MICROBIAL DIVERSITY OF THE ŽITAVA RIVER AND WETLANDS IN THE ŽITAVSKÝ LUH NATURE RESERVE

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Abstract

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Over 2003–2004 a study was conducted to evaluate microbial quality of surface water of the Žitava river, its branches and adjacent wetlands in the Žitavský luh Nature Reserve. This paper characterizes and describes the most significant microbial communities occurring in this specific environment. The presence of coliform bacteria, myxobacteria, actinomycetes and soil microscopic fungi in the plankton and benthos areas of flowing water (the new Žitava river-bed), stagnant water (adjacent old bed of the Žitava) and wetlands. The most numerous communities in the locality under investigation in the area of benthos were coliform bacteria (with the maximum occurrence value of $4.1.10^4$ KTJ. g^{-1} of benthos) and microscopic fungi (with the maximum occurrence value of $3.5.10^3$ KTJ. g^{-1} of benthos). From microscopic fungi, species of the genera *Aspergillus, Penicillium, Trichoderma, Cladosporium* and *Fusarium* occurred in particular. In the area of wetlands were dominant the species of the genus *Trichoderma* and in the Žitava river (both new and old river-beds) the species of the genera *Penicillium* and *Aspergillus.*

Key words: wetlands, surface waters, microorganisms, water quality, water monitoring

Introduction

Wetlands usually develop in places which are periodically inundated by water, especially along rivers, lakes, in coastal areas. They can also occur on slopes where underground water soaks. According to the definition, a wetland is an interface between dry terrestrial ecosystem and aquatic ecosystem, where the underground water level is usually near the soil surface or it is covered with shallow water, stands are inundated or saturated with water, and characteristic wetland plants and hydrophormic soils are present (www.fns.uniba.sk). Wetlands are of great importance for the conservation of biodiversity of organisms. The most numerous community in both natural and artificial wetlands is bacteria community. Among dominant bacteria are mainly the species of the genera *Bacillus, Clostridium, Mycoplasma, Eubacterium* and *Proteobacteria,* originally isolated from the mammal gastro-intestinal truct (Ibekve et al., 2003). In these ecosystems bacteria play an important role as an indicator of substance decomposition in an environment, because in regard to a small volume they have the largest body surface of all organisms (Merkley et al., 2004).

Situated in the north of Nové Zámky district, a nature reserve called Žitava luh meadow can be classed as the so-called river wetland system, since it is found in the vicinity of the Žitava river, which depth in some places is more than 2 m. With respect to the fact that this area was and still is monitored from the viewpoint of the occurrence of characteristic flora and animals, our aim was to study and describe the typical microbial biocoenosis in this specific environment. In available literature we have found no information about possible microbial research in this ecologically interesting territory.

Material and methods

Characteristic of the study area

The Žitavský luh Nature Reserve stretches on the left bank of the river Žitava between the villages of Maňa, Michal nad Žitavou and Kmeťovo, on the boundary of Nitra and Nové Zámky districts, in a place called Gedrianske lúky (meadows). It is located at an altitude of 132–133 m, below deforested ridges of Hronská vrchovina (highland). The territory arose and was gradually developed and formed by human activities in accordance with yearly floods. Žitavský luh of a total area of 74.69 ha was declared the nature reserve on 29th February 1980, which helped to ensure at least partial prevention of the Žitava river from the consequences of its regulation. The river flow was modified in 1980–1981. A new water body of the Žitava river was directed across the most valuable parts of meadows, causing that about two thirds of wet meadow area and 90% of the total amount of plants and animals have disappeared. The flood system of a newly created nature reserve was based on building up a system of lock gates and a protective, artificially made embankment. In 2001, drainage was built up for the purposes of draining the water infiltrated behind the embankment. Regulation of the Žitava river caused the underground water to decrease, thus limiting natural spring floods. Water regime has rather stagnant than flowing character. In the spring (February–April), a larger area of the locality is inundated. During the vegetation period, the lowermost southern part is more or less permanently inundated but out of this period, the territory is practically without water. According to the climate classification of Slovakia, the nature reserve territory has lowland, rather dry climate with mild temperature inversions. The Žitava floodplain belongs to warm areas, having more than 50 summer days with the average daily air temperature over 25 °C. Mean annual air temperature is 9.5 °C, the warmest month is July (average daily temperature of about 19.5 °C) and the coldest ones are January and February (-1 to -4 °C). Based on an amount of precipitation, this territory is among the driest ones in Slovakia. The total sum of precipitation in the Žitavský luh Nature Reserve varies from 530 to 650 mm per year. The total precipitation sum is unstable. Most rain falls in summer, whereas in March, February and January there is minimum rain. Average precipitation sum in this area in 87 days is more than 1 mm.

