COMPARATIVE INVESTIGATIONS ON REED-SURFACE EPIPHYTIC BACTERIAL POPULATIONS IN DIFFERENT REGIONS OF LAKE BALATON (W. HUNGARY)

(A preliminary report)

by

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20.04.1982. Received:

Introduction

The Lake Balaton in W. Hungary belongs to the type of large shallow lakes. Its littoral zone has a relatively large surface area, especially considering all stands of macrophytic vegetation rooting in the bottom sediment as parts of this region (L e n z 1928, R u t t n e r 1940 cit. in S e b e s t y é n 1943). Reeds *(Phragmitetum)* from the largest plant communities in the littoral zone, and at the same time in the whole lake-environment, too. Considering their particular physico-chemical parameters and biological characteristics the reeds as habitats for toher organisms sharply differ from all other true littoral (in the strict sense of the word: eulittoral, S e b e s t y é n 1943) and open water milieus.

Investigating the local distribution of aquatic mosses *Fontinalis antipyretica* and *F. hypnoides* (F e l f ö l d y – T ó t h 1957) and the phenology of reeds in the Balaton (T ó t h 1960a) the cited workers stated that these reeds may be classified into different types or subtypes. The possible existence of various reeds-types was suposed earlier also by M e s c h k a t (1934) furthermore E n t z – S e b e s t y é n (1940), although exact structural-coenological data were not available for them. Closed reeds of large extent may show both in structural and functional point of view pregnant horizontal inhomogenities (M e s c h k a t 1934). Perhaps the most important one among them is the interesting distribution pattern between the clear and the turbid (rich in suspended calcium-carbonate and partly polluted) water masses within the individual reeds stands. The turbid and polluted water streaming from the open water region into the reeds passes through the stand in the direction of the coast and becomes clear (free from $CaCO_3$) and detoxified. It is a very characteristic phenomenon that within the individual reeds-stands there is a more or less sharp border line between the turbid and polluted but O_2-rich water masses and the CO_2-rich, O_2-poor, clear ones. F e l f ö l d y – T ó t h (1957) stated that *Fontinalis* spp. are living even in this border region. Besides this T ó t h (1960b) distinguished individual reeds-types on the basis of the occurence

of *Fontinalis* spp. as community members. He studied intensively the ecology of „*Scirpo-Phragmitetum fontinalosum*"-type in which *F. antipyretica* is a commonly occuring, characteristic *Fontinalis* species. According to the data of Tóth the oxygen and carbonate content of the lake water gradually decreases as it passes through the reeds. Finally the suspended carbonates disappear and the water becomes clear and rich in free CO_2. All these changes may be attributed to microbial activities. The direct effect of the open water of the lake cannot be observed already in that inner reeds-zone where *Fontinalis* spp. occur.

Otherwise, in the reeds, the physical and chemical parameters of the water are changing not only horizontally but vertically, too (Entz 1981). Studying the hydrobiology of the reeds in the Hungarian part of the Lake Fertő characterized by another type of hydrochemistry such dynamics of the water self-purification were not possible to observe (Tóth – Szabó 1962).

In the inner part of reeds-stands where the intensity of the water movements is relatively low the submerse surface of the individual reeds is covered by a living crust. The members of this crust population (bacteria, algae, nematodes, copepodes, trichoptera, etc.) are connected by a very complex web of routes of the community metabolism which is, unfortunately only little known at present. Meschkat (1934), Entz – Sebestyén (1942) and Sebestyén (1963) published important data on this crust-biota. I. h. a. r. o. s. (1964) showed that the population dynamics of this crust inhabiting Tardigrada is deeply influenced by locally acting physical and chemical factors (light intensity, oxygen-supply, etc.).

The so called filtration effect of the reeds is of outmost importance. The polluted water which is streaming through the reeds-stands gradually becomes poor in phosphorous and nitrogen futhermore in suspended particulate materials Lesenyei – Szabó 1953, Tóth 1972, Oláh et al. 1977, Kovács et al. 1979, Lakatos 1979, Dobolyi et al. 1980) and microorganisms (Lesenyei – Szabó 1953).

At present there are many data in the literature making undoubtedly that microorganisms living epiphytically on the reed-surface are responsible for this filtering effect. In the present study an attempt is made to analyse bacterial communities living epiphytically in the crust-material of the reed surface region.

