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ON SOME CHEMICAL PROPERTIES OF *WOLFFIA ARRHIZA* (L.) WIMM

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Wolffia arrhiza was found on August 2, 1960, in the reservation area of Lake Velence (PRISZTER 1961 p. 120) while exploring the reed banks. Beside the specimens for the herbarium we secured living ones and planted them in the experimental concrete-lined pond of the Biological Research Institute of Tihany.

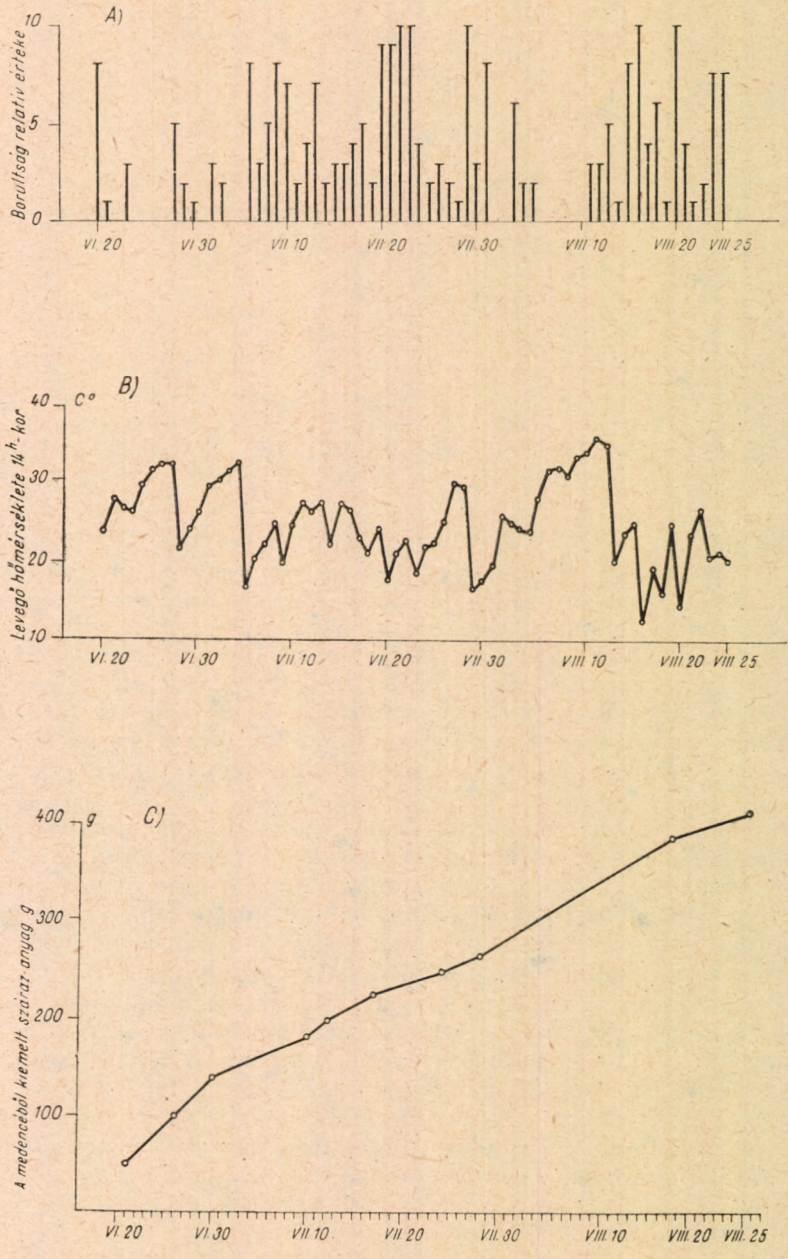
This species, very rare in Hungary (ALMÁDI 1961 pp. 112—3) and regarded in the past years as becoming extinct, proved to have an excellent faculty of propagation and was found to be suitable in experiments concerning the role of makrophytic aquatic vegetation.

The various duckweeds are widely used in phytobiological experiments (HILLMAN 1961), especially the *Lemna minor* both in Hungary and abroad (E. BÖSZÖRMÉNYI and Z. BÖSZÖRMÉNYI 1957). *Wolffia* has so far been used plant beside the duckweeds which are known also to be used in practice (STEFANOVITS 1949, LAUTNER and MÜLLER 1954, NIKOLAEVA 1956, FASSETT 1957, etc.).

