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Allelopathic activity of Characeae

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13 different Characeae species were screened for allelopathic activity using agar-diffusion assays. Nine different Cyanobacteria, one diatom species and three Chlorophyceae were applied as target organisms. Whereas cyanobacteria were strongly inhibited by certain stoneworts, surprisingly no clearing of eukaryotic target strains was noted in any of the screening assays. *Chara aspera*, *C. globularis* and *Nitellopsis obtusa* were proven to be the most active test organisms and therefore selected for further analyses. In order to test whether the allelochemicals are soluble in a hydrophilic or in an increased lipophilic solution, aqueous and 60% methanol extracts were performed. The lipophilic methanol extracts exhibited a stronger clearing of target organisms indicating a more lipophilic behavior of the allelochemical.

Key words: Characeae, *Chara, Nitellopsis*, allelopathic activity.

Introduction

In aquatic ecosystems, macrophytes and benthic algae have to compete for light and space, while nutrient limitation is only of minor importance due to the ability of benthic plants in taking up nutrients from the sediment (Gross, 1999; Wetzel, 2001). Especially in the littoral, light availability is reduced by epiphytes overgrowing photosynthetic active parts, scums of filamentous algae floating on the water surface, and by high phytoplankton densities (Philips et al., 1977). Light intensity decreases exponentially with depth and the light spectrum changes, forcing certain species back into more shallow environments. The littoral zone is therefore often characterized by the occurrence of dense macrophyte beds. Submersed living water plants have few possibilities to overcome the shading effect, either enhancing growth, luxury uptake of nutrients (Balls et al., 1978), formation of dissected leaves, low light and CO_2 compensation points (Gross, 1999), and the release of allelopathic active compounds (Wium-Andersen, 1987). The high density of plants and the shallowness of the water body result in a limited water exchange, which supports the discharge of allelochemicals by the host macrophyte as a powerful strategy against shading epiphytes and phytoplankton.

Characeae spread a huge rhizoidal system through the sediment and are able to form widespread monospecific underwater meadows. Especially shallow calcareous lakes in lime rich regions are preferred habitats and have consequently been termed as "Chara lakes" (Almquist, 1929). The plants are characterized by a pungent smell and have therefore long been regarded as repellent to herbivorous larvae or even toxic to some mosquito larvae (for references see Zaneveld, 1940). The usual scarcity of epiphytes on Characeae and the low phytoplankton density (Horecká, 1991) in *Chara* dominated systems has long been attributed to allelopathy (Wium-Andersen et al., 1982). Evidence for

the allelopathic activity of Characeae has already been proven. ANTHONI et al. (1980) and WIUM-ANDERSEN et al. (1982) isolated two thermolabile, sulfur containing compounds from *Chara globularis*, 4-methylthio-1,2-dithiolane and 5-hydroxy-1,2,3-trithiane. The two compounds have additionally implicated herbicidal and insecticidal effects. These two sulfur-holding allelochemicals were also detected in *Chara baltica*, *C. hispida*, *Nitella translucens* (for references see KLEIVEN, 1991) and *Tolypella nidifica* (WIUM-ANDERSEN et al., 1987).

Another allelochemical from *Chara globularis* with a strong antibiotic activity was isolated by ANTHONI et al. (1986) six years later. The compound Charamin (4-azo-niaspiro[3,3]heptane-2,6-diol) represents one of the few nitrogen holding allelopathic substances, which are generally costly for plants and therefore presented in very low concentrations (BRYANT et al., 1983; WIUM-ANDERSEN et al., 1982).

Furthermore, KLEIVEN & SZCZEPANSKA (1988) showed that extracts of *Chara tomentosa* inhibit shoot growth and leaf development of *Lepidium sativum*. The allelopathic compounds were proven to be of low molecular weight and temperature stable. HOOTSMANS & BLINDOW (1994) concluded from their investigations that "Characeae excrete substances that influence the growth of test algae either directly or via interaction with bacteria". It was proven that growth of *Scenedesmus communis* was stimulated or not affected by *Chara tomentosa, C. hispida* and *C. delicatula* while it was significantly inhibited by *C. globularis*.

