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INFLUENCE OF BARLEY STRAW AND SUBMERGED MACROPHYTES ON FISHPOND WASTEWATER QUALITY

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ABSTRACT

Barley straw has received, recently, considerable attention as an algicide, which could be used to control algal growth in fishponds. It is regarded as cost-effective, user friendly and environmentally sounds. On the other hand, the submerged wetland macrophytes are crucial for the stabilization of clear water state in shallow lakes, by their active production of anti-algal compounds (allelochemicals) through allelopathy. Microcosm experiments using barley straw and the submerged macrophytes: *Najas marina* L., *Potamogeton pectinatus* L. and *Ceratophyllum demersum* L. in culture media with fishpond water were carried out outdoors to simulate the situation as natural as possible. Nutrients concentrations were measured beside the analysis of phytoplankton and zooplankton communities were analyzed during nineteen days incubation period. Nitrogen and phosphorus elements were determined in the tissues of used aquatic macrophytes at the beginning and by the end of the experiment. Multivariate statistical assessment using matrix correlation and principal components analysis was applied for interpretation of the experimental data. Principal component PC_1 in barley straw medium accounted for about 47% of the total variance with strong correlation between selected parameters and a positive coefficient (r = 0.94 and 0.84) for diatoms – PO_4 and diatoms – SiO_4 respectively. On the other hand, PC_1 in the status of *N. marina* and *C. demersum* contributed to 46% and 58% of the total variance, respectively with high positive loading between diatoms and green algae, while in case of *P. pectinatus* PC_1 accounted for 62% of the variance with positive loading for chlorophyll – *a*, green algae and rotifers. Thus, rotted barley straw stimulated the growth and dominance of diatoms, rather than the green algae, throughout the incubation period. In the mean time, the macrophytes had allelopathic impact on the diatoms community, particularly *Najas*, while stimulated growth of the green algae. Zooplankton community was dominated by rotifers in all macrophytes media, while protozoan dominated barley straw medium. Complete depletion of nutrients was detected after few days in all media, while oxygen sustained acceptable levels. Phytoplankton assemblages shifting from green algae dominance to diatoms in barley straw medium is recommended as an invention to provide an integrated bioremediative product with ready-to-use. Active biological compounds (algicides) derived from barley straw were more reliable than allelochemicals from aquatic macrophytes. Through their beneficial effects they would render aquatic environments such as, ponds, lagoons, aquaria, aquaculture systems, wastewater treatment, holding or conveying systems more aesthetically pleasing, efficient in aquatic animal production, and less susceptible to algae and other undesirable aquatic plants. They would thus require less maintenance. It is a tentative suggestion to use barley straw for field manipulation experiments.

1. INTRODUCTION

Algal growth during summer seasons in farm ponds can pause a number of problems and the control of algae with mechanical or chemical means can be costly and ineffective. Generally, the warmer the climate, the worse the ponds quality problems and the more efforts needed for algae control.

Managing pond conditions to prevent algal blooms and resultant fish kills is a challenge for all pond owners, during the summer months when blooms most frequently occur. Most algal blooms lead to an oxygen reduction within pond

The use of barley straw to control algae is a cost-effective, user-friendly and environmentally sounds (Boylan and Morris, 2003). The anti-algal activity is only produced when straw is rotting in a well-oxygenated environment. The decomposition of barley straw in water produces and releases many chemical compounds one of which may control algal populations. Twelve simple free phenolic acids were identified in straw from barley, (Kil Ung Kim, 1993)

When straw decomposes (rots) under aerobic conditions, phenolic compounds, such as lignins and specially oxidized phenolics, are slowly leached into the surrounding water (Everall and Lees, 1997). The chemical compounds do not eliminate existing algal cells, but interferes with and prevent the growth of new cells. Barett *et al*., (1999) mentioned that, populations of cyanobacteria, diatoms and unicellular green algae in a potable supply reservoir have been suppressed by repeated treatments with barley straw. The decomposition products, released when barley straw rots, are transformed into fulvic and humic acids, which then form hydrogen peroxide (Everall & Lees, 1997), that demonstrated algae growth inhibition (CEH, 2004).

On the other hand, submerged macrophytes are crucial for the stabilization of the clear water state in shallow, mesotrophic and eutrophic lakes. Lake restoration research has demonstrated that in many cases it is possible to change a stable situation with turbid water and dominance by phytoplankton into an alternative stable state with clear water and dominance by macrophytes (Hansson *et al*., 1998).

Allelopathy is one of the 'buffer' factors that stabilizes a planted system and keeps its water clear of algae. Field evidence and laboratory studies indicated that, aquatic angiosperms are capable of producing and releasing allelopathically active compounds (Gross, 2003), affecting phytoplankton and periphyton (Jasser, 1995) and perhaps also higher trophic levels (Burks *et al*., 2000). The impact of different submerged macrophytes or their extracts on natural phytoplankton assemblages was also studied, under experimental conditions by Jasser (1995).

Frequently, the impact of surplus or limiting nutrients has been shown to affect the overall production of allelochemicals, by macrophytes, and their effect on target species. However, the net effect of the submerged vegetations on water column nutrients, algae and bacterioplankton are still not well understood (Rooney and Kalff, 2003). Macrophytes are part of the food web and compete for nutrients and other resources with phytoplankton and periphyton (Van Donk, 1998).

Outdoor microcosm experiment was established to evaluate the impact of barley straw and aquatic submerged macrophytes on fishpond wastewater quality. The relationship between macrophytes, phytoplankton, chlorophyll-*a* and zooplankton, their effects on water column nutrient budgets, the contribution of compounds produced by straw and plants, to change in nutrient budget, phytoplankton and zooplankton community structures were studied.

2. MATERIALS AND METHODS

2.1. Experimental design

Wastewater derived from a fishpond during autumn was used for the experiment. About 20 liters were used for each of the duplicate glass basins. Chopped pieces of barley straw, weighing 25g/20 L medium, following Newman (1999) recommended dosage, were packed loosely in netting, best configured as long tube, which was kept near the water surface of the glass aquarium, usually by attaching float to the netting.

Another three (replicates) glass aquaria were provided each with about 25g/20 L medium of the submerged macrophytes *Najas marina, Potamogeton pectinatus* and *Ceratophyllum demersum.*

The control medium contained fishpond wastewater without any treatment.

