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Elemental flux between algae and duckweeds (*Lemna gibba*) during competition

Sándor Szabó[1], Mihály Braun[2] and Gábor Borics[3]

With 4 figures and 1 table

Abstract: Axenic *Lemna gibba* fronds were co-cultured with five green algal, three diatom and three cyanobacterial species and were grown separately and in mixed combination on autoclaved communal wastewater. These species were obtained from a local minipond containing wastewater covered by duckweeds. The impact of selected algal species on bioproduction of *Lemna* cultures was measured. The flux of elements during competition for nutrients between algae and duckweeds was examined. When the duckweed cover was incomplete, duckweeds had no impact on the multiplication of algae. Under this condition, algae showed a much stronger competitive effect for nutrients against *Lemna*, because algal nutrient removal was much more intensive than the nutrient removal of the fronds. The elemental composition of the water was mainly determined by algae. In *Lemna*-alga cocultures, the elemental concentration of the water was lowered to below 0.01 mg/l for phosphorus and for iron due to the algal activity. They were below the minimal concentrations for growth of duckweeds. Algae increased the pH of the water to above 10, which resulted in 5.5 mg/l NH_3-*N* in the medium. The elemental concentration of the algal treated fronds decreased by 87 % for phosphorus and by 90 % for iron. Results suggested that during competition with algae, iron and phosphorus became a potential minimum factor for duckweeds.

Introduction

It has been shown that duckweeds (Lemnaceae family) may be used for removing considerable quantities of nutrients from sewage effluent. Therefore, they are used in post-treatment pond systems (Zirschky & Reed 1988,

[1] **Authors' addresses:** Department of Botany, György Bessenyei College, H 4401 Nyíregyháza, P.O. Box 155, Hungary; E-mail: SZABOS@AGY.BGYTF.HU

[2] Department of Inorganic and Analytical Chemistry, Lajos Kossuth University, H 4010 Debrecen, P.O. Box 21, Hungary; E-mail: BRAUN@TIGRIS.KLTE.HU

[3] Environmental Protection Authority for Trans Tiszanian Region, H 4025 Debrecen, Piac u. 9/B, Hungary

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ALAERTS et al. 1996). However, as a result of high organic and inorganic loading, algae may develop in the wastewater pond-system, especially when the duckweed cover is incomplete. In many cases, algal bloomed wastewater was allowed into the duckweed covered lagoons. Some of these algal species may inhibit the growth of the duckweed species. It is well known that extracts of some cyanobacteria (blue-green algae) inhibit the growth of duckweed species in laboratory conditions (CHAUHAN et al. 1992). In field conditions, the development of *Anabaena* caused the disappearance of *Lemna trisulca* (KRULL 1969). Once the duckweed cover has disappeared, algal competition which leads to the removal of nutrients, might prevent re-establishment of the system (ZIRSCHKY & REED 1988).

In a preliminary investigation, the influence of three green algal, one diatom and five blue-green algal species on the growth of *L. gibba* was investigated in various conditions (SZABÓ et al. 1998). The algal species were obtained from a local minipond containing wastewater covered by duckweeds. With incomplete (<50 %) duckweed cover in communal wastewater, seven algal species significantly lowered the growth of duckweeds. *Chlorella pyrenoidosa* was found to have the most severe influence on the bioproduction of *Lemna* cultures. The causes of inhibition by the examined algae (nutrient removal and/or allelopathy) have not been tested.

The purpose of this study was to examine the elemental dynamics between algae and duckweed when the duckweed cover is incomplete, in order to find those key factors that are responsible for the growth inhibition of *L. gibba* during competition with algae. Knowing the key factors (elements) may help us to re-establish the sytems effectively in case of algal blooms.

