Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2006
Volume 9
Issue 2
Topic
ENVIRONMENTAL DEVELOPMENT

BŁASZAK M. , NOWAK A. 2006. MICROORGANISMS RESISTANCE TO PESTICIDES REGULARLY APPLIED IN CROPS PROTECTION (TRIFLUROTOX 250 EC, MIEDZIAN 50 WP, SIARKOL EXTRA 80 WP) **Electronic Journal of Polish Agricultural Universities**, Environmental Development, Volume 9, Issue 2.
Available Online http://www.ejpau.media.pl/volume9/issue2/art-28.html

MICROORGANISMS RESISTANCE TO PESTICIDES REGULARLY APPLIED IN CROPS PROTECTION (TRIFLUROTOX 250 EC, MIEDZIAN 50 WP, SIARKOL EXTRA 80 WP)

Magdalena Błaszak, Andrzej Nowak
Department of Microbiology and Environmental Biotechnology, Agriculture University of Szczecin, Poland

ABSTRACT

The impact of long term application of pesticides for crops protection on bacteria and fungi resistance has been studied. At first the microbes have been isolated from two soil samples, namely a soil sample contaminated with pesticides, and a control one. Subsequently, under *in vitro* conditions with the same pesticides present, microbes growth capacities were observed. Growth capabilities of microorganisms isolated from the soils contaminated with Miedzian 50WP, Siarkol 80 extra WP, Triflurotox 250EC have proven to be less impacted (by a few to several %) with these xenobiotics present in the substrates than the capabilities of the microbes from the control soil. The only exception were bacteria from the soil samples treated with Siarkol 80WP whose resistance has been found lower (by app. 10%) than the resistance of microbes from the control soil.

Key words: pesticides, microorganisms, resistance.

INTRODUCTION

A process of developing resistance to pesticides is a complex physiological and genetic phenomenon, on the base of which a temporary and permanent resistance can be distinguished [4]. Tolerance to a contaminated environment observed for many organisms, has various aspects. On the one hand microorganisms that developed resistance to xenobiotics, such as pesticides, are frequently capable of biodegrading them [3] and are able to bioremediate soil [9], but still the risk is run that phytopathogenic microbes and weeds can develop resistance to crops protection products [7, 8, 18]. Physiologic changes inducing temporary resistance can consist in establishing a new metabolic pattern, which tends to bypass a biochemical reaction inhibited by a specific toxin [6]. Equally likely, however, it can lead to an enhanced capability for producing metabolites or enzymes inhibiting pesticides action by their detoxification [16], namely creating chelate bondings. In microbes resistance to pesticide, when they are transferred from a substrate containing a pesticide to another substrate free of this specific compound, tends to drop to zero after average three transfers, whereas permanent resistance results from genetic changes, inherited by the subsequent generation of microbes. The rate of genetic variations is particularly high in microbes, and the frequency of evolved resistance depends on the gene mutation frequency [4].

In the environment quality changes within the microbes colonies are induced by pesticides, since they act selectively on microbes [19, 20]. Consequently, microbes strains resistant to pollutants tend to dominate over the sensitive ones [1, 16, 15, 17]. The phenomenon of replacing the less resistant microbes with other microbes, frequently having no specific ecologic function to serve, can bring serious consequences onto the disturbed functioning of the ecosystem [2].

The subject of pesticides impact on microbes though extensively covered in the literature, seems insufficient with regards to the resistance-related issues. Long term exposition to pollutants in selected groups of microbes can lead to developing induced resistance [1, 19]. The objective of the present experiment was to determine whether microbes response to a pesticide exposure is stronger when re-contacting a pesticide, or if the earlier microbe-xenobiotic contact has no influence on its subsequent reaction.

