately enter the soil. Therefore, organisms colonising this environment are usually the first to be exposed to the toxic action of various substances contained in pesticide formulations. Introduction of biocides into soil has a significant effect on soil microorganisms, reflected in changes in their abundance, biochemical activity, and diversity (Cycoń and Kaczyńska 2004). Therefore, it is necessary to recognise and monitor the negative effects of these substances on the soil microflora and the microbially driven processes.

Many parameters can be used in assessment of the effect of anthropogenic factors on the soil environment; they contribute to expanding knowledge and understanding of soil processes operating under their impact. Observation of biocide-induced changes in soil exclusively regarding its chemical and physical properties is incomplete and insufficient. Moreover, these indicators change at a low rate, therefore, observation of significant changes requires a considerably longer time. Hence, assessment, monitoring, and characterisation of soil treated with pesticides is often based on biological indicators, such as the microbial and enzyme activity of soil. These parameters are more sensitive than the chemical and physical soil properties; they respond to all changes in the soil environment more readily, and de-

scribe soil condition more adequately (Smith and Papendick 1993, Pascual *et al.* 2000, Masciandaro *et al.* 2004). Additionally, they are largely modified by environmental and anthropogenic factors and can be potential indicators of ecological stress (Jezierska-Tys and Frąc 2008, Nannipieri *et al.* 2003).

One of the methods for determination of the microbial activity in soil treated with pesticides is evaluation of the abundance of various groups of soil microorganisms and measurement of the intensity of respiration, ammonification and nitrification processes, and enzymatic activity (Kucharski *et al.* 2009b, Niewiadomska *et al.* 2009, Przybulewska and Nowak 2004a, Wyszkowska 2004). Pesticides also affect microbial communities occurring in the environment by reducing their biodiversity. Therefore, beside soil microbiological activity, the functional activity of the diversity of microbial communities should be monitored and assessed on the basis of analysis of the community-level physiological profiles (CLPP) (Ros *et al.* 2006).

2.5.1. The impact of pesticides on selected microbiological properties of soil

Soil is a natural habitat for living and development of a variety of organisms, including microorganisms. Literature data indicate that soil microorganisms constitute ca. 85% of the biomass of all organisms that are present in this environment (Martyniuk 2011). Owing to their specific biochemical capacities, soil microorganisms are one of the key factors determining soil quality and fertility.

Microorganisms play a key role in many basic processes occurring in soil, contributing to proper soil function and plant growth (Paul and Clark 1996). According to Martyniuk *et al.* (2007), their high activity in soil indicates not only good soil quality but also proper function of processes carried out by soil organisms. One of the most important roles of soil microbes is decomposition and mineralisation of the organic matter as well as enrichment of soil in nitrogen, and growth-promoting, antibiotic, and biologically active substances (Corstanje and Reddy 2006, Janvier *et al.* 2007).

Among the many functions that microorganisms serve, their role in degradation and detoxification of a variety of soil contaminants (Chowdhury *et al.* 2008), including chemical plant protection products (pesticides), should be emphasised. However, according to Niewiadomska *et al.* (2005), not all microbial species exhibit the ability to degrade these substances. According to Onet (2009), detoxification catabolism and metabolism occur when soil microorganisms are capable of utilising organic compounds contained in pesticides as a carbon and energy source. As reported by De Schrijver and De Mot (1999), fungi, bacteria, and actinomycetes have the highest capacity of transformation and degradation of pesticides. The highest xenobiotic degrading abilities are ascribed to fungi due to their greater resistance to adverse envi-

ronmental conditions (Niewiadomska *et al.* 2005). Similarly, Gianfreda and Rao (2004) report that fungi are the main microorganisms inducing biotransformation of pesticides and xenobiotics in soil through changing the structure of their components and thereby contributing to loss of toxicity. Next, transformed pesticides are released into soil and are subjected to further bacterial degradation.

The intensity of degradation of pesticides by soil microorganisms is largely dependent of the content of organic matter as well as climatic conditions, e.g. humidity and appropriate temperature. According to many authors (Wu *et al.* 1997, Zhang *et al.* 2006, Briceño *et al.* 2007), an increased soil content of organic matter, which provides nutrients for microorganisms, leads to an increase in microbial biomass and, hence, enhanced intensity of pesticide degradation. Therefore, Navarro *et al.* (2004) report that degradation of pesticides by soil microorganisms occurs most efficiently in the surface layers of soil due to the higher content of organic matter.

Soil microorganisms are not only involved in degradation of pesticides but are also influenced by these biocides. Hence, they are widely used as biological indicators of soil quality due to their high sensitivity to various agricultural practices (Bossio *et al.* 2005, Epelde *et al.* 2008). In turn, evaluation of changes in the abundance of microorganisms is one of the parameters demonstrating the side effects of the pesticide action on the soil environment (Baćmaga *et al.* 2007, Baćmaga *et al.* 2006). According to Mandić *et al.* (2005), while monitoring soil contamination with pesticides, it is important to assess their effect on fungal abundance, since this microbial group is able to restore its normal metabolism rapidly. Therefore, fungi are regarded as important indicators of the negative effects exerted by pesticides in the entire agroecosystem.

After penetration of soil, active substances contained in chemical preparations can disturb the development and activity of soil microorganisms (Chowdhury *et al.* 2008, Lo 2010). According to Błaszak and Przybulewska (2011), this is mainly related to the fact that not all microorganisms tolerate the presence of pesticides in soil. Application of agrochemicals frequently leads to quantitative and qualitative changes in the microflora and alters important microbiological processes.

The available literature concerning the wide issue of soil contamination by chemical plant protection products presents investigations of both the adverse and beneficial effects of chemical formulations on microbial abundance (Rola and Kieloch 2001, Wyszkowska 2002, Kucharski and Wyszkowska 2008). However, the extent and type of such changes depends on a variety of factors, including the type and dose of the active substance, the timing of application of the chemicals (Jastrzębska 2010, Jezierska-Tys and Rutkowska 2013), the physico-chemical properties of soil, weather conditions, and the sensitivity of the microflora to the pesticides applied (Przybulewska and Nowak 2004a).

Wyszkowska (2004) showed that the abundance of microorganisms in soil depended on the dose of the chemical formulation used. In her investigations of the impact of the Triflurotox 250 EC herbicide, the author found that introduction of high doses of this preparation into the soil led to reduction of the mean abundance of bacteria, actinomycetes, and fungi. In turn, it was found in another study (Błaszak and Nowak 2006) that fungi were susceptible to the effects of the herbicide to a much lesser extent. The research of Wyszkowska and Kucharski (2004) concerning the effect of the Chwastox Trio 540 SL herbicide on various groups of soil microorganisms confirmed the inhibitory effect of higher doses of this preparation on cellulolytic, oligotrophic, and copiotrophic bacteria and their endospores and on bacteria from the genus *Azotobacte*r and fungi. The authors also demonstrated that application of an optimal dose caused changes in the abundance of soil microorganisms. In their investigations of the effect of a field dose of the Funaben T fungicide and Pivot 100SL herbicide on the abundance of *Rhizobium* and *Azotobacter* bacteria and total numbers of bacteria, fungi, and actinomycetes, Niewiadomska *et al.* (2005) found both a stimulatory and inhibitory action of these preparations on the development of the analysed soil microflora. The authors also reported that the fungicide rather than the herbicide applied exhibited substantially higher toxicity against the analysed microbial groups. Moreover, the investigations conducted by the authors showed that fungi and actinomycetes were the least sensitive of all the analysed microbial groups to the action of the tested formulations. In turn, Ahmed and Ahmad (2006) demonstrated that insecticides also exerted a negative effect on the total abundance of bacteria. In their investigations, Kucharski *et al.* (2006) reported substantial reduction in the abundance of fungi caused by application of a higher dose of herbicides. Research conducted by Pampulha *et al.* (2007) showed that ammonium glufosinate exerted both an inhibitory and stimulating effect on soil microorganisms and the type of this activity depended on the concentration of the substance and incubation time. Vig *et al.* (2008) proved that application of insecticides at doses recommended by the manufacturer might have a negative effect on the number of bacteria from the *Azotobacter* genus. According to Krzyśko-Łupicka (2008), the field dose of the Roundup herbicide can also significantly decrease the abundance of diazotrophs in soil even as early as 30 days of application thereof. Similarly, Jastrzębska (2010) demonstrated that the use of such plant protection products as Unix 75 WG, Nomolt 150 SC, and Durban 480 EC could contribute to changes in bacterial growth in soil. Furthermore, the author emphasised that the effect of these formulations on the examined microorganisms was determined by their type, dose, and date of analyses. Investigation results obtained by Baćmaga *et al.* (2010) demonstrated that active substances (phenmedipham, desmedipham, and etofumesat) contained in a commercial herbicide Akord 180 OF inhibited microbial prolifera-

tion in soil. The authors observed a particular inhibitory effect of the tested formulation on bacteria from the genus *Azotobacter*. Research conducted by Błaszak and Przybulewska (2011) showed that active substances such as atrazine and simazine exerted an inhibitory effect not only on the abundance of bacteria but also soil-inhabiting fungi.

However, available literature provides reports in which no negative effect of a pesticide on the abundance of selected microbial groups was found. As explained by the authors, pesticides may serve as nutrients for microorganisms, since the organic substances contained therein may be used as a source of energy, carbon, or nitrogen (Kaszubiak and Durska 2000, Michalcewicz 2001). The ability of some microorganisms to utilise these compounds may stimulate their proliferation, thereby increasing their abundance and enhance their activity in soil (Das and Mukherjee 2000). In her investigations of the effect of chemical plant protection formulations (herbicides, fungicides, insecticides) on soil microorganisms, Michalcewicz (1995) demonstrated that a 10-fold increase in the dose of the tested chemicals applied resulted in increased abundance of bacteria in soil. Stimulation of the development of soil microorganisms through application of various chemical substances were also reported by Kaszubiak and Durska (2000). The authors found that the fungicide Oxafun T used in the experiment caused an increase in the analysed abundance. Increased growth of bacteria and actinomycetes caused by application of the herbicide Brominal in low doses was also reported by Omar and Abdel-Sater (2001). Investigations carried out by Araujo *et al.* (2003) evidenced that application of herbicides containing glyphosate as an active substance led to increasing abundance of both fungi and actinomycetes in soil. Similarly, Baćmaga *et al.* (2010) reported stimulation of proliferation of bacteria and actinomycetes as well as oligotrophic and copiotrophic bacteria by application of herbicides into soil. In their investigations of the effect of three different pesticides (insecticide, herbicide, and fungicide) on the abundance of heterotrophic bacteria as well as fungi and bacteria involved in nitrogen metabolism, Cycoń and Piotrowska-Seget (2007) showed stimulation of fungal growth promoted by application of herbicides with linuron as an active substance.

2.5.2. The effect of pesticides on selected soil biochemical properties

Pesticides, particularly those characterised by long-term retention and long half-life, pose a serious threat to the natural soil environment, as they can lead to disturbances in the biochemical processes occurring in the soil ecosystem (Kucharski *et al.* 2006, Das *et al.* 2003, Seghers *et al.* 2004, Wyszkowska 2002, Chowdhury *et al.* 2008). However, it should be emphasised that biocides applied in accordance with manufacturers' recommendations do not always exert a negative impact on

soil enzymatic activity (Wyszkowska 2004), although when used in excess, they may lead to disturbances in the biological balance in soils (Wyszkowska and Kucharski 2004).

A parameter that reflects the state of the soil environment is the enzymatic activity, which can be regarded as an indicator of total microbiological activity (Alkorta *et al.* 2003, Nannipieri *et al.* 2003, Uziak and Steinbrich 2005). According to many authors (Jezierska-Tys and Frąc 2008, Wang *et al.* 2009), high activity of soil microorganisms demonstrates not only good soil quality but also normal function of processes carried out by soil organisms. Furthermore, the level of soil enzymatic activity is a sensitive indicator of soil fertility and productivity (Russel 2005). The enzymatic activity is also an early and sensitive indicator of the degree of soil degradation; therefore, it can be used for assessment of the effects of natural and anthropogenic factors on the quality of soil (Kumar *et al.* 2013).

The activity of enzymes in soil is influenced by a number of factors, including the organic matter content, moisture content, pH, temperature, soil type, and vegetation cover (Zahir *et al.* 2001), which are largely dependent on the soil management system (Bielińska and Mocek 2003, Natywa *et al.* 2010). Additionally, agricultural practices such as fertilisation (Barabasz *et al.* 2002) and application of plant protection products may modify soil biological activity (Wyszkowska and Kucharski 2004).

Soil enzymes respond rapidly to all changes occurring in the soil environment. Therefore, analysis of their quality not only provides information about the biochemical processes taking place in soil (González *et al.* 2007) but also allows observation of the direction of metabolic transformations induced by biocides (Baćmaga *et al.* 2007). Measurement of the activity of soil enzymes is often employed for diagnosis of soils subjected to pesticide pressure (Nasreen *et al.* 2012, Tejada 2009). According to Mocek-Płóciniak (2010), assessment of soil quality is primarily based on determination of the activity of four enzymes, i.e. dehydrogenases, phosphatases, proteases, and ureases. However, dehydrogenases and phosphatases are most commonly analysed in soil since, unlike other enzymes, they respond considerably faster to stress factors. According to some authors (Bielińska 2002, Nowak *et al.* 2003), phosphatase activity may be more useful for assessment of changes in the soil environment than dehydrogenase activity. Phosphatases catalyse hydrolysis of phosphorus bonds and hence they are used for assessment of the potential rate of mineralisation of these compounds in soil (Januszek 1999). The activity of these enzymes is positively correlated with respiration and microbial biomass, thereby serving as an efficient indicator of the activity and population size of soil microorganisms. According to Bielińska (2005), phosphatase activity is a good indicator of changes in the soil environment induced by e.g. agrochemicals.

Available literature shows that investigation on the effect of biocides on soil enzyme activity is still a major interest of many researchers. The common use of chemical plant protection products and their adverse side effects on the environment reaffirms the need for monitoring all pesticide-induced changes in soil. Many authors (Kucharski *et al.* 2009a, Niewiadomska *et al.* 2009, Rasool and Reshi 2010, Wyszkowska and Kucharski 2004) have studied the impact of biocides on the activity of soil enzymes. Their investigations sometimes demonstrated highly diverse effects of chemicals included in pesticide formulations on soil enzymatic activity. Pesticides applied to the soil can have both a stimulatory or inhibitory effect. The differences observed in the activity of individual enzymes may be related to their varied response to different groups of pesticides (Rasool and Reshi 2010). The intensity and direction of enzyme activity changes depends on the type, dose, properties, and decomposition rate of the agent applied as well as the properties of the soil environment and the analysed enzyme (Krzyśko-Łupicka 2008, Onet 2009).

Investigations conducted by Niewiadomska *et al.* (2009) proved that even optimal pesticide doses induced changes in the soil enzymatic activity. The authors examined the effect of optimal doses of the herbicide Fox 480 (active substance - bifenox) and a seed dressing fungicide (active substance flutriafol) on the activity of dehydrogenases and acid phosphatase. The analyses proved that the plant protection formulations used were not neutral to the activity of the examined enzymes. Application of the herbicide to soil in the initial investigation period stimulated the activity of dehydrogenases, whereas the fungicide significantly reduced dehydrogenase activity. The plant protection products applied exerted an impact on the activity of acid phosphatase as well; they increased its levels in the initial period of the investigations and significantly reduced them in the final stage. The authors consider the type of the plant protection product as a differentiating parameter. In turn, Sebiomo *et al.* (2011) investigated the effect of four herbicides, i.e. atrazine, primeextra, paraquat, and glyphosate, on the activity of soil dehydrogenases. The results obtained demonstrated a decline in the enzyme activity induced by the tested herbicides. According to Rasool and Reshi (2010), fungicides caused considerable modifications in soil biological activity. The authors tested the impact of various concentrations of the fungicide Mancozeb on the activity of dehydrogenases, alkaline phosphatase, protease, urease, amidase, and asparaginase. They observed that the activity of the analysed enzymes was influenced by the dose of the formulation applied, incubation time, and their mutual interactions. Contamination of soil with the fungicide generally inhibited urease and asparaginase activity and increased the activity of dehydrogenases, protease, and amidase. In turn, the alkaline phosphatase activity exhibited a variable response to the different concentrations of the tested pesticide. For instance, application of a pesticide dose that was ten-fold

higher than the recommended one (60 kg ha^{-1}) increased the soil phosphatase activity by approx. 41% after 14-day incubation. The research conducted by Wyszkowska and Kucharski (2004) showed that application of the herbicide Chwastox Trio 540 SL resulted in not only increased activities of dehydrogenases and urease but also decreased the activities of acid and alkaline phosphatases. Additionally, the authors observed that the activity of the analysed enzymes was generally lower in soil treated with high herbicide doses than in the control soil. In turn, in their investigations, Kucharski et al. (2009a) reported a significant reduction in the activity of not only urease but also acid and alkaline phosphatases after application of the optimal dose of the Harpun 500 S.C. formulation into soil. Inhibition of urease activity was also reported by Wang *et al.* (2009), however, after soil treatment with copper-based fungicides. The authors explain the inhibitory effect of pesticides on the activity of this enzyme with soil acidification. In their analyses of the impact of glyphosate, atrazine, carbaryl, and paraquat on the activity of invertase, urease, and acid and alkaline phosphatases, Sannino and Gianfreda (2001) observed a clear effect of these substances on soil activity. Introduction of the insecticide into the soil usually resulted in an increase in the activity of urease and phosphatase in soil. Noteworthy, the impact of pesticides on the enzymes analysed was also determined by the organic carbon content and soil type. Nasreen *et al.* (2012) analysed protease and urease activity in loamy black soil treated with two different insecticides (endosulfan and profenofos) applied in field doses and significantly increased (1.0, 2.5, 5.0, 7.5, 10.0 kg ha^{-1}) doses. The investigations demonstrated that the effect of the tested pesticides on the enzymes was primarily determined by the dose of the preparation and incubation time. The use of insecticides in the doses of 2.5 and 5.0 kg ha^{-1} had a positive effect on the activity of both enzymes analysed. Moreover, the authors observed the highest increase in their activity, compared with the control, during the first 20 days after the insecticide treatment. However, within longer incubation periods, i.e. after 30 and 40 days, the activity of both protease and urease declined, but the differences were insignificant in relation to the control. Similarly, an increase in urease activity persisting for the first 10 days of the experiment was observed by Mohiddin *et al.* (2011) in soil treated with acephate and imipdacloprid insecticides. Nevertheless, along the experimental time, i.e. after 30 and 40 incubation days, the authors reported a significant decline in the activity.

The parameters used in assessment of the impact of pesticides on the function of the soil ecosystem and soil quality include processes associated with nitrogen transformations in soil, such as ammonification and nitrification. These processes are closely related to the nitrogen cycle in nature and play an important role in the primary production of biomass in various ecosystems and in transformation of

bio-elements (Barabasz 1992). Due to their role and the fact that they are carried out by microorganisms that are highly sensitive to the action of agrochemicals, these processes are regarded as important indicators of soil biological activity. Therefore, measurement of the intensity of these processes can be used for estimation of the side effects of biocides on the soil environment (Kucharski and Wyszkowska 2008).

In their investigations of the impact of various herbicides (Harpun 500 S.C., Fawory 300 SL, Akord 180 OF, Mocarz 75 WG) on the ammonification process in soil, Kucharski *et al.* (2009b) demonstrated that the course of the process is largely determined by the type and dose of the herbicide, type of the ammonified compound, and the length of the experiment. Additionally, the authors observed that all biocides used in the experiment exerted a significant effect on the course of the process. Among all the herbicides tested, Mocarz 75 WG, containing active substances tritosulfuron and dicamba, was found to be the most potent inhibitor of ammonification. The other herbicides did not have such a distinct effect on the amount of ammonified nitrogen. In turn, Krzyśko-Łupicka (2008) reported that introduction of the Roundup herbicide, containing the active substance glyphosate, into soil enhanced the intensity of the ammonification process in soil.

The impact of pesticides on the course of the nitrification process in soil was investigated by Przybulewska and Nowak (2004b). The authors observed that contamination of heavy and light soil with the Lontrel 300 SL herbicide and the Champion WP fungicide exerted an effect on nitrification intensity, typically inhibiting the process. However, the authors emphasised that the degree of the induced changes depended on the type and dose of the formulation, duration of action, incubation temperature, and soil type. Contamination of heavy soil with the fungicide, particularly in the dose of 10 mg kg^{-1} in the initial period of the experiment, increased the intensity of the nitrification process. However, the stimulating effect of the Champion WP formulation decreased with time and, in the final experimental stage, the nitrogen content was at a similar level to that in the control soil. In contrast, the herbicide applied in the heavy soil reduced nitrification intensity. Lang and Cai (2009) assessed the effect of field and increased doses of the chlorothalonil and carbendazim fungicides on the nitrification process in 6 types of soil. The authors found that the type and dose of the fungicide applied as well as soil properties determined the course of the analysed process. Additionally, they observed that a field dose of chlorothalonil practically did not disturb nitrification and had a slight inhibitory effect in only one of the six types of soil analysed. In turn, application of higher doses significantly reduced nitrification in all the soils. Even when applied in higher doses, carbendazim did not exert any effect on intensification of the process. Cycoń and Kaczyńska (2004)

reported that pesticides increased nitrification intensity in soil as well. In their investigations of the impact of herbicides, fungicides, and insecticides on the process, the authors observed increased nitrification intensity in soil induced only by the linuron herbicide and the diazinon insecticide. Similarly, the investigations conducted by Cycoń et al. (2006) demonstrated a significant increase in the nitrification level in soil treated with tebuconazoles and l-cyhalothrin.

2.6. Summary

The presented literature review indicates that chemical formulations commonly used in agricultural practices, i.e. chemical plant protection products, deserve special attention, given their diverse effects on the biological life of soils. Excessive use of chemical plant protection products often leads to disturbances in the abundance and biodiversity of microorganisms and biochemical processes occurring in the soil environment. It should be emphasised that investigations of the impact of chemicals on the biological life of soils is crucial from the ecological, ecotoxicological, and agricultural point of view, as they provide information about the risks associated with inappropriate use of agrochemicals. Moreover, the investigations provide complementary knowledge of the response of soil microorganisms to introduction of common chemical formulations into crop cultivation.

3. AIM OF STUDY

The aim of this study was to investigate the effects of various chemicals used in cultivation of winter rapeseed on the abundance of some groups of microorganisms and their biochemical activity in soil.

4. RESEARCH SCOPE AND METHODOLOGY

4.1. Characteristics of chemicals used in the experiments

4.1.1. Characteristics of the Roundup 360 SL, Reglone 200 SL, and Basta 150 SL formulations used in field experiment 1 and in the pot experiment

In field experiment 1, the Roundup 360 SL, Reglone 200 SL, and Basta 150 SL chemical formulations were used, whereas Avans Premium 360 SL, Spodnam 555 SC, and Caramba 60 SL were used in field experiment 2. Chemicals Roundup 360 SL and Reglone 200 SL were tested in the pot experiment. The basic information about the tested formulations is derived from the description of their characteristics provided by the manufacturers.

- Roundup 360 SL is produced by Monsanto Europe S.A. The formulation has a form of a concentrate used for preparation of aqueous solutions. Glyphosate (aminophosphonate compound) is the biologically active substance contained therein. The active substance in the herbicide is present in the form of the isopropylamine salt in an amount of 360 g dm^{-3} of the formulation. The dose recommended by the manufacturer is 3 dm^3 ha^{-1} in 100-150 dm^3 of water or 4 dm^3 ha^{-1} in 200-300 dm^3 of water. In winter rapeseed plantations, the chemical agent should be used at seed moisture below 30%.
- Reglone 200 SL is produced by Syngenta Crop Protection AG. The formulation has a form of a concentrate used for preparation of aqueous solutions. The content of diquat ion (9,10-dihydro-8a,10a-diazonia phenanthrene cation), i.e. its bioactive substance, is 200 g dm^{-3} of the chemical agent. The technological dose recommended by the manufacturer is 2-3 dm^3 ha^{-1}. In winter rapeseed plantations, it is applied when 70% of pods on the main shoots are yellowish-celadon, and the seeds are dark-brown in the apical part of the shoot and black in the lower part of the shoot. Plants should be harvested 4-10 days after application of the herbicide.
- Basta 150 SL from Bayer CropScience S.A. The formulation has a form of a concentrate used for preparation of aqueous solutions. The content of glufosinate ammonium (aminophosphonate compound), i.e. the biologically active substance contained therein is 150 g dm^{-3} of the chemical agent. The technological dose recommended by the manufacturer is 2.5 dm^3 ha^{-1}. In winter rapeseed plantations, the formulation is applied when pods are green in the apical part and change colour from light green to straw-yellow. Plants should be harvested 8-15 days after application of the chemical agent.

4.1.2. Characteristics of the Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL formulations used in field experiment 2

- Avans Premium 360 SL is manufactured by Syngenta Limited. The formulation has a form of a concentrate used for preparation of aqueous solutions. Glyphosate (aminophosphate acid compound) is the biologically active substance contained therein. The biologically active substance contained in the herbicide is ammonium salt in an amount of 360 g dm^3 of the agent in a unique technology known as System 4 Technology. The technological dose recommended by the manufacturer is 3 dm^3 ha^{-1} in 100-150 dm^3 of water or 4 dm^3 ha^{-1} in 200-300 dm^3 water. In winter rapeseed plantations, the herbicide should be applied in the technological maturity phase when a majority of pods turn yellow, the seeds are formed, and the moisture level is below

30%, 2/3 of pods on the main shoots are yellow-celadon, and 70% of seeds are red-brown or brown. Rapeseed harvesting with a combine harvester is recommended to be carried out 14-21 days after the treatment in order to obtain full dryness of the seeds.

- Spodnam 555 SC is produced by Mandops Limited. The formulation has a form of a concentrate used for preparation of aqueous solutions and is designed for application by ground and agro-aviation apparatus. Di-1-P-menthene is the biologically active substance contained therein in an amount of 555 g dm^{-3}. The technological dose recommended by the manufacturer for two-step harvesting is 0.6 dm^3 ha^{-1} in the first stage and 0.75 dm^3 ha^{-1} in the second stage. In the case of one-step harvest, the recommended dose is 1.2 dm^3 ha^{-1} without desiccation and 0.6 dm^3 ha^{-1} with desiccation. During the two-step rapeseed harvest, the formulation is applied twice. The first treatment is conducted 2-3 weeks before swathing when the pods turn light green, are flexible, and crack slightly without seed release when bent in a V-shape. The second treatment is applied 24 hours after swathing. In the one-step harvesting method without desiccation, the herbicide should be applied when a majority of the pods are greenish-yellow, flexible, and crack slightly without seed ejection when bent in a V-shape. In the treatment with desiccation, the pods are still flexible, and crack slightly releasing single seeds when bent in a V-shape. Desiccation should then be delayed by 7 days after the traditional term.
- Caramba 60 SL is produced by BASF Agro B.V. Arnhem – the Netherlands. The fungicide is used as prophylaxis or at the first disease symptoms in winter rapeseed cultivation. The formulation has a form of a concentrate used for preparation of aqueous solutions with systemic activity and has a preventive, intervention, and detrimental effect in the control of winter and spring cereals as well as winter and spring rapeseed in fungal diseases. Metconazole (triazole compound) is the biologically active substance contained therein in an amount of 60 g dm^{-3}. Application of the agent in spring (from the early phase of shoot growth to the green-bud phase) contributes to shortening of the stem length and limits plant lodging. The technological dose recommended by the manufacturer is 1 dm^3 ha^{-1}.