Characteristics of intake points and samplings

Intake points in the Žitavský luh Nature Reserve were chosen to reflect objectively the water quality in different parts and different biotopes of the river being investigated, its branches and wetlands subjected to specific conditions of the environment. In cooperation with the Department of Environment and Zoology, Faculty of Agriculture and Food Resources, Slovak Agricultural University, Nitra, six intake points have been chosen (Fig. 1). In the 1st

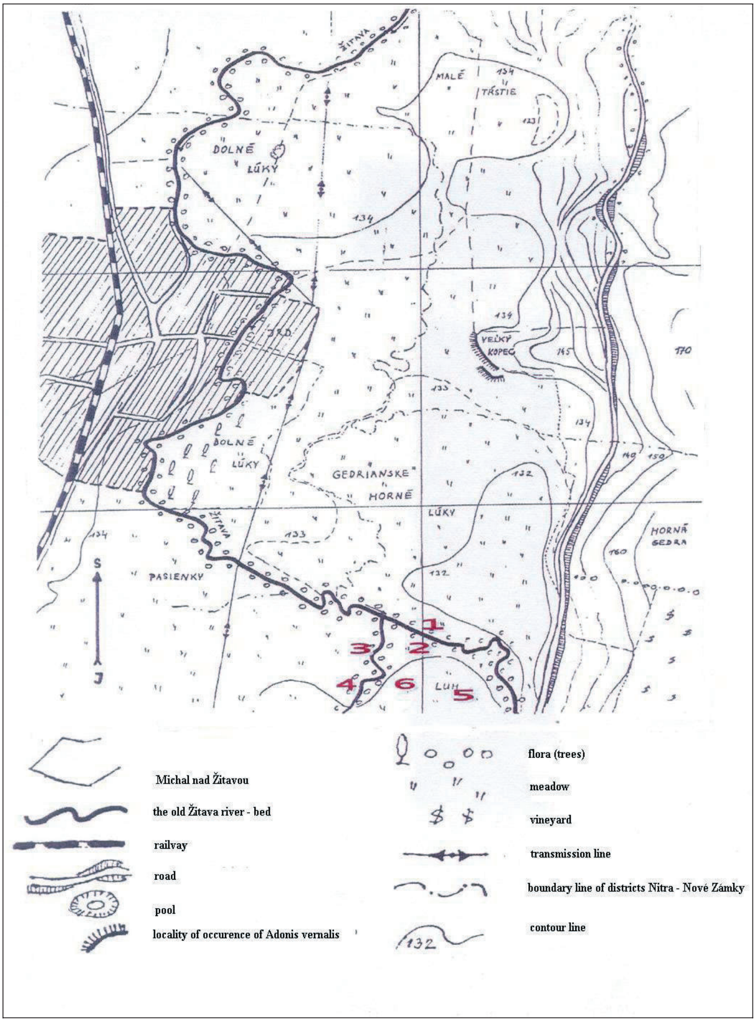

Fig. 1. Investigated points in the Žitavský luh.

intake point water samples were collected direct from the Žitava river-bed.. The 2nd intake point was located in the beginning of the old (original) bed of the said river and from this point, in spring and summer, water enters the bed l through the underground channel from the Žitava river. The 3rd and 4th intake points represented the old Žitava river-channel – continuation of the meander of the old course of river from the south (3rd intake point) and from the south-west (4th intake point), ending with a water outlet.

Typical wetlands were only represented by the 5th and 6th intake points, surrounded by a belt of cultivated soil.

Water samplings in which we measured temperature and pH were collected in the areas of plankton and benthos (bottom) over a period 2003–2004 once per quarter, in March, June, September and December (Table 1).

