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RESEARCH NOTE

Determining the nutrient removal capacity of duckweed *Wolffia globosa* under artificial conditions

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ABSTRACT

Aquatic vegetation like duckweed (*Wolffia globosa*) can eliminate contaminant from wastewater, which also can be commercial and possible options for wastewater treatment. Thus, this study aimed to estimate the nutrient removal capability of *Wolffia globosa* under artificial culture conditions. The nutrient removal capacity of *W. globosa* was evaluated in a 12-day growth trial with mineral mixture containing 173.6 mg/litre nitrogen; 40.3 mg/litre phosphorous; 100 mg/litre potassium and 0.6 g/litre as a reference fertilization rate (RF) along with five other different [RF/2; RF/4; RF/8; RF/16; RF/20 and Control (no fertilizers)] NPK fertilization rates under natural sunlight. In all the treatments, the concentrations of nitrate-nitrite, ammonia, nitrate and ortho-phosphate, decreased over the experimental period in a statistically significant (p=0.05) manner. At the end of the experiment, the total dissolved inorganic nitrogen (T-DIN) in the culture media was reduced by 99. 57% (RF/20), 100% (Control group - no fertilizers) while Ortho-phosphate (OP) by 100% in RF/16, RF/20 and control group, respectively. It was concluded that the *Wolffia globosa* is a suitable aquatic plant for nutrient removal under natural sunlight.

Keywords: Duckweed**,** Fertilization rates, Nutrient removal efficiency, *Wolffia globosa*

In India and China, around 50% of the population face the problem of water scarcity (WWAP 2017). Over 80% of wastewater is discharged into the environment without adequate remedy around the world (WWAP 2017). Domestic wastewater contains high levels of nitrogen and phosphate which accelerates the eutrophication and pollution in the aquatic environment (Verma and Suthar 2014). In view of the huge demand for water, it has become extremely important to manage the waste water by treating it properly. An ecologically affable and cost-effective solution is required for it. Aquatic plants, such as duckweed, water hyacinth, giant reed, microalgae and water lettuce are used to remove the pollutants from the wastewater (Li *et al.* 2018). Duckweeds are simple plants which have no stems or leaves (Iqbal *et al.* 2019). The abnormal leaf-like body is called a frond (Sirirustananun and Jongput 2021). Accordingly, it grows faster than most different plant life and be able to double its biomass in 2 days (Iqbal *et al.* 2019). Duckweed (*Wolffia globosa)* is capable to grow on the surface of wastewater and eliminate pollutants (particularly, nitrogen and phosphorous) from wastewater at high rates (Sirirustananun and Jongput 2021, Sirirustananun and Jongput 2021). Because of this potential, duckweed has already been used for the treatment of domestic, industrial and swine wastewaters (Gaur and Suthar 2017). Nitrate and ammonium are the principal forms of available nitrogen for the growth of duckweeds, however; the absorption of ammonium is 3 to 11 times greater than nitrates (Iqbal *et al.* 2019). Duckweed indicates best growth at phosphorus concentration of 4 and 22 mg P/l of growth medium (Al Nozaily 2000). Phosphorus removal efficiencies by duckweed ranged from of 14 to 99% and it depends on the growth rate, harvesting frequency and the available ortho-phosphate (Korner *et al.* 2003). Despite the aforementioned information from various duckweed studies, there is limited data on nutrient removal efficiency by *W. globosa*. Hence this study was conducted to quantify the removal of nitrate-nitrite, ammonia, nitrate and ortho-phosphate in the culture media by *W. globosa*.

The experiment was carried out for 12 days in September month (2017) in twenty-one thermocol fish icebox (58 x 39 cm x 30 cm) at the College of Fisheries, Central Agricultural University, Lembucherra, Tripura, India. The inner side of each thermocol box was lined with transparent plastic film and used as an experimental tank. The surface area in each box was 0.226 sq. m. The boxes were cleaned and washed copiously and were filled with groundwater to a 20 cm water depth, giving a volume of 50 litres. All boxes were set up under shade which