Materials and methods

Cultures of duckweed and algal species for experiments

The axenic *L. gibba* clone was produced according to BOWKER et al. (1980). The clone was grown on a twice diluted Hutner medium (LANDOLT & KANDELER 1987) supplemented by 200 mg/l of peptone, 200 mg/l of beef extract, 200 mg/l of yeast extract and 0.5 % glucose to ensure that microbial infection would become visible. Planktonic and epiphytic algal species were isolated from three plastic miniponds (0.4 m^2 of surface, 0.5 m depth of water) containing communal wastewater covered partially (25 % and 50 %) and completely (100 %) by duckweeds. The isolated algal species grown on Allen's agar (ALLEN 1968), were observed by light microscopy and identified according to FELFÖLDY (1972, 1985) and KRAMMER & LANGE BERTALOT (1986, 1988). The cultures were purified according to RIPPKA (1988). These axenic algal species were then grown in Allen's liquid medium for the further inoculations of experiments. Inoculation was done by adding 1 ml of algal suspension into 1 litre of medium. Incubation of

algae and duckweed cultures was done under the following conditions: 54 μmol $m^{-2} s^{-1}$ photon flux density, 16-h light/8-h dark, temperature 25 ± 1.5 °C.

Lemna-alga co-cultures study

In this study, the impact of selected algal species on *Lemna* cultures was measured. In experiment 1, the impact of 11 algal species inoculated separately on *Lemna* cultures was measured under complete (100 %) and low duckweed cover. The purpose of experiment 2 was to investigate the influence of the three dominant algal species (*Chlorella pyrenoidosa, Chlamydomonas ehrenbergii* and *Limnothrix redekei*) incubated together on duckweed cultures under low duckweed cover; furthermore, to describe the elemental dynamics between water, algae and duckweeds during algal competition. For the mixed algal incubation, the main selection criterion was that the chosen species should be dominant among the algae living in the described wastewater minipond and should have significant inhibitory impact on *Lemna* cultures.

Experiment 1

One hundred ml of communal wastewater was placed in Erlenmeyer flasks, and a further 20 ml of the same medium was put into test tubes. The chemical composition of autoclaved wastewater was as follows (values in mg/l): pH (6.75), NH_4^+-N (20.6), NO_3^--N (0.23), total P (6.94), PO_4^{3-}-P (6.91), Fe (0.266), Mn (0.049). The vessels were autoclaved (121 °C for 20 min) and 12 axenic *L. gibba* fronds were placed into each vessel. Complete (100 %) and low (<50 %) duckweed cover was prepared in the vessels as described by SZABÓ et al. (1998). The vessels were inoculated by various algal species separately (11 species). The codes of the algal species used in the experiments are: *Chlorella pyrenoidosa* (Z1), *Protococcus viridis* (Z4), *Sphaerellopsis* sp. (Z5), *Chlamydomonas ehrenbergii* (Z6), green filamentous algal species (Z13), *Achnanthes hungarica* (D1), *Nitzschia palea* (D3), *Navicula venata* (D4), *Limnothrix redekei* (K1), *Oscillatoria neglecta (laaginema neglectum)* (K2), *Lyngbya limnetica* (K5). The control cultures remained axenic and contained the same quality of wastewater. *Lemna*-alga co-cultures were grown for ten days before harvesting. Chlorophyll content of the fronds was extracted in 95 % ethanol and determined by spectrophotometry according to LICHTENTALER (1987). The total chlorophyll production of the *Lemna* cultures was the indicator of the effect of algal treatments on the growth of duckweeds. The total chlorophyll production of the cultures equals the total chlorophyll content after 10 days minus the initial chlorophyll content.

Experiment 2

One hundred ml of communal wastewater each were placed in 72 Erlenmeyer flasks which were autoclaved (121 °C for 20 min). The chemical composition of wastewater was the same as in experiment 1. One part of the flasks (18) was inoculated by *C. pyrenoidosa, C. ehrenbergii* and *L. redekei* together (algal treatment). The second part of the flasks (18) remained axenic and 12 axenic *L. gibba* fronds were placed into them (*Lemna* treatment). The third part of the flasks (18) inoculated by *C. pyrenoidosa, C.*

