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Lemna gibba and *Azolla filiculoides* for sewage treatment and plant protein production

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ABSTRACT

Application of duckweed (*Lemna gibba*) and water fern (*Azolla filiculoides*) for nutrients recovery and biomass production using municipal sewage supplemented with nitrogen and phosphorous has been investigated. At a temperature range of 15-26 ^{o}C results of daily biomass production showed a significantly higher rate for *Azolla* (4.8-5.9 g dry weight (dw)/m^2.d compared to the duckweed (2.1- 5.3 g dw/m^2.d) with corresponding monthly dry weight production of 1.3 and 0.9 ton/ha for *Azolla* and duckweed, respectively. The estimated value of the monthly dry weight production is LE 3250 and 2250 respectively (US $ = LE 7.8). An average protein content of duckweed and *Azolla* is 26.9% and 25.6%, respectively which make them competitive for many commercial fish and poultry feed ingredients in Egypt. The duckweed and *Azolla* can be used interchangeably in Summer (duckweed) and Winter (*Azolla*) to get temperature range suitable for each plant.

Keywords: *Azolla filiculoides*, *Lemna gibba*, nutrients removal, treatment, wastewater.

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INRTODUCTION

Egypt is one of the water scarce countries in the middle-east since the annual per capita share of water had dropped below 1,000 cubic meters. Even though, Egypt has reached a stage of water poverty, with an average yearly water share of 663 cubic meters per capita, and is expected to fall below 582 cubic meters by 2025. Around 70% of the people in rural Egypt has some type of on-site septic tanks of house vaults [1]. The people are emptying their septic tanks into the nearest water bodies. Even many villages have already built an informal or formal sewer system and discharge the collected untreated effluent into the drains. The deterioration of irrigation water quality deprives Egyptian crops of competitive opportunities in the global markets. The water pollution and its scarcity are the main challenge to the Egyptian government to secure food and water for the rapidly growing population. The Egyptian government has committed itself to reduce poverty and secure food for rural people.

Reuse of the drainage water and treated sewage in irrigation was officially approved and documented in the water resource management plan (2017-2037). Reuse of the treated effluent is one of the solutions to overcome economic constrains and enhance cost recovery for sanitation services [2] in rural Egypt.

Sustainable development of animal, poultry and fish production sectors could be achieved by affording low cost feed. Aquatic plants that have high uptake rates and contain considerable amount of protein, represent alternatives for imported fish feed ingredients. The imported feed ingredients have been accounted for 99% of soybean cake, 97% of soybean seeds and 50% of maize consumed in Egypt [3]. The duckweed plant has been investigated for nutrients recovery and biomass production using municipal sewage [4] and swine wastewater [5].

The duckweed biomass has been used solely as fish feed for tilapia in duckweed/tilapia production system using municipal sewage [6 and 7]. It has also been used as a feed ingredient up to 40% without considerable negative impacts on growth performance of Nile tilapia in intensive production systems [8]. The Azolla cultivation practice is going to be widely considered as low cost cattle feed by dairy farmers [9]. The potential of duckweed (*Lemna gibba*) and water fern (*Azolla filiculoides*) for nutrients recovery and biomass production was explored in this study.

MATERIALS AND METHODS

Experimental setup

In a semi-batch experiment, settled municipal sewage was used to propagate duckweed and Azolla in the outdoor area at National Research Centre (NRC) for a period of 49 days. The experiment was carried out in triplicate using six plastic containers. Each plastic container has a 27.6 cm diameter and 12 cm total depth. Each tank was filled with five litres of settled sewage providing 8.35 cm water depth. A sample of the settled sewage was taken and subjected to laboratory analyses. Plant biomass were collected separately from a polluted water canal located in Giza Governerate. Prior to use the plants were purified from debris and impurities using running tap water and a plastic sieve followed by a total drain of free water from the biomass. Samples of the biomass were taken for laboratory analysis. The duckweed and Azolla biomass were distributed randomly between the culture tanks. Each tank was stocked with the specified individual plant at stocking density of 1000 g fresh biomass/m^2 for Azolla and at stocking density of 1200 g fresh biomass/m^2 for duckweed.