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made by using transparent polythene sheet and bamboo poles. A completely randomized design (CRD) with three replications was used. A modified Schenk-Hildebrandt medium (Appenroth *et al.* 2017) was used as reference fertilization (RF) to prepare different concentrations of N, P and K and of minerals (**Table 1**). A single dose of fertilization [173.6 mg/litre nitrogen; 40.3 mg/litre phosphorous; 100 mg/litre potassium and 0.6 mg/litre with vitamins and minerals mixture namely 'Agrimin Fort India' to fulfil the requirement of minerals for their growth] was done as a reference fertilization rate (RF) and five serially diluted (0-20 times) (RF/2; RF/4; RF/8; RF/16; RF/20) concentrations were prepared. Inoculums samples of Wolffia fronds were obtained from the College of Fisheries, Lembucherra (Tripura) and inoculated at a rate of 400 g /m^2 (90.4 g in each tank) in each treatment. Harvesting was done at two-day intervals.

During the cultivation period, water samples were collected on 0, 3rd, 6th, 9th and 12th day of culture for analysis of nutrients concentration. The collected water samples were passed through a glass fibre filter (pore size, 10 µm) to remove suspended materials**.** Nitrate-nitrite, ammonia, nitrate and ortho-phosphate in the culture media were measured using SKALAR Auto analyser (Model no. SA 1100, SKALAR). Water from each experimental unit was sampled for analysing pH with a glass electrode in a digital pH meter (Model FEP-20). Total alkalinity and hardness of water were also measured by the standard methodology of APHA (2005). Total chlorophyll contents in water were measured by using EXO-multi-parameter sonde. The temperature in the water was measured every day, using a digital thermometer (YSI ProODO). The sunlight intensity also measured every day in five places using a digital lux meter (model no. D. 33979).

The data obtained were analysed statistically and interpreted by using Statistical Package for Social Sciences (SPSS, version 16.0 for windows). Analysis of variance (one way - ANOVA) was performed to determine the differences between the mean values of different treatments. Differences in means were compared by Duncan's New Multiple Range test (multiple range test) at p=0.05 level.

Nutrient removal efficiency of *W. globosa*

The concentrations of nitrate-nitrite, ammonia, nitrate and ortho-phosphate in the culture media, decreased from day zero to the twelfth day (**Table 2**). Macrophytes are expected to take up nutrients to build up their biomass over time, which is why nitrates and nitrites concentration were expected to reduce over the study period (Sirirustananun and Jongput 2021). *W. globosa* prefered NH_4^+"N to NO_3^- "N as the nitrogen resource (Suppadit 2011). When *W. globosa* were grown in different treatment, the nitrate-nitrite (NO_3^- " NO_2^-) levels were also different ($P < 0.05$). After the experiments were completed, the remaining NO_3^- " NO_2^- levels ranged from 0.02 (RF) to 0.00 (control) mg N /l, down from the initial NO_3^- " NO_2^- value of 2.65 (RF) to 0.08 (control) mg N/l, depending on nutrient concentration in the culture media (**Table 2**). This might be because the *W. globosa* adsorbed NO_3^- " NO_2^- for its growth (Suppadit 2011). The nitrate (NO_3^-) concentration in the culture media were also reduced by *W. globosa*, the remaining NO_3^- levels ranged from 1.23 (RF) to 0.00 (control) mg N /l, down from the initial NO_3^- value of 43.83 (RF) to 0.27 (control) mg N/l **(Table 2**). Our results confirmed Suppadit (2011) findings on nutrient removal rate of *W. arrhiza*.

The ammonia removal showed significant differences (p=0.05) between treatments. From the initial concentration of ammonia, which was 25.55 (RF) to 0.85 (control) mg N/l, the ammonia tended to decrease as the biomass and the treatment time increased and the remaining value of ammonia was from 3.10 (RF) to 0.00 (control) mg N/l (**Table 2**). Our results are similar to those of Suppadit (2011) and Sirirustananun and Jongput (2021). The removal of total dissolved inorganic nitrogen (T-DIN) and Ortho-phosphate showed significant differences ($p<0.05$). The nutrient removal capabilities of *W. globosa* were estimated using temporal changes in nitrate-nitrite, ammonia, nitrate and ortho-phosphate concentrations in the culture media. The T-DIN removal rate (mg/l/day) of *W. globosa* was highest in RF (5.64 mg/l/day) and RF/2 (5.45 mg/l/day), as nutrient concentration in the culture media was also higher in both treatments. Similarly, ortho-phosphate removal rate (mg/l/day) of *W. globosa* was also

