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Biodegradation of Carbendazim by Epiphytic and Neustonic Bacteria of Eutrophic Chełmżyńskie Lake

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Abstract

The paper presents a study on biodegradation of carbendazim (1 mg/l) by homogeneous cultures of epiphytic (n = 25) and neustonic (n = 25) bacteria and heterogeneous (n = 1) cultures containing a mixture of 25 bacterial strains isolated from epidermis of the Common Reed (*Phragmites australis*, (Cav.) Trin. ex Steud.) and surface microlayer (SM ≈ 250 μm) of eutrophic lake Chełmżyńskie. Results indicate that epiphytic bacteria are characterized by higher average capacity to decompose carbendazim than neustonic bacteria ($p < 0.05$). The half-life of carbendazim in epiphytic bacterial cultures equaled an average of 60 days. In the same period, neustonic bacteria reduced the concentration of the fungicide by 31%. The level of carbendazim biodegradation in mixed cultures of epiphytic and neustonic bacteria after 20-day incubation was lower than the biodegradation level in homogeneous cultures. Sixty-day homogeneous cultures of epiphytic and neustonic bacteria were characterized by a higher mean level of carbendazim biodegradation than mixed cultures. After 40-day incubation, mean values of biodegradation of the fungicide in homogeneous and mixed cultures were similar. It was demonstrated that among epiphytic bacteria, *Pseudomonas luteola* was the most efficient organism in reducing the concentration of carbendazim. Among neustonic bacteria, *Burkholderia cepacia* and *Aeromonas hydrophila* were the most effective in degradation of the fungicide.

Key words: biodegradation, carbendazim, epiphytic bacteria, neustonic bacteria, pesticides

Introduction

Due to their common usage, stability, toxic properties, low selectivity, and ability to accumulate, pesticides are particularly hazardous to living organisms, including humans. Their mutagenic, carcinogenic, and teratogenic properties have been widely reported (Hayes, 1991). Pesticides may cause damage to the immune (Culliney *et al*., 1992), nervous, or endocrine (LeBlanc, 1995; Perreault *et al*., 1992; Nakai *et al*., 1993; Sarrif *et al.*, 1994) systems. Pesticides from the organic halogen hydrocarbons impair water self-purification properties negatively affecting bacteria participating in nitrogen transformations (Biziuk *et al*., 1996).

Microbiological degradation is one of the most important processes that determine the fate of pesticides in the environment. This process is used in bioremediation, which is a strategy that utilizes biological processes to remove hazardous chemicals from the environment (Gianfreda and Rao, 2004). Microorganisms are highly effective in transforming organic pollutants and modifying their structure and toxic properties; also, they can completely mineralize organic compounds to non-organic products (Zipper *et al*., 1996).

Carbendazim is a common fungicide in the world, including Poland. It is used against various diseases caused by fungi. This compound is an active substance in the following commercial agents: Arrest, Bavistin, Cerezim, Desrosal, Funaben, Hinge, Kolfugo Super, Occidor, Sabendazim, Volzim, or Zen (http://agrochemchina.com/carbendazim.htm). Moreover, carbendazim is the primary degradation product of two other pesticides, benomyl and methyl thiophante (Mazellier *et al*., 2002) and may remain in locations it was applied for an extended period of time. According to numerous studies, this substance was detected in fruits and leaves of plants even after they had been harvested (Chiba and Veres, 1980; Kiigemagi *et al*., 1991). Its mutagenic and teratogenic effects on mammals have also been confirmed, even when the substance was applied in a single

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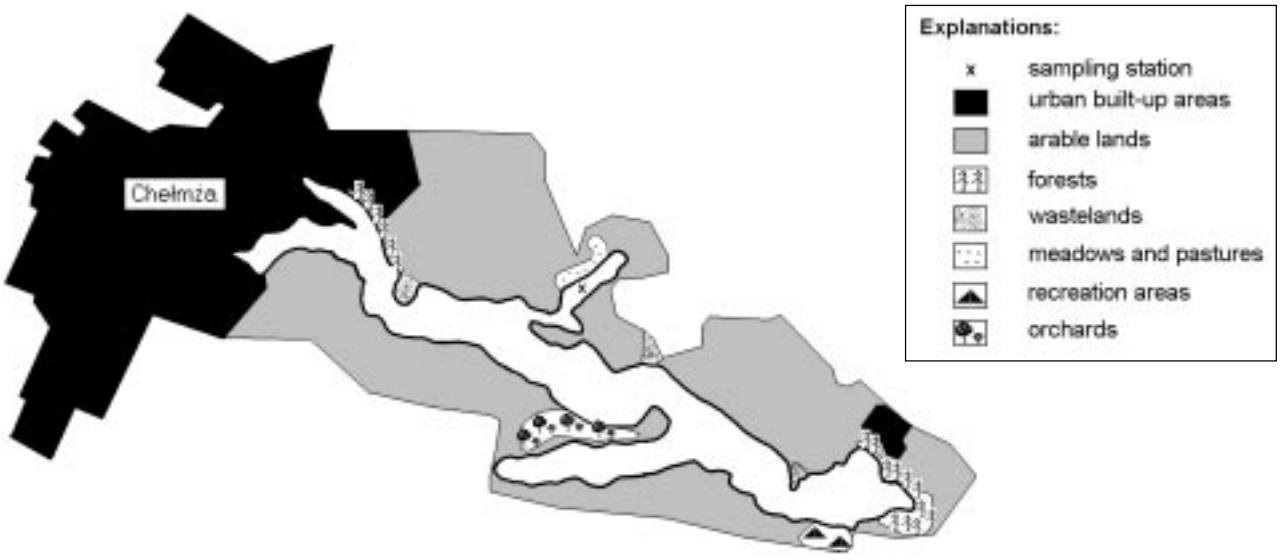