ehrenbergii and *L. redekei* together, and they received 12 axenic *L. gibba* fronds each (*Lemna*-alga treatment). Further non-treated flasks (18) containing 100 ml autoclaved wastewater were incubated as control flasks. The cultures were grown for ten days. At the initial, 2nd, 4th, 6th, 8th and 10th day, vessels were taken randomly in three replicates from the incubation room, then the contents of the vessels (duckweed fronds, water, sediment and algal biomass) were analysed. The fronds of the *Lemna*-treated cultures were counted, the wet and dry weight of the fronds were determined by analytical balances, and they were used for chemical analysis. In the algal treated flasks, cell numbers of the three inoculated algal species (*C. pyrenoidosa, C. ehrenbergii* and *L. redekei*) were determined by light microscopy. The water was centrifuged for ten minutes (5000 RPM). The supernatants, the sediments and the centrifuged algal pellets were used for chemical analysis (see analytical methods).

Analytical methods

In the fronds and in the algal pellets, nitrogen content was analysed using a VARIO EL elemental analyser. For the multi-elemental analysis of the frond, algal pellet and sediment, samples were digested with 5 ml 65 % (m/m) HNO_3 and 2 ml 30 % (m/m) H_2O_2 at 100 °C. The digested dried sample was dissolved with 2 ml nitric acid and 6 ml distilled water, then the concentration of the solution for P, S, Na, K, Mg, Ca, Sr, Ba, Fe, Mn, Zn, Cu, Mo and B was determined by inductively coupled plasma atomic emission spectrometry (ICP AES) using a SPEKTROFLAME instrument.

In the water, the pH and the redoxpotential were recorded. Ortho-phosphate was determined colorimetrically by the molybdate method. Nitrate was determined by nitrate sensitive electrodes using the standard addition method (SMART et al. 1983). Ammonium was determined acidimetrically after the Kjeldahl distillation. For the determination of the concentrations of P, S, Na, K, Mg, Ca, Sr, Ba, Fe, Mn, Zn, Cu, Mo and B, 80 ml of supernatant were digested by 5 ml 65 % HNO_3 and 2 ml 30 % H_2O_2 at 100 °C until the water had evaporated. The digested dried sample was dissolved in 2 ml nitric acid and 6 ml distilled water, then analysed by ICP AES.

Statistical procedures

The treatments of the experiments were repeated three times meaning that 72 flasks were used in both experiments. The SPSS/Pc + 4.0 program package (SPSS 1990) was used for statistical calculations. A t-test was used to compare the total chlorophyll production of the cultures to the controls in experiment 1 and the frond and the dry weight production of the axenic *Lemna* cultures with frond and dry weight production of algal treated *Lemna* cultures in experiment 2. Principal component analysis (PCA) was used to estimate the flux of elements during competition for nutrients between algae and duckweeds. Before PCA, data of the chemical components in the water, in the duckweed fronds and in the centrifuged algal pellet were transformed using log (x+1) transformation. PCA was based on a correlation matrix and a Varimax rotation was applied.

Results

Experiment 1

With low (<50 %) duckweed cover in communal wastewater, all of the 11 algal species except *Nitzschia palea* significantly ($P < 0.05$) lowered the chlorophyll production of duckweeds. The examined algae reduced the chlorophyll production of duckweeds by 24–73 %. Unicellular green-algal species had the most inhibitory influence on the *Lemna* cultures. With complete duckweed cover, the examined algal species did not significantly lower the chlorophyll production of the cultures.

Experiment 2

Bioproduction of duckweed and of algae

During the first 6 days of incubation, the three dominant algal species (*C. pyrenoidosa, C. ehrenbergii* and *L. redekei*) incubated together had no significant ($P > 0.05$) effect on the frond production and dry weight production of the *Lemna* cultures. However, after six days, the examined algae had a significant ($P < 0.05–0.01$) inhibitory effect on the growth of duckweeds. On the 10th day the frond and dry weight production of the duckweed cultures were lowered by 83 % and 82 % respectively (Fig. 1). Dry weight production of the cultures indicated the influence of algal species more sensitively than frond production.

The most intensive propagation of unicellular green algae took place between the 2nd and 4th days and then it slowed down. The cell number of *L. redekei* increased most intensively after 4th day (Fig. 2). The presence of *Lemna* did not significantly ($P > 0.05$) modify the propagation of algae.