4.2. Characteristics of the experimental models

The investigations on the impact of the chemical agents (Roundup 360 SL, Reglone 200 SL, Basta 150 SL, Avans Premium 360 SL, Spodnam 555 SC, Caramba 60 SL) applied for desiccation of rapeseed on the microbiological and biochemical soil properties were conducted in two field experiments and a pot experiment.

- **Location of the field experiments**

The field experiments (referred to as field experiment 1 and field experiment 2) were set up in 2010-2012 in the Experimental Station for Variety Assessment in Głębokie, Kujawsko-Pomorskie Province (52 °38'41"N, 18° 26'18"E).

- **Soil**

The experiments were established on soil from the class of black earths (Mollic Gleysols) formed from sandy loam, which contained 65% of the sand fraction (2-0.5 mm), 23% of the loam fraction (0.05-0.002 mm), and 12% of floating particles (<0.002 mm). The characteristics of the soil used in the experiments are provided in Table 1.

Table 1. Chemical characteristics of soil used in the experiment

Parameter	Unit	Value
Reaction	pH_{KCl}	6.1
C_{org}	g kg^{-1} d.m.	9.8
Total N	g kg^{-1} d.m.	1.3
C:N		7.5
Total P	g kg^{-1} d.m.	0.7
K	g kg^{-1} d.m.	0.1
Zn	mg kg^{-1} d.m.	34.0
Cd	mg kg^{-1} d.m.	0.15
Cu	mg kg^{-1} d.m.	10.8
Pb	mg kg^{-1} d.m.	9.6
Ni	mg kg^{-1} d.m.	7.5
Cr	mg kg^{-1} d.m.	14.4
Hg	mg kg^{-1} d.m.	0.1

4.2.1. Characteristics of the model of field experiment 1

The investigations on the impact of the Roundup 360 SL, Reglone 200 SL, and Basta 150 SL on the microbiological and biochemical soil activity were conducted in a three-year field experiment. It was set up with the split-block method on soil from the class of black earths (Mollic Gleysols) formed from sandy loam. The basic characteristics of the soil are summarised in subsection 4.2.

The experimental model comprised the following objects:

- with the chemical agent **Roundup 360 SL**:

1a – control soil without herbicide,
2a – soil treated with the optimal (4 $dm^3 ha^{-1}$) dose of Roundup 360 SL,
3a – soil treated with a 10% higher (4.4 $dm^3 ha^{-1}$) dose of Roundup 360 SL,

- with the chemical agent **Reglone 200 SL:**

1b – control soil without herbicide;
2b – soil treated with the optimal (2 $dm^3 ha^{-1}$) dose of Reglone 200 SL,
3b – soil treated with a 10% higher (2.2 $dm^3 ha^{-1}$) dose of Reglone 200 SL,

- with the chemical agent **Basta 150 SL**

1b – control soil without herbicide,
2b – soil treated with the optimal (2.5 $dm^3 ha^{-1}$) dose of Basta 150 SL,
3b – soil treated with a 10% higher (2.75 $dm^3 ha^{-1}$) dose of Basta 150 SL.

All the objects were sown with "Californium" cv. winter rapeseed in the first experimental year, with sugar beet in the second year, and with barley in the third year. The choice of the plant (rapeseed) in the first year of the field experiment was determined by the cultivation of the plant, since it requires very high expenditure for pest control and, hence, application and introduction a variety of chemical agents, e.g. desiccants, into cultivation. In the first experimental year, technological doses recommended by the manufacturer and 10% higher doses of Roundup 360 SL, Reglone 200 SL oraz Basta 150 SL were applied. The application of the chemicals was performed using a backpack sprayer. The spraying with Roundup 360 SL, Reglone 200 SL, and Basta 150 SL was done 7, 5, and 8 days, respectively, before the winter rapeseed harvest. Basic agricultural practices in accordance with the recommendations for cultivation of the plant were applied in all the objects. Additionally, in order to meet the nutritional requirements of rapeseed plants, a uniform scheme of fertilisation: N – 160 kg ha^{-1}, P – 27.50 kg ha^{-1}, K – 96.30 kg ha^{-1}, S – 36 kg ha^{-1}, was applied. The surface area of the harvest plots was 12 m^2.

Soil was sampled for analyses eight times during the three years of the experiment from the arable layer of each plot, i.e.:

- I term – after winter rapeseed harvest (early August 2010),
- II term – after 2 months of the experimental period (October 2010),
- III term – after 10 months of the experimental period (May 2011),
- IV term – after 12 months of the experimental period (August 2011),
- V term – after 14 months of the experimental period (October 2011),
- VI term – after 22 months of the experimental period (May 2012),
- VII term – after 24 months of the experimental period (August 2012),
- VIII term – after 26 months of the experimental period (October 2012).

In addition, in each experimental year, i.e. 2010, 2011, and 2012, the contents of organic carbon and total nitrogen in the soil samples were determined after the microbiological and biochemical analyses. The chemical analyses were performed at the Central Laboratory of Chemical Analysis (GLACH) IUNG in Puławy.

4.2.2. Characteristics of the model of field experiment 2

The investigations on the impact of the Avans Premium 360 SL, Spodnam 555 SC, and Caramba 60 SL on the microbiological and biochemical soil activity were conducted in a three-year field experiment (2010-2012) as well. The field experiment was set up with the split-block method on soil from the class of black earths (Mollic Gleysols) formed from sandy loam. The basic characteristics of the soil are summarised in subsection 4.2.

The experimental model comprised the following objects:
1 – control soil without chemicals,
2 – soil treated with the optimal (3 $dm^3 ha^{-1}$) dose of Avans Premium 360 SL,
3 – soil treated with the optimal (1.2 $dm^3 ha^{-1}$) dose of Spodnam 555 SC,
4 – soil treated with the optimal (1 $dm^3 ha^{-1}$) dose of Caramba 60 SL.

The experimental objects were sown with "Californium" cv. winter rapeseed. The application of the chemicals was performed using a backpack sprayer. In the case of Caramba 60 SL, the spraying was performed in the initial shoot growth phase, and the Avans Premium 360 SL and Spodnam 555 SC formulations were applied 20 and 5 days, respectively, before the winter rapeseed harvest. Basic agricultural practices and a uniform scheme of fertilisation were applied in the experiment. In all the experimental objects, the plants were supplemented with the following nutrient doses: N – 140 kg ha^{-1}, P – 18.33 kg ha^{-1}, K – 58.12 kg ha^{-1}, S – 36 kg ha^{-1}. The surface area of the harvest plots was 12 m^2 as well.

Soil was sampled for analyses eight times during the three years of the experiment from the arable layer of each plot, i.e. immediately after winter rapeseed harvesting (early August) and next after 2, 10, 12, 14, 22, 24, and 26 months of the experiment (the description of the sampling dates are explained in subsection 4.2.1. in the methodology section).

Additionally, in each experimental year, i.e. 2010, 2011 and 2012 the contents of organic carbon and total nitrogen in the soil samples were determined after the microbiological and biochemical analyses.

The chemical analyses were performed at the Central Laboratory of Chemical Analysis (GLACH) IUNG in Puławy.

4.2.3. Characteristics of the model of the pot experiment

The aim of the three-factor pot experiment was to investigate the impact of the Roundup 360 SL and Reglone 200 SL agents as well as the dose and duration of the experiment on soil microbial and biochemical activity.

The pot experiment was established on soil from the class of black earths formed from sandy loam. The basic soil characteristics are summarised in subsection 4.2. The three-factor experiment was conducted in pots filled with 4 kg of soil in triplicate using the complete randomization method. Soil was sampled from the Ap layer and kept in the laboratory in order to determine its biological balance. After 7 days, the soil was thoroughly mixed and sieved through a 2-mm mesh. Before establishment of the experiment, the soil was mixed with mineral fertilisers and with Roundup 360 SL or Reglone 200 SL in the respective objects. Both herbicides were applied to the soil in the form of aqueous emulsion in doses recommended by the manufacturer (optimum doses) and 10-, 50-, and 100-fold increased doses. Soil without addition of the herbicide was the control. Additionally, mineral fertilisation in identical doses of N – 187 kg ha^{-1}, P – 24.45 kg ha^{-1}, K – 77.46 kg ha^{-1}, and S – 48 kg $ha^{-1}$was applied. Next, the soil was placed in 4.2 dm^3 pots. Spring rapeseed was cultivated.

The experiment comprised the following objects:

- with the chemical agent **Roundup 360 SL**:
  1a – control soil without chemicals;
  2a – soil supplemented with the optimal dose of Roundup 360 SL (1.3 mm^3 kg^{-1}),
  3a – soil supplemented with a 10-fold increased dose of Roundup 360 SL (13 $mm^3 kg^{-1}$),
  4a – soil supplemented with a 50-fold increased dose of Roundup 360 SL (65 $mm^3 kg^{-1}$),
  5a – soil supplemented with a 100-fold increased dose of Roundup 360 SL (130 $mm^3 kg^{-1}$).
- with the chemical agent **Reglone 200 SL:**
  1b – control soil without chemicals,
  2b – soil supplemented with the optimal dose of Reglone 200 SL SL (0.6 mm^3 kg^{-1}),
  3b – soil supplemented with a 10-fold increased dose of Reglone 200 SL (6 $mm^3 kg^{-1}$),
  4b – soil supplemented with a 50-fold increased dose of Reglone 200 SL (30 $mm^3 kg^{-1}$),
  5b – soil supplemented with a 1000-fold increased dose of Reglone 200 SL (60 $mm^3 kg^{-1}$).

Additionally, 60% total water capacity was maintained in the soil throughout the experiment. The incubation was carried out at a temperature of ca. 20°C for 200 days in controlled humidity conditions. Microbiological and biochemical analyses were performed after 25, 50, and 200 days of the experiment. The contents of organic carbon and total nitrogen were determined in the soil samples at the end of the experiment. The chemical analyses were performed at the Central Laboratory of Chemical Analysis (GLACH) IUNG in Puławy.

4.2.4. Preparation of the material for microbiological and biochemical analyses

100 g of soil from the pot experiment were sampled from all the pots and mixed thoroughly. In field experiments soil samples for the analyses were taken from the arable soil layer of each plot. 20-30 combined soil samples were taken from each experimental treatment and their total mass was equal to 6 kg. Soil samples were transported in plastic bags with thermal insulation inserts at low temperature. In the laboratory the soil material was thoroughly mixed, dried, and passed through a sieve with 2-mm mesh. Soil subsamples for the microbiological and biochemical analyses were taken from the soil material. Until the completion of all analyses, the prepared soil samples were stored at temperature of 4°C.

4.3. Soil analyses

The microbiological and biochemical analyses were performed in the pot experiment and field experiments 1 and 2. Chemical analyses of the soils were carried out in the final stage of the field (1 and 2) and pot experiments.

4.3.1. Microbial analysis

Microbial analysis of soil samples included: determination of the total number of bacteria by the plate method on a substrate with the soil solution (Trolldenier 1995) and of the total numbers of fungi by the Martin method (1950). The determination of the numbers of proteolytic bacteria and fungi was carried out by the plate method on a substrate with gelatine (Trolldenier 1995).

4.3.2. Biochemical analysis

Biochemical analysis of soil samples included: The determination of dehydrogenase activity was done according to the Thalmann (1968) method modified by Alef and Nannipieri (1995). The measurement of protease activity was made using the Ladd and Butler (1972) method modified by Alef and Nannipieri (1995). Urease activity was determined by the Zantua and Bremner (1975) modified method, while acid and alkaline phosphatase activity was measured by the Tabatabai and Bremner (1969) method. The rate of the process of ammonification was determined on the basis of the content

of NH_4^+ ions, with the Nessler method (Nowosielski 1981). The intensity of the process of nitrification was determined on the basis of the content of NO_3^-, with the brucine method (Nowosielski 1981). Respiratory activity of the soil was assayed with the method of respiration induction through the addition of substrate (glucose) to the soil, according to Rühling and Tyler (1973).

4.3.3. Chemical analyses

Chemical analyses included determination of the content of organic C by the Tiurin method; total N by the Kjeldahl method; total P spectrophotometrically; K by atomic emission spectrometry; Zn, Cd, Cu, Pb, Ni, Cr, Hg by atomic absorption spectrometry. The soil pH in 1M KCL was determinated potentiometrically.

4.4. Statistical analyses

The results obtained were processed statistically using the analysis of variance (ANOVA). The least significant differences were calculated with the Tukey test at significance level of $\alpha = 0.05$. To demonstrate the existence of interrelationships between the microorganisms and their biochemical activity in the soil subjected to the effects herbicides, the analysis of correlation was performed. The statistical analyses of the results were performed with the use of the program STATISTICA 7.1. (StatSoft 2005).

5. INVESTIGATION RESULTS

5.1. Field experiment nr 1 – comparison of the effect of Roundup 360 SL, Reglone 200 SL and Basta 150 SL herbicides on the abundance of selected groups of microorganisms and phosphatases activity in soil

5.1.1. The effect of Roundup 360 SL and Reglone 200 SL herbicides on soil microorganisms

5.1.1.1. Total bacterial abundance

The results concerning the total bacterial abundance in the soil are presented in Figures 1, 2, and 3. The bacterial abundance depended on the type of the chemical agent applied as well as the dose and the duration of its action.

The results obtained showed that, after application of the optimum dose of the Roundup 360 SL preparation, the most intense bacterial growth in the soil was noted immediately after the winter rapeseed harvest (stage I) and in analysis stage VII. In turn, compared with the control soil, significantly lower bacterial abundance was found in stages II, III, V, and VIII. In the other experimental stages, bacterial abundance was comparable to the values obtained in the control soil.

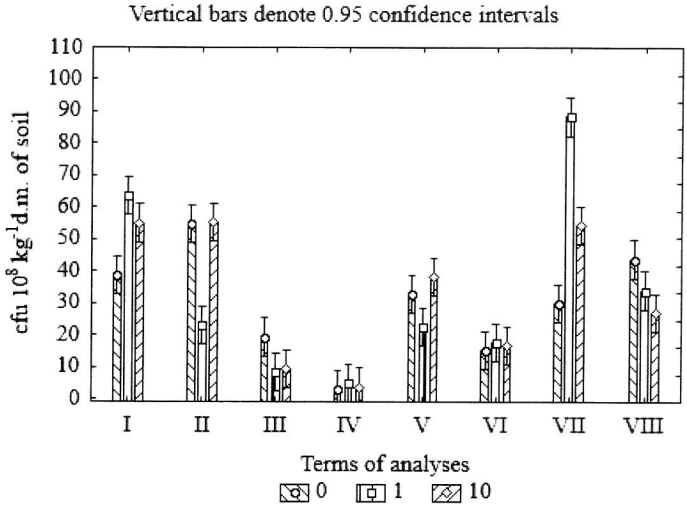

Fig. 1. Temporary bacteria numbers in soil contaminated with herbicide Roundup 360 SL. Explanations: 0 – control soil without herbicide addition; 1 – soil + Roundup 360 SL at the optimum dose (4 dm^3 ha^{-1}); 10 – soil + Roundup 360 SL at the 10% higher dose (4.4 dm^3 ha^{-1})

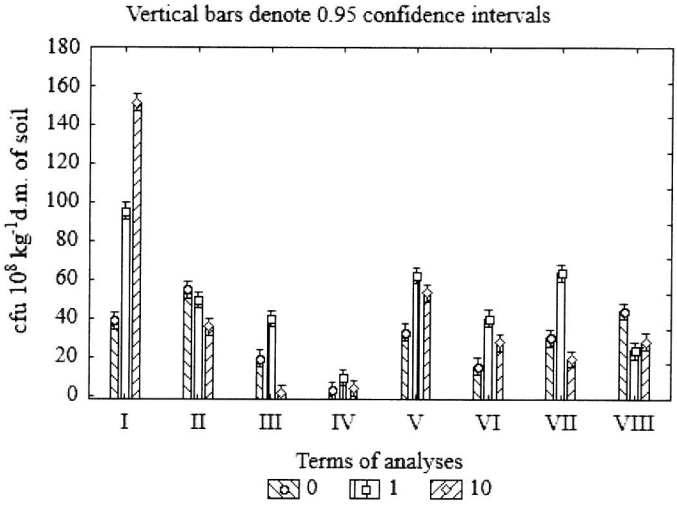

Fig. 2. Temporary bacteria numbers in soil contaminated with herbicide Reglone 200 SL. Explanations: 0 – control soil without herbicide addition; 1 – soil + Reglone 200 SL at the optimum dose (2 dm^3 ha^{-1}); 10 – soil + Reglone 200 SL at the 10% higher dose (2.2 dm^3 ha^{-1})

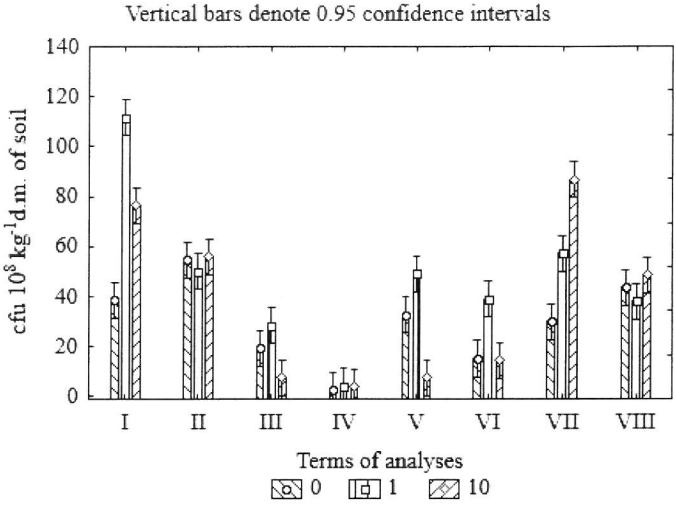

Fig. 3. Temporary bacteria numbers in soil contaminated with herbicide Basta 150 SL.
Explanations: 0 – control soil without herbicide addition; 1 – soil + Basta 150 SL at the optimum dose (2.5 dm^3 ha^{-1}); 10 – soil + Basta 150 SL at the 10% higher dose (2.75 dm^3 ha^{-1})

The increased Roundup 360 SL herbicide dose applied in the experiment stimulated proliferation of the investigated microbial group in the initial stage of the analyses and stages V and VII. In the other stages, the bacterial abundance persisted at the level of control values or was significantly lower.

After application of the optimum dose of the Reglone 200 SL herbicide, lower abundance than that in the control was found only in stage II and VIII of the analysis, whereas the bacterial abundance increased in the other stages of the analysis.

The increased dose of Reglone 200 SL stimulated bacterial growth in the initial stage of the analyses and in stages V and VI. A significant decline in bacterial abundance was noted in analysis stages II, III, VII, and VIII. Importantly, application of both herbicides (Roundup 360 SL and Reglone 200 SL) in doses recommended by the manufacturer and in 10% higher doses resulted in a significant increase in proliferation of the investigated microbial group in the initial stage of the analyses and a significant decrease in the total bacterial abundance in the final stage, i.e. stage VIII.

The total bacterial abundance in soil contaminated with the Basta 150 SL formulation is presented in Figure 3.

The chemical agent applied in both the optimum and increased doses induced a significant increase in the abundance in the initial stage of the experiment and after 24 months (stage VII). A stimulatory effect of the optimum dose of the prep-

aration was also noted after 10, 14, and 22 months of the experiment (i.e. stages III, V, and VI). The increased dose induced significant inhibition of bacterial growth in stages III and V of the experiment.

The statistical analysis of the mean values of the results obtained during the three-year experiment (Fig. 4) revealed that the optimum and increased doses of the Roundup 360 SL herbicide did not cause significant disturbances in bacterial abundance, compared with the control values. The optimum and increased doses of Reglone 200 SL and Basta 150 SL stimulated the investigated abundance.

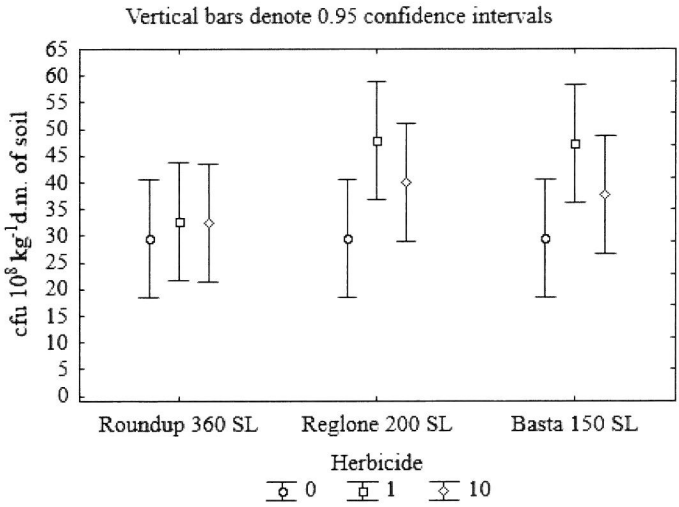

Fig. 4. Means numbers of bacteria in soil contaminated with herbicides Roundup 360 SL, Reglone 200 SL and Basta 150 SL. Explanations: 0 – control soil without herbicide addition; 1 – soil + herbicide at the optimum dose ((Roundup 360 SL: 4 dm^3 ha^{-1}, Reglone 200 SL: 2 dm^3 ha^{-1}, Basta 150 SL: 2.5 dm^3 ha^{-1}); 10 – soil + herbicide at the 10% higher dose (Roundup 360 SL: 4.4 dm^3 ha^{-1}, Reglone 200 SL: 2.2 dm^3 ha^{-1}, Basta 150 SL: 2.75 dm^3 ha^{-1})

5.1.1.2. Total fungal abundance

The impact of the Roundup 360 SL, Reglone 200 SL and Basta 150 SL herbicides on the total fungal abundance in the examined soil is presented in Figures 5, 6, and 7.

The investigations showed that the fungal abundance in soil was influenced by the type and dose of the herbicide applied as well as the duration of the experiment. Compared with the control object, the optimum and increased doses of the Roundup 360 SL herbicide significantly increased the total fungal abundance only in stage VII of the analyses.

In the other stages of the analyses, the formulation caused a decline in the total fungal abundance in comparison with the control soil.

The optimum and 10% higher doses of the Reglone 200 SL herbicide generally caused significant inhibition of fungal growth, which is presented in Figure 6.

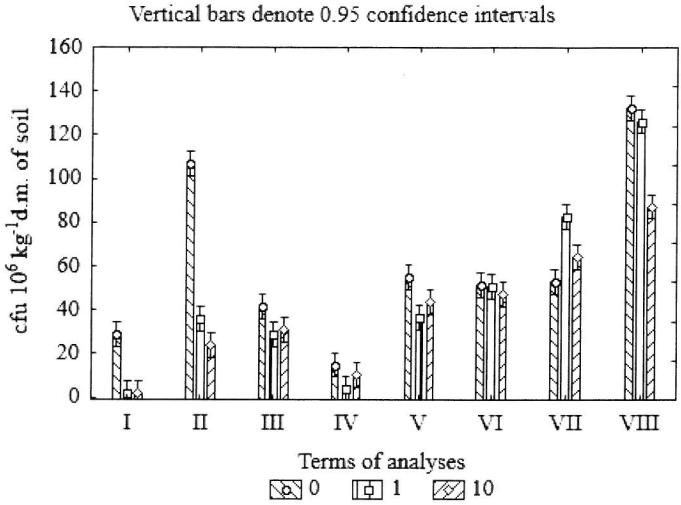

Fig. 5. Temporary fungi numbers in soil contaminated with Roundup 360 SL. Explanations as in Figure 1

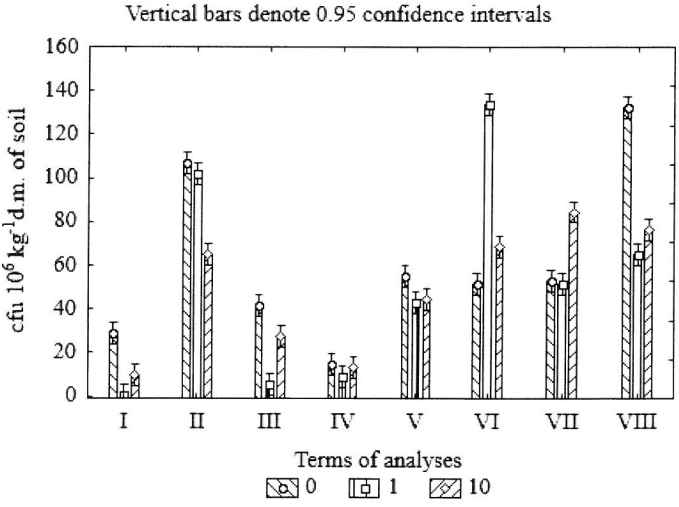

Fig. 6. Temporary fungi nubmers in soil contaminated with Reglone 200 SL. Explanations as in Figure 2

A stimulatory impact of both Reglone 200 SL doses on bacterial abundance was found only after 22 months of the experiment (stage VI), and a significant increase in fungal growth was observed in stage VII after application of the increased dose of the formulation.

In the investigations, the Basta 150 SL preparation was also applied in the optimum and 10% higher doses. The study results presented in Figure 7 indicate that the optimum dose of the Basta 150 SL herbicide induced an insignificant increase in fungal abundance in the initial stage of the experiment (immediately the after winter rapeseed harvest) and in stages III, IV, and V as well as a significant increase in analysis stages VI and VII. The herbicide dose increased by 10% caused significant inhibition of fungal growth (stimulation only in stage VII) during the experiment.

The analysis of variance performed for the three-year experiment showed that the Roundup 360 SL and Reglone 200 SL herbicides inhibited the total fungal abundance, even when applied in the technological dose. Interestingly, fungal growth in the soil was inhibited more substantially by the increased dose of both formulations. The Basta 150 SL herbicide applied in the optimum dose did not cause changes in fungal abundance, whereas the increase dose applied to the soil induced insignificant inhibition of microbial proliferation (Fig. 8).

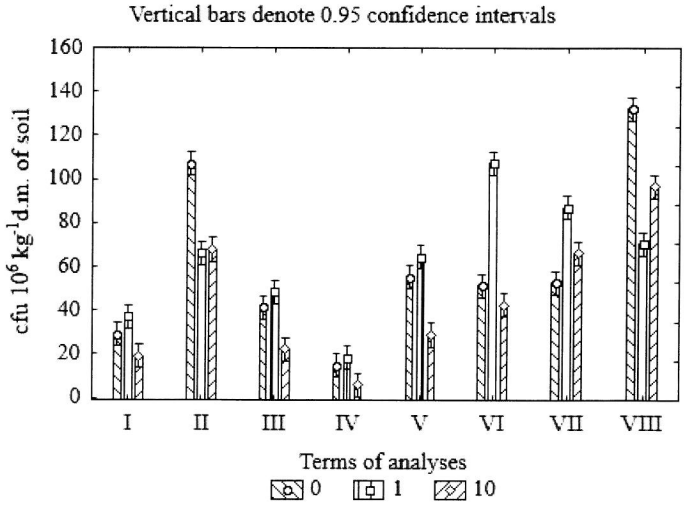

Fig. 7. Temporary fungi numbers in soil contaminated with Basta 150 SL. Explanations as in Figure 3

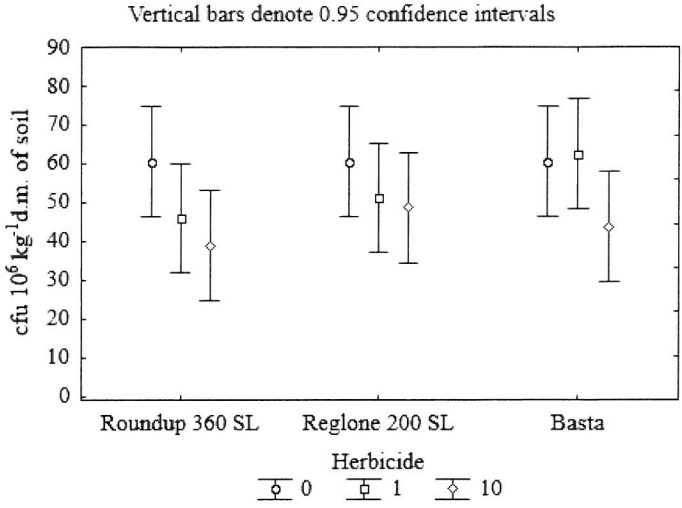

Fig. 8. Means numbers of fungi in soil contaminated with herbicides Roundup 360 SL, Reglone 200 SL and Basta 150 SL. Explanations as in Figure 4

5.1.1.3. Abundance of bacteria with proteolytic activity

The effect of the Roundup 360 SL, Reglone 200 SL and Basta 150 SL herbicides on the abundance of bacteria with proteolytic abilities is presented in Figures 9, 10, and 11.

