

Phytotoxicological Assessment of Two Backwater Wetlands in Kannamaly, Ernakulam Using Aquatic Macrophyte - *Spirodela Polyrhiza*.

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Abstract

In the current study, the duckweed aquatic macrophyte *Spirodela polyrrhiza* was employed for assessing the toxicity of two backwater wetlands in the Kannamaly, Chellanam panchayath, Ernakulam district, Kerala, South India. The assessments were made according to OECD guidelines for testing (2006). The studies involve study of growth parameters, Growth Index, Biomass and changes in productivity. The water samples were collected from two different wetland sites at the same time. The *spirodela* plants were introduced into several dilutions of wetland water samples. The parameters were measured after 7 days of exposure. All samples except control affected all parameters. The results of this study emphasize the significance of duckweeds as standard and reliable testing material for biological parameters in polluted aquatic ecosystem.

Keywords: Growth inhibition; *Spirodela polyrrhiza*; Growth Index; Macrophyte; Duckweed

Introduction

Wetlands support a wide array of flora and fauna and deliver many ecological, climatic and societal functions. Scientists often refer to wetlands as the "kidneys" of the earth. Kerala is well known for its wetlands. The Kerala coast is bordered by 29-backwaters running parallel to the shoreline. The water quality of these backwaters is deteriorating due to population explosion, rapid industrialization, silting, tourism and agricultural activities. Effluents from industries are major cause of pollution in coastal area. The waste water/effluents from seafood processing plant have high organic content. The effluents from Shrimp processing plants locates at Kannamaly, Chellanam panchayath, Ernakulam district directly discharge the waste water to the neighboring water bodies. Apart from raising the BOD at immediate vicinity, limited effluents do not cause any severe damage to the system. But at high levels, often cause severe pollution and adversely affect the aquatic flora and fauna. Standardised ecotoxicity test methods frequently uses duckweed species *Spirodela polyrrhiza* due to their advantages such as rapid vegetative propagation, sensitivity to toxicants, easy culturing under axenic conditions [1]. *Spirodela polyrrhiza* has tolerance to moderate saline conditions. Duckweeds are salinity tolerant, adapt with time to high salinity, remove salinity, and have a potential for desalination in agricultural detention ponds [2].

Objectives

The objective of current study to assess the toxicity of water from two wetlands by standard testing procedure that includes growth analysis and photosynthetic pigment analysis.

Materials and Methods

Duckweed *Spirodela polyrrhiza* were obtained from an unpolluted natural pond near Fort Kochi, Kerala, India. It is a floating aquatic macrophyte belonging to the family Lemnaceae and can be found worldwide on the surface of fresh and brackish waters [3]. The duckweeds are among the most standardized test organisms in aquatic ecotoxicology [4-7]. One sample (Wetland 1) was collected approximately 50 meters south of the Kannamaly pilgrim centre and close to India Seafoods Factory, at a location approximately Lat 9.8704°N and Long 76.2665°E) (Figure 1).

The second sample (Wetland 2) was collected from the wetlands south to the " wetland I area, approximately 1.8 km away and located at Lat 9.8612°N Long 76.2642°E) (Figure 2).

Wetland water samples collected from two sites were analysed according to APHA standards [8]. Metals were analysed using AAS. Test solutions were prepared by diluting water samples of wetland 1 and 2 with distilled water. The solutions were prepared in 100%, 50%, 25%, 10%, 5% and 0.5% concentrations of wetland water plus a control (water taken from an unpolluted site near Kumbalangi, 3 km away from Kannamaly. After seven days of exposure, plants were harvested, washed with double distilled water, blotted and used for the study of various parameters. The parameters include study of vegetative characters, growth parameters and study photosynthetic pigments. All the tests were conducted in six replicates.



Figure 1: One sample (Wetland 1) was collected approximately 50 metres south of the Kannamaly pilgrim centre and close to India Seafoods Factory.

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Figure 2: The second sample (Wetland 2) was collected from the wetlands south to the wetland 1 area, approximately 1.8 km away.

Analysis of Parameters

Physio chemical analysis of wetland water

Study of growth parameters:

1.a. Dry weight: All colonies are collected from each of the test vessels and rinsed with distilled or deionised water. They are blotted to remove excess water and then dried at 60°C to a constant weight. Any root fragments should be included. The dry weight should be expressed to an accuracy of at least 0.1 mg.