Changes in elemental composition

The elemental concentration of the water was not markedly reduced by duckweed nutrient removal in flasks containing only axenic *Lemna* fronds. The change of elemental concentration was related to physico-chemical processes (precipitation, adsorption and sedimentation of P and trace metals) rather than to nutrient removal of the fronds.

In algal cultures and in *Lemna*-alga co-cultures, the elemental concentration of the water (N, P, Mg, Fe, Mn, Cu) was markedly reduced during ten days of incubation and showed a similar pattern. The sharpest drop in elemental concentration of the wastewater took place between the 2nd and the 6th day of incubation. In algal cultures and in *Lemna*-alga co-cultures, the concentration of the water was lowered to below 0.01 mg/l after 6 days for phosphorus and after 8 days for iron. The manganese concentration of the solution was lowered to below 0.001 mg/l after 6 days. Concentration of ammonium nitro-

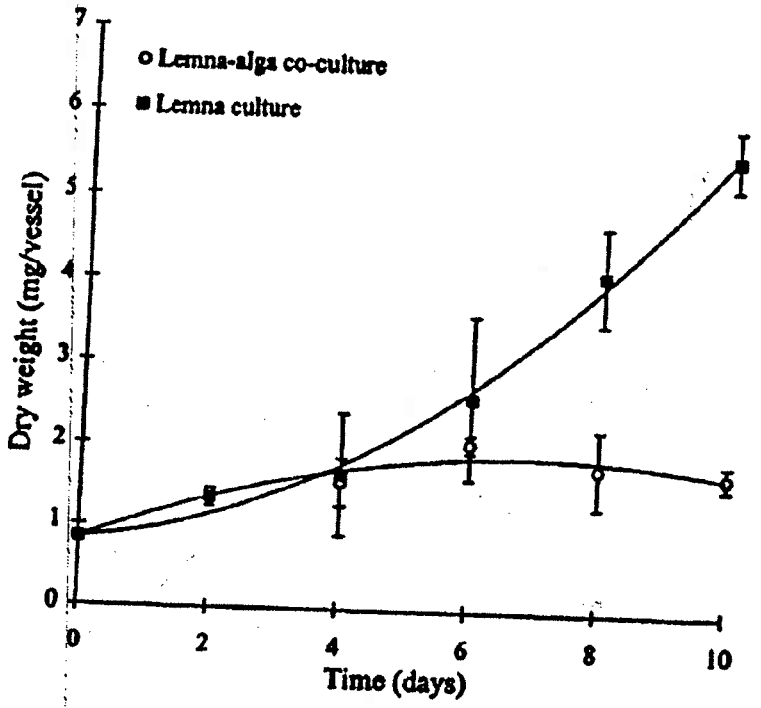

Fig. 1. Dry weight of the *Lemna* fronds in *Lemna* cultures and in *Lemna*-alga co-cultures with treatment of *C. pyrenoidosa*, *C. ehrenbergii* and *L. redekei*. Error bars indicate the standard deviations of the data.

Table 1. Elemental composition of the frond in *Lemna*-alga co-cultures and in *Lemna* cultures. Data are in mg/g dry weight ± standard deviations.

Time (days)	P	K	Fe
	Lemna-alga co-culture		
0	7.1±0.59	59± 4.9	0.8±0.07
2	6.4±0.32	28± 1.4	0.4±0.02
4	3.8±0.71	11± 2.1	0.2±0.05
10	0.9±0.07	7± 0.6	0.2±0.02
	Lemna culture		
2	6.6±0.52	33± 2.6	0.4±0.03
4	5.4±2.43	24±10.7	0.9±0.42
10	7.0±0.44	22± 1.4	1.9±0.12

gen was reduced from 20.6 mg/l to 0.3 mg/l during 10 days of algal incubation. The pH of the growth medium was increased to above 10.0 after the 4th day of the treatment. Elevated pH resulted in 5.5 mg/l free ammonia in the medium on the 4th day of incubation.