MATERIAL AND METHODS

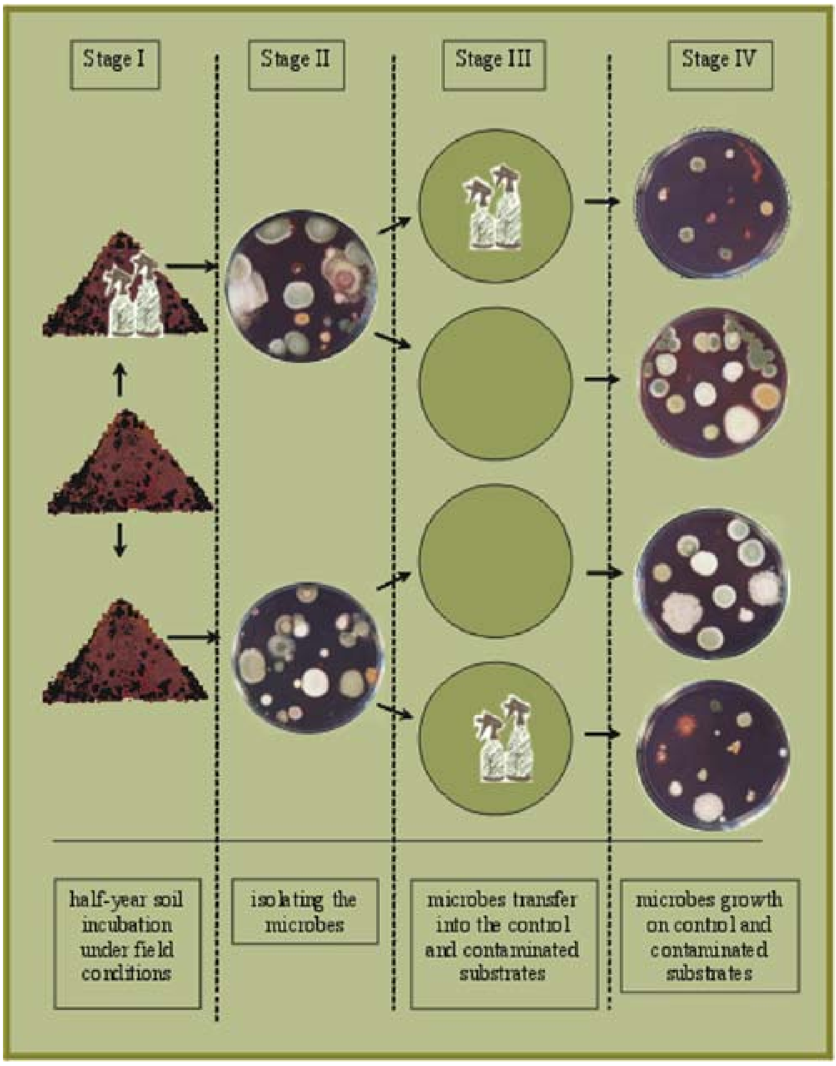
The soil (a light, dusty clayey sand of pH 7.9) originating from the experimental field, both contaminated with pesticides for 6 months, and the control sample, free of pollutants were collected and transported to the laboratory. While preparing soil solutions bacteria and fungi were isolated; the microbes originated from the soil contaminated with a dose exceeding a 100 times the recommended, admissible dose for Miedzian 50 WP, Siarkol 80 extra WP, and Triflurotox 250 EC (Table 1), as well as from the control soil sample. Bacteria were cultured on a solid substrate [5], whereas fungi on Martina solid substrate [11]. Since from each soil, both in case of bacteria and fungi, only 10 strains among the species most widely occurring in the culture were isolated, the scope of the experiment shall be regarded as rather limited, and thus the experiment itself can be considered solely a pilot experiment, targeted at testing a new, simple methodology.

Table 1. Characteristics for pesticides applied in the experiment

PESTICIDES						
TRADE NAME OF PESTICIDE	TYPE OF PESTICIDE	BIOLOGICALLY ACTIVE MATTER		FIELD DOSE	TOXICITY CLASS	PRODUCER
		name	%*, $g \cdot dm^{-3}$			
Miedzian 50WP	Traditional fungicide without adjuvant	copper oxychloride	50*	5 $kg \cdot ha^{-1}$	III	Z. Ch. “Organik - Azot” S.A. Jaworzno
Siarkol extra 80WP	Traditional fungicide without adjuvant	sulphur	80*	2.4 $kg \cdot ha^{-1}$	IV	Z. Ch. “Organik - Sarzyna” S.A. Nowa Sarzyna
Triflurotox 250EC	Traditional herbicide without adjuvant	trifluralin	250	4 $l \cdot ha^{-1}$	IV	Z. Ch. “Organik - Sarzyna” S.A. Nowa Sarzyna

Microbes originating from the soil contaminated with a pesticide were transplanted onto the substrate to which a dose of 100 $mg \cdot l^{-1}$ of the pesticide was added, as well as to a substrate free from it (control). After few days incubation at 25°C temperature, on 2 dishes “twin” colonies (i.e. of the same source of origin, namely the “parent” colony) of microbes were obtained (Fig. 1).

