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Original Research

Biodegradation of Carbendazim by Planktonic and Benthic Bacteria of Eutrophic Lake Chełmżyńskie

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Abstract

This study evaluates the biodegradation of carbendazim (1 mg/l) by homogeneous cultures of planktonic (N=25) and benthic (N=25) bacteria as well as by heterogeneous cultures (N=1) containing a mixture of 25 bacterial strains. The bacteria were collected from a 25 cm subsurface water layer and a 5-10 cm surface layer of bottom sediments of lake Chełmżyńskie.

Results indicate that bacterioplankton are better able to decompose carbendazim than benthic bacteria ($p<0.05$). In the case of decomposition by planktonic bacteria, the mean half-life of carbendazim equaled 40 days. Benthic bacteria, on average, required 60 days to reduce the concentration of fungicide by 37%. The level of biodegradation of carbendazim in mixed cultures of benthic and planktonic bacteria after a 20-day incubation period was lower than the average value of its biodegradation in homogeneous cultures. Forty- and 60-day homogeneous cultures of planktonic bacteria were characterized by a higher mean level of carbendazim biodegradation than that of the mixed culture. Decomposition of the fungicide in 40- and 60-day mixed cultures of benthic bacteria was higher than the mean value of biodegradation of this compound in homogeneous cultures. We demonstrated that among planktonic bacteria, the species *Sphingomonas paucimobilis, Aeromonas hydrophila,* and *Pseudomonas fluorescens* were the most efficient in reducing the concentration of carbendazim, while among benthic bacteria, *Burkholderia cepacia* and two unidentified strains of bacillus were the most efficient.

Keywords: bacterioplankton, benthic bacteria, biodegradation, carbendazim, lake, pesticides

Introduction

Pesticides are a group of pollutants that are very common in surface waters. They have been found in drinking water, ground water, the troposphere, and even in water and ice of the polar regions. Due to the common usage of pesticides in agriculture and their properties as stability, toxicity, and ability to accumulate, these compounds are particularly hazardous to living organisms, including humans. Mutagenic, carcinogenic, and teratogenic characteristics of pesticides have been reported multiple times [1-3].

However, the environment has a unique, internal resistance and ability to neutralize hazardous substances. Microbiological degradation is one of the most important processes that determine the fate of pesticides in the environment.

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Carbendazim (methyl-benzimidazole-2-yl-carbamate) is a commonly used fungicide in Poland and in the world. It belongs to the benzimidazole group of compounds, which are used as a remedy against various fungal diseases. Moreover, carbendazim is the main degradation product of two other pesticides: benomyl and methyl thiophene [4, 5]. The global use of carbendazim exceeds 12,000 tons of active substance annually, which corresponds to over USD 200 million [6]. In Poland, 42 agents containing carbendazim are in use. This compound is used as an active substance in the following commercial agents: Alert, Duett, Funaben, Sarfun, Siarkol, and Sarbawit [7].

Carbendazim can be treated as a hazardous substance. Numerous studies indicate that this substance is detectable in fruits and leaves of plants even after they have been harvested [8, 9]. Its mutagenic and teratogenic effects on mammals have also been confirmed, even when the substance was applied in a single and relatively small dose [10-12]. The effect of carbendazim on the reproductive ability is related to the compound's interaction with microtubules of the mitotic spindle, which leads to aberration in number of chromosomes in cells of mammals exposed to this substance *in vivo*.

The literature on pesticide biodegradation lacks comparative studies that present the problem from the ecological perspective and on the level of natural communities of microorganisms. The purpose of this study was to address the following questions: whether any planktonic or benthic bacteria isolated from a eutrophic lake are capable of degrading carbendazim, and which group of these microorganisms has a higher ability to neutralize this xenobiotic; and what is the level of carbendazim degradation caused by pure and mixed cultures of these microorganisms.

Materials and Methods

Isolation of Strains

The analyzed strains of bacteria were isolated from the samples of subsurface water (planktonic bacteria) and the surface layer of bottom sediments (benthic bacteria), which were collected from a sampling station located in the extra-urban section of lake Chełmżyńskie surrounded by arable land.

Lake Chełmżyńskie, located ca. 20 km from Toruń, belongs to the Fryba–Vistula river basin and is a typical eutrophic water body. The lake has the following characteristics: surface area – ca. 271 ha, capacity – 16,451.9 thousand m^3, maximum depth – 27.1 m, average depth – ca. 6 m. The watershed of the lake primarily includes arable lands, which constitutes 72% of the immediate watershed. The urbanized areas of Chełmża are located in the northwest section of the lake.

The subsurface water sample was collected from a depth of ca. 20 cm with a sterile glass pipette using an automatic Pipet-Boy pipettor (Biotechnology, De Ville). The bottom sediment sample was collected at a depth of 10 cm using a tube scoop (custom made) with a diameter of 5 cm. The samples of water and bottom sediments were transported to a laboratory in a cold container at below +4ºC. The time between the sample collection and the microbiological analyses usually did not exceed 4 hours.