Fig. 1. Outlook of lake Chełmżyńskie.

and relatively small dose (Perreault, 1992; Holtman and Kobayashi, 1997).

Although, the literature on the pesticide biodegradation is quite extensive, it lacks comparative studies that would present the problem from the ecological perspective and on the level of natural communities of microorganisms such as neustonic and epiphytic bacteria. The first group of those organisms occurs in the external, boundary layer of water (surface microlayer SM), which separates the water column from the atmosphere. This layer contains naturally occurring organic compounds, such as: lipids, proteins, and polysaccharides (Hardy, 1982; Garabetian *et al*., 1993). Sedimentation and fallout of various aerosols, including pesticides, dust and gases occurring in the atmosphere, play an important role increasing the concentration of chemical substances in the surface microlayer (Norkrans, 1980). Persistent organic pollutants (POPs) of anthropogenic origin, such as: pesticides, polynuclear aromatic hydrocarbons (PAHs), or polychlorobiphenyls (PCBs) tend to hyperaccumulate in that zone (Wania *et al*., 1998; Southwood *et al*., 1999; Wurl and Obbard, 2005; Manadori *et al*., 2006). Epiphytic bacteria grow on aquatic macrophytes with their greatest numbers occurring in littoral zones of water bodies; this zone is particularly affected by pesticides due to the proximity of farmland. It was demonstrated that only 1–3% of the applied dose of pesticide reaches its target (Plimmer, 1990). The majority of the applied compound is wasted and reaches surface waters through runoff, gets dispersed by wind over vast surfaces, or evaporates.

The purpose of this study was to address the following questions: whether any of epiphytic or neustonic bacteria are capable of decomposing carbendazim, which group of these microorganisms has a higher capacity to neutralize this carbendazim, and what is the level of carbendazim degradation in pure and mixed cultures of these microorganisms.

Experimental

Materials and Methods

Isolation of strains. The analyzed strains of bacteria were isolated from the samples of epidermis of the Common Reed (*Phragmites australis*, (Cav.) Trin. ex Steud.) and surface microlayer (≈250 μm), which were collected in the extra-urban section of lake Chełmżyńskie surrounded by farmland (Fig. 1). Lake Chełmżyńskie is located 20 km from Toruń, belongs to the Fryba-Vistula river basin, and is a typical eutrophic water body (Table I). The watershed of the lake primarily includes ploughland, which constitutes 72% of the immediate watershed. The urbanized areas of Chełmża are located in the northwestern section of the lake.

Table I
Morphometric and trophic characteristics of lake water sampled from pelagic zone (1m beneath the water level) of studied lake during summer (July)[(1)]

Characteristic	Value
Area (ha)	271.1
Maximal depth (m)	27.1
Mean depth (m)	6.0
Length of shore line (m)	20985
Total phosphorus (μg l^{-1})	30.0–99.0
Total nitrogen (mg l^{-1})	0.94–1.76
Chlorophyll *a* (mg l^{-1})	26.4 – 56.9
Water transparency (m)	1.2–1.9

(1) data supplied by Provincial Inspectorate of Environmental Service in Bydgoszcz, data for 2000

A sample of epidermis was collected with a sterile scalpel. Samples of surface microlayer were collected with a nylon Garret net with a pore diameter of 65 mm and an active surface of 50 × 50 cm, which allowed us to collect 250 ± 50 μm thick samples (Garret, 1965). The samples of water and epidermis were placed in glass jars and transported to a laboratory in a cold container at below +4°C. The time between the sample collection and the beginning of the microbiological analyses did not exceed 4 hours.

Ten grams of epidermis of the Common Reed was placed in 90 ml of a sterile buffer (Daubner, 1967) and was homogenized (Unipan 329) for 2 minutes at 4000 rpm. Both, the homogenate of the Common Reed epidermis and a sample of surface microlayer were diluted with sterile buffer water according to Daubner (1967), inoculated on peptone-iron agar (IPA), according to Ferrer *et al*. (1963), and incubated at 20°C in order to isolate bacterial colonies for further analyses. Twenty five bacterial strains were isolated from the samples of the Common Reed epidermis and surface water microlayer.