Fig. 1. Experimental set up



Subsequently, the growth of two strains on these two substrates, with pesticides addiction and the control one, was examined. Due to differences in growth of the twin colonies on two dishes, their growth diameters were measured [mm] with a scale. By comparing the size of the respective colonies on the two dishes, a percentage of the growth capability was determined for each of the strains from the soil contaminated with pesticide, with respect to the growth observed in the control soil sample.

In that way 10 results were obtained (the size for 10 pairs of strains originating from the soil contaminated with a specific pesticide). These 10 obtained values (%) provided data for one an average value, which corresponded to the *resistance percentage* of a studied pesticide.

In parallel, a similar experiment with microbes from the control sample was carried out. With the same methodology as presented above, the *resistance percentage* was determined for the microbes originating from the control sample. By means of variance analysis statistically significant differences were detected in microbes growth under *in vitro* conditions (at the significance level $\alpha = 0.05$).

RESULTS

Performed one-factor variance analysis showed no statistically significant difference to occur between the growth under *in vitro* conditions in bacteria (fungi) originating from the soil contaminated for half a year with Miedzian 50 WP (Siarkol extra 80 WP) and the microbes from the control soil.

Bacteria resistance to Triflurotox 250EC

All the bacteria originating from the control soil, and then transplanted onto the substrate contaminated with trifluraline did not show any growth capability, while on the control soil, i.e. under the conditions optimal for growth, 10 strains grew (Table 2).

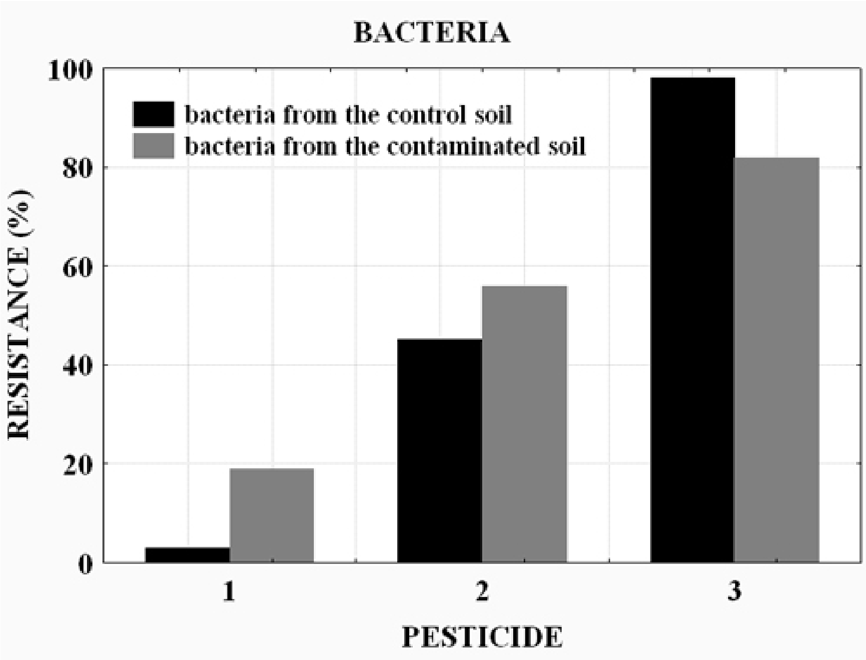
Table 2. Strains diameters [mm] for microbes cultured on the control substrate with pesticides added