The elemental concentration of the fronds in *Lemna*-alga co-cultures decreased by 87 % for phosphorus, 90 % for iron, 70 % for potassium (Table 1)

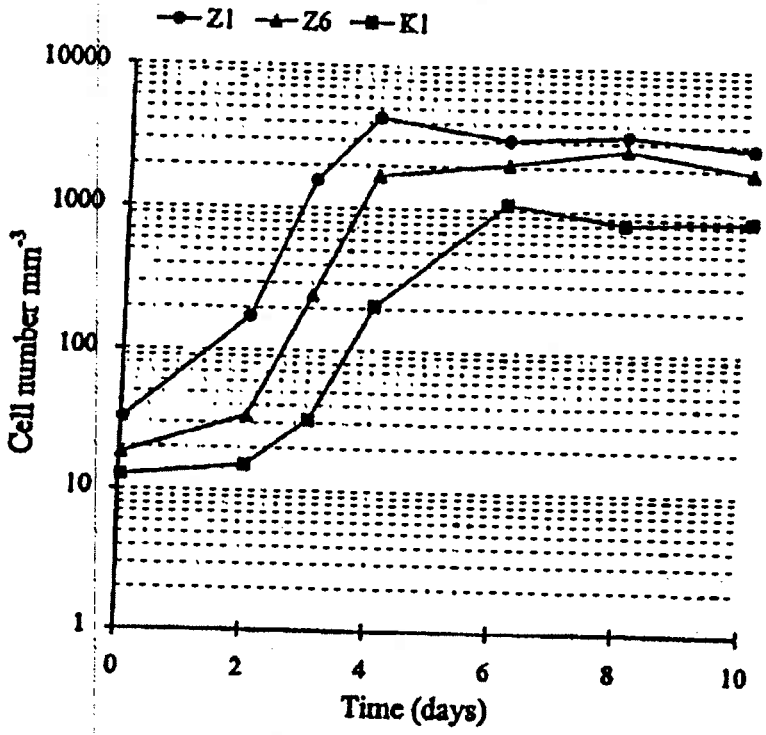

Fig. 2. Cell number of *C. pyrenoidosa* (Z1), *C. ehrenbergii* (Z6) and *L. redekei* (K1) in *Lemna*-alga co-cultures during 10 days of incubation.

and by 56 % for manganese. It increased by 193 % for calcium and 120 % for strontium on the 10th day of incubation compared with the non-treated *Lemna* cultures. The sharpest change took place on the 4th day for iron, manganese and potassium and between the 8th and 10th days for calcium and phosphorus.

Elemental dynamics

The flux of nutrients between compartments (water, algal biomass and duckweed fronds) was followed through the analysis of water, sediment, algal biomass and duckweed fronds. The relative amounts of nutrients contained in water, algal biomass, duckweed fronds and sediment were calculated for each vessel. On the initial day, the examined elements (nitrogen, phosphorus, magnesium, manganese and zinc) were mainly present in the water (>90 %). The *Lemna* fronds and the inoculated algal cells contained less than 2 %. The remaining amount of nutrients were stored in the sediment.

In *Lemna* cultures, elements were removed from the water both by uptake by the fronds and by precipitation. After 10 days of incubation, 9 % of the total amount of nitrogen, 4 % of total phosphorus, 2 % of magnesium, 3 % of copper, 2 % of zinc, 25 % of iron and 18 % of manganese were converted into

duckweed biomass. This elemental flux did not result in a major drop of elemental concentration in the water (Fig. 3).

In algal cultures and *Lemna*-alga co-cultures, the elemental dynamics were mainly related to the activity of the inoculated algae (Fig. 3). After four days of incubation, there was a rapid elemental flux from the water and from the sediment into the algal fraction. Elemental flux from the sediment into the water fraction was stimulated by the exhaustion of nutrients in the water. After 10 days, 83 % of total nitrogen, 79 % of phosphorus, 78 % of total magnesium, 61 % of iron, 63 % of manganese and 80 % of zinc had been transferred from the water and from the sediment into the algal fraction. The algal related elemental flux reduced the elemental concentrations in the water by 82–92 % for magnesium nitrogen, iron, manganese and zinc, and 99.9 % for phosphorus. The remaining elemental reduction of the medium was mainly related to the sedimentation processes.

