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Letter to Editor

The Influence of Heavy Metals on the Activity of Chitinases Produced by Planktonic, Benthic and Epiphytic Bacteria

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Abstract

Research was carried out on the influence of heavy metals on the activity of chitinases produced by chitinolytic bacteria isolated from Moty Bay in Lake Jeziorak. In tests on the concentration of heavy metal ions in the surface water, the highest concentration of copper and zinc ions was found in July in the littoral zone, of cobalt ions in September in the pelagic zone and of lead ions in August, also in the pelagic zone. Studies on the number of chitinolytic bacteria have shown that the highest number of chitinolytic bacteria among the total number of heterotrophic bacteria occurred on macrophytes and in bottom sediments. The highest percentage of chitinolytic bacteria was found in the surface water, and the lowest in the bottom sediments.

As follows from research on the influence of heavy metals on the activity of chitinases , in the majority of the strains tested, heavy metal ions inhibited chitinolytic activity as concentration increased.

Keywords: chitinolytic bacteria, chitinases, heavy metals

Introduction

Chitin is a polysaccharide, a linear polymer composed of N-acetylglucosamine monomers connected by glycoside β-1, 4 bonds [1]. It makes part of the outer arthropod skeletons of insects and crustaceans [2, 3] and also is one of components of fungi and some yeast cell walls [4]. Biological degradation of chitin is accompanied by endo- and exo- enzymes known as chitinases (EC 3.2.1.114) and β-N–acetylhexaminidases (EC 3.2.1.52) [5]. Tronsmo and Harman [6] isolated three groups of chitinolytic enzymes: chitinases cutting the chitin at random at different points of N-acetylglucosamine polymer: chitobiosidases operating after chitinases and releasing chitobiasis off spilt fragments, and N-acetyl-β-glucosamidases hydrolyzing chitobiasis for monomers.

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Due to incessant industrial development, heavy metal contaminants have been introduced into water environments. Undoubtedly, they affect growth and enzymatic activity of existing groups of bacteria. Low concentrations of heavy metals do not exert a distinct influence on bacteria enzymatic activity. However, during inflow from the catchment basin or ecological disasters, metal concentration suddenly grows and becomes possible to disturb the course of the mineralization processes of organic matter in which one of the parts is chitin. Hence, the principal aim of the present work was to discover the influence of some heavy metals in amounts exceeding their acceptable concentrations, on bacteria chitinolytic activity as well as to find differences in chitinase activity among planktonic, benthic and epiphytic bacteria (depending on current concentrations of particular metals).

Materials and Methods

Object of Study

The study was carried out in Moty Bay of lake Jeziorak. Lake Jeziorak is located in the Iławskie Lake District and makes part of the Vistula-Drwęca catchment area. It is a post-glacial lake of a meridian-like placement. The lake surface is 32.3 km^2, length – 27.5 km, mean width – about 1.2 km and maximum depth – 12.0 m; mean depth is about 5.7 m. The lake is a eutrophic water body [7].

Sampling

The study material was made of bacteria isolated from water surface water, bottom sediments of the sublitoral and pelagic zones, and macrophyt (lesser reedmace). The water was sampled from the depth of 10-20 cm using a pipette automatic pump (Pipet – boy, De Ville), and then poured into sterile bottles sealed with rubber stoppers. The bottom sediments were sampled by means of a tube scoop and the surface layer (down to 5 cm) was aseptically transferred into sterile jars. Also, 15 cm long lesser reedmace collected for examination and placed into sterile glass jars. All samples were placed in thermoisolated containers with ice (not exceeding ± 7°C), and brought to a laboratory where they were immediately analyzed. The study material was collected between June and November 2000.

Heterotrophic Bacteria Number

The number of heterotrophic bacteria in the samples of water, bottom sediments and macrophytes were determined by means of the spread plates method, inoculating the material from respective solutions in three parallel repetitions on the iron-peptone agar medium, according to Ferrer, Stapert, Sokolski [8]. Buffered water after Daubner [9] was used as solvent. After 6 days of incubation at 20°C, the grown colonies (CFU) were counted by converting the result into 1 cm^3 water or 1 g dry weight (bottom sediments).

