Contemporary Problems of Management and Environmental Protection
Vol. 5, 2010. Influence of the Pesticide Dump on the Environment: 43-53

Chapter 4

Ewa Jastrzębska

The Effect of Crop Protection Chemicals on Soil-Dwelling Microorganisms

Microorganisms are an important component of the soil environment, and they play a key role in the decomposition of organic matter, the circulation of elements in the natural environment and the preservation of soil fertility. Microbes enhance the overall health of plants. In the rhizoplane, ectorhizosphere and endorhizosphere, high populations of microbes dwell around plant roots which secrete biogenic substances. Soil-dwelling organisms protect the plant against pathogens. Bacteria and actinomycetes are important contributors to plant health – by forming symbiotic associations with roots, they supply the plant with nitrogen. Symbiotic fungi also exert a beneficial effect – they facilitate the supply of water and minerals, as well as protect the plant against pathogens and heavy metals. One gram of soil contains thousands of prokaryote species whose populations can be measured in billions.

Soil is not only a habitat of macroorganisms and microorganisms, but it is also an environment that absorbs man-made xenobiotics that affect its physical, chemical and biological properties to a varied extent. This group of substances is inclusive of pesticides.

The use of crop protection chemicals, collectively referred to as pesticides, dates back to the late 19^{th} century. Owing to intensified agricultural production and the need to protect crops against pest, microorganisms and their metabolites, such as mycotoxins, the use of pesticides became widespread, contributing to an increase in the number and the diversity of the available products. To maximize crop yield, farmers around the world use herbicides, fungicides, insecticides and nematicides, often during the same growing season.

Crop protection chemicals contain substances that do not occur naturally in the environment. They are composed of one or more chemical compounds, which are active substances of the commercial product, as well as additives – adjuvants. Pesticides are applied to prevent, damage, repel and alleviate the harmful effects of pests and weeds. They are classified in view of their purpose, time and type of action as well as chemical composition. Pesticides contain both organic and inorganic substances. Many pesticides persist in the environment for years, the most notable examples being DDT and chloroorganic insecticides which have been present in

water and soil for more than 20 years, accumulating in food products and living organisms.

The stability of pesticides in soil differs significantly due to their varied chemical structure. Pesticides undergo chemical and biological degradation, and the rate of their decomposition is determined by many physical, chemical and biological factors, such as:

- the product's chemical properties: chemical structure, solubility, volatility, concentration, method, time and frequency of application;
- soil type: structure, texture, organic and mineral content, moisture, pH;
- climate conditions: temperature, sun exposure, precipitation, wind;
- the type of environment affected by pesticides: cultivation methods, the species composition of crops, soil fauna and microbes;
- the content of other xenobiotics, including heavy metals, in soil.

The above factors also affect the direction and the strength of the effects exerted by pesticides on microorganisms. The soil environment, climate conditions and the applied cultivation system determine the population size, distribution and the species composition of soil-dwelling microbes.

The ability of soil microbes to biodegrade various man-made toxins, including pesticides, is a very important feature from the environmental point of view. This process takes place with the involvement of different bacterial groups. The species composition, population size and metabolic pathways of microorganisms colonizing a given environment determine the rate of biodegradation and the type of the produced indirect metabolites which may have an even more detrimental effect on microbes than the toxin itself.

Crop protection chemicals affect various species, physiological and taxonomic groups of microorganisms as well as their biochemical and physiological attributes. Studies investigating the effect of pesticides on soil-dwelling bacterial populations determine the abundance of each species and physiological group, their biomass, phospholipid fatty acid profiles (PLFAs) and genetic structure.