Despite these findings, evidence about growth inhibition caused by stoneworts in situ is still conflicting. The low primary production observed in *Chara* lakes is more likely explained by both low phosphorus levels limiting growth of phytoplankton (FORSBERG et al., 1990) and photoinhibition (KLEIVEN, 1991). Characeae are able to cope with phosphorus concentrations below 20 μg L^{-1} (FORSBERG, 1965) and develop large underwater meadows. FORSBERG (1990), additionally referred the scarcity of phytoplankton to the immense storage capacity and the high uptake rate of phosphorus by Characeae and not to the release of allelopathic compounds.

One purpose of this study was to screen a number of different Characeae species for allelopathic activity that suppresses growth of microalgae. This study represents a basis for further detailed investigations dealing with single Characeae species and bioactive nature of their compounds. Bioassays were used for the evaluation of the allelopathic potential of living material and extracts of the investigated species. Screening assays such as plate-diffusion-tests (FLORES & WOLK, 1986) deploying target organisms, have been proven to be a simple method to detect bioactive substances of cultured organisms. This method seems to be suitable as it reflects the diffusion of an allelopathic agent released by a benthic organism and simultaneously gives an overview of its effective radius.

Material and methods

Characeae specimens used for the screening tests were taken from different lakes located in Eastern Austria and Hungary (Tab. 1). Thirteen different *Chara*

Table 1. Characeae populations investigated in screening tests and their origin.

Test organisms	Origin
Chara aspera DETHARDING ex WILLDENOW	gravel pond in Neudörfl (Austria)
Chara contraria A. BRAUN ex KÜTZING	Weißensee (Austria)
Chara delicatula AGARDH	Erlaufsee (Austria)
Chara globularis THUILLIER	gravel pond in Neudörfel (Austria)
Chara globularis THUILLIER	sterile culture derived from plants grown in the gravel pond in Neudörfel (Austria)
Chara polyacantha RABENHORST & STITZENBERGER	Weißensee (Austria)
Chara rudis A. BRAUN in LEONHARDI	Erlaufsee (Austria)
Chara tomentosa LINNÉ	Neusiedlersee (Ilmitz, Austria)
Chara vulgaris LINNÉ	rivulet Fischa-Dagnitz, (Austria)
Nitella cf. *gracilis* AGARDH	garden pond in Vienna (Austria)
Nitella opaca AGARDH	Lunzer Untersee (Austria)
Nitellopsis obtusa J. GROVES	Neue Donau, Vienna (Austria)
Nitellopsis obtusa J. GROVES	Plattensee (Hungary)

Table 2. Algae used as target organisms in bioassays (ASW = Culture collection at the Institute of Ecology and Conservation Biology, Vienna University. Culture media according to KUSEL-FETZMANN & SCHAGERL (1992). a Soos, Austria; b Neusiedlersee, Austria; c Alte Donau, Austria; d Museumsteich, Austria; e Figurteich, Austria; f Eliasteich, Austria. JÜ = Jüttner; DES = nutrient solution for Desmediaceae; CHU = medium according to CHU; E = soil extract

Target organisms	Strain	Origin	Medium
Cyanobacteria			
Anabaena cylindrica LEMMERMANN	ASW 0103	a	JÜ
Anabaena torulosa (CARM.) LAGERHEIM	ASW 01023	b	JÜ
Anabaenopsis elenkini V. MILLER	ASW 01027	b	JÜ
Aphanizomenon flexuosum MORREN	ASW 01033	c	JÜ
Cylindrospermum sp. KÜTZING	ASW 01016	d	JÜ
Microcystis aeruginosa KÜTZING	ASW 01002	b	JÜ
Microcystis flos-aquae (WITTR.) KIRCHN.	ASW 01004	b	JÜ
Planktothrix rubescens (DE CANDOLLE) ANAQN. et KOMÁREK	ASW 01059	e	JÜ
Planktothrix agardhii (GOMONT) ANAQN. et KOMÁREK	ASW 01060	e	JÜ
Bacillariophyceae			
Fragilaria sp. LYNGBYE	ASW 03018	b	DES+E
Chlorophyceae			
Ankistrodesmus fusiformis CORDA	ASW 05021	d	CHU+JÜ
Scenedesmus armatus (CHOD.) G. M. SMITH	ASW 05021	f	CHU
Scenedesmus acutus MEYEN	ASW 05031	f	CHU+JÜ

populations were tested for allelopathic activities. Nine cyanobacteria species, three Chlorophyta and one Bacillariophyceae were selected as target organisms (Tab. 2). Strains are indicated by the acronym ASW (Algensammlung Wien) followed by their strain number.