Preliminary cultures and preparation of bacterial inoculum. Bacterial strains isolated from individual samples and cultured on peptone-iron agar slants (IPA) were tested for purity and then used for preparation of preliminary cultures. Subsequently, bacterial inoculum for the analyses of carbendazim degradation was obtained from these preliminary cultures. Five ml of sterile mineral medium, containing (g/l of distilled water) KH_2PO_4 – 1, KNO_3 – 0.5, $MgSO_4 \times 7H_2O$ – 0.4, $CaCl_2$ – 0.2, NaCl – 0.1, $FeCl_3$ – 0.1, glucose – 1, was poured into test tubes, inoculated with pure bacterial cultures, and incubated at 20°C for 3 days in a rotary shaker (WL-2000, JW Electronic). Bacterial inoculum was retrieved from the culture with 2 inoculation loops. The medium contained carbendazim with the final concentration of 0.1 mg/l. For each bacterial strain, we also carried out control analyses, which involved culturing bacteria on a mineral medium without the pesticide.

In order to prepare the inoculum, the preliminary cultures were brought to identical optical density, $A = 0.2 \pm 0.05$. The spectrophotometric measurements of the culture absorbance was conducted with a spectrophotometer "Marcel s 330 Pro" at a wavelength of $\lambda = 560$ nm. In addition to bacterial inocula containing homogeneous bacterial cultures, mixed bacterial cultures, containing 25 strains of pure strains, were prepared for each of the analyzed ecological groups of lacustrine microorganisms. The cultures were brought to the appropriate optical density with the same method as the homogeneous cultures.

The measurement of the culture optical density. In order to determine the adaptability of the microorganisms to growth in the presence of the xenobiotic, we measured the optical density of preliminary cultures in a medium with and without carbendazim after 3 days of incubation in accordance with the above method.

Proper cultures. Five hundred micro liter samples of bacterial inocula were poured into Erlenmeyer flasks containing 75 ml of sterile mineral medium with the above composition. The final concentration of carbendazim in cultures equaled 1 mg/l. Sterile mineral medium without pesticide was used to dilute the samples. All samples were analyzed in two replicates. Simultaneously, control analyses were conducted for each bacterial strain. These analyses involved culturing bacteria in 75 ml of uninoculated mineral medium with the addition of the xenobiotic. Bacterial cultures were incubated for 60 days at 20°C in the dark in the rotary shaker (WL-2000, JW Electronic).

Analysis of reduction of carbendazim concentration (presence of 2-AB) in the bacterial cultures. Degradation of carbendazim and the presence of 2-AB (a primary product of carbendazim decomposition) was monitored with a high performance liquid chromatograph (HPLC). The analyses were conducted with a Perkin-Elmer HPLC set with a SERIES 200 LC pump and a SERIES 200 UV/VIS detector. In order to determine the concentration of the pesticide, 1 ml samples of cultures were collected after 20, 40, and 60-day incubation and filtered through 0.22 μm syringe filters (Fisherbrandt) in order to remove cells. The obtained filtrates were kept at – 20°C.

The chromatographic analyses were conducted with a 5 μm, 25 cm × 4.6 mm column (LC-18, Supelcosil™; SUPELCO) and a 5 μm, 2 cm × 4 mm pre-column (Supelcosil™ Supelguard® SUPELCO) at a wavelength of 254 nm. The size of analyzed samples equaled 50 μl. A solution (40:45:15) of acetonitrile (J.T. Baker), spectrally pure water (J.T. Baker), and a buffer solution containing 0.067 M Na_2HPO_4 and 0.067 M KH_2PO_4 (2:3) constituted the mobile phase. The isocratic separation was carried out at a flow rate of 1ml/min; the amount of the pesticide in a sample was determined based on the peak absorption during the retention time obtained from the standard. The pesticide concentration in the sample was determined based on the standard curve.

In order to obtain the analytical curve, a basic solution of carbendazim was prepared based on a 10 mg/l analytical standard of fungicide (Sigma – Aldrich). The basic solution of carbendazim was diluted with the mobile phase (composition as above) in preparation for HPLC. The following concentrations were obtained (mg/l): 0.1; 0.25; 0.5; 0.75; and 1.0. The prepared standards underwent chromatographical analysis; each concentration was analyzed in three replicates. The analytical curve with a linear correlation coefficient of <0.99 was graphed using spreadsheet Excel (Microsoft Office Professional Edition, 2003).