The effect of pesticides cannot be described as entirely negative or positive owing to the great chemical diversity of the relevant products and the properties of soil where pesticidal substances are deposited. The matter is further complicated by the presence of adjuvants which increase the product's efficacy by enhancing the performance of the spray liquid. According to Włodarczyk, adjuvants contribute to the product's persistence and mobility in the environment. They slow down the decomposition of the active substance and prolong its deposition in the ground. The active substance is prevented from leaching into deeper soil horizons, and it is deposited for longer periods of time in the surface layer. For this reason, studies investigating the effect of crop protection chemicals on soil-dwelling microbes are usually limited to several commercially available products or their active substances. They contain a detailed specification of the tested soil, including its granulometric composition, pH, content of organic substances and biogenic elements. This data are vital for the determination of the microbes' natural habitat, soil's sorptive capacity which plays a key role in the transformation and bioaccumulation of pesticides, as well as their susceptibility to microbiological decomposition. Lewandowska found that xenobiotics may be retained by the soil adsorption complex, bound in the

fraction of fulvic acids, humic acids and humin, owing to adsorption and chemical reactions taking place on the surface of humus and mineral layer.

DDT, i.e. 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, is one of the oldest pesticides which was first used in 1874 and banned many years ago. In Poland, the following DDT-containing products were used to fight the potato beetle and rape pests: Azotox, Ditox and Tritox. Although the products had been pulled out of the market more than 30 years ago, traces of DDT are still detected in soil samples around the world, including DDD 1,1,1-dichloro-2,2-bis(4-chlorophenyl)ethane, DBP (4,4'-dichlorobenzophenone) and DDE (1,1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene).

DDT is the best known pesticide, which is why the results of studies investigating the effect of that biocide and its metabolites on soil–dwelling microbes are worth reviewing. It is toxic for bacteria, fungi and algae and it reduces their biomass. In their study, fungi's response to soil contamination was determined by the applied DDT dose. Their abundance was stimulated under low contamination conditions, and reduced when contamination reached a high level. The highest resistance to DDT is displayed by white root fungi which are capable of accumulating the toxin and decomposing DDT to DDD and DBP. Fungal species such as *Gloeophyllum trabeum*, *Fomitopsis pinicola* and *Daedalea dickinsii* also exhibit a high ability to metabolize DDT, while *Saccharomyces cerevisiae* yeast transforms DDT to DDD. *Proteus vulgaris* is a bacterial species capable of decomposing DDT to DDE.

Microalgae are an important and a widely distributed component of the soil environment. Microalgae produce oxygen, they enrich the soil with organic substances and as free-living microorganisms, they fix and transform soil nitrogen. Algae are capable of metabolizing DDT. The predominant metabolite is DDD, but members of the genus *Chlorococcum* produce mainly DDE. Although algal cells contain metabolic pathways supporting the decomposition of DDT, the substance has a toxic effect on these organisms. From among the group of algae present in non-contaminated soil, i.e. *Chlorococcum* spp., *Chlorella* spp. (genus *Chlorophyceae*), *Anabaena* spp. and *Nostoc* spp. (genus *Cyanobacteria*), only *Chlorella* spp. was detected in samples highly contaminated with DDT.

The contemporary pesticides are more biodegradable and are applied in smaller doses. Nevertheless, pesticide pollution still affects the soil's microbiological equilibrium, leading to an increase or a decrease in the biomass of soil-dwelling microbes, and in the abundance of physiological groups, taxonomic groups or even species. Due to the ability of microbes to rely on pesticides as the only source of carbon and energy, the recommended or a slightly higher doses of the product stimulates the proliferation of soil-dwelling microbes, resulting in an increase in their biomass or abundance, while concentrations that significantly exceed the recommended dose have an inhibitory effect.

An increase in the total counts or the biomass of soil-dwelling microorganisms is not always indicative of a given xenobiotic's positive impact on all microbes. Some soil-dwelling bacteria are less resistant to changes in environmental homeostasis, among them bacteria participating in the nitrogen cycle: *Azotobacter* spp. responsible for non-symbiotic nitrogen fixation, members of the genera *Rhizobium*

and *Bradyrhizobium* which fix nitrogen as part of a symbiotic relationship with legumes, and nitrifying bacteria. Their abundance in soil treated with pesticides is often lower than in control samples.