Water samples from each aquarium were taken every 2-3 days intervals to measure the dissolved inorganic nutrient salts (nitrite, nitrate, ammonia, phosphate and silicate) and chlorophyll-*a* following Strickland and Parsons (1972), using a Shimadzu double beam spectrophotometer UV-150-02. The pH value was measured using a pocket pH meter (model 201/digital pH meter). Dissolved oxygen was estimated according to the Winkler method (Strickland and Parson, 1972).

Phytoplankton and zooplankton identifications and counts were also estimated every 2-3 days intervals. Phytoplankton samples were examined by placing a known volume of well-mixed sample into a settling chamber for 24 hours. Algal cells were counted at 400x magnification using a Zeiss inverted compound microscope. Zooplankton count was determined by counting 5-ml sub-samples in a Bogorov tray at 25x magnification using a Leica stereomicroscope.

The macrophytes initial nutrient contents (nitrogen and phosphorus) were determined at the beginning and by the end of the experiment. Ten random entire plant samples were dried at 80°C for 48 hours, then were macerated and three random sub-samples were analysed for nutrient concentration. Plant nitrogen (N) concentration was determined by the Kjeldahl method (APHA, 1992), while phosphorus (P) concentration was determined according to Murphy & Riley (1962).

2.2. Statistical analysis

2.2.1. Multivariate Statistical Assessment

Multivariate approaches (Matrix correlation and principal components analysis PCA) were applied for interpretation of the data in experimental studies (Liu *et al.,* 2003). The STATGRAPH plus 4.0 software package was employed for data treatment. The principle components score plot can not only interpret the variation by clustering the samples, but can also describe their different characteristics and help to find out the relationship between different variables by the parameter lines (Liu *et al*., 2003).

3. RESULTS

3.1. Nutrient salts

The results indicate that at the beginning of the experiments, high values of nitrates, ammonia, silicates as well as reactive phosphates and lower values of nitrites and chlorophyll-*a* were measured in the raw fishpond water media. Air temperature did not exceed 20°C ± 1. After 4 days incubation period with straw and the macrophytes, NO_2-N, NO_3-N, NH_4-N and PO_4-P were highly reduced from all media, while SiO_4 increased considerably in barley straw medium. Thereafter, such nutrients remained at constant low concentrations till the end of the experiment.

Dissolved oxygen and pH remained high during the experiment in all media. Chlorophyll-*a* increased continuously in the

media with straw and the macrophytes till day 17 of the onset of the experiment after which it decreased in the final 2 days (Fig. 1).

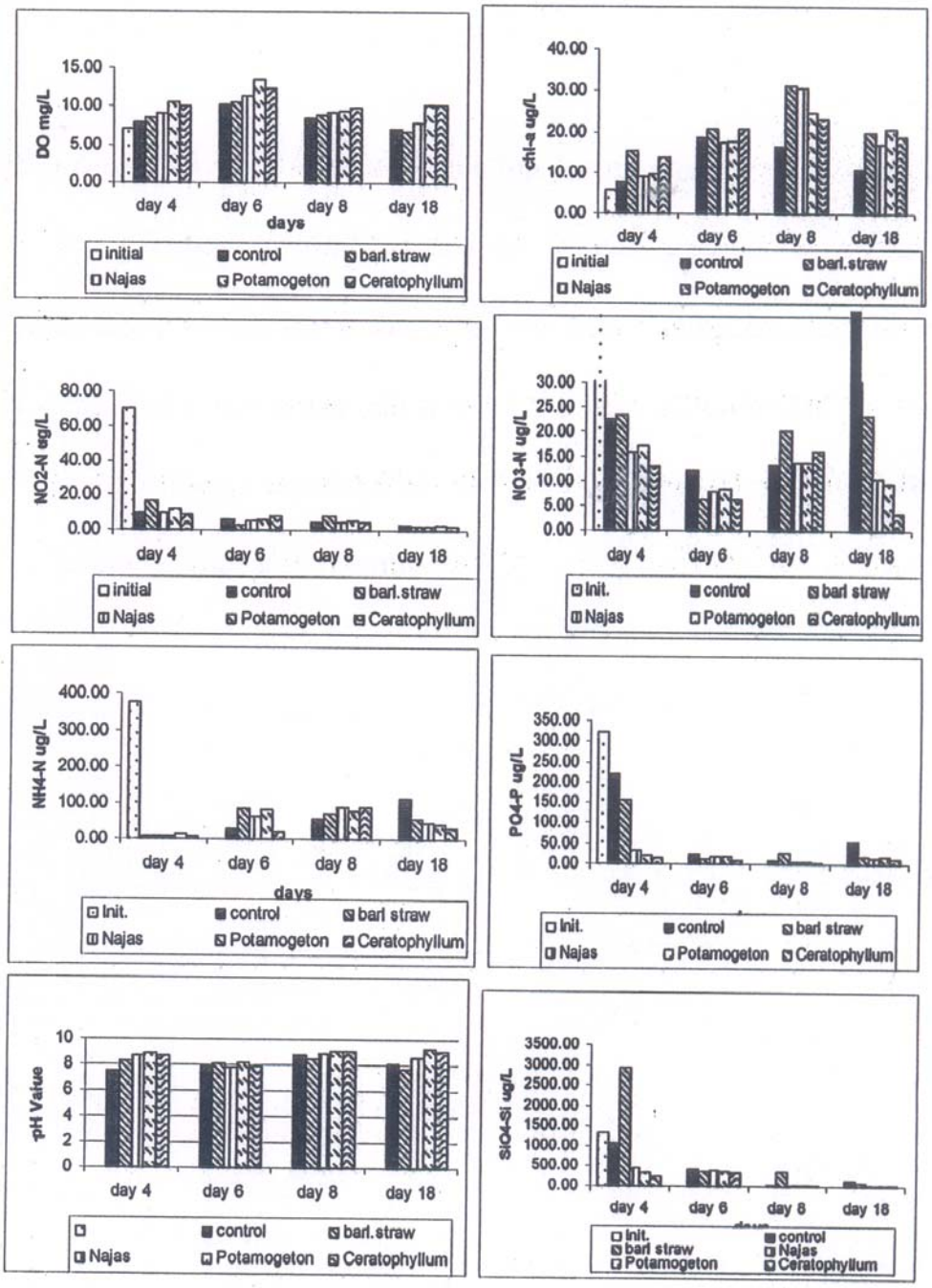


Fig. (1): Average nutrient salts concentrations, pH, chlorophyll-*a* and dissolved oxygen during 18 days outdoor incubation.