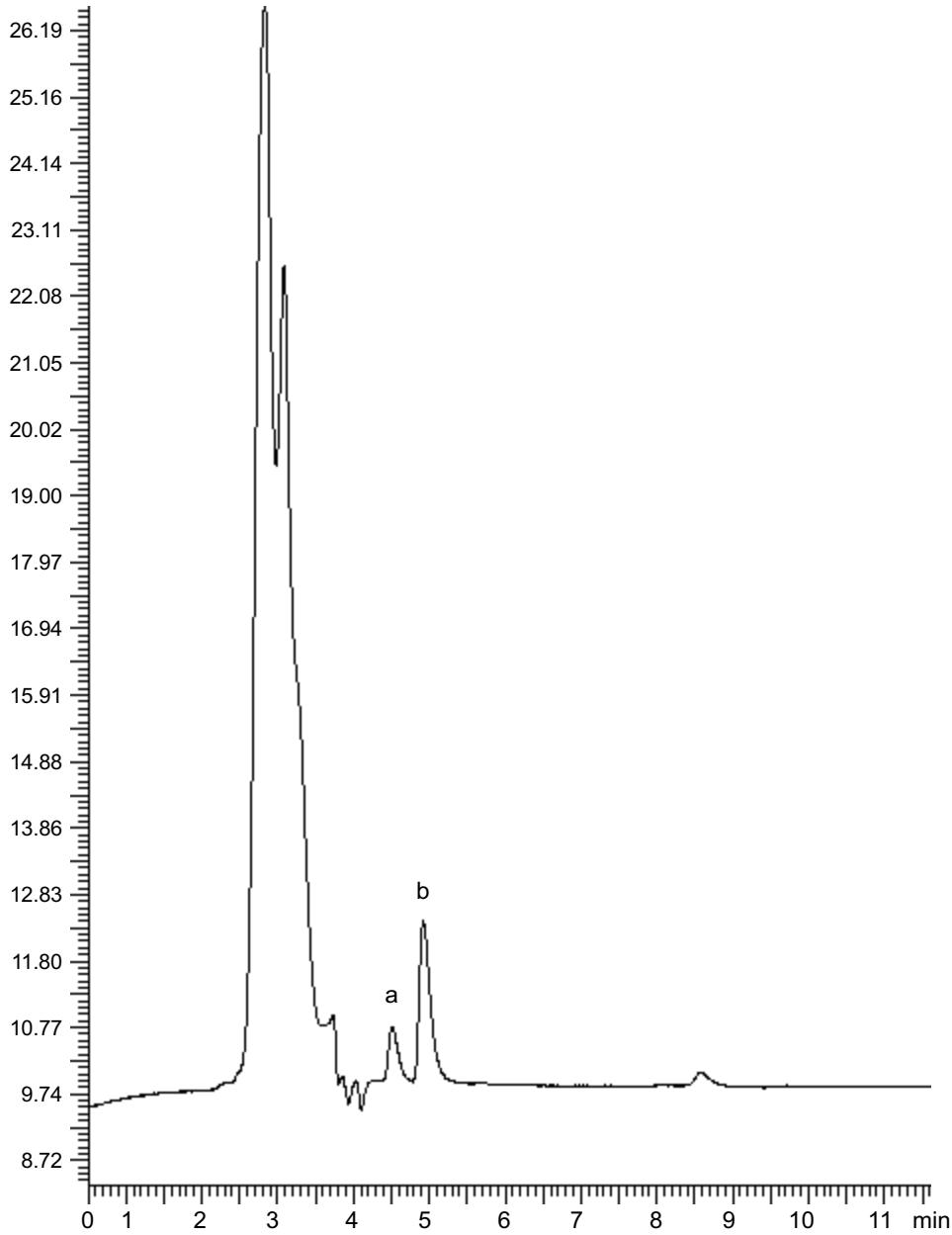

Fig. 2. HPLC plot of culture filtrate.
Explanations: a – 2-AB peak at 3.91; b – carbendazim peak at 4.38.

Retention time for carbendazim was recorded at 3.91 min and for 2-AB at 4.18 min (Fig. 2).

The level of carbendazim biodegradation (%) was calculated from the equation:

$$B = \frac{a - b}{a} \times 100$$

where: B – biodegradation (%), a – concentration of carbendazim in a culture after t_0, b – concentration of carbendazim in a culture after $t_{20, 40, 60}$

Identification of strains. Isolated bacterial strains capable of decomposing carbendazim were identified with the Analytical Profile Index (API 20NE, API 50CH, API 20E, API Staph, API Strep test kit, bioMeriéux). Tests were conducted in three replicates as recommended by the producer.

Statistical analysis. Statistical analyses were conducted in STATISTICA 6.0, 2001. T-test was used in order to determine the statistically significant differences in average ability of the analyzed bacteria to break down carbendazim after 20, 40, and 60 days of incubation.