Pesticides also modify the physiological and biochemical properties of microorganisms. Enzymes produced by microbes play an important role in pesticide decomposition as well as in the synthesis and decomposition of humic compounds. Soil enzymes are produced mostly by plants and microorganisms – bacteria and fungi. Some of them are present inside microbial cells, for example, dehydrogenases are found in the plasma membrane of bacteria and in the mitochondrial membrane of fungi. Others, such as urease, phosphatase, cellulase and pectinase, are excreted outside the cell. Regardless of their place of origin, those enzymes are a product of the metabolic activity of microbes and a sensitive indicator of that activity. Xenobiotic contamination also affects the soil's enzymatic activity. Pesticides stimulate or inhibit enzymatic activity, and the direction of the induced changes is determined by a number of physical and chemical factors, the properties of active substances and adjuvants found in pesticides as well as by soil attributes. The activity of live cells in ecosystems may be also determined by measuring the concentrations of ATP, a high-energy compound which participates in all metabolic reactions in microbial cells. Crop protection chemicals may lower ATP levels in soil.

Experimental conditions

Fungicides are a widely applied group of pesticides which protect crops from fungal diseases and contamination with fungal mycotoxins. A pot experiment was carried out in four replications in the greenhouse of the University of Warmia and Mazury in Olsztyn, Poland to determine the effect of the fungicide Riza 250 EW (active substance – tebuconazole) on the abundance of soil-dwelling microbes. The experimental variables were: a) fungicide dose in terms of active substance content per $mg \cdot kg^{-1}$ d.m. soil: 0.05, 0.5, 5.0, 50 and 500, b) soil type: loamy sand - pH_{KCl} = 6.6 and light loam - pH_{KCl} = 6.7, c) crop species: spring rapeseed cv. *Huzar* and white lupine cv. *Butan*, d) date of soil sampling for microbiological analyses: I (day 10) and II (day 50). The control sample consisted of non-contaminated soil (0).

Soil samples were mixed with the applied fungicide, macronutrients and micronutrients in quantities corresponding to the fertilizer requirements of crops. Soil samples weighing 3.2 kg each were placed in polyethylene pots and sown with crops. The moisture content of soil was maintained at 60% capillary water capacity throughout the entire experiment. Soil samples were collected on experimental day 10 and 50. The abundance of the following bacteria was determined on selective media with the use of the plate-count method, in three replications: oligotrophic bacteria [Olig], copiotrophic bacteria [Cop] on Onta and Hattori medium, ammonifying bacteria [Am], nitrogen immobilizing bacteria [Im], cellulolytic bacteria [Cel] on Winogradski medium, actinomycetes [Act] on Küster and Williams medium containing the antibiotics nystatin and actidion, *Azotobacter* spp. on Fenglerowa medium, and fungi [Fun] on Martin medium. Microbes were incubated at a temperature of 28ºC. Copiotrophic, ammonifying, nitrogen immobilizing

bacteria and actinomycetes were incubated for 7 days, *Azotobacter* spp. and fungi – for 3 days, cellulolytic bacteria – for 14 days, and oligotrophic bacteria – for 21 days.

The results were processed statistically with the use of Duncan's multiple range test and a four-factorial analysis of variance. Correlation coefficients were determined between the abundance of each microbial group and the applied fungicide dose, soil type, crop species and date of microbiological analyses.

The effect of soil contamination with Riza 250 EW fungicide on microbial counts

The results of the study indicate that soil contamination with the fungicide Riza 250 EW affected the soil's microbiological balance (Fig. 1 and 2). Even the smallest dose of the applied biocide's active substance (0.05 mg·kg^{-1}) elicited a response from the tested microorganisms. Regardless of soil type and the applied crop species, the average counts of oligotrophic bacteria, copiotrophic bacteria and actinomycetes isolated from soil samples treated with low fungicide doses (0.05 and 0.5 mg·kg^{-1}) were higher in comparison with the control sample (non-contaminated soil).