Materials and methods

Reed samples were collected at three areas of different productivity of the Lake Balaton (1. Balatonkenese, 2. Bay of Bozsa, 3. Bay of Keszthely), on the lst and the 28th October, 1980. The samples were taken in each case in the border region of the reeds-stands at about 4 – 5 m far from the bordering free water.

At sampling we cut the submerse parts of the single reed to approximately 10 cm pieces using sterile shears and tweezers, than we transported

them into the laboratory in sterilized test tubes filled with untreated lake-water. More than one year old and fresh reeds were collected at each site. A part of the samples consisted of reed material being cut from about 20—30 cm below water surface, another part of them was taken from approximatevely 10—20 cm above the mud surface near the bottom. Reed samples were stored in refrigerator at 4 °C untill processing having taken place in 36 hours. Pieces of reed were washed three times with sterile tap water to remove organisms adhering loosely. Subsequently scrapes were taken aseptically from the reed-surface. Dilution series were made from the suspension of the homogenized crust material. Plating was carried out on four kinds of media, as follows: nutrient agar (Cowan—Steel, 1965), starch-caseine agar (Waksman 1961), ISP—9 basal medium completed with glucose (Pridham—Gottlieb 1948) and a synthetic agar enriched in yeast-extract (Szabó 1974). Inoculated plates were incubated on 28 °C for six days, then colonies were isolated non-selectively to slants composed of the same media as plates. 2126 isolates were obtained this way. They were stored at 4 °C in refrigerator. After this from among our isolates true eubacterial ones capable of growing on nutrient agar were selected and isolates turning out not to be bacteria or not to be maintainable on laboratory media, were neglected. So we obtained 1147 isolates altogether from the three sampling sites (232 from Balatonkenese, 223 from Bay of Bozsa and 692 from Bay of Keszthely).

Thereupon a tentative grouping (on the basis of cultural-morphological features of diagnostic value) and selection was done among our isolates obtained from reederust matter taken from the very same sampling site. In this manner several groups of similar isolates were formed in relation of all milieus. Finally we compared all of the members of all groups also each with other. Similar groups were united into larger ones and representative strains were selected from all of them. Isolates which remained outside the large clusters and represented only rarely occuring types or species (altogether 13, 24 and 59 ones from the three habitats, respectively) were excluded from the futher work. At the end 190 isolates, or more exactly representative strains belonging to 20 different similarity groups were subjected to detailed analyses.

Chemical analyses were carried out in laboratory and at the sampling sites, on samples collected from the characteristic regions (*Hydrocharis*-zone, *Fontinalis*-zone, open-water side of the reeds' border) of the reeds-stands and from the open water of Bay of Bozsa, as well, between 8th and 10 th of July 1981, twice a day (6.30 A.M. and 14 P.M.).

Measurements of water pH, O_2 saturation and temperature were carried out using Aquachek field-instrument and that of transparency with Secchi-discs at the site. Quantity of floating material, dissolved reactive P, NH_4-N, NO_2-N, $CO^2{}_3{}^-$, $HCO^-{}_3$ total dissolved iron and oxygen consumption via permanganete (COD_{Mn}) were determinated as by Felföldy (1980), quantity of chlorophyll-a and phaeopigment as by Tett et al. (1977). Dissolved organic carbon content of water samples was investigated by Beckman organic carbon analysing instrument.

Table I.

Results of comparative investigations on 148 representative bacterial strains isolated and selected from epiphytic microbial communities of three reeds of the lake Balaton

Tests	Sampling sites											
	Balatonkenese				Bay of Bozsa				Bay of Kszthely			
	+	%	±, −	%	+	%	±, −	%	+	%	±, −	%
Gram staining	11	17	8 (47)	12 (71)	5	16	8 (19)	25 (59)	17	34	0 (33)	0 (66)
Shape: rod	37	56	29	44	21	66	11	34	30	60	20	40
Coccus or coccoidal	29	44	37	56	11	34	21	66	20	40	30	60
Gelatinase activity	33	50	33	50	19	59	13	41	36	72	14	28
Starch hydrolysis	29	44	37	56	9	28	23	72	23	46	27	54
Arginine hydrolysis	25	38	41	62	12	37	20	63	26	52	24	48
Aesculin hydrolysis	25	38	41	62	11	34	21	66	38	76	12	24
Reduction of methylenblue	22	33	44	67	7	22	25	78	12	24	38	76
Utilization of citrate	19	29	47	71	4	13	28	87	9	18	41	82
Catalase	52	79	14	21	27	84	5	16	40	80	10	20
Motility	14	21	52	79	7	22	25	78	33	66	17	34
Casease activity	32	48	34	52	20	62	12	38	32	64	18	36
Voges-Proskauer test	9	14	57	86	5	16	27	84	15	30	35	70
Nitrate reduction ($NO_3^- - NO_2^-$)	11	17	55	83	7	22	25	78	6	12	44	88
Nitrate reduction ($NO_3^- - N_2$)	0	0	66	100	0	0	32	100	1	2	49	98
Tyrosine decomposition	38	58	28	42	14	44	18	56	24	48	26	52
Indole production	3	5	63	95	1	3	31	97	4	8	46	92