The Roundup 360 SL herbicide applied in the technological and 10% higher doses generally decreased the abundance of the investigated microorganisms throughout the experimental period. Compared with the control soil, stimulation of proliferation of the examined microbial group was reported immediately after the winter rapeseed harvest (insignificant) and in the 24th month (stage VII) of the analyses (significant stimulation). Furthermore, Roundup 360 SL applied in the optimum and increased doses significantly inhibited the growth of proteolytic bacteria in stages III, VI, and VIII.

The optimum and 10% higher doses of the Reglone 200 SL herbicide did not induce significant changes in the abundance of proteolytic bacteria immediately after the winter rapeseed harvest, i.e. in stage I of the analyses and after 2, 10, and 12 months of the experiment (stages II, III, and IV).

In turn, significant inhibition of the growth of this bacterial group was noted in stage VI after application of the optimum dose, and both doses of the herbicide in the final stage of the experiment, i.e. in the 26th month of the analyses (stage

VIII). The analysis of the other experiment stages showed that the increased dose of this herbicide caused significant stimulation in stage V.

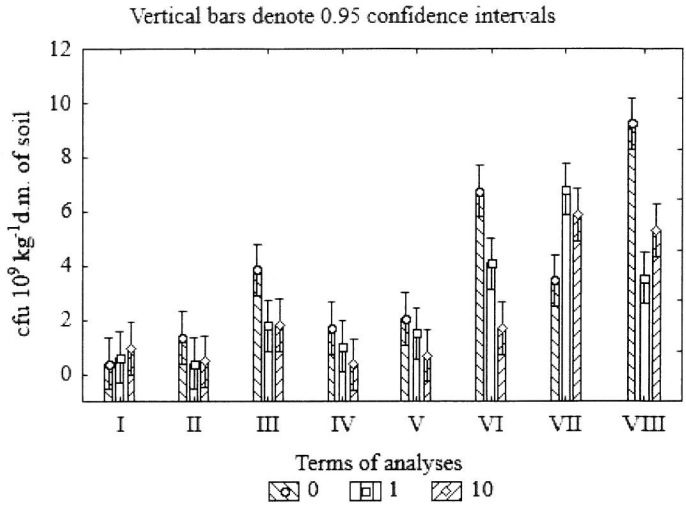

Fig. 9. Temporary numbers of bacteria with proteolytic capabilities in soil contaminated with herbicide Roundup 360 SL. Explanations as in Figure 1

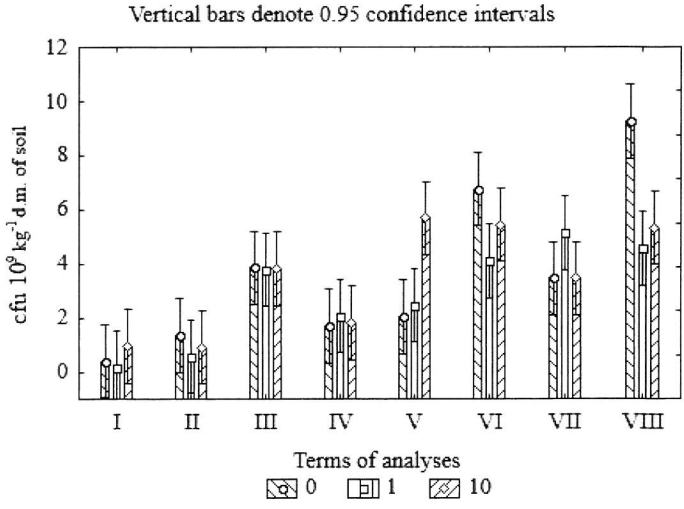

Fig. 10. Temporary numbers of bacteria with proteolytic capabilities s in soil contaminated with herbicide Reglone 200 SL. Explanations as in Figure 2

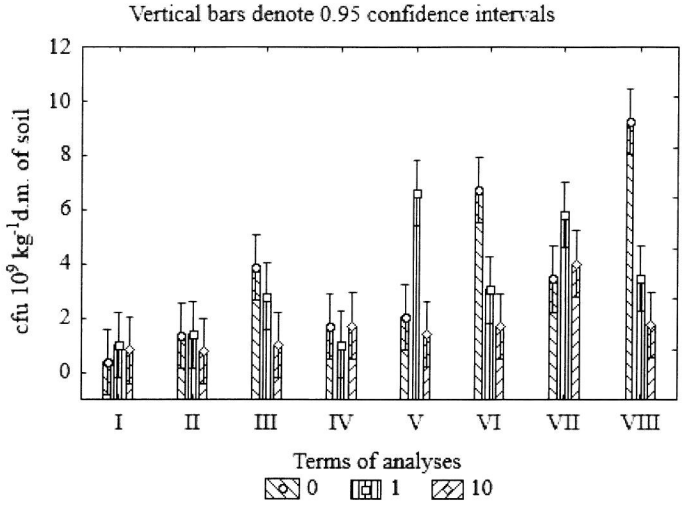

Fig. 11. Temporary numbers of bacteria with proteolytic capabilities in soil contaminated with herbicide Basta 150 SL. Explanations as in Figure 3

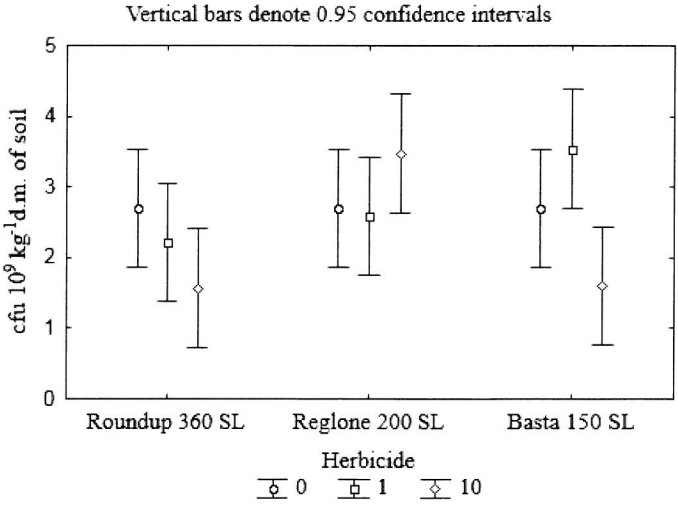

Fig. 12. Means numbers of bacteria with proteolytic capabilities in soil contaminated with herbicides Roundup 360 SL, Reglone 200 SL and Basta 150 SL. Explanations as in Figure 4

The optimum dose of the Basta 150 SL formulation significantly increased the abundance of protein-degrading microorganisms in stages V and VII of the analyses. In stages VI and VIII, both the optimum and increased doses of the herbicide caused a significant decline in the abundance of this microbial group. In the other experimental stages, the 10% higher dose of the formulation had a stimulatory effect on the growth of microorganisms with proteolytic abilities.

The analysis of the mean abundance of bacteria with proteolytic abilities indicated that the Reglone 200 SL herbicide used in the technological and increased doses did not change significantly the proliferation of the analysed microbial group in the soil. In contrast, the Roundup 360 SL and Basta 150 SL herbicides applied in the 10% higher doses lowered the abundance of proteolytic bacteria, compared with the values obtained in the control, as presented in Figure 12.

5.1.1.4. Abundance of fungi with proteolytic activity

The results shown in Figures 13, 14, and 15 present the abundance of fungi with proteolytic abilities in soil supplemented with optimum and 10% higher doses of the Roundup 360 SL, Reglone 200 SL and Basta 150 SL herbicides. Roundup 360 SL applied to the soil in the technological dose stimulated proliferation of proteolytic fungi in stage I (immediately after the winter rapeseed harvest) and stage V of the analyses.

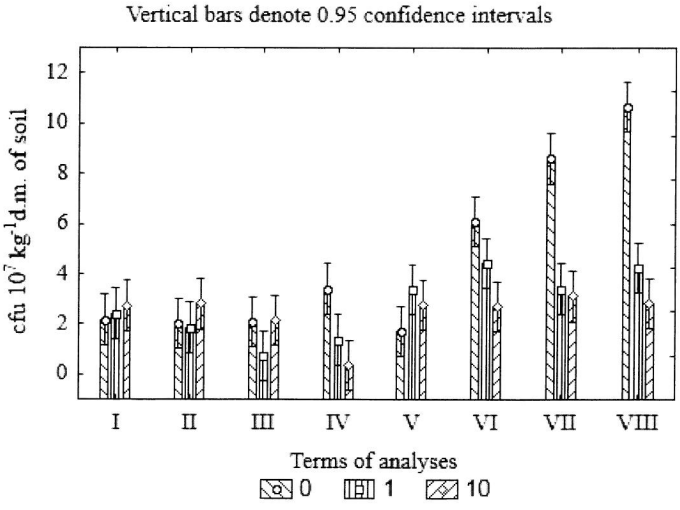

Fig. 13. Temporary numbers of fungi with proteolytic capabilities in soil contaminated with herbicide Roundup 360 SL. Explanations as in Figure 1

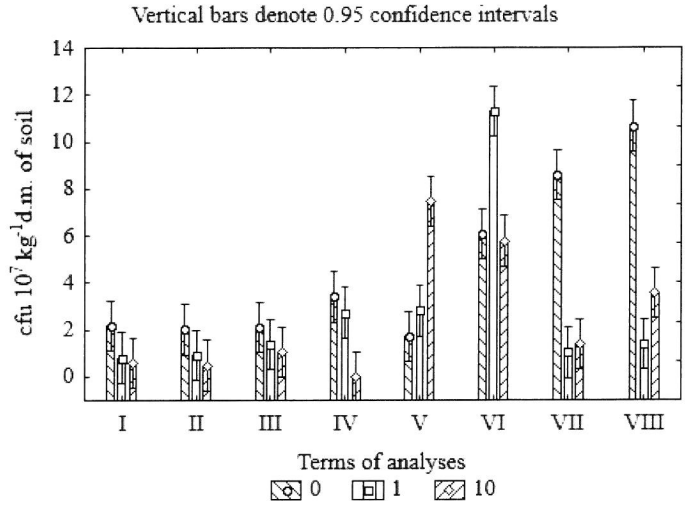

Fig. 14. Temporary numbers of fungi with proteolytic capabilities in soil contaminated with herbicide Reglone 200 SL. Explanations as in Figure 2

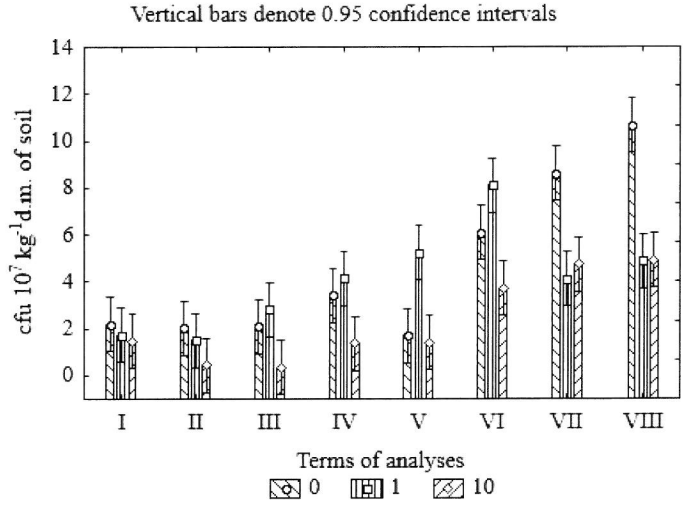

Fig. 15. Temporary numbers of fungi with proteolytic capabilities in soil contaminated with herbicide Basta 150 SL. Explanations as in Figure 3

However, it should be emphasised that the values obtained were not statistically significant, compared with the values noted for the control soil. In stages IV, VI, VII, and VIII of the analyses, Roundup 360 SL applied in both the optimum and increased doses significantly inhibited the growth of proteolytic fungi.

Compared with the control object, the Reglone 200 SL herbicide used in the dose recommended by the manufacturer caused the highest significant increase in the abundance of fungi with proteolytic abilities in stage VI and, when applied in the increased dose, in stage V of the analyses. After 24 and 26 months (stages VII and VIII), the growth of proteolytic fungi was found to be significantly inhibited by both doses of the herbicide.

The Basta 150 SL herbicide used in the optimum dose caused a statistically insignificant stimulation of fungal growth between stages III and VI, which was only significant in stage V of the experiment. Between stage I and VI, soil supplemented with the 10% higher dose of the examined formulation exhibited lower abundance of proteolytic fungi than that in the control soil. After 24 and 26 months (stages VII and VIII), the abundance of the investigated microorganisms in soil treated with the optimum and increased doses was significantly lower than that in the control.

The statistical analysis of the mean values of the abundance of proteolytic fungi presented in Figure 16 revealed that both doses of the Roundup 360 SL, Reglone 200 SL and Basta 150 SL herbicides inhibited the growth of the investigated microorganisms. However, the increased dose caused statistically confirmed inhibition of growth of fungi with capabilities of mineralisation of nitrogenous organic matter.

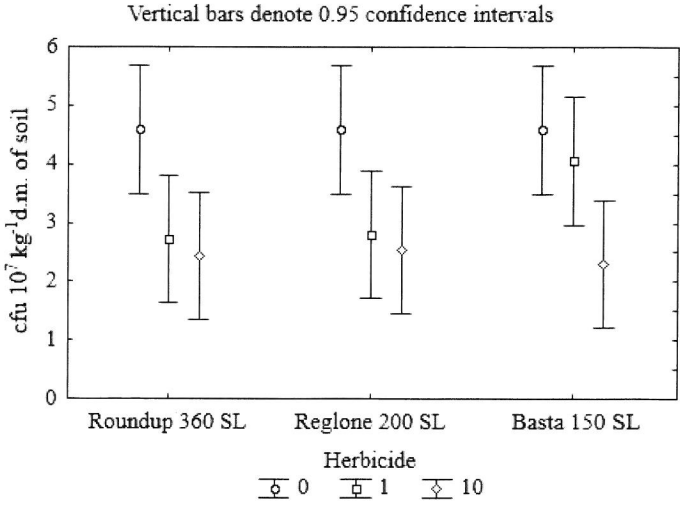

Fig. 16. Means numbers of fungi with proteolytic capabilities in soil contaminated with herbicides Roundup 360 SL, Reglone 200 SL and Basta 150 SL. Explanations as in Figure 4

5.1.2. Effect of the Roundup 360 SL, Reglone 200 SL and Basta 150 SL herbicides on phosphatases activity in soil

5.1.2.1. Acid phosphatase activity

The periodic activity of acid phosphatase after application of the Roundup 360 SL, Reglone 200 SL and Basta 150 SL herbicides is presented in Figures 17, 18, and 19.

Treatment with the technological dose of the Roundup herbicide significantly enhanced the activity of the analysed enzyme in the soil, which exceeded the values obtained in the control in the initial stage of the experiment, i.e. in stage I, and in the 12th month (stage IV) of the analyses.

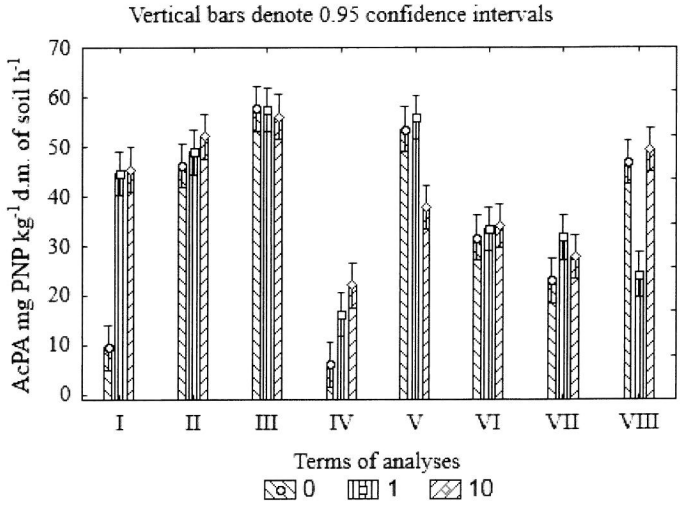

Fig. 17. Temporary acid phosphatase activity (AcPa) in soil contaminated with herbicide Roundup 360 SL. Explanations as in Figure 1

A significant decline in the acid phosphatase activity to the value of 23,158 mg PNP kg^{-1} d.w. soil h^{-1} induced by the optimum herbicide dose was observed in the final stage of the experiment (stage VIII). The 10% higher herbicide dose significantly enhanced the activity of the examined enzyme immediately after the winter rapeseed harvest (stage I) and after 12 months (stage IV). In contrast, a significant decline in the activity of acid phosphatase induced by the optimum dose of the herbicide was found in the final stage of the experiment, i.e. in stage VIII. In comparison with soil that was not supplemented with the herbicide, the increased dose of the herbicide significantly reduced the acid phosphatase activity in stage V of the experiment. In the other analysis stages, the Roundup 360 SL herbicide applied in both the dose recommended by the manufacturer and the

increased dose did not exert a significant effect on the activity of the enzyme, and the values corresponded to those obtained for the control.

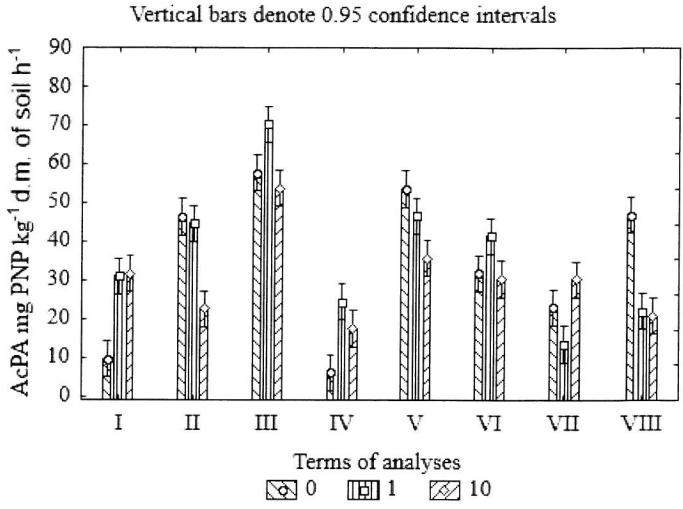

Fig. 18. Temporary acid phosphatase activity (AcPa) in soil contaminated with herbicide Reglone 200 SL. Explanations as in Figure 2

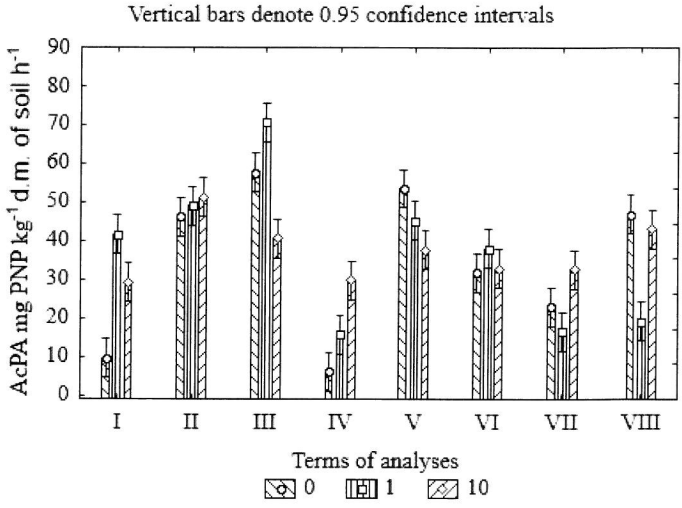

Fig. 19. Temporary acid phosphatase activity (AcPa) in soil contaminated with herbicide Basta 150 SL. Explanations as in Figure 3

The herbicide applied in the optimum dose significantly stimulated the acid phosphatase activity in analysis stages I, III, IV, and VI.

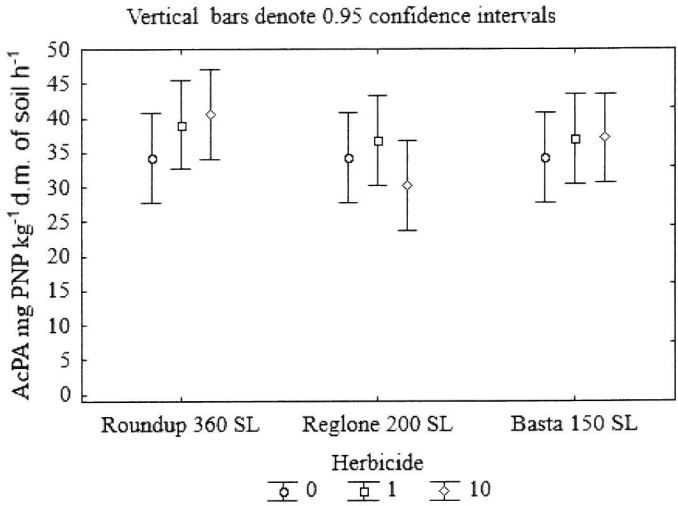

Fig. 20. Means acid phosphatase activities (AcPa) in soil contaminated with herbicides Roundup 360 SL, Reglone 200 SL and Basta 150 SL. Explanations as in Figure 4

The inhibitory effect of the optimum dose of the agent was significant in stages VII and VIII of the analyses. In turn, the higher dose of the herbicide stimulated the examined activity immediately after the winter rapeseed harvest and in stages IV and VII of the experiment. The higher dose significantly reduced the activity of acid phosphatase below the values obtained for the control in analysis stages II and V and in the final stage of the experiment, i.e. in stage VIII.

The Basta 150 SL herbicide applied in the dose recommended by the manufacturer induced a significant increase in the activity of acid phosphatase in stage I of the analysis and after 10 months (stage III).

A significant inhibitory effect of the optimum dose of the Basta 150 SL herbicide on the analysed enzymatic activity was only found in the final stage of the analyses (stage VIII). In the other stages of the experiment, the activity of acid phosphatase in soil treated with the optimum dose of the agent differed insignificantly from the values obtained in the control object. Application of the 10% higher dose of the herbicide resulted in significant stimulation of the acid phosphatase activity in stages I and IV of the analyses. Significant inhibition of the activity of the enzyme induced by the herbicide dose applied was found in stages III and V of the analyses. In the other analysis stages, the differences in the enzyme activity were statistically insignificant.

The analysis of the mean values of the biochemical activity for the Roundup 360 SL, Reglone 200 SL and Basta 150 SL herbicides presented in Figure 20 indicates that the application of the technological and the 10% higher doses did not cause significant differences in the activity of acid phosphatase in the examined soil. The increased dose of Reglone 200 SL caused an insignificant reduction of the activity of the analysed enzyme in the soil.

5.1.2.2. Alkaline phosphatase activity

Changes in the activity of alkaline phosphatase in the soil induced by the applied doses of the Roundup 360 SL, Reglone 200 SL and Basta 150 SL herbicides are illustrated in Figures 21, 22, and 23.

The highest significant activity of alkaline phosphatase, compared with the control, was found in soil treated with the optimum dose of the Roundup 360 SL herbicide in stages II and IV of the analyses. In comparison with the values obtained in the control object, a significant reduction in the analysed activity induced by the dose applied was evident at the end of the experimental period, i.e. in stages VII and VIII of the analyses.

Application of the increased dose resulted in significant stimulation of the alkaline phosphatase activity in stage I of the analyses, i.e. immediately after the winter rapeseed harvest, and in stages II and VII. In contrast, a significant decline in the activity of alkaline phosphatase in soil treated with the increased dose of the Roundup 360 SL herbicide was only reported in the final stage of the experiment, i.e. in analysis stage VIII. In the other stages, the alkaline phosphatase activity differed significantly from the values obtained for the control.

In the technological dose, Reglone 200 SL significantly enhanced the activity of the analysed enzyme at the beginning of the experimental period, i.e. in the first stage of the analyses as well as in stages IV, V, and VII. In turn, a significant reduction of the alkaline phosphatase activity below the control values caused by the application of the optimum dose was only found in the final stage of the experiment, i.e. in stage VIII of the analyses.

The 10% higher dose of the Reglone 200 SL herbicide induced significant stimulation of the investigated activity between the beginning of the experiment and stage VII. Significant inhibition of the investigated activity was evident in the final stage of the experiment, i.e. in the 26^{th} month (stage VIII).

After application of the optimum dose of the Basta 150 SL herbicide, statistically higher alkaline phosphatase activity than that in the control soil was reported in a majority of the analysis stages (I, II, III, IV, VI). Significantly lower activity of the investigated enzyme was noted in the control object in the final stage of the experiment (stage VIII).