In the following we shall briefly sum up our experience gained in growing *Wolffia* in 1961 and some chemical properties of its mass culture.

The major part of the experimental material was grown in a 275 cm long, 105 cm wide, 45 cm deep basin (made of about one cubic metre concrete) in the garden of the Biological Research Institute of Tihany, which was fed beside rain by the "outlet water" of the algal mass cultures in the green-house of the Institute. The "outlet water" was a solution remaining from algal suspension after eliminating the cells by supercentrifuging. The alga cultures were grown in tap water enriched by the addition of KNO_3, KH_2PO_4 and K_2SO_4. Owing to uneven anion adsorption, the water, after being centrifuged, contained a large amount of potassium cations in the form of $KHCO_3$ and K_2CO_3. The residual nitrate and phosphate in it served as anorganic food for the *Wolffia* culture, whereas the metabolic products of the algae represented the organic food. (For the anorganic nutriment of the duckweeds, see HILLMAN 1961 pp. 247 and 251.)

At the outset the „outlet water" coming from the algae had a favourable effect (see curve 1a in *Fig. 1)* but later, since it replaced the evaporating water and owing to the accumulation of a considerable amount of salt, to the uneven anion adsorption of the algae and the *Wolffia* plants living in the basin and to the consumption of phytoplantonic carbon dioxide, a strong alkalization took place (above pH 10.5). Beside the meteorological factors prevailing in



1. ábra. A meteorológiai tényezők (A = borultság, B = hőmérséklet) és a *Wolffia* tenyészet szaporodási görbéje (C).

Figure 1. Growth rate of out door culture of *Wolffia arrhiza* (C) in comparison with cloudiness (0—10 relative grades) (A) and with air temperature at 14h (B) from 20th June to 25th August.

the autumn, this shift in the content of the solution was also responsible for lessening the growth rate of the culture.

According to ERNŐ SZABÓ's analysis and kind information, the chemical composition of the water on June 20, *i. e.* in the phase of the rapid growth of *Wolffia* was as follows: $Ca^{++} = 21.8$ mg/l; $Mg^{++} = 36.7$ mg/l; $Na^{+} = 42$ mg/l; $K^{+} = 152$ mg/l; $CO_3^{--} = 47.5$ mg/l; $HCO_3^{-} = 370.9$ mg/l; $NO_3^{-} = 4.5$ mg/l; $PO_4^{---} = 0.4$ mg/l; $NO_2 = 0$; $NH_3 = 0$. Its electrical conductivity was 744. The large amount of K ions was conspicuous as early as that.

When the whole surface of the basin was covered with the mass vegetation of *Wolffia*, the majority of the plants were scooped of with a sieve, dried at a temperature of 105 °C and weighed. The removal took place invariably at 11 a. m. The one twentieth to one fiftieth part of the original mass was left on the surface of the water.

The quantitative increase of the dry matter obtained from the pond is shown in the function of time in *Fig. 1* (C), where also the overcast (A) and temperature (B) conditions of the growth period are plotted as measured at 2 p. m. daily by the meteorologic station set up in the park of the Biological Institute. The relative value of overcast conditions is expressed by grades going from 0 to 10, *i. e.* from cloudless sky to complete cloudiness, each column representing thus one tents of cloudiness.

The growth rate of the mass vegetation of the *Wolffia* decreased with time. If the curve of the increase of dry substance in *Fig.* 1 C is collated with the graphs of the meteorological data (A, B), it is found that at the end of June, when the rate of propagation of the *Wolffia* was high, the meteorological conditions were most favourable: air temperature 24—32 °C, clear sky. The low temperatures and the almost permanent overcast in July retarded growth. Between Aug. 1 and 12, owing to the favourable temperature conditions (24—36 °C, clear sky) mass propagation was resumed.

Beside the shift in the content of the feeding solution, meteorological factors also influenced the rate of propagation of the *Wolffia*.

Between June 21 and August 25, 414 g of dry substance was collected from our pond of 3 m^2. Out of this amount 95 g was recovered between June 21 and 30, which is almost one fourth of the total (23 per cent). As this was collected during nine days, *i. e.* during one seventh of the breeding time observed (66 days), with the maintenance of the favourable June conditions, the amount of dry substance would have been 693 g instead of 414.