Results

Table II presents basic descriptive statistics (average and standard deviations) that characterize the optical density in epiphytic and neustonic bacterial cultures on medium with and without carbendazim. The results demonstrate that both ecological groups of lacustrine

Table II
Culture optical density in the medium with (Ac) and without (A_0) carbendazim

Group of bacteria	Statistics	A_0	A_C
		t_{168}	
Epiphytic	Average*	0.150	0.137
	SD	0.03	0.08
Neustonic	Average*	0.088	0.087
	SD	0.04	0.05

Explanations: * – average (N = 26), SD – standard deviation

bacteria were developing well in the presence of carbendazim. The fungicide at the concentration of 1 mg/l did not hinder the growth of bacteria in cultures. In the first 3 days of incubation, no statistically significant differences between the absorbance in cultures with (A_C) and without (A_0) carbendazim were found.

The data regarding the reduction of carbendazim concentration in cultures of epiphytic and neustonic are presented in the Table III and Figures 3–4. The results demonstrate that the isolated bacterial strains had different capacity to reduce the concentration of the pesticide (1 mg/l) during 20, 40, and 60 days of the experiment. The differences in reduction of carbendazim concentration in bacterial cultures from different ecological groups of lacustrine microorganisms were statistically significant ($p < 0.05$, Table IV).

The obtained results demonstrate that the greatest reduction in pesticide concentration, that is, the highest rate of biodegradation was observed during the first 20 days of culturing. Epiphytic bacteria were found to be more effective in decomposing carbendazim than neustonic bacteria. In 60-day cultures of epiphytic bacteria, the concentration of carbendazim ranged from 0.26 mg/l to 0.64 mg/l with the average of 0.50 mg/l. Planktonic bacteria degraded pesticide to 39–77% of its initial concentration. The average reduction of the fungicide concentration after 60 – day incubation equaled 53%. After 60 days of incubation, the concentration of the pesticide in cultures of neustonic bacteria remained at 0.55 m/l – 0.92 mg/l with the average of 0.72 mg/l. Bacteria occurring in bottom sediments were less effective in decomposing carbendazim with the effectiveness ranging from 11% to 48%, on average 31%.

Table V presents results of analyses of carbendazim biodegradation in pure strain cultures of epiphytic and neustonic bacteria and in mixed cultures containing 25 strains. According to the results, the effectiveness of carbendazim degradation by mixed cultures was variable during the experiment in comparison to that of homogeneous cultures. The level of carbendazim

Table III
Biodegradation of carbendazim by epiphytic and neustonic bacteria

Group of bacteria	Statistics	C (mg l^{-1})	B (%)	C (mg l^{-1})	B (%)	C (mg l^{-1})	B (%)
		t_{20}		t_{40}		t_{60}	
Epiphytic	Average	0.71	32	0.59	44	0.50	53
	Min.	0.48	14	0.45	30	0.26	39
	Max.	0.89	55	0.73	58	0.64	77
	SD	0.09	9.6	0.08	8.2	0.09	8.7
Neustonic	Average	0.83	17	0.78	25	0.72	31
	Min.	0.62	0.2	0.60	9	0.55	11
	Max.	1.02	40	0.94	43	0.92	48
	SD	0.09	9.9	0.08	8.3	0.09	9.1

Explanations: C – carbendazim concentration; B – biodegradation; $t_{20, 40, 60}$ – incubation time 20, 40, 60 – days

Table IV
Differences between the rate of biodegradation of carbendazim in 20, 40 and 60-day cultures of epiphytic (EPI) and neustonic (NEU) bacteria

Variable	EPI 20	EPI 40	EPI 60	NEU 20	NEU 40	NEU 60
EPI 20		*	*	*	*	*
EPI 40			*	*	*	*
EPI 60				*	*	*
NEU 20					*	*
NEU 40						*
NEU 60						

Explanations: * – difference statistically significant ($p < 0.05$)

Table V
Biodegradation of carbendazim in mixed (MC) and homogenous (HC) cultures of bacteria

Group of bacteria	Type of culture	Biodegradation (%)		
		t_{20}	t_{40}	t_{60}
Epiphytic	MC*	43	44	46
	HC**	31	44	53
Neustonic	MC	24	26	27
	HC	16	25	31

Explanation: * – average (N = 25); ** (N = 1);
$t_{20, 40, 60}$ – incubation time 20, 40, 60 days