The samples, which had been diluted with sterile buffer water according to Daubner [13], were inoculated on the peptone-iron agar (IPA), a recommended medium for the isolation of bacteria from freshwater environments [14], and incubated at 20ºC in order to isolate bacterial colonies for further analyses. Twenty-five bacterial strains were isolated from both the water and bottom sediment samples.

Preliminary Cultures and Preparation of a Bacterial Inoculum

Bacterial strains isolated from individual samples and cultured on peptone-iron agar plates (IPA) were tested for purity and then used for preparation of preliminary cultures. Subsequently, bacterial inoculum for the analyses of carbendazim degradation were 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, $MgSO_4 \cdot 7H_2O$ – 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 20ºC for 168 hours in a rotary shaker (WL – 2000, JW Electronic). Bacterial inoculum were 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 “Marcel s 330 Pro” spectrophotometer 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 analyzed ecological group 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 grow in the presence of a xenobiotic, we measured the optical density of preliminary cultures in a medium with and without carbendazim after 168 hours of incubation using a “Marcel s 330 Pro” spectrophotometer at a wavelength of $\lambda = 560$ nm.

Proper Cultures

Five hundred µl samples of bacterial inocula were added into Erlenmeyer flasks containing 75 ml of sterile mineral medium with the above composition. The final concentration of carbendazim in cultures was 1 mg/l.

Table 1. Culture optical density in the medium with (A_c) and without (A_0) carbendazim.

Group of bacteria	Statistics	A_C	A_0
		t_{168}	
Planktonic	Average *	0.147	0.137
	SD	0.02	0.03
Benthic	Average	0.168	0.157
	SD	0.04	0.09

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

Sterile mineral medium without pesticide was used to dilute the samples. All samples were analyzed in triplicates. 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. There were also control for abiotic degradation of carbendazim.

Bacterial cultures were incubated for 60 days at 20ºC in the dark in the rotary shaker (WL – 2000, JW Electronic).

Analysis of Reduction of Carbendazim Concentration and Presence of 2-aminobenzimidazole (2-AB) in the Cultures

Degradation of carbendazim (presence of 2-AB – the main product of carbendazim decomposition) was monitored by measuring the reduction of the pesticide concentration in the cultures with a method of high performance liquid chromatography (HPLC). HPLC equipment (Perkin-Elmer) with pomp - SERIES 200 LC and detector - SERIES 200 UV/VIS was applied. One ml culture were collected after 20, 40, and 60 days of incubation and filtered through a 0.22µm syringe filter (Fisherbrandt) in order to remove the cells. Filtrates were stored at -20ºC for further analysis.

The analysis were conducted with a column Supelcosil™, LC-18 (25 cm x 4.6 mm, 5 µm; SUPELCO) and pre-column Supelcosil™ Supelguard® (2 cm x 4 mm, 5µm; SUPELCO) at a wavelength of λ= 254 nm. A solution (40:45:15) of acetonitrile (J.T. Baker), spectrally pure water (J.T. Baker), and buffer containing 0.067 M Na_2HPO_4 and 0.067 M KH_2PO_2 (2:3) constituted the mobile phase. Fifty µl samples of filtrate were chromatographed in triplicates.

The isocratic separation was carried out at a flow rate of 1ml/min; the quantity of pesticide in the 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/1 analytical standard of fungicide (Sigma – Aldrich, purity of carbendazim 99.1%, purity of 2-AB 99.9%). The basic solution of carbendazim diluted with the mobile phase (composition as above) in preparation for HPLC. The following concentrations were obtained (mg/1): 0.1; 0.25; 0.5; 0.75; 1.0. Standard of 2- aminobenzimidazole (Sigma – Aldrich) was made at a concentration of 0.25 mg/1. The prepared standards underwent chromatographical analysis; each concentration was analyzed in three replicates. The analytical curve for carbendazim with a linear correlation coefficient of < 0.99 was graphed. Retention time for carbendazim was recorded at 3.91 min and for 2-AB at 4.18 min.

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). The 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 1 presents basic descriptive statistics (average and standard deviations) that characterize the optical density in planktonic and benthic bacterial cultures on medium with and without carbendazim. Results demonstrate that both ecological groups of lacustrine bacteria were developing well in the presence of carbendazim. The 1 mg/l solution of fungicide did not hinder the growth of bacteria in cultures. In the first 168 hours of incubation, we found no statistically significant differences between the absorbance in cultures with (A_k) and without (A_0) carbendazim.

The data regarding the reduction of carbendazim concentration in cultures of planktonic and benthic are presented in Table 2 and Figs. 1, 2. Results demonstrate that the isolated bacterial strains had different abilities in reducing the concentration of the pesticide (1mg/l) during 20, 40, and 60 days of the experiment. The differences in reduction of carbendazim concentration in bacterial cultures that belong

Table 2. Biodegradation of carbendazim by planktonic and benthic bacteria.

