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Assessment of growth and composition of aquatic plant *Wolffia Globosa* (L.)

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Abstract
A genus of nine to 11 species, *Wolffia globosa* (L.) is the smallest of the world's flowering flora. These aquatic plants usually refer to as watermeal or 'duckweed' is similar to cornmeal spots which float on the water. Wolffia species are thalli, green or yellow leaf free-floating and rootedless. The aquatic plant *Wolffia globosa* (L.) was grown for phytoremediation treatment in range 0 (control), 20, 40, 60, 80 and 100% waste water. Fresh matter yield and dry matter yield of 7, 15 and 21 days growth of *Wolffia globosa* (L.) showed maximum value at 40% level of waste water. Chlorophyll content of 7 days growth of *Wolffia globosa* (L.) was found maximum at 40% level while 20% level of waste water showed maximum chlorophyll content of 15- and 21-days growth of *Wolffia globosa* (L.) plant. Maximum ascorbic acid content and catalase activity was recorded at 40% level of waste water in 7 and 15 days and 20% level of waste water in 21 days growth of *Wolffia globosa* (L.) plant.

Keywords: *Wolffia Globosa* (L.), wastewater, FMY, DMY, ascorbic acid

1. Introduction

A small water herb, known as Asian meal [1], *Wolffia globosa* (L.) belongs to the lemnaceae or duckweed family. Central root system is not found in *Wolffia globosa* (L.). The leaves and stem called a "frond"[2], are fused together. The thickness is ellipsoid-sphere, 0.5 to 0.8 mm long, 0.4 to 0.6 mm high, and on the surface is green-pale [3]. With approximately four days of generation in controlled laboratory conditions [4], *Wolffia globosa* (L.) develops easily and rapidly. It also contains high levels of chlorophyll, carotenoids and B12 flavonoids [5], and is also suitable for various consumer goods such as animal feed, alcoholic drinks and even biologically degradable plastics [1].

Wolffia globosa (L.) has been used for the last two decades to handle wastewater flowing from the factories [6]. Because of its ability to expand at any temperature, pH and nutrient level, it is often used in wastewater treatment [7]. Duckweed also decreased the development of pond algae [8,9]. As with various species, duckweeds extract nutrients from their surroundings [10]. This potential has been used to balance excess nutrients from effluents from swine lagoons [11]. The flowers that grow will then be processed to eliminate the surplus phosphorus and nitrogen. Nevertheless, the effectiveness of duckweed in regeneration [12] and reduction of phosphorus and nitrogen from swine lagoon water was found to be important to concentrations of water and seasonal climatic conditions because the primary function is to assimilate such vitamins in the climate, the correct mild depth and leading temperature are main criteria for duckweed in the elimination of excess nutrients [11], and duckweed tends to consume NH^{4+} as compared to NO^{3-} from frond.

2. Material and Methods

Waste water samples were collected from waste water channel of Ganga Barrage, Kanpur in wide large plastic containers and bottle cork immediately. All the samples were brought to settle in an open plastic tubes for one week to allow microorganism to break down solid organic waste and then added to ice cube were transferred to the lab where it was placed in the refrigerator.

For culture of aquatic plants *Wolffia globosa* (L.), water sample were collected from the pond near Maswanpur, Kalyanpur, Kanpur. After collecting plants in plastic bags with some amount of water from the same site were transported to the laboratory where it were washed with fresh tap water and then finally with distilled water to remove any soil or sediments particles attached to plant surface. The wastewater collected then filtered through multifolded cloths and brought the laboratory and stored in a refrigerator till before treatment investigation was completed.