Chitinolytic Bacteria Number

The number of chitinolytic bacteria in the examined samples were determined with the use of media consisting of the following: peptone (peptobak) – 1.0 g, iron sulphate – 0.1 g, ammonia sulphate – 0.1 g, iron gluconate – 0.1 g, yeast extract – 0.1 g, colloidal chitin – 7.0 g of dry mass, agar – 15.0 g, tap water – 1.0 dm^3, pH 7.2-7.4. The colloidal chitin had been prepared according to Lingappa and Lockwood [10]. The plates with inoculation were incubation at 20°C and after 14 days of the diameter, light-coloured patches around the colonies were measured to asses the bacteria ability to decompose chitin. Those strains were then used for further study on chitinolytic activity. They were refrigerated and inoculated onto a fresh semi-liquid medium every 2 months.

Identification of Chitinolytic Bacteria

The identification of the strains under study was done according to the pattern suggested by Allen, Austin and Colwell [11] for freshwater bacteria.

Examination of Chitinolytic Activity

Bacterial strains displaying high chitinolytic activity isolated from water, bottom sediments and the surface of lesser reedmace were used for the tests. They were grown in 100 cm^3 Erlenmeyer flasks containing 50 cm^3 of liquid medium composed of peptone (peptobak) – 1.0 g; yeast extract – 0.1 g; ammonium sulphate – 0.1 g; ferrous sulphate – 0.1 g; iron gluconate – 0.1 g; colloidal chitin – 2.0 g dry mass; and tap water – 1 dm^3, pH – 7.4. The medium was seeded with 0.5 cm^3 of inoculum. After 4 days of incubation at 20°C the culture was centrifuged at 15,000 g/min for 30 min. The temperature during centrifugation did not exceed +4°C.

Supernatant was used to determine the activity of chitinases. The measurement of the activity of chitinases was conducted at 30°C. For this purpose, test tubes with a reactive mixture containing 1 cm^3 of supernatant and 1 cm^3 of colloidal chitin suspension (10 mg (cm^3) in phosphate buffer with a pH of 7.0 were placed in a water bath with water circulation. Test tubes for the control contained the same components as the studied test tubes, except that the supernatant was inactivated before introducing it into the test tubes by boiling them in the water bath for 15 min. Enzymatic reactions were conducted for 48 h, after which they were interrupted by placing the test tubes in the boiling water bath for 15 min. After cooling, the contents of the test tubes were centrifuged at 15,000 g/min for 30 min., and then the content of released N-acetylglucosamine was determined in a clear liquid using the method by Reissig, Strominer, Leloir [12].

The chitinase activity was determined on the basis of the amount of µmols of released N-acetylglucosamine calculated per 1 mg bacterial protein, per hour (specific activity). The amount of bacterial proteins was determined using Bradford's method [13]. Results were calculated for the coefficient of chitinolytic activity:< 100 – stimulation, <100 – inhibition, = 100 – no influence.

The Influence of Heavy Metals on Chitinase Activity

Chitinase activity was tested in the presence of heavy metal ions: Cu^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+} at the following concen-

trations: 0.25 mg/l; 0.5 mg/l; 1.0 mg/l; 1.5 mg/l. The source of copper was $CuSO_4$, that of zinc was $ZnSO_4$, that of lead was $(CH_3COO)_2Pb$ and cobalt was $CoCl_2$. The reactive mixture contained 1 cm^3 of supernatant and 0.5 cm^3 of colloidal chitin suspension (10 mg/cm^3) in 0.05 M phosphate buffer with a pH of 7.0 and 0.5 cm^3 of the appropriate concentration of metal. The studied concentration of heavy metals ions was higher than the concentration of heavy metals ions in the surface water in the littoral and pelagic zone in Moty Bay of Lake Jeziorak.