Group of bacteria	Statistics	C [mg/l]	B [%]	C [mg/l]	B [%]	C [mg/l]	B [%]
		t_{20}		t_{40}		t_{60}	
Planktonic	Average	0.58	**43**	0.51	**49**	0.45	**58**
	Min.	0.50	27	0.45	29	0.32	41
	Max.	0.73	50	0.71	55	0.62	71
	SD	0.07	6.8	0.06	6.1	0.06	6.3
Benthic	Average	0.85	**18**	0.80	**23**	0.76	**27**
	Min.	0.71	9	0.70	14	0.63	16
	Max.	0.93	32	0.90	35	0.87	40
	SD	0.06	5.7	0.06	5.9	0.08	7.6

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

to different ecological groups of lacustrine microorganisms were statistically significant ($p<0.05$, Table 3).

Results demonstrate that the greatest reduction of pesticide concentration, that is, the highest rate of biodegradation, was observed during the first 20 days of culturing.

Planktonic bacteria proved to be more effective in decomposing carbendazim than benthic bacteria.

In 60-day cultures of planktonic bacteria, the concentration of carbendazim ranged from 0.32 mg/l to 0.62 mg/l with the average of 0.45 mg/l. Planktonic bacteria degraded pesticide to 41-71% of its initial concentration. The average reduction of the fungicide concentration after 60-day incubation equaled 58%.

After 60 days of incubation, in cultures of benthic bacteria, the concentration of the pesticide remained at 0.63 mg/l – 0.87 mg/l with the average of 0.76 mg/l. Bacteria occurring in bottom sediments were less effective in decomposing carbendazim with the effectiveness ranging from 16% to 40%, on average 27%.

The microorganisms' ability to decompose the pesticide was confirmed by the presence of the main product of carbendazim degradation, 2-aminobenzimidazole (2-AB) in all examined cultures of bacteria (Fig. 3).

Table 4 presents results of analyses of carbendazim biodegradation caused by pure strains of planktonic and benthic bacteria and by 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 biodegradation in mixed cultures of benthic and planktonic bacteria after 20-day incubation was lower than that in homogeneous cultures. Forty- and 60-day homogeneous cultures of planktonic bacteria were characterized by higher mean value of carbendazim biodegradation than that of mixed cultures. After 40 and 60 days of experiments, strains of benthic bacteria demonstrated on average a higher ability to degrade the fungicide in mixed cultures than in homogeneous cultures.

Table 5 presents results of analyses of genus composition of the microorganisms capable of decomposing carbendazim.

Among the 25 bacterial strains isolated from the lacustrine water, 21 (84%) strains have been identified, while among the 25 benthic bacterial strains, 19 (76%).

Table 3. Differences between the rate of biodegradation of carbendazim in 20, 40 and 60-day cultures of planktonic (PL) and benthic (BENT) bacteria.

Variable	PL20	PL40	PL60	BENT20	BENT40	BENT60
PL20		*	*	*	*	*
PL40			*	*	*	*
PL60				*	*	*
BENT20					*	*
BENT40						*
BENT60						

* - difference statistically significant ($p < 0.05$)

Table 4. Biodegradation of carbendazim in mixed (Cm) and homogenous (Ch) cultures of bacteria.

Group of bacteria	Type of culture	Biodegradation		
		t_{20}	t_{40}	t_{60}
Planktonic	Cm	27	29	41
	Ch*	43	49	59
Benthic	Cm	15	25	37
	Ch	18	23	27

* - average (N=25); $t_{20,40,60}$ - incubation time 20, 40, 60 days.

Among the identified planktonic bacteria capable of degrading carbendazim, there were 5 strains of the *Sphingomonas paucimobilis* genus, 5 strains of the *Aeromonas hydrophila* genus, and 4 strains of the *Pseudomonas* genus (*P. fluorescens* – 2 strains, *P. luteola* – 2 strains). Among the benthic bacteria capable of decomposing the fungicide, *Burkholderia cepacia* (10 strains), *Aeromonas hydrophila* (4 strains), and *Bacillus megaterium* (3 strains) were the most common. The remaining species of bacteria degrading fungicide were represented by single strains.

It was demonstrated that among the planktonic bacteria, the species *Sphingomonas paucimobilis*, *Aeromonas hydrophilas*, and *Pseudomonas fluorescens* were the most effective in reducing the concentration of carbendazim.

Table 5. Generic composition of planktonic and benthic bacteria used for the study on biodegradation of carbendazim.