Ta b l e 1. Temperature (the first line) and pH (the second line) in the time of intake samplings.

Sampling	Date of sampling	Intake points					
		1.	2.	3.	4.	5.	6.
1.	19. 03. 2003	12 7.72	12.1 8.48	12.1 8.56	12.6 8.55	13 7.07	13.3 6.88
2	19. 06. 2003	22.9 7.24	23.4 7.8	23.1 7.45	24.2 7.82	23.8 6.78	24 6.7
3.	17. 09. 2003	18.2 7.74	18.4 7.9	18.4 8.04	18.7 7.64	19.2 7.95	18.6 7.91
4.	10. 12. 2003	5 7.21	5.4 7.21	5.7 7	5.5 6.95	4.1 6.99	5.3 6.95
5.	18. 03. 2004	16.4 7.5	16.5 7.4	14.7 7.6	15.5 7.7	15 6.9	15.4 6.8
6.	16. 06. 2004	20.9 7.5	20.4 7.4	20.1 7.35	19.9 7.3	19.7 6.8	19.9 6.7
7.	17. 09. 2004	23 7.9	23 8.1	22.8 7.8	23 7.7	ww ww	ww ww
8.	09. 12. 2004	7.58 4.6	8.4 4.6	7.25 5.6	7.3 4.8	6.94 4.8	6.95 5.4

Note: ww - waterless of wetlands in intake points 5 and 6

Studied microbiological indicators

Using a plate method, we determined in three replicates the occurrence of 4 physiological groups of microorganisms in surface water samples:

- coliform bacteria on tergitol agar (TA) according to STN ISO 93 08-1-2
- myxobacteria on champignon agar (ChA) according to Miklošovičová, 1987
- soil microscopic fungi on Czapek-Dox´s (Cz-DA) and sweet wort agars (SWA) according to Häusler, 1995
- actinomycetes on Krainsky´s (KA) agar and Waksman´s agar (WA) according to Häusler, 1995.

The quantitative analysis of coliform bacteria was carried out in compliance with STN 75 7221, actinomycetes according to Brysa (as given by Häusler, 1995), and myxobacteria according to Bernátová (1983). The identification of microscopic fungi was performed according to mycologic clues (Fassatiová, 1979; Niemi et al., 1982; Pitt, Hocking, 1997).

Statistical analysis of studied indicators

Variation-statistical parameters were calculated from the obtained counts of microorganisms (including replicates) in $CFU.mL^{-1}$, and the sets of values measured were tested according to the calculated value χ^2. Statistical significance of differences in the groups of microorganisms under study between year, intake points, samplings, as well as replicates were verified by Kruskal-Wallis variance analysis and by testing differences as described by Dunn (not shown in the tables because of length). For graphic representation the average values of the occurrence of microorganisms were used.

Results and discussion

The Žitavský luh Nature Reserve is not only unique because of the occurrence of rare birds whose species are often endangered, but also it is an interesting area from the botanical aspect (Svobodová, 1992) and an unexplored one from the microbiological viewpoint. Microorganisms, especially bacteria, response most sensitively to adverse changes in an environment and are considered to be a bioindicator of the condition of wetlands (Lemley, King, 2000; Merkley et al., 2004). While other organisms such as algae, macrophytes and inverterbrates have a different communicative value, e.g. algae indicate a phosphor redundance, invertebrates did not response to phosphor abundance.

In addition to the artificial regulation of the Žitava river, among the main factors endangering the Žitavský luh reserve are arable land in the east of the territory (above and below the national road, Fig. 1), sewage, fertilizers, chemicals causing soil erosion of terrain depressed areas, closed water gates in the winter, and irregular mowing out. Wetlands belong to the most endangered ecosystems due to continual drainage, soil cultivation, pollution and excessive exploitation of their resources.

Among the hygienically most important communities with the highest abundance in the Žitava river were coliform bacteria. Their most considerable incidence (Fig. 6), which is also statistically significant, was observed in the surface water of wetlands and in the flowing water in the new Žitava river channel. The water flowing in the old river channel and in the intake points 2–4 showed no substantial contamination from the hygienic aspect (Fig. 2). On the contrary, a bigger number of coliform bacteria was found in benthos even in the old river-bed, as compared to wetlands. The highest number of the said bacteria in the area of benthos reached a value of $4.08 .10^4$ $KTJ.g^{-1}$.