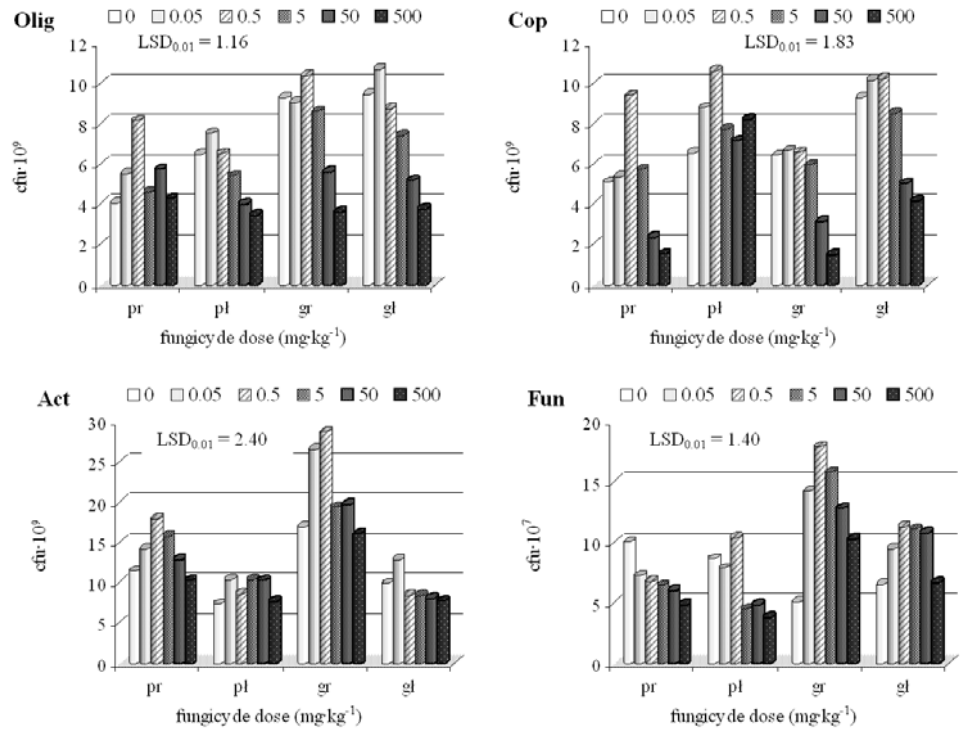

Figure 1. Counts of oligotrofic bacteria (Olig), copiotrofic bacteria (Cop), actinomycetes (Act) and fungi (Fun) in fungicide Riza 250 EW-contaminated soil (cfu·kg^{-1} d.m.)
pr – loamy sand cropped with spring rapeseed, pł – loamy sand cropped with white lupine, gr – light loam cropped with spring rapeseed, gł – light loam cropped with white lupine

The absence of fungicide's adverse effect on soil-dwelling microbes has been also observed by other authors. In a field experiment, Lupwayi et al. (2009) investigated the effect of the fungicide vinclozolin and the insecticide λ-cyhalothrin, applied in doses recommended by the manufacturer, on soil microorganisms. These authors observed no significant impact of the tested biocides on microbial biomass and bacterial diversity, but they noted changes in the microbiological, genetic and taxonomic structure of bacteria during the experiment. In a study by Vig et al. (2008), covering a period of up to 120 days and investigating the effect of seven insecticide products, the recommended dose did not affect the activity of soil-dwelling microbes, including fungi. If such an impact was observed, it was always short-lasting and it affected only bacteria of the genus *Azotobacter*, although in the final phase of the experiment the abundance of this bacterial group was similar to that noted in the control sample of unpolluted soil.