Planktonic bacteria			Benthic bacteria		
No	Gender/Species	B [%]	No	Gender/Species	B [%]
1	*Pseudomonas fluorescens*	61	1	*Bacillus mycoides*	26
2	*Sphingomonas paucimobilis*	59	2	*Burkholderia cepacia*	20
3	*Staphylocooccus intermedius*	54	3	*Burkholderia cepacia*	40
4	*Sphingomonas paucimobilis*	64	4	*Burkholderia cepacia*	20
5	*Micrococcus luteus*	60	5	*Bacillus megaterium*	33
6	*Sphingomonas paucimobilis*	71	6	*Burkholderia cepacia*	16
7	*Staphylococcus sciuri*	60	7	*Aeromonas hydrophila*	35
8	*Aeromonas hydrophila*	70	8	u.s.	18
9	u.s.	59	9	*Burkholderia cepacia*	32
10	*Pseudomonas luteola*	53	10	u.s.	28
11	*Aneuribacillus aneurinilyticus*	56	11	*Aeromonas hydrophila*	28
12	*Geobacillus thermoglucosidasus*	56	12	*Burkholderia cepacia*	22
13	*Aeromonas hydrophila*	48	13	*Aeromonas hydrophila*	29
14	*Moraxella sp.*	57	14	u.s.	40
15	*Pseudomonas fluorescens*	68	15	*Pseudomonas luteola*	16
16	*Sphingomonas paucimobilis*	60	16	*Burkholderia cepacia*	28
17	u.s.	58	17	*Burkholderia cepacia*	28
18	*Aeromonas hydrophila*	58	18	*Aeromonas hydrophila*	24
19	*Aeromonas hydrophila*	56	19	u.s.	24
20	*Sphingomonas paucimobilis*	60	20	*Bacillus megaterium*	27
21	*Pseudomonas luteola*	59	21	u.s.	40
22	*Aeromonas hydrophila*	52	22	*Burkholderia cepacia*	19
23	*Staphylocooccus intermedius*	59	23	*Burkholderia cepacia*	36
24	*Brevundimonas vescicularis*	51	24	*Bacillus megaterium*	18
25	*Vibrio metchnikovii*	59	25	u.s.	23

No – number of strain, B – biodegradation, u.s. unidentified strain

Among the analyzed benthic bacteria, the strains of *Burkholderia cepacia* and two unidentified strains of bacillus were the most effective in degrading carbendazim.

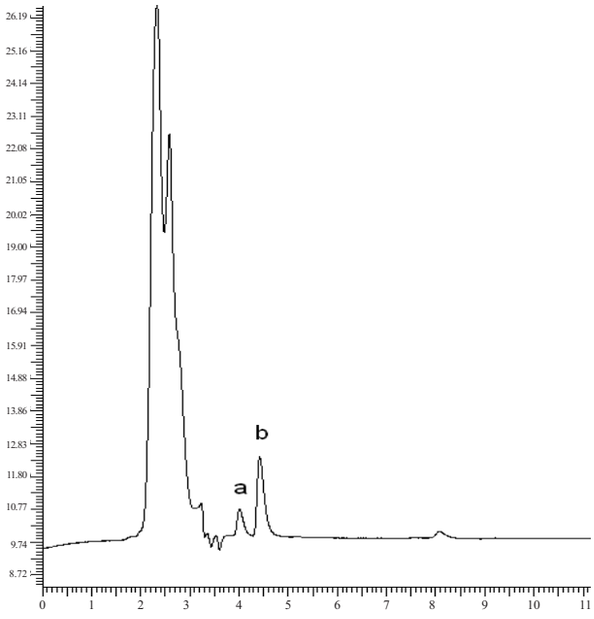
Discussion

Microbiological degradation is one of the most important processes that determine the fate of pesticides in the environment. 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 [15].

Occurrence of a "new" foreign organic substance in the environment usually leads to selection of certain sensitive microorganisms, while the remainder adapts to the new environmental conditions. In the course of the adaptation, microorganisms initiate enzymatic processes that enable them to utilize compounds that are not the products of their own metabolic transformation as energetic and building substrates (microbiological metabolism), or these substances are incorporated in the metabolic cycle of microbial cells and are not used as an energy source (microbiological cometabolism) [16].

In the natural environment, carbendazim primarily undergoes the microbiological degradation [17]. 2-aminobenzimidazole (2-AB) is the most important product of carbendazim decomposition; the former substance is also degraded by microorganisms [18].

On the basis of results of this study, it was concluded that after 168 hours 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 1mg/l.



a – 2-AB peak at 3.91; b – carbendazim peak at 4.38.

Fig. 1. HPLC plot of culture filtrate.

Thus, the carbendazim concentration of 1 mg/l did not have a germicidal or bacteriostatic effect on the analyzed microorganisms. However, certain organic micropollutants may be toxic to the population of microorganisms and inhibit their metabolism; as a result, degradation of these compounds is possible only to a small degree [16]. 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. The microorganisms' ability to decompose the pesticide was confirmed by the presence of the main product of carbendazim degradation, 2-aminobenzimidazole (2-AB). Yarden et al. [19] and Aharonson and Katan [20] observed the phenomenon of accelerated degradation of carbendazim after its single introduction to the soil environment.