Results

Studies on the concentration of heavy metals ions in surface water (Table 1) showed the highest concentration of copper and zinc ions to be in July in the littoral zone, that of cobalt ions to be in September in the pelagic zone and that of lead to be in August, also in the pelagic zone.

Studies on the number of chitinolytic bacteria are presented in Table 2. As follows, the highest number of chitinolytic bacteria among the total number of heterotrophic bacteria occurred on the surface of lesser reedmace (on average 2275.4 x 10^3 cells/g dry plant mass) and in bottom sediments. In surface water, the highest number of chitinolytic bacteria was found in June and July (4.7 x 10^3 cells/cm^3, 3.8 x 10^3 cells/cm^3, respectively), in bottom sediments in August (456.1 x 10^3 cells/g dry mass), and on lesser reedmace in July and in September (3866.1 x 10^3 cells/gram of dry plant, 3446.7 x 10^3 cells/gram of dry plant, respectively). In November, no chitinolytic bacteria were found in any of the habitats studied.

The highest percentage of chitinolytic bacteria occurred in surface water with the maximum in July (on average 12.9%), and the lowest in bottom sediments (on average 6.6%).

The results of research on the influence of heavy metals ions on the activity of chitinases are presented in Tables 3-6 and Figures 1-4. As follows, the studied heavy metal ions inhibited chitinolytic activity as concentration increased in the case of the majority of strains. Zn^{2+} ions inhibited the activity of chitinases in the majority of the studied strains. *Aeromonas sp*. isolated from the water did a concentration of 0.25 mg/l and 0.5 mg/L stimulate the activity of chitinases. *Bacillus megaterium* isolated from the macrophytes did a concentration of 0.25 mg/L stimulate the activity of chitinases (Table 5, Figure 1). Cu^{2+} ions inhibited chitinolytic activity in the majority of the studied strains. However, in two of the strains isolated from the water (*Vibrio fluvialis*, *Cytophaga salmonicolor*) and two strains isolated from the bottom sediments (*Alcaligenes denitryficans* and *Aeromonas hydrophila*), a concentration of 0.25 mg/l stimulated chitinase activity

Table 1. The concentration of heavy metals (mg/L) in the littoral and pelagic zones in Moty Bay, Lake Jeziorak.

Place of sampling	Copper			Cobalt			Lead			Zinc		
	24.07.00	22.08.00	21.09.00	24.07.00	22.08.00	21.09.00	24.07.00	22.08.00	21.09.00	24.07.00	22.08.00	21.09.00
Littoral	0.120	0.080	0.085	0.053	0.034	0.048	0.010	0.012	0.012	0.025	0.024	0.010
Pelagic	0.080	0.103	0.091	0.050	0.046	0.066	0.002	0.028	0.022	0.020	0.017	0.009

Table 2. Number of heterotrophic and chitinolytic bacteria inhabiting Moty Bay, Lake Jeziorak.

Date of sampling	Surface water•		Bottom sediments ••		Macrophytes ••	
17.06.00	* 23.6 ** 4.7	(20.1)	3075.0 307.5	(10.1)	21166.7 1693.3	(8.0)
24.07.00	14.0 3.8	(27.1)	4062.0 207.2	(5.1)	32217.1 3866.1	(12.0)
22.08.00	9.0 0.3	(3.3)	13820.0 456.1	(3.3)	28666.7 946.0	(3.3)
21.09.00	14.7 1.9	(13.3)	880.0 67.8	(7.7)	15666.7 3446.7	(22.0)
20.10.00	8.3 1.1	(13.3)	660.0 87.8	(13.3)	12833.3 1925.0	(15.0)
25.11. 00	2.0 0.0	(0.0)	480.3 0.0	(0.0)	ns	
Average	11.9 1.97	(12.9)	3829.5 187.7	(6.6)	22119.1 2375.4	(12.1)

Explanations: * – number of heterotrophic bacteria, ** – number of chitinolytic bacteria, • – number of bacteria x 10^3 (cm^3, •• – number of bacteria x 10^3/g dry weight plant or dry weight bottoms, ( ) – bacteria in percent, ns – non studies.