Sample for estimation of dissolved oxygen was collected in 250 ml bottle and fixed immediately for each treatment. For culture of *Wolffia globosa* (L.) aquatic plant 6" x 9" enamal disc were used. The concentrations of waste water were 0, 20, 40, 60, 80 and 100%. For the control (0%) pond water was used. For study, fresh matter yield, dry matter yield, chlorophyll and ascorbic acid content and catalase activity are recorded at 7, 15- and 21-days growth of *Wolffia globosa* (L.). Entire data have been statistically analyzed and tested for significance at 5% and 1% probability levels subjected to the analysis of variance according to Steel and Torrie [13]. Fresh matter yield was determined by weight first washing with running tap water, rinsing with distilled water and absorbing surface with clean white blotting paper. Dry matter yield was analyzed by drying and finely chopped and mixed plants samples in a forced draught oven at 65^{o} C for 1 day to constant weight. The samples were taken out from the oven and placed in a desiccators, cooled for about an hours and weighed for the determination of yield.

Chlorophyll was determined by the method of Petering *et al* [14] and the chlorophyll content was measured by estimating the absorption of the acetone extract in. Elico-CL- 20A-Photo-electric-calorimeter used red filter and referring the reading to the standard calibration curve prepared by the method of Comer and Zscheile [15]. Ascorbic acid content was estimated titrimetrically by the method of Harris and Roy [16] and Catalase was assayed by the permanganate titration method of the Euler and Josephson [17].

3. Result

3.1 Fresh Matter Yield

With the increase in waste water level upto 40 % level fresh matter yield of *Wolffia globosa* (L.) at 7, 15 and 21 days of growth increased. Further increase in waste water level decreases the fresh matter yield of 7, 15- and 21-days growth of *Wolffia globosa* (L.) plant was observed. In comparison to control, the increase in fresh matter yield of 7, 15- and 21-days growth of *Wolffia globosa* (L.) was found to be highly significant (P=0.01) at all the level tested upto 80% level of waste water. 100% waste water level over control was found to be toxic effect for fresh matter yield of 7, 15 and 21 days of *Wolffia globosa* (L.).

Increase in fresh matter yield of 7, 15 and 21 days growth of *Wolffia globosa* (L.) at 20% over control and 40% over 20% waste water level was found to be highly significant (P=0.01). Decrease in fresh matter yield of 7, 15- and 21-days growth of *Wolffia globosa* (L.) was found to be highly significant (P=0.01) at 60% over 40%, 80% over 60% and 100 over 80% waste water level. Maximum fresh matter yield of 7, 15- and 21-days growth of *Wolffia globosa* (L.) was found at 40 % waste water level.

3.2 Dry Matter Yield

Increase in dry matter yield of 7, 15- and 21-days growth of *Wolffia globosa* (L.) was observed with the increase in waste water level upto 40%. Further increase in waste water level decreases dry matter yield of 7, 15 and 21 days of *Wolffia globosa* (L.) was observed. As compared to control 20%, 40%, 60% and 80% waste water level showed highly significant (P=0.01) increase in dry matter yield of 7, 15- and 21-days growth of *Wolffia globosa* (L.). 100% waste water level showed toxic effect for dry matter yield of 7, 15- and 21-days growth of *Wolffia globosa* (L.). Increase in dry matter yield of *Wolffia globosa* (L.) at 7, 15- and 21-days growth was found to be highly significant (P=0.01) at 40% over 20% and 20% waste water level over control. Decrease in dry matter yield of 7, 15- and 21-days growth was found to be highly significant (P=0.01) at 60% over 40%, 80% over 60% and 100% over 80% waste water level. 40 % waste water level showed maximum dry matter yield of 7, 15- and 21-days growth of *Wolffia globosa* (L.).