Changes in principal components

Because of the high number of chemical components, the trends of the changes were easier to survey by the examination of the scores of principal components (PC). Principal component analysis (PCA) groups the variables based on their correlation to each other. Components changing similarly in time were loaded with large weight into the same principal component.

In the water, PCA grouped the variables into three principal components which represented 87 % of the variance. Nutritive elements (N, P), alkaline earth elements (Ca, Sr, Ba, Mg) and trace-elements (Fe, Mn, Cu) were weighted into the first PC, which included 57.2 % of the variance. In *Lemna* cultures, the first PC showed only a slight decrease. In the algal cultures and in the *Lemna*-alga co-cultures, the first PC showed a similar pattern and markedly decreased between the 2nd and the 4th day (Fig. 4a).

In the centrifuged algal pellet, the variables were grouped into two PC (91.9 % of the variance). All the examined elements but Na were weighted into the first PC, which included 83.5 % of the variance. In the algal cultures and in the *Lemna*-alga co-cultures, the first PC continuously increased during the incubation period and showed a similar pattern (Fig. 4b).

In duckweed fronds the variables were grouped into two PC (85.7 % of the variance). Wet weight, dry weight, N, Na, Ca, Mg, Sr, Ba, B, Mn and Cu were weighted into the first PC, which included 76.1 % of the variance. Both in *Lemna* cultures and in *Lemna*-alga co-cultures, the first principal component increased during the incubation but it was higher in the first case (Fig. 4c). The results indicated that *Lemna* accumulated these elements, however, the process was inhibited with the presence of algae. K, P, S and Fe were weighted into the second PC which included 9.6 % of the variance. This PC increased in