Oxidative decompensation of glucose	29	44	37	56	18	56	14	44	37	74	13	26
Fermentative decompensation of glucose ..	38	58	28	42	19	59	13	41	41	82	9	18
Growth on MacConkey agar	20	30	46	70	8	25	24	75	11	22	39	78
Oxydase test	43	65	23	35	26	81	6	19	39	78	11	22
Production of H_2S	0	0	66	100	2	6	30	94	2	4	48	96
Phosphatase activity	44	67	22	33	23	72	9	28	43	86	7	14
Acid production (TSI agar)	10	15	56	85	1	3	31	97	15	30	35	70
Acid production (NH_4 – salt + glucose)	35	53	31	47	21	66	11	34	38	76	12	24
Acid production (nutrient broth + glucose)	27	41	39	59	13	41	19	59	31	62	19	38
Gas production (TSI agar)	1	2	65	98	0	0	32	100	2	4	48	96
Gas production/nutrient broth + glucose ..	1	2	65	98	1	3	31	97	2	4	48	96
Urease activity	1	2	65	98	1	3	31	97	0	0	50	100
Utilization of anorganic N	13	20	53	80	10	31	22	69	5	10	45	90
Tolerance of NaCl 3%	46	70	20	30	23	72	9	28	40	80	10	20
Tolerance of NaCl 7%	24	36	42	64	17	53	15	47	29	58	21	42
Tolerance of NaCl 11%	18	27	48	73	13	41	19	59	22	44	28	56
Growth at 10°C	51	77	15	23	28	88	4	12	49	98	1	2
Growth at 37°C	43	65	23	35	16	50	16	50	40	80	10	20
Growth at 45°C	5	8	61	92	5	16	27	84	0	0	50	100
Treatment with moist heat at 60 °C	50	76	16	24	26	81	6	19	34	68	16	32
Treatment with moist heat at 80 °C	4	6	62	94	5	16	27	84	0	0	50	100
Utilization of Na-propionate	25	38	41	62	10	31	22	69	6	12	44	88
Phenylalanine desamination	3	5	63	95	0	0	32	100	10	20	40	80

Results and discussion

In contrast to the composition of bacterial communities of the open water characterised by the predominance of micrococci (K o t s i s 1982), in the crust of the reed gramnegative rood-shaped bacteria are the most common organisms (Table 1.). This fact clearly shows, that species of high ecological tolerance and biochemical capacities are the prominente members in this community. Table 1. also shows, that more, than 50% of strains of all the three milieus are able to degrade glucose fermentatively. Therefore the epiphytic bacterial communities of the reed posses a potential capacity to tolerate and remain active at low oxygen levels. Presumably the internal layer of reed crust is colonized permanently by facultative anaerobes and microaerophiles and anaerobiosis may occure ther frequently. A characteristic interspecies metabolism of this reed-surface community might be responsible for the fact that a number of isolates cannot be maintained under laboratory conditions for long time and die out. They cannot exist without their natural partners.

The composition of epiphytic bacterial populations of these three compared reeds proved not to be identical. A differentiation of epiphytic bacterial communities in various bays of Balaton is induced by the local factors of these distinct trophic environments. Species and varieties adapted better to these factors are selected by the locally acting environmental stresses. Differences were showed among the members (species) of these three communities both in taxonomic composition and in biochemical properties. According to our taxonomical analyses in progress certain types or species of bacteria occur in all the three reeds, some only in two and others only in one.