According to the data describing 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 [17, 21]. On the basis of results of this study, it was concluded that the highest level of reduction of the pesticide content, that is, the highest rate of carbendazim biodegradation was observed during the initial 20 days of culturing.

Microorganisms inhabiting various ecological niches of a water body are characterized by a different metabolic activity and a different response to xenobiotics introduced to the environment. According to numerous studies related to the metabolic activity of heterotrophic bacteria that occur in the water column and bottom sediments, bacterioplankton is characterized by higher metabolic and biochemical activity than benthic bacteria [22-26]. In spite of a relatively high abundances of microorganisms inhabiting bottom sediments [27, 28] and the fact that bacterial cells that originate from deep sediments are capable of growth on solid mediums in laboratory conditions (viable bacteria) [29, 30], the activity of benthic microorganisms is often low, which indicates a low cell-specific activity. It is probable that precisely this low cell-specific activity of bacteria inhabiting bottom sediments is responsible for their limited ability to decompose carbendazim.

The lower ability of the analyzed strains to decompose carbendazim in mixed cultures of planktonic bacteria could be a result of antagonistic relations or a competition for the substrate. Wiliams and Vickers [31] argued that antagonistic relations of microbes associated with production of antibiotic substances may occur in heterogeneous bacterial mixtures. Strzelczyk et al. [32] and Lemos et al. [33] indicated that certain bacteria are capable of secreting bacteriocytes, functioning as inhibitors of the bacterial respiration activity. Chodyniecki [34], when analyzing various combinations of bacteria from the *Pseudomonas*, *Aeromonas*, *Vibrio*, and *Flavobaterium* genera, found that 12% of bacterial interactions were of antagonistic character, and 8% of stimulating. This latter type of interaction and probably also bacterial synergism occurred in heterogeneous mixtures of benthic bacteria, and as a result these cultures decomposed carbendazim more effectively than the single strains of these bacteria.

Analysis of microorganisms with particularly high abilities to degrade carbendazim is very important in the aspect of bioremediation of this type of pollution. In spite of this, reports regarding isolation and identification of pure bacterial cultures capable of degrading carbendazim are rare and primarily pertain to the soil environment. Fuchs and de Vries [35] demonstrated that rod-shaped gram negative bacteria mainly from the *Pseudomonas* genus isolated from soil and aquatic environments were capable of degrading carbendazim. Also, Holtman and Kobyashi [36] found that the gram-positive *Rhodococcus erythropolis* obtained from irrigated fields in Japan effectively participates in biodegradation of this fungicide. According to Zhang et al. [37], the gram-negative *Ralstonia sp*. inhabiting the soil environment could be a new bacterial source useful in biodegradation of carbendazim. Pattanasupong et al. [38] demonstrated that cells of soil-derived bacterial consortium immobilized on polyester fibers are highly effective in degrading carbendazim with concentrations reaching its solubility threshold (8mg/l).

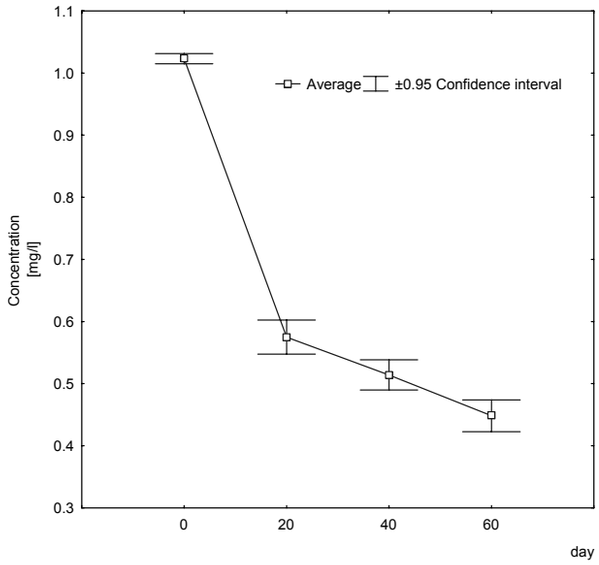


Fig. 2. Reduction of carbendazim concentration in the cultures of planktonic bacteria.

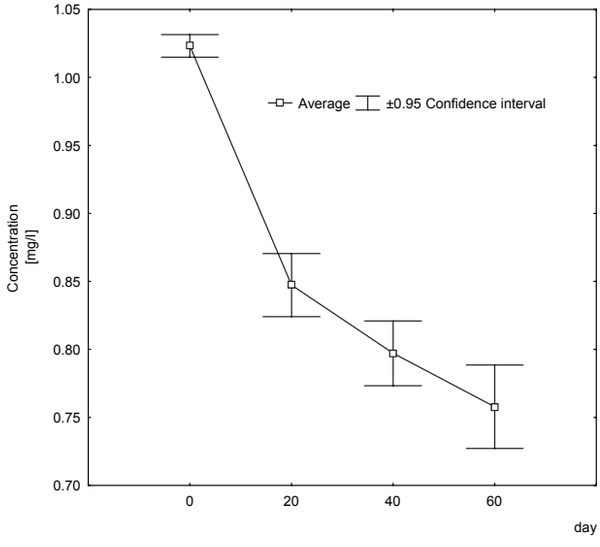


Fig. 3. Reduction of carbendazim concentration in the cultures of benthic bacteria.