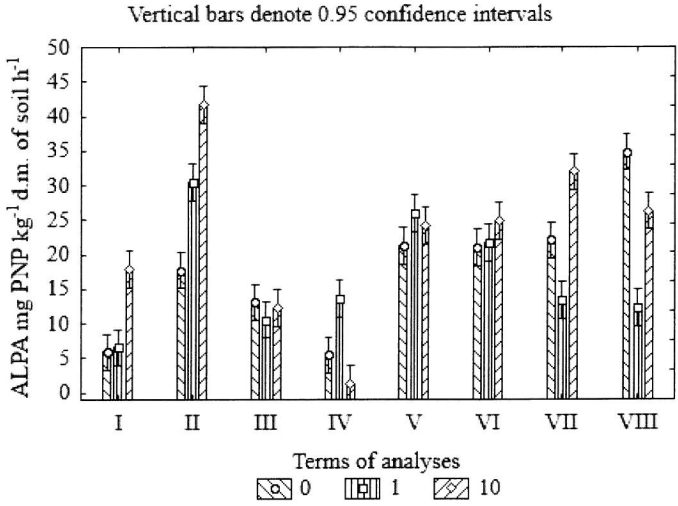

Fig. 21. Temporary alkaline phosphatase activity (ALPA) in soil contaminated with herbicide Roundup 360 SL. Explanations as in Figure 1

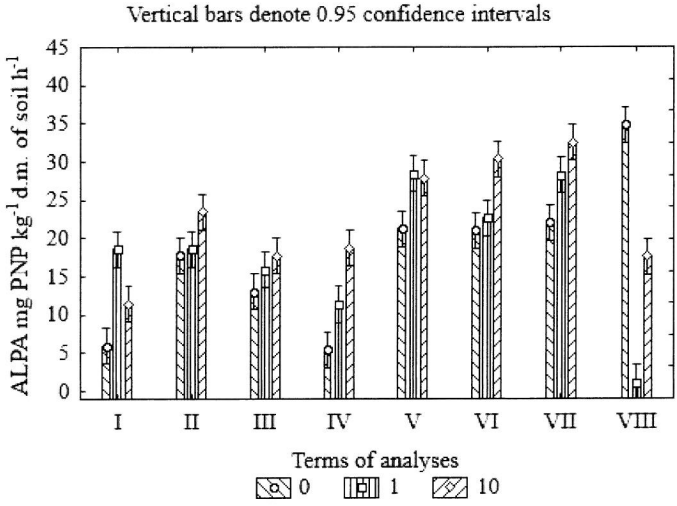

Fig. 22. Temporary alkaline phosphatase activity (ALPA) in soil contaminated with herbicide Reglone 200 SL. Explanations as in Figure 2

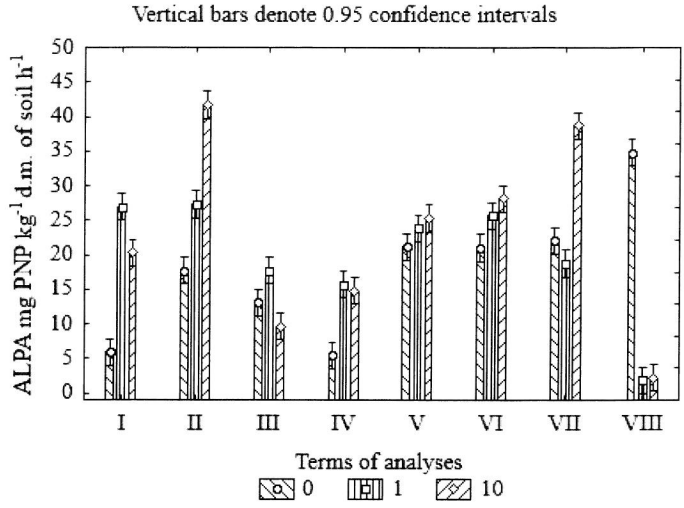

Fig. 23. Temporary alkaline phosphatase activity (ALPA) in soil contaminated with herbicide Roundup 360 SL. Explanations as in Figure 3

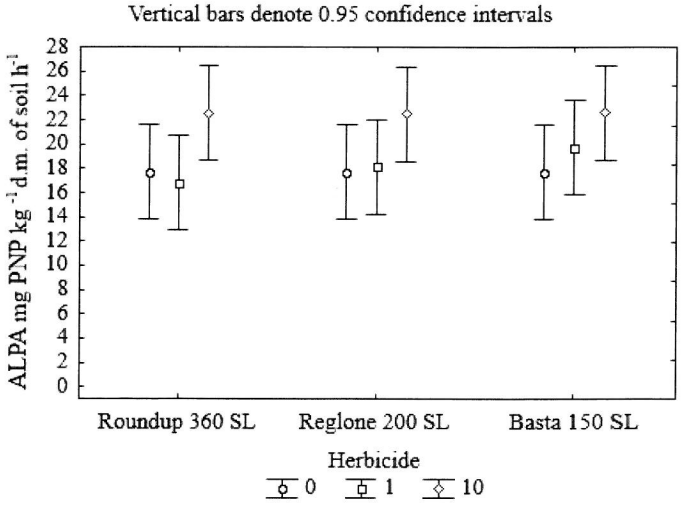

Fig. 24. Means alkaline phosphatase activity (ALPA) in soil contaminated with herbicide Roundup 360 SL, Reglone 200 SL and Basta 150 SL. Explanations as in Figure 4

Similarly, the increased dose of the Basta 150 SL herbicide induced significant stimulation of the analysed activity in stages I, II, IV, V, VI, and VII. The lowest alkaline phosphatase activity was found in this object in stage VIII.

The analysis of the mean values of the alkaline phosphatase activity obtained throughout the experimental period presented in Figure 24 indicates that the 10% higher doses of the Roundup 360 SL, Reglone 200 SL and Basta 150 SL herbicides had a positive impact on the soil enzymatic activity.

5.1.3. Effect of the Roundup 360 SL, Reglone 200 SL and Basta 150 SL herbicides on soil chemical properties

In the final stage of the experiments in 2010, 2011, and 2012, the content of organic C and total N in the experimental objects was analysed and it is presented in Table 2. The analyses indicate that the Roundup 360 SL herbicide increased the organic carbon and total nitrogen content in the soil in all the investigation stages only when it was applied in the optimum dose. In contrast, the higher dose of the herbicide caused a decline in the organic carbon (exception: 2012) and total nitrogen content, compared with the level of this element in the control. Application of both doses of the Reglone 200 SL herbicide to the soil increased the organic carbon content in all the study stages. Moreover, under the impact of the optimum and increased doses of the formulation, the content of total nitrogen in the soil generally corresponded to the values obtained for the control soil (exception: 2010).

When applied in the dose recommended by the manufacturer, the Basta 150 SL herbicide increased the content of organic carbon and total nitrogen in the soil in all the experimental stages. The 10% higher dose of the herbicide caused a decline in the organic carbon content (exception: 2010) and total nitrogen, compared with its content in the control.

The changes in the carbon and nitrogen content had an impact on the C:N ratio in the experimental objects. The results obtained indicate that the increased doses of the Roundup 360 SL herbicide increased the C:N ratio, compared with the control object, and the optimum dose decreased the ratio in 2010 and 2012. The herbicide applied in both doses increased the carbon-to-nitrogen ratio in all the experimental periods. The Basta 150 SL herbicide applied in the optimum dose decreased the C:N ratio, compared with the results obtained in 2010 and 2011. In comparison with the results for the soil in the control object, the increased dose of the herbicide elevated the C:N ratio.

Table 2. Content of organic carbon, total nitrogen and C:N in the individual experimental objects during the experiment (g kg^{-1} d.m. of soil)

Experimental objects	Year	Soil properties		C:N
		C-organic	N-total	
K	2010	9.8	1.3	7.5
	2011	10.8	1.2	9.0
	2012	11.6	1.3	9.0
Mean		10,7	1.3	8.2
RD	2010	10.4	1.4	7.4
	2011	11.8	1.3	9.1
	2012	12.0	1.4	8.6
Mean		11.4	1.4	8.1
RD 10	2010	9.4	1.2	7.8
	2011	10.5	1.1	9.5
	2012	11.9	1.2	9.9
Mean		10.9	1.2	8.8
RG	2010	10.8	1.3	8.3
	2011	11.6	1.2	9.7
	2012	12.2	1.3	9.4
Mean		11.5	1.3	8.8
RG10	2010	10.0	1.2	8.3
	2011	11.0	1.2	9.2
	2012	14.8	1.3	11.4
Mean		11.9	1.2	9.9
B	2010	10.3	1.4	7.4
	2011	11.5	1.3	8.8
	2012	12.8	1.4	9.1
Mean		11.5	1.4	8.2
B10	2010	10.9	1.3	8.4
	2011	10.6	1.1	9.6
	2012	11.3	1.1	10.3
Mean		10.9	1.2	9.0

Explanations: K – control soil, without herbicide addition; RD – soil + herbicide Roundup 360 SL (4 dm^3 ha^{-1}); RD10 – soil + herbicide Roundup 360 SL (4.4 dm^3 ha^{-1}); RG – soil + herbicide Reglone 200 SL (2 dm^3 ha^{-1}); RG10 – soil + herbicide Reglone 200 SL (2.2 dm^3 ha^{-1}); B – soil + herbicide Basta 150 SL (2.5 dm^3 ha^{-1}); B10 – soil + herbicide Basta 150 SL (2.75 dm^3 ha^{-1})

5.1.4. Correlations between the microbiological and biochemical properties of the examined soil

In order to identify the relationships between microorganisms and their biochemical activity and chemical agents, a correlation analysis between the investigated parameters was performed. The correlation coefficients (r) are presented in Table 3. They indicate positive correlations between the microbiological, biochemical, and chemical properties analysed.

The data presented imply that the total fungal abundance was positively correlated at the highest significance level ($\alpha = 0.001$) with the abundance of proteolytic bacteria and fungi. Furthermore, there was a significant positive correlation ($\alpha = 0.01$) between the abundance of proteolytic fungi and the abundance of proteolytic bacteria, which indicates that these microbial groups were able to develop in parallel, and higher proliferation rates in one group accompanied growth of the other. Additionally, a positive correlation was found between alkaline phosphatase and the total abundance of fungi and bacteria as well as the abundance of proteolytic fungi. Moreover, the analysis indicated that the activities of acid phosphatase and alkaline phosphatase were positively correlated.

Table 3. Correlation coefficients (R) between examined microbial and biochemical parameters of soil

Object	1	2	3	4	5	6
1	–	0.40**	n.i.	0.44***	n.i.	n.i.
2		–	n.i.	0.52***	n.i..	0.20**
3			–		n.i..	0.20**
4				–	n.i.	0.25**
5					–	0.20**
6						–

Eksplanations: *** – correlation coefficient significant at significance level $\alpha = 0.001$,
** – correlation coefficient significant at significance level $\alpha = 0.01$,
* – correlation coefficient significant at significance level $\alpha = 0.05$,
n.i. – non significant.
1 – number of "proteolytic" bacteria; 2 – number of "proteolytic" fungi; 3 – total number of bacteria; 4 – total number of fungi; 5 – acid phosphatase activity; 6 – alkaline phosphatase activity.

5.2. Field experiment nr 2 - comparison of the effect of Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL herbicides on the abundance of selected groups of microorganismsand phosphatases activity in soil

5.2.1. The effect of Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL herbicides on soil microorganisms

5.2.1.1. Total bacterial abundance

The chemical formulations Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL were applied in the optimum dose, as recommended by the manufacturer. The results of the investigations are presented in Figure 25. In the initial stage of the analyses, the herbicides used in the experiment inhibited bacterial growth. In stage II, the bacterial abundance was significantly higher in soil treated with the Caramba 60 SL and Spodnam 555 SC formulations. Spodnam 555 SC stimulated bacterial growth in soil after 14 and 24 months (stage V and VII) and inhibited it in stage III of the analyses. In the other stages, the bacterial abundance in the soil treated with the chemical agents differed insignificantly from the results obtained for the control.

The statistical analysis of the mean values obtained throughout the experimental period showed that the formulations used did not exert a significant effect on the fungal abundance, which is illustrated in Figure 26. The presented data imply that only Avans Premium 360 SL contributed to the insignificantly lowest bacterial abundance during the three experimental years.

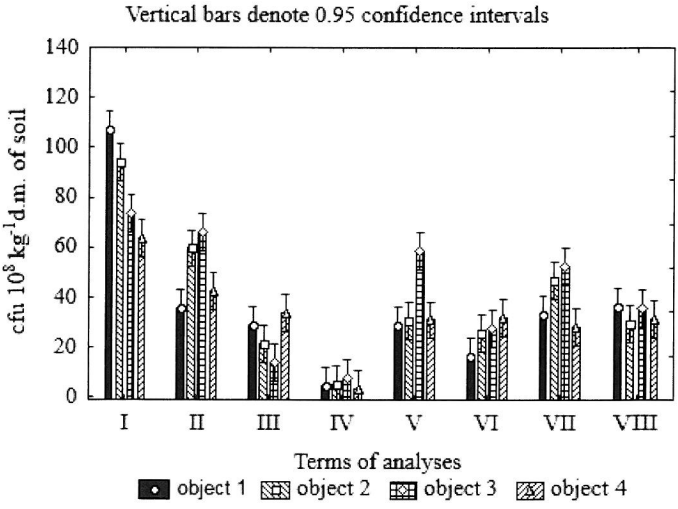

Fig. 25. Temporary numbers of bacteria in particular objects. Explanations: object 1 – control – no chemicals; object 2 – soil + Avans Premium 360 SL (3 dm^3 ha^{-1}); object 3 – soil+ Spodnam 555 SC (1.2 dm^3 ha^{-1}); object 4 – soil + Caramba 60 SL (1 dm^3 ha^{-1})

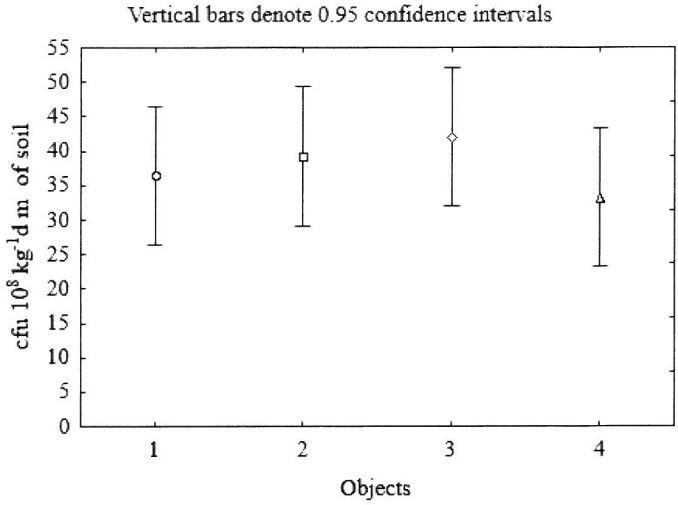

Fig. 26. Means numbers of bacteria in particular object. Explanations as in Figure 25

5.2.1.2. Total fungal abundance

Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL used in the experiment induced periodic changes in the fungal abundance, which is illustrated in Figure 27. Stages II and VI of the analysis are of particular interest. In stage II, all the chemical agents used in the field research significantly inhibited fungal growth in the examined soil, compared with the results obtained in the control. In stage VI, they significantly stimulated fungal growth. In stage I of the analysis, the Spodnam 555 SC formulation contributed to the lowest fungal abundance in the soil. In the other analysis stages, the changes in the fungal abundance induced by the application of the formulations were insignificant in comparison with the results obtained in the control.

The statistical evaluation of the mean values obtained during the three-year field experiment showed no significant effect of the application of these formulations on the fungal abundance, which is illustrated in Figure 28. Avans Premium 360 SL was the only chemical agent that caused an insignificant decline in the fungal abundance in this experiment, compared to the values obtained for the control.

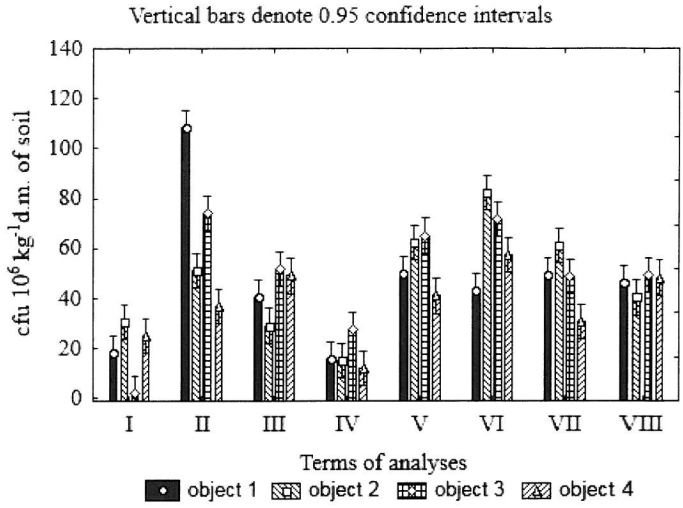

Fig. 27. Temporary fungi numbers in particular treatment. Explanations as in Figure 24

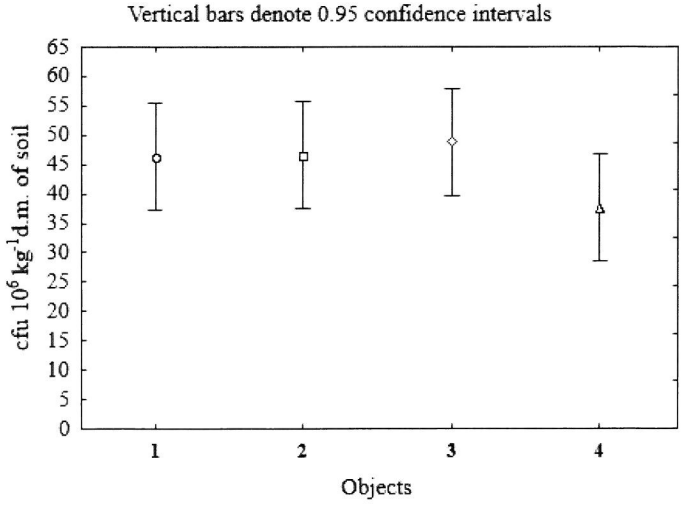

Fig. 28. Means numbers of fungi in particular treatment. Explanations as in Figure 24

5.2.1.3. Abundance of bacteria with proteolytic activity

The results presented in Figure 29 show the abundance of proteolytic bacteria in soil treated with the optimum doses of the Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL herbicides. They indicate significant inhibition of

proliferation of proteolytic bacteria in stage I of the analyses induced by Spodnam 555 SC and in stage V in soil treated with the Avans Premium 360 herbicide. In turn, in the final stage of the analysis (stage VIII), all the herbicides applied in the field experiment decreased the abundance of this microbial group.

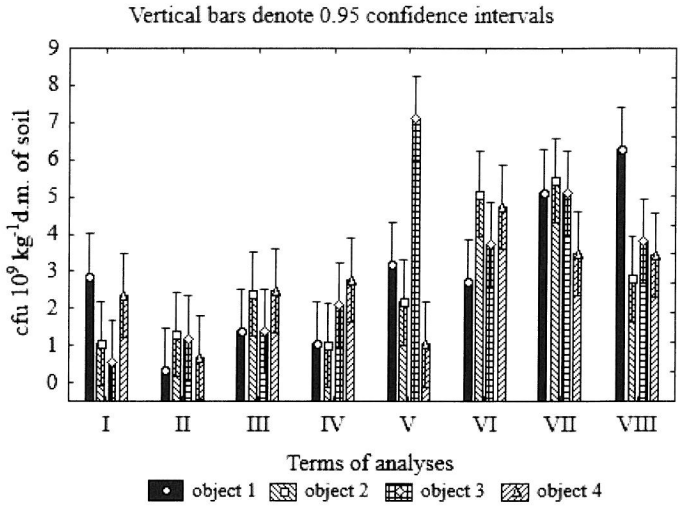

Fig. 29. Temporary numbers of bacteria with proteolytic capabilities in particular treatment. Explanations as in Figure 24

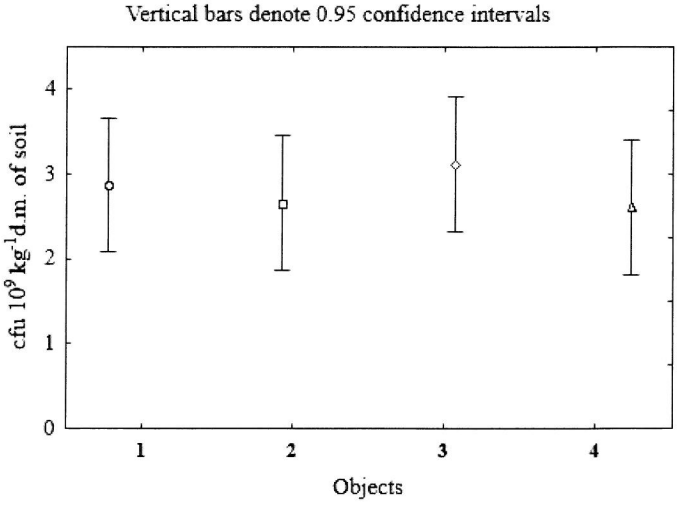

Fig. 30. Means numbers of bacteria with proteolytic capabilities in particular treatment. Explanations as in Figure 24

Noteworthy, in stage V, significant stimulation of the growth of proteolytic bacteria induced by the Spodnam 555 SC herbicide as well as a significant decline in the abundance of the investigated microbial group in the soil treated with Avans Premium 360 SL were observed. The Caramba 60 SL chemical agent significantly stimulated proliferation of these microorganisms in stage VI of the experiment.

Analysis of the statistical means of the abundance of proteolytic bacteria obtained throughout the experimental objects and presented in Figure 30 reveals that Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL disturbed insignificantly the growth of proteolytic bacteria in the tested soil.

5.2.1.4. Abundance of fungi with proteolytic activity

In the field experiment, the Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL chemical agents were applied in doses recommended by the manufacturer. The results obtained are illustrated in Figure 31. After the application of the tested chemical agents, the abundance of proteolytic fungi underwent periodic changes. The Caramba 60 SL formulation significantly reduced the fungal abundance in stages V, VII, and VIII of the analyses in comparison with the abundance of proteolytic fungi in the control soil. In the other stages, the abundance of the analysed microbial group after application of Caramba 60 SL differed insignificantly. The Spodnam 555 SC herbicide contributed to significant growth of this group of microorganisms in stage II and induced a significant decline in the final stage of the experiment (stage VIII). No significant differences were detected in the other stages after application of this herbicide. The application of the Avans Premium 360 SL herbicide caused a significant decline in the abundance of fungi with proteolytic activity in stage V of the analyses; furthermore, after 22 and 26 months (stages VI and VIII) of the experiment, this abundance was found to be significantly lower than that in the control.

Figure 32 presents statistical analysis of the mean values of the abundance of proteolytic fungi obtained throughout the experimental period from soil treated with the Spodnam 555 SC and Avans Premium 360 SL formulations. They indicate that the Caramba 60 SL and Spodnam 555 SC chemicals inhibited statistically insignificantly the growth of these fungi, whereas Avans Premium 360 SL insignificantly stimulated their proliferation.

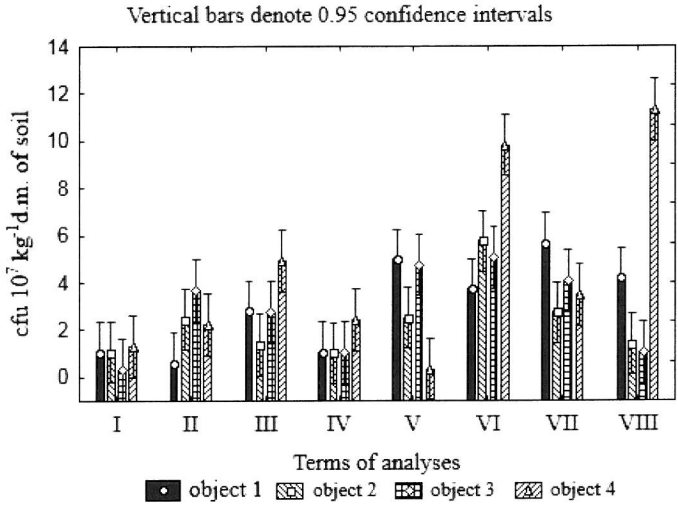

Fig. 31. Temporary numbers of fungi with proteolytic capabilities in particular treatment. Explanations as in Figure 25

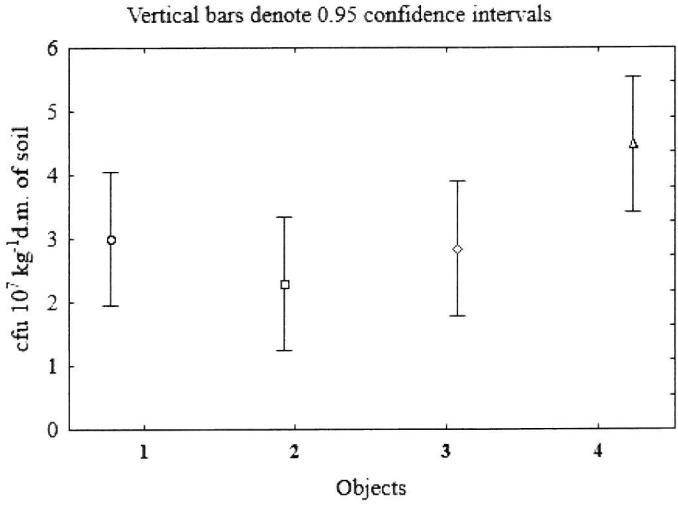

Fig. 32. Means numbers of fungi with ptoteolytic capabilities in particular treatment. Explanations as in Figure 25

5.2.2. Effect of the Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL herbicides on phosphatases activity in soil

5.2.2.1. Acid phosphatase activity

The Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL herbicides tested in the experiment modified the activity of acid phosphatase, which is presented in Figure 33.

The Caramba 60 SL formulation induced a significant increase in the activity of acid phosphatase in analysis stages I, II, III, V, and VII and a significant decrease in stages IV and VIII. In the other analysis stages, the acid phosphatase activity in the soil treated with this herbicide was at the level reported in the control soil. A stimulatory effect of the Spodnam 555 SC herbicide on the activity of acid phosphatase was observed in stages I, III, V, and VIII. A statistically significant reduction of the activity induced by the tested herbicide was noted in analysis stage VII. The Avans Premium 360 SL formulation stimulated the acid phosphatase activity throughout the 3-year-long experiment. Significant differences were evident in stages I, II, III, and VIII of the analyses.

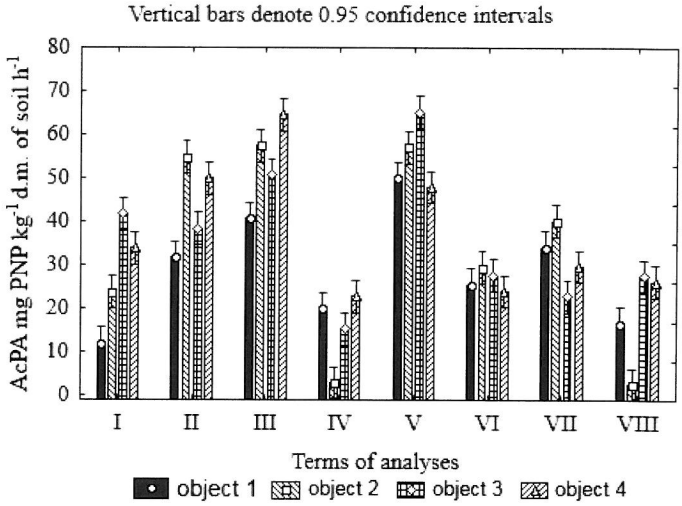

Fig. 33. Temporary acid phosphatase activity (AcPA) in particular treatment. Explanations as in Figure 25

Figure 34 presents the mean values of the acid phosphatase activity reported from the experimental objects over the entire period of the research, which indicate that the Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL used in the field experiment did not cause significant differences in the analysed activity.

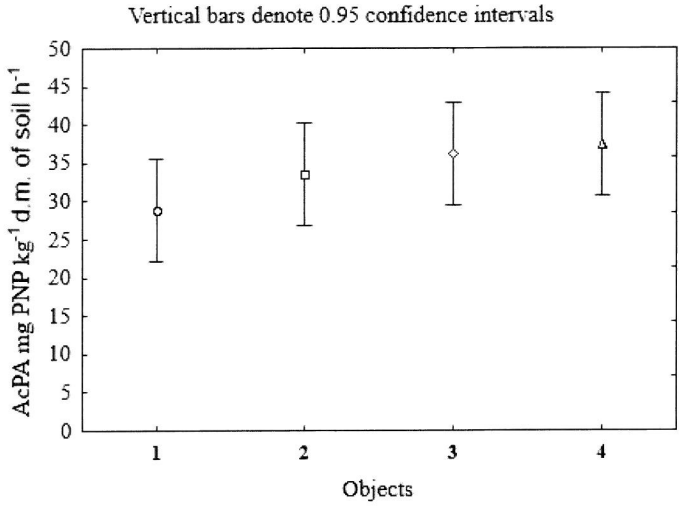

Fig. 34. Means acid phosphatase activities (AcPA) in particular treatment. Explanations as in Figure 25

5.2.2.2. Alkaline phosphatase activity

Changes in alkaline phosphatase activity induced by the Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL chemicals applied are presented in Figure 35. The Caramba 60 SL preparation caused a significant decline in the activity of alkaline phosphatase in stage I of the analyses as well as in the final phase of the experiment (stages VI, VII, and VIII). A statistically insignificant increase in the activity of the examined enzyme was only observed in stage IV. The Caramba 60 SL formulation significantly inhibited the activity of alkaline phosphatase throughout the experimental period, with the exception of stage IV, when the analysed activity exhibited values similar to those obtained in the control soil. The Avans Premium 360 SL herbicide caused a significant decline in alkaline phosphatase activity in stages I, II, and VI and significantly enhanced the activity of the enzyme in stage III. In the other stages, the formulation did not induce statistically significant differences.