As far as ecological indicators are concerned, over 2003–2004 we observed the presence of myxobacteria, microscopic fungi and actiomycetes were observed in (Tables 2, 3) in this locality. According to average values, the occurrence of these groups was always greater in the area of benthos (Figs 2–7).

In the surface flowing and stagnant water (the new and old Žitava river-beds) we determined in benthos about 10-fold lower values for actinomycetes, 12.08±37.25 $KTJ.ml^{-1}$ than those reported by Tržilová, Miklošovičová (1985) for the Nitra river. However, the presence of actinomycetes confirmed that the course of the river was also contaminated by organic matters difficult to degrade. Differences in determined values between different intake points in the benthos were found to be statistically significant only at the significance level $P < 0.05$ (Table 4). In the area of plankton there were even statistically more significant differences ($P < 0.01$) between individual intake points. In particular, their substantial greater occurrence was observed in wetlands in the 5th intake point (Figs 3, 6). An increased incidence of actinomycetes was found mainly in the winter (2004) and in spring (2003, 2004). Like in all groups of microorganisms under investigation, we recorded statistically significant differences ($P < 0.01$) between different samplings (Table 4). According to Brys (Häusler, 1995), however, we can evaluate the plankton area as very clean water (78% of samples). There was only one sample (2%) collected in March in the 5th intake that indicated very

T a b l e 2. The basic variance-statistical characteristics and results from distribution fitting of occurrence of microorganisms ($CFU.ml^{-1}$) in the plankton of surface water – Žitavský luh.

Statistical characteristics	Observed microorganisms				
	coliform bacteria	myxobacteria	microscopic fungi Cz-DA	microscopic fungi SWA	actinomycetes WA+KA
n	138	137	138	137	138
x	24.70	12.04	2.88	8.36	12.08
median	10	5	1	5	1.75
mode	0	1	0	3	0
minimal value	0	0	0	0	0
maximal value	190	86	24	68	275
s	37.47	18.75	4.44	9.81	37.25
s_x	3.19	1.6	0.38	0.84	3.17
s^2	1403.7	351.73	19.74	96.24	1387.52
v%	151.67	155.72	154.46	117.38	308.27
Distribution fitting					
χ^2	189.28	324.34	336.26	126.25	108.66
P-value	0	0	0	0	0

Notes: Cz-DA – Czapek-Dox´s agar, SWA – sweet wort agar, WA – Waksman´s agar, KA-Krajinsky´s agar, n – number of observation, x – average, s – standard deviation, sx – standard mean error, s^2 – variance, v – coefficient of variation, χ^2 – chí square test

T a b l e 3. The basic variance-statistical characteristics and results from distribution fitting of occurrence of microorganisms ($CFU.g^{-1}$) in the benthos – Žitavský luh.

Statistical characteristics	Observed microorganisms				
	coliform bacteria	myxobacteria	microscopic fungi Cz-DA	microscopic fungi SWA	actinomycetes WA+KA
n	136	125	137	138	135
x	3734.12	116.64	326.28	155.41	341.67
median	1275	70	80	110	220
mode	0	0	0	80	0
minimal value	0	0	0	10	0
maximal value	40800	920	3500	880	2400
s	6032.9	156.92	680.23	153.84	392.87
s_x	517.32	14.04	58.12	13.10	33.81
s^2	3.64	24622.5	462718.0	23665.9	154343.0
v %	161.56	134.53	137.30	98.99	114.99
Distribution fitting					
χ^2	216.86	87.68	485.63	99.46	108.66
P-value	0	0	0	0	0