1.b. Fresh weight: All colonies are transferred to pre-weighed plastic tubes with small (1 mm) holes in the rounded bottoms. The tubes are then centrifuged at 3000 rpm for 10 minutes at room temperature. Tubes, containing the now dried colonies, are re-weighed and the fresh weight is calculated by subtracting the weight of the empty tube.

1.c. Dry weight-Fresh weight ratio can be determined from above estimations. The plant growth index was calculated as follows

$$\text{Growth Index} = \frac{\text{Biomass} (t = 7 \text{ days})}{\text{Biomass} (t = 0)}$$

1.d. Doubling time: To determine the doubling time (T_d) of frond number and adherence to this validity criterion by the study, the following formula is used with data obtained from the control vessels:

$$T_d = \ln 2 / \mu$$

Where μ is the average specific growth rate.

1. e. Average specific growth rate: this response variable is calculated on the basis of changes in the logarithms of frond numbers, and in addition, on the basis of changes in the logarithms of another measurement parameter (total frond area, dry weight or fresh weight) over time (expressed per day) in the controls and each treatment group. It is sometimes referred to as relative growth rate. The average specific growth rate for a specific period is calculated as the logarithmic increase in the growth variables -frond numbers and one other measurement variable (total frond area, dry weight or fresh weight) - using the formula below for each replicate of control and treatments:

$$\mu_{i-j} = \frac{\ln(N_j) - \ln(N_i)}{t}$$

where:

μ_{i-j} : average specific growth rate from time i to j

N_i : measurement variable in the test or control vessel at time i

N_j : measurement variable in the test or control vessel at time j

t: time period from i to j

1.f. Percentage of growth inhibition: Percent inhibition of growth rate (I_r) may then be calculated for each test concentration according to the following formula

$$\%I_r = \frac{(\mu C - \mu T)}{\mu C} \times 100$$

where:

% I_r : percent inhibition in average specific growth rate

μC : mean value for μ in the control

μT : mean value for μ in the treatment group

Estimation of photosynthetic pigments

The chlorophyll estimation is an important study parameter for the estimation of impact of pollution on photosynthetic activity. About 200mg of treated plants were weighed. This is taken in a mortar with 5ml of 90% acetone and 1ml of Magnesium carbonate. It is then ground thoroughly with pestle. This is then kept at 4°C for 4 hours for the pigments to elute. The solution is then centrifuged at 2500 rpm for 15 minutes. The extract is then decanted to a volumetric flask and the volume is made up to 50 ml with 90% acetone. The absorbance at 750, 663, 645, 510 and 480 were measured in the spectrophotometric analysis using Hitachi-U-2000 spectrophotometer.

Statistical analysis

Analysis of variance for each test was conducted using STATISTICA software (One way Anova). The significant difference between treatments were determined by Duncan's multiple range test ($P < 0.05$). Each test was conducted in six replicates.

Results and Discussions

Physiochemical properties of water from both wetlands are given in Table 1. The values of BOD, Nitrate, phosphate, Ammonia, TDS, TSS, Turbidity and heavy metals Cu, Pb, Zn and Cd were significantly high in wetland 1 in comparison to the control.

Sl.No	Parameter (Unit)	Control wetland	Wetland 1	Wetland 2
1	Temp(°C)	28.8	29.1	28.8
2	Conductivity(mS)	1.67	4.1	2.3
2	pH	7.9	8.2	7.8
3	Salinity (ppt)	1.8	1.8	1.8
4	DO(mg O ₂ /L)	5	2.65	3.2
5	BOD(mg O ₂ /L)	8	112	12.24
6	Nitrate(mg/L)	45	12.28	0.91
7	Phosphate(mg/L)	6.2	14.42	1.57
8	Ammonia	1.2	28.09	0.75
9	TDS	2100	2811	2220
10	TSS	112	55.42	24.89
11	Turbidity	11	413.2	13.8
12	Cu(mg/L)	2.4	2.43	2.2
13	Pb(mg/L)	1	3.71	2.68
14	Zn(mg/L)	3.2	112.21	23.2
15	Cd(mg/L)	not detected	2.33	0.47

