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Short communication

Influences of nine algal species isolated from duckweed-covered sewage miniponds on *Lemna gibba* L.

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Abstract

Axenic *Lemna gibba* L. (duckweed) cultures grown on autoclaved communal wastewater were inoculated by nine algal species isolated from a minipond containing wastewater covered by duckweeds. After 10 days of incubation, the total chlorophyll content of the duckweed cultures were measured as an indicator of the physiological condition of duckweeds. With low ($< 50\%$) duckweed cover, all of the nine algal species except *Nitzschia palea* and *Anacystis nidulans* significantly lowered the chlorophyll content of *Lemna* cultures. Algae reduced the chlorophyll content of duckweeds by 21–64%. With complete duckweed cover, the examined algal species did not significantly lower the chlorophyll content of the cultures. According to the result of the factorial experiment, we may state that *Chlorella pyrenoidosa*, *Chlamydomonas ehrenbergii* and *Oscillatoria redekei* reduce the chlorophyll content of *L. gibba* at low but not at high duckweed cover. However, we observed that interaction between *C. pyrenoidosa–C. ehrenbergii*, *C. pyrenoidosa–O. redekei*, *C. ehrenbergii–O. redekei* increased the chlorophyll content of *Lemna* cultures. Inhibitory influence of algal species was higher at high organic concentration of the nutrient solution. Under these condition and low duckweed cover, the presence of *C. pyrenoidosa*, *O. redekei* and *Oscillatoria pseudogeminata* was lethal to *Lemna.* © 1998 Elsevier Science B.V.

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1. Introduction

Studies have shown that duckweeds (Lemnaceae) might be a desirable plant for removing nutrients from sewage effluent (Zirschky and Reed, 1988; Ornon, 1994; Alaerts et al., 1996). However, in several situations, high organic and inorganic loading cause microalgae to develop intensively and bloom in the wastewater pond-system, in cases of low duckweed cover. Some of these algal species have inhibitory influence on the growth of the duckweed species. Extracts of some cyanobacteria are known to inhibit growth of *Lemna* species (Entzeroth et al., 1985), for example cyanobacterin which is released by *Scytonema hofmanni* (Gleason and Case, 1986). Other reports have shown that antibiotic from *Oscillatoria* sp. killed *Spirodela polyrrhiza* (Chauhan et al., 1992). Furthermore, once the duckweed cover has been lost, algal competition (nutrient removing) might prevent duckweed to be re-established (Zirschky and Reed, 1988). On the other hand, *Lemna minor* and other water plant are able to prevent the development of planktonic and epiphytic algae through shading of the water column and by the excretion of allelochemicals (Fitzgerald, 1969; Allert et al., 1985).

Thus, it appears that the interactions between Lemnaceae and algal species is complex. Little information is available about these interactions in wastewater purification pond systems. Thus, the purpose of this study is to investigate the influence of selected algal species on the growth of the duckweed *Lemna gibba* in various conditions and to identify algal species with the greatest impact on duckweed growth.

2. Materials and methods

Axenic *L. gibba* clone was used for the experiments, and was grown on a two-time diluted Hutner medium (Landolt and Kandeler, 1987). Planktonic and epiphytic algal species were isolated from three wastewater miniponds (surface area: 0.4 m^2, depth: 0.5 m) covered by duckweeds. The isolated algal species were grown on Allen's agar (Allen, 1968). For the preparation of axenic cyanobacteria species, the Allen's medium was supplemented with 50 mg l^{-1} cycloheximide, for the eukaryotic algae the medium was supplemented with 50 mg l^{-1} penicillin. These axenic algal species were then grown on Allen's liquid solution for further inoculation of experiments. Incubation of algae and duckweed cultures was carried out under the following conditions: 54 μmol m^{-2} s^{-1} photon flux density, 16-h light/8-h dark, temperature 25 $\pm$ 1.5°C.