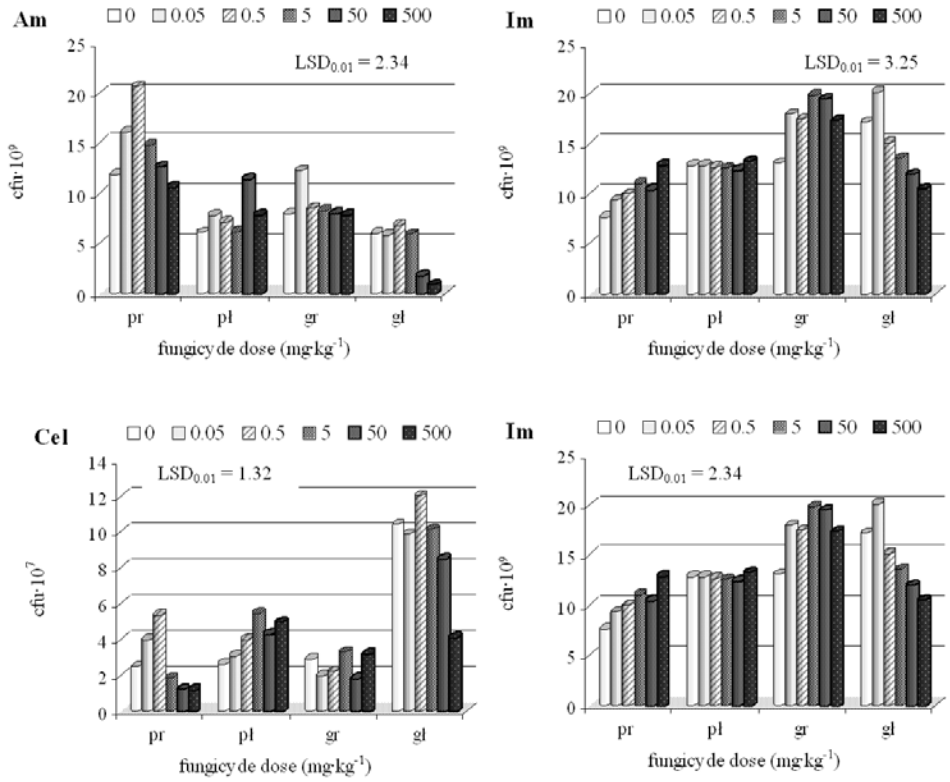

Figure 2. Counts of ammonifying bacteria (Am), nitrogen immobilizing bacteria (Im), cellulolytic bacteria (Cel) and *Azotobacter* spp. (Az) in fungicide Riza 250 EW-contaminated soil (cfu·kg^{-1} d.m.)

pr – loamy sand cropped with spring rapeseed, pł – loamy sand cropped with white lupine, gr – light loam cropped with spring rapeseed, gł – light loam cropped with white lupine

High biocide doses applied in this experiment (50 and 500 $mg \cdot kg^{-1}$) not only failed to stimulate but, in fact, inhibited the proliferation of the discussed microbial groups, as well as *Azotobacter* spp. Fungi's response to soil contamination was clearly determined by the type of soil. A negative response was noted in loamy sand, whereas in light loam samples the investigated biocide stimulated fungal growth. The response of ammonifying, nitrogen immobilizing and cellulolytic bacteria to increased doses of the pollutant was determined by the remaining experimental factors.

When introduced to the environment, chemical substances, regardless of type, lead to the selection of species which are resistant to their harmful effects. Soil contamination with pesticides limits microbial biodiversity, but it also increases the abundance of bacteria which are more resistant to changes in the environmental homeostasis. In an experiment performed by Wang et al. (2008), methamidophos lowered the genetic diversity of soil-dwelling bacteria, it reduced the biomass of bacteria and fungi, but it also stimulated the proliferation of Gram-negative bacteria and it significantly increased their catabolic activity. An increase in the biomass and catabolic activity of Gram-negative bacteria indicates that the studied microbes play a significant role in methamidophos decomposition in the soil. The negative effect of high fungicide doses on soil-dwelling microbes was validated by a study by Jastrzębska (2006) in which the fungicides Unix 75 WG and Swing Top 183 SG applied in quantities exceeding the recommended dose 100-fold had an inhibitory effect on copiotrophic bacteria and actinomycetes.