Table 1: Results of physio chemical analysis of Wetland 1 and 2 water before exposure. Each values are means of triplicates. The significant difference between treatments is $P < 0.05$

Duckweeds show great tolerance to changes in physiochemical parameters of water. The growth rate of duckweed is favoured by organic pollutants as well as inorganic nutrients. Gopal and Chamanlal [9] reported the maximum biomass of *L. perpusilla* and *S. polyrrhiza* from roadside pools and ditches in India within an electrolyte conductivity range of 650-1 000 $\mu\text{S}/\text{cm}$.

The concept of average specific growth rate is based on the general exponential growth pattern of duckweed in non-limited cultures, where toxicity is estimated on the basis of the effects on the growth rate, without being dependent on the absolute level of the specific growth rate of the control; slope of the concentration-response curve or on test duration. The use of average specific growth rate for estimating toxicity is scientifically preferred. In the current study ASGR and frond doubling time (Td) of the control and treatment with 0.5% and 5% concentration I WI yield the same result. WII water shows different result ASGR and Td remains same as control up to 25% concentration. The inhibition of growth in this concentration is negligible. As the concentration of effluent increases, all the parameters vary. For the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h) (OECD guidelines) [7]. When the frond doubling time exceeds 2.5, the test solution is considered toxic. In the study only un dilutes 100% concentration water from wetland 1 shows Td values more than 2.5, thus found to be toxic. The values are given in Table 2.

Plant growth index were measured after 7 days of exposure with different dilutions of water from both wetlands. At 0.5 % and 5% dilution GI is greater than control values in both waters. At 10% W2 treatment shows less GI than control but W1 still has values above control which shows the water quality of W1 is better than W2. From 25% GI values shows sharp decline in both treatments. The finding is given in Table 3.

The growth index of both wetland water is similar in lowest concentration (0.5%). From 5% dilution onwards it is quite clear that growth of *Spirodela* is enhanced more in wetland I. This may be due to the increase in crude protein content of duckweed however, seems to increase to a maximum of ~40 percent DM over the range from trace ammonia concentrations to 7-12 mg N/L [10]. Khondker, Islam and

Site	Medium	Mean Nj	Mean ASG (μ)	Td	%lr
Wet I	control	16	4.258	1.272	0
	0.5	16	4.258	1.272	0
	5	16	4.258	1.272	0
	10	15	3.871	1.4	9.08
	25	14	3.484	1.555	18.177
	50	13	3.097	1.75	27.26
	100	10	1.935	2.801	34.21
Wet 2	0.5	16	4.258	1.272	0
	5	16	4.258	1.272	0
	10	16	4.258	1.272	0
	25	16	4.258	1.272	0
	50	15	3.871	1.4	9.08
	100	15	3.871	1.4	9.08

Table 2: ASGR, Td and lr% of *S. polyrrhiza* after 7 days of treatment with various dilutions of wetland I and 2. Mean N(i)=5, Mean T(j)=7 and T(i)=0. Standard deviations were presented by error bars. Each value is means of six replicates. The significant difference between treatments is P<0.05.

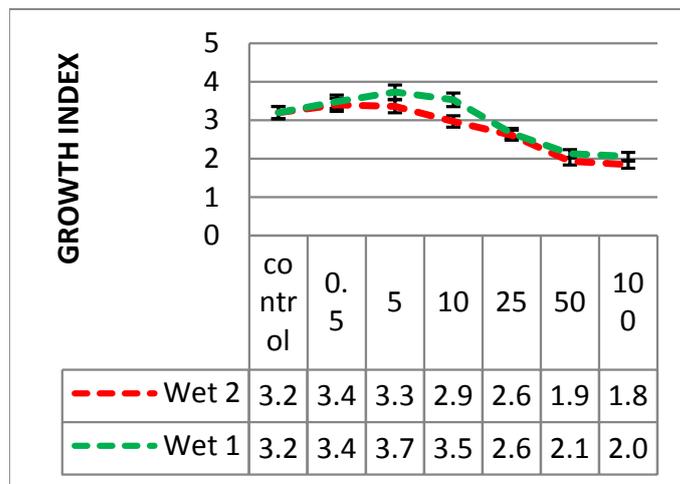


Table 3: Growth Index of *Spirodela* plant in different dilutions of Wetland 1 and 2 after 7 days of exposure. Standard deviations were presented by error bars. Each values are means of six replicates. The significant difference between treatments is P<0.05.