These proportions inevitably suggest the idea that the "outlet water" of alga-growing plants to be established in the future could be utilized for growing *Wolffia* or some other aquatic plants (e. g. *Lemna minor*) of small size and of high propagating faculty.

With a view to investigating the function of aquatic vegetation of higher order, our further aims are to start mass cultivation in natural conditions and to produce bacterium-free laboratory cultures.

The results of the chemical analyses can be summed up as follows:

The samples dried at 105 °C up to constant weight were powdered in a mill and the dry substance was kept in desiccators (paper bags) filled with $CaCl_2$. Our computations refer to this substance.

For determining chlorophyll, carotenoids and sugars, living material was used. To be able to refer the results to dry substance, water was extracted from the mass of living plants by blotting paper (taking care not to squash

them), and the content of dry matter was weighed in an aliquot part of the sample thus obtained and serving as the basis of our computations.

The examination of ash ingredients.

The samples were heated at 650 °C up to weight constancy (about three hours). The heating remainder is given in the percentage of the dry substance (raw ash).

The total amount of nitrogen.

The organically bound nitrogen was determined by means of the modified KJELDAHL method, with a microdistiller of the PARNAS—WAGNER type.

In the presence of 5 ml sulphuric acid (s. w. 1.84) and 0.1 g selenium calatyzer, 250 gm of dry substance was decomposed in a 50 ml KJELDAHL flask until complete discolouration. The content of the decomposing flask was transferred quantitatively into a 50 ml weighing flask, from the 2×10 ml of solution filled up to the mark, after its alkalization with sodium hydroxide (40 per cent), the released ammonia was distilled through a 10 ml boric acid of about 4 per cent and weighted with a 0.1 N hydrochloric acid solution in the presence of a GROÁK indicator.

One ml 0.1 N hydrochloric acid yields 1.4 mg nitrogen. The series $N \times 6.25$ yields the protein content of the substance investigated.

Crude fibres.

The crude fibres were determined by the SCHARRER—KÜRSCHNER method based on acidic hydrolysis and oxidation (OROSZLÁN, SZOLNOKI and FELFÖLDY 1952).

With a mixture of 36 ml acetic acid (70 per cent), 2.5 ml nitric acid (s. w. 1.4) and 0.5 g of trichloroacetic acid, 1.5 g dry matter was boiled in a 100 ml ERLENMEYER flask for 30 minutes with a reflux condenser, then dried at 105 °C and filtered through a quantitative filtering paper of known weight. The filter was washed with 7—10 ml of the above hot acidic mixture, with hot water, 20 ml of ethanol and finally with 5—10 ml of ether. The filtering paper was dried on 105°C together with the remnants of filtering to weight constancy and then weighed. Deducting the wieght of the filtering paper from the result of weighing, the value obtained was somewhat higher than that of the actual fibre material, the difference being due to the amount of incrustation substance insoluble during hydrolysis and concomitant to cellulose. Therefore the filtering remainder was incinerated together with the filtering paper. and the actual value was computed from the decrease of weight thus obtained (TÓTH and SZABÓ 1958 p. 368). In case of the *Wolffia*, however, the amount of ash is so small (0.5 per cent) that it can safely be neglected.

Lipoid (petroleum ether soluble) substances.

For determining the lipoids the samples were extracted for 24 hours with pure petroleum ether in a SOXHLET device. After drying, the remainder in the filtering paper bags was weighed.

The above two dates were selected for publication because then extreme values were obtained. The nitrogen and protein content, compared to other plants, can be regarded as mean values. The crude-fibre content is rather low. The high ash content of the samples is conspicuous. At a first glance one may think of the feeding solution of high salt concentration adhering to the plants yet the very thorough washing of the plants with distilled water resulted in no essential change (20.5 and 19.8 per cent). Further experiments will have to reveal whether the ash content of the *Wolffia* as was found here should be

Table 1 — 1. Táblázat

Ash, protein, crude-fibre and lipoid content (per cent of dry matter) in *Wolffia arrhiza**

Wolffia arrhizza hamu protein, nyersrost és lipoid tartalma (SzA %)

Date Datum	Ash Hamu	Total N Nyersrost	Protein 6.25 N	Crude fibre Összes N	Petroleum ether soluble substances
26 VI	26.7	2.65	16.58	13.50	—
12 VII	23.1	3.30	20.62	10.60	6.85

ascribed to the feeding solution being different from the normal or else to the capacity of the species of accumulating large amounts of salt.