Table 3. The influence Zn^{2+} on activity of chitinase production by planktonic, benthic and epiphytic bacteria inhabiting Moty Bay, Lake Jeziorak.

Name of bacteria	Habitat	Zn^{2+} [mg (L]				
		0.0	0.25	0.5	1.0	1.5
Vibrio fluvialis	surface water	* 0.011 * * 100	0.0058 52.7	0.0030 27.3	0.0029 26.4	0.0013 11.8
Cytophaga salmonicolor		0.0084 100	0.0067 76.8	0.0029 34.5	0.0013 15.5	0 0
Aeromonas sp.		0.0033 100	0.0094 163.6	0.0043 130.3	0.0014 42.4	0 0
Acinetobacter sp.		0.021 100	0.0040 19.0	0.0032 15.2	0.0023 10.9	0.0013 6.2
Pseudomonas fluorescens		0.010 100	0.0025 25.0	0.00031 3.1	0.00031 3.1	0 0
Agrobacterium	bottom sediments	0.023 100	0.0045 19.6	0.0019 8.3	0.0013 5.7	0 0
Aeromonas sp.		0.023 100	0.0094 36.5	0.0069 30.0	0.0028 12.2	0 0
Aeromonas hydrophila		0.014 100	0.0073 52.1	0.0069 47.9	0.0037 26.4	0.0020 14.3
Bacillus megaterium		0.023 100	0.0056 24.3	0.0028 12.2	0.0020 8.7	0 0
Alcaligenes denitryficans		0.011 100	0.0069 62.7	0.0036 32.7	0.0014 12.7	0 0
Aeromonas hydrophila		0.023 100	0.0078 33.9	0.0044 19.1	0.0028 12.2	0 0
Aeromonas salmonicida	macrophytes	0.027 100	0.010 37.0	0.0087 32.2	0.0053 19.6	0 0
Pseudomonas fluorescens		0.015 100	0.011 73.3	0.0023 15.3	0.0010 6.7	0 0
Bacillus megaterium		0.0083 100	0.010 120.5	0.0039 47.0	0.0032 38.6	0.00081 9.8
Bacillus pumilus		0.026 100	0.0079 30.4	0.0036 13.8	0.0025 9.6	0 0

Explanations: * - specific activity of chitinases (in mol GlcNAc/mg protein/h), ** - coefficient of chitinolytic activity: > 100 - stimulation, < 100 - inhibition, = 100 - no influence.

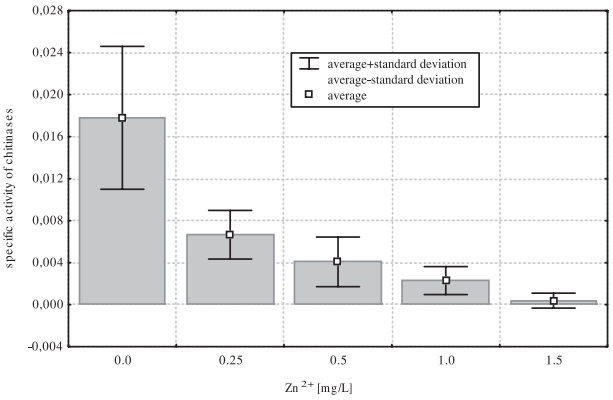

Fig. 1. The influence of Zn^{2+} on activity of chitinase production by planktonic, benthic and epiphytic bacteria inhabiting Moty Bay, Lake Jeziorak.

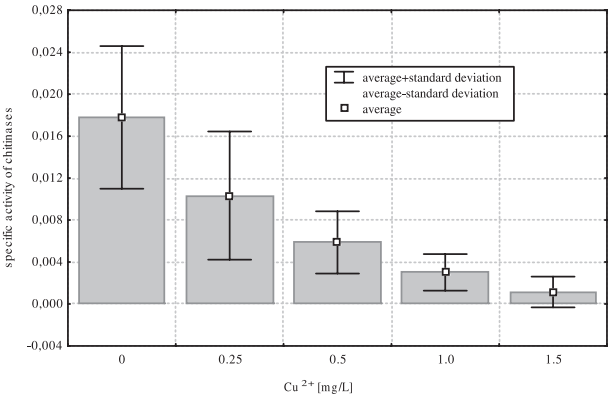

Fig. 2. The influence of Cu^{2+} on activity of chitinase production by planktonic, benthic and epiphytic bacteria inhabiting Moty Bay, Lake Jeziorak.