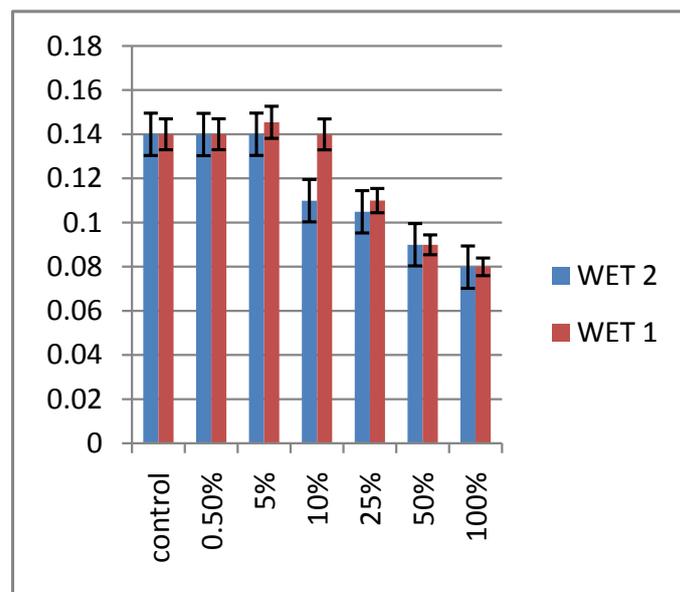


Table 4: Biomass DW/FW ratio of *S. polyrrhiza* after 7 days of exposure in water from Wetland 1 and 2. Standard deviations were presented by error bars. Each values are means of six replicates. The significant difference between treatments is P<0.05.

Makhnun [11] observed that both phosphate and silicate concentrations had significant positive correlation with the biomass of *L. perpusilla* in Bangladesh. But at 25% dilution GI is surprisingly similar. At extreme concentrations again GI vary. The differences in GI under different concentrations are illustrated in Table 4.

Changes in Dry weight fresh weight ratio indicate that in exposed *Spirodela* plants, growth retardation takes place in comparison to the control. At 0.5 % concentration, the biomass yield is same as that in control. In 5% concentration of wetland 1 and 2 water, slight increase in DW/FW ratio recorded in wetland 2 effluents than control. At 10% and 25% solutions, the DW/FW ratio is higher in wetland water 1 in comparison with Wet 2. But in 50% and 100% the ratio shows sharp decline. It was noted that at 50% concentration, *spirodela* growing in highly polluted wetland 2 water yield higher FW/DW ratio than those growing in wetland 1 (Table 5).

The decline in biomass ratio may be due the presence of excess heavy metals present in wetland. It has been shown that accumulation of heavy metals disturb the plant water status which eventually results in osmotic stress and growth reduction [12,13]. Water especially in wetland 2. In wetland 1 the biomass (DW/FW ratio) percentage was 100, 100.7, 103.9, 100, 78.5, 64.27, and 57.1 for dilutions 0.5, 5, 10, 25, 50 and 100 respectively. Similarly for wetland 2 it was 100, 99.9, 100.07, 78.4, 74.9, 64.2, and 57.04 for dilutions 0.5, 5, 10, 25, 50 and 100 respectively.

Chlorophyll and carotenoids are the central part of the energy manifestation of every green plant system and therefore, any significant alteration in their levels is likely to cause a marked effect on the entire metabolism of the plant. The productivity of plants is directly related with changes in the content of photosynthetic pigments chlorophyll a, chlorophyll b and Carotenoids. Industrial wastewater not only affects the chlorophyll content but the chlorophyll activity also [14-16]. In the study photosynthetic pigments may be inhibited due to metal toxicity. Industrial wastewater not only affects the chlorophyll content but the chloroplast activity also [14-16].