This study demonstrated that among the planktonic bacteria, the species *Sphingomonas paucimobilis*, *Aeromonas hydrophila* and *Pseudomonas fluorescens* were characterized by the highest ability to decompose carbendazim. Among benthic bacteria, *Burkholderia cepacia* and unidentified strains of bacilli were the most effective in decomposing the analyzed fungicide.

The bacterial species *Sphingomonas paucimobilis*, *Aeromonas hydrophila*, *Pseudomonas fluorescens* and *Burkholderia cepacia*, characterized by the ability to decompose a wide spectrum of organic pollutants, including pesticides, are useful in bioremediation. For instance, *Sphingomonas paucimobilis* is capable of degrading hexachlorocyclohexane and biosorption of cadmium [39,40] and phenyl-methyl-ethers [41]. *Aeromonas hydrophila*, which is commonly found in surface water [42], has a wide spectrum of exoenzymes (amylase, protease, lipase, nuclease, and others), active in degradation of many organic compounds [43]; it is also capable of decomposing a common herbicide, propanil [44] and textile dyes [45], among other agents. Microorganisms from the *Pseudomonadaceae* family are particularly active and decompose biotic and xenobiotic hydrocarbon substrates. Microorganisms forming the *Pseudomonas* genus are characterized by an unprecedented tolerance for toxic compounds. *P. fluorescens* grows in soils polluted with petroleum [46] and is able to grow on a substrate containing polynuclear aromatic hydrocarbons [47], while *Pseudomonas sp*. P166 grows in the presence of biphenyl [48]. Microorganisms that belong to the *Burkholderia cepacia* species demonstrate exceptional abilities to decompose many structurally complex organic compounds. The abilities of this microorganism to decompose 2, 4, 5 – trichloroacetic acid [49], benzo(a)pyrene, dibenz(a,h)anthracene, coronene [50], p-nitrophenol [51], and other polyaromatic hydrocarbons [52] are substantiated.

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References

1. AQUARELLA J., DOES J., TOMENSON J., CHESTER G., COWELL J., BLOEMEN L. Epidemiologic studies of occupational pesticide exposure and cancer: regulatory risk assessments and biologic plausibility, Annals Epidemiol. **13** (1), 1, **2003**.
2. CROSS P., JONES G.-E. variation in pesticide hazard from arable crop production in Great Bitain from 1992-2002: pesticide risk indices and policy analysis, Crop Protect. **25** (10), 1101, **2006**.