A similar effect may be exhibited by other pesticides. In a study by Kucharski et al. (2008), the herbicide Granstar 75 WG affected the soil's microbiological equilibrium, and when applied in doses recommended by the manufacturer and higher, it inhibited the proliferation of selected soil-dwelling bacteria. Herbicide doses 5- and 10-times higher than that recommended by the manufacturer ere significantly negatively correlated with the abundance of oligotrophic bacteria and their spore forms, spore-forming copiotrophic bacteria, *Azotobacter* spp., cellulolytic bacteria, actinomycetes and fungi.

In this study, the counts of the studied microbial groups were also determined by the date of microbiological analyses, crop species and the type of soil from which the microbes were isolated (Fig. 3). The time of fungicide deposition in soil was a factor which significantly modified the responses of microbes to soil pollution. Average results show that deposition time significantly modified the abundance of all analyzed bacterial groups, excluding cellulolytic bacteria. The increase in the total counts of soil-dwelling bacteria observed on day 50, in comparison with day 10 of the experiment, was probably affected by the specific properties of the studied microbial groups, their ability to adapt to or decompose the fungicide, as well as by the crop's species and development stage.

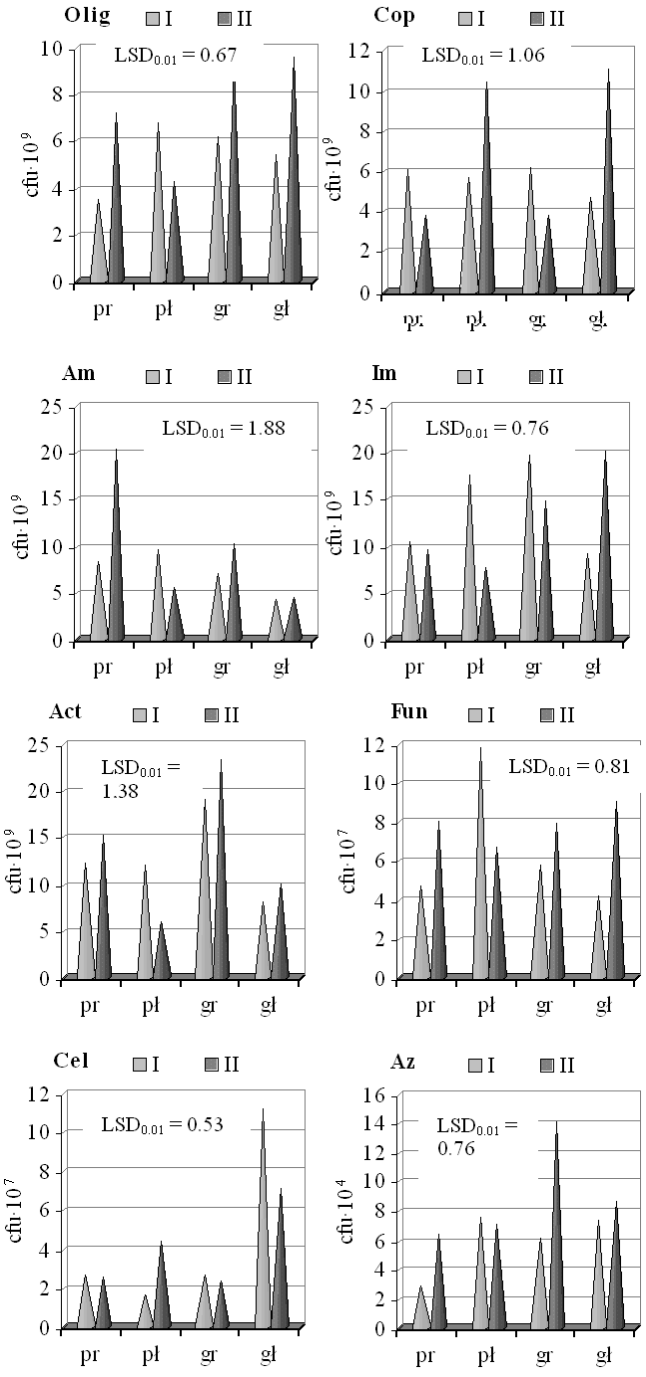

Figure 3. Counts of oligotrofic bakteria (Olig), copiotrofic bacteria(Cop), actinomycetes (Act), fungi (Fun), ammonifying bacteria (Am), nitrogen immobilizing bacteria (Im), cellulolytic bacteria (Cel) and *Azotobacter* spp. (Az) in fungicide Riza 250 EW-contaminated soil (cfu·kg^{-1} d.m.) depending on analysis term, crop species and soil type
I (day 10) and II (day 50) - date of soil sampling for microbiological analyses

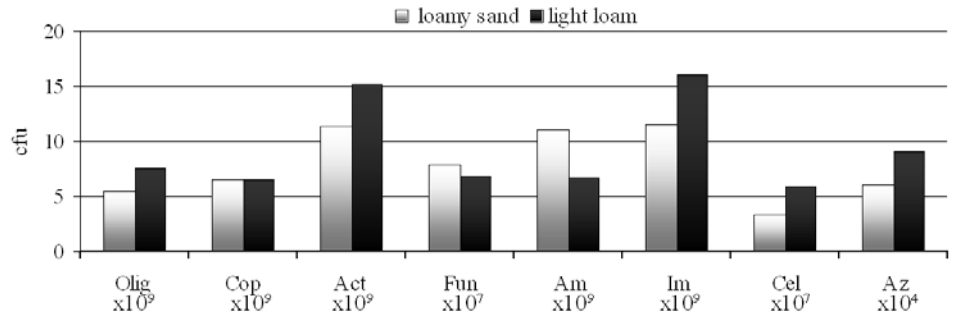

Figure 4. Counts of microorganisms depending on soil type (cfu·kg^{-1} d.m.)