Notes: abbreviations see in Table 2

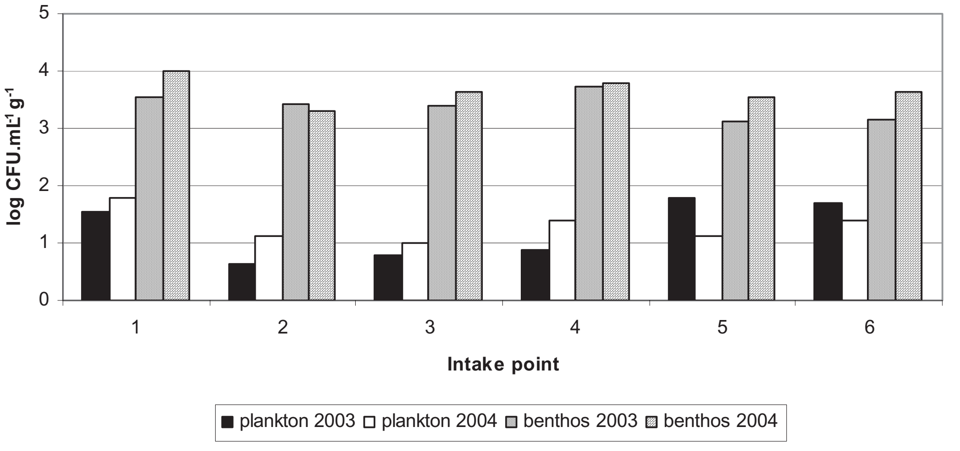

Fig. 2. Occurrence of coliform bacteria in the Žitavský luh from march 2003 to December 2004.

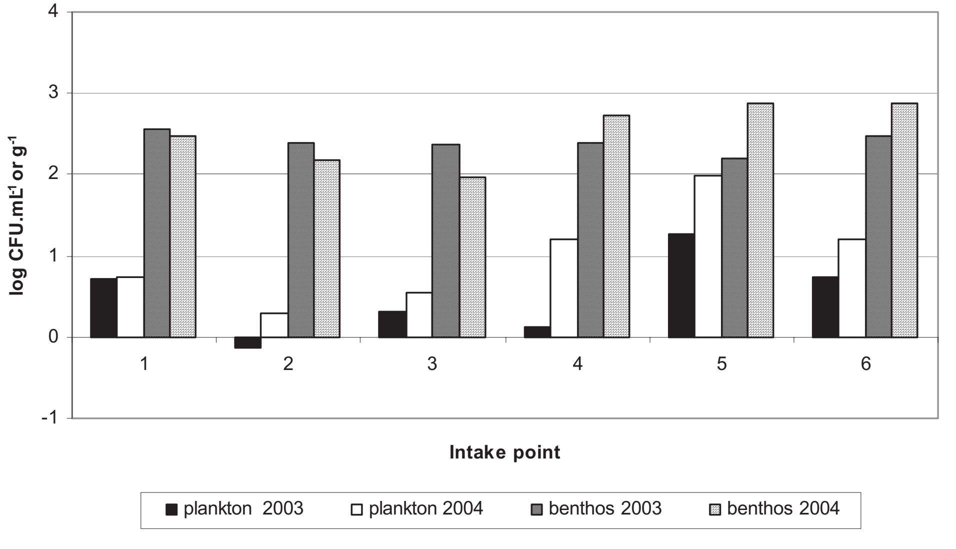

Fig. 3. Occurrence of actinomycetes in the river Žitava from march 2003 to December 2004.

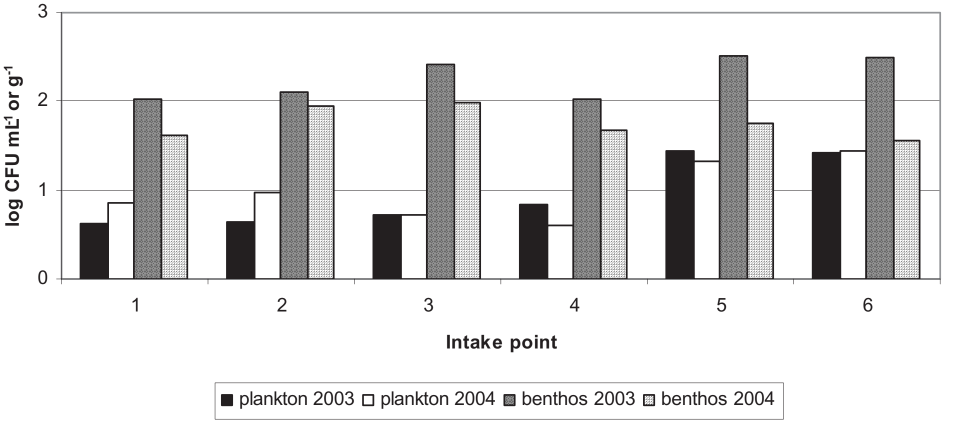

Fig. 4 . Occurrence of myxobacteria in the Žitavský luh from march 2003 to December 2004.