3.3 Chlorophyll

Chlorophyll content of *Wolffia globosa* (L.) increased with the increase in waste water level upto 40 % level in 7 days growth and upto 20 % level in 15- and 21-days growth. Beyond these respective level further increase in waste water level decreases the chlorophyll content of *Wolffia globosa* (L.). As compared to control 20%, 40% and 60% waste water in 7 days growth of *Wolffia globosa* (L.) and 20% waste water in 15- and 21-days growth showed highly significant (P=0.01) increase. 40% waste water in 15 days showed insignificant increase in chlorophyll content of *Wolffia globosa* (L.). 80% waste water in 7 days showed significant (P=0.05) decreases while 100% waste water in 7 days and 60%, 80% and 100% waste water in 15 and 21 days growth and 40% waste water in 21 days growth was found to be toxic effect for chlorophyll content of *Wolffia globosa* (L.). Increase in chlorophyll content of *Wolffia globosa* (L.) was found to be highly significant (P=0.01) at 20% over control in 7, 15- and 21-days growth and at 40% over 20% waste water level in 7 days growth of *Wolffia globosa* (L.). Decrease in chlorophyll content of *Wolffia globosa* (L.) was highly significant (P=0.01) 60% over 40% in 7 days, 80% over 60% and 100% over 80% waste water in 7, 15- and 21-days growth, and 60% over 40% waste water in 15- and 21-days growth of *Wolffia globosa* (L.). Maximum chlorophyll content of 7 days growth at 40 %, and 15- and 21-days growth of *Wolffia globosa* (L.) at 20 % level was observed.

3.4 Ascorbic Acid

Ascorbic acid content of *Wolffia globosa* (L.) increased with increase in waste water level upto 40 % at 7 and 15 days growth and up to 20 % level at 21 days growth. Further increase in waste water level decrease in ascorbic acid content of *Wolffia globosa* (L.) was observed. As compared to control 20%, 40% and 60 % waste water in 7 and 15 days growth and 20 % waste water in 21 days growth showed highly significant (P=0.01) increase, and 80% waste water in 7 days and 40 % waste water in 21 days showed significant (P=0.05) increase in ascorbic acid content of *Wolffia globosa* (L.). 80% in 15 days and 100 % waste water in 7 days showed insignificant decrease in ascorbic acid content. 60% waste water in 21 days showed significant (P=0.05) decrease while 80% waste water at 21 days showed highly significant (P=0.01) decrease. 100% waste water 15- and 21-days growth showed toxic effect for ascorbic acid content of *Wolffia globosa* (L.). Increase in ascorbic acid content at 20% over control in 7, 15- and 21-days growth, 40% over 20% in 15 days growth showed highly significant (P=0.01) increase in ascorbic acid content of *Wolffia globosa* (L.). 40% over 20% in 7 days growth showed significant (P=0.05) increase in ascorbic acid content of *Wolffia globosa* (L.). A highly significant (P=0.01) decrease in ascorbic acid content at 60% over 40%, 80% over 60% and 100% over 80% waste water in 15 days, 80 over 60% and 100% over 80 % waste water in 7 days and 60% over 40% in 21 days. 100% over 80% waste water in 21 days showed significant (P=0.05) decreases. However, 60% over 40% in 7 days and 40% over 20 % waste water in 21 days showed insignificant decreases. Maximum ascorbic acid content of 7- and 15-days growth at 40% level and at 21 days of *Wolffia globosa* (L.) at 20% level was observed.