Table 1. Fertilization rates of different treatments

Treatment	Nitrogen (mg/l)	Phosphorous (mg/l)	Potassium (mg/l)	Mineral mixture (g/l)
Reference fertilization (RF)	173.6	40.3	100	0.6
RF/2	86.8	20.15	50.0	0.3
RF/4	43.4	10.07	25.0	0.15
RF/8	21.7	5.03	12.5	0.075
RF/16	10.85	2.51	6.25	0.037
RF/20	8.68	2.015	5.0	0.03

highest in RF (2.22 mg/l/day) and RF/2 (1.14 mg/l/day) (**Table 3**). This might be because the *W. globosa* used phosphorus in the form of orthophosphate for its growth. Whereas, at the end of the experiment, it was seen, T-DIN removal efficiency of *W. globosa* in the culture media was highest in control group (100 %) and RF/20 (99.57 %) while ortho-phosphate removal efficiency was 100 % in RF/16, RF/20 and control group, as the nutrient concentration in the culture media was also low (**Table 3**). The results of this study confirmed findings of Soda *et al.* (2013), Suppadit (2011), Fujita *et al.* (1999).

Physicochemical parameters and chlorophyll content

During the experiment, the water temperature recorded daily in the afternoon and it was within a normal range (31.21-31.59 ºC), which was suitable for the growth of *W. globosa* (**Table 4**). The duckweed species exhibit optimum growth between 17.5°C to 34°C (Hasan and Chakarbarti 2009; Soda *et al.* 2013). Sirirustananun and Jongput (2021) reported the water temperature 28.25±0.07 to 31.85±2.19 °C, optimal for the growth of *Wolffia arrhiza*. Our results are similar to those of Sirirustananun and Jongput (2021) who reported that the light intensity of 4,560±463.86 to 9,795±265.76 lux, were optimal for the growth of *W. arrhiza.* The temperature and light intensity observed in this experiment was in a productive range. During the experimental period, the pH value varied from 6.2 to 10.3 (**Table 4**). Duckweed survives at pH's among 5 and 9 but grows greatest above the pH range of 6.5-7.5 (Hasan and Chakrabarti 2009). The pH values reported in this study were in the optimal range but in later period of the culture it becomes up to 10.3. As a consequence, for the final pH, the culture media was in a slightly basic state. A similar value of pH (7.50±0.24 to 7.79±0.007) was reported by Sirirustananun and Jongput (2021) for the growth of *W. arrhiza.* Muvea *et al.* (2019) reported that the ammonia oxidation again contributed to the increase of pH from 7 to 10. During the experimental period, the total alkalinity and total hardness of the culture media varies from 38.67 to 96 mg/l and 33.33-150 mg/l, respectively (**Table 4**).

The chlorophyll content in culture medium varied from 1.55-189 µg/l indicating an increase in the later period of cultivation (**Table 4**), due to the infestation of algae in the medium. Unicellular algae are the primary competitors of duckweed for nutrients and space. Algae domination will result in a swing toward high pH and making of free ammonia, which is lethal to duckweed. The algae may also reduce the growth of *W. globosa* by inhibiting nutrient uptake and can be more dangerous to *W. globosa*, as it clogged and wrapped itself around fronds, causing shrivel and in the end die (Soda *et al.* 2013, Fujita *et al.* 1999). But, when algal infestation become excessive, it becomes important to clear the pond and restock with clean duckweed. *W. globosa* can compete with or coexist with algae and other aquatic plants if operated for long periods in open environments (Soda *et al.* 2013).

Table 2. The nutrients concentrations (means ±SE) in the culture media during different sampling periods