Pigment.

The total amount of chlorophyll components and of the carotenoids was measured in simple axetone ectract (MacKinney 1941). Portions of fresh plants, weighed with a permissible error of +0.01 g, mixed with a small quantity of powdered glass, magnesium oxide and acetone, were ground into a homogeneous pulp. The extraction with 90 per cent acetone was carried on until, after drying, the mass remaining on the asbestos filter became white. Filled up to 50 ml, the extract was treated spectrophotometrically (with a Beckman DU spectrophotometer) at wave lengths 665, 645 and 480 m μ. The specific extinction coefficients of Richard and Thompson (Barnes 1959, p. 323, Table 44) were used in the computations.

The results are compiled in Table 2.

Table 2 — 2. táblázat

Results of chlorophyll and total-carotenoid estimations of *Wolffia arrhiza* cultures grown outdoors (W_I) and in laboratory conditions (W_{II})

A *Wolffia arrhiza* szabadban (W_I) és laboratóriumban (W_{II}) nevelt tenyészeteinek klorofill és össz-karotinoid tartalma

Dátum Date	Jelzés Sign.	a-klorofill sza % % of dry matter	b-klorofill sza % % of dry matter	össz-karotinoida SPU in 100 g dry matter
19 IX	W_I	0.49	0,14	0,21
29 IX	W_I	0,35	0,11	0,15
5 X	W_I	0,41	0,15	0,15
	W_{II}	0,63	0,24	0,16

The values of the laboratory samples (cultivated near light, window) are 20—40 per cent higher than those of the outdoor samples. This is probably due to the fact the laboratory conditions were more favourable for the *Wolffia arrhiza* living in the shadow of other plants outdoors. Excessive sunshine may result in the decomposition of chlorophyll (Montfort 1941, Zurzycki 1957).

For the determination of β-carotene playing the part of A-provitamin, so important in practice, the fresh plant substance was homogenized with about 20 ml of ethanol, powdered glass, mixed with 4 ml 60 per cent KOH solution and digested for five minutes ina water bath not exceeding 40 °C.

This was centrifuged at a low r. p. m., the pure liquid was poured off and the extraction was continued first with ethanol, then with water and acetone. Then the unsaponified pigments were shaken into pure ether. washed, dried and chromatographed on a 1 cm dia and about 14 cm long column made of a 1 : 1 mix of magnesium-Hyphlo supercel. The β-carotene could be well separated with petroleum ether containing 4 per cent of acetone (BOOTH 1957). No α-carotene was found.

For determining the quantity of β-carotene TIEW's (1959) extinction coefficient (on 452 mμ $E^{1\%}_{1cm}$ = 2,600, in hexane) was used, whereas the other carotenoids were computed with the coefficient (on 445 mμ $E^{1\%}_{1cm}$ = 2,500, in hexane) of GOODWIN (1955).

The results are summed up in Table 3.

Table 3 — 3. táblázat

β-carotene and mixed xanthophylls in *Wolffia arrhiza* (mgs of 100 g dry matter)

A *Wolffia arrhiza* β-karotin és xantofill tartalma (mg %)

Date Dátum	β—carotene β—karotin	Mixed xanthophylls xantofill keverék
28 XI	24,9	78,0
30 XI	29,0	95,1
	28,4	86,0

The differences between the results of the two methods (the value of total-carotene content weighed in acetone extract is always higher than the amount obtained in hexane) can be explained by methodologic reasons (FELFÖLDY SZABÓ and TÓTH 1962.)

Sugar analysis.

For the sake of orientation the quality of sugars to be found in the *Wolffia* was examined with the paper-chromatographic method. For a starting point living matter was used, here too, (for the determination of dry matter see above). It was extracted with tepid 70 per cent ethanol, distilled in vacuum at a temperature not exceeding 30 °C until it was dry and then solved in 1 ml 70 per cent ethanol. A 0.02 ml portion of this extract was chromatographed on a 40 cm long SCHLEICHER and SCHÜLL 2043 B paper with a solvent of butanol : ethanol : water (4 : 1 : 5). The sugar was made visible by basic silver nitrate (the saccharose was inverted on a band with a mix of trichloroacetic acid and ethanol) (LINSKENS 1959). The sugars were identified by means of the corresponding standards.