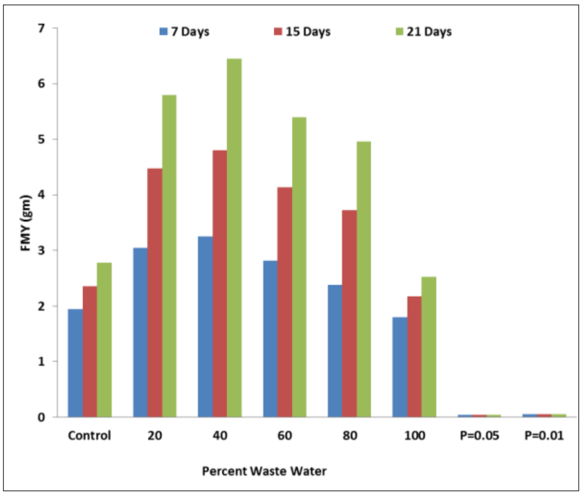
3.5 Catalase

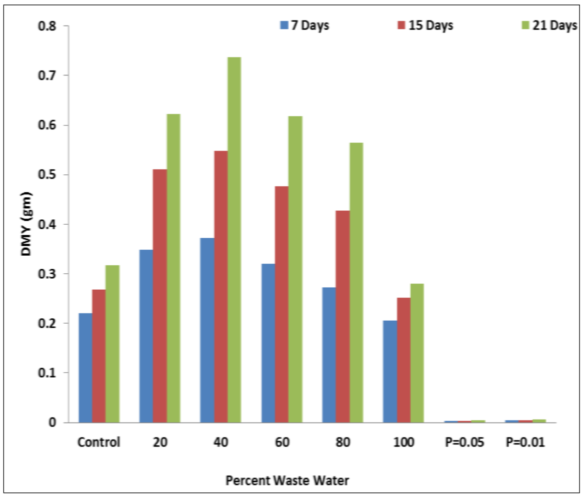
Catalase activity of *Wolffia globosa* (L.) increased with the increase in waste water levels upto 40% level at 7- and 15-days growth and up to 20% level at 21 days growth. Beyond these respective levels further increase in waste water levels decrease the catalase activity of *Wolffia globosa* (L.). As compared to control 40% wastewater in 7 days growth showed highly significant (P=0.01) while, 20% over control in 7 days, 40% over control in 15 days and 20% waste water in 21 days growth showed significant (P=0.05) increase while 80% over control in 21 days, 100% over control in 15 days growth of *Wolffia globosa* (L.) showed significant (P=0.05) decrease in catalase activity. However, 60% and 80% in 7 days over control, 20% over control in 15 days showed insignificant increase while, 100% over control in 7 days, 60% and 80% in 15 days and 60% over control in 21 days showed insignificant decrease in catalase activity of growth of *Wolffia globosa* (L.). 100% waste water over control in 21 days growth showed toxic effect of catalase activity of *Wolffia globosa* (L.). Increase in catalase activity was found to be significant (P=0.05) at 20% over control in 7- and 21-days growth of *Wolffia globosa* (L.). 40% over 20% in 7 days, 20% over control and 40% over 20% showed insignificant increase in 15 days growth of *Wolffia globosa* (L.).

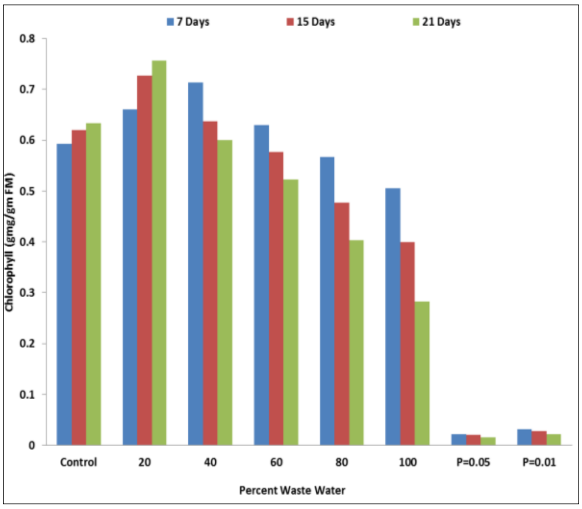
Decrease in catalase activity of *Wolffia globosa* (L.) was found to be highly significant

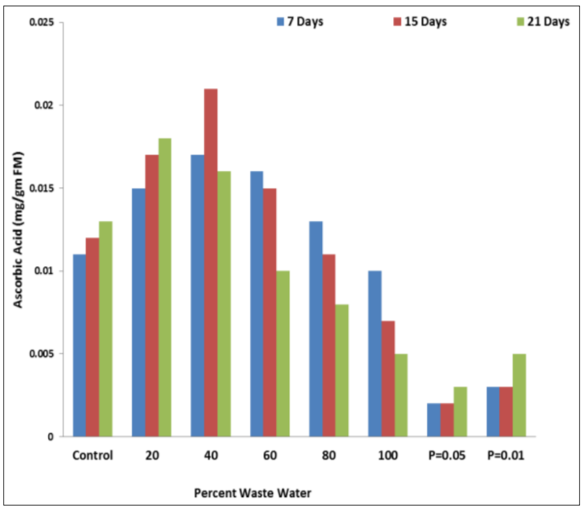
Table 1: Effect of wastewater on Growth and Composition of *Wolffia globosa* (L.)