Sampling Occasion	Physicochemical parameters	Treatment						
		RF	RF/2	RF/4	RF/8	RF/16	RF/20	Control (no. fertilizers)
Baseline/Zero day	Nitrate-nitrite (mg N/l)	2.65 ±0.07^{e}	1.96±0.11^{d}	0.96±0.01^{c}	0.49±0.03^{b}	0.25±0.02^{a}	0.23±0.03^{a}	0.08±0.01^{a}
	Ammonia (mg N/l)	25.55±1.50^{e}	22.89±0.64^{d}	13.13±0.19^{c}	5.29±0.02^{b}	2.18±0.06^{a}	2.26±0.08^{a}	0.85±0.06^{a}
	Nitrate (NO_3^-) (mg N/l)	43.83±0.96^{g}	41.72±0.38^{f}	25.57±0.06^{e}	7.06±0.46^{d}	3.96±0.19^{c}	2.18±0.18^{b}	0.27±0.08^{a}
	T-DIN (mg/l)	72.03±0.93^{f}	66.57±1.09^{e}	39.66±0.13^{d}	12.85±0.50^{c}	6.39±0.23^{b}	4.67±0.23^{b}	1.20±0.15^{a}
	Ortho-phosphate (mg P/l)	27.12±0.22^{f}	13.68±0.70^{e}	6.51±0.34^{d}	3.45±0.21^{c}	1.38±0.08^{b}	1.17±0.04^{ab}	0.32±0.03^{a}
3rd day	Nitrate-nitrite (mg N/l)	2.68±0.02^{f}	0.54±0.04^{d}	1.16±0.01^{e}	0.31±0.03^{c}	0.15±0.01^{b}	0.10±0.01^{b}	0.01± 0.00^{a}
	Ammonia (mg N/l)	46.42±0.77^{f}	42.59±0.43^{e}	18.50±0.41^{d}	7.35±1.50^{c}	3.80±0.09^{b}	2.28±0.31^{ab}	0.45± 0.00^{a}
	Nitrate (mg N/l)	1.77±0.03^{f}	0.50±0.05^{d}	0.70±0.09^{e}	0.30±0.03^{c}	0.16±0.03^{b}	0.13±0.01^{ab}	0.00± 0.00^{a}
	T-DIN (mg/l)	50.87±0.76^{f}	43.63±0.49^{e}	20.37±0.48^{d}	7.96±1.46^{c}	4.11±0.08^{b}	2.51±0.30^{ab}	0.47± 0.00^{a}
	Ortho-phosphate (mg P/l)	16.59±0.74	7.19±0.39	3.26±0.23	1.68±0.28	0.79±0.09	0.30±0.07	0.17± 0.01
6th day	Nitrate-nitrite (mg N/l)	0.08±0.00^{d}	0.09±0.00^{d}	0.07±0.00^{c}	0.08±0.00^{d}	0.01±0.00^{ab}	0.02±0.00^{b}	0.01±0.00^{a}
	Ammonia (mg N/l)	10.06±0.54^{f}	6.05±0.09^{e}	4.54±0.18^{d}	2.28±0.10^{c}	0.90±0.03^{b}	0.94±0.00^{b}	0.00± 0.00^{a}
	Nitrate (mg N/l)	15.47±0.77^{f}	10.68±0.21^{e}	2.76±0.25^{d}	1.31±0.06^{c}	1.04±0.03^{bc}	0.10±0.03^{ab}	0.00±0.00^{a}
	T-DIN (mg/l)	25.61±0.65^{g}	16.81±0.27^{f}	7.37±0.28^{e}	3.68±0.06^{d}	1.96±0.04^{c}	1.06±0.02^{b}	0.02± 0.00^{a}
	Ortho-phosphate (mg P/l)	7.22±0.30^{e}	2.73±0.08^{d}	1.15±0.01^{c}	0.58±0.12^{b}	0.28±0.04^{ab}	0.14±0.02^{a}	0.01± 0.00^{a}
9th day	Nitrate-nitrite (mg N/l)	0.05±0.01^{bc}	0.05±0.00^{bc}	0.05±0.00^{bc}	0.07±0.01^{c}	0.03±0.01^{b}	0.04±0.01^{b}	0.00± 0.00^{a}
	Ammonia (mg N/l)	5.10±0.25^{d}	3.46±0.12^{c}	1.42±0.16^{b}	1.11±0.03^{b}	0.15±0.03^{a}	0.14±0.01^{a}	0.00± 0.00^{a}
	Nitrate (mg N/l)	5.19±0.03^{d}	3.55±0.46^{c}	1.12±0.06^{b}	0.20±0.05^{a}	0.17±0.01^{a}	0.03±0.01^{a}	0.00± 0.00^{a}
	T-DIN (mg/l)	10.34±0.25^{e}	7.05±0.38^{d}	2.59±0.16^{c}	1.37±0.06^{b}	0.35±0.02^{a}	0.20±0.02^{a}	0.01± 0.00^{a}
	Ortho-phosphate (mg P/l)	2.23±0.06^{d}	0.34±0.06^{c}	0.13±0.00^{b}	0.10±0.01^{ab}	0.06±0.02^{ab}	0.03±0.01^{ab}	0.00±0.00^{a}
12th day	Nitrate-nitrite (mg N/l)	0.02±0.01^{d}	0.01±0.00^{bcd}	0.01 ±0.00^{bcd}	0.02±0.00^{cd}	0.01±0.00^{bc}	0.00±0.00^{ab}	0.00± 0.00^{a}
	Ammonia (mg N/l)	3.10±0.04^{c}	0.74±0.33^{b}	0.32±0.05^{a}	0.15±0.03^{a}	0.05±0.02^{a}	0.01±0.00^{a}	0.00± 0.00^{a}
	Nitrate (mg N/l)	1.23±0.03^{c}	0.38±0.09^{b}	0.02±0.01^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.00± 0.00^{a}
	T-DIN (mg/l)	4.35±0.07^{c}	1.13±0.35^{b}	0.35±0.06^{a}	0.17±0.04^{a}	0.07±0.02^{a}	0.02±0.00^{a}	0.00±0.00^{a}
	Ortho-phosphate (mg P/l)	0.49±0.09^{b}	0.04±0.01^{a}	0.02±0.01^{a}	0.01±0.01^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00± 0.00^{a}