Experiments 1–4 were designed to determine the influences of nine isolated algal species on axenic duckweed cultures in various nutrient solutions and illumination conditions. The brief design of these experiments are shown in Table 1. Some 40 ml of nutrient solution was placed in Erlenmeyer dishes, and a further 20 ml of the same solution was put in test tubes. The vessels were autoclaved (121°C for 20 min) and inoculated by the isolated and classified algal species according to the experiment design detailed in Table 1. During the inoculation, 0.04 ml of algal suspension was added to the Erlenmeyer dishes and 0.02 ml algal suspension was added to the test tubes. The control cultures remained axenic and contained the same quality of nutrient solution detailed in Table 1. The vessels were incubated for 3 days then 12 axenic *L. gibba* fronds were

Table 1
Brief description of the experiments 1–4

Experiment	Nutrient solution	Inoculated algal species
1	Wastewater[a]	A, B, C, D, E, F, G, H, I separately[b]
2	Wastewater supplemented by 0, 200, 400, 800 mg l^{-1} of peptone	A, B, C, D, E, F, G, H, I together
3	Wastewater supplemented by 400 mg l^{-1} of peptone	A, B, C, D, E, F, G, H, I separately
4	Wastewater	A×C×D×E using full factorial design

[a]Chemical composition of autoclaved wastewater used in experiments was in the following range (data are in mg l^{-1}). COD (320–480), NH_4–N (32.5–65.0), NO_3–N (0.08–1.96), Organic N (2.7–7.8), P (9.3–11.2). Wastewater used for the experiments was diluted twice with distilled water.
[b]Codes of the algal species: *C. pyrenoidosa* (A), *Sphaerellopsis* sp. (B), *C. ehrenbergii* (C), *N. palea* (D) *O. redekei* (E) *O. neglecta* (F), *O. pseudogeminata* (G), *Lyngbya limnetica* (H), *A. nidulans* (I).

placed into each vessel. Following this, *Lemna* cultures were grown for 10 days before harvesting. Chlorophyll was extracted in 95% ethanol and the content determined by spectrophotometry according to Lichtentaler (1987). The total chlorophyll content of the duckweed cultures was the indicator of the influence of algal treatments on *L. gibba*. The cell number of unicellular algae was also measured at the end of the treatments by light microscopy using Burker chamber in experiments 1, 2 and 3.

In the case of complete (100%) duckweed cover, test tubes (25 ml, 15 mm in diameter) were used which contained 20 ml of nutrient solution. The sides of the vessels were covered and only the surface of water and duckweed fronds received maximal light intensity. Initial fronds of duckweed resulted in an 80–90% cover of the surface. The value of duckweed cover reached 100% on the 3rd day of incubation. In the case of low ($< 50\%$) duckweed cover, Erlenmeyer dishes (50 ml) were used which contained 40 ml of nutrient solution. The side of vessels were left uncovered to ensure duckweeds and algae received maximal light intensity. Vessels of each kind were used in all experiments.

The experiments were replicated three times. The total chlorophyll contents of the cultures were compared to the axenic cultures using the *t*-test in experiments 1–3. In experiment 4, the main effects and the interaction effects of the four biotic factors on the chlorophyll content of *L. gibba* cultures were estimated according to SPSS/PC + software (SPSS, 1990) and the significance levels of these effects were evaluated by analysis of variance (ANOVA) using the *F*-test.

3. Results and discussion

3.1. Experiment 1

With low ($< 50\%$) duckweed cover in communal wastewater, all of the nine algal species except *Nitzschia palea* and *Anacystis nidulans* significantly ($^{*}P < 0.05$) lowered the chlorophyll content of duckweeds. The examined algae reduced the chlorophyll

content of duckweeds by 21–64%. *Chlorella pyrenoidosa* had the most inhibitory influence on the *Lemna* cultures. With complete duckweed cover, the examined algal species did not significantly lower the chlorophyll content of the cultures (Table 2).

3.2. Experiment 2

With low ($< 50\%$) and complete (100%) duckweed cover, the chlorophyll content of axenic cultures of duckweed was increased by 167% and by 169%, respectively, by the addition of 800 mg l^{-1} concentration of peptone. With low ($< 50\%$) duckweed cover, the treatments of mixed algal species significantly ($^{*}P < 0.05$) lowered the chlorophyll content of duckweeds. Moreover, *L. gibba* cultures were killed by mixed algal treatments in solution, with an increase of organic loading above an addition of 200 mg l^{-1} peptone. With complete duckweed cover, the treatments of mixed algal species did not significantly ($P > 0.05$) reduce the chlorophyll content of the duckweed cultures. Among the nine inoculated species, *C. pyrenoidosa* was the dominant species during low and complete duckweed cover, therefore, interspecific competition could have occurred between the inoculated species.

