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EPIPELON STUDIES ON THE UPPER TISZA RIVER AND ON ITS TRIBUTARIES

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ABSTRACT

The role of sediment and periphyton is significant in the life of aquatic and wetland habitats. The recent sediment covering the surface of the bottom has a significant role in the process which influence on water quality. Investigation of the epipelon, the periphytic association can be found on or in the fine-grained sediment is our recent task which is a current topic in international view, because the organisms creating the epipelon have strong interaction with the physical, chemical, biochemical and microbiological processes of the sediment. As a result of the activity of the periphyton and the sediment organisms the metabolism pathways come into being in the aquatic ecological systems that can indicate or influence the habitat characteristics. For analysis of the epipelon the measuring of the ETS (Electron Transport System) - activity can be used. By this parameter we would like to increase number of the non-taxonomic periphyton index determining the characteristic ecological status of waters within the scope of WFD (Water Framework Directive).
Keywords: sediment, epipelon, ETS-activity, Tisza River

INTRODUCTION

According to the Water Framework Directives (WFD) of EU, common principals are needed in order to coordinate Member States' efforts to improve the protection of waters in terms of quantity and quality. Standardisation of monitoring, sampling and analysis methods is required. Common definitions of the status of water in terms of quality should be established and common water quality standards should be laid down. Aquatic ecosystems should be monitored on a systemic and comparable basis throughout the Community. It is necessary to reveal the principals lying behind the evaluation of present classification systems of water quality and there is a need for optimalizing monitoring systems through developing the monitoring methods of ecological indicators and seeking for new indicators or new indicative parameters.

Sediment and periphyton play a significant role in wetlands and aquatic ecosystems. The upper layer of the sediment has a great influence on the water quality. Exchange of ions takes place on the sediment-water interface and particles of the sediment and those of the surrounding medium are constantly in a physical contact and form a dynamic equilibrium. Our study focused on the investigation of the epipelon (organisms living on the surface of or in the fine-sized sediments), which is a current topic and gained a great international interest, since the organisms forming the epipelon are involved in the physical, chemical, biochemical and microbiological processes of the sediment. The autotrophic organisms of the periphyton produce oxygen and synthesize organic compounds by taking up inorganic plant nutrients from the soil and absorbing light energy. The organic matter produced this way is an essential food source for heterotrophic organisms of the periphyton (zootecton) just like for higher heterotrophic organisms such as fish (Lakatos et al., 2001). The activity of organisms of periphyton and epipelon in aquatic ecosystems leads to activation of metabolic pathways, which can determine or greatly influence the features of a given habitat.

Investigating the structure and function of epipelon is of a great importance, since its structure and complexity characterises the conditions of different habitats. Changes in the quality or quantity of epipelon indicate changes in water quality. From the point of view of nature conservation it can characterize a natural status or indicate the degree of disturbance (degradation). Considering the Water Framework Directives, the epipelon index is an important and useful parameter, as the results of epipelon studies can be applied successfully for determining or revealing the ecological status of waters.

MATERIALS AND METHODS

River Tisza is the second largest river of Hungary, the whole length of which is 966 km. The source of the river is in Rahó in Máramaros, Ukraine. Hungarian reach of River Tisza can be divided into three sections: Upper-Tisza (from the frontiers to Tokaj), Middle-Tisza (from Tokaj to Tiszazug) and Lower-Tisza (from Tiszazug to the southern frontier of the country).

The river has a remarkably diversified catchment area which consists of variegated natural and seminatural habitats. Vegetation of inundation areas and flood plains directly depends on the river as well as on the quantity and quality of water. As it is determined by the frequency and period of inundations, different levels of inundation areas have different vegetation cover.

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Natural values of the region are threatened by several factors, such as river control and drainage of river systems, supplantation of wetlands, pollution of surface waters and urbanisation. On the catchment area of River Tisza more than 90 percent of the surface waters come from foreign countries which decisively influence the water quality in the Hungarian reach of the river. Besides external loads, water quality in the riverbed is also determined by the transformational processes. These processes comprise those physical, chemical, biological and biochemical processes (e.g. degradation, sedimentation, reproduction and biological uptake of substances etc.) the effects of which modifies the quantity of a given substance in a unit volume of water.