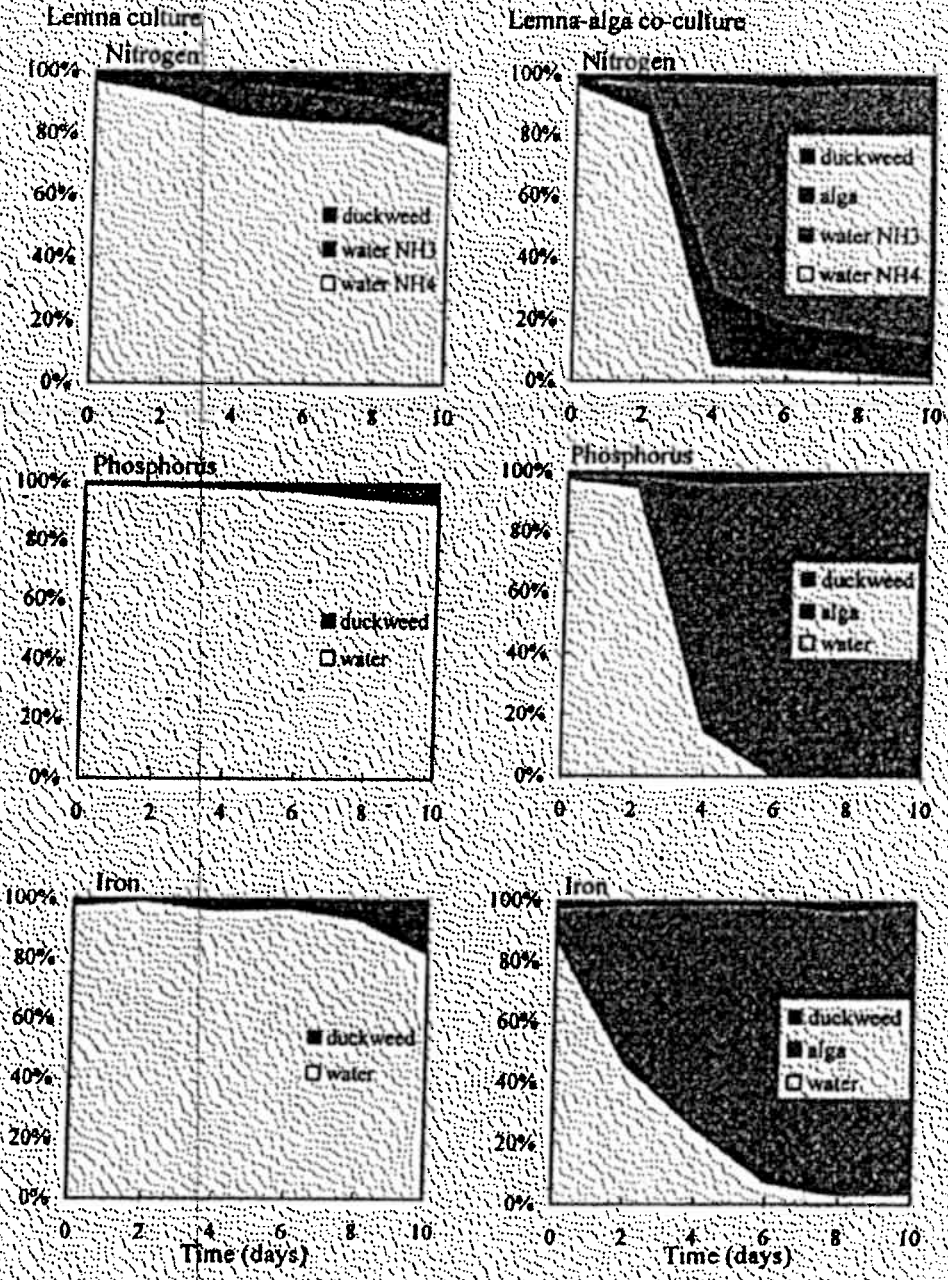

Fig. 3. Elemental distribution over *L. gibba* fronds, algae (*C. pyrenoidosa*, *C. ehrenbergii* and *L. redekei*) and water in *Lemna* cultures and in *Lemna*-alga co-cultures during competition.