Screening of living material

The screening tests were carried out as plate diffusion assays following a modified protocol of FLORES & WOLK (1986). Sterile Petri dishes were filled with 30 mL 1.0% (distilled water) agar (Merck 1614), which formed a basic layer. A second agar suspension layer (0.2%, culture medium) enriched with target algae was added after 2 hours (height about 5 mm). Following an incubation time of two days one or two shoots of a non-axenic *Chara* specimens were placed in the middle of the Petri dish half covered in the second agar layer. Before adding the macrophytes, shoots were carefully rinsed with water to wash off epiphytes and animals without harming the test organism (microscopical control). Each bioassay was carried out in triplicates and analyzed after further 14 days by comparing the zone of growth retardation or inhibition of target organisms around the *Chara* shoots (Fig. 1).

Fig. 1. Screening assays of *Nitellopsis obtusa*. Target organism: *Anabaena cylindrica*.

Bioactive extracts

To gain more information about the nature of allelochemicals, fresh plant material from highly bioactive *Chara globularis*, *Chara aspera* and *Nitellopsis obtusa* was lyophilized for 14 days and afterwards grinded in a mill. 1g of the dry pulverous plant material was homogenized in 100 mL solvent composed of either 60% methanol (Merck) or distilled water. The extraction process was carried out under permanent shaking in the dark at a constant temperature of 15 °C. In order to limit the risk of bacterial growth the extraction times of 24 and 48h were selected for the aqueous and methanol extracts respectively. The extracts were centrifuged subsequently (2500 rpm, 20 °C, 20 min) and filtered through membrane filters (Sartorius 0.45 μm, prefilter Whatman GF/C). The supernatants were restricted with a rotation evaporator and re-dissolved in 5 mL 98% methanol (Merck). The extracts were stored at −30 °C.

Table 3. Allelopathic activities of the characean species *Chara globularis* (C glob), *Chara aspera* (C asp), *Nitellopsis obtusa* origin Neue Donau (N obt ND), *Nitellopsis obtusa* origin lake Plattensee (N obt P), *Chara delicatula* (C del), *Chara contraria* (C con), *Chara tomentosa* (C tom), *Chara vulgaris* (C vul), *Chara rudis* (C rud), *Nitella opaca* (N opa), *Nitella gracilis* (N gra) and *Chara polyacantha* (C poly) [a]no effect observed (–); weak clearing (+); intermediate clearing (++); strong clearing (+++), not determined (nd).

Target strains[a]	C glob	C asp	N obt ND	N obt P	C del	C con	C tom	C vul	C rud	N opa	N gra	C poly
Cyanobacteria												
Anabaena cylindrica	+++	++	++	++	+	+	–	–	++	+	+++	–
Anabaena torulosa	+++	++	+	+	+	–	+	+	–	+	++	–
Anabaenopsis elenkinii	++	+	+	–	+	–	–	+	–	+	++	–
Planktothrix agardhii	–	nd	–	–	nd	–	–	–	–	nd	–	–
Planktothrix rubescens	+++	–	–	–	–	–	–	–	–	++	++	–
Microcystis aeruginosa	++	+	–	–	+	+	–	+	–	–	–	–
Microcystis flos-aque	++	+++	++	–	+	–	–	–	–	–	–	–
Cylindrospermum sp.	++	–	++	+	+	+	–	–	+	+	++	–
Aphanizomenon flexuosum	++	–	++	nd	+	–	+	–	–	+	+++	–
Chlorophyta												
Ankistrodesmus fusarum	–	–	–	nd	–	–	–	–	–	–	–	nd
Scenedesmus armatus	–	–	–	nd	–	–	–	–	–	–	–	nd
Scenedesmus acutus	–	–	–	nd	–	–	–	–	–	–	–	nd
Bacillariophyceae												
Fragilaria sp.	–	–	–	nd	–	nd	nd	nd	nd	–	nd	nd

The Characeae extracts and the biomass pellet received from the centrifugation process were tested for allelopathic activity using agar-diffusion assays. The bioassays were performed in a similar way as the screening tests described before. 100 μL of the extract was employed on the basic agar layer (1% agar), and dried with sterile air before the second agar layer (0.2% agar, culture media) inoculated with target strains was coated.