The analysis of the mean values of the alkaline phosphatase activity from each experimental object shows that the Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL agents had an inhibitory effect on the value of the biochemical parameter analysed, with a significant decline observed after application of Caramba 60 SL and Spodnam 555 SC (Fig. 36).

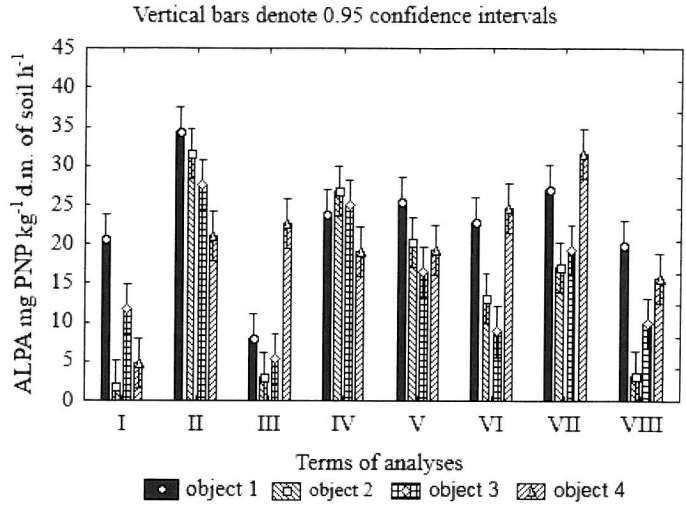

Fig. 35. Temporary alkaline phosphatase activity (ALPA) in particular treatment. Explanations as in Figure 25

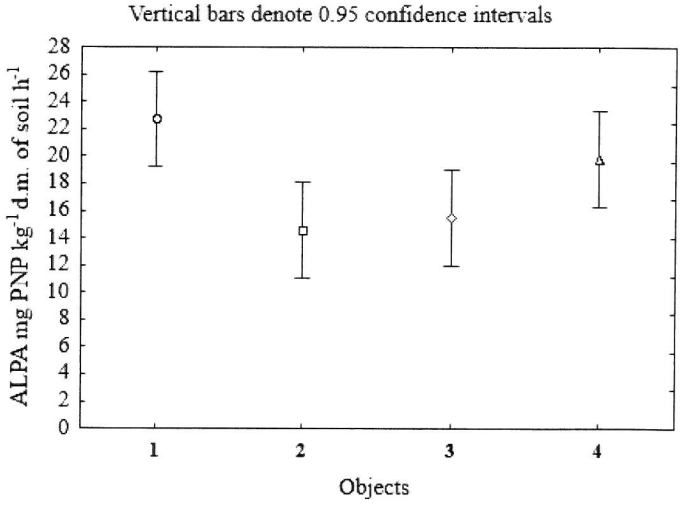

Fig. 36. Means alkaline phosphatase activities (ALPA) in particular treatment. Explanations as in Figure 25

5.2.3. Effect of the Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL herbicides on soil chemical properties

The content of organic C and total N were assessed in the final stage of the experiments in 2010, 2011, and 2012. The results are presented in Table 4.

Table 4. Content of organic carbon, total nitrogen and C:N in the individual experimental objects during the experiment (g kg^{-1} d.m. of soil)

Experimental objects	Year	Soil properties		C:N
		C-organic	N-total	
1	2010	9.8	1.3	7.5
	2011	10.8	1.2	9.0
	2012	11.6	1.3	9.0
	Mean	10.7	1.3	8.2
2	2010	9.9	1.1	9.0
	2011	13.3	1.3	10.2
	2012	14.1	1.4	10.1
	Mean	12.4	1.3	9.8
3	2010	12.2	1.2	10.2
	2011	12.0	1.2	10.0
	2012	13.2	1.3	10.2
	Mean	12.5	1.2	10.1
4	2010	10.1	1.2	8.4
	2011	12.1	1.3	9.3
	2012	14.8	1.3	11.4
	Mean	12.3	1.3	9.5

Explanations: 1 – control soil without herbicide addition; 2 – soil + chemical preparation Avans Premium 360 SL (3 dm^3 ha^{-1}); 3 – soil + chemical preparation Spodnam 555 SC (1.2 dm^3 ha^{-1}), 4 – soil + chemical preparation Caramba 60 SL (1 dm^3 ha^{-1})

The analysis of the data indicates that the Avans Premium 360 SL, Spodnam 555 SC and Caramba 60 SL herbicides used in the experiment increased the content of organic carbon, in comparison with that in the control soil in all the analysis stages. Furthermore, it was found that, compared with the content of the ele-

ment in the control, the total nitrogen level was reduced by the herbicides applied only in 2010. As far as the other experimental years are concerned, an increase in the total nitrogen content was reported after application of Avans Premium 360 SL and Caramba 60 SL in 2011. The content of total N in soil treated with the Spodnam 555 SC and Caramba 60 SL agents (2012) reached values that were obtained in the control

The content of organic carbon and total nitrogen had an impact on the C:N ratio in the investigated soil. All the chemical agents introduced into the soil elevated the values of the carbon-to-nitrogen ratio above those reported for the control soil.

5.2.4. Correlations between the microbiological and biochemical properties of the examined soil

To check the relationships between the microorganisms and biochemical activity in soil treated with herbicides, the correlations between the investigated parameters were analysed. The results are presented in Table 5. This analysis shows a positive correlation within the microbial groups studied between the total abundance of fungi and the abundance of proteolytic bacteria and fungi as well as a highly significant correlation ($\alpha = 0.001$) between bacteria with proteolytic activity and the abundance of proteolytic fungi. A positive correlation was also found between the total abundance of fungi and acid phosphatase activity.

Table 5. Correlation coefficients (R) between examined microbial and biochemical parameters of soil

Object	1	2	3	4	5	6
1	–	0.48***	n.i.	0.23*	n.i.	n.i.
2		–	n.i.	0.32**	n.i.	n.i.
3			–	n.i.	n.i.	n.i.
4				–	0.26*	n.i.
5					–	n.i.
6						–

Explanations as in Table 3.

5.3. Pot experiment - comparison of the effect of different doses of Roundup 360 SL and Reglone 200 SL herbicides on the abundance of selected groups of microorganisms and their biochemical activity in soil

5.3.1. The effect of different doses of Roundup 360 SL and Reglone 200 SL herbicides on soil microorganisms

5.3.1.1. Total bacterial abundance

Changes in the bacterial abundance induced by the application of the different Roundup 360 SL and Reglone 200 SL doses during the experiment are presented in Figure 37.

The analysis performed showed that the application of the optimum dose of the Roundup 360 SL herbicide to the soil did not affect bacterial proliferation and the total bacterial abundance significantly. A significant increase in the abundance was found from day 25 of soil incubation to the end of the experiment in soil supplemented with a 10-fold higher dose than the recommended one. In turn, the 50-fold higher dose significantly stimulated an increase in the bacterial abundance after days 50 and 200 of soil incubation. Moreover, application of a 100-fold higher dose of Roundup 360 SL stimulated bacterial growth throughout the experimental period. This effect was statistically confirmed after 200 days of soil incubation. At a dose recommended by the manufacturer (optimum), the Reglone 200 SL herbicide introduced into the soil stimulated bacterial proliferation significantly only in the final stage of the analyses. The 10-fold higher herbicide dose did not induce significant changes in bacterial abundance, which was similar to that in the control soil in all the analysis stages. Reglone 200 SL introduced into the soil in the 50-fold higher dose significantly increased the number of microorganisms on days 50 and 200 of the experiment. A significant stimulatory effect of the 100-fold higher dose of this herbicide was only found on day 25 of soil incubation. Throughout the study period, the significant inhibitory effect of the Reglone 200 SL formulation was only evident on day 50 of the analyses in soil supplemented with the highest (100-fold) dose.

Statistical analysis of the results obtained demonstrated the greatest bacterial abundance in soil supplemented with the 10-fold higher dose of the Roundup 360 SL herbicide. Soil receiving the 50- and 100-fold higher doses exhibited significantly greater bacterial abundance than that in the control soil and soil supplemented with the optimum dose. In turn, Reglone 200 SL introduced into the soil in both the optimum and the 50-fold higher doses stimulated bacterial proliferation significantly. The total number of bacteria after the application of the 10- and 100- fold doses of the Reglone 200 SL herbicide was similar to that in the control soil (Fig. 38).

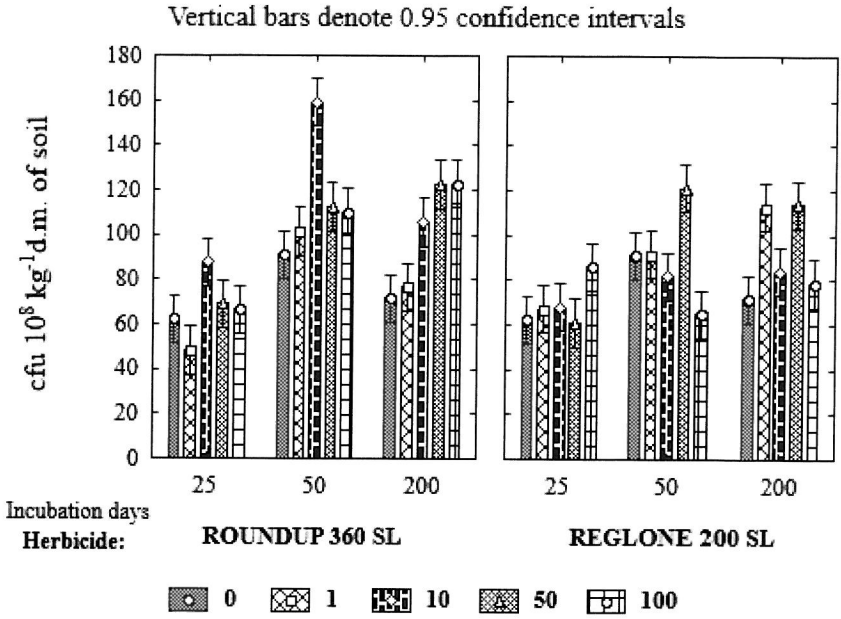

Fig. 37. Temporary bacteria numbers in soil contaminated with herbicide Roundup 360 SL and Reglone 200 SL. Explanations: 0 – control soil without herbicide addition; 1 – soil + herbicide at dose recommended by the manufacturer; 10 – soil + herbicide at the dose 10 – times higher than recommended by the manufacturer; 50 – soil + herbicide at the dose 50 – times higher than recommended by the manufacturer; 100 – soil + herbicide at the dose 100 – times higher than recommended by the manufacturer

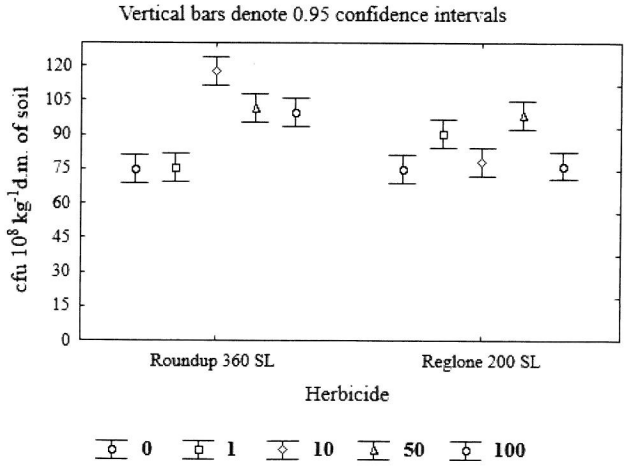

Fig. 38. Means numbers of bacteria in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

5.3.1.2. Total fungal abundance

The periodic abundance of fungi after application of the different doses of the Roundup 360 SL and Reglone 200 SL herbicides is illustrated in Figure 39.

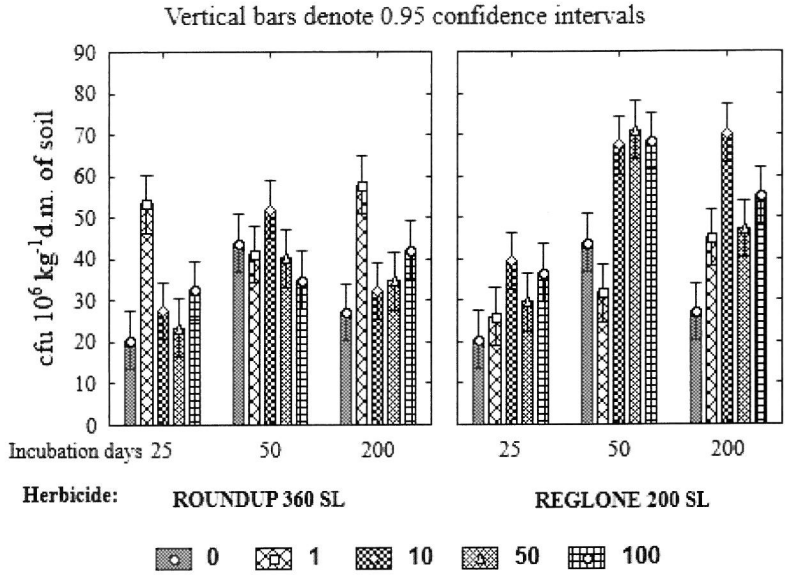

Fig. 39. Temporary fungi numbers in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

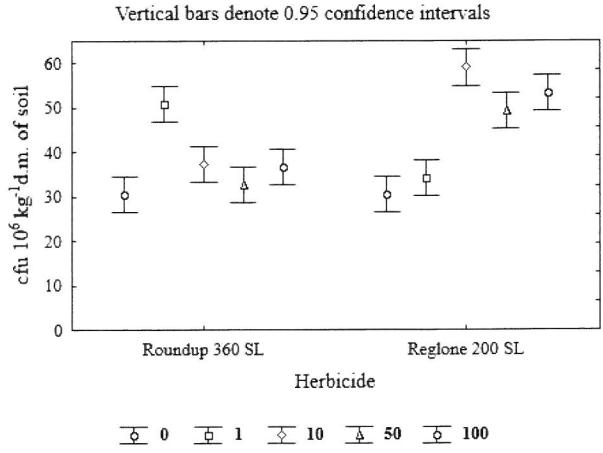

Fig. 40. Means numbers of fungi in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

The investigations revealed periodic fluctuations in the total abundance of fungi induced by the doses of both herbicides applied. In the first and third stage of the analyses, the optimum dose of the Roundup 360 SL herbicide applied to the soil caused a significant increase in the total abundance of fungi, compared with the control soil and the other increased doses of the chemical. The highest (100-fold) dose of the herbicide increased the fungal abundance during the final stage of the analyses, i.e. after 200 days. The other increased doses of the herbicide typically led to greater fungal abundance in the first and last stages of the analyses, compared with that in the control soil. In turn, on incubation day 50, the investigated abundance exhibited a lower level than in the control soil. However, the results obtained were not statistically confirmed. Application of the optimum dose of the Reglone 200 SL herbicide to the soil resulted in a significant increase in the abundance of fungi only in the final analysis stage (after 200 days of incubation). The 10- and 100-fold higher herbicide doses also significantly stimulated microbial proliferation in the soil in all the analysed stages, whereas the 50-fold higher dose had a stimulatory effect on the microbial abundance on days 50 and 200 of the analyses.

The mean number of fungi in the soil of each experimental object is illustrated in Figure 40. The analysis of variance performed showed that only the optimum dose of the Roundup 360 SL herbicide applied to the soil enhanced significantly the growth of the total fungal abundance, compared with the values reported from the control and the other increased doses of this herbicide. Upon application of the 10-, 50-, and 100-fold higher doses of Reglone 200 SL, the soil exhibited significantly greater abundance of the investigated microorganisms than that in the control soil and soil supplemented with the optimum dose.

5.3.1.3. Abundance of bacteria with proteolytic activity

The results shown in Figure 41 present the effect of the Roundup 360 SL and Reglone 200 SL herbicides on the abundance of "proteolytic" bacteria in soil.

The investigations showed that application of all the doses of the Roundup 360 SL herbicide significantly stimulated proliferation of bacteria with proteolytic activity after days 25 and 50 of soil incubation. Importantly, the 10-fold higher dose caused the most intense development of the analysed microbial group on day 50 of the entire experiment. Application of the 100-fold higher dose of the Roundup 360 SL herbicide in the final stage of the experiment resulted in a significant increase in the abundance of "proteolytic" bacteria, compared with the control soil. A stimulatory effect on the microbial abundance was also observed in the soil treated with the Reglone 200 SL herbicide. The results indicate that in the first analysis stage (after 25 days) almost all the herbicide doses used (with the exception of the

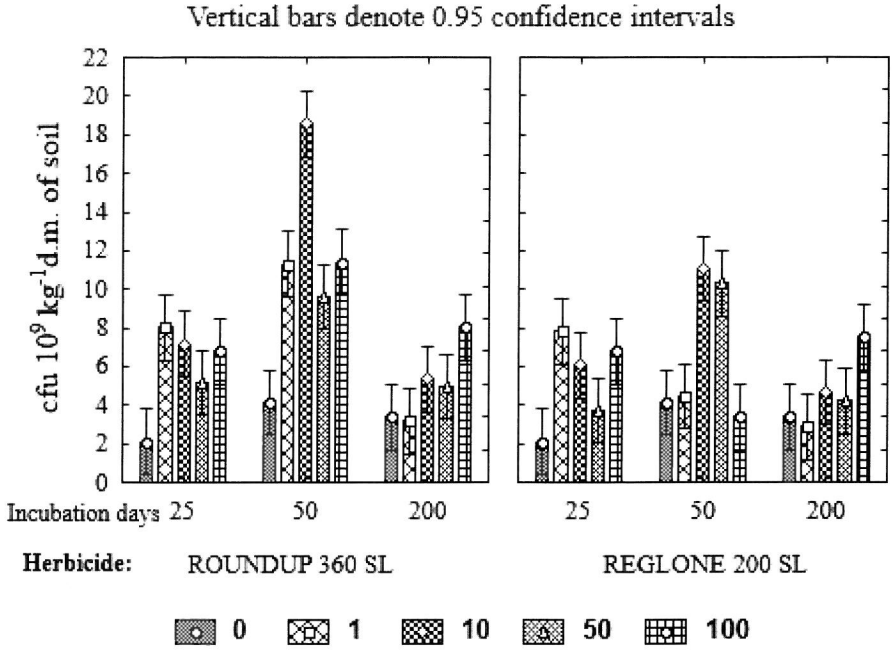

Fig. 41. Temporary numbers of bacteria with proteolytic capabilities in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

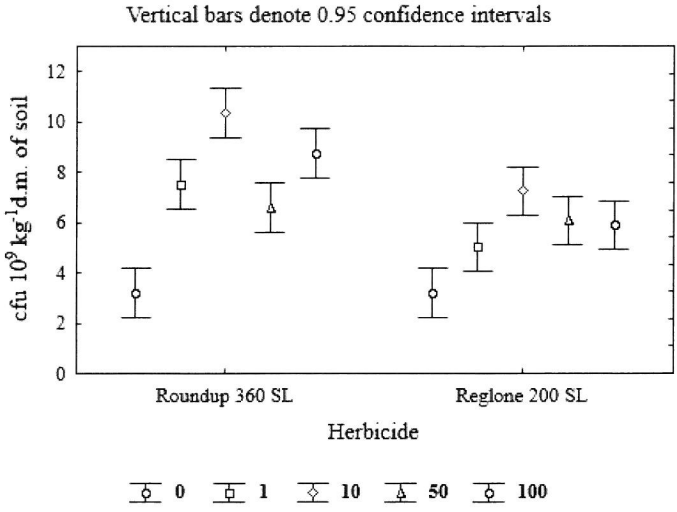

Fig. 42. Means numbers of bacteria with proteolytic capabilities in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

50-fold higher dose) induced a significant increase in the abundance of bacteria with proteolytic activity. Similarly, in another stage of analysis, i.e. after 50 days, the 10- and 50- fold higher doses had a significant effect on the increase of the microbial abundance. In turn, in the final stage of the experiment, only the highest herbicide dose applied to the soil significantly increased the abundance of the "proteolytic" bacteria, which exceeded the values reported for the control soil.

Based on the 95% Tukey's confidence interval, it was found that introduction of all the doses of the Roundup 360 SL and Reglone 200 SL herbicides into the soil stimulated proliferation of bacteria with proteolytic activity (Fig. 42).

5.3.1.4. Abundance of fungi with proteolytic activity

The periodic changes in the abundance of fungi with proteolytic activity induced by application of the different Roundup 360 SL and Reglone 200 SL doses is illustrated in Figure 43.

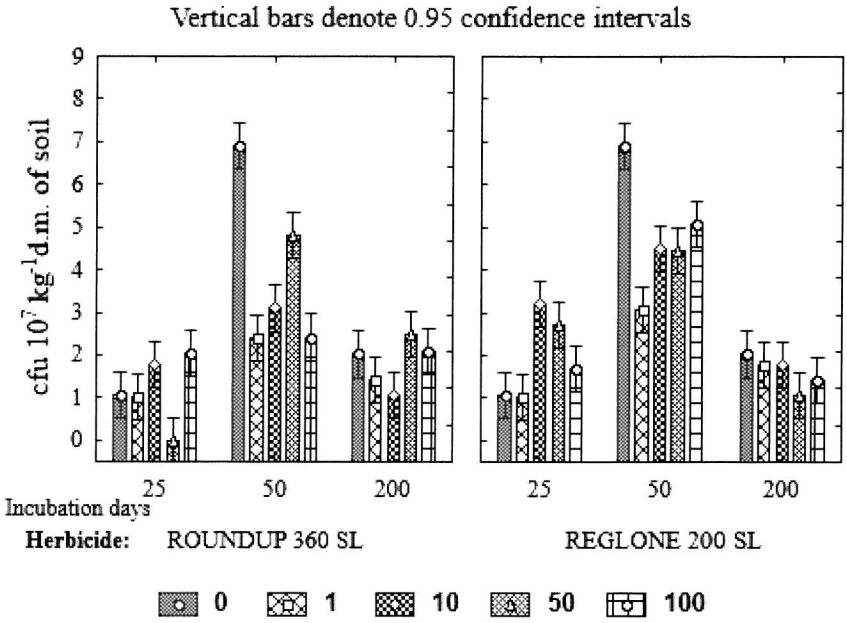

Fig. 43. Temporary numbers of fungi with proteolytic capabilities in soil contaminated with herbicide Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

Already within the first 25 incubation days, the introduction of the Roundup 360 SL herbicide in a 100-fold higher dose than the recommended one caused the largest increase in the abundance of "proteolytic" fungi, in comparison with the

control soil. Interestingly, a significant decline in the abundance of the examined fungi was found on day 50 of the experiment, when of the Roundup 360 SL herbicide applied even in the technological dose significantly decreased the abundance in this microbial group. During the final experiment stage, i.e. after 200 days of incubation, both stimulation (by the 50- and 100-fold higher doses) and inhibition (by the optimum and 10-fold higher doses) of the proliferation rate of "proteolytic" fungi were induced by the Roundup 360 SL herbicide. However, these results were not statistically confirmed. Introduction of the 10- and 50-fold higher dose of Reglone 200 SL into the soil resulted in significant stimulation of growth of the fungi with proteolytic activity only within the first 25 days of soil incubation. In turn, in the second analysed period (after 50 days), all the herbicide doses applied caused a decrease in the number of these microorganisms to a significantly lower level than the values observed in the control soil. In the final stage of the experiment, both the optimum and the increased doses of the Reglone 200 SL herbicide insignificantly reduced the abundance of "proteolytic" fungi to values below those obtained in the control soil.

The analysis of the mean values of the abundance of fungi with proteolytic activity indicates that all the Roundup 360 SL doses applied to the soil induced a significant decrease in the analysed abundance, compared with that in the control soil. Similarly, application of the Reglone 200 SL herbicide inhibited the growth of fungi with proteolytic activity. This effect was particularly evident at the application of the optimum dose to the soil (Fig. 44).

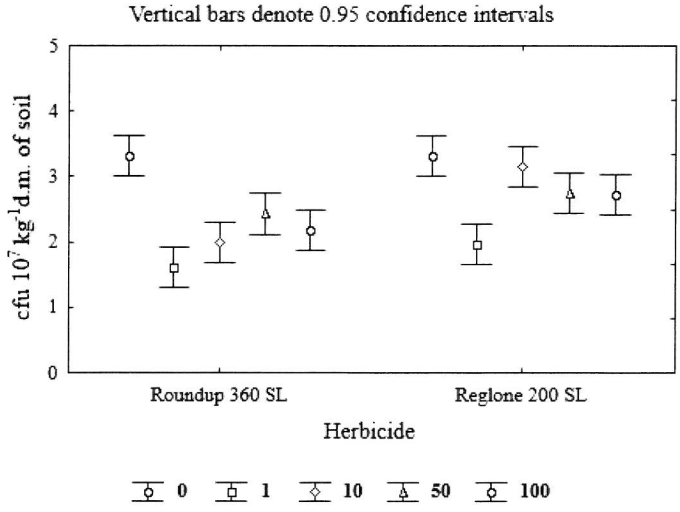

Fig. 44. Means numbers of fungi with proteolytic capabilities in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

5.3.2. Effect of the different doses of the Roundup 360 SL and Reglone 200 SL herbicides on soil biochemical properties

5.3.2.1. Dehydrogenase activity

The results presented in Figure 45 show the effect of the doses of the Roundup 360 SL and Reglone 200 SL herbicides on dehydrogenase activity in the soil. On incubation day 25, the 50- and 100-fold higher doses of the Roundup 360 SL herbicide introduced into the soil stimulated the activity of the enzyme, compared with the control. In the other stages of the analysis, i.e. after 50 and 200 days, the dehydrogenase activity significantly increased after the application of all the tested Roundup 360 SL doses. In turn, application of all the tested doses of the Reglone 200 SL herbicide caused a significant increase in the soil dehydrogenase activity on day 25 of the analyses. In the other stages of the analysis (after 50 and 200 days), a significant stimulatory effect of the Reglone 200 SL herbicide on dehydrogenase activity was noted, particularly after application of the optimum as well as the 5- and 100-fold higher doses. Throughout the study period, the optimum dose of the herbicide applied to the soil was noteworthy, as it induced the highest increase in dehydrogenase activity in all the stages of the analysis.