3. BOLONGESI C. Genotoxitcity of pesticides: a review of human biomonitoring studies, Mut. Res./Rev. Mut. Res. **534** (3) 251, **2003**.
4. MONTGOMERY H.J. Benomyl. In: Agrochemicals Desk Reference, 2nd edn., New York, Lewis Publishers, ISBN:**1**, 56670, 168, **1997**.
5. MAZELLIER P., LEROY E., LEGUBE B. Photochemical behavior of fungicide carbendazim in dilute aqueous solutions, J. Photochem. Photobiol. A: Chemistry **153**, 221, **2002**.
6. Carbendazim. In: Pesticide News, **57**, 20, **2002**.
7. http://www.zch.sarzyna.pl/firma/index.html, viewing date: March 01. **2007**.
8. CHIBA M., VERES D.F. HPLC method for simultaneous determination of residual benomyl and methyl-2-benzimidazole carbamate in apple foliage without cleanup, J. Assoc. Off. Anal. Chem., **63**, 1291, **1980**.
9. KIJEMAGI U., INMAN U., MELLENTHIN W.M., DEINZER M.L. Residues of benomyl (determined as carbendazim) and captan in post harvest – treated pears in cold storage, J. Agric. Food Chem., **39**, 400, **1991**.
10. PERREAULT D.S., JEFFAY S., POSS P., LASKEY J.W. Use of the fungicide carbendazim as a model compound to determine the impact of acute chemical exposure during oocyte maturation and fertilization on pregnancy outcome in the hamster, Toxicol. Appl. Pharmacol., **114**, 225, **1992**.
11. SARRIF A.M., ARCE G.T., KRAHN D.F., O,NEIL R.M., REYNOLDS V.L. Evaluation of carbendazim in the presence of some normal soil constituents with photodiode-array detection, J. Chromatogr., **538**, 480, **1994**.
12. NAKAI B.M., MOORE B.J., HESS R.A. Epithelial reorganization and irregular growth following carbendazim – induced injury of the efferent ductales of the rat testis, Anat. Rec., **235**, 51, **1993**.
13. DAUBNER I. Water microbiology, Slov. Akad. Vied., Bratislava, **1967**.
14. FERRER E.B., STAPERT E.M., SOKOLSKI W.T. A medium for improved recovery of bacteria from water, Can. J. Microbiol., **9**, 420, **1963**.
15. ZIPPER CH., NICKEL K., ANGST W., KOHLER H.P. Complete microbial degradation of both enantiomers of the chiral herbicide mecoprop [(RS)-2-(chloro-2-methylphenoxy) propionic acid] in an Enantioselective Manner by Sphingomonas herbicidovorans sp. nov., Appl. Environ. Microb., **12**, 4318, **1996**.
16. WARREN N., ALLAN I.J., CARTER J.E., HOUSE W.A., PARKER A. Pesticides and other micro-organic contaminants in freshwater sedimentary environments-a review., Appl. Geochem., **18**, 159, **2003**.
17. WHO, Environment Health Criteria 149: Carbendazim, Geneva: World Health Organization, (http://www.inchem.org/documents/ehc/ehc/ehc149.htm, viewing date: March 01.2007) **1993**.
18. HELWEG A. Degradation and adsorption of carbendazim and 2-aminobenzimidazole in soil, Pestic Sci., **8**, 71, **1977**.
19. YARDEN O., SALOMON R., KATAN J., AHARONSON N. Involvement of fungi and bacteria in enhanced and non enhanced biodegradation of carbendazim and other benzimidazole compounds in soil, Canadian J. Microbiol., **36**, 15, **1990**.
20. AHARONSON N., KATAN J. Delayed and enhanced biodegradation of soil-applied diphenamid, carbendazim, and aldicarb, Arch. Insect Biochem. Physiol., **22**, 451, **1993**.
21. CUPPEN J.G.M., BRINK P.J. van der, CAMPS E., UIL K.F., BROCK T.C.M. Impact of the fungicide carbendazim in freshwater microcosms. I. Water quality, breakdown of particulate organic matter and responses of macroinvertebrates, Aquat. Toxicol., **48**, 233, **2000**.
22. STRZELCZYK E., MIELCZAREK A. Comparative studies on metabolic activity of planktonic, benthic and epiphytic bacteria, Hydrobiol., **38**, 67, **1971**.
23. DONDERSKI W., SYRZELCZYK E. The ecology and physiology of aerobic heterotrophic bacteria in lakes of different trophy. In: Some Ecological Biological Systems in North Poland, Ed. Bohr R., Nienartowicz A., Wilkoń-Michalska J., N. Copernicus University Press, Toruń, **1992**.
24. MUDRYK Z. Heterotrophic bacteria in the processes of transformation of organic matter in estuary lakes, Rozprawy, WSP, Słupsk, **1994**.
25. FISCHER H., WANNER S.C., PUSCH M. Bacterial abundance and production in river sediments as related to the biochemical composition of particulate organic matter (POM), Biogeochem. **61**, 37, **2002**.
26. HAGLUND A.L., LANTZ P., TÖRNBLOM E., TRANVIK L. Depth distribution of active bacteria and bacterial activity in lake sediment, FEMS Microb. Ecol., **46**, 31, **2003**.
27. NIEWOLAK S. Seasonal changes of the heterotrophic microflora of the Iława lakes bottom sediments. Pol. Arch. Hydrobiol., **3**, 211, **1968**.
28. KALWASIŃSKA A., DONDERSKI W. Benthic bacteria of Chełmżyńskie Lake (Poland), Polish J. Environ. Stud., **14**(6), 761, **2005**.
29. MISKIN I., RHODES G., LAWLOR K., SAUNDERS J.R., PICKUP R.W. Bacteria in post-glacial freshwater sediments, Microbiol. **144**, 2427, **1998**.
30. PARKES R.J., CRAGG B.A., BALE S.J., GETLIFF J.M., GOODMAN K., ROCHELLE P.A., FRY J.C., WEIGHTMAN A.J., HARVEY S.M. Deep bacterial biosphere in Pacific Ocean sediments, Nature, **371**, 410, **1994**.
31. WILLIAMS S.T., VICKERS J.C. The ecology of antibiotic production, Microbiol. Ecol., **12**, 43, **1986**.
32. STRZELCZYK E., STOPIŃSKI M., MYZYK G. Studies on metabolic activity of single and mixed cultures of planktonic and benthic bacteria of two lakes of different trophy, AUNC Toruń, Limnol. Papers, **16**, 3, **1988**.
33. LEMOS M.L., DOPAZO C.P., TORANZO A.E., BARJA J.L. Competitive dominance of antibiotic-producing marine bacteria in mixed cultures, J. Appl. Bacteriol., **71**, 228, **1991**.
34. CHODYNIECKI A. Antibiosis and symbiosis among freshwater bacteria, Szczecin, **1968**.
35. FUCHS A., VRIES F.W. de, Bacterial breakdown of benomyl. I. Pure cultures, Antonie van Leeuwenhoek, **44**, 283, **1978**.
36. HOLTMAN M.A., KOBAYASHI D.Y. Identification of *Rhodococcus erythropolis* isolates capable of degrading the fungicide carbendazim, Appl. Microbiol. Biotechnol., **47**, 578, **1997**.
37. ZHANG G.SH., JIA X.M., CHENG T.F., MA X.H., ZHAO Y.H. Isolation and characterization of new carbendazim – degrading *Ralstonia sp*. strain, World J. Microb. Biotechnol., **21**, 256, **2005**.
38. PATTANASUPONG A., NAGASE H., INOUE M., HIRATA K., TANI K., NASU M., IYAMOTO K. Ability of a microbial consortium to remove pesticide, carbendazim and 2,4-dichlorophenoxyacetic acid, World J. Microbiol. Biotechnol., **20**, 517, **2004**.
39. PAL R., BALA SH., DADHWAL M., KUMAR M., DHINGRA G., PRAKASH O., PPRABTGARAN S.R., SHIVASI S., CULLUM J., HOLLIER CH., LAL R. Hexachlorocyclohexane – degrading bacterial strains