Beside a small amount of maltose, a medium amount of glucose, fructose and a large quantity of saccharose were found. As far as the changes in the amount of sugars during the twenty-four hours of a day is concerned, saccharose was found to be compiled in large quantities toward the evening, reducing to the minimum in the morning. These changes indicate the importance of the disaccharides for the metabolism of *Wolffia*.

I wish to express my thanks to the Head of Department DR LAJOS FELFÖLDY for the assistance I received from him during my work. For the water analysis I am indepted to MR ERNŐ SZABÓ, scientific research worker,

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Summary

Our observations made during the cultivation of the *Wolffia arrhiza* (L.) Wimm. transplanted in the concrete basin in the park of the Biological Institute of Tihany from the reed banks of Lake Velence and the chemical analysis of the mass here obtained have been treated.

A medium amount of protein (2.5—3.3 per cent), petroleum-ether-soluble substance (6.9 per cent), a small amount of crude fibres (10—13 per cent) and a strikingly large quantity of ash (23—28 per cent) were found in the dry matter of the *Wolffia*.

In the plants grown under laboratory conditions there was much more a- and b-chlorophyll (0.6, resp. 0.2 per cent) than in the outdoor samples (0.4 resp. 0.1 per cent). No α-carotene was found, and the β-carotene content varied between 24 and 30 mg per cent. The quantity of the other carotene was 80—100 mg per cent. The sugar analysis of the *Wolffia* yielded a small amount of maltose, a medium amount of glucose and fructose, and much saccharose.

The results of outdoor cultures reflect the impact of meteorologic conditions: the highest rate of propagation was observed during the warm, sunny days between June 20 and 30, and between Aug, 1 and 12.

Later the rate of propagation was undoubtedly influenced by the mass of algae in the "outlet water" (feeding solution left over from algal suspension, from which the cells were removed by centrifuging) which, owing to the uneven anion adsorption of the algae living in the pond and of the *Wolffia* plants and to the consumption of phytoplankton carbon dioxide, became strongly alkaline.

In the most favourable period, *i. e.* between June 21 and 30, 95 g of dry matter was produced in the 3 m^2 basin, that is during one seventh (66 of the total period (66 days) 23 per cent (414 g) of the total crop.

We have set ourselves further tasks, such as to examine how the so-called "outlet" water of an alga-growing apparatus can be best utilized for *Wolffia* cultures and to create a bacterium-free culture for investigating the function of the aquatic vegetation of higher order.

IRODALOM

Almádi L. (1961): A Wollffia arrhiza (L.) Wimm. szarvasi előfordulása. — *Bot. Közl.* **49,** 112—113.

Booth, V. H. (1957): Carotene, its determination in biological materials. — *Heffer and Sons Ltd., Cambridge* 1—119.

Böszörményi, E. and Z. Böszörményi (1957): N and P nutrition and the physiological age of Lemna minor L. — *Acta. Bot. Acad. Sci. Hung.* **3,** 1—7.

Fassett, N. C. (1957): A manual of aquatic plants. — *The University of Wisconsin Press, Madison, 1*—405.