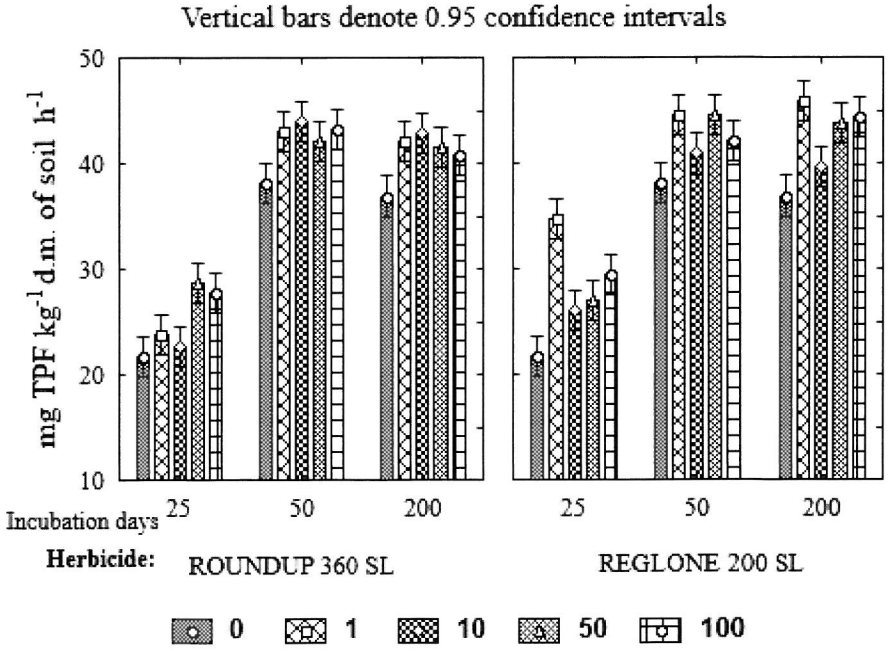

Fig. 45. Temporary dehydrogenase activity in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

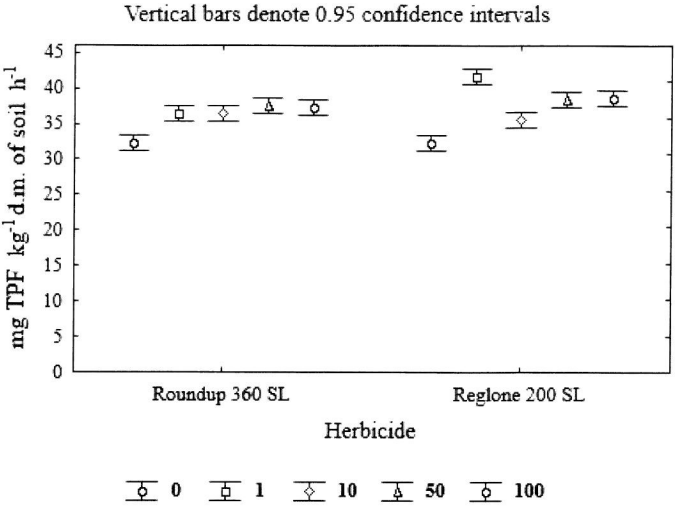

Fig. 46. Mean dehydrogenase activity in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

The results concerning the mean dehydrogenase activity demonstrated that the Roundup 360 SL and Reglone 200 SL herbicides used in the experiment stimulated the activity of oxidoreductases (Fig. 46). All the doses of the Roundup 360 SL herbicide tested caused a significant increase in soil dehydrogenase activity to values higher than those found in the control soil. In turn, the stimulatory effect of Reglone 200 SL on the enzyme activity analysed was most pronounced in soil supplemented with the optimum dose of the herbicide and least visible after application of the 10-fold dose.

5.3.2.2. Protease activity

The periodic protease activity in the soil observed after application of the different doses of the Roundup360 SL and Reglone 200 SL herbicides is presented in Figure 47. The results obtained indicate a significant increase in the activity on day 25 of the analyses after the application of the optimum and the 50-fold Roundup 360 SL doses. Furthermore, the positive effect of the herbicide doses persisted in the second stage of the analyses as well. Application of the 10-fold dose resulted in a significant decrease in soil protease activity on day 50 of the experiment. After 200 days, soil proteolytic activity significantly increased and reached the value of 6.13 mg tyrosine·kg $^{-1}$ d.w. soil·h $^{-1}$ only in soil supplemented with the 100-fold higher dose of the formulation. Application of the optimum

dose of the Reglone 200 SL herbicide to the soil stimulated the enzyme activity in all the analysis stages. The 50-fold higher dose also significantly increased the investigated activity in the soil, although only in the initial stage of the experiment (day 25 of the analyses). Application of the highest (100-fold) dose of the herbicide resulted in a significant increase in the proteolytic activity only in the final stage of the experiment, i.e. on day 200. The Reglone 200 SL herbicide exhibited an inhibitory effect on protease activity only upon application of the 50-fold higher dose to the soil on day 50 of the experiment.

The investigations demonstrated that the increased amounts of the Roundup 360 SL herbicide applied to the soil significantly stimulated the proteolytic activity of the analysed soil. The Reglone 200 SL herbicide also induced an increase in the proteolytic activity in the soil, however, only in the optimum and 50- and 100-fold higher doses (Fig. 48).

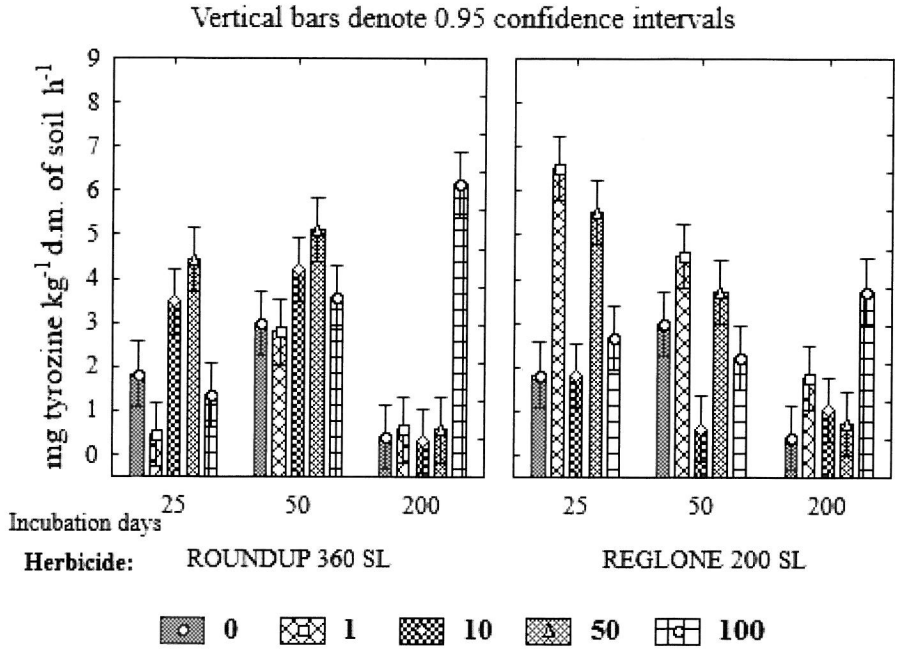

Fig. 47. Temporary protease activity in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

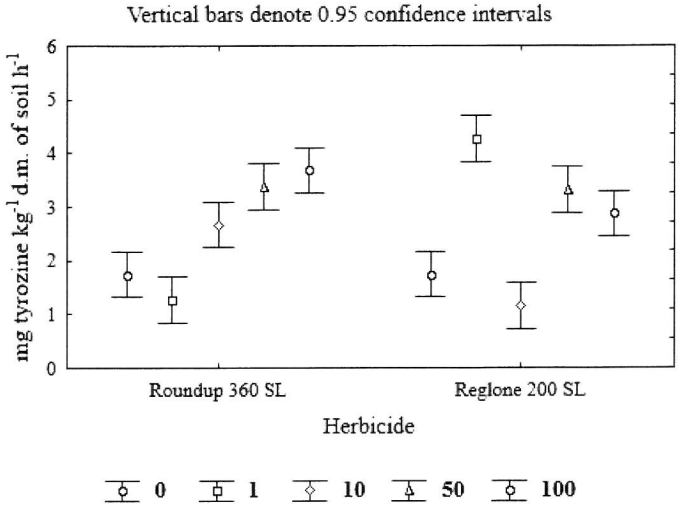

Fig. 48. Mean protease activity in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

5.3.2.3. Urease activity

Data presented in Figure 49 demonstrate that the different doses of the Roundup 360 SL and Reglone 200 SL herbicides applied to the soil had a significant effect on ureolytic activity. In the initial stage of the experiment, i.e. on day 25, only the 100-fold higher dose of the Roundup 360 SL herbicide induced a significant decline in the ureolytic activity, compared with the values obtained in the control soil. In contrast, in the second period of the analyses, soil urease activity was significantly stimulated only by the 50-fold higher dose. The Roundup 360 SL herbicide applied to the soil both in the optimum and increased doses (10-, 50-, and 100-fold) significantly enhanced urease activity after 200 days of soil incubation. The analysed activity was higher in soil supplemented with the highest (100-fold) dose of the herbicide than with the other doses. Introduction of the optimum dose of the Reglone 200 SL herbicide into the soil had a significant inhibitory effect on soil ureolytic activity only on day 25 of the analyses. A significant stimulatory effect of the Reglone 200 SL herbicide on the activity of the investigated enzyme in the second stage of the experiment was only found after application of the highest, i.e. 100-fold dose. In the final phase of the experiment (after 200 days), clear enhancement of the ureolytic activity was observed in soil supplemented with all the Reglone 200 SL herbicide doses.

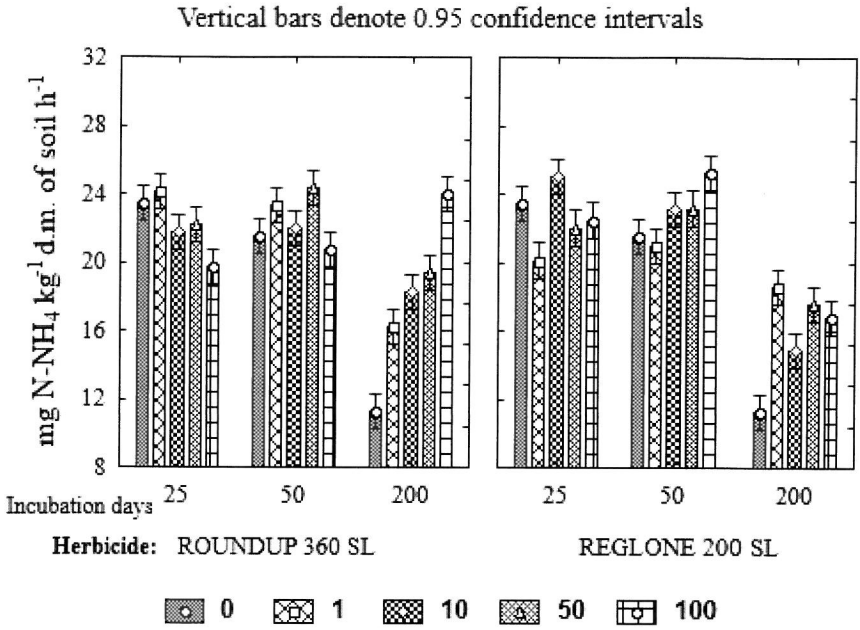

Fig. 49. Temporary urease activity in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

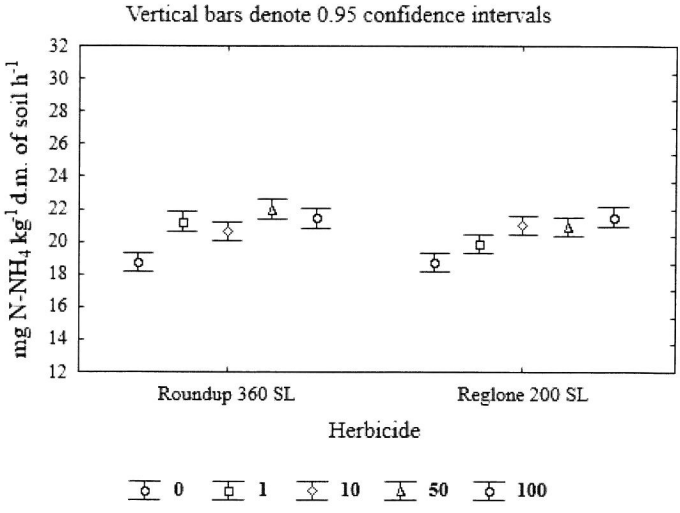

Fig. 50. Mean urease activity in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

Analysis of the mean values of the ureolytic activity determined for each dose of both herbicides implies that all the doses of the Roundup 360 SL herbicide exerted a stimulatory effect on the value of the investigated parameter. Similarly, Reglone 200 SL enhanced soil ureolytic activity, particularly when applied in the 10-, 50-, and 100-fold doses (Fig. 50).

5.3.2.4. Intensity of the ammonification process

The periodic ammonification rate induced by the applied doses of the Roundup 360 SL and Reglone 200 SL herbicides is illustrated in Figure 50.

The data obtained demonstrate that all the doses of the Roundup 360 SL herbicide applied to the soil decreased the intensity of organic nitrogen mineralisation only on day 25 of the experiment. A significant decrease in ammonification intensity below the control values was visible in the soil receiving the 10- and 50-fold increased doses. After 50 days, a significant stimulatory effect of the Roundup 360 SL herbicide was only found after the application of the highest i.e. 100-fold higher dose of the formulation. In the final period of the analyses, i.e. after 200 days, the intensity of the process increased significantly under the impact of the 50- and 100-fold higher Roundup 360 SL doses. Application of the 10- and 50-fold higher Reglone 200 SL herbicide doses resulted in a significant decline in the intensity of the ammonification process on day 25 of the analyses. In the subsequent analysis stage (after 50 days), only the 50- and 100-fold doses had a stimulatory effect on the soil ammonification activity. Furthermore, the results obtained indicate that the stimulatory effect of all the Reglone 200 SL herbicide doses introduced into the soil was visible in the final stage of the experiment, i.e. after 200 days of incubation. Application of the optimum dose of this agent resulted in the highest increase in the ammonification activity, compared with the control values. Throughout the study period, the highest (100-fold increased) dose of the Reglone herbicide applied to the soil was noteworthy, as it induced a significant increase in the intensity of the analysed process in all the experimental periods.

The analysis of the mean values of the examined biochemical activity revealed significantly higher ammonification intensity in soil treated with Reglone 200 SL than in soil receiving Roundup 360 SL. These herbicides applied to the soil only in the 10-fold higher dose induced a significant decrease in the ammonification intensity, whereas the highest (100-fold) dose had a significant stimulatory effect on the investigated activity in the soil (Fig. 51).

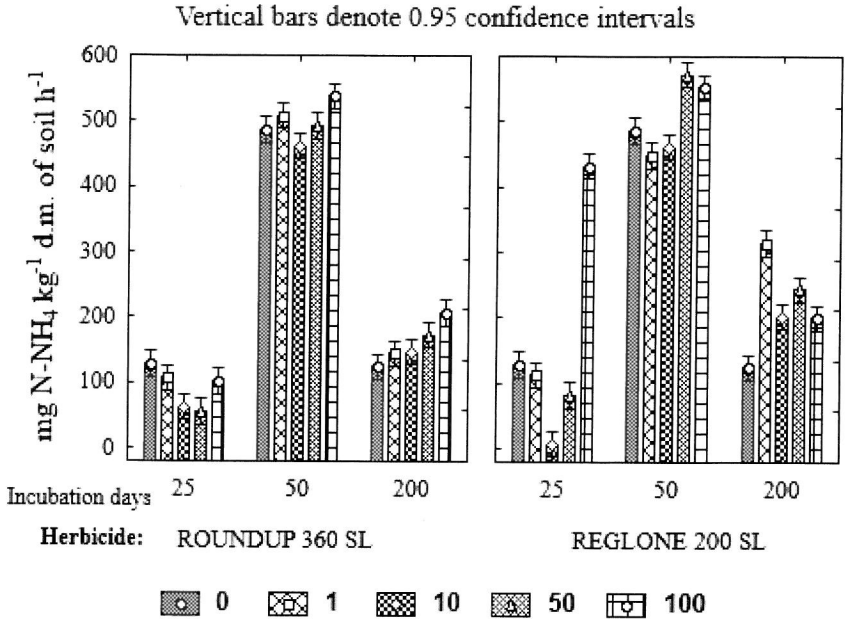

Fig. 51. Temporary amonification rate activity in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

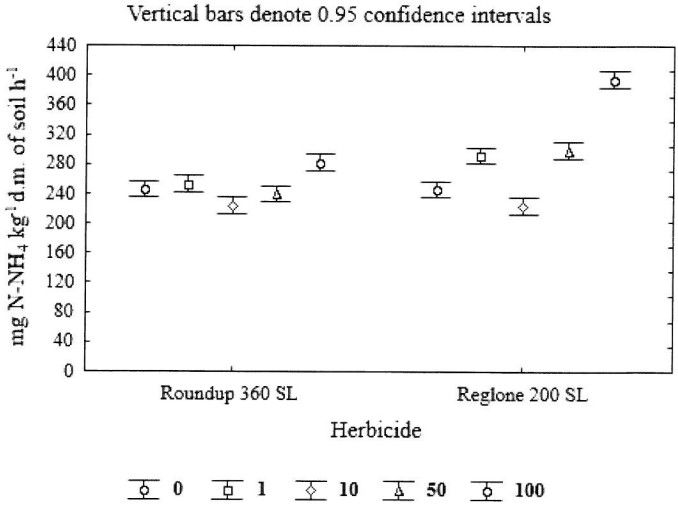

Fig. 52. Mean values of amonification rate activity in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

5.3.2.5. Intensity of the nitrification process

The data presented in Figure 53 indicate that the intensity of the nitrification process underwent periodic fluctuations.

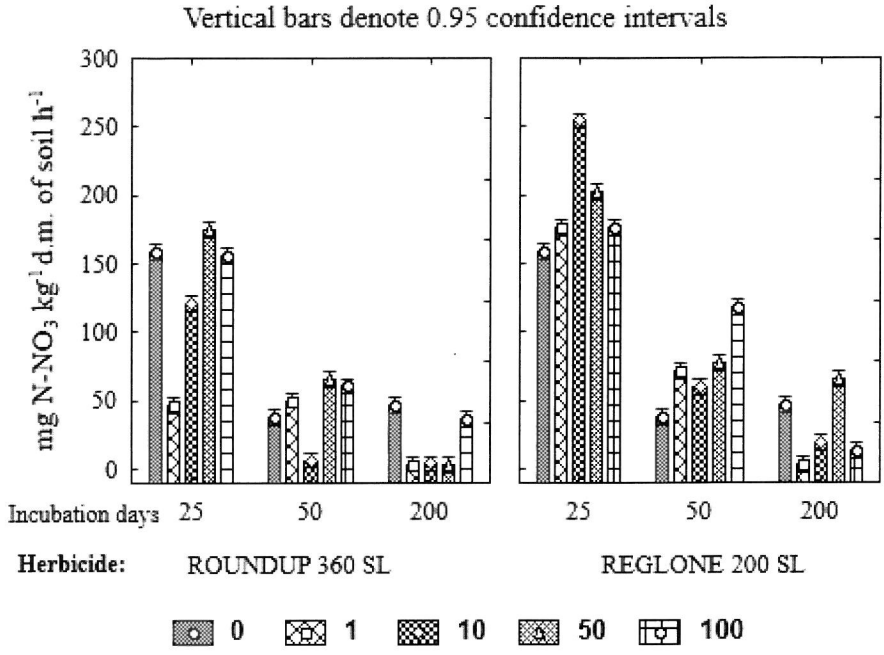

Fig. 53. Temporary nitrification rate activity in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

Application of the optimum dose of the Roundup herbicide to the soil caused the highest decline in the nitrification activity below the value obtained in the control soil on days 25 and 200 of the experiment. The 10-fold higher dose of this herbicide had an inhibitory impact on the analysed activity also on day 25, and the effect persisted until the end of the experiment and was significantly lower than that in the control soil. The highest level of the analysed activity was noted in the object treated with the 50-fold increased herbicide dose on days 25 and 50 of the experiment. Roundup 360 SL introduced into the soil in the 100-fold increased dose induced a significant increase in the intensity of the analysed process only on day 50 of the experiment. Treatment of the soil with the Reglone 200 SL herbicide contributed to changes in nitrification intensity in the soil. In the initial and second stages of the analyses, all the herbicide doses applied exerted a stimu-

latory effect on the course of the analysed process in the soil. The nitrification intensity on day 25 of the analyses (the first stage) was the highest in soil treated with the 10-fold herbicide dose, whereas on day 50 it was the highest in soil supplemented with the 100-fold greater dose. In the final stage of the experiment on incubation day 200, the nitrification rate in soil treated with the 50-fold greater Reglone 200 SL herbicide dose was higher than in the control. In contrast, application of the optimum and 10- and 100-fold higher Reglone 200 SL doses led to a significant decline in the nitrification intensity below the values obtained in the control soil.

Analysis of the mean values of the analysed biochemical activity for the Roundup 360 SL herbicide showed that the application of the optimum and 10-fold higher doses reduced nitrification intensity, compared with the soil receiving the higher doses of the herbicide and with the control soil. Application of the 10-, 50-, and 100- fold Reglone 200 SL herbicide doses significantly stimulated the analysed process in the soil (Fig. 54).

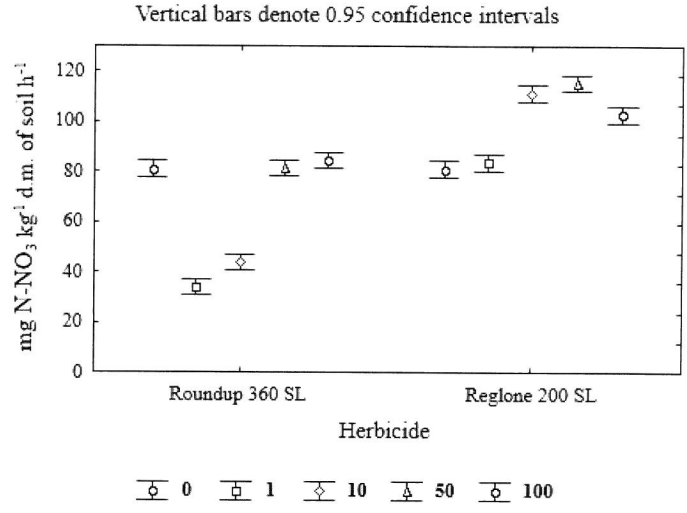

Fig. 54. Mean values of nitrification rate activity in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

5.3.2.6. Acid phosphatase activity

Changes in the acid phosphatase activity induced by the applied doses of the Roundup 360 SL and Reglone 200 SL herbicides are illustrated in Figure 55.

After the application of the different doses of the Roundup 360 SL herbicide, the periodic acid phosphatase activity was generally similar. Importantly, there was a rapid decline in the analysed activity in soil supplemented with the 50-fold

dose on day 50 of the analyses and with the highest dose of the herbicide on days 25 and 50 of incubation. Given this periodic increase, the analysis of variance revealed a significant difference in the mean acid phosphatase activity in the case of the Roundup 360 SL herbicide, in comparison with the value obtained in the control soil and the values for the other doses. A periodic significant increase in the analysed activity was also noted after 200 days of the experiment when the 10- and 100-fold higher Roundup 360 SL doses were applied. In turn, the elevated doses of the Reglone 200 SL herbicide (10-, 50-, and 100-fold) applied to the soil induced a significant decline in the activity of the tested enzyme in the soil on day 25 of the experiment. In the subsequent stage of the analysis, i.e. after 50 days of incubation, a significant decrease in the acid phosphatase activity was observed after application of all the herbicide doses. This effect was most pronounced in soil supplemented with the optimum (technological) dose. Moreover, only in the final stage of the analyses, i.e. after 200 days, all the doses of the Reglone 200 SL herbicide applied to the soil significantly increased the analysed activity, compared with that in the control soil. The highest increase in the activity in this period was reported after application of the 10- and 100-fold higher doses to the soil.

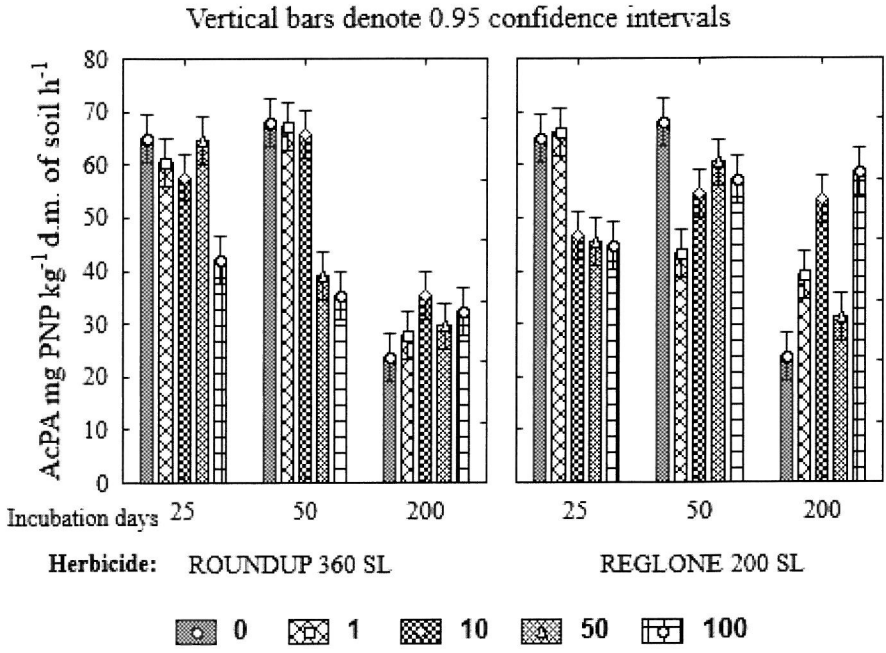

Fig. 55. Temporary acid phosphatase activity (AcPA) in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

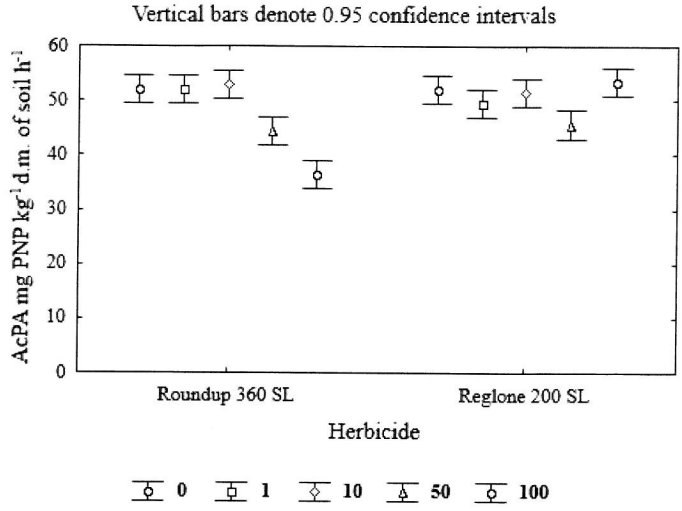

Fig. 56. Mean acid phosphatase activity (AcPA) in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

The mean values of the acid phosphatase activity presented in Figure 56 indicate that application of the 10- and 100-fold higher doses of the Roundup 360 SL herbicide resulted in a significant decline in the activity of the enzyme. In turn, application of the 50-fold higher dose of the Reglone 200 SL formulation reduced the enzymatic activity analysed to lower values than those in the control soil.

5.3.2.7. Alkaline phosphatase activity

The periodic results obtained in the analyses of the impact of the applied doses of the Roundup 360 SL and Reglone 200 SL herbicides on the activity of alkaline phosphatase is presented in Figure 57. Throughout the experimental period, the herbicides introduced into the soil caused substantial periodic fluctuations in the biochemical activity analysed.

Application of the optimum doses of the Roundup 360 SL herbicide stimulated the activity of alkaline phosphatase on days 50 and 200 of the experiment. The results obtained also demonstrated a significant increase in the analysed enzymatic activity on days 25 and 50 of incubation induced by application of the 10-fold Roundup 360 SL dose. Furthermore, the higher (50-fold) dose of the chemical agent caused a significant reduction in alkaline phosphatase activity below the values obtained in the control only in the final stage of the experiment, i.e. after 200 days of incubation. In turn, a significant decline in the activity of alkaline phosphatase was noted in the first stage of the analysis (day 25) after application of the 100-fold

dose. Throughout the experiment, this effect persisted at a considerably lower level than that reported for the control soil. The Reglone 200 SL herbicide used in the experiment modified the activity of the analysed enzyme as well. Its lowest (optimum) dose caused a significant increase in the activity of the enzyme on days 25 and 50 of the experiment. Interestingly, the 10-fold higher dose was the only one that induced a significant increase in the alkaline phosphatase activity throughout the investigation period. In the initial phase of the experiment, i.e. after 25 days, the highest 50- and 100-fold doses significantly inhibited the activity of the analysed enzyme, compared with the control soil and soil supplemented with the other doses. After 50 days, only the 100-fold higher Reglone 200 SL dose still inhibited the alkaline phosphatase activity. In the final stage of the analysis, i.e. after 200 days, all the doses of the chemical agent used in the experiment stimulated the analysed enzyme activity in the soil; in comparison with the other doses, the 100-fold higher dose induced the highest increase in the alkaline phosphatase activity.