Bacteria from the control soil		Bacteria from the contaminated soil		Fungi from the control soil		Fungi from the contaminated soil	
Growth [mm] on the control and contaminated substrate [mm]							
control substrate	contami-nated substrate	control substrate	contami-nated substrate	control substrate	contami-nated substrate	control substrate	contami-nated substrate
Triflurotox 250EC							
3	-	3	-	107	14	13.5	12
5.5	-	8	-	4	3	32	15
15	-	11	-	65	11	33	7
16	-	12	12	11	5	16.5	3
18	-	12	-	22	-	52.5	10
19.5	-	13	-	32.5	-	15	-
22	-	13	-	64	-	28	-
22	-	16.5	-	31	-	41	-
25	-	20	-	12	-	8	-
30	-	24	18	8	-	10	-
Miedzian 50WP							
8	10	4	6.5	16	7	21	11
9	3	9	7	19	17	13	8
10	5	10	4	10	5	44.5	32
12.5	2	10	4	16.5	3	18	13
12.5	3	11	7	21	10	16.5	7
13	6	12	4	10.5	2	11.5	4
13	13	12	4	10.5	7	17	14.5
13	3	12	5	23	21	30	30
16	5	17	3.5	36.5	11	49	21
24	2	20	10	53	13	34	21
Siarkol 80WP							
3	3	3	4	28	28	4	4
4	3	4	3	12.5	12.5	28	28
5	5	5.5	7	7	8	19.5	19.5
7.5	9	10.5	2	18.5	12	11	11
9	10.5	11	2	35	33	60	120
10	13	12	12	26.5	23	13	15
12	12	12.5	13	18	19	29	33
12	5	16.5	12	16	20	21	17
12	11	16	13	32	38	18	15
15	15	18	18	60	3	8	5

The bacteria isolated from the soil contaminated with trifluraline were found able to grow in its presence only in two cases. The first bacteria diameter reached 12 mm, i.e. equalled the value for the control soil (Table 2), whereas the other reached 75% of the value of the control bacterium.

The resistance (average value over 10 strains) in bacteria isolated from the soil contaminated with trifluraline was equal to 18%, while the bacteria that were never contacted with the substance before, were found not to grow at all (Fig. 2).

Fig. 2. Percentage resistance in bacteria (mean value over all the studied strains) to: 1 – Triflurotox 250EC, 2 – Miedzian 50WP, 3 – Siarkol 80WP



Fungi resistance to Triflurotox 250EC

Six strains isolated from the control soil did not show growth capability on the substrate with trifluraline. Efficiency of growth for one strain, however, reached 70%. Another three fungi showed a stronger reaction to trifluraline present in the culture medium, since their growth was reduced by more than twice in comparison to the substrate with no herbicide added, and their diameters were equal to 5, 11, and 14 mm.

The fungi from the soil contaminated with trifluraline for over six months, in five cases were found to be sensitive to Triflurotox 250 EC. One of the strains was insignificantly smaller than the "twin fungi" on the control soil. Three microbes proved to reduce their growth substantially, and their diameters reached 20% of the respective diameters of the control microbes. One fungi strain reached 15 mm in diameter, which was smaller by circa 15 mm than the fungi on the culture medium containing no Triflurotox 250 EC (Table 2).

For the fungi from the soil contaminated with trifluraline an average resistance for ten strains reached 20%, while for the microbes deprived of such contact it amounted to 15% (Fig. 3).

Bacteria resistance to Miedzian 50WP

In general, ten bacteria isolated from the control soil were able to grow on the substrate with copper oxychloride. One strain of bacteria was found totally resistant, but also only one strain proved to grow intensively with Miedzian 50 WP present, namely the growth was by 30% greater in comparison to the growth on the control substrate. Colonies diameters in seven cases were registered smaller by over 50% than the diameters of the control colonies (one bacterium growth reached a mere 7%), whereas only one case was observed with the diameter greater than 50%. The diameters for bacteria colonies on the contaminated substrate were equal to 6 mm, i.e. were by 4 mm smaller than in the control sample (Table 2).

Overall, ten bacteria isolated from the soil contaminated with Miedzian 50WP proved to grow on the substrate containing this pesticide. In one of the studied strains growth was intensified and the diameter was greater by circa 65% than the diameter of the "twin" control colony. Six strains were found to reduce their size by more than 50% when exposed to Miedzian 50WP presence, diameters of one strain were smaller by 80% when compared to the control sample. Three strains, however, were resistant to the pesticide presence in the substrate in a higher degree, their colonies diameters varied between 51% and 77% of the control diameters.