Days	Percent Waste Water						LSD	
	Control	20	40	60	80	100	P=0.05	P=0.01
Fresh Matter Yield (gm)								
7	1.94±.01	3.05±.02	3.25±.02	2.82±.01	2.38±.01	1.80±.01	0.04	0.06
15	2.35±0.1	4.47±.02	4.80±.01	4.14±.02	3.73±.01	2.18±.01	0.04	0.05
21	2.78±.01	5.80±.01	6.45±.01	5.40±.02	4.96±.01	2.53±.01	0.04	0.05
Dry Matter Yield (gm)								
7	0.220±.001	0.348±.002	0.372±.001	0.320±.001	0.272±.001	0.206±.001	0.003	0.004
15	0.268±.001	0.511±.002	0.548±.001	0.476±.001	0.428±.001	0.252±.001	0.003	0.005
21	0.317±.002	0.622±.002	0.737±.001	0.618±.001	0.565±.001	0.280±.001	0.004	0.006
Chlorophyll (mg/gm FM)								
7	0.593±.009	0.660±.006	0.713±.003	0.630±.006	0.567±.009	0.506±.009	0.022	0.031
15	0.620±.006	0.727±.003	0.637±.007	0.577±.009	0.477±.007	0.400±.006	0.020	0.028
21	0.633±.003	0.757±.003	0.600±.006	0.523±.009	0.403±.003	0.283±.003	0.016	0.022
Ascorbic Acid (mg/gm FM)								
7	0.011±.001	0.015±.002	0.017±.002	0.016±.001	0.013±.002	0.010±.001	0.002	0.003
15	0.012±.001	0.017±.001	0.021±.001	0.015±.002	0.011±.002	0.007±.001	0.002	0.003
21	0.013±.002	0.018±.001	0.016±.002	0.010±.002	0.008±.001	0.005±.001	0.003	0.005
Catalase (Unit/gm FM)								
7	0.87±.12	1.23±.12	1.33±.09	1.10±.06	1.07±.15	0.80±.06	0.32	0.45
15	1.00±.06	1.29±.12	1.40±.17	0.90±.06	0.80±.06	0.67±.09	0.31	0.44
21	1.03±.12	1.33±.09	1.20±.12	0.83±.09	0.73±.09	0.50±.06	0.29	0.41









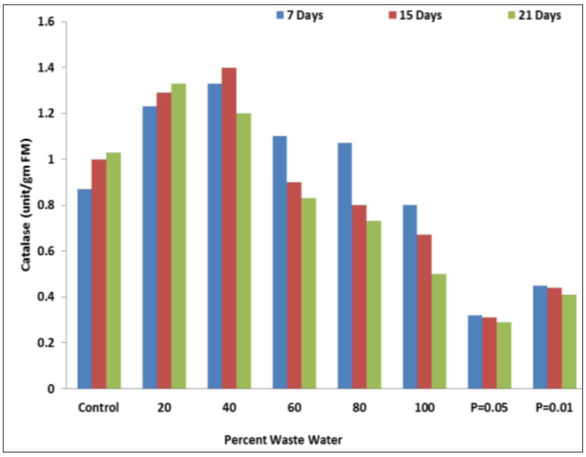


Fig 1: Effect of wastewater concentration on growth and composition of *Wolffia globosa* (L.)

(P=0.01) at 60% over 40% in 15 days growth. Significant (P=0.05) decrease at 60 over 40 % in 21 days growth of *Wolffia globosa* (L.). 100% over 80% waste water in 7 days, 80% over 60% and 100% over 80% waste water in 15 and 21 days, 40% over 20% showed insignificant decrease in 21 days growth of *Wolffia globosa* (L.). Maximum catalase activity of 7 and 15 days growth at 40% and of 21 days growth of *Wolffia globosa* (L.) at 20% level was observed.