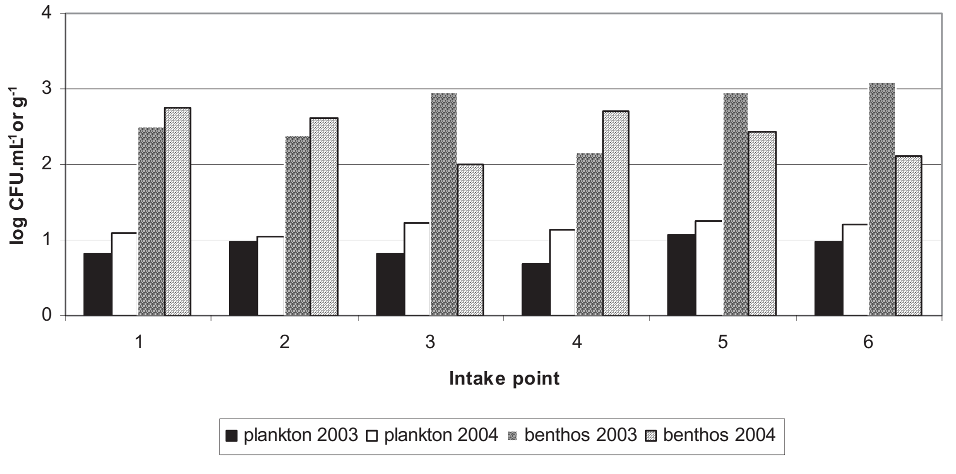

Fig. 5. Occurrence of microscopic fungi in the Žitava from march 2003 to December 2004.

strongly contaminated surface water. Fifteen per cent of samples can be characterized as clean water and 4% as contaminated water. In the area of benthos where there are most accumulated substances which are more difficult to decompose, over half samples (54.3%) indicated very strongly contaminated water, and very clean water was identified only in 19.6% of samples (Table 5).

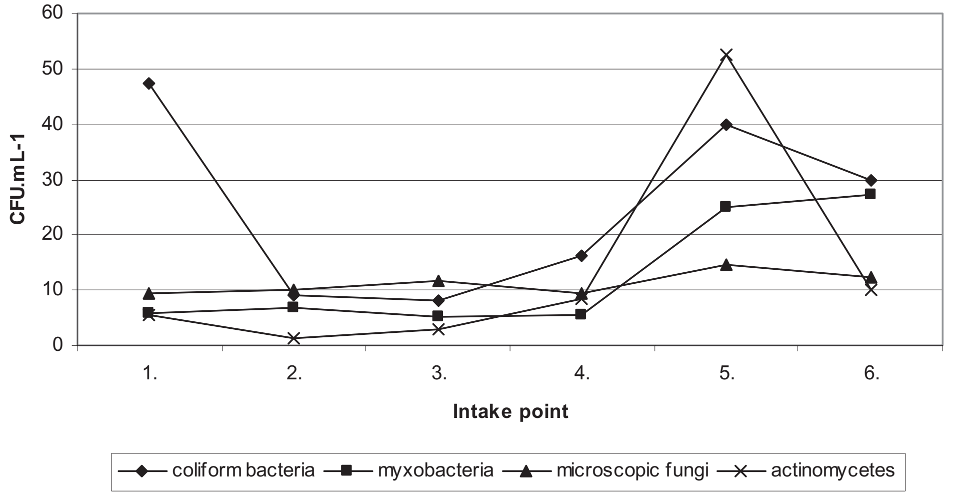

Fig. 6. Occurrence of microorganisms in the plankton – river Žitava from march 2003 to December 2004.