3.2. Communities in different media

3.2.1. Control medium (without treatment)

The raw fishpond water medium was rich in green algae assemblages representing about 56.5% (775,304 cells/l), while diatoms contributed about 36% (495,797 cells/l) of the total phytoplankton counts (1,371,136 cells/l). The blue- green algae and euglenoids constituted about 6% (82,080 cells/l) and 1.3% (179,55 cells/l), respectively. Diatoms were represented by five taxa, dominated by *Cyclotella*, contributed up to about 72% of the total algal counts after 6 days from the onset of the system, and dropped sharply (4.4%) at the end (Table 1). On the other hand, a considerable drop in green algae (represented by 3 taxa) was observed after 4 days incubation in microcosm system with a dominance shift from *Coelastrum* - *Scenedesmus* to *Ankistrodesmus* - *Scenedesmus*. This was followed by consequent increases of green algae counts up to 92.2% of the total phytoplankton with the dominance of *Scenedesmus* (Tables 1 & 3). The cyanophyte community recorded 82,080 cells/l at the beginning of the experiment, then fluctuations between decrease and increase occurred during the incubation period, recording 92,137 cells/l, at the end of the experiment.

Rotifers recorded a maximum of 81% of the total zooplankton community in the raw fishpond water, due to the dominance of *Brachionus calyciflorus* (95% after 4 days). The dominance shifted towards the marine species *B. plicatilis* which formed 82% of the rotifers by the end of the incubation period (Table 3). Protozoans were represented by two taxa.

3.2.2. Barley straw medium

Diatoms sustained high populations (from 100,440 to 1,406,728 cells/l) along the experiment compared with that of the control (from 22,275 to 342,720 cells/l). They were represented by 6 taxa. *Cyclotella* was the dominant genus at the beginning of the experiment, while *Navicula* dominated only after 4 days incubation and was accompanied by absolutely highest silicate records (108.12 mg/l). Green algae (2 taxa) showed decreasing affinity in the straw medium along the progressive days of the experiment, where *Scenedesmus* was dominated (Tables 1 & 5). The blue green algae disappeared during the first incubation period, but appeared again with counts ranging from 3,376 to 92,137 cells/l.

Rotifers exhibited increasing counts, after a considerable drop within the first 4 days, reaching a peak 91.5% at the end of the experiment, with the dominance of *B. calyciflorus* at the beginning, followed by *B. plicatilis*. Protozoans sometimes recorded higher counts, compared with rotifers and with those in control medium and dominated by *Heterophrys* sp. (Tables 4 & 6).

3.2.3. Potamogeton medium

The medium with *Potamogeton* supported the highest diatoms population (between 65,880 and 660,713 cells/l) , among all tested plant media. *Cyclotella* and *Navicula* dominated the diatoms community. The medium was occupied by green algae populations with percentages ranged from 10.74 to about 68% of the total phytoplankton and were dominated by *Scenedesmus* (Tables 1 & 7). Blue green algae counts ranged from 5,782 to 128,993 cells/l.

Rotifers count dropped to the minimum during the first 6 days, after which they recorded considerable increases till the end, dominated by *Monostyla closterocerca*. Protozoan (mainly ciliophores) behaved inversely, where they reach a peak during the first 6 days, then a pronounced drop occurred, till disappearance at the end (Table 3 & 8). Loss of the initial N and P contents in the tissue of macrophyte was detected by the end

of the experiment (from 17.03 to 12.69µgN/g dry weight and from 0.03 to 0.007 µg P/g dry weight).

3.2.4. Ceratophyllum medium

The diatoms counts ranged from 81,090 to 370,271 cells/l, while the green algae assemblages displayed increased tendency towards the end of the experiments with counts ranging from 14,310 to 1,560,487 cells/l. *Cyclotella*, *Navicula* and *Scenedesmus* dominated during the whole incubation period (Table 9). The blue green algae population ranged from 4,770 to 187,542 cells/l.

Zooplankton showed the same trend as that occurred in the *Potamogeton* medium. Rotifers recorded the lowest count on the 6th day, due to (*B. plicatilis*), accompanied by highest protozoan population (ciliophores) (Tables 3 & 10). *Ceratophyllum* medium lost its initial N and P in its dry tissues (from19.28 to 16.19 µg N/g dry and from 0.28 to 0.17 µg P/g dry).

3.2.5. Najas medium

This medium occupied the last order concerning diatoms counts (from 71,174 to 179,550 cells/l), over all the macrophytes media. Over the 6 taxa of diatom population *Bacillaria*, *Synedra* and *Cyclotella* dominated. In contrast, green algae displayed the highest counts reaching a peak (90.6%) at the end of the experiment, with the dominance of *Scenedesmus* (Tables 1 & 11). The blue green assemblage recorded maximum count 86,580 cells/l.

Rotifers population increased considerably along the incubation period being dominated by *B. calyciflorus* during the first period, and by *B. plicatilis* and *Lecane luna* during the last 8 days. Protozoan count was the highest after 4 days incubation mainly due to ciliophores, then dropped markedly during the rest period of incubation (Tables 3 &12). The initial N content (19.48 µg/g dry), in the macrophyte decreased into 13.64 µg/g dry weight at the end of experiment, while P content decreased from 0.01 to 0.071 µg/g dry weight.

3.3. Statistical analyses

The multivariate analysis (Principal Component Analysis PCA) was used to reduce the dimensionality of the data set from 8 original chemical variables as well as phytoplankton (diatoms and green algae) and zooplankton (rotifers and protozoans) to three new components (PCA) for barley straw and the macrophytes (*Najas*, *Potamogeton* and *Ceratophyllum* spp.) used in the experimental study. These variables were built by means of a linear combination of the original data and the eigenvalues, and could be plotted to obtain graphical pictures for straw and the three macrophytes (Fig. 2)

In barley straw medium, three significant components accounting for 100% of the variance were distinguished (Table 13 & Fig. 2). The first component (PC_1) described the general loading of the phytoplankton and zooplankton with the chemical parameters, which accounted for 47% of the variance. There was a strong correlation between the selected parameters with a positive coefficient (r = 0.94 and 0.84) for diatoms-PO_4 and diatoms- SiO_4, respectively, as internal structure. The second component (PC_2) accounted for 40% of the total variance, with a positive loading for PO_4, NO_3, diatoms, green algae, rotifers and protozoa. The third component (PC_3) accounted for 13% of the total variance including chl-*a*, NO_3 and NH_4. Chlorophyll-*a* showed positive correlation with NO_3 (r = 0.51) and negative correlation with NH_4 (r = -0.61), indicating the direct relationships of phytoplankton with NO_3 and reverse one with NH_4.