Blanks with methanol (98%) were conducted for each test series to secure that clearing effects were not caused by traces of the solvent.

Results

Screening of living material

13 different *Chara* populations were screened against microalgal target strains including cyanobacteria and eukaryotic microalgae. Ten species inhibited growth of the cyanobacterial target organisms. Interestingly, no clearing effects against the eukaryotic strains could be observed (Tab. 3).

Compared to the other species, *Chara globularis*, *C. aspera* and *Nitellopsis obtusa* (Neue Donau) exhibited the highest bioactivity. *Chara globularis* cleared all cyanobacterial target strains except *Planktothrix agardhii* at intermediate to strong level whilst other *Chara* species inhibited only two or three of the target algae (Tab. 3). The most sensitive target strains which were growth-inhibited with great potency by almost all Characean species were the diazotrophic filamentous cyanobacteria *Anabaena cylindrica*, *A. torulosa* and *Anabaenopsis elenkinii*. No bioactivity was observed in the rugged *Chara polyacantha*.

Screening of Chara extracts

Extracts were obtained from *Chara aspera*, *C. globularis* and *Nitellopsis obtusa* (ND). The biotests were exclusively performed with cyanobacterial target strains due to the results of the preceding screening tests (Tab. 4). All methanol as well as aqueous extracts of the three species tested showed allelopathic effects. The plant extracts inhibited growth of the same cyanobacterial species as the shoots did before.

Generally, the methanol extracts exhibited a stronger bioactivity than the aqueous extracts. The extracted biomass pellets of the methanol extracts were free of any allelochemicals, thus permitting unconstrained growth of the target algae. A harming effect was only caused by the pellet of the aqueous *Nitellopsis obtusa* extract (not presented), which showed a weak clearing of *Anabaena torulosa* and *Anabaenopsis elenkinii*. No clearing effects were noticed for the blanks prepared for each charge of bioassays.

Table 4. Bioactivities of the Characeae extracts against eight cyanobacterial species. Test organisms used for extracts: *Chara aspera* (C asp), *Chara globularis* (C glob), *Nitellopsis obtusa* (N obt), aqueous extract (W), 60% methanol extract (M), no effect observed (–), weak clearing (+), intermediate clearing (++), strong clearing (+++), not determined (nd).

Extracts Target strains	C asp W	C asp M	C glob W	C glob M	N obt W	N obt M
Anabaena cylindrica	+	+	+++	+++	++	+++
Anabaena torulosa	+	++	++	++	–	–
Anabaenopsis elenkinii	–	+	+	++	+++	+++
Planktothrix rubescens	nd	nd	nd	nd	nd	nd
Microcystis aeruginosa	+	+	+	+	+	+
Microcystis flos-aque	+	+++	+	+	+	+
Cylindrospermum sp.	–	–	+	+	++	+++
Aphanizomenon flexuosum	nd	–	++	++	++	++

Discussion

The compounds involved in allelopathic processes are secondary metabolites, by-products of metabolic pathways with various chemical structures and strong bioactivity (Todorova, 1996). Polyphenolic compounds of *Myriophyllum spicatum* were implicated to reduce phytoplankton and epiphyte development in the early growth period of the macrophyte (Gross, 2000; Planas et al., 1981). Wium-Andersen et al. (1983) suggested that a labile compound, which easily releases elemental sulfur, is responsible for the low epiphyte content of *Ceratophyllum demersum*. Allelopathic active compounds were also found in several microalgae with emphasis on cyanobacteria (Schagerl et al., 2001; Todorova, 1996; Smith & Doan, 1999; Doan et al., 2000; Carmichael, 1991). Many of these substances isolated exhibited anti-microbial, toxic and cytotoxic effects and are therefore of great pharmacological, biotechnological, medical and agricultural interest.