3.3. Experiment 3

During higher organic loading, the inhibitory influences of examined algae were more intensive on the chlorophyll content of *Lemna* cultures especially with low cover of duckweed. Each of the nine examined algal species significantly ($^{*}P < 0.05$) lowered the chlorophyll content of duckweeds in low ($< 50\%$) duckweed cover (Table 2). The

Table 2
Influence of nine algal species on total chlorophyll content of *L. gibba* on communal wastewater (experiment 1) and on high organic loaded communal wastewater (experiment 3) with complete (100%) and with low ($< 50\%$) duckweed cover. The mean of total chlorophyll content values (CHL) were presented as total chlorophyll μg/vessel during 10 days of incubation. The initial chlorophyll content of 12 fronds was 13.2 μg

Algal species	On communal wastewater				On high organic loaded communal wastewater			
	Cover 100%		Cover $< 50\%$		Cover 100%		Cover $< 50\%$	
	CHL	% of control	CHL	% of control	CHL	% of control	CHL	% of control
Control	64.6		31.9		61.1		37.4	
C. pyrenoidosa	62.8	97	11.4	36**	53.4	87	0	0***
Sphaerellopsis sp.	55.8	86	14.5	45**	30.5	50	14.5	39**
C. ehrenbergii	47.8	74	15.6	49*	58.4	96	23.4	63**
N. palea	48.3	75	22.7	71	66.8	109	16.1	43*
O. redekei	41.3	64	16.7	52*	38.7	63*	0	0***
O. neglecta	39.3	61	20.3	64*	37.4	61	16.5	44**
O. pseudogeminata	47.0	73	19.8	62*	59.3	97	0	0***
Lyn. limnetica	42.4	66	22.5	71*	43.5	71	13.0	35**
A. nidulans	50.6	78	25.3	79	78.7	129*	12.7	34**

Significance levels according to *t*-test: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

inhibiting effects varied between 37–100%. A considerable mass of *Sphaerellopsis* sp. and *Chlamydomonas ehrenbergii* alga floated up to the surface of the nutrient solution, covering it entirely. The addition of peptone also caused more intensive cyanobacteria propagation. Biofouling was formed on the side of vessels at the beginning of the treatment, following which two species (*Oscillatoria redekei*, *Oscillatoria pseudogeminata*) wove into the fronds of duckweeds. With high organic loading and low duckweed cover, the following algal species examined caused the decay of *Lemna* cultures: *C. pyrenoidosa*, *O. redekei*, *O. pseudogeminata*. The cause of the stronger inhibiting influence of algae is that their higher density resulted in more intensive nutrient removal from the medium, so the concentration of certain elements were below a critical minimum value for the duckweeds. Another cause of higher inhibiting influences of algae was probably that they excreted metabolites causing autoinhibition, which probably also inhibited the growth of *Lemna* as well (Allert et al., 1985). No such intensive inhibitory effects were observed in the case of complete duckweed cover when the algae were supplied by low light intensity. Only *O. redekei* significantly decreased the chlorophyll content of *Lemna* cultures. After the treatment with the *A. nidulans*, the chlorophyll content of *Lemna* cultures was significantly increased ($^{*}P < 0.05$).