For the microbes from the contaminated soil the average resistance for 10 strains reached 55%, whereas for bacteria having no previous contact with the soil, it reached 46% (Fig. 2).

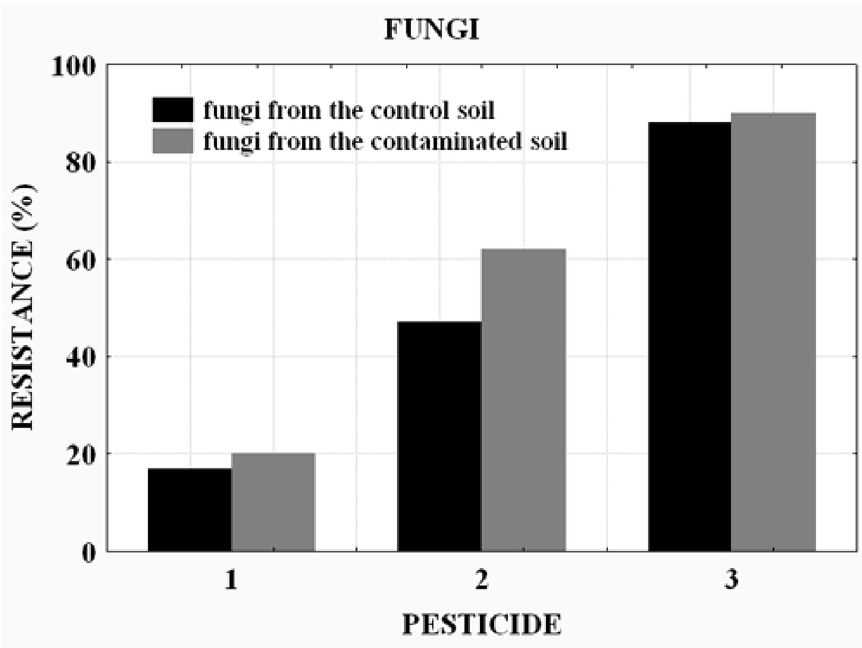
Fungi resistance to Miedzian 50WP

The fungi from the control soil, when transplanted onto the microbiological substrate with copper oxychloride, proved to grow on the applied substrate. In comparison to the size of the fungi on the control substrate, the diameters of two fungi were smaller by circa 10%. The growth of five microbes was restrained by 56–82% in comparison to the control. The diameters of the remaining fungi reached the size of 5, 7 and 10 mm, and were smaller by circa 50% in comparison to the size observed on the control substrate (Table 2).

All of the microbes originating from the soil contaminated with Miedzian 50WP grew on the substrates into which fungicide was introduced. One of the studied fungi was found totally resistant. Three studied microbes occurred to reduce their size significantly, namely by over 50%; one of them was reduced even by circa 65% in comparison to the control. The remaining microbes were less sensitive to copper present in the substrate, and their diameters ranged between ca 50% to 80% of the size of the control diameters.

For fungi from the soil contaminated with Miedzian 50WP the resistance averaged over 10 strains reached 62%, whereas for the fungi from the control soil it was equal to 48% (Fig. 3).

Fig. 3. Percentage resistance in fungi (mean value over all the studied strains) to:
1 – Triflurotox 250EC, 2 – Miedzian 50WP, 3 – Siarkol 80WP.



Bacteria resistance to Siarkol 80WP

All the bacteria isolated from the control soil proved to grow on the substrate contaminated with Siarkol 80WP. Growth on the substrate containing sulphur was particularly intense for three strains, and the microbes colonies diameters were bigger than the control diameter by 30%, 18% and 17%, respectively. Four bacteria were found not to react to the fungicide presence, while the growth capabilities for the remaining ones were reduced in comparison to the control substrate, and their size were respectively smaller by ca 30%, 60% and 10% which corresponded to their diameters measuring 3, 5, and 11 mm.

Sulphur content in the culture nutrient stimulated the development of three microbes that originated from the soil contaminated with Siarkol 80WP, whose diameters were equal to 13 mm, 4 mm and 7 mm, i.e. were bigger by ca. 5%, 30% and 25% than diameters obtained under the control conditions. Two bacteria did not react to the sulphur present in the substrate. The remaining microbes were detected to reduce their size when exposed to Siarkol 80WP presence by ca 20% – 80%.