For example we detected considerable differences in the distribution of strains of the three habitats according to their tolerance to moist heat treatment at 80 °C and growth activities at a temperature of 45 °C. Bacteria being able to tolerate 80 °C heat and to grow at 45 °C occur in the reeds of Kenese and the Bay of Bozsa, but not in the Bay of Keszthely.

Generally, the individual epiphytic bacterial communities are characterised by the predominance of a few species or types and the presence of many sporadically occuring or less frequent ones. Predominantes occur generally in large masses. Probably these are responsible for chemical and biological self-purification of streaming water above all. We have to concentrate our attention to these species in the future.

On the basis of the data presented in Table 1. the physiological-biochemical abilities of the strains isolated from the three crust habitats can be compared. So it is conspicuous that only 21% of the strains of Kenese and 22% of these of Tihany were motile, but 66% of the Keszthely-Bay ones were able to change their positions actively to chemical stimuli. This may be connected with the fact that in the highly polluted water of Bay of Keszthely microbes are more frequently exposed to intensive positively and negatively acting chemical influences (stimuli) and these force the selection of flagellated forms.

A certain correlation can be observed between the biochemical abilities and ecological tolerance of our strains and the characteristics of their original, natural reedhabitat. The bacterial strains of the reeds of the Bay of Bozsa are biochemically more active and withstand wider ranges of ecological stresses than those which were isolated from the Bay of Kenese, and the strains of the Keszthely-Bay are in this respect the most potent organisms. This statement is corroborated by the distribution of positive strains (given in percent) among the Balatonkenese-, Bozsa- and Keszthely-ones, respectively, regarding the following tests: gelatinase activity: 50%, 59%, 72%; arginine hydrolysis: 38%, 37%, 52%; casease activity: 48%, 62%, 64%; oxidative decomposition of glucose: 44%, 56%, 74%; fermentative decomposition of glucose: 58%, 59%, 82%; phosphatase activity: 67%, 72%, 86%; acid production on NH_4-salt-glucose medium: 53%, 66%, 76%; growth in the presence of 11% NaCl: 27%, 41%, 44%; growth at 10 °C: 77%, 88%, 98%; etc. All these lead to the accurate conclusion, that in what proportion the pollution of Balaton water is increasing in one region, in that proportion increases the biochemical activity and tolerance of the epiphytic bacterial flora of reeds.

It is to be mention that the composition of the epiphytic microbial population of the surface of the more than one year old reed differs from that of the fresh reed. It seems that certain types of bacteria colonize only reeds of definite state and age.

One of the most important tasks of our investigations would be to clarify the species structure of these epiphytic communities. This work is in progress now with help of computer analysis.

On the basis of the results of water analyses (Table 2.) it seems to be obvious, that in the water of reeds the dissolved reactive phosphorous, HCO_3^- and organic carbon concentration, as well as transparency of the water are gradually decreasing, approximating the border line between the open water and the reeds. This tendency is also shown in changes in the total amounts of NH_4-N, NO_2-N and dissolved iron futhermore in permanganate oxygen consumption. The measure of oxygen saturation, amount of floating material, chlorophyll-a content, CO_3^{2-} concentration and temperature of water of the reeds increase in the direction from the shore to the open water. Such characteristic change in the concentration of phaeopigments in a given direction, cannot be established at all.

Important diurnal changes were measured in many water chemical characteristics, as e.g. in pH, oxygen saturation, transparency, dissolved reactive phosphorous, CO_3^{2-}, HCO_3^-, NO_2-N, NH_4-N and also regarding the water temperature. Besides these, the diurnal amplitudo of these parameters is rapidly decreasing from the shore to the open water. This diurnal rhytm may be caused by organic matter production and decomposition processes. In daytime HCO_3^- concentration of water decreases, its oxygen saturation, pH and CO_3^{2-} concentration increases due to the intensive photosynthesis. At the same time vigorous biogen calcification reduces the transparency of the water. At night organic material decomposing processes dominate, oxygen saturation decreases, CO_2 arises and after this pH

Table II.