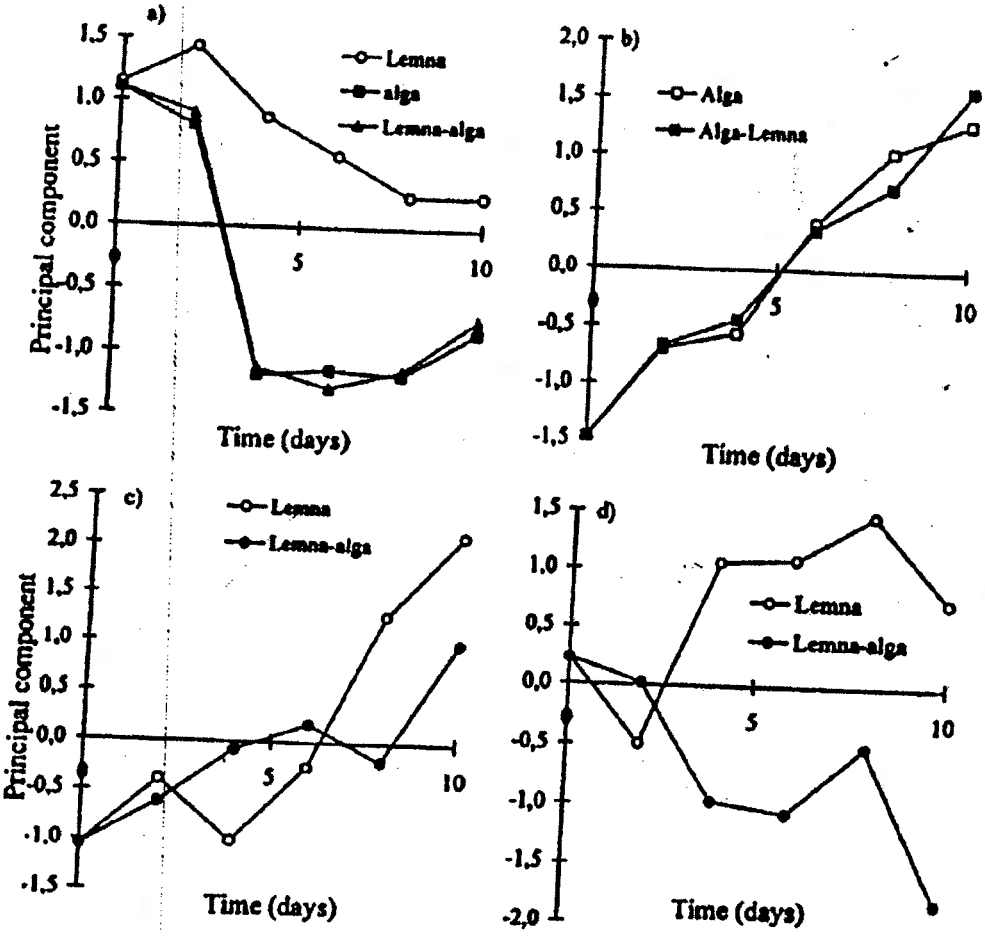

Fig. 4. Principal components of the water-alga-*Lemna* system in *Lemna* culture, in alga culture and in *Lemna*-alga co-culture: a) 1st PC in the water, b) 1st PC in algae, c) 1st PC the fronds, d) 2nd PC in the fronds. See text for the meaning of the principal components.

Lemna cultures and showed a rapid drop after two days in *Lemna*-alga co-cultures (Fig. 4 d). Among these variables, the phosphorus and iron concentrations were also reduced in the water. This fact indicated that iron and phosphorus could be potential key factors that are responsible for the inhibition of growth of *L. gibba* during competition.

Discussion

When the duckweed cover was incomplete (<50 %), the fronds of *Lemna* did not have a significant effect on the growth of the three examined algae as the algal biomass in algal cultures and in *Lemna*-alga co-cultures showed a similar

pattern. *C. pyrenoidosa* became the dominant algal species during the incubation. The increase in algal biomass especially between the 2nd and the 6th day coincided well with the exhaustion of nutrients in the water.

In the *Lemna*-alga co-culture study, algae showed a strong competitive effect against *Lemna* during competition for nutrients, because algal nutrient removal was much more intensive than the nutrient removal of the fronds. The elemental composition of the water was mainly determined by the algae.

In the mixed algal incubation of *C. pyrenoidosa, C. ehrenbergii* and *L. redekei,* the concentration of S, Na, K, Ca, Mg, Cu and Mo were sufficient for the optimal growth of duckweeds (YOSHIMURA 1943, EYSTER 1966). The concentration of ammonium was suboptimal for the duckweed after 4 days (REJMANKOVA 1981). After 6 days, the phosphorus and after 8 days, the iron were exhausted in the water and they were below the minimal concentrations needed for the growth of duckweed species (EYSTER 1966, LANDOLT 1986). The algal treatment increased the pH so that it was too high for the optimal growth of Lemnaceae (LANDOLT & WILDI 1977, LANDOLT & KANDELER 1987). As the medium became more and more alkaline, the ammonium ion deprotonated into free ammonia which is known to be toxic for duckweeds (WANG 1991). During algal incubation, elevated pH resulted 5.5 in mg/l NH_3-N in the growth solution. Consequently, algae can inhibit the growth of duckweeds directly by their alkalisation activity.

Our laboratory results suggest that the three dominant algal species inhibit the growth of duckweeds by their alkalisation activity and by the removal of iron, phosphorus and nitrogen from the medium. The elemental deficiency in the water was manifested as a drop in elemental concentration of algal treated fronds (Table 1). During algal treatment, the P concentration in the fronds was reduced from 7.1 to 0.9 mg/g. This concentration was considerably below the range (5.7–26 mg/g) reported for optimally growing duckweed species (KOLES 1986, PORTIELJE & ROIJACKERS 1995, VERMAAT & HANIF 1998). As a consequence of algal growth iron and phosphorus became potential minimum factors for duckweeds. To confirm the existence of these limiting factors in *Lemna*-alga competition, further nutrient supplement experiments are required.

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