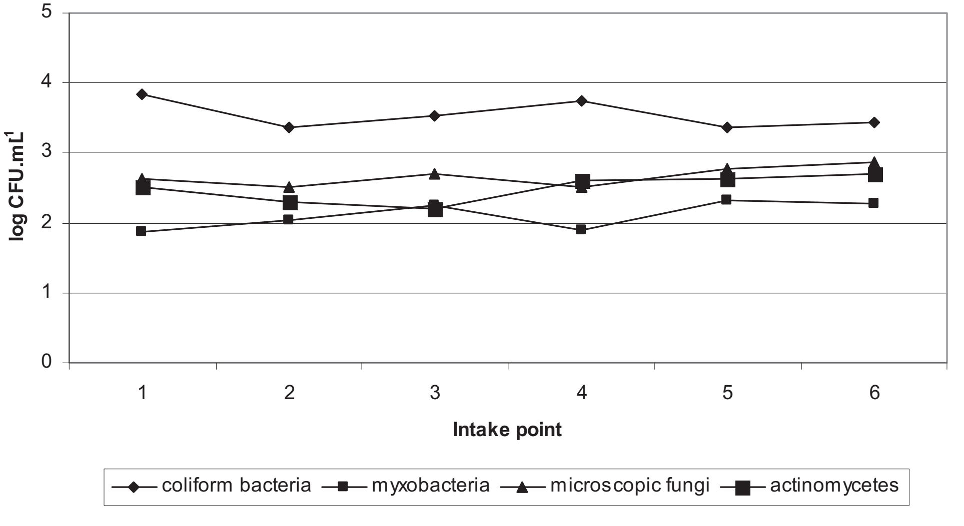

Fig. 7. Occurrence of microorganisms in the benthos – river Žitava from march 2003 to December 2004.

A greater supply of plant mass in wetlands and in the old Žitava river-bead has been confirmed by the presence of myxobacteria. Their incidence was considerable mainly in the 2003 spring in benthos (Fig. 4), where its average values amounted to 600–787 KTJ.g^{-1}, T a b l e 4. Variance analysis occurrence of microorganisms in the Žitavský luh according to Kruskal-Wallis in the plankton (the first line) and benthos (the second line) of surface water.

Observed micro-organ-isms	Statistical parameters							
	T-value				P-value			
	Intake Point	Year	Sam-pling	Replica-tion	Intake point	Year	Sampling	Replica-tion
coliform bacteria	33.06 5.07	1.05 0.33	37.89 105.76	0.15 0.15	$3.65.10^{-6++}$ 0.41	0.31 0.56	$3.18.10^{-6++}$ 0^{++}	0.93 0,93
myxobacteria	11.18 8.80	2.91 20.57	53.51 40.12	1.08 0.10	0.05 0.12	0.09 $5.75.10^{-6++}$	$2.94.10^{-9++}$ $1.20.10^{-6++}$	0,58 0.10
microscopic fungi Cz-DA	23.40 5.73	0.08 4.87	45.09 102.42	0.07 $1.94.10^{-3}$	$2.83.10^{-4++}$ 0.33	0.78 0.03^{+}	$1.31.10^{-7++}$ 0^{++}	0.07 0.99
microscopic fungi SWA	8.17 13.37	25.45 8.05	49.78 44.80	1.47 0.43	0.15 0.02^{+}	$4.54.10^{-7++}$ $4.55.10^{-3++}$	$1.60.10^{-8++}$ $1.50.10^{-7++}$	0.48 0.81
actinomycetes WA+KA	20.16 13.77	$5.95.10^{-3}$ 1.95	63.44 82.51	4.36 0.01	$1.17.10^{-3++}$ 0.02^{+}	0.94 0.16	$3.10.10^{-11++}$ $4.22.10^{-15++}$	0.11 0,99

Notes: abbreviations see in Table 2

T a b l e 5. Quality [%] of plankton of surface water (the first line) and part receiving on the benthos (the second line) in the Žitavský luh.