Duckweed leaves started to show signs of chlorosis (pigment loss) following 7d exposure to surface water samples. At 50% and 100% dilutions necrosis could also seen. The Carotenoids contents are found to be less affected. At the end of 7 days of exposure 0.5% dilution of wetland 1 and 2 shows slight increase in chlorophyll a content (Hormesis) while at the same dilution and Carotenoids content remain unchanged. From 5% to 100% all pigments shows gradual decrease in concentration. Chl b degraded at a much slower rate than chl a which indicates that greater damage of pollutants present in water samples on chl a. The loss of photosynthetic pigment content has been reported in duckweed plants following exposure to Cu, Pb and Ni [17-19]. The destruction of photosynthetic pigments by heavy metals could be due to: impairment of ETC, replacement of Magnesium ions associated with chlorophyll ring, inhibition of important enzymes [20] associated with chlorophyll synthesis or peroxidation processes in chloroplast membrane lipids by reactive oxygen species [21] (Table 6).

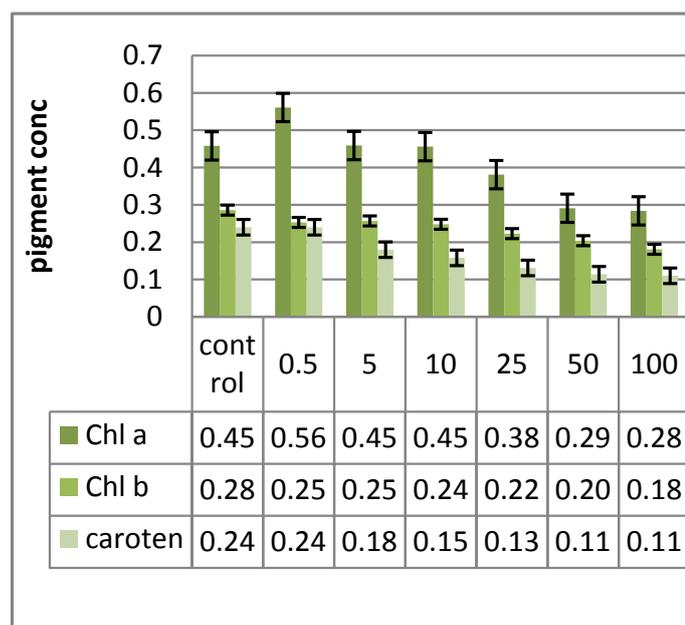


Table 5: Relative photosynthetic pigment concentrations after 7 days of exposure in different dilutions of wetland 1. Standard deviations were presented by error bars. Each values are means of six replicates. The significant difference between treatments is P<0.05.

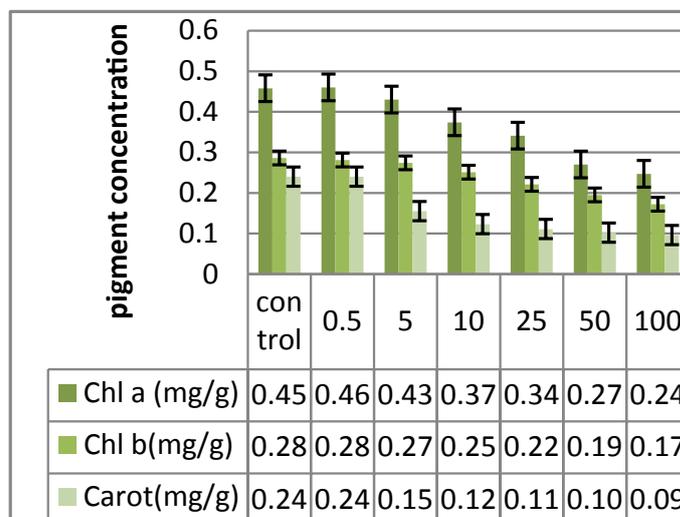


Table 6: Relative photosynthetic pigment concentrations after 7 days of exposure in different dilutions of wetland 2. Standard deviations were presented by error bars. Each values are means of six replicates. The significant difference between treatments is P<0.05.

Conclusion

The study revealed that both wetlands are highly polluted. Wetland 2 has more pollutants compared with wetland 1 which is evident from the assessment of vegetative, growth and photosynthetic pigment parameters. The study also points towards the importance of conservation of wetlands in the area.

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