Upper-Tisza and its tributaries have been examined by us for years. In the last years, we attached significant importance to the examination of epipelon i. e. to the biotecton on the surface of the sediment (Szabó et al. 2002). 14 sampling sites were marked out in 2000 on the Upper-Tisza and on the tributaries. Samplings for present results were carried out in 2003, 2004 and 2005. The analysed epipelon samples were taken with a Hargrave-sampler from underwater, the 2-3 cm thick upper layer of the sediment (G-Tóth et al., 1994).

Sampling sites:

C1 Szamos, Csenger	C8 Tisza, Lónya
C2 Szamos, Tunyogmatolcs	C9 Tisza, Tuzsér
C3 Tisza, Tiszabecs	C10 Tisza, Dombrád
C4 Túr, Sonkád	C11 Tisza, Tiszabercel
C5 Kraszna, Kocsord	C12 Tisza, Balsa
C6 Tisza, Kisar	C13 Tisza, Tokaj
C7 Tisza, Aranyosapáti	C14 Bodrog, Tokaj

Geo-coordinates of sampling sites, water depth and transparency, water temperature, pH, dissolved oxygen content and oxygen saturation were measured on the site, while taxa list of identified plants was also drawn up. Chlorophyll-a %, dry matter %, organic matter % and ETS activity (which characterises the metabolic intensity of epipelon) of epipelon samples were determined in a laboratory (Felföldy,1987).

Non-taxonomic periphyton index refers to the structure and functioning of biotecton and enables the classification of that. For Hungarian initiatives, such systems are used by hydrobiologists not only in Hungary but in foreign countries as well (Lakatos et al., 1999; Pizarro et al., 2000; Lakatos et al., 2003, 2004). Non-taxonomic indices such as organic matter weight or ETS activity values can be used to determine the effects, which cannot be pointed out by taxonomic analyses. For example, sublethal or physiologic damages which caused by toxins, cannot be detected directly by taxonomic parameters, but smaller quantities of biomass or lower oxygen concentrations may indicate a modified status (Lakatos et al., 2002).

ETS tests can detect the presence of every compound that stimulates or inhibits the cytochrom system. If an ETS inhibitor is present in the surrounding water body, it can possibly get into the cytosol, and as a response to the inhibition of the specific activity of ETS multienzyme complex, the synthesis of enzymes is enhanced. Thus ETS tests are suitable for indicating environmental stress.

ETS tests are used widely for measuring the metabolic activity of various prokaryotic and eukaryotic aquatic organisms. With the help of an empiric factor, the value of maximal oxygen consumption can be estimated from the ETS-activity, during field studies. Originally, ETS tests were worked out for sea-plankton (Kenner et al., 1975; Owens et al., 1975; Christensen et al, 1979).

Several studies confirmed that there is a direct connection between ETS-activity and the intensity of respiration in bacteria, phyto- and zooplankton, and sediment (Packard, 1971, 1985; Packard et al., 1988; Vosjan et al., 1987, 1990; Span, 1986, 1988; G-Tóth et al., 1991, 1995).

Samples were stored at –30 ºC in a deep-freeze, this way the ETS-activity of the samples remains unchanged for two or three weeks (Yamashita et al., 1990).

ETS-activity gives the maximal rate of respiration, therefore ETS tests are suitable for measuring the intensity of metabolism. The measurement itself is simple and reproducible.

ETS in organisms functions as a link element between molecular oxygen and biological oxidation of organic compounds. The occurrence of ETS in aerobic organisms is universal, this way 90% of biological oxidation in biosphere is the result of the activity of ETS (Packard, 1985; G-Tóth et al., 1995)

Samples were stored in a deep-freeze till measurement of ETS-activity. First we optimalized the reaction circumstances (technique and time of homogenisation, separation of ETS fractions). Then we determined the saturation level of substrates (NAD, NADP and succinate) and reagent (INT). The effect of pH, incubation time and homogenisation on formazane production was determined as well and the rate of breakdown of formazane was measured. All examinations were carried out in the laboratory, at 20-24 ºC room temperature. Frozen epipelon samples weighed 20-80 mg (wet weight) were used for the analysis.