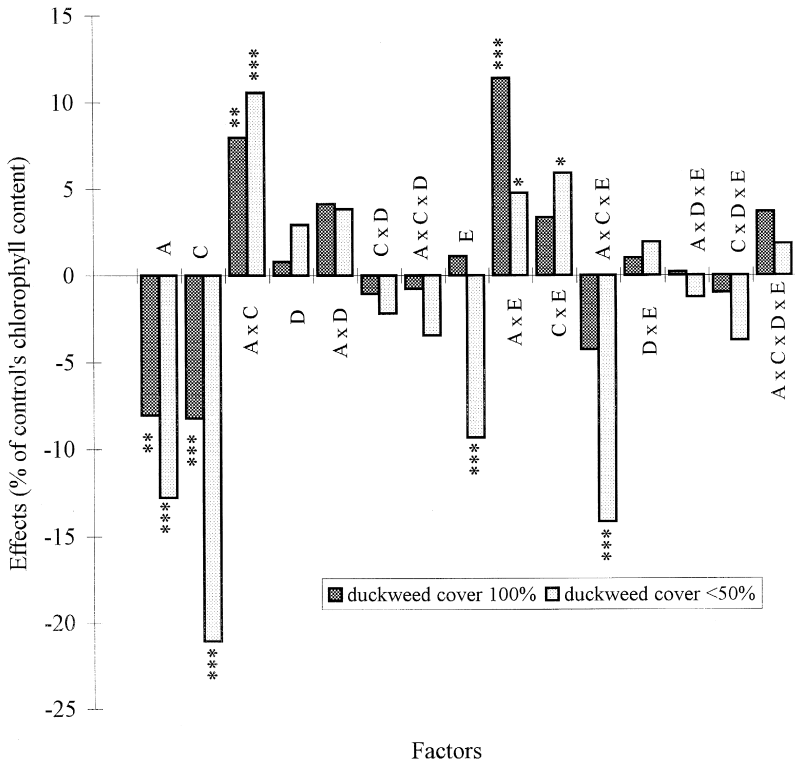

Fig. 1. Influence of four algal species on the chlorophyll content of *L. gibba* in communal wastewater according to full factorial design. The effects of factors and their interactions were evaluated as a % of the control cultures' total chlorophyll content. Codes of algae: *C. pyrenoidosa* (A), *C. ehrenbergii* (C), *N. palea* (D), *O. redekei* (E) Significance levels: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3.4. Experiment 4

This experiment was designed to determine the influences of algal species in different combinations on duckweed cultures. There is a significant ($P < 0.001$) difference among the treatments with low (< 50%) and complete (100%) duckweed cover. Under 50% cover of duckweed, *C. pyrenoidosa* (A), *C. ehrenbergii* (C) and *O. redekei* (E) reduced the chlorophyll content of *Lemna* cultures significantly ($^{***}P < 0.001$). *N. palea* (D) did not have significant ($P > 0.05$) influence on the chlorophyll content of *Lemna* cultures. With low (< 50%) duckweed cover, among the 12 possible interactions only four interaction of factors had a significant effect on the chlorophyll content of *Lemna* cultures (Fig. 1). With complete (100%) cover of duckweed, *C. pyrenoidosa* (A) and *C. ehrenbergii* (C) significantly ($^{**}P < 0.01$) lowered the chlorophyll content of duckweeds. With complete (100%) duckweed cover, among the 12 possible interactions only two interactions of factors had significant effect on the chlorophyll content of *Lemna* cultures. With the exception of interaction A × C × E with low duckweed cover, the significant interactions had a positive effect on the chlorophyll content of *L. gibba* with low and complete duckweed cover. The cause of these promoting interaction effects of algae is probably that there is an interspecific competition between the examined algal species. They probably reduce the density of each other, therefore, the interaction effects between the noted algal species increased the chlorophyll content of *Lemna* cultures. The inhibitory influence of algae were more intensive with low (< 50%) than with complete (100%) duckweed cover (Fig. 1).

4. Conclusions

On the basis of observed effects of algae on *L. gibba*, we may state that inhibitory effects of isolated algae are more intensive in conditions of low cover than of complete duckweed cover especially during higher organic loading. The result of our laboratory experiments corresponds to field studies by Zirschky and Reed (1988). In their study, decline of duckweeds occurred frequently in the case of high organic loading of wastewater. To make use of our laboratory results in wastewater pond systems covered by duckweeds, we suggest care be taken during the harvesting of the duckweed mat. The cover of duckweeds must not be lowered below 50%, otherwise, the decline of duckweeds could take place more frequently. Furthermore, we propose that wastewater be not highly organic-loaded during the settlement of the duckweed mat into a new wastewater pond system.

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