Observed	Running surface water					Benthos				
microorganisms	Class quality of surface water									
	I.	II.	III.	IV.	V.	I.	II.	III.	IV.	V.
coliform bacteria	6.5 0	41.3 13.0	47.8 13.0	4.3 4.3	0 0	6.0 4.3	0 0	21.7 4.3	23.9 6.5	47.8 15.2
actinomycetes	78.3 17.4	15.2 8.7	4.3 2.2	0 0	2.2 2.2	19.6 4.3	4.3 0	15.2 2.2	6.5 4.3	54.3 19.6
myxobacteria	78.3 15.2	13.0 6.5	4.3 4.3	4.3 4.3	– –	8.7 4.3	10.9 2.2	2.4 4.3	56.5 19.6	– –

the maximum value being 920 $KTJ.g^{-1}$ (Table 3). In the area of benthos, in total as much as 56.5% of samples showed water quality class IV. Any lower incidence, not reaching order ten, was observed in the new Žitava river-bed (1^{st} intake point). There were no statistically significant differences between different intake points (Table 4). The myxobacteria occurrence was only statistically significantly influenced by the month of sampling and in the area of benthos it was also affected by the year of monitoring. Based on the occurrence of myxobacteria, in terms of water quality as many as 78.3% of samples collected in both of the new and old Žitava river-beds indicated water quality class I (Table 5).

Average values of microscopic fungi in the Žitava river ranged from 10 to 3103 KTJ.g^{-1} (together on both cultivating media) in the benthos area and from 0 to 31 KTJ.ml^{-1} in the plankton one. Although the abundance of microscopic fungi was found to be considerably greater in benthos (Fig. 5), statistically significant differences ($P < 0.01$) were only observed in the area of plankton. Substantially higher values of the occurrence of microscopic fungi particularly manifested themselves in wetlands, compared to those observed for the new and old channels of the Žitava river. A spectrum of the identified and most frequent species and genera of microscopic fungi (*Aspergillus, Penicilliumn, Trichoderma, Cladosporium* and *Fusarium*) was similar to that in the Danube river reported by Tóthová (1999). A newly identified species was *Clonostachys rosea*, which was observed in the new Žitava channel.

T a b l e 6. The most common genera (in % from all samples with microscopic fungi identfication) occurrences in the running surface water (the first line) and benthos (the second line) in the investigation points.

Genus	Investigated point					
	1.	2.	3.	4.	5.	6.
Acremonium	0.8	0.8	–	–	4.7	0.8
	0.1	–	–	0.2	0.4	0.4
Alternaria	1.3	2.0	5.8	4.5	0.9	1.2
	12.6	3.5	12.0	4.3	–	9.7
Aspergillus	20.0	44.7	14.5	1.8	3.0	1.2
	2.8	2.1	1.9	2.1	2.7	2.7
Cladosporium	4.3	4.7	9.3	2.2	–	3.9
	1.84	–	–	0.2	–	–
Fusarium	5.6	3.9	4.5	2.2	3.0	13.8
	15.2	13.1	21.3	5.5	2.3	8.2
Humicola	–	1.2	1.9	0.4	–	1.2
	18.0	23.3	17.8	0.2	–	0.3
Mucor	–	0.4	0.4	4.5	3.0	0.4
	10.8	2.5	4.8	25.1	19.5	4.9
Penicillium	50.0	29.6	36.1	29.0	25.8	25.2
	23.0	28.6	22.4	27.0	37.0	44.6
Rhizopus	2.2	1.6	1.5	2.2	–	1.6
	0.7	1.1	1.1	–	0.4	2.1
Trichoderma	13.5	7.9	21.2	49.1	58.8	50.7
	14.6	25.7	18.2	34.8	37.0	26.8
Other genera	1.0	0.4	2.6	0.6	0.8	–
	0.2	0.1	0.3	0.1	0	0.3
Non sporulated mycelia	1.3	2.8	2.2	4.0	–	–
	0.2	–	0.2	0.2	0.8	–

A spectrum of isolated and identified 13 species of the genus *Penicillium* was interesting as well. It mainly concerned the species *Penicillium atramentosum, P. aurantiogriseum, P. crustosum, P. carneum, P. corylophilum, P. chrysogenum, P. janczewskii, P. madriti, P. manginii, P. olsonii, P. piscarium, P. polonium* and *P. restrictum*. In wetlands dominated the species of the genus *Trichoderma,* whilst in the Žitava river (new bed) did those of the genera *Penicillium* and *Aspergillus* (Table 6).

As to a group of soil fungi, we revealed the occurrence of representatives belonging to imperfect fungi and the so-called sugar fungi (the class *Zygomycetes*). In terms of hygiene and toxicology, the toxinogenic species *Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, A. clavatus* were detected in the Žitava river.

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