Samples were decomposited in 4 ml of homogenizing solution constituted of 0,1 M phosphate buffer pH 8.4, 75 μM $MgSO_4$, 1.5 % (v/v) polyvinyl-pyrolidine (PVP), 0.5 % (v/v) Triton-X-100. The composition of reaction mixture is known from enzyme kinetics measurements, this way the saturation levels of subsequent substrates and reagents can be determined. Sonication (Cole-Palmer Ultrasonicator, 60 W, 1-5 min., 0-4 ºC) is an essential step for determining turbidity and ETS-

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activity, then centrifugation is needed for a proper and precise measurement of ETS-activity. 0.5 ml of the 4 ml homogenized sample was used for incubation in 1.5 ml substrate solution with 0.5 ml INT added. Measurements were carried out with three parallel reactions. The reaction was stopped with 0.5 ml stop solution/quench (cc. H_3PO_4 : formaline = 1:1).

The absorbance of formazane produced was measured at a wavelength of 490 nm with spectrophotometer. Calibration curve was made with the help of INT solutions of different turbidity. Standard solutions approximated the overall composition of the reaction mixture except homogenized solution, which was added later. Incubation time was the same as in the case of paralelly-incubated samples; the homogenized solution was added after stopping the reaction. Results were expressed in terms of absorbance or as µl/O_2/g wet weight/h (Kenner et al., 1975).

RESULTS AND DISCUSSIONS

Results of water analyses can be seen in Table 1. The average water depth was 40-50 cm in all three years, the number of sampling sites is much deeper than the bottom, and water was transparent everywhere. Each year, the water temperature corresponded to the summer situation. The alkaline pH values reflect a state in 2003, between pH 7.23 and pH 8.62, in 2004, between pH 7.68 and pH 8.83, in 2005, pH 7.96 and pH ranged 6:36.
The conductivity measurement of each year and the individual differences observed among the sampling sites. The highest conductivity in all three years was measured in Csenger, Tunyogmatolcs and Tiszabecs sampling points, while in the following three sampling locations (Sonkád, Kocsord, Kisar) we measured decreased conductivity. On the other sampling sites conductivity was higher than in Sonkád, Kocsord and Kisar, but lower or comparable to the first three sites.

In 2003, the average oxygen saturation was above 100%, in 2004, it did not reach 50%, and increased again in 2005, when an average value of around 65% was measured. The low O_2 content, and oxygen saturation of the floating vegetation and emergent shielding effect, and the very low concentration of planktonic chlorophyll-a may be the explanation. Floating material content of water generally decreased in the direction of flow. Contrary, low floating material contents were measured only on the sampling sites of Kocsord and Kisar.

Table 1: Results of water analyses

Samples	water depth (cm)	transparency (cm)	temperature (^{O}C)	pH	conductivity (uS)	O_2 (mg/l)	O_2 saturation %	floating matter (mg/l)
2003.07.07-08.								
C1	110	60	23,1	7,41	880	10,2	119	52,0
C2	40	40	23,1	8,17	971	13,4	156	45,0
C3	50	10	22,6	8,40	994	13,4	156	48,0
C4	40	40	22,1	7,52	376	10,8	126	41,0
C5	-	-	23,1	8,02	365	16,0	186	2,0
C6	40	40	23,4	7,93	344	16,0	186	1,0
C7	-	-	23,2	8,32	620	17,3	201	35,0
C8	30	10	23,7	8,62	582	17,9	209	37,0
C9	40	20	22,8	7,29	580	15,3	179	17,0
C10	50	50	22,8	7,23	562	7,6	89	7,0
C11	40	40	24,5	7,26	566	11,5	134	7,0
C12	40	40	25,0	7,47	581	12,1	141	9,0
C13	30	30	24,7	7,34	380	14,1	164	2,0
C14	50	50	25,5	7,31	565	14,7	171	10,0
2004.06.24-25.								
C1	50	50	22,4	7,84	892	1,8	22	3,0
C2	30	30	26,0	8,15	641	3,4	36	22,0
C3	50	30	25,6	8,22	633	3,8	37	27,0
C4	40	40	22,2	7,68	347	3,1	27	20,0
C5	30	30	25,3	7,96	347	3,5	30	7,0
C6	30	30	23,6	7,98	316	3,1	22	8,0
C7	30	30	23,4	8,20	429	3,5	36	18,0
C8	60	50	24,0	8,33	424	3,8	40	11,0