Felföldy L., Szabó E. és Tóth L. (1962): Egysejtű algák pigment tartalmáról. — *Annal. Biol. Tihany* **29,** 101—106.
Goodwin, T. W. (1955): Carotenoids. — In Paech, K. and M. V. Tracey (ed): Modern methods of plant analysis. — *Springer, Berlin,* Vol. III, 272—311.
Hillman, S. W. (1961): The Lemnaceae, or Duckweed. A reviev of the descriptive and experimental literature. — *Bot. Rev.* **27,** 221—287.
Landolt, E. (1957): Physiologische und ökologische Untersuchungen an Lemnaceen. — *Ber. Schweiz. Bot. Ges.* **67,** 271—410.
Lautner V. und Z. Müller (1954): Die Futterwerte einiger unseren Wasserpflanzen. I. — *Sborn. Ceskoslov. Akad. Zemedel Ved.* a. **27,** 333—354. (Csehül német összefogl.)
Linskens, H. F. (1959): Papierchromatographie in der Botanik. Zweite Auflage. *Springer, Berlin* 1—408.
Montfort, C. (1941): Die Beziehungen zwischen Stagnationseffekt, photooxydativer Chlorophyllzerstörung und funktionellem Sonnenstich bei Wasserpflanzen. — *Naturwiss.* **1941,** 238.
Nikolaeva, A. M. (1956): Duckweed as feed for water fowl. — *Ptitsevodstvo* **1956,** 24—27. (Orosz nyelven angol összefoglalással)
Oroszlán I., Szolnoki J. és Felföldy L. (1952): Évelő füveink kémiai vizsgálata. I. Tarlómaradványok és földalatti részek. — *MTA. Biol. Oszt. Közl.* **1,** 213—222.
Priszter Sz. (1961): Megjegyzések adventív növényeinkhez. — *Bot. Közl.* **49,** 115—121.
Stefanovits P. (1949): Egy kecskeméti mezőségi talaj humuszának biológiai vizsgálata. — *Agrártudomány* **1,** 190—192.
Tiews, J. (1959): Zur Analytik des Vitamins A. — *Vitamine u. Hormone* **8,** 185—242. f
Tóth L. és E. Szabó (1958): Über die chemische Zusammensetzung verschiedener Schilproben vom Balaton-See. — *Annal. Biol. Tihany* **25,** 363—374.
Zurzycki, J. (1957): The destructive effect of intense light on the photosynthetic apparatus. — *Acta Soc. Bot. Polon.* **26,** 157—175.

A *WOLFFIA ARRHIZA* (L.) Wimm NÉHÁNY KÉMIAI TULAJDONSÁGÁRÓL

Tóth László

Összefoglalás

A Velencei-tó nádasaiból származó és a tihanyi Biológiai Intézet udvarán levő beton-medencébe telepített *Wolffia arrhiza* (L.) Wimm. tenyésztése során kapott tapasztalatainkat és az itt nyert tömeg kémiai analízisét ismertettük.

A *Wolffia* szárazanyagában közepes mennyiségű fehérjét (2,5—3,3%) és petróleum éter oldékony anyagot (6,9%) kevés nyersrostot (10—13%) és feltűnően sok hamut (23—28%) találtunk.

A laboratóriumban termett növényekben lényegesen több (0,6, ill. 0,2%) a-, illetve b-klorofill volt, mint a szabadföldi mintákban (0,4, ill. 0,1%). α-karotint nem találtunk, a β-karotin tartalom 24—30 mg% között változott. A többi karotinoid mennyisége 80—100 mg%. A *Wolffia* cukor-analízise során kevés maltózt, közepes mennyiségű glukózt és fruktózt és sok szaharózt találtunk.

A szabadföldi tenyésztés eredményei a meteorológiai tényezők hatására mutatnak, a leggyorsabb szaporodás a jún. 20, jún. 30 és az aug. 1, aug. 12 közötti meleg, napos időszakra esett.

A későbbiek folyamán a szaporodás gyorsaságát feltétlenül befolyásolta az az alga „szennyvíz" tömeg is (az algaszuszpenzióból visszamaradó tápoldat, amiből a sejteket szupercentrifugával távolították el) — mely a medencében élő algák és *Wolffia* növények egyenlőtlen anion felvétele és a fitoplankton széndioxid felhasználása miatt erősen ellúgosodott és egyoldalúvá vált —, amit az elpárolgó víz helyébe adtunk.

A jún. 21 és jún. 30 közötti legkedvezőbb periódusban — 3 m² felületű tenyésztő medencénkben — 9 nap alatt 95 g szárazanyag termelődött, vagyis a vizsgált tenyészidő (66 nap) egyhetede alatt az össztermés (414 g) 23%-a.

*Wolffia*val való vizsgálataink további célkitűzése, egyrészt, hogy megvizsgáljuk, hogy egy alga-tenyésztő berendezés ún. „szennyvizét" hogy hasznosíthatjuk a legjobban *Wolffia* tenyésztésével, másrészt, hogy baktériummentes kultúrát állítsunk elő a magasabbrendű vízi vegetáció szerepének kutatása céljából.