In *Najas* medium Figure (2), 84% of the data variations explained by three components. First component, accounting for 46% of the variance, reflects the relationship between NO_2, SiO_4, diatoms, rotifers, green algae and Protozoa. High positive correlation

was recorded between diatoms and green algae ($r = 0.98$ at $p<0.05$). The PC_2 accounted for 25% of the total variance, with positive loading for pH, DO, NO_3 and Green algae (Fig.2). The green algae showed positive correlation ($r = 0.64$) with both pH and DO, and negative correlation with NO_3($r = –0.54$). The third principal component for *Najas* sp. revealed positive loading (13.7%) for NO_3, NH_4, Chl-*a* and diatoms.

In *Potamogeton* sp. the three components accounted for 100% of the total variance. The loading distribution of the variables in PC_1 represent 62% of the variance, including Chl-*a*, green algae and rotifers. The second component was responsible for 25% of the data variation, with positive loading for pH and NO_3 (0.41 & 0.49), and negative loading for DO and NH_4 (–0.17 & –0.49). The third component was positive for PO_4 and green algae and negative for diatoms with both NO_3 and NH_4 (Table 13).

In the medium containing *Ceratophyllum*, three components were obtained with Eigenvalues > 1, representing 100% of the total variance. The first PC_1 accounted for 58% of the variance that was correlated with pH, NO_2, SiO_4, diatoms, green algae, rotifers and protozoans, but they have negative loading scores for NO_2, SiO_4, diatoms and protozoans. High positive correlation was reported between diatoms and green algae ($r = 0.98$ at $p < 0.05$). This might explain the influence of pH, NO_3 and SiO_4 affecting phytoplankton growth. The PC_2 for *Ceratophyllum* had relatively high and positive scores for $NO_3$and NH_4, while negative and significant correlation was recorded between them ($r = –0.82$, at $p < 0.05$). The PC_3 was loaded positively for DO and chl-*a* with negative score for PO_4 (Table 13).

Table (1): Relative abundance (percentage) of dominant phytoplankton groups in the control, barley straw, *Potamogeton*, *Ceratophyllum* and *Najas* media during the incubation period.

Incubation periods	Control		Barley straw		*P. pectinatus*		*C. demersum.*		*N. marina.*	
	Diatoms	Green algae	Diatoms	Green algae	Diatoms	Green algae	Diatoms	Green algae	Diatoms	Green algae
Day 0	36.16	56.55	36.16	56.55	36.16	56.55	36.16	56.55	36.16	56.55
Day 4	48.21	14.29	79.12	-	88.01	10.74	79.07	13.95	50.71	43.61
Day 6	71.93	23.69	71.21	23.49	71.86	16.17	61.15	33.66	63.07	29.23
Day 8	36.97	55.80	80.72	17.22	52.41	41.18	43.77	41.21	41.42	52.35
Day11	23.97	72.45	85.29	11.56	23.67	67.89	42.29	36.29	7.92	84.52
Day 14	13.54	79.90	77.62	14.50	39.87	47.38	31.07	55.64	13.58	75.19
Day 18	4.42	92.22	60.23	35.45	21.82	67.72	13.87	83.54	4.78	90.62

Table (2): Relative abundance (percentages) of phytoplankton species to their classes in the control medium during the incubation period.

Incubation period	*Cyclotella*	*Navicula*	*Bacillaria*	*Nitzschia*	*Synedra*	*Cymbella*	*Scenedesmus*	*Ankistrodesmus*	*Coelastrum*
Day 0	81.9	7.4	0.6	5.8		0.6	28.6	2.0	44.5
Day 4	26.5	18.5		22.2		7.4	33.3	50.0	
Day 6	89.0			3.7		1.2	59.3	22.2	
Day 8	62.2	3.4		10.1		9.2	52.3	6.1	26.7
Day 11	88.5	4.5		3.7		2.5	79.2	0.8	6.6
Day 14	80.0	2.0		6.0		6.0	59.2	1.4	5.6
Day 18	40.0			40.0		20.0	91.1	1.7	3.8

Table (3): Relative abundance (percentage) of dominant zooplankton groups in the media of control, barley straw, *P. pectinatus*, *C. demersum* and *N. Armatas*, during the incubation period.

Incubation period	Control		Barley straw		*P. pectinatus*		*C. demersum*		*N. Armata*	
	Rotifera	Protozoa	Rotifera	Protozoa	Rotifera	Protozoa	Rotifera	Protozoa	Rotifera	Protozoa
Day 0	81.25	16.67	81.25	16.67	81.25	16.67	81.25	16.67	81.25	16.67
Day 4	30.07	69.93	34.06	65.84	26.69	73.31	37.21	62.79	27.15	72.84
Day 6	69.09	30.91	44.09	55.91	25.71	74.08	33.96	66.04	52.37	46.89
Day 8	77.14	22.86	24.24	75.76	97.10	2.90	68.29	31.71	93.33	5.83
Day 11	51.78	18.05	46.67	53.33	89.61	3.90	79.24	20.34	82.43	9.46
Day 14	46.51	53.49	57.78	42.22	95.56	2.22	65.22	34.78	96.30	------
Day 18	74.44	25.56	91.53	6.78	83.33	------	98.19	8.11	92.19	1.56

Table (4): Relative abundance (percentages) of zooplankton species to their classes in the control medium during the incubation periods.

Incubation period	Rotifers					Protozoa	
	Brachionus calyciflorus	*B. plicatilis*	*Lepadella patella*	*Monostyla closterocerca*	*Lecane luna*	*Heterophrys* sp.	*Euplotes patella*
Day 0	92	2	0	0	0	0	100
Day 4	95	1	0	0	0	6	92
Day 6	92	3	5	0	0	82	18
Day 8	44	7	37	0	0	100	0
Day11	50	23	2	0	0	3	97
Day 14	5	60	35	0	0	83	17
Day 18	0	82	15	3	0	100	0

Table (5): Relative abundance (percentages) of phytoplankton species to their classes in barley straw medium during the incubation periods.