Results of chemical analyses of water samples obtained from different zones of reeds in the Bay of Bozsa

Time		Zone of Hydrocharis	Zone of Fontinalis	Border line between open water and reeds	Open water
		Water depth (cm)			
08. 07. 81.	6^{30}	20	60	120	250
	14^{00}	20	60	120	250
09. 07.	6^{30}	20	60	120	250
	14^{00}	20	60	120	250
10. 07.	6^{30}	20	60	120	250
		Floating material ($g \cdot m^{-3}$)			
08. 07. 81.	6^{30}	6.4	2.3	2.1	8.6
	14^{00}	151.0	4.1	5.4	6.6
09. 07.	6^{30}	3.6	2.7	4.3	19.2
	14^{00}	2.0	5.6	6.8	7.9
10. 07.	6^{30}	3.3	4.3	5.2	15.2
		Water temperature (°C)			
08. 07. 81.	6^{30}	15.3	19.4	19.4	19.4
	14^{00}	20.5	21.4	21.7	21.7
09. 07.	6^{30}	16.9	21.0	21.2	21.6
	14^{00}	20.5	23.4	23.4	23.5
10. 07.	6^{30}	18.3	21.5	21.9	22.2
		pH			
08. 07. 81.	6^{30}	7.70	7.83	8.24	8.59
	14^{00}	8.08	8.66	8.66	8.65
09. 07.	6^{30}	7.60	8.36	8.35	8.53
	14^{00}	9.00	8.71	8.71	8.75
10. 07.	6^{30}	7.58	8.00	8.17	8.56
		Secchi transparency (cm)			
08. 07. 81.	6^{30}	t.m.*	t.m.	t.m.	74
	14^{00}	t.m.	t.m.	80	80
09. 07.	6^{30}	t.m.	t.m.	t.m.	60
	14^{00}	t.m.	t.m.	98	80
10. 07.	6^{30}	t.m.	t.m.	t.m.	72
		Oxygen saturation (%)			
08. 07. 81.	6^{30}	0	4	74	126
	14^{00}	65	130	110	115
09. 07.	6^{30}	0	82	83	107
	14^{00}	65	128	130	120
10. 07.	6^{30}	0	35	52	104
		Dissolved organic carbon ($\mu g \cdot m^{-3}$)			
08. 07. 81.	6^{30}	11.0	10.0	9.3	9.3
	14^{00}	9.3	8.3	9.0	8.3
09. 07.	6^{30}	11.0	9.0	9.3	8.3
	14^{00}	10.5	8.3	8.0	8.0
10. 07.	6^{30}	10.5	9.0	10.5	8.3

Table 2. (Continued):

Time	Zone of Hydrocharis	Zone of Fontinalis	Border line between open water and reeds	Open water
	Dissolved reactive P ($g \cdot m^{-3}$)			
08. 07. 81. 6^{30}	0.043	0.014	0.004	0.002
14^{00}	0.030	0.000	0.003	0.004
09. 07. 6^{30}	0.091	0.009	0.005	0.002
4^{00}	0.030	0.008	0.002	0.000
10. 07. 6^{30}	0.052	0.009	0.004	0.005
	NH_4-N ($g \cdot m^{-3}$)			
08. 07. 81. 6^{30}	0.037	0.032	0.051	0.003
14^{00}	0.052	0.047	0.027	0.000
09. 07. 6^{30}	0.077	0.013	0.015	0.027
14^{00}	0.024	0.017	0.017	0.000
10. 07. 6^{30}	0.037	0.014	0.037	0.032
	NO_2-N ($g \cdot m^{-3}$)			
08. 07. 81. 6^{30}	0.009	0.022	0.018	0.012
14^{00}	0.000	0.000	0.000	0.000
09. 07. 6^{30}	0.017	0.007	0.018	0.000
14^{00}	0.000	0.000	0.000	0.000
10. 07. 6^{30}	0.022	0.018	0.023	0.014
	CO_3^{2-} ($g \cdot m^{-3}$)			
08. 07. 81. 6^{30}	0.0	0.0	0.0	18.0
14^{00}	0.0	9.0	15.0	15.0
09. 07. 6^{30}	0.0	12.0	15.0	30.0
14^{00}	21.0	12.0	12.0	15.0
10. 07. 6^{30}	0.0	1.8	3.0	18.0
	HCO_3^- ($g \cdot m^{-3}$)			
08. 07. 81. 6^{30}	259.3	244.0	231.8	195.2
14^{00}	256.2	210.5	192.2	192.2
09. 07. 6^{30}	253.2	204.4	170.8	146.4
14^{00}	192.2	210.5	213.5	207.4
10. 07. 6^{30}	244.0	222.0	213.5	180.0
	Chlorophyll – a ($mg \cdot m^{-3}$)			
08. 07. 81. 6^{30}	3.6	1.7	1.3	4.2
14^{00}	3.9	4.4	6.5	5.3
09. 07. 6^{30}	0.0	1.3	0.6	3.5
14^{00}	0.0	0.2	0.8	3.1
10. 07. 6^{30}	0.4	0.9	0.0	4.0
	Phaeopigment ($mg \cdot m^{-3}$)			
08. 07. 81. 6^{30}	9.1	4.7	4.6	10.0
14^{00}	25.2	4.3	2.4	4.0
09. 07. 6^{30}	4.9	3.7	4.4	10.0
14^{00}	5.7	6.2	5.1	3.2
10. 07. 6^{30}	3.3	3.4	3.6	3.8