Sphingomonas paucimobilis B90A, UT26 and Sp+, having similar genes, represent three distinct species, *Sphingobium indicum sp*., nov., *Sphingobium japonicum* sp., nov., and *Sphingobium francense sp*. nov., and reclassification of [*Sphingomonas*] chungbukensis as *Sphingobium chungbukense* comb. nov., Int. J. Evol. Microbiol., **55**(5), 1965, **2005**.

40. TANGAROMSUK J., POKETHITIYOOK P., KRUATRACHUE M., UPATHAM E.S. Cadmium biosorption by *Sphingomonas paucimobilis* biomass, Biosource Technol., **85**(1), 103, **2002**.
41. NISHIKAWA S., SONOKI T., KASAHARA T., OBI T., KUBOTA S., KAWAI S., MOROHOSHI N., KATAYAMA Y. Cloning and sequencing of the *Sphingomonas* (*Pseudmonas*) *paucimobilis* gene essential for o-demethylation of vanillate and syringate, Appl. Environ. Microbiol., **63**(3), 836, **1998**.
42. SZEWCZYK U., SZEWCZYK R., MANZ W., SCHLEIFER K.H. Microbial safety of drinking water, Annu. Rev. Microbiol., **54**, 81, **2000**.
43. PEMBERTON J.M., KIDD S.P., SCHMIDT R. Secreted enzymes of *Aeromonas*, FEMS Microbiol. Lett., **152**, 1, **1997**.
44. DILEK F.B., ANDERSON G.K., BLOOR J. Investigation into the microbiology of the rate jet-loop activated sludge reactor treating brewery wastewater, Wat. Sci. Tech., **43**(5-6), 107, **1996**.
45. CHEN K.-H., WU J.-Y., LIOU D.-J., HWANG SZ.-CH. J. Decolorization of the textile dyes by newly isolated bacterial strains. J. Biotechnol., **101**(1), 57, **2003**.
46. BARATHI S., VASUDEVAN N., Utilization of petroleum hydrocarbons by Pseudomonas fluorescens isolated from a petroleum – contaminated soil., Environ. Int., **26**, 413, **2001**.
47. SOROKA Y.M., SAMOILENKO L.S., GVOZDYAK P.I. Strains of *Pseudomonas fluorescens* 3 and *Atrhrobacter sp*. 2 degrading polycyclic aromatic hydrocarbons. Microbiol. Zh., **63**, 65, **2001**.
48. JUNG K.-J., KIM E., SO J.-S., KOH S.-CH. Specific biodegradation of polychlorinated biphenyls (PCBs) facilitated by plant terpenoids, Biotechnol. Bioproc. Eng., **6**, 61, **2001**.
49. DAUBRAS D.L., DANGANAN C.E., HŰBNER A., YE R.W., HENDRICKSON W., CHAKRABARTY A.M. biodegradation of 2,4,5-trichlorophenoxyacetoc acid by Burkholderia cepacia strain AC1100: evolutionary insight, Gene, **179**(1), 1, **1996**.
50. JUHASZ A.L., BRITZ M.L., STANLEY G.A., Degradation of benso(a)pyrene, dibenz(a,h)anthracene and coronene by *Burkholderia cepacia*, Wat. Sci. Tech., **36**(10), 45, **1997**.
51. BHUSHAN B., CHAUHAN A., SAMANTA S.K., JAIN R.K. Kinetics of biodegradation of p-nitrophenol by different bacteria, Bioch. Biophis. Res. Communic., **274**, 626, **2000**.
52. KIM T.J., LEE E.Y., KIM Y.J., CHO K.-S., RYU H.W. Degradation of polyaromatic hydrocarbons by *Burkholderia cepacia* 2A-12, World J. Microb. Biot., **19**(4), 411, **2003**.