Table 4. The influence Cu^{2+} on activity of chitinase production by planktonic, benthic and epiphytic bacteria inhabiting Moty Bay, Lake Jeziorak.

Name of bacteria	Habitat	Cu^{2+} [mg (L]				
		0.0	0.25	0.5	1.0	1.5
Vibrio fluvialis	surface water	* 0.011 * * 100	0.013 118.2	0.0061 55.5	0.0035 31.8	0.00065 5.9
Cytophaga salmonicolor		0.0084 100	0.0107 127.4	0.0098 116.7	0.0029 34.5	0 0
Aeromonas sp.		0.0033 100	0.0029 87.9	0.0013 39.4	0.0013 39.4	0.00036 10.9
Acinetobacter sp.		0.025 100	0.0067 31.9	0.0030 14.3	0.0030 14.3	0.0017 8.1
Pseudomonas fluorescens		0.010 100	0.0074 74.0	0.0040 40.0	0.0014 14.0	0.00080 8.0
Agrobacterium	bottom sediments	0.023 100	0.014 60.9	0.010 43.5	0.0022 9.6	0.00045 2.0
Aeromonas sp.		0.023 100	0.0075 32.6	0.0050 21.7	0.0023 10.0	0.0010 4.3
Aeromonas hydrophila		0.014 100	0.010 71.4	0.0070 50.0	0.0045 32.1	0.00090 6.4
Bacillus megaterium		0.023 100	0.0044 19.1	0.0036 15.7	0.0020 8.7	0.00081 3.5
Alcaligenes denitryficans		0.011 100	0.012 109.1	0.0094 85.5	0.0033 30.0	0.0004 3.6
Aeromonas hydrophila		0.023 100	0.027 117.4	0.0082 35.6	0.0077 34.5	0.0056 24.3
Aeromonas salmonicida	macrophytes	0.027 100	0.0053 19.6	0.0020 7.4	0.0020 7.4	0.0010 3.7
Pseudomonas fluorescens		0.015 100	0.0060 40.0	0.0023 15.3	0 0	0 0
Bacillus megaterium		0.0083 100	0.0044 53.0	0.0020 24.1	0.00081 9.7	0 0
Bacillus pumilus		0.026 100	0.0040 15.4	0 0	0 0	0 0

Explanations: * - specific activity of chitinases (in mol GlcNAc/mg protein/h), ** - coefficient of chitinolytic activity: > 100 - stimulation, < 100 - inhibition, = 100 - no influence.

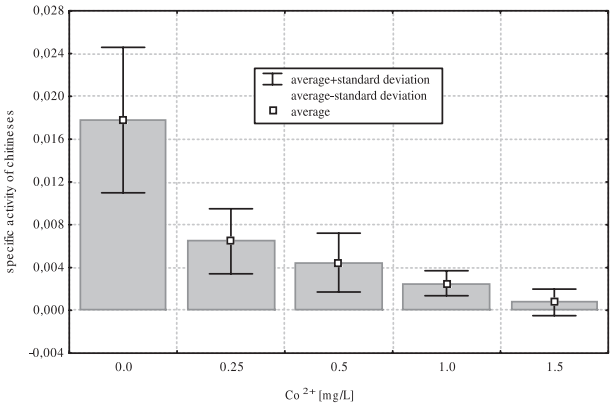

Fig. 3. The influence of Co^{2+} on activity of chitinase production by planktonic, benthic and epiphytic bacteria inhabiting Moty Bay, Lake Jeziorak.