The average resistance (mean value over 10 strains) for microbes originating from the soil contaminated with Siarkol 80WP amounted to 82%, while for bacteria that had not contacted such soil it was equal to 98% (Fig. 2).

Fungi resistance to Siarkol 80WP

Fungi originating from the soil contaminated with Siarkol 80WP, cultured on a substrate with this fungicide added were capable of growing. The size for four strains were identical as the size obtained under control conditions. Two fungi on the substrate with the fungicide addiction developed diameters of 15 and 33 mm (Table 2), i.e. bigger than the control ones by ca 12%. The diameters of all the remaining fungi were smaller and ranged between ca 50% to ca 16% of the diameter reached on the control substrate.

Three microbes originating from the control soil did not react to sulphur present in the substrate, and two were observed to grow intensively on the substrate contaminated with sulphur, with their colonies diameters bigger than the control ones by 14% and 25%. One strain was found to nearly entirely inhibit its growth, and was smaller than the control by a factor of over 90%. For the remaining fungi growth had not been observed to be inhibited that drastically by Siarkol 80WP admixture into the substrate as their diameters were reduced by ca 12 – 35%.

The average resistance (man value over 10 strains) for the microbes originating from the soil contaminated with Siarkol 80WP amounted to 91%, whereas for the fungi that most likely had no contact with such soil the value reached 88% (Fig. 3).

DISCUSSION

The microbes isolated from the soil which have been contaminated with Miedzian 50WP for half a year proved more tolerant to oxychlorine present in the microbiological substrate than the bacteria and fungi originating from the control soil. Microbiological experiments performed by Niklińska and Chmiel [13] have confirmed our observations, since microbes originating from the soils heavily contaminated with copper proved to be more tolerant to this chemical element under *in vitro* conditions (5000 mg $Cu \cdot kg^{-1}$) than the microbes from the control soil. Prolonged exposure to pollutants can then lead to developing an induced resistance in some microbes groups [1]. Microbes capable of adapting quickly to copper fungicides present in soil was also reported by Borecki [4].

Siarkol Extra 80 WP, subject to the current study, was found not to induce significantly the growth capabilities of microbes originating from both the control and contaminated soil. Sulphur fungicides show low toxicity, which was confirmed by the reported study, therefore they are known to be applied in preventive treatment [10].

The microbes originating from the soil contaminated with trifluraline proved to be more resistant to biologically active substance present in the microbiological substrate than the microbes isolated from the soil free from pesticides. In another experiment where trifluraline [3] had been applied for four decades on a farming land, the microbes resistant to this herbicide were isolated from the soil and then their capabilities to biodegrade the active substance were studied. The observed efficiency of the biodegradation process varied for bacteria strains between 24.6%, 25%, 21%, and 16% of the initial trifluraline content. Herbicides introduced to soil can inhibit sensitive soil microbes development or even result in their atrophy, which in turn can provide enzymes and other nutrients and thus, cause lavish development of microbes from selected systematic or physiological groups [12, 15]. Still, Triflurotox 250EC applied in the experiment at a dose of 100 $mg \cdot l^{-1}$ have proven to be the strongest microbes growth inhibitor of all the applied pesticides, with respect to the microbes originating from both the contaminated and control soil.

SUMMARY

The microbes, i.e. fungi and bacteria, isolated from soils contaminated for half a year prior to the experiment with Miedzian 50WP, Siarkol 80WP, and Triflurotox 250EC have proven more tolerant (by a factor of few to several %) to the xenobiotics presence in the microbiological substrates than the microbes originating from the control soil. Slightly reduced growth capability, i.e. by circa 10%, was observed only for bacteria from the soil treated with Siarkol 80WP on the substrate with this fungicide, in comparison to the bacteria originating from the control soil.