The abundance of soil microbes is also determined by the type, structure, texture, organic substance content, moisture and pH of soil. Soil samples examined in this experiment were characterized by a similar pH and moisture content, therefore, the counts of the isolated microbes were affected only by soil texture and organic substance content. In comparison with loamy sand, light loam was more conducive to the proliferation of oligotrophic, nitrogen immobilizing and cellulolytic bacteria, actinomycetes and *Azotobacter* spp. (Fig. 4), although as regards nitrogen immobilizing bacteria, the above was not validated by the coefficient of correlation (Table 1). When analyzing the degradation of pencycuron in soil, Pal et al. (2005) noted that this fungicide was decomposed faster in soils with a higher silt and clay content which could both minimize the biocide's toxic effect on microbes and stimulate the production of more toxic indirect metabolites.

Table 1

Coefficients of correlation between dose of fungicide, analysis term, soil type, crop species and counts of microorganisms (N = 144)

Variable	Olig	Cop	Act	Fun	Am	Im	Cel	Az
Dose	-0,45**	-0,35**	-0,12	-0,25**	-0,11	-0,15	-0,12	-0,29**
Term	0,34**	0,22**	0,05	0,16	0,22**	0,08	0,01	0,44**
Soil	0,35**	-0,01	0,26**	-0,13	-0,35**	0,17	0,33**	0,43**
Plant	-0,00	0,40**	-0,58**	0,16	-0,43**	-0,17	0,53**	0,03

** correlation coefficients significant difference for $p<0.01$

Highly significant coefficients of correlation (Table 1) testify to the effect of fungicide and other experimental factors on the proliferation of the studied microbial groups. The counts of oligotrophic and copiotrophic bacteria, *Azotobacter* spp. and fungi were significantly negatively correlated with the applied fungicide dose, while

the date of microbiological analyses was positively correlated with the abundance of oligotrophic, copiotrophic and ammonifying bacteria and well as *Azotobacter* spp. Crop species also had a significant impact on the counts of copiotrophic, ammonifying and cellulolytic bacteria as well as on actinomycetes.