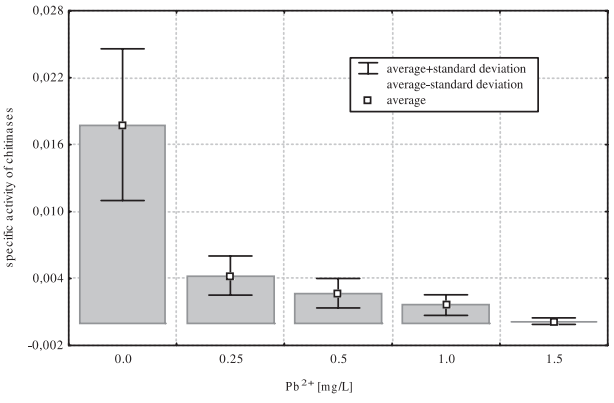

Fig. 4. The influence of Pb^{2+} on activity of chitinase production by planktonic, benthic and epiphytic bacteria inhabiting Moty Bay, Lake Jeziorak.

(Table 2, Fig. 2). Co^{2+} ions stimulated chitinolytic activity only in *Vibrio fluvialis* (Table 6, Fig. 3). Pb^{2+} ions inhibited chitinolytic activity in all of the studied strains (Table 3, Figure 4).

Discussion

Among heterotrophic bacteria, which are of fundamental significance in the flow of matter and energy in nature, it is possible to distinguish a group of bacteria capable of mineralizing chitins. This complex organic compound can be hydrolyzed only by microorganisms that have the appropriate enzymatic equipment. The decomposition of chitin and its inclusion of carbon and nitrogen into circulation is conducted using chitinolytic enzymes called chitinases [3]. Research on the number of chitinolytic bacteria have shown that their number was several times higher in bottom deposits and on the surface of macrophytes than in the water. The cause can be taken to be the very high content of sedimenting nutritive substances and the stability of the conditions in comparison with the water, permanently undergoing mixing. Donderski [14] believes that the smaller number of chitinolytic bacteria in the water of a eutrophic lake is connected with the greater variety of nutritive substances in this water body, amongst which are compounds that are more readily and more easily metabolized than chitin. As a percentage, however, more chitinolytic bacteria were found in the water. This is probably linked to the fact that fewer heterotrophic bacteria occur in the water in general, and thus the proportion of chitinolytic bacteria among the total number of heterotrophs may be higher. According to Donderski [14, 15], only 8% of the number of heterotrophs were capable of decomposing chitin in the bottom sediments of Lake Jeziorak, compared with 15% in the water. As follows from this research, the highest percentage of chitinolytic bacteria in the water was found in

Table 5. The influence Co^{2+} on activity of chitinase production by planktonic, benthic and epiphytic bacteria inhabiting Moty Bay, Lake Jeziorak.

Name of bacteria	Habitat	Co^{2+} [mg (L]				
		0.0	0.25	0.5	1.0	1.5
Vibrio fluvialis	surface water	* 0.011 * * 100	0.014 127.3	0.012 109.1	0.0025 22.7	0.0013 11.8
Cytophaga salmonicolor		0.0084 100	0.0042 50.0	0.0029 34.5	0.0013 15.5	0 0
Aeromonas sp.		0.0033 100	0.0014 42.4	0 0	0 0	0 0
Acinetobacter sp.		0.021 100	0.0057 27.1	0.0037 17.6	0.0023 10.9	0.00068 3.2
Pseudomonas fluorescens		0.010 100	0.0028 28.0	0.0012 12.0	0 0	0 0
Agrobacterium	bottom sediments	0.023 100	0.0055 23.9	0.0032 13.9	0.0022 9.5	0 0
Aeromonas sp.		0.023 100	0.0069 30.0	0.0038 16.5	0.0019 8.3	0 0
Aeromonas hydrophila		0.014 100	0.0053 37.9	0.0033 23.6	0.0013 9.3	0.0008 5.7
Bacillus megaterium		0.023 100	0.010 43.5	0.0048 20.9	0.0036 15.6	0 0
Alcaligenes denitryficans		0.011 100	0.0043 39.1	0.0040 36.4	0.0029 26.4	0.00074 6.7
Aeromonas hydrophila		0.023 100	0.0081 35.2	0.0072 31.3	0.0056 24.3	0.0044 19.1
Aeromonas salmonicida	macrophytes	0.027 100	0.0047 17.4	0.0033 12.2	0.0017 6.3	0.0010 3.7
Pseudomonas fluorescens		0.015 100	0.0060 40.0	0.0042 28.0	0.0023 15.3	0.0011 7.3
Bacillus megaterium		0.0083 100	0.0063 75.9	0.0044 53.0	0.0028 33.7	0.0016 19.3
Bacillus pumilus		0.026 100	0.0069 26.5	0.0051 19.6	0.0025 9.6	0.00074 2.8