Samples	water depth (cm)	transparency (cm)	temperature (^{O}C)	pH	conductivity (uS)	O_2 (mg/l)	O_2 saturation %	floating matter (mg/l)
C9	-	-	23,8	8,72	418	4,3	44	14,0
C10	20	20	23,1	8,83	416	4,3	47	14,0
C11	150	50	23,0	8,81	423	3,1	41	15,0
C12	200	50	23,1	8,71	402	3,0	32	7,0
C13	200	100	23,2	7,83	440	2,8	32	3,0
C14	40	40	23,2	8,44	417	3,7	44	5,0
2005.07.12-14.								
C1			21,4	6,36	563	4,6	45	75,5
C2	30	30	21,8	7,68	595	6,7	77	40,0
C3	30	30	22,9	7,78	568	6,4	73	46,0
C4	50	30	22,2	7,84	234	6,1	68	17,5
C5	30	30	22,9	7,81	388	6,2	71	35,5
C6	30	30	21,2	7,77	387	5,5	63	18,0
C7	-	-	21,7	7,81	518	5,5	65	29,0
C8	-	-	21,7	7,85	545	7,1	78	35,5
C9	30	30	21,5	7,73	535	6,3	68	40,0
C10	25	25	21,2	7,79	512	6,4	76	23,5
C11	-	-	21,2	7,96	590	5,3	59	23,0
C12	-	-	21,7	7,89	522	4,3	54	22,0
C13	-	-	21,7	7,56	392	4,4	50	17,0
C14	-	-	21,4	7,70	705	7,3	83	28,0

Plant species of sampling sites were identified in all examined years. We could identify 22 species in 2003, 24 species in 2004 and 45 species in 2005. According to Simon (1992) natural protection values (TVK) were determined for the identified plant species in all three years. During the classification of natural protection values (TVK), we separated the species which refer to natural status from those species which refer to degraded status. Proportion of species which refer to natural status was higher in all years. In 2003 and in 2005, only one species (*Iris pseudacorus* L.) can be classified as protected species. Among weed species, *Xanthium sp*. is worth to mention, which could be identified more and more sites from year by year. It can be found on one site in 2003, while on six sites in 2004 and on seven sites in 2005.

In the biotekton, metabolic processes occurring in the intensity of the ETS-activity data points to provide clarification. ETS activity of the wet epipelon samples relative to the material values of bar graph plotted. On the variety of habitats, the measured ETS activity values are also very diverse (Table 2.). Usually 10 ml O_2/g wet wt/h are the typical values. Twice during the three years, peak activity was observed in 2003, and 2005, in Tuzsér and Tiszabecs sampling locations. In these places, the epipelon chlorophyll-a content was high.

We also measured the correlation between the epipelon ETS - activity levels and chlorophyll-a% patterns (Fig 1.). The result did not show correlation between the activity of epipelon samples and chlorophyll-a %. The organic content of sediment derived from autochton and allochton sources. The kl-a % of the results, taking into account the relationship of organic allochton be held responsible for ETS activity results. In order to compare the epipelon samples of the three years, paired t-test was completed. On the basis of the results, we can say that there was no significant difference between the years of the Upper Tisza.

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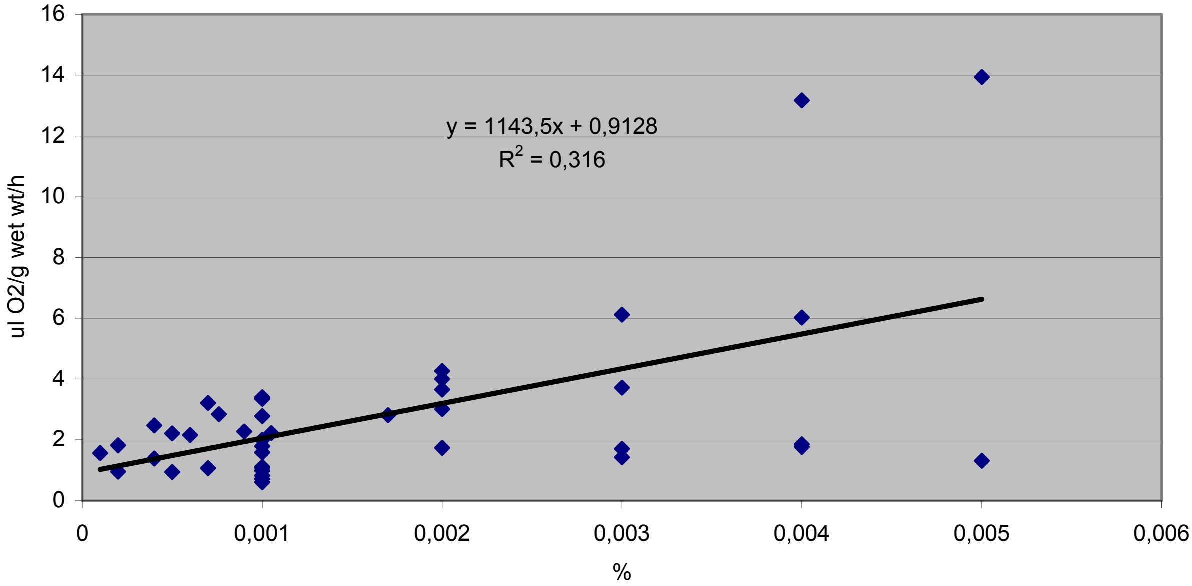
Table 2: Dry weight, ash and AFDW content, chlorophyll-a concentration and ETS-activity of epipelon samples