In the present work 10 species out of 11 tested, caused growth inhibition of the target algae. Allelopathic compounds have already been isolated from *Chara globularis* by Anthoni et al. (1979, 1980) and Wium-Andersen et al. (1982), but evidence has never been found that these compounds are also released by intact *Chara* plants. In the present study, bioactivity of intact material was shown by means of plate diffusion assays for the first time.

Attempts were made to obtain axenic material derived from surface – sterilized oospores, but only a few specimens sprouted. Therefore non-axenic stoneworts from the field were used after careful rinsing. According to investigations by Keating (1978) the presence of bacteria does not qualitatively influence the results of screening experiments. Microscopical controls proved that only small quantities of epiphytes occurred on the stoneworts shoots. Therefore it is unlikely that the strong bioactivity was caused by bacteria or epiphytes.

In the previous studies it has been reported that extracts of *Chara globularis* inhibit photosynthesis of *Nitzschia palea* (Anthoni et al., 1979) and damaged cells of *Chara tomentosa* decrease biomass of *Scenedesmus* spp. (Kleiven & Szczepanska, 1988). In contrast to this observation, no bioactivity against eukaryotes (*Fragilaria* sp., *Scenedesmus armatus, S. acutus*) could be detected in our bioassays. Additionally, the low epiphyte cover of *Chara globularis* used in screening assays consisted almost exclusively of the diatom *Nitzschia palea*. In a further test series, *Chara globularis* shoots were proven to exhibit no bioactivity against this diatom and no toxic effects were noticed from *Nitzschia palea* directed against the target strains *Anabaena cylindrica* and *A. torulosa* (data not shown).

As mentioned above, only cyanobacterial target organisms were inhibited by certain stoneworts. Cyanobacteria are known to form extensive blooms in eutrophicated waters and many toxic species represent a considerable threat to the public health in all parts of the world (Carmichael, 1992, 1997). One practical aspect of investigations dealing with allelochemicals is to find a new substance with a great growth inhibition effect against cyanobacteria, which could be used as a management tool in lake restoration. Also other macrophytes have been reported to be a source of such bioactive compounds. *Myriophyllum spicatum* contains the hydrolysable polyphenol tellimagradin II, which exhibited a strong in-

hibitory effect against various coccoid and filamentous cyanobacteria and to a less extend against chlorophytes and diatoms (GROSS et al., 1996). One of the most sensitive target strains of this study is *Anabaena torulosa*, which itself is able to produce allelochemicals directed against other cyanobacterial species (SCHAGERL, 2001).

It is noteworthy that bioactivity against cyanobacteria was very strong in *Chara aspera*, *Chara globularis*, *Nitellopsis obtusa* and *Nitella gracilis* while other species exhibited a far lower activity. The clearing specificity of the target strains is also of high interest. None of the test organisms inhibited all cyanobacteria (Tab. 3). Nine Characean populations cleared the target strains *Anabaena cylindrica* and *A. torulosa*. Growth of *Cylindrospermum* sp. was suppressed by eight of the test organisms and *Anabaenopsis elenkinii* as well as *Aphanizomenon* sp. were inhibited by six different stoneworts. Growth of *Microcystis aeruginosa* and *M. flos-aque* was inhibited by four of the Characeae tested, *Planktothrix rubescens* once and *P. agardhii* never. This specificity may be caused by different reasons. One explanation might be that the test organisms produce more than one allelopathic active substance, pointed against different biochemical processes of the target organism. Specific enzymatic reactions at the cell surface of the target organism are responsible whether an allelochemical can develop its effect. Different permeability of the test organism to the active substances may also influence the specificity of the allelopathic effect caused by each *Chara* species.

As already mentioned in the results, low variation of allelopathic activities was also observed in replicates prepared for each species tested. As is generally known, the production of secondary metabolites such as allelopathic active compounds depends on physiological factors such as stage of growth and optimal light and nutrient conditions, which would need to be evaluated for each species separately. Different physiological conditions of shoots and also of different species might be the reason for the strength of bioactivities observed.