4. Discussion

In the present study, aquatic plant *Wolffia* globosa (L.) were used as phytoremediation of waste water. The growth and composition of *Wolffia globosa* (L.) in wastewater reveals the following observation:

Maximum fresh matter yield was found at 40 % level of waste water at 7, 15 and 21 days of growth of *Wolffia globosa* (L.) plant. The findings are in support of Abou *et al* [18] who studied that fresh weight of *Lemna gibba* increased with increasing treatment period at certain level. In Louisiana, under summer conditions, with heavy fertilization, upto 44 t/ha/yr have been obtained [19]. Average yields of around 10-20 t/ha can be obtained where nutrients are generally not limiting and frequent harvesting is practiced to avoid overcrowding of plants [20]. Singh and Singh [21] studied fresh and dry weight and indicate significant decrease in industrial wastewater and sewage wastewater during the different seasons of the year.

Maximum dry matter yield were recorded at 40% level of waste water in 7, 15 and 21 days growth of *Wolffia globosa* (L.). The findings are in support of Chikuvire *et al*[22] who studied that dry matter increase by 23-45% compared to the negative control whereas, Nassar *et al* [23] investigated dry matter yield were about 108 kg/ha/d on average, respectively. The dry matter values were ranged from 5.5 to 7.2 with an average value of 6.1%. Leng *et al* [24] reported dry matter yields per hectare and year of up to 23 t under sub-optimal and up to 79 t under near-optimal conditions. Furthermore, duckweeds can be very rich in protein with up to 455 g crude protein (CP) per kg dry matter (DM) [25, 26].

Industrial waste water not only affects the chlorophyll content but the chloroplast activity also [27, 28]. Maximum chlorophyll content in 7 days growth at 40 % and 15 and 21 days growth of *Wolffia globosa* (L.) at 20 % level were observed. Chlorophyll content of *Wolffia globosa* (L.) were gradually decreases with increase in the waste water concentration. The findings are in support of Singh and Singh [21].

Catalase activity was found to be maximum at 40% level of waste water in both 7- and 15-days growth of *Wolffia globosa* (L.), and at 20% level of waste water in 21 days growth of *Wolffia*.

Heavy metal-induced H_2O_2 accumulation is prevented by catalase and peroxidases. Hydrogen peroxide is a very toxic reactive oxygen species [29]. The increased activity of CAT indicates effective scavenging of H_2O_2. However, in this study, activities of CAT once inceased then gradually decreased.

The reason for this decrease may be due to increased activities of peroxidases which are also detoxifiers of H_2O_2. Additionally, the inhibition of CAT activity has been stated by Piotroska *et al* [30] as possibly caused by H2O2 and other ROS' toxic effects. In plants under Cd stress (*Bacoppa monnieri*, *W. arhiza* and *G. denses*), Cu (*L. minor, S. polyrhiza*), Pb (*W. arhiza*) and Cr (cauliflower), decreased CAT behavior has been recorded in a similar way to the findings of this study [31, 32, 33].

Maximum ascorbic acid content was recorded at 40% level of waste water in both 7- and 15-days growth of *Wolffia globosa* (L.) while at 20% level of waste water in 21 days growth of *Wolffia globosa* (L.). Ascorbic Acid, a naturally occurring antioxidant, creates free heavy-metal radicals [34]. The amount of ascorbic acid was linked positively and substantially in the plant and metal concentrations to those of Cd, Pb, Zn and Ni. Ascorbic acid production in fenugreek crops grown in the soil with a tannery sludge was also documented by Sinha *et al* [35], as a result of the negatives of heavy metals being nullified. The production of ascorbic acid under waste water irrigation was increased by *Culocasia esculentum* and *Raphanus sativus* in the wastewater irrigated region of Durgapur, western Bengal [36].

5. Conclusion

Heavy metals in our ecosystem as a chronic pollutant require total removal for a fully remedial reason.

The use of phytoremediation tends to be a less harmful, economical and environmentally sound clean-up technology. The choice of suitable plant is the most critical aspect of phytoremediation. Aquatic plants play very vibrant functions in the remediation of heavy metals from the contaminated site with the same ease as other hyperaccumulator plants. The successful introduction of aquatic plants with living plant biomass for the eradication of heavy metals.

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