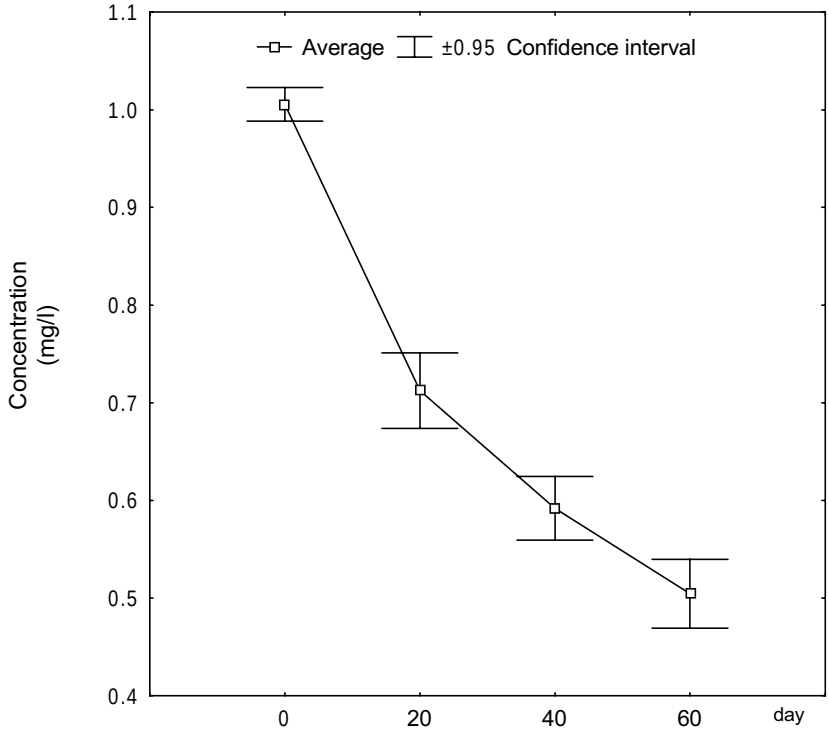

Fig. 3. Reduction of carbendazim concentration in the cultures of epiphytic bacteria.

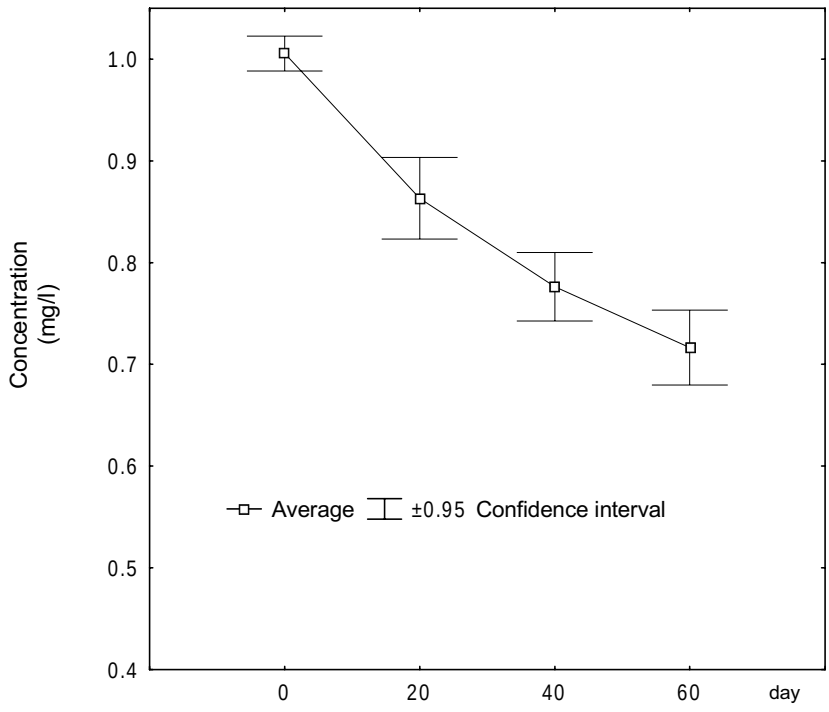

Fig. 4. Reduction of carbendazim concentration in the cultures of neustonic bacteria.

biodegradation in mixed cultures of epiphytic and neustonic bacteria after 20-day incubation was lower than that in homogeneous cultures. Sixty-day homogeneous cultures of epiphytic and neustonic bacteria were characterized by a higher mean level of carbendazim biodegradation than mixed cultures. After 40 days of the experiment, both epiphytic and neustonic bacteria demonstrated similar mean ability to decompose the fungicide in homogeneous and heterogeneous cultures.

Table VI presents analyses of a genus composition of microorganisms capable of decomposing carben-

Table VI
Generic composition of epiphytic and neustonic bacteria used for the study on biodegradation of carbendazim

Epiphytic bacteria		
No	Species	B (%)
1.	*Bacillus megaterium*	60
2.	n.i.	55
3.	*Aeromonas hydrophila*	42
4.	*Staphylococcus lentus*	48
5.	*Pseudomonas luteola*	56
6.	*Pseudomonas luteola*	48
7.	n.i.	53
8.	*Enterococcus faecium*	45
9.	*Pseudomonas luteola*	59
10.	*Leusconostoc* sp.	57
11.	*Pseudomonas luteola*	60
12.	*Pseudomonas luteola*	52
13.	*Aeromonas hydrophila*	45
14.	*Pseudomonas luteola*	77
15.	*Bacillus megaterium*	46
16.	*Aerococcus viridans*	39
17.	*Enterobacter sakazakii*	47
18.	*Aeromonas hydrophila*	44
19.	*Pseudomonas luteola*	59
20.	*Bacillus licheniformes*	60
21.	*Pseudomonas luteola*	56
22.	*Pseudomonas fluorescens*	55
23.	*Pseudomonas luteola*	66
24.	*Bacillus megaterium*	42
25.	*Aeromonas hydrophila*	55