The extract bioassays showed similar results. The concentration of the methanol and the aqueous extracts (20%) of *Chara aspera*, *Chara globularis* and *Nitellopsis obtusa* was high enough to note growth inhibition of cyanobacterial target algae. The more lipophilic methanol extracts exhibited stronger clearing than the aqueous extracts derived from the same test organisms. This can be explained by a lower concentration of active substances in the aqueous extract. The residue of the aqueous extracts also caused a weak inhibition of target algae, indicating a more lipophilic behavior of the allelochemical.

The existence of allelopathic effects caused by Characeae is expected to be a light stimulated process. During light periods competition for limiting nutrients and light sources with co-occurring organisms such as cyanobacteria is enforced. Cyanobacteria are adapted to low light conditions similarly as Characeae, which are frequently known to be the deepest living benthic plants in lakes (SCHWARZ et al., 1996). Since competition for light is a major factor for benthic algae it would be advantageous for Characeae to increase the production of allelochemicals under low light conditions. This theory is corroborated by ANTHONI et al. (1979) who reported that photosynthesis of the diatom *Nitzschia palea* was completely inhibited at a concentration of 3 μM dithiane and trithane.

The major question is whether or not the allelochemicals are produced in quantities large enough to be effective and whether inhibition under test conditions means that these effects can also be observed in nature. An adequate concentration of the bioactive substances may not occur in the pelagic zone but can be present in benthic microhabitats directly at the cell surface of the producing organism. SCHAGERL et al. (2000) showed that the epiphyte biomass on young *Chara* shoots is small compared to other macrophytes such as *Myriophyllum spicatum* or *Potamogeton perfoliatus*. In addition no cyanobacteria epiphyte cover was demonstrated by HPLC-pigment-analysis. This may indicate the production of allelochemicals, which is probably responsible for the epiphytic species composition and succession.

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References

ALMQUIST, E. 1929. Upplands vegetation and flora. Acta Phytogeogr. Suecica **1**.

ANTHONI, U., CHRISTOPHERSEN, C., MADSEN, J. O., WIUM-ANDERSEN, S. & JACOBSEN, N. 1980. Biologically active sulfur compounds from the green alga *Chara globularis*. Phytochem. **19:** 694–695.

ANTHONI, U., NIELSEN, P. H., SMITH-HANSEN, L., WIUM-ANDERSEN, S. & CHRISTOPHERSEN, C. 1986. Charamin, a quaternary ammonium ion antibiotic from the green alga *Chara globularis*. J. Org. Chem. **52:** 694–695.

Balls, H., Mos, B. & Irvine, K. 1989. The loss of submerged plants with eutrophication I. Experimental design, water chemistry, aquatic plant and phytoplankton biomass in experiments carried out in ponds in the Norfolk Broadland. Freshwat. Biol. **22:** 71–87.

Bryant, J. P., Chapin, F. S. & Klein, D. R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos **40:** 357.

Carmicheal, W. W. 1992. Cyanobacteria secondary metabolites-the cyanotoxins. J. Appl. Bacteriol. **72:** 445–459.

Carmichael, W. W. 1997. The cyanotoxins. Adv. Bot. Res. **27:** 211–256.

Doan, T. N., Rickards, R. W., Rothschild, J. M. & Smith, G. D. 2000. Allelopathic actions of the alkaloid 12-epi-hapalindole E isonitrile and calothrixin A from cyanobacteria of the genera *Fischerella* and *Calothrix*. J. Appl. Phycology **12:** 409–416.

Flores, E. & Wolk, C. P. 1986. Production by filamentous, nitrogen fixing cyanobacteria of a bacteriocin and of other antibiotics that killed related strains. Arch. Microbiol. **145:** 215–219.

Forsberg, C. 1965 b. Nutritional studies of *Chara* in axenic cultures. Physiol. Plant. **18:** 275–290.

Forsberg, C., Kleiven, S. & Willen, T. 1990. Absence of allelopathic effects of *Chara* on phytoplankton in situ. Aquat. Bot. **38:** 289–294.

Gross, E., Meyer, H. & Schilling, G. 1996. Release and ecological impact of algicidal hydrolysable polyphenols in *Myriophyllum spicatum*. Phytochem. **41:** 133–138.

Gross, E., M. 1999. Allelopathic interaction, pp. 179–199. In: Inderjit Dakshini, K. M. M. & Foy, C. L. (eds), Principles and practices in plant ecology CRC Press. ISBN 0-8493-2116-6.