Incubation period	*Cyclotella*	*Navicula*	*Bacillaria*	*Nizschia*	*Synedra*	*Cymbella*	*Scenedesmus*	*Ankistrodesmus*
Day 0	81.9	7.4	0.6	5.8		0.6	28.6	2.0
Day 4	30.6	43.1		9.7	1.4	5.6		
Day 6	81.9	2.1	6.4	2.7		1.6	90.3	1.0
Day 8	65.0	5.1		6.1	0.3		59.7	6.0
Day 11	61.8	2.6		2.9		13.2	62.3	3.9
Day 14	53.9	1.2		2.7		28.1	89.2	1.0
Day 18	41.6		0.2	16.5		30.1	90.2	1.2

Table (6): Relative abundance (percentages) of zooplankton species to their classes in barley straw medium during the incubation periods.

Incubation period	Rotifers					Protozoa	
	Brachionus calyciflorus	*B. plicatilis*	*Lepadella patella*	*Monostyla closterocerca*	*Lecane luna*	*Heterophrys* sp.	*Euplotes patella*
Day 0	92	2	0	0	0	0	100
Day 4	99	1	0	0	0	1	92
Day 6	98	1	1	0	0	93	7
Day 8	63	8	25	0	0	99	1
Day11	8	13	65	1	0	85	15
Day 14	16	35	46	0	0	68	32
Day 18	2	85	13	0	0	100	0

Table (7): Relative abundance (percentages) of phytoplankton species to their classes in *Potamogeton* medium during the incubation periods.

Incubation period	*Cyclotella*	*Navicula*	*Bacillaria*	*Nitzschia*	*Synedra*	*Cymbella*	*Scenedesmus*	*Ankistrodesmus*	*Coelastrum*
Day 0	81.9	7.4	0.6	5.8		0.6	28.6	2.0	44.5
Day 4	6.1	46.0	9.2	13.0	2.0	19.9	61.4	2.8	
Day 6	35.0	30.0	4.2	10.0	9.2	10.0	44.0	0.8	
Day 8	21.4	28.6	16.3	6.1	8.2	6.1	46.8		41.6
Day 11	37.8	37.8	1.4	4.2	6.3	12.6	41.9	1.5	46.8
Day 14	43.0	26.6	10.2	4.1	4.1	2.0	72.4		1.4
Day 18	12.6	16.8	18.9	11.6	6.3	31.6	46.1	0.7	1.4

Table (8): Relative abundance (percentages) of zooplankton species to their classes in *Potamogeton* medium during the incubation periods.

Incubation period	Rotifers					Protozoa	
	Brachionus calyciflorus	*B. plicatilis*	*Lepadella patella*	*Monostyla closterocerca*	*Lecane luna*	*Heterophrys* sp.	*Euplotes patella*
Day 0	92	2	0	0	0	0	100
Day 4	99	0	0	0	0	6	87
Day 6	97	2	0	0	0	12	88
Day 8	42	4	22	3	0	100	0
Day11	14	9	25	16	1	17	83
Day 14	14	12	14	30	0	100	0
Day 18	0	0	0	100	0	0	0

Table (9): Relative abundance (percentages) of phytoplankton species to their classes in *Ceratophyllum* medium during the incubation periods.

Incubation period	*Cyclotella*	*Navicula*		*Nitzscha*	*Synedra*	*Cymbella*	*Scenedesmus*	*Ankistrodesmus*	*Coelastrum*
Day 0	81.9	7.4		5.8		0.6	28.6	2.0	44.5
Day 4	26.5	29.4	*Bacillaria*	29.4		2.9	33.3	16.7	28.6
Day 6	20.8	60.4	0.6	9.4	4.7	3.8	61.7	1.3	
Day 8	53.6	17.4		17.4	2.9	1.4	66.1	3.1	
Day 11	26.8	35.4		25.2	3.4	5.0	86.0	2.0	0.3
Day 14	18.3	52.9	2.3	6.9	2.3	11.5	71.8	2.6	20.5
Day 18	15.5	42.7	0.4	25.2		15.5	45.0	0.2	27.0

Table (10): Relative abundance (percentages) of zooplankton species to their classes in *Ceratophyllum* medium during the incubation periods.

Incubation period	Rotifers					Protozoa	
	Brachionus calyciflorus	*B. plicatilis*	*Lepadella patella*	*Monostyla closterocerca*	*Lecane luna*	*Heterophrys* sp.	*Euplotes patella*
Day 0	92	2	0	0	0	0	100
Day 4	94	1	0	1	0	0	99
Day 6	81	11	6	0	0	43	57
Day 8	32	4	25	4	0	77	23
Day11	5	19	59	3	0	94	0
Day 14	7	93	0	0	0	100	0
Day 18	3	61	18	15	3	67	33

Table (11): Relative abundance (percentages) of phytoplankton species to their classes in *Najas* medium during the incubation periods.

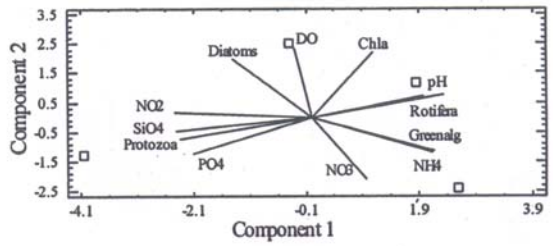
Incubation period	*Cyclotella*	*Navicula*	*Bacillaria*	*Nitzschia*	*Synedra*	*Cymbella*	*Scenedesmus*	*Ankistrodesmus*	*Coelastrum*
Day 0	81.9	7.4	0.6	5.8		0.6	28.6	2.0	44.5
Day 4	18.7	9.3	25.2	15.0	8.4	16.8	26.1	1.1	
Day 6	24.4	14.6	2.4	4.9	29.3		42.1		
Day 8	19.2	6.8	24.8	9.0	23.7	6.8	82.1	2.7	
Day 11	3.6	14.4	25.2	18.0	18.0	14.4	69.6	1.0	2.7
Day 14	39.2	11.1	2.1	2.8	19.6	16.8	74.7	0.5	16.2
Day 18		15.4	15.4	2.6	30.9	3.9	62	4.9	26.1

Table (12): Relative abundance (percentages) of zooplankton species to their classes in *Najas* medium during the incubation periods.