Neustonic bacteria		
No	Species	B (%)
1.	*Stentrophomonas maltophila*	24
2.	*Stentrophomonas maltophila*	30
3.	*Aeromonas hydrophila*	37
4.	*Burkholderia cepacia*	30
5.	*Vibrio mimicus*	22
6.	n.i.	11
7.	*Burkholderia cepacia*	38
8.	n.d.	35
9.	*Aeromonas hydrophila*	44
10.	*Stentrophomonas maltophila*	35
11.	*Chromatium violaceum*	22
12.	*Burkholderia cepacia*	46
12.	*Vibrio fluvialis*	25
12.	*Burkholderia cepacia*	48
12.	*Staphylococcus cohnii*	25
12.	*Micrococcus luteus*	37
12.	*Burkholderia cepacia*	30
12.	n.i.	43
12.	*Pseudomonas luteola*	40
12.	*Chromatium violaceum*	36
12.	*Burkholderia cepacia*	24
12.	*Chromatium violaceum*	23
12.	*Vibrio fluvialis*	21
12.	*Vibrio fluvialis*	24
12.	*Burkholderia cepacia*	25

Explanations: n.i. – not identified

dazim. Twenty three (92%) out of 25 bacterial strains isolated from epidermis of the Common Reed were identified, while among neustonic bacteria – 22 (88%) strains. The genera *Pseudomonas* (*P. luteola* – 9 strains, *P. fluorescens* – 1 strain), *Aeromonas (A. hydrophila* – 4 strains), and *Bacillus* (*B. megaterium* – 3 strains, *B. licheniformes* – 1 strain) were particularly abundant among the identified epiphytic bacteria capable of degrading carbendazim. The following genera were the most abundant among neustonic bacteria capable of decomposing the fungicide: *Burkholderia* (*B. cepacia* – 7 strains), *Vibrio* (*V. fluvialis* – 3 strains, *V. mimicus* – 1 strain), *Stentrophomonas* (*S. maltophila* – 3 strains), *Chromatium* (*C. violaceum* – 3 strains), and *Aeromonas* (*A. hydrophila* – 2 strains). The remaining species of bacteria degrading fungicide were represented by single strains.

It was demonstrated that among epiphytic bacteria, *P. luteola* was the most efficient microorganism reducing the concentration of carbendazim. Among the analyzed neustonic bacteria, the strains of *B. cepacia* and *A. hydrophila* were the most effective in degrading carbendazim.

Discussion

In the natural environment, microbiological degradation is the main process responsible for reducing the concentration of carbendazim (WHO, 1993). Two-AB (2-aminobenzimidazole) is the most important product of carbendazim decomposition; 2-AB is also degraded by microorganisms (Helweg, 1977).

This study demonstrated that after 7 days from the moment the xenobiotic was added, both pure and mixed cultures of analyzed groups of lacustrine bacteria were developing relatively well in the presence of carbendazim with the concentration of 1 mg/l. Thus, the carbendazim concentration of 1 mg/l did not have a germicidal or bacteriostatic effect on the analyzed microorganisms. Certain organic micropollutants may have a toxic effect on populations of microorganisms and inhibit their metabolism; as a result, degradation of these compounds is possible only to a small degree (Warren *et al*., 2003). Furthermore, the study showed that all of the analyzed strains of bacteria and their mixed cultures were capable of reducing the concentration of carbendazim in cultures containing this

pesticide. This finding was confirmed by the presence of the main product of carbendazim degradation, 2-aminobenzimidazole (2-AB).

According to data available in literature describing the rate of carbendazim degradation, this compound is stable in water. In the aquatic environment, microbiological decomposition takes from 2 to 25 months (environment with and without oxygen, respectively) depending on the amount of oxygen present in water (WHO, 1993; Cuppen *et al*., 2000). According to this study, the highest reduction of the pesticide concentration, that is, the highest rate of carbendazim biodegradation was observed during the initial 20 days of culturing. Epiphytic bacteria reduced the pesticide concentration by 32%, and neustonic bacteria, by 17%.