Samples	ETS ($\mu l\ O_2$/g wet wt/h)	dry w. %	ash %	AFDW %	chl-a %
2003.07.07-08.					
C1	1,997	58,27	92,33	7,67	0,001
C2	6,119	77,17	99,37	0,63	0,003
C3	3,406	50,47	94,73	5,27	0,001
C4	3,654	37,50	92,32	7,68	0,002
C5	1,857	59,53	99,61	0,39	0,004
C6	1,705	68,74	97,31	2,69	0,003
C7	1,591	66,44	98,14	1,86	0,001
C8	1,121	65,98	96,58	3,42	0,001
C9	13,934	42,56	93,80	6,20	0,005
C10	4,262	50,22	97,15	2,85	0,002
C11	0,615	69,24	97,75	2,25	0,001
C12	3,718	45,33	94,36	5,64	0,003
C13	1,087	62,79	97,06	2,94	0,001
C14	1,791	74,05	97,94	2,06	0,001
2004.06.24-25.					
C1	1,317	37,27	85,68	14,32	0,005
C2	1,765	83,24	99,40	0,60	0,004
C3	1,073	68,46	97,63	2,37	0,0007
C4	2,160	76,53	98,89	1,11	0,0006
C6	1,429	66,37	98,43	1,57	0,003
C7	1,826	68,72	98,21	1,79	0,0002
C8	2,473	68,34	98,16	1,84	0,0004
C9	1,395	64,64	96,33	3,67	0,0004
C10	0,946	64,61	95,86	4,14	0,0005
C11	2,208	72,43	98,25	1,75	0,0005
C12	2,223	71,56	98,08	1,92	0,00105
C13	2,842	67,22	98,15	1,85	0,00076
C14	2,817	63,37	96,72	3,28	0,0017
2005.07.12-14.					
C1	6,029	55,31	90,71	9,29	0,004
C2	3,354	78,26	99,57	0,43	0,001
C3	13,168	44,27	93,00	7,00	0,004
C4	2,786	62,61	97,51	2,49	0,001
C5	3,017	64,52	97,97	2,03	0,002
C6	0,717	70,63	98,25	1,75	0,001
C7	4,004	59,82	96,37	3,63	0,002
C8	0,830	61,63	93,86	6,14	0,001
C9	0,987	68,63	97,29	2,71	0,001
C10	1,740	61,27	97,01	2,99	0,002
C11	0,962	60,56	96,55	3,45	0,0002
C12	1,572	74,57	98,72	1,28	0,0001
C13	3,215	61,97	95,24	4,76	0,0007
C14	2,278	62,85	95,56	4,44	0,0009

Fig. 1. Correlation analysis between ETS-activity and chlorophyll-a concentration at Upper Tisza River and its tributaries



CONCLUSIONS

In this paper, we presented the results of epipelon and water examinations, which were carried out in 2003, 2004 and 2005. Among our research methods, we consider ETS test important, because ETS analysis is a tractable test, which indicates the biochemical background of exterior effects on the level of cell oxidation. ETS-test is also proved to be suitable for detecting the materials, which cause damages in the environment, therefore ETS analyses have biomonitoring value.

Organisms of the epipelon contribute to the self-purification of waters and consequently to modifications of water quality. However, their role in the food chain is significantly important as well. According to our results, differences in the structure and functioning of epipelon indicate different environmental habitats, while its characteristic qualitative and quantitative changes indicate the status and modifications of water quality. Therefore epipelon examinations can be effectively used in monitoring of nature conservation and environmental protection. Monitoring of River Tisza has to be continued in order to get more exact information about living species and community structures and to meet the requirements of our contry and the Europian Union on international level as well.

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