REFERENCES

1. Balicka N.; 1983. Różne formy wzajemnego oddziaływania drobnoustrojów z herbicydami. [Various forms of microbes-herbicides interactions]. Post. Mikrobiol.; 22; 3/4: 291-298; [in Polish].
2. Bellen P., Doelman P.; 1997. Significance and adaptation of microbial toxicity tests in assessing ecotoxicological risks of contaminations in soil and sediment. Chemosphere; 34: 455-499.
3. Bellinaso M., Greer C. W., Peralba M.; 2003. Biodegradation of the herbicide trifluralin by bacteria isolated from soil. FEMS Microb. Ecol., Elsevier, 43: 191-194.
4. Borecki Z.; 1984. Fungicydy stosowane w ochronie roslin. [Fungicides applied in plant protection]. PWN Warszawa [in Polish].
5. Bunt J.S., Rowir A.D.; 1955. Microbiological studies of some susubantarctic soil. J. Soil Sci. 6. (1): 119-128.
6. Digrak M., Özcelik S.; 1998. Effect of some pesticides on soil microorganisms. Bull. Environ. Contam. Toxicol, 60: 916-922.
7. Dzienia S., Romek B., Wrzesiński E.; 1999. Agroekologiczne podstawy produkcji roslinnej. [Agroecological basis for plant production] Wyd. AR Szczecin. [in Polish].
8. Górska-Poczopko J., Ptaszkowska J.; 1995. Czy pestycydy siarkowe sa przestarzałe? [Are sulphur pesticides outdated?] Pestycydy. 1: 51-54. [in Polish].

9. Juhasz A., Smith E., Smith J., Naidu R.; 2002. Biosorption of organochlorine pesticides using fungal biomass, J. of Industrial Microbiology & Biotechnology, 29: 163-169.
10. Ławecki A.; 1998. Jak działaja preparaty grzybobójcze. [How do fungicides act?]. Agrochemia. 5: 20-12. [in Polish].
11. Martin J.P.; 1950. Use of acid, rose bengale and streptomycin in the plate method for estimating soil fungi. Soil Sci. 6.
12. Mitterer M., Bayer H., Schinner F.; 1981. Der Einfluβ von Fungiziden auf die mikrobielle Aktivität eines Bodens, Z. PflErnaehr. [Fungicides influence on microbiological activity in soil]. Z. PflErnaehr. Bodenk., 144, s. 463-471. [in German].
13. Niklińska M., Chmiel M.; 1997. Porównanie odpornosci na metale ciężkie u mikroorganizmów glebowych z rejonów silnie zanieczyszczonych miedziš lub cynkiem. [Comparison of resistance to heavy metals in soil microbes from regions heavily contaminated with copper or zinc. Microbes in the environment, occurrence, activity and role]. Drobnoustroje w □rodowisku, występowanie, aktywnosc i znaczenie. Barabasz W. (ed.) AR Kraków: 491-504. [in Polish].
14. Nowak A.; 1998. Mikrobiologia. Wyd. AR Szczecin. [in Polish].
15. Perucci P., Scarponi L.; 1992. Interference on soil microbial biomass and persistence of trifluralin in a clay soil, Proceedings of the international symposium on environmental aspects of pesticide microbiology. Sigtuna. Sweden: 129-134.
16. Poprawski T., Majchrowicz J., 1995. Effects of herbicides on *in vitro* vegetative growth and sporulation of entomopathogenic fungi. Crop Protection, 14: 81-87.
17. Różański L.; 1992. Przemiany pestycydów w organizmach żywych i srodowisku. [Pesticides transformation in living organisms and environment]. PWRiL Warszawa. [in Polish].
18. Sobótka W.; 1999. Herbicydy – wczoraj i dzis [Herbicides – past and present]. Post. Ochr. Ro□l. 39: 218-223. [in Polish].
19. Wainwright M.; 1977. Effects of fungicides on the microbiology and biochemistry of soils – a review. Z. PflErnaehr. Bodenkd., 140: 587-603.
20. Werf H. M. G.; 1996. Assessing the impact of pesticides on the enviroment. Agriculture Ecosyst. & Envir., 60: 81-96.

Accepted for print: 07.06.2006

Magdalena Błaszak
Department of Microbiology and Environmental Biotechnology,
Agriculture University of Szczecin, Poland
Słowackiego 17, 71-434 Szczecin, Poland
email: mblaszak@agro.ar.szczecin.pl

Andrzej Nowak
Department of Microbiology and Environmental Biotechnology,
Agriculture University of Szczecin, Poland
Słowackiego 17, 71-434 Szczecin, Poland
Phone: +48 91 42 50 234
email: anowak@agro.ar.szczecin.pl