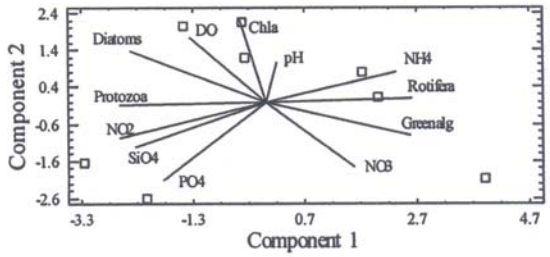
Incubation period	Rotifers					Protozoa	
	Brachionus calyciflorus	*B. plicatilis*	*Lepadella patella*	*Monostyla closterocerca*	*Lecane luna*	*Heterophrys* sp.	*Euplotes patella*
Day 0	92	2	0	0	0	0	95
Day 4	95	2	0	0	0	0	80
Day 6	90	3	7	0	0	20	0
Day 8	31	6	19	4	1	100	0
Day11	29	23	3	15	7	29	0
Day 14	19	25	10	17	13	0	0
Day 18	3	5	14	22	34	0	0

Table (13): Principal component analysis showing component loading of chemical parameters, phytoplankton and zooplankton (I: control, II:straw, III:Najas sp., IV:*Potamogeton* sp.and V: *Ceratophylum* sp.)

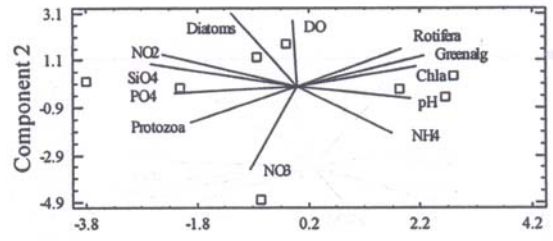
	Component-1					Component-2					Component-3				
Macrophytes / Parameters	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Eigenvalue	7.31	5.13	5.53	7.46	6.97	4.13	4.42	2.99	3.02	2.86	-	1.43	1.65	1.52	2.17
Variance %	60.91	46.66	46.05	62.20	58.05	34.41	40.30	24.94	25.12	23.83	-	13.04	13.71	12.68	18.12
PH	0.29	0.35	-0.08	0.26	0.30	0.14	-0.27	0.31	0.41	0.12	-	0.19	0.22	0.09	-0.39
DO	-0.05	0.10	0.05	-0.21	-0.21	0.47	-0.37	0.47	-0.45	-0.17	-	-0.50	-0.28	0.17	0.53
NO2-N	-0.37	0.13	0.39	-0.31	-0.37	0.04	0.10	0.16	0.27	0.09	-	0.04	0.06	-0.20	-0.05
NO3-N	0.15	-0.31	0.24	-0.12	-0.04	-0.42	0.30	0.48	0.49	0.53	-	0.59	0.45	-0.34	-0.28
NH4-N	0.32	0.38	-0.26	0.14	0.23	-0.23	-0.34	-0.37	-0.48	0.45	-	-0.44	0.33	-0.34	0.15
PO4-P	-0.32	0.39	0.04	-0.22	-0.13	-0.25	0.33	0.23	0.11	-0.46	-	-0.12	0.07	0.63	-0.36
SiO4-Si	-0.36	-0.06	0.36	-0.36	-0.36	-0.09	0.20	0.15	-0.10	-0.08	-	-0.21	0.07	0.02	0.17
Chl-*a*	0.16	0.38	-0.16	0.32	0.17	0.44	-0.36	-0.20	-0.22	0.29	-	0.53	0.65	-0.22	0.50
Diatoms	-0.21	0.43	0.32	-0.33	-0.35	0.39	0.34	-0.33	0.05	0.19	-	0.08	0.36	-0.34	-0.17
Greenalgae	0.32	-0.34	0.48	0.34	0.32	-0.24	0.45	0.40	0.04	-0.29	-	-0.12	-0.19	0.32	0.17
Rotifera	0.35	0.38	-0.36	0.36	0.37	0.15	0.31	0.15	0.10	-0.02	-	0.03	0.14	-0.14	-0.04
Protozoa	-0.35	-0.32	0.36	-0.36	-0.37	-0.15	0.36	-0.18	-0.10	0.12	-	0.07	-0.11	0.01	0.04



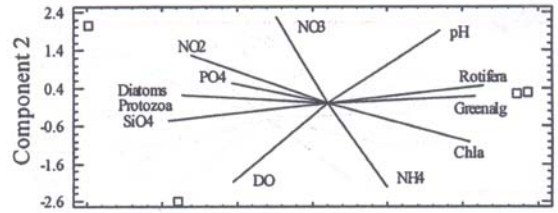
Control medium



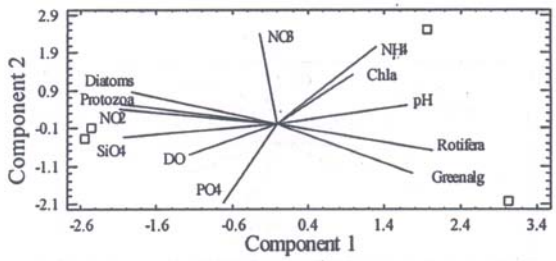
Barley Straw medium



Najas marina medium



Potamogeton pectinatus medium



Ceratophyllum demersum medium

Figure (2): Principal component loading of phytoplankton, zooplankton and chemical parameters in different treated media.

4. DISCUSSION

4.1. Barley straw medium

Fishpond water, used in the present work, was rich in diatom assemblage after applying barley straw and had insignificant levels of soluble nitrite and ammonia, along the investigation period. Also, phosphorus levels were considerably reduced from the straw medium. Anhorn (2005) indicated that, decaying barley straw results into phosphorus limitation for algae, not inhibition by a chemical compound. However, Stan *et al*. (2005) pointed out that, it is still unclear whether barley straw may interact significantly with nutrients (N & P) or metals. These essential substances are made unavailable to algae and fail to restore their growth.