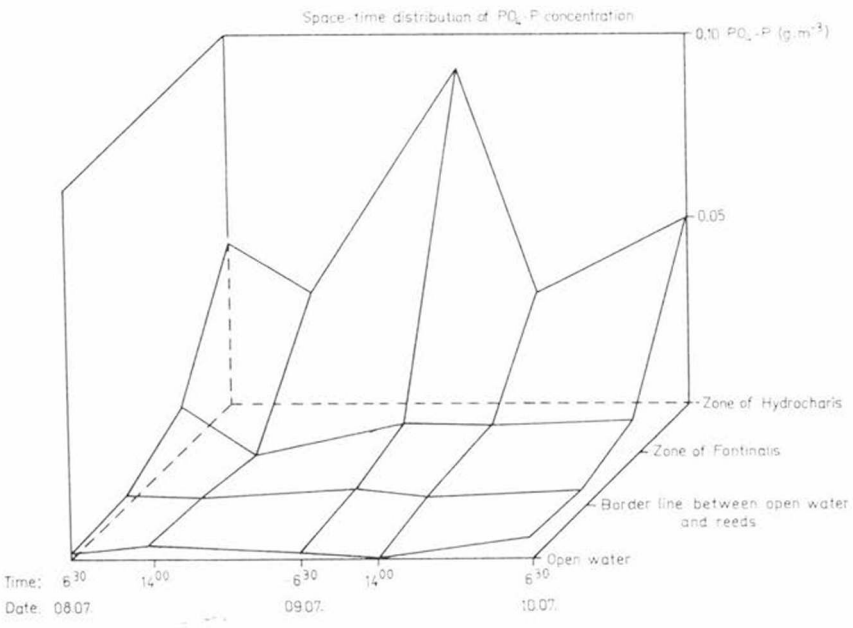
Table 2. (Continued):

Time		Zone of Hydrocharis	Zone of Fontinalis	Border line between open water and reeds	Open water
		COD_{Mn} ($g \cdot m^{-3}$)			
08. 07. 81.	6^{30}	10.2	5.2	6.0	6.4
	14^{00}	20.2	3.6	6.6	6.7
09. 07.	6^{30}	7.0	5.7	5.6	6.0
	14^{00}	6.8	6.4	6.0	6.2
10. 07.	6^{30}	9.9	6.1	7.4	7.0
		Total dissolved iron ($g \cdot m^{-3}$)			
08. 07. 81.	6^{30}	0.030	0.000	0.004	0.004
	14^{00}	0.032	0.000	0.008	0.008
09. 07.	6^{30}	0.042	0.014	0.006	0.003
	14^{00}	0.021	0.004	0.009	0.010
10. 07.	6^{30}	0.034	0.008	0.012	0.005

* t.m.: transparency to the mud-surface

of the water decreases as well. Biogen lime partly dissolves again, concentration of HCO_3^- increases and transparency of water rises.

Reeds are very complex biological systems showing rapid changes in biochemical activities both in time and space. It is interesting, that the reactive phosphorous does not emerge in measurable quantities from the reeds to the open water (Figure 1.). Streaming of ammonia and nitrit into the



direction of open water is possible only at night. The streaming of dissolved phosphorous is limited by the reactive phosphorous uptake of living organisms and by the phosphate binding activity of $CaCO_3$ precipitate arising by CO_2 uptake during photosynthesis. The biological and chemical self-purification mechanisms of Balaton-water in reeds-filter must be studied in more detail in the future.

Fig. 1 Diagrammatic representation of the results of chemical analyses of water samples obtained along a cross-section of reeds in the Bay of Bozsa.

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