Microorganisms inhabiting various ecological niches of a water body are characterized by different metabolic activity levels and, in consequence, a different response to xenobiotics introduced to the environment. The results indicate that epiphytic bacteria are characterized by higher average capacity to decompose carbendazim than neustonic bacteria ($p<0.05$). The half-life of carbendazim in epiphytic bacterial cultures equaled on average 60 days (Table IV). Neustonic bacteria needed on average 60 days to reduce the concentration of fungicide by 31%. Numerous researchers (Griffith *et al*., 1995; Biddanda and Benner 1997; Mudryk, 1998) point out the differences in respiration activity of bacteria occurring in different sections of a water column. According to Williams *et al*. (1986), Maki and Herwig (1991) and Mudryk (1998), neustonic bacteria are less active metabolically than planktonic bacteria. Similarly, benthic bacteria inhabiting lacustrine bottom sediments are less active metabolically than planktonic bacteria (Strzelczyk and Mielczarek, 1971; Donderski and Strzelczyk, 1992). According to the research on respiration activity of epiphytic and planktonic bacteria from Jeziorak Lake, bacteria growing on upper (near-surface) and bottom (near-bottom) sections of reeds are more active metabolically than planktonic bacteria (Lalke-Porczyk and Donderski, 2001). Despite the fact that heterotrophic bacteria inhabiting water bodies are able to metabolize various organic compounds and in spite of the availability of numerous techniques (enzymatic, respirometric, radiographic, or fluorescent) used to measure their metabolic activity, our knowledge on the subject is still fragmentary. The absence of comparative data describing metabolic activity of neustonic and epiphytic bacteria prevents a more detailed examination of the subject.

Comparing pure strains and mixed cultures of bacteria, it was concluded that during the initial 20 days of the experiment, heterogeneous cultures were on average more effective in decomposing carbendazim than pure strains. More effective biodegradation of carbendazim in mixed cultures is attributed to the prevalence of stimulating interaction over antagonistic interactions between organisms from different genera and species and most probably is also related to synergistic interactions. According to research conducted by Pattanasupong *et al*. (2004) on biodegradation of carbendazim and 2,4-D, the bacterial „consortium" (not isolated strains) obtained by the authors from the soil of irrigated fields in Japan had a relatively high capacity to decompose various pesticides – 100 mM for carbendazim and 3 mM for 2,4-D during 36 h and 24 h, respectively. Lower capacity of homogeneous cultures to decompose carbendazim in comparison to that of the homogeneous cultures after 60 days of incubation can be explained by an increased competition for the nutrient substrate in cultures containing cells of various bacterial strains.

Screening microorganisms with particularly high capacities to degrade carbendazim is very important in bioremediation of this type of pollution. In spite of the above, the reports regarding isolation and identification of pure bacterial cultures capable of degrading carbendazim are scarce and primarily pertain to the soil environment. Fuchs and de Vries (1978) demonstrated that rod-shaped Gram-negative bacteria, primarily, from the *Pseudomonas* genus isolated from soil and aquatic environments were capable of degrading carbendazim. Also, Holtman and Kobyashi (1997) found that the Gram-positive *Rhodococcus erythropolis* obtained from irrigated fields in Japan effectively biodegraded this fungicide. According to Zhang *et al*. (2005), the Gram-negative *Ralstonia* sp. inhabiting the soil environment could be a new bacterial source useful in biodegradation of carbendazim. Pattanasupong *et al*. (2004) demonstrated that cells of soil-derived bacterial consortium immobilized on polyester fibers are highly effective in degrading carbendazim with concentration reaching its solubility threshold (8 mg/l).

This study demonstrated that among the epiphytic bacteria, organisms from the *P. luteola* species had the highest capacity to decompose carbendazim, while, *B. cepacia* and *A. hydrophila* were the most effective among neustonic bacteria. The bacterial genera *Pseudomonas, Burkholderia* and *Aeromonas*, characterized by ability to decompose a wide spectrum of organic pollutants, including pesticides, are useful in bioremediation. Microorganisms from the *Pseudomonadaceae* family are particularly productive and decompose biotic and xenobiotic hydrocarbon substrates. Microorganisms form the *Pseudomonas* genus are characterized by an unprecedented tolerance for toxic compounds – *P. fluorescens* grows in soils polluted by petroleum (Barathi and Vasudevan, 2001) and is able to grow on a substrate containing polynuclear aromatic hydrocarbons (Soroka *et al*., 2001), while *Pseudomonas* sp. P166 grows in the presence of biphenyl

(Jung *et al*., 2001). *B. cepacia* species demonstrates exceptional ability to decompose many structurally complex organic compounds. The ability of this microorganism to decompose 2, 4, 5-trichloroacetic acid (Daubras *et al*., 1996), benzo(a)pyrene, dibenz(a,h) anthracene, coronene (Juhasz *et al*., 1997), p-nitrophenol (Bhushan *et al*., 2000), and other polyaromatic hydrocarbons (Kim *et al*., 2003) have been confirmed. *A. hydrophila*, which is common in surface water (Szewczyk *et al*., 2000), has a wide spectrum of exoenzymes (amylase, protease, lipase, nuclease, and others), which are active in degradation of many organic compounds (Pemberton *et al*., 1997); it is also capable of decomposing a common herbicide, propanil (Dilek *et al*., 1996) and textile dyes (Chen *et al*., 2003) among other agents.

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