Although inorganic nitrogen forms and phosphorus concentrations were reduced from the straw medium there was still nitrogen and phosphorus to support the growth of diatoms and green algae. This may be attributed to that dried straw released N and P into the water, which might promote algae growth. Center of Ecology and Hydrology (CEH, 2004) explained also that, anaerobic decomposition of straw produce chemicals which actually stimulate the growth of algae, because the algae can use them as a source of carbon.

Dissolved oxygen sustained levels ranging from 6.72 to 10.53mg/l during the investigation, lead us considering the used weight 25g chopped pieces of barley straw per 20 liters fishpond water as reliable for the application for fishpond algal treatment, at current temperature of 20°C.

Dominance shift from green algae to diatoms in barley straw medium, was supported by silicate and phosphate as shown from the high positive correlations (r = 0.94 and 0.84) for diatoms-PO_4 and diatoms- SiO_4, thus turning the medium more aesthetically pleasing and required for fish production. However, Brownlee *et al.* (2003) showed susceptibility of freshwater and brackish phytoplankton to barley straw exposure, including species – specific responses and shifts in species dominance in mixed assemblages. In the present investigation barley straw reduced the yield of *Ankistrodesmus falcatus* and inhibited *Coelastrum* sp. while no effect was observed on *Cyclotella* and *Scenedesmus*. These results were supported by Brownlee *et al.* (2003) for *Ankistrodesmus* but were in contrast to Ball *et al* (2001) for *Scenedesmus*. According to Geiger *et al*. (2005), different species of algae have been found to vary widely in their susceptibility to the effects of barley straw. On the other hand, in laboratory studies, they reported that some types of algae could be controlled effectively by barley straw extracts, but the main target, green mat-forming algae, did not seem to be inhibited. However, a natural pond needs some algae - almost nothing is more effective in reducing ammonia as the fuzzy green algae.

Generally, zooplankton grazers (ciliated-protozoan and rotifers) dominated both grazing on phytoplankton and bacteria and subsequent nutrient recycling might explain continuous increases in phytoplankton biomass.

The zooplankton community structure dominated by rotifers (*Brachionus*) and protozoan (*Heterophrys*) might be attributed to that, specific algal groups possibly could have favored the maintenance of high densities, biomass, and production of *Brachionus* and *Heterophrys* spp.

The high negative loading of green algae was met by high positive loading of rotifers in straw medium could be explained that, soluble humatic products released from fermented straw had not inhibition potential to green algae and rotifers. Generally, these results could be referred to the non-consistent degree of algal growth inhibition and the non-affected zooplankton community structure, after treatment with barley straw (CEH, 2004).

4.1.1. Potamogeton medium

All dissolved nitrogen forms and PO_4-P and SiO_4 were highly consumed from *Potamogeton* medium and accompanied by pronounced increases of chlorophyll-*a* and dissolved oxygen. Eriksson and Weisner (1997) confirmed the importance of epiphytic denitrifying bacteria on *P. pectinatus* in removing N from a shallow nutrient-enriched freshwater ecosystem. In addition, *P. pectinatus* and the attached epiphytes can act as a sink for phosphorus (Howard-Williams, 1981).

The positive loading (PC1) of chl-*a*, green algae in the present work might prove that macrophytes allelopathic impact on phytoplankton was not actively exhibited, may be because the initial biomass was so small. However, *Potamogeton* might have exerted allelopathic impact on *Ankistrodesmus* and *Cyclotella* to some extent, while favoured the growth and development of other diatom species. Kantrud (1990) suggested that *P. pectinatus* was seldom limited by nutrients, but loss of N and P contents of the plants tissues might have stimulated the growth of green algae by additional nutrients source.

On the other hand, low light, high nutrient conditions of the turbid water state (caused by green algae and diatoms) reduce defensive plant phenolics then nutrients bound in plant tissue should be released more readily, and algicidal compounds should decrease. These conditions would tend to decrease macrophyte-standing stock and potentially increase phytoplankton production, stabilizing the turbid water state (Greg and David, 2003). This was most probably the case characterizing the present fishpond water with *Potamogeton* media, particularly proved by the nutrients (N & P) decreases in the *Potamogeton* tissues by the end of the experiment.

Therefore, nutrients and organic substances are passively released in the water (Pomogyi, 1984), when living parts of the plants are damaged by means of autolysis, leaching and microbial breakdown. This is supported by Van Donk and Gulati (1995) who observed that, *Potamogeton* sp. showed a progressive coverage of epiphytes, causing *Potamogeton* to decline.

4.1.2. Ceratophyllum medium

All estimated nutrients were highly consumed from the medium with *Ceratophyllum.* Increased DO concentrations was healthy sign for fishpond water, supported by results given by Pokorný and Rejmanková (1983) who gave a net oxygen production up to 5.7 mg/l daily in dense *Ceratophyllum* stands in small fishpond.

Studies proved that, sulfur containing extracts of *C. demersum*, which have strong algicidal properties, inhibit the photosynthesis of natural phytoplankton communities (Wium Andersen, 1987). These authors mentioned also that, this macrophyte acts as an allelopathic agent which account for the low epiphytic growth on it. In the present work, the increased chl-*a* in *Ceratophyllum* medium could not indicate that allelopathy was not exhibited. This is in agreement with Körner and Nicklisch (2002) who observed that *C. demersum* inhibited the photosystem II activity of the microalgae without a parallel effect on chl-*a* concentration and that the increase in chl-*a* cannot be interpreted as growth.

High positive loading of green algae in *Ceratophyllum* medium in the present study was also reported by Jasser (1995) that, aqueous extracts of *C. demersum* caused a decline of cyanobacteria and increase of chlorophytes, in growth assays with natural phytoplankton.

Cyr and Downing (1988) demonstrated that, *Ceratophyllum demersum* in general do not support more invertebrates per unit plant biomass than broad-leaved plants, but in the present investigation this macrophyte medium supported high rotifers population. However, *Ceratophyllum* could have got colonized by epiphytes, therefore, production

of allelochemicals for attracting grazers might have occurred (Gopal and Goel, 1993).

4.1.3. Najas medium

The highest positive load (PC1) of green algae and diatoms in *Najas* media, compared with the other test macrophytes media, revealed undetectable allelopathic activity. In contrast, Gross *et al*. (2003) showed that *Najas marina* produce and release allelopathically active compounds.