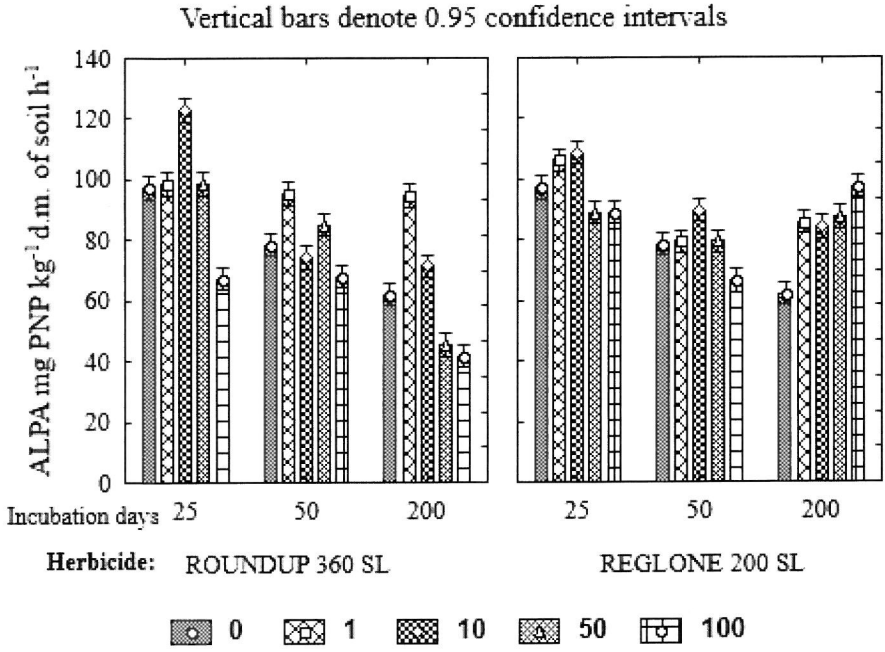

Fig. 57. Temporary alkaline phosphatase activity (ALPA) in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

The analysis of variance revealed that the lowest doses of the Roundup 360 SL herbicide introduced into the soil, i.e. the optimum and 10-fold doses, induced a significant stimulation of the alkaline phosphatase activity in the soil. In con-

trast, increased amounts of the formulation, particularly the 100-fold doses, exerted an inhibitory effect on the soil activity of the enzyme.

Application of Reglone 200 SL to the soil both in the optimum and increased doses significantly increased the mean values of the alkaline phosphatase activity. More pronounced stimulation of the activity was noted at the lower, i.e. optimum and 10-fold increased, doses of the herbicide (Fig. 58).

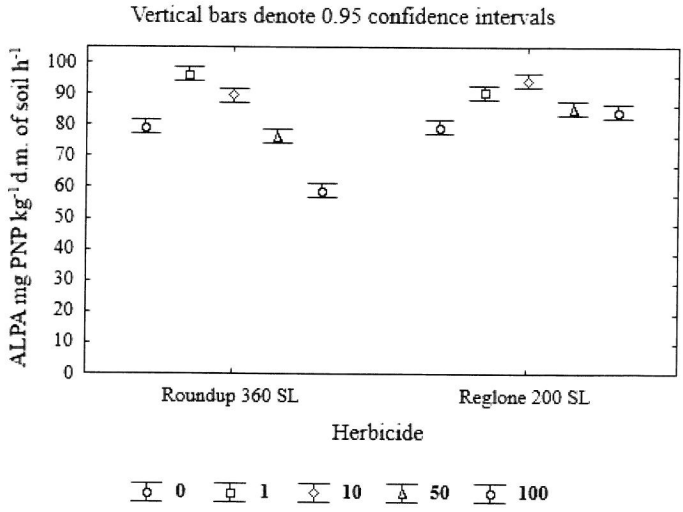

Fig. 58. Mean alkaline phosphatase activity (ALPA) in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

5.3.2.8. Soil respiration activity

The periodic results of the analyses of the impact of the Roundup 360 SL and Reglone 200 SL doses used on the soil respiration activity are presented in Figure 59. Throughout the study period, soil respiration activity was characterised by certain dynamics of changes. All the doses of the Roundup 360 SL herbicide introduced into the soil caused a significant increase in the analysed activity on days 25 and 200 of the experiment. The highest activity was noted on experimental day 25 in soil treated with the 10- and 50-fold higher dose. In the subsequent stages of the analysis, i.e. on day 50, soil respiration activity increased significantly only in the soil receiving the 10-fold dose. The other herbicide doses (optimum, 50-, and 100-fold) induced a significant decline in the amount of emitted CO_2 below the values obtained in the control soil. In the final experimental period, i.e. after 200 days of incubation, all the Roundup 360 SL doses again caused a significant increase in the activity of the analysed soil process. Analysis of the dynamics of the

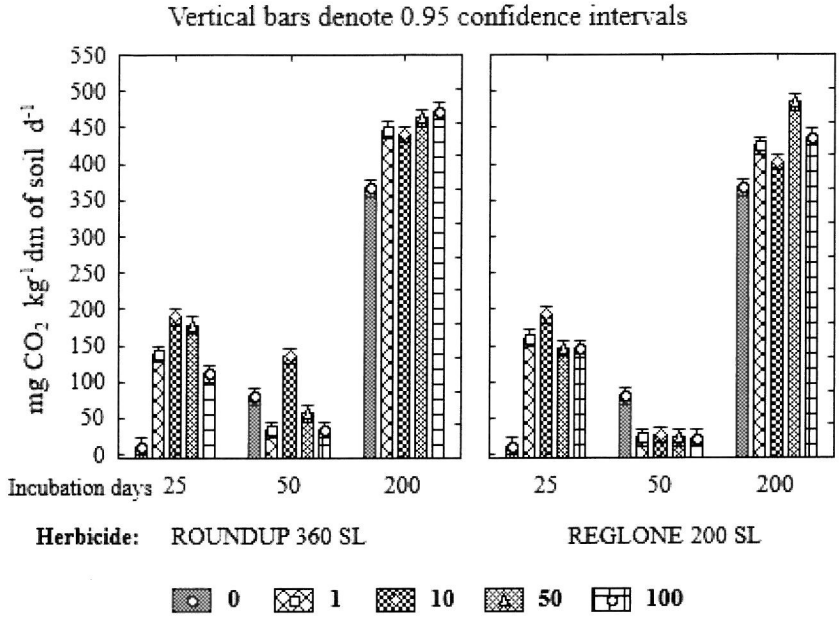

Fig. 59. Temporary respiration activity in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

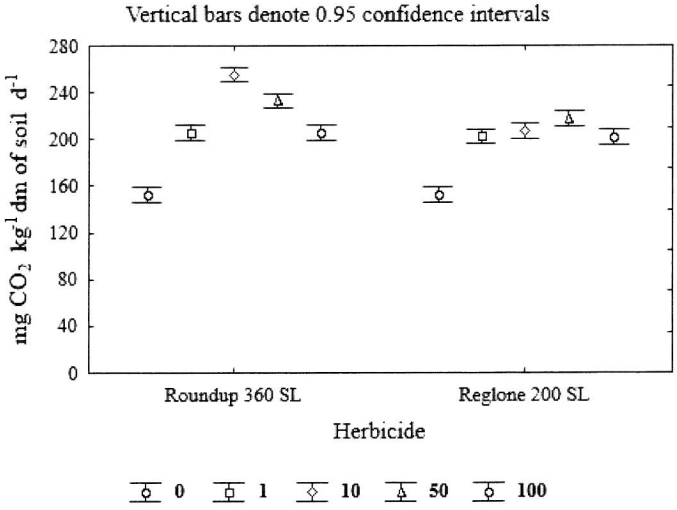

Fig. 60. Mean respiration activity in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL. Explanations as in Figure 37

soil respiration activity after application of all the doses of the Reglone 200 SL herbicide revealed a significant increase in the investigated activity on days 25 and 200 of the experiment and a significant decline on day 50 of incubation. Application of the 10-fold increased herbicide dose to the soil caused the highest stimulation of the respiration activity on days 25 and 200 of the experiment. The results also indicate that on day 50 the effect of the applied doses of the Reglone 200 SL herbicide on soil respiration activity persisted at a more uniform level, although it was lower than in the control soil.

Based on the Tukey's 95% confidence interval, it was found that application of the Roundup 360 SL and Reglone 200 SL herbicides to the soil resulted in a significant increase in the mean rate of the analysed activity. More pronounced stimulation of respiration activity induced by the doses used was noted in soil treated with Roundup rather than Reglone 200 SL (Fig. 60).

5.3.3. Effect of the Roundup 360 SL and Reglone 200 SL formulations on soil chemical properties

In the experiment, determination of the chemical parameters involved soil reaction in the respective investigation periods and the content of organic C and total N in the final stage of the experiment.

The results presented in Table 6 demonstrate that all the Roundup 360 SL and Reglone 200 SL doses introduced into the soil did not induce changes in the soil reaction in the initial stage of the experiment (after 25 days). In contrast, on day 50 of the experiment, the optimum and increased doses of the Roundup 360 SL and Reglone 200 SL formulations increased the values of soil pH by 0.20 and 0.30, respectively. The investigations demonstrate that after 200 days of the experiment the Roundup 360 SL herbicide applied to the soil at the 10- and 100-fold higher doses decreased the reaction of the analysed soil compared with the control (pH = 6.58). In turn, the other doses used in the experiment, i.e. the optimum and the 50-fold higher doses increased the soil pH. Application of the Reglone 200 SL to the soil in the final stage of the experiment increased the soil reaction, with the largest increase (by 0.11) induced by the 10-fold higher dose. The lowest increase in the soil reaction (by 0.02) was found after application of the 100-fold higher dose of the formulation.

The content of organic C and total N in the experimental objects is presented in Table 7. The analyses performed revealed that the Roundup 360 SL herbicide induced an increase in the content of organic carbon and total nitrogen even when doses that exceeded the recommended ones substantially were applied. Additionally, the levels of organic carbon and total nitrogen were found to increase together with the increasing soil contamination caused by the Roundup 360 SL herbicide. Application of the Reglone 200 SL herbicide generally increased the content of organic carbon and total nitrogen as well. The analyses also showed that the

content of organic carbon and total nitrogen was higher in the soil treated with Roundup 360 SL than in soil supplemented with Reglone 200 SL.

The investigation results indicate that the value of the carbon-to-nitrogen ratio was lower in the soil contaminated with Roundup 360 SL than in the control soil. Similarly, application of the Reglone 200 SL herbicide (except for the 10-fold dose) caused a decline in the C:N ratio.

5.3.4. Correlations between the microbiological, biochemical, and chemical properties of the examined soil

For better understanding of the interrelations between microorganisms and their biochemical activity and chemical factors, the correlations between these characteristics were analysed (Tab. 8).

The correlation analysis revealed a strong positive correlation of the total abundance of bacteria with the abundance of bacteria with proteolytic activity, dehydrogenase activity, and soil pH and negative correlations with the intensity of the nitrification process and alkaline phosphatase activity. The total fungal abundance was positively correlated with the abundance of bacteria and fungi with proteolytic activity as well as dehydrogenase activity and soil pH. Furthermore, strong positive correlations were found between the total abundance of bacteria and fungi as well as the abundance of "proteolytic" bacteria and fungi and the intensity of the ammonification process. In turn, a negative value of the correlation coefficient was obtained between the total abundance of fungi and the intensity of the nitrification process. The abundance of bacteria with proteolytic activity was significantly positively correlated with the dehydrogenase, protease, urease, and acid phosphatase activities and the intensity of the ammonification process. Positive values of the correlation coefficient were found between the abundance of "proteolytic" fungi and the dehydrogenase, urease, and acid phosphatase activities as well as the intensity of the ammonification process. In contrast, there was a negative correlation between the abundance of "proteolytic" fungi and the alkaline phosphatase activity and soil respiration. A high and highly significant correlation coefficient was obtained between dehydrogenase activity and the intensity of the ammonification process and soil pH. Moreover, there was a strong negative correlation between the dehydrogenase and acid phosphatase activities and the intensity of the nitrification process. A significant positive correlation ($\alpha = 0.001$) was found between the protease activity and urease activity as well as between acid phosphatase activity and the intensity of the nitrification process. The urease activity was significantly positively correlated at the highest significance level $\alpha = 0.001$ with acid phosphatase activity and intensity of the nitrification process. The analysis performed also demonstrates that the abundance of proteolytic bacteria and the activity of the analysed enzymes were negatively correlated with soil respiration activity. Additionally, a high and highly significant correlation coefficient was obtained between the respiration activity and soil pH.

Table 6. Changes of pH_{KCl} in soil contaminated with herbicides Roundup 360 SL and Reglone 200 SL during the experiment

Dose of herbicides	Terms of analyses (days)		
	25	50	200
Herbicide Roundup 360 SL			
0	6.20	6.20	6.58
1	6.20	6.40	6.67
10	6.20	6.40	6.55
50	6.20	6.40	6.60
100	6.20	6.40	6.54
Herbicide Reglone 200 SL			
0	6.20	6.20	6.58
1	6.20	6.50	6.64
10	6.20	6.50	6.69
50	6.20	6.50	6.62
100	6.20	6.50	6.60

Explanations: 1 – soil + herbicide at dose recommended by the manufacturer; 10 – soil + herbicide at the dose 10 – times higher than recommended by the manufacturer; 50 – soil + herbicide at the dose 50 – times higher than recommended by the manufacturer; 100 – soil + herbicide at the dose 100 – times higher than recommended by the manufacturer

Table 7. Content of organic carbon, total nitrogen and C:N in the individual experimental objects at the end of the experiment (g kg^{-1} d.m. of soil)

Dose of herbicides	Soil properties		C:N
	C-organic	N-total	
Herbicide Roundup 360 SL			
0	9.2	1.4	6.6
1	9.3	1.5	6.2
10	9.4	1.6	5.9
50	9.6	1.8	5.3
100	9.6	1.9	5.0
Herbicide Reglone 200 SL			
0	9.2	1.4	6.6
1	9.4	1.6	5.9
10	9.0	1.2	7.5
50	9.4	1.7	5.5
100	9.4	1.7	5.5

Explanations as in Table 6.

Table 8. Correlation coefficients (R) between microbial and biochemical parameters of soil

Explanatons: 1 – total number of bacteria; 2 – total number of fungi; 3 – number of "proteolytic" bacteria; 4 – number of "proteolytic" fungi; 5 – dehydrogenase activity; 6 – protease activity; 7 – urease activity; 8 – amonification rate; 9 – nitrification rate; 10 – acid phosphatase activity; 11 – alkaline phosphatase activity; 12 – respiration activity; 13 – pH; 14 – doses of herbicide

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	–	n.i.	0.51 ***	n.i.	0.63 ***	n.i.	n.i.	0.48 ***	0.52 ***	n.i.	0.40 ***	n.i.	0.39 ***	0.24 *
2		–	0.28 **	0.36 ***	0.49 ***	n.i.	n.i.	0.46 ***	– 0.40 ***	n.i.	n.i.	n.i.	0.45 ***	0.25 *
3			–	n.i.	0.28 **	0.30 **	0.31 **	0.41 ***	n.i.	0.22 *	n.i.	0.25 *	n.i.	0.31 **
4				–	0.30 **	n.i.	0.29 **	0.66 ***	n.i.	0.22 *	0.23 *	0.42 ***	n.i.	n.i.
5					–	n.i.	0.27 *	0.60 ***	0.70 ***	0.28 **	0.47 ***	0.30 **	0.76 ***	n.i.
6						–	0.40 ***	n.i.	0.27 *	0.30 **	n.i.	0.29 **	0.33 **	0.27 **
7							–	0.30 **	0.42 ***	0.52 ***	0.24 *	0.64 ***	0.57 ***	0.25 *
8								–	0.33 **	n.i.	0.25 *	0.47 ***	n.i.	n.i.
9									–	0.26 *	0.45 ***	0.46 ***	0.72 ***	n.i.
10	Explanations: *** – correlation coefficient significant at significance level α = 0.001									–	0.51 ***	0.60 ***	0.57 ***	n.i.
11	** – correlation coefficient significant at significance level α = 0.01										–	0.28 **	0.45 ***	0.22 *
12	* – correlation coefficient significant at significance level α = 0.05 n.i. – non significant											–	0.65 ***	n.i.
13													–	n.i.
14														–

6. SUMMARY OF RESULTS AND DISCUSSION

The growing global population size and the increasing demand for food necessitate permanent development of agricultural production through intensified crop cultivation (Chowdhury *et al.* 2008). However, production of high yields with desired quality traits requires not only adequate fertilisation, but also intensive crop protection (Łozowicka and Bułatowicz 2009, Mandić et al. 2005, Wyszkowski and Wyszkowska 2004). Despite its clear benefits related to raising the level of agricultural production (Doruchowski 2005), chemical intensification often brings about numerous adverse environmental effects (Błaszak and Nowak 2006, Das *et al.* 2003). Many studies have shown that prolonged, intense use of pesticides poses a threat to not only weeds and pests of crops, but also to soil organisms (Adamczewski and Banaszak 2000). The most frequent side effect of agrotechnical treatments is penetration of soil by a variety of toxic substances and elements contained in pesticides (Przybulewska and Nowak 2004 ab, Vig *et al.* 2008). This is particularly important in the case of application of biocides characterised by a slow degradation rate and a long period of retention in soil, as they raise the risk of contamination with the agents themselves but also with their transformation products. Excessive accumulation of toxic substances in the environment can modify many metabolic processes in soil and lead to disturbances in the soil biological equilibrium (Cycoń and Kaczyńska 2004, Kieliszewska-Rokicka 2001).

Pesticides are regarded as substances with high biological activity; therefore, after penetrating the soil, they can exert various impacts on its biological life (Baćmaga *et al.* 2010, Das and Mukherjee 2000, Wyszkowska 2002). According to Przybulewska and Nowak (2004ab), the response of microorganisms to pesticides depends on several factors, among which the type of the active substance contained in preparations, the sensitivity of the microflora, and soil conditions are of great importance. Furthermore, the soil microflora may exhibit differences in sensitivity to pesticides between microbial groups and within species and strains.

Our results have shown that application of the chemical formulations Roundup 360 SL, Reglone 200 SL, Basta 150 SL Avans Premium 360 SL, Spodnam 555 EC, and Caramba 60 SL to the soil disturbed the soil microbial balance. The results obtained indicate varied effects of the plant protection products on the analysed microbial groups, as both stimulation and inhibition of microbial growth by the tested chemicals were observed. This effect can be explained by the ability of some microorganisms to grow in the presence of pesticides as well as degrade them and utilise organic compounds contained therein, as highlighted by many authors (Adamczewski and Banaszak 2000). A majority of chemical plant protection products contain carbon, hydrogen, oxygen, nitrogen, and chlorine (Johnsen et al. 2001, Soulas and Lagacherie 2001), which can be utilised by certain micro-

organisms as a source of energy and nutrients (Baćmaga et al. 2010, Błaszak and Przybulewska 2011). Therefore, biocides can be an excellent substrate increasing the abundance of microorganisms in soil (Johnsen et al. 2001) or they can exert a toxic effect on other microorganisms by inhibition of microbial growth and decreasing microbial abundance (Das and Mukherjee 2000). Pesticides applied in recommended doses do not typically cause significant changes in microbial abundance in soil (Wyszkowska 2002, Wyszkowska and Kucharski 2004, Jastrzębska 2006). In turn, application of excessive amounts of chemical agents to soil may have an adverse effect on soil microflora, which was supported by our research and other authors (Niewiadomska *et al.* 2005).

Our field and pot investigations have indicated a significant effect of the type, dose, and duration of action of the applied plant protection products on the level and direction of changes in bacterial and fungal abundance. The Roundup 360 SL formulation applied in a dose recommended by the manufacturer and the 10% higher dose did not induce disturbances in the bacterial abundance in the soil. The optimum and increased doses of Reglone 200 SL and Basta 150 SL stimulated bacterial growth in the examined soil. Additionally, increased abundance of this microbial group was observed after application of the Avans Premium 360 SL and Spodnam 555 SC formulations and there was an insignificant decline in the bacterial abundance induced by application of the Caramba 60 SL herbicide. Our findings from the field experiments showed that the optimum and increased doses of the Roundup 360 SL, Reglone 200 SL, and Basta 150 SL preparations and the Avans Premium 360 SL and Spodnam 555 SC doses recommended by the manufacturers did not have a negative impact on fungi. A slight decrease in fungal abundance, although not confirmed statistically, was evident only in soil treated with the optimum dose of the Caramba 60 SL formulation. Out pot experiment demonstrated a positive effect of the Roundup 360 SL and 200 SL Reglone herbicides on the bacterial and fungal abundance. The stimulatory effect of the chemical agents on the analysed abundance may have been caused by the ability of these microorganisms to degrade the tested pesticides and utilise nutrients contained therein. Among all microorganisms, fungi exhibit a relatively high ability to degrade biocides, which is associated with the activity of their constitutive enzymes. Substances contained in plant protection products can also be a source of nutrition for these microorganisms (Kaszubiak and Durska 2000, Jastrzębska and Kucharski 2005). The positive effect of the chemicals on soil microbial abundance was confirmed by other authors as well (Jezierska-Tys and Rutkowska 2013). The authors cited herein reported that 10- and 100-fold higher doses than the recommended ones were able to inhibit proliferation of bacteria of the genus *Azotobacter* and *Pseudomonas* as well as oligotrophic bacteria and actinomycetes. In their investigations of the effect of the glyphosate herbicide, Araujo *et al.*

(2003) observed that application of 2.16 mg kg^{-1} of the chemical to soil caused a decrease in bacterial abundance and stimulated growth of actinomycetes and fungi. The analysis of the effect of the Chwastox Trio 540 SL herbicide on different microbial groups carried out by Wyszkowska and Kucharski (2004) revealed that the optimum doses of the chemical increased the abundance of copiotrophic bacteria and actinomycetes but inhibited fungal growth. Moreover, the authors reported that, in comparison with the optimum dose, the 5- and 10-fold higher doses of the herbicide had a negative effect on fungi, cellulolytic, oligotrophic, copiotrophic, and sporulating bacteria, and on genus *Azotobacter* bacteria. Krzyśko-Łupicka and Grata (2008) investigated the effect of the phosphoorganic Roundup herbicide on the abundance and nitrogen fixation capacity of soil non-symbiotic microorganisms (diazotrophs). After 30 days, the tested chemical caused periodic stimulation of the abundance of the non-symbiotic diazotrophs. After 90 days, a 10-fold decrease in the abundance was reported. Jezierska-Tys and Rutkowska (2013) demonstrated that the optimum doses of the Reglone 200 SL and Elastiq 550 SC plant protection products applied to soil increased bacterial and fungal abundance.

In our investigations carried out in both field and laboratory conditions, evident response of proteolytic bacteria and fungi to the applied pesticides was reported. The rate and direction of the changes observed in the analysed microbial groups depended on the type and dose of the chemical formulation applied as well as the duration of its impact. The field experiments showed that the abundance of "proteolytic" bacteria was decreased by the Roundup 360 SL formulation. In the case of the Reglone 200 SL herbicide, a slight increase in the abundance of the examined microbial group was found in soil treated with the 10% higher dose. The same dose of the Basta 150 SL chemical in soil caused a significant decline, in comparison with the soil treated with the recommended dose. The optimum doses of Caramba 60 SL, Spodnam 555 SC, and Avans Premium 360 SL had an insignificant modifying effect of the growth of "proteolytic" bacteria in soil. The abundance of fungi with proteolytic activity was significantly decreased by the Roundup 360 SL, Reglone 200 SL, and Basta 150 SL herbicides. This effect was particularly evident in the final period of the experiment, i.e. after 22, 24, and 26 months, which implies that prolonged biocide retention in soil does not lead to reduction in their effect on protein-degrading fungi. The abundance of the investigated microorganisms decreased together with application of higher amounts of these chemicals in soil. The increase in the abundance of "proteolytic" fungi was caused by the Caramba 60 SL herbicide. Avans Premium 360 SL and Spodnam 555 SC inhibited fungal growth in a statistically insignificant manner. In laboratory conditions at constant humidity and temperature, the Roundup 360 SL and Reglone 200 SL herbicides applied in substantially higher amounts than the optimum doses stimulated proliferation of bacteria with proteolytic activity. In turn,

in controlled conditions, the abundance of protein-degrading fungi declined under the impact of all the doses of these formulations. In our previous field investigations (Jastrzębska 2010), application of the optimum dose of the Reglone 200 SL herbicide in the soil was shown to stimulate proliferation of "proteolytic" bacteria and fungi. The Elastiq 550 EC herbicide contributed to a decrease in the abundance of protein-degrading bacteria. Błaszak and Nowak (2006) observed that a 100 mg dm^{-3} dose of the Miedzian 50 WP fungicide elicited a similar response of bacteria and fungi with proteolytic activity to the herbicides used. Similarly, Michalcewicz (2001) reported such changes in the abundance of these microbial groups after application of Pikot 100 SL and Sencor 70 WG formulations.

The effect of biocides on soil microorganisms changes in time. This is primarily related to the gradual degradation of pesticides in soil (Niewiadomska *et al.* 2009). A longer time of retention of plant protection products in soil can both increase and reduce their toxic effects. This relationship was observed in our investigations. In the field experiment, the recommended and increased doses of the Roundup 360 SL and Reglone 200 SL herbicides induced a significant increase in bacterial abundance immediately after application, and inhibited the growth of this microbial group after 26 months (stage VIII). In soil treated with the Reglone 200 SL and Basta 150 SL herbicides, the fungal abundance was reduced in analysis stage VIII. In turn, both doses of the Roundup 360 SL, Reglone 200 SL, and Basta 150 SL herbicides applied in the soil decreased the abundance of bacteria and fungi with proteolytic activity after 26 months of interaction in soil. Inhibition of microbial growth after such a long period may be associated with accumulation of products of decomposition of the active substance contained in these formulations. According to Jastrzębska (2010), intermediate metabolites can exhibit considerably greater toxicity than primary compounds, which intensifies their negative impact on soil microorganisms. The results obtained in the pot experiment indicate that the Roundup 360 SL and Reglone 200 SL formulations stimulated bacterial and fungal proliferation and growth of bacteria with proteolytic activity even after 200 days. The tendency towards the intensified growth of these microbial groups reported in the final stage of the pot experiment implies slow decomposition of nutrients introduced into the soil with the herbicides and utilisation thereof by microorganisms. This resulted in stimulation of proliferation of these groups of microorganisms. Noteworthy, the effects of the Roundup 360 SL and Reglone 200 SL herbicides on the same soil microbial groups observed in the field experiment differed from that found in the laboratory conditions. Our investigations revealed that in natural conditions Roundup 360 SL and Reglone 200 SL caused a decrease in the so-called total fungal abundance and in the abundance of

bacteria with proteolytic activity. In turn, in controlled conditions, both herbicides stimulated proliferation of these microbial groups in the examined soil. The different impact of the chemicals on the same group of microorganisms observed in the field and pot experiments was likely to be dependent on environmental factors such as humidity or temperature. Throughout the pot experiment, the soil was maintained at a constant moisture level (60% of total water capacity) and incubated at a constant temperature (ca. 20°C). In natural conditions, the soil was influenced by variable weather conditions, which may have resulted in periodic changes in humidity and temperature. According to Przybulewska *et al.* (2004), temperature, humidity, and nutrient contents have a significant impact on microbial growth in soil and determine the impact of pesticides on microorganisms and their activity in the soil. Przybulewska and Nowak (2004b) emphasise that in the natural conditions of variable temperature the impact of biocides on soil microflora and microbial processes may differ significantly from the effects exerted at the constant temperature of laboratory conditions. In turn, Krzyśko-Łupicka and Grata (2008) argue that soil microorganisms are more responsive to environmental changes caused by climate conditions as well as agrotechnical treatments and plant vegetation than to the pesticide itself. According to these authors, investigations on the effect of biocides on microorganisms and soil properties should be carried out in controlled conditions using laboratory tests. Since chemical plant protection products are designed for application in crop fields, it is advisable that their impacts should be tested in the real conditions of field experiments.