Table 3. Total dissolved inorganic nitrogen (T-DIN) and ortho-phosphate (OP) removal by *W. globosa* in different treatments

Parameter	Treatment						
	RF	RF/2	RF/4	RF/8	RF/16	RF/20	Control
T-DIN (Zero day) (mg/l)	72.03	66.57	39.66	12.85	6.39	4.67	1.20
T-DIN (End of experiment) (mg/l)	4.35	1.13	0.35	0.17	0.07	0.02	0.00
T-DIN removal (mg/l)	67.68	65.44	39.31	12.68	6.32	4.65	1.2
T-DIN removal per day (mg/l/day)	5.64	5.45	3.28	1.06	0.53	0.39	0.10
T-DIN removal rate (%/day)	7.83	8.19	8.26	8.22	8.24	8.30	8.33
T-DIN removal efficiency (%)	93.96	98.30	99.12	98.68	98.90	99.57	100.00
OP (Zero day) (mg/l)	27.12	13.68	6.51	3.45	1.38	1.17	0.32
OP (End of experiment) (mg/l)	0.49	0.04	0.02	0.01	0.00	0.00	0.00
OP removal (mg/l)	26.63	13.64	6.49	3.44	1.38	1.17	0.32
OP removal per day (mg/l/day)	2.22	1.14	0.54	0.29	0.12	0.10	0.03
OP removal rate (% / day)	8.18	8.31	8.31	8.31	8.33	8.33	8.33
OP removal efficiency (%)	98.19	99.71	99.69	99.71	100.00	100.00	100.00

Table 4. The physicochemical parameters of water and chlorophyll content as affected by different treatments

Treatment	Ranges of physico-chemical parameters				
	Temperature (ºC)	pH	Total alkalinity (mg/l)	Total hardness (mg/l)	Chlorophyll (µg/l)
RF	31.21	6.2-8.4	96.00-92.67	150.00	1.55-5.96
RF/2	31.43	6.3-8.9	83.33-76.67	120-108.67	4.29-22.50
RF/4	31.54	6.3-10.0	73.33-60.00	86.67-72.00	35.97-157.05
RF/8	31.46	6.3-9.9	73.33-41.33	74.67-68.00	133.20-189.04
RF/16	31.48	6.3-10.3	76.00-40.00	60.67-50.67	31.35-109.93
RF/20	31.59	6.3-10.0	77.33-40.00	52.67-48.00	58.06-171.70
Control	31.50	6.3-8.0	73.33-38.67	34.67-33.33	22.81-5.45

It is concluded that *W. globosa* is capable of nutrient removal from the culture media. The high nutrient removal efficiency by vegetative fronds was 99.57-100% T-DIN and 100% Ortho-phosphate. Thus, *W. globosa* can grow very well in artificial conditions under natural sunlight and it is a useful weed, suitable for high nutrient removal due to its rapid growth rate.

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