To conclude, it should be noted that soil pollution with Riza 250 EW fungicide affected the soil's microbiological equilibrium, leading to changes in the abundance of the tested microbial groups: oligotrophic, copiotrophic, ammonifying and nitrogen immobilizing bacteria, *Azotobacter* spp., actinomycetes and fungi. The biocide's effect on each of the analyzed bacterial groups was determined by the applied dose, soil type, crop species and date of microbiological analyses. Small fungicide doses (0.05 and 0.5 $mg \cdot kg^{-1}$) stimulated the proliferation of oligotrophic bacteria, copiotrophic bacteria and actinomycetes. High doses (50 and 500 $mg \cdot kg^{-1}$) inhibited the growth of the investigated microbial groups, including *Azotobacter* spp.

Acknowledgements

This study was financed by a grant of the State Committee for Scientific Research, No. N305 111 32/4006, **4006/P01/2007/32**

Literature

Aon M.A., Colaneri A.C. 2001. Temporal and spacial evolution of enzymatic activities and physico-chemical properties in en agricultural soil. Appl. Soil Ecol., 18: 255-270.

Arias-Estévez M., López-Periago E., Martinez-Carbaloo E., Simal-Gàndara J., Garcia-Rio L. 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agric. Ecosyst. Environ., 123: 247-260.

Huang Y., Zhao X., Luan S. 2007. Uptake and biodegradation of DDT by 4 ectomycorrhizal fungi. Sci. Total Environ., 385: 235-241.

Jastrzębska E. 2006. The effect of soil contamination with fungicydes on microorganisms counts. Pol. J. Natur. Sc. 21(2): 487-498.

Kucharski J., Wyszkowska J., Borowik A. 2008. Microbiological properties of soil contaminated with the herbicide Granstar 75 WG (in Polish). Ekol. Tech. 16(4): 160-164.

Lupwayi N.Z., Harker K.N., Dosdall L.M., Turkington T.K., Blackshow R.E., O'Donovan J.T., Cárcamo H.A., Otano J.K., Clayton G.W. 2009. Changes in functional structure of sol bacterial communitie due to fungicide and insecticide applications in canola. Agric. Ecosyst. Environ., 130: 109-114.

Megharaj M., Kantachote D., Singleton I., Naidu R. 2000. Effects of long-term contamination of DDT on soil microflora with special reference to soil algae and algal trasformation of DDT. Environmen. Poll., 109:35-42.

Pal. R., Chakrabarti K., Chakraborty A., Chowdhury A. 2005. Pencycuron application to soils: degradation and effect on microbiological parameters. Chemosphere, 60: 1513-1522.

Purnomo A.S., Kamei I., Kondo R. 2008. Degradation of 1,1,1-Trichloro-2,2-Bis(4-chlorophenyl)Ethane (DDT) by brown_rot fungi. J. Biosci. Bioeng., 105(6): 614-621.

Vig K., Singh D.K., Agarval A.K., Dhawan A.K., Dureja P. 2008. Soil microorganisms in cotton fields sequentially treated with insecticides. Ecotoxicol. Environmen. Safety, 69: 263-276.

Wang M., Liu Y., Wang Q., Gong M., Hua X., Pang Y., Hu S., Yang Y. 2008. Impacts of methamidophos on the biochemical, catabolic and genetic characteristics of sol microbial communities. Soil Biol. Biochem., 44: 778-788.

Dr. Ewa Jastrzębska

University of Warmia & Mazury in Olsztyn
Faculty of Environmental Management & Agriculture
Department of Microbiology
Plac Łódzki 3, 10-727 Olsztyn
e-mail: ewa.jastrzebska@uwm.edu.pl