During investigations, considerable differences in the interactions of the same pesticides in soil and in their effect on microorganisms can frequently be observed. This is associated with the variability of the soil environment induced by the content of specific organic and mineral compounds, humidity temperature, pH, and agrotechnical factors (Baćmaga *et al.* 2010). Our field experiments indicated that, although the Roundup 360 SL and Avans Premium 360 SL herbicides belong to the same group of pesticides and contain the same active substance (glyphosate), they exerted significantly different effects on the so-called total abundance of bacteria and fungi with proteolytic activity. Both field experiments were established and conducted at the same time and in the same experimental station. Additionally, all experimental plots were established on the same type of soil and sown with winter rapeseed cv. "Californium". The basic agricultural treatments and fertilisation followed the recommendations developed for the cultivation of the plant. This helped to exclude, to some extent, the impact of all these factors on the different interactions between the pesticides and abundance of the microbial groups. The differences in the microbial abundance reported in our

study were probably induced by the presence of additional surfactants and carrier substances in the formulations used. According to Skoczko (2013), glyphosate is a herbicide with low toxicity and persistence, hence it does not accumulate in the food chain and poses no threat to the environment. In addition, it is quickly adsorbed in the soil and decomposed by soil organisms. However, commercial preparations containing glyphosate as the active substance and additional surface-active compounds and carrier substances can exhibit substantially higher toxicity. Therefore, the observed differences in the effects of glyphosate-containing pesticide formulations on the total bacterial abundance and the abundance of "proteolytic" bacteria and fungi were probably associated with the different composition of these pesticides.

Enzymatic activity is one of the widely used indicators of changes induced by chemical substances in the soil environment (Jezierska-Tys and Rutkowska 2013, Kucharski *et al.* 2009a). The effect of biocides on this activity is varied and strongly dependent not only on the type of the analysed enzyme but also on the type, dose, and length of retention of the chemical product in soil, which was confirmed by our investigations and other authors' studies (Siwek *et al.* 2008).

The activity of enzymes involved in phosphorus metabolism in the soil can serve as an indicator in assessment of the effect of pesticides on changes in the soil environment (Niewiadomska *et al.* 2009). Our investigations carried out in field conditions showed that the optimum doses of the Roundup 360 SL, Reglone 200 SL, Basta 150 SL, Avans Premium 360 SL, Spodnam 555 SC, and Caramba 60 SL herbicides caused a statistically insignificant increase in the activity of acid phosphatase in the examined soil. Avans Premium 360 SL, Spodnam 555 SC, and Caramba 150 SL generally stimulated the activity of the enzyme not only in the initial stage of the experiment, but also in the final (VIII) stage of the analyses, i.e. 26 months after application thereof. The 10% higher dose of the Reglone 200 SL herbicide significantly inhibited the activity of the enzyme. Our investigations carried out in field conditions indicated that the activity of alkaline phosphatase was enhanced by the application of the increased dose of the Roundup 360 SL and Reglone 200 SL herbicides, whereas both doses of Basta 150 SL stimulated the alkaline phosphatase activity. Significant inhibition of the enzyme activity was noted in the soil treated with the Avans Premium 360 SL and Spodnam 555 SC herbicides, while Caramba 60 SL insignificantly reduced the activity of alkaline phosphatase. In turn, in controlled (laboratory) conditions, significant inhibition of acid phosphatase in soil was observed after application of the 50-fold higher dose of Reglone 200 SL and the 50- and 100-fold higher Roundup 360 SL doses. It was also demonstrated that the 50- and 100-fold higher Roundup 360 SL

doses significantly inhibited the activity of alkaline phosphatase. The technological and 10-fold higher doses significantly stimulated this activity. The activity of alkaline phosphatase was also significantly enhanced after application of all the Reglone 200 SL doses tested to the soil. A vast majority of investigations focused on assessment of the effect of various biocides on the activity of phosphatases in soil indicate an adverse impact of these substances on the discussed activity (Jezierska-Tys and Rutkowska 2013). A toxic effect of the glyphosate herbicide on the activity of phosphatases was reported by Sannino and Gianfreda (2001). A clear decline in the activity of phosphatases in soil was also noted by Bielińska and Pranagal (2007). Lowered phosphatase activity was reported by Kucharski et al. (2009a) after application of the Harpun 500 SC formulation to soil. Investigations conducted by Baćmaga *et al.* (2010) in the conditions of a pot experiment demonstrated a 16% reduction in the activity of acid phosphatase induced by a 200-fold higher dose of the Akord 180 OF herbicide. In turn, after application of some herbicides (Izoturon 500 SC, Aminopielik Super 464 SL, and Rokituron D 470 SC), Nowak *et al.* (2006) reported a stimulatory effect of the chemicals on phosphatase activity in soil. Increased doses of Izoturon 500 SC and Rokituron D 470 SC had an inhibitory effect on the activity of alkaline phosphatase in soil. Niewiadomska *et al.* (2009) observed that application of a herbicide and fungicide to soil evidently stimulated acid phosphatase activity in the initial stage of their experiment. However, with time, i.e. after 60 days, the activity was significantly reduced. According to the authors, the significant reduction in the acid phosphatase activity was probably related to an increase in the content of mineral phosphorus in the soil triggered by microorganisms.

The activity of dehydrogenases is another good indicator of transformations occurring in soil under the impact of pesticides. As indicated in the study of Niewiadomska *et al.* (2009), these enzymes are characterised by high sensitivity to chemicals applied to soil, in particular herbicides and fungicides. Similarly, Kieliszewska-Rokicka (2001) emphasizes the very high sensitivity and distinct responses of dehydrogenases to biocides. Our analyses performed in laboratory conditions showed that application of chemical plant protection products to soil both stimulated and inhibited dehydrogenase activity. Both herbicides tested, i.e. Roundup 360 SL and Reglone 200 SL, applied in the technological and higher doses significantly enhanced dehydrogenase activity in the soil. In the case of the Roundup 360 SL herbicide, the activity increased along with the amount of the chemical applied in the soil. In their analyses of acetamipirid, a pesticide from the group of insecticides, Singh and Kumar (2008) demonstrated a similar 22% increase in dehydrogenase activity. A stimulatory effect of the mancozeb fungicide

was also reported by Rasool and Reshi (2010). Earlier investigations (Mocek-Płóciniak 2010) confirmed the stimulation of the activity of this enzyme by the field dose of the Reglone 200 SL herbicide. Dehydrogenases are common soil enzymes and exhibit abilities to decompose soil organic compounds (Januszek 1999). The enhancement of their activity observed in our study may have been caused by organic compounds contained in the pesticides. Additionally, soil dehydrogenase activity indicates the presence of physiologically active microorganisms (Kumar 2011), which are likely to have the ability to degrade and utilise the chemical components of pesticides. The high activity of these enzymes in soil treated with biocides may be related to the stimulatory effect of the chemical substances on the growth of some microbial groups and hence the increased amounts of secreted enzymes. In our investigations (conducted in laboratory conditions), a positive correlation was found between bacterial and fungal abundance and the activity of dehydrogenases, which indicates enhancement of the activity accompanying increased microbial growth. According to Bielińska (2001), dehydrogenase activity is strongly correlated with the content of organic C and total nitrogen in soil. Since the herbicides used in the pot experiment increased the content of organic C and total nitrogen, the enhancement of the dehydrogenase activity may have been caused by the increased levels of the two components in the examined soil.

Protease is an enzyme that reacts to pesticides penetrating the soil. Its activity may indicate the soil biological capacity of enzymatic conversion of the substrate. One of the major functions of the enzyme is involvement in nitrogen mineralisation, which determines the availability of this element to plants (Jezierska-Tys and Rutkowska 2013). Our pot experiment showed that high doses of Roundup 360 SL and SL 200 Reglone (50- and 100-fold higher) significantly stimulated the proteolytic activity of the soil. In an experiment with two different pesticides (endosulfan and chlorpyrifos), Kumar (2011) observed enhanced soil proteolytic activity induced by low doses (1-10 mg kg^{-1}) of these pesticides. Higher concentrations (50 mg kg^{-1}) of both these chemicals caused a significant reduction in the activity. Enhancement of protease activity induced by field doses of chlorothalonil and propiconazole fungicides was also reported by Ramudu *et al.* (2012). Earlier studies (Baćmaga *et al.* 2010) confirm the stimulatory effect of the optimum dose of the Reglone 200 SL herbicide on soil proteolytic activity. In turn, Srinivasulu *et al.* (2010) reported that such pesticides as monocrotophos, mancozeb, chlorpyrifos, and carbendazim applied in higher than the recommended doses significantly decrease the proteolytic activity. Similar results were obtained by Rasool and Reshi (2010) in their experiment on the Mancozeb formulation.

Another enzyme involved in the nitrogen conversion in soil and reacting to soil contamination is urease (Jezierska-Tys and Rutkowska 2013). According to Ramudu *et al.* (2012), reduction of the activity of the enzyme in soil may depend not only on the decline in ion concentration and pH but also on pesticide contamination. Our laboratory investigations showed that the Roundup 360 SL 200 and SL Reglone formulations applied in field and substantially increased doses had a positive effect on the activity of the enzyme. Stimulation of urease activity by the optimum dose of the Reglone 200 SL herbicide was also found in our previous study (Barabasz 1992, Emmerling *et al.* 2002). According to Cycoń *et al.* (2010), urease is strongly bound with organic matter and soil mineral particles, thereby being protected against degradation and denaturation. Therefore, Ramudu *et al.* (2012) argue that the enzyme may be more stable and exhibit greater resistance to the environmental stress. However, a majority of available investigations of the effect of various pesticides on urease activity in the soil environment indicate that large doses of biocides significantly reduce activity of the enzyme. In their tests of the Harpun 500 SC formulation, Kucharski *et al.* (2009a) reported a significant reduction in urease activity induced by the optimum dose of the chemical. Similarly, Baćmaga *et al.* (2010) reported a negative effect of herbicides, especially when applied in excessive doses, on the enzyme activity. In their analyses of the Akord 180 OF formulation on urease activity, the authors observed a reduction by 19.0% and 41.1% caused by application of 10- and 200-fold higher doses of the chemical agent, respectively. Cycoń *et al.* (2010) found a negative impact of high concentrations of fungicides (mancozeb and dimetomofr) on soil urease activity as well. Nitrogen is one of the most important biogenic elements in nature, and the processes of ammonification and nitrification indicate its transformations in soil (Jezierska-Tys 2002, Shi *et al.* 2004). These processes play a significant role in the nitrogen cycle in soil and serve as important indicators of its biological activity. Measurement of the ammonification and nitrification rate is a common method for identification of the impact of various factors on the biological status of the soil environment (Johnsen *et al.* 2001). Our investigations indicate that chemical plant protection products used in agricultural practice exert an effect on the biochemical processes involved in the C and N cycle in soil. Experiments carried out in laboratory conditions showed that the highest intensity of ammonification in soil treated with the 100-fold higher dose of the Roundup 360 SL and Reglone 200 SL formulations. The optimum and 50-fold higher doses of the Reglone 200 SL herbicide yielded a significant increase in the intensity of ammonification as well. In our pot experiment, an inhibitory effect of both these formulations on the intensity of the process was noted. Strong inhibition of ammonification intensity was caused by soil application of the 10-fold higher dose of both Roundup 360 SL and Reglone 200 SL. In contrast, Kara *et al.* (2004) ob-

served that the Topogard 50 WP herbicide increased the intensity of ammonification in neutral and alkaline soils and reduced the intensity of the process in acidic soils. A stimulatory effect of pesticides from the fungicide group on the ammonification process in soil was also reported by Monkiedje and Spiteller (2002). An increased concentration of ammonia nitrogen in soil persisting at a constant, high level throughout the 90-day experimental period was noted by Krzyśko-Łupicka (2008) in an experiment with the Roundup herbicide used in the field dose. Laboratory assessment of the effect of applied chemicals on the intensity of nitrification showed that the optimum and 10-fold higher doses of Roundup 360 SL significantly reduced the intensity of the process. In contrast, application of the Reglone 200 SL herbicide, particularly in excessive doses, i.e. 10-, 50-, and 100-fold higher than the recommended dose, resulted in significant enhancement of soil nitrification. Research conducted by Kara *et al.* (2004) showed a decline in the activity of nitrifying bacteria in soil and, hence, reduction in the amount of $N\text{-}NO_3$ in soils treated with the Topogard 50 WP herbicide. Similarly, Krzyśko-Łupicka (2008) found a significantly decreased nitrate nitrogen concentration, however, only during the first 30 days after application of the Roundup herbicide to soil. Kucharski et al. (2009c) reported that the Faworyt 300 SL formulation increased the nitrification rate, whereas contamination of soil with the Harpun 500 SC, Akord 180 OF, and Mocarz 75 WG herbicides reduced the amount of nitrate nitrogen in the soil.

An important indicator commonly used for assessment of the condition of the soil environment is the respiration activity, measurement of which facilitates estimation of potential disturbances in carbon transformation processes caused by the adverse effect of pesticides and other xenobiotics. In our pot experiments, no decrease in the level of CO_2 emitted from soils contaminated with plant protection products was observed. The results obtained indicate that in controlled conditions with a constant temperature and humidity the tested Roundup 360 SL and Reglone 200 SL preparations applied in significantly higher (10-, 50-, and 100-fold) doses than the recommended ones significantly stimulated the respiration activity of the soil. The stimulation was higher in soil treated with the Roundup 360 SL herbicide rather than Reglone 200 SL. All doses of these preparations induced a substantial increase in the amount of emitted CO_2 in the initial and final stages of the experiment, i.e. after 25 and 200 days, respectively. Plant protection products contain e.g. organic compounds, which can be a source of energy and nutrients for some microorganisms (Jezierska-Tys and Rutkowska 2013). The enhancement of soil respiration activity observed was probably related to rapid microbial growth resulting from decomposition of biocides and gradual utilisation of carbon and nitrogen contained in these preparations. Investigations carried out by Kara *et al.* (2004) demonstrated that the 50 WP Topogard herbicide decreased the amounts of CO_2 emitted in acidic

soil. When applied to neutral and alkaline soils, the herbicide stimulated soil respiration activity in the initial stage of the experiment. In their analyses of samples of soil contaminated with insecticides (triazophos and endosulfan), Vig *et al.* (2008) did not find a negative effect of the formulations on soil respiration. Černohlávková *et al.* (2009) reported that application of low doses of mancozeb and dinocap fungicides in soil genrally stimulated the respiratory activity. In turn, Yao *et al.* (2006) found an adverse impact exerted by even the field dose of acetamipirid on soil respiration. Similar results were obtained by Niewiadomska *et al.* (2009) in their experiment with the Fox 480 herbicide (active substance – bifenox).

Chemical plant protection products have an effect on not only soil microorganisms and enzymes but also soil chemical properties. Higher pH values were reported throughout the experimental period in the soil treated with the Reglone 200 SL herbicide than in the soil supplemented with Roundup 360 SL.

The analyses indicated that the content of organic C in all the experiments increased after application of all the plant protection products, whereas the content of total N in the field experiments was increased by the optimum doses of Roundup 360 SL, Avans Premium 360 SL, and Spodnam 555 SC. The content of total nitrogen in the pot experiment was also increased by the Roundup 360 SL and Reglone 200 SL herbicides. The research conducted by Jastrzębska (2010) showed increased soil pH after application of the Unix 75 WG fungicide and a slight decrease induced by Nomolt 150 SC and Durban 480 EC. The author observed that none of the above products significantly affected the levels of organic carbon in the soil.

A correlation analysis was performed in order to demonstrate the relationships between microorganisms and their biochemical activity in soil treated with chemical plant protection products. It revealed relationships between microorganisms and their biochemical activity and soil chemical properties. Within the microbial groups examined, there were correlations indicating interactions between the microorganisms. A positive correlation was found between the total fungal abundance and the abundance of bacteria and fungi with proteolytic activity, which implies that the increase in the total fungal abundance was accompanied by an increase in the abundance of "proteolytic" bacteria and fungi. Similarly, a positive correlation between the abundance of "proteolytic" bacteria and fungi was observed in the field experiments. A correlation between the abundance of both these microbial groups and the activity of alkaline phosphatase was found in the field experiment and laboratory conditions. Additionally, positive correlation coefficients were obtained between the activity of acid and alkaline phosphatase in the field experiment. Previous investigations of Jezierska-Tys and Rutkowska (2013) revealed correlations between the microorganisms and their biochemical activity in soil treated with the chemical agents as well. In these analyses, a strong positive correlation was found between the total bacterial abundance and the

abundance of bacteria and fungi with proteolytic activity. The abundance of fungi with proteolytic activity was positively correlated with the activity of protease, acid and alkaline phosphatases, and soil pH.

7. CONCLUSIONS

1. The present investigations showed that contamination of soil with the Roundup 360 SL, Reglone 200 SL, Basta 150 SL, Avans Premium 360 SL, Spodnam 555 SC, and Caramba 60 SL plant protection products led to disturbances in the microbial abundance and enzyme activity.

2. The Roundup 360 SL, Reglone 200 SL, and Basta 150 SL herbicides applied in optimum and slightly increased (by 10%) doses increased bacterial and fungal abundance and caused a decline in the abundance of "proteolytic" fungi.

3. The application of the increased doses of the Roundup 360 SL and Reglone 200 SL doses (10-, 50-, and 100-fold higher than the recommended ones) did not induce negative changes in the abundance of bacteria, fungi, and bacteria with proteolytic activity. The highest doses of these formulations had an inhibitory effect only on the abundance of fungi with proteolytic activity.

4. The Spodnam 555 SC herbicide exerted a stronger effect of the analysed microbial groups than the Avans Premium 360 SL formulation. The optimum dose of Spodnam 555 SC stimulated proliferation of bacteria, fungi, and "proteolytic" fungi in soil.

5. The Reglone 200 SL herbicide applied in the technological dose increased the activity of protease and acid and alkaline phosphatases. Additionally, the increased doses of the agent, in particular the 50- and 100-fold higher doses, significantly stimulated the activity of dehydrogenases, urease, and proteases in the analysed soil.

6. The Roundup 360 SL herbicide introduced into the soil in doses that were 50- and 100-fold higher than the recommended ones stimulated the activity of dehydrogenases, protease, and urease and inhibited the activity of acid and alkaline phosphatases.

7. The analyses indicate that the Avans Premium 360 SL, Spodnam 555 SC, and Caramba 60 SL chemicals applied in the soil in doses recommended by the manufacturer (optimum dose) caused inhibition of alkaline phosphatase activity and an insignificant increase in the activity of acid phosphatase.

8. The Roundup 360 SL, Reglone 200 SL, Basta 150 SL, Avans Premium 360 SL, Spodnam 555 SC, and Caramba 60 SL plant protection products contributed to the increase in the organic carbon content.

9. The microbiological and biochemical tests used proved to be sensitive indicators of changes occurring in the soil environment under the impact of chemical plant protection products.

10. The soil microorganisms and processes carried out by them were characterised by varied sensitivity to the plant protection products applied to the soil. The impact of all the chemical agents on the examined microbial groups and soil biochemical properties was determined by the type of the chemical applied as well as its dose, analysis stage, and the type of the microbiological and biochemical indicator.

11. The stimulation of some of the microbial groups in soil contaminated with the chemical plant protection products implies a possibility that microorganisms utilise organic compounds contained in biocides.

12. The plant protection products tested are characterised by a long-term effect on the analysed microbial groups, soil enzyme activity, and the intensity of biochemical processes.

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9. SUMMARY

The aim of this study was to investigate the effects of various chemicals used in cultivation of winter rapeseed on the abundance of some groups of microorganisms and their biochemical activity in soil.

The investigations on the impact of the chemical agents (Roundup 360 SL, Reglone 200 SL, Basta 150 SL, Avans Premium 360 SL, Spodnam 555 SC, Caramba 60 SL) applied for desiccation of rapeseed on the microbiological and biochemical soil properties were conducted in two field experiments and a pot experiment. Soil was sampled for analyses eight times during the three years of the experiment from the arable layer of each plot, i.e. immediately after winter rapeseed harvest(early August), and next after 2, 10, 12, 14, 22, 24 and 26 months of the experimental period. The experiment no 1 comprised the following objects: soil without chemicals, soil + optimal dose of Roundup 360 SL, soil + 10% higher dose of Roundup 360 SL, soil + optimal dose of Reglone 200 SL, soil + 10% higher dose of Reglone 200 SL. The experiment no 2 comprised the following objects: soil without chemicals, soil + optimal dose of Avans Premium 360 SL, soil+ optimal dose of Spodnam 555 SC, soil + optimal dose of Caramba 60 SL.

The pot experiment was established on soil from the class of black earths formed from light clay loam. The aim of the three-factor pot experiment was to investigate the impact of the Roundup 360 SL and Reglone 200 SL agents as well as the dose and duration of the experiment on soil microbial and biochemical activity. Both herbicides were applied to the soil in the form of aqueous emulsion in doses recommended by the manufacturer (optimum doses) and 10-, 50-, and

100-fold increased doses. Soil without addition of the herbicide was the control. Microbial analyses both field experiments comprised determination of the following: total number of bacteria, total number of fungi, number of bacteria with proteolytic capabilities, number of bacteria with proteolytic capabilities. Biochemical analyses on field experiments included determinations of acid and alkaline phosphatase activity. On pot experiment analyses included determinations of dehydrogenase, protease, urease, acid phosphatase, alkaline phosphatase, amonification and nitrification intensity and respiratory activity of the soil. Chemical analyses included determination of the content of organic C, total N and the soil pH.

The present investigations showed that contamination of soil with the Roundup 360 SL, Reglone 200 SL, Basta 150 SL, Avans Premium 360 SL, Spodnam 555 SC, and Caramba 60 SL plant protection products led to disturbances in the microbial abundance and enzyme activity. The soil microorganisms and processes carried out by them were characterised by varied sensitivity to the plant protection products applied to the soil. The impact of all the chemical agents on the examined microbial groups and soil biochemical properties was determined by the type of the chemical applied as well as its dose, analysis stage, and the type of the microbiological and biochemical indicator. The plant protection products tested are characterised by a long-term effect on the analysed microbial groups, soil enzyme activity, and the intensity of biochemical processes.

Keywords: soil, microbial activity, enzymatic activity, plant protection products

10. SUMMARY IN POLISH (STRESZCZENIE)

WPŁYW ŚRODKÓW CHEMICZNYCH STOSOWANYCH W UPRAWIE RZEPAKU NA MIKROORGANIZMY I ICH AKTYWNOŚĆ W GLEBIE

Celem przeprowadzonych badań było poznanie wpływu różnych środków chemicznych stosowanych w uprawie rzepaku ozimego na liczebność wybranych grup mikroorganizmów i ich aktywność biochemiczną w glebie.

Badania nad oddziaływaniem środków chemicznych: Roundup 360 SL, Reglone 200 SL, Basta 150 SL, Avans Premium 360 SL, Spodnam 555 SC, Caramba 60 SL wykorzystywanych podczas zabiegu desykacji rzepaku na mikrobiologiczne i biochemiczne właściwości gleby przeprowadzono w oparciu o dwa doświadczenia polowe oraz doświadczenie wazonowe.

Doświadczenia polowe (opisane, jako doświadczenie polowe nr 1 oraz doświadczenie polowe nr 2) zostały założony w latach 2010-2012 na terenie Stacji Doświadczalnej Oceny Odmian w Głębokim w województwie kujawsko- pomorskim. Glebę do analiz pobierano ośmiokrotnie w ciągu trzech lat trwania doświadczenia z warstwy ornej każdego poletka tj. bezpośrednio po zbiorze rzepaku

ozimego (początek sierpnia), a następnie po 2, 10, 12, 14, 22, 24 i 26 miesiącach trwania doświadczenia. Doświadczenie nr 1 obejmowało następujące obiekty: gleba bez preparatu chemicznego, gleba + Roundup 360 SL w dawce optymalnej, gleba + Roundup 360 SL w dawce zwiększonej o 10%; gleba + Reglone 200 SL w dawce optymalnej, gleba + Reglone 200 SL w dawce zwiększonej o 10%. Doświadczenie nr 2 obejmowało następujące obiekty: gleba bez preparatu chemicznego, gleba + Avans Premium 360 SL w dawce optymalnej, gleba + Spodnam 555 SC w dawce optymalnej, gleba + Caramba 60 SL w dawce optymalnej.

Doświadczenie wazonowe założono na glebie należącej do czarnych ziem właściwych wytworzonych z glin lekkich pylastych. Celem doświadczenia wazonowego trzyczynnikowego było poznanie wpływu zastosowanych herbicydów Roundup 360 SL i Reglone 200 SL, ich dawki oraz czasu trwania eksperymentu na aktywność mikrobiologiczną i biochemiczną gleby. Obydwa herbicydy zostały zaaplikowane do gleby w postaci emulsji wodnej w następujących dawkach: dawki zalecane przez producenta (dawki optymalne) oraz dawki większe od zalecanych: 10-, 50-, 100-krotnie. Kontrolą była gleba bez dodatku środka chwastobójczego.

Analizy mikrobiologiczne wykonane w dwóch doświadczeniach polowych oraz w doświadczeniu wazonowym obejmowały oznaczenie: ogólnej liczebności bakterii, ogólnej liczebności grzybów, liczebności bakterii o uzdolnieniach proteolitycznych, liczebności grzybów o uzdolnieniach proteolitycznych. Analizy biochemiczne w doświadczeniach polowych obejmowały oznaczenie aktywności aktywności fosfatazy kwaśnej i fosfatazy zasadowej. W doświadczeniu wazonowym analizy obejmowały oznaczenie aktywności dehydrogenaz, aktywności proteazy, aktywności ureazy, aktywności fosfatazy kwaśnej i fosfatazy zasadowej, intensywności amonifikacji i nitryfikacji oraz aktywności respiracyjnej gleby. Analizy chemiczne obejmowały oznaczenia: zawartość węgla organicznego, zawartość azotu ogólnego i pH.

Przeprowadzone badania wykazały, że zanieczyszczenie gleby środkami ochrony roślin: Roundup 360 SL, Reglone 200 SL, Basta 150 SL, Avans Premium 360 SL, Spodnam 555 SC i Caramba 60 SL spowodowało zaburzenia w liczebności mikroorganizmów oraz aktywności enzymów. Mikroorganizmy glebowe oraz przeprowadzane przez nie procesy charakteryzowały się zróżnicowaną wrażliwością na wprowadzane do gleby środki ochrony roślin. Wpływ wszystkich testowanych preparatów chemicznych na badane grupy mikroorganizmów oraz biochemiczne właściwości gleby był uwarunkowany rodzajem preparatu, jego dawką, terminem badań oraz rodzajem mikrobiologicznego i biochemicznego wskaźnika. Testowane środki ochrony roślin charakteryzują się długim okresem oddziaływania na analizowane grupy mikroorganizmów, aktywność badanych enzymów glebowych i intensywność procesów biochemicznych.

Słowa kluczowe: gleba, aktywność mikrobiologiczna, aktywność enzymatyczna, środki ochrony roślin

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