Explanations: * - specific activity of chitinases (in mol GlcNAc/mg protein/h), ** - coefficient of chitinolytic activity: > 100 - stimulation, < 100 - inhibition, = 100 - no influence.

July, while in September it was still fairly high and remained the same until October. Chitinolytic bacteria reached their highest percentage in the bottom sediments in October. Studying the microflora of Lake Gardno, Mudryk [16] also found the highest percentage of chitinolytic bacteria in October, and an only slightly lower percentage in July. In turn, Donderski [14] says that the maximum percentage of chitinolytic bacteria in Lake Jeziorak occurred in November in the water and in May in the bottom sediments. The total lack of bacteria that decompose chitin observed in this paper in November can be explained by the low temperature of the water, which impedes the production of enzymes, and by the fact that chitin is a compound that is decomposed slowly [15, 17]. Some authors, however, have observed the presence of chitinolytic bacteria in lakes in winter [16], which could be linked with a local increase in temperature or with a generally warm winter at that time.

The speed of organic matter mineralization, including chitin, depends on numerous factors: activity of enzymes produced by different groups of microbes and abiotic factors that directly affect that activity. As a result of industrial development and an increase in strongly urbanized areas, water environments are seeing increasing amounts of chemical contaminants that affect the mineralization process. The concentration of toxic substances, which are brought into water reservoirs, are frequently low and have no distinct impact on the speed of organic matter degradation. Conducted investigations allowed us to notice low concentrations of heavy metals in Lake Jeziorak. Previous investigations revealed that they do not affect growth and chitinolytic activity of bacteria. However, in extreme circumstances resulting in sudden inflow of heavy metals (disasters, poor wastes management); the concentration of these substance can suddenly rise. Therefore, it is crucial to find metal concentrations at which their restraining effect on enzymatic activity, including chitinolytic one, of bacteria becomes noticeable. Our investigations proved

Table 6. The influence Pb^{2+} on activity of chitinase production by planktonic, benthic and epiphytic bacteria inhabiting Moty Bay, Lake Jeziorak.

Name of bacteria	Habitat	Pb^{2+} [mg (L]				
		0.0	0.25	0.5	1.0	1.5
Vibrio fluvialis	surface water	* 0.011 * * 100	0.0074 67.3	0.0042 38.2	0.0026 23.6	0.00031 2.8
Cytophaga salmonicolor		0.0084 100	0.0046 54.8	0.0017 20.2	0.00085 10.1	0.00042 5.0
Aeromonas sp.		0.0033 100	0.0010 30.3	0.00081 24.5	0.00041 12.4	0 0
Acinetobacter sp.		0.025 100	0.0010 47.6	0.0009 42.9	0.00076 36.2	0 0
Pseudomonas fluorescens		0.010 100	0.0037 37.0	0.0012 12.0	0.00031 3.1	0 0
Agrobacterium	bottom sediments	0.023 100	0.0048 20.9	0.0032 13.9	0.0017 7.4	0.00065 2.8
Aeromonas sp.		0.023 100	0.0031 13.5	0.0019 8.3	0.0017 7.4	0 0
Aeromonas hydrophila		0.014 100	0.0047 33.6	0.0043 30.7	0.0033 23.6	0 0
Bacillus megaterium		0.023 100	0.0048 20.9	0.0036 15.7	0.0012 5.2	0.00081 3.5
Alcaligenes denitryficans		0.011 100	0.0062 56.4	0.0036 32.7	0.0029 23.4	0 0
Aeromonas hydrophila		0.023 100	0.0056 24.3	0.0044 15.1	0.0017 7.4	0 0
Aeromonas salmonicida	macrophytes	0.027 100	0.0027 10.0	0.0017 6.3	0.0017 6.3	0 0
Pseudomonas fluorescens		0.015 100	0.0026 15.3	0.0015 8.8	0.00077 4.5	0 0
Bacillus megaterium		0.0083 100	0.0048 57.8	0.0016 19.3	0.00038 4.6	0 0
Bacillus pumilus		0.026 100	0.0022 8.5	0.00074 2.8	0.00036 1.4	0 0