Gross, E. M. 2000. Seasonal and spatial dynamics of allelochemicals in the submersed macrophyte *Myriophyllum spicatum* L. Verh. Internat. Verein. Limnol. **27:** 2116–2119.

Hootsmans, M. J. M. & Blindow, I. 1994. Allelopathic limitation of algal growth by macrophytes, pp. 175–192. In: Van Viersen, W., Hootsmans, M. J. M. & Vermaat, J. E. (eds), Lake Veluwe, a macrophyte-dominated system under eutrophication stress. Geobotany 21, Kluwer Academic Publishers.

Horecká, M. 1991. The significant role of *Chara hispida* – growth in water regime of a gravel pit lake at Senec. Arch. Potistenkd. **139:** 275–278.

Keating, K. I. 1978. Blue-green algal inhibition of diatom growth: transition from mesotrophic to eutrophic community structure. Science **199:** 971–973.

Kleiven, S. & Szczezpanska, W. 1988. The effect of extracts from *Chara tomentosa* and two other aquatic macrophytes on seed germination. Aquat. Bot. **32:** 193–198.

Kleiven, S. 1991. An analysis of allelopathic effects of *Chara* on phytoplankton development. Acta Univ. Ups., Comprehensive Summaries of Uppsala Diss. from the Fac. of Sci. 313, 20 pp. Uppsala, ISBN 91-554-2725-1.

Kusel-Fetzmann, E. & Schagerl, M. 1992. Verzeichnis der Sammlung von Algen-Kulturen an der Abteilung für Hydrobotanik am Institut für Pflanzenphysiologie der Universität Wien. Phyton (Horn, Austria) **32:** 209–234.

Molisch, H. 1937. Der Einfluß einer Pflanze auf die andere. Allelopathie. Jena, Fischer.

Philips, G. L., Eminson, D. & Moss, B. 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquat. Bot. **4:** 103–126.

Planas, D., Sarhan, F., Dube, L., Godmaire, H. & Cadieux, C. 1981. Ecological significance of phenolic compounds of *Myriophyllum spicatum*. Verh. Internat. Verein. Limnol. **21:** 1492–1496.

Schagerl, M., Unterrieder, I. and Angeler, D. (2001): Allelopathic interactions among *Anabaena torulosa* (Cyanoprokaryota) and other algae isolated from lake Neusiedlersee (Austria). Algological Studies **103** (Cyanobacterial Research 2)**:** 117–130.

Schagerl, M., Berger, J., Fesl, C., Pichler, C., Riedler, P. & Zinocker, M. 2000. Interaktionen zwischen Characeae und anderen Algen – Untersuchungen zu allelopathischen Effekten. Endbericht zum Projekt 7470.

Schwarz, A. M., Hawes, I. & Howard-Williams, C. 1996. The role of photosynthesis/light relationships in determining lower depth limits of Characeae in South Island, New Zealand lakes. Freshwater Biology **35:** 69–80.

Smith, G. D. & Doan, N. T. 1999. Cyanobacterial metabolites with bioactivity against photosynthesis in cyanobacteria, algae and higher plants. J. Appl. Phycology **11:** 337–344.

Todorova, A. K. 1996. *Nostocyclamid*, ein neuartiger Naturstoff aus *Nostoc* (Cyanobacteria) mit allelopathischen Eigenschaften. Inaugural-Dissertation, Zürich.

Wetzel, R. G. 2001. Limnology, 3rd Ed. Academic Press, 1006 pp.

Wium-Andersen, S., Anthoni, U. & Houen, G. 1982. Allelopathic effects on phytoplankton by substances isolated from aquatic macrophytes (Charales). Oikos **39:** 187–90.

Wium-Andersen, S., Anthoni, U. & Houen, G. 1983. Elemental sulphur, a possible allelopathic compound from *Ceratophyllum demersum*. Phytochem. **22:** 2613.

Wium-Andersen, S. 1987. Allelopathy among aquatic plants. Arch. Hydrobiol. Beih. **27:** 167–172.

Zanefeld, J. S. 1940. The Charophyta of Malaysia and adjacent countries. Blumea **4:** 1–41.

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