However, Troeger, (1978) indicated that, *Najas* sp. in four Bryan County, Oklahoma ponds supported diverse populations of epiphytic diatoms, dominated by *Navicula* spp. and *Nitzschia* spp. In the present experiment, probably the initial biomass of *Najas* was not enough to sustain allelopathy and the phytoplankton were supported by nutrients released from the plant tissue (N) and organic detritus.

4.2. Macrophytes media in general

Submerged macrophytes are excellent choice as shade, and as "nutrient sponges" in tanks that are experiencing algae problems. They also produce allelochemicals that act directly against algae. Either way, they are useful plants in the battle against algae. However, there is a clear lack of controlled field experiments because few allelochemicals have been identified (Legrand *et al*. 2003).

Several mechanisms may contribute to the impact of submerged macrophytes on the planktonic food web. Firstly, macrophytes are themselves part of the food web, and compete for nutrients and other resources with phytoplankton and periphyton (Ozimek *et al*., 1990; Van Donk *et al*., 1993). Furthermore, the conditions inside macrophyte beds may increase denitrification (e.g. Weisner *et al*., 1994), contributing to a decreased availability of nitrogen for phytoplankton growth. Effects on phytoplankton may subsequently affect higher trophic levels—zooplankton and fish.

However, several studies showed that macrophytes do not necessarily affect nutrient availability for phytoplankton and that nutrient competition between macrophytes and phytoplankton may be relatively unimportant (Schriver *et al*. 1995, Beklioglu & Moss, 1996, Van Donk & Van de Bund, 2002).

High phytoplankton densities and thick epiphyte layers attenuate the light availability up to 90%. Counter adaptive strategies, that should exist, are fast growing shoots and the production of allelochemicals which inhibit algae and cyanobacteria, but that was not prevailed in the present investigation. The effects of allelopathic substances seem to be linked with the abundance of the organisms that secrete them. Besides, when the abundance of macrophytes is low, P and N enrichment favored chlorophytes as well as periphytic algae (Kirsi, 2005), that might be the general case prevailing in the macrophytes media in the present work.

On the one hand, aquatic macrophytes actively excrete nutrients and organic substances which are passively released in the water (Pomogyi, 1984). Nutrients are released when living parts of the plants are damaged by animals, or by means of autolysis, leaching and microbial breakdown. These excretions seemed favoring the growth of diatoms and green algae in the macrophytes media, but definitely these communities will be needed as fish food resources.

Although, zooplankton grazing in macrophyte beds may play a major role in controlling phytoplankton, other factors may often be important (Ellen and Wouter, 2002).

Schriver *et al*. (1995) and Beklioglu and Moss (1996) observed in enclosure experiments with *Potamogeton* sp. and *C. demersum* that the phosphate and ammonium concentrations were unaffected by the presence or absence of such macrophytes, but strongly and positively correlated with the grazing pressure from zooplankton. Generally, zooplankton (loaded by rotifers) characterized the media treated with the three

used macrophytes and the nutrients depleted from the media, probably exhausted by the green algae assemblages rather than by the macrophytes.

However, allelopathic effects are described as being species-specific, for e.g. *Scenedesmus* might have an allelopathic-like direct negative effect on other green algae (Florence *et al*., 2001). Thus, further research is needed to understand the nature of this process and the effect of submerged vegetation on other phytoplankton species.

Many different levels of algal monitoring and assessment exist. Metrics based on indicator taxa such as qualitative estimates of relative dominance of algal divisions. For example, dominance by diatoms might be rated "good". Therefore, the treated media, whether with macrophytes or straw where diatoms dominated for the whole experiment duration (barley straw) or even for a short period (macrophytes), might indicate good water quality, aesthetically pleasing for fishpond aquaculture.

Whether macrophytes have an impact on zooplankton via allelopathy is not very clear (Van Donk and Van de Bund, 2002). Increase in the biomass of rotifers was generally associated with an increase in chl- *a*, indicating the low ability of these specialized suspension feeders to control total phytoplankton biomass.

Rotifers dominated the different macrophytes media over ciliates. They did not prefer any specific plant species. In this respect, Arndt (1991) revealed that, ciliates should be a common part of the food of most rotifer species. Also, Gilbert and Jack (1991) found that, in the absence of edible algal food, rotifers might extensively prey ciliates in natural plankton communities.

The grazing activity of zooplankton benefits the smaller edible phytoplankton species that grow faster because of more efficient nutrient uptake. If the larger zooplankton species become scarce (for whatever reason) the phytoplankton community will change to slow-growing bigger, mainly unedible blue-green species. The small zooplankton as well as small phytoplankton species were prevailing the media with different treatments in the present investigation.

5. CONCLUSIONS

It is recommended that barley straw be best applied in the autumn, winter or very early spring when the water temperature is low. The straw will usually become active within one month and will continue to inhibit algal growth for about 6 months. At water temperatures above 20°C straw has been effective in controlling algal blooms. Avoid applying straw during prolonged periods of hot weather as the combined effect of the dying algae and the rotting straw may increase the risk of de-oxygenation.

Metrics based on indicator taxa such as qualitative estimates of relative dominance of algal divisions indicated that dominance by diatoms, as occurred in barley straw medium, might be rated "good".

Live *P. pectinatus*,*C. demersum* and *N. marina* had quite similar effects on the phytoplankton: whereas the percentage contribution of cyanobacteria to total algal biomass was low, those of green algae, especially, increased. Precautions against an algae pond should start already in autumn and after the winter evergreen underwater plants that clean the water during the whole year are an additional step to enrich the water with oxygen and to reduce string algae.

Potential products in such systems include algal protein for direct consumption by fish or zooplankton that, in turn, are important food organisms for larval and several adult fish.

We advice to follow all these natural and ecological useful precautions, before trying to get rid of green algae with chemical substances. To stop algae with a chemical clarifier should always be the last choice, after all natural and biological treatments have failed as well as all technical devices

like pond bio-filters UV-devices and pond pumps.

The balance of oxygen and water quality in aquatic ecosystems depends also a lot on the proper amount of fish and other pond biota in relationship to pond volume and water flora

Future research in analyzing the presence of allelopathic substances should focus on studies with intact macrophytes and experimental setups in which it is possible to make a clear distinction between allelopathy and other growth limiting factors.

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