Explanations: * - specific activity of chitinases (in mol GlcNAc/mg protein/h), ** - coefficient of chitinolytic activity: > 100 - stimulation, < 100 - inhibition, = 100 - no influence.

that at least as high as 0.5 mg/l concentrations distinctly restrained chitinolytic activity, and that chitinolytic enzymes produced by planktonic bacteria are less sensitive to higher concentrations of heavy metals. The higher concentration applied, the stronger the observed restrain. In some strains this was even a stimulating influence. Conceivably, these bacteria can be of allochthonous origin and get to the lake from the catchment area or from the air or even from sewage. In such environments they could have adapted and become more resistant to their operation than other groups of bacteria. However, in more strains heavy metals ions in this paper distinctly inhibited this activity.

Heavy metals that influence the development of cells have a considerable influence on enzymatic activity. The degree of inhibition by heavy metals of enzymatic activity depends on many factors, including the degree of their solubility and the degree of oxidation. Salts of the same element can cause inhibition of enzymes differently at different degrees of oxidation. Another factor is the resistance of the cells to a given type and concentration of heavy metal ions, which consequently results in the strengthening or weakening of their enzymatic activity. Some strains are very sensitive, others less so. Enzymatic inhibition could also involve masking the catalytically active subunits of the enzyme, degrading proteins, changing the conformation of the particles of the enzyme and competing with cation activators connected with the formation of a substrate-enzyme complex [18]. The studies in the present paper demonstrate that zinc ions and lead inhibited the activity of chitinases by as much as 100% at a concentration of 1.5 mg/l. Studying the activity of chitinases, Tominga and Tsujiska [19] showed that the majority of the heavy metals they studied also inhibited the activity of chitinases. Zinc inhibited the activity of chitinases by 15.8%, lead 21.1%, and copper up to 23.7%, although the concentration of ions used they was 50 times higher than the highest concentration used in the present paper. Probably the reason for such great differences in the activity of chitin may be the presence of other inhibing compounds. Laboratory research in the present paper was conducted on crude enzymes. Kańska, Łebkowska and Peszta [20] determined the influence of copper, chrome, cadmium and lead on the proteolytic and dehydrogenase activity of microorganisms of biological membranes in deposits that clean organic sewage. The studies showed that the heavy metals tested reduced the activity of the studied enzymes from 75 to 100% at a concentration of 1.5 mg/l, with the greatest inhibition being caused by zinc and lead. In this paper, the majority of heavy metal ions inhibited the activity of the enzyme by 100% at a concentration of 1.5 mg/l in many of the strains, while lead and zinc inhibited chitinase activity in as many as 11 strains.

Conclusion

1. The highest number of chitinolytic bacteria among the total number of heterotrophic bacteria occurred on the surface of lesser reedmace x 10^3 cells/g dry and in bottom sediments.
2. In surface water, the highest number of chitinolytic bacteria was found in June, in bottom sediments in August and on lesser reedmace in July. In November, no chitinolytic bacteria were found in any of the habitats studied.
3. The highest percentage of chitinolytic bacteria occurred in surface water with the maximum in July, and the lowest in bottom sediments.
4. The studied heavy metal ions inhibited chitinolytic activity as concentration increased in the case of the majority of strains.

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