Water sampling and fertilization regime

On weekly basis, the tanks were completed to the initial water level using tap water followed by collecting water samples for laboratory analyses. The tanks were fertilized on days 12, 22, 32 and 42 using nutrient solution containing 19.5 mgNH_4-N/ml and 5 mg P/ml at rate of 5 ml/tank. The nutrient solution was prepared by using ammonium chloride and ammonium dihydrogen phosphate. Analyses of water samples were carried out according to the Standard Methods for the Examination of Water and Wastewater [10].

Harvesting and analyses of biomass and sediment

The duckweed and Azolla biomass were harvested individually on weekly basis using plastic net. One layer of the biomass was left on the water surface. Free water retained in the harvested biomass was totally removed using plastic sieve. Fresh weight of each sample was recorded and dry matter content of the biomass was measured by drying at 70 oC overnight. The biomass was ground and homogeneously mixed. A sample of 0.1 g of the biomass powder was taken and subjected for the analysis of organic nitrogen (ON) using macro-Kjeldahl method [10]. The protein content was calculated using the nitrogen content of the biomass. Protein content = ON content × 6.25 [4]. Another 0.1 g of the biomass powder was taken and subjected for the analysis of total phosphorus using persulfate digestion method followed by vanadomolybdate colorimetric method [10]. By the end of the trial, biomass harvesting was carried out as on regular base to estimate the growth rate followed by total harvesting of the remaining biomass. After complete harvesting of the biomass sediments in the tanks were collected separately and subjected to laboratory analysis. Total dry matter, total nitrogen, and total phosphorus of the sediments were carried out according to the standard methods [10].

Statistical analysis

One-way ANOVA was used to investigate the significance differences ($p<0.05$) between the two treatments including biomass production, quality of biomass, sediment analysis and nutrients mass balance.

RESULTS AND DISCUSSION

Water characteristics

The data in Table (1) show water quality at start up (settled sewage) and end of the experiment. The results show medium strength municipal wastewater with COD and TSS values of 355 mgO_2/L and 167 mg/L, respectively. The concentration of TKN was 51.6 mgN/L with around 66% present as ammonia nitrogen (34.2 mgN/L). The water quality of the tanks at the end of the experiment shows considerable variation in pH between duckweed and Azolla tanks. The duckweed ponds were more vulnerable for algae growth which negatively affect growth performance of the duckweed. The results of TSS and COD which have significant higher values in duckweed ponds indicate the presence of algae and periphyton in the water column of the duckweed ponds. On the other hand, the water quality in the Azolla pond was more better than in duckweed ponds (Table 1) which is attributed to the better growth performance of Azolla and it's inhibition effects on the algae growth in the pond. .

Table 1: Characteristics of settled sewage at start up and end of the experiment

Water quality	Unit	Settled sewage	End of the experiment	
			Lemna gibba	*Azolla filiculoides*
pH	-	7.7	8.0-8.6	7.3-7.8
DO	mgO_2/L	0.8	6.6±0.5	5.8±0.3
TSS	mg/L	167	$105±4^a$	$62±6^b$
COD	mgO_2/L	355	$135±2^a$	$79±5^b$
Ammonia nitrogen	mgN/L	34.2	$2.0±0.3^a$	$1.6±0.2^b$
Nitrate nitrogen	mgN/L	traces	$0.5±0.1^a$	$1.3±0.2^b$
TKN	mgN/L	51.6	$15.1±1.8^a$	$10.8±0.9^b$
TP	mgP/L	2.13	$3.6±0.2^a$	$4.0±0.2^b$

* nm is not measured

Parametric values in the same raw with different superscript letters are statistically significant different

Growth performance and biomass production

It takes one week to start harvesting from the Azolla while in case of duckweed it takes two weeks which indicates one week delay in the first harvest of the duckweed. The results depicted in Figure (1) show better growth performance of Azolla with daily average production range of 73-99 g fresh weight/m^2.d comparing to 39-74 g fresh weight/m^2.d for duckweed. The corresponding total average production rate is significantly higher for Azolla with 86 g fresh weight/m^2.d than duckweed (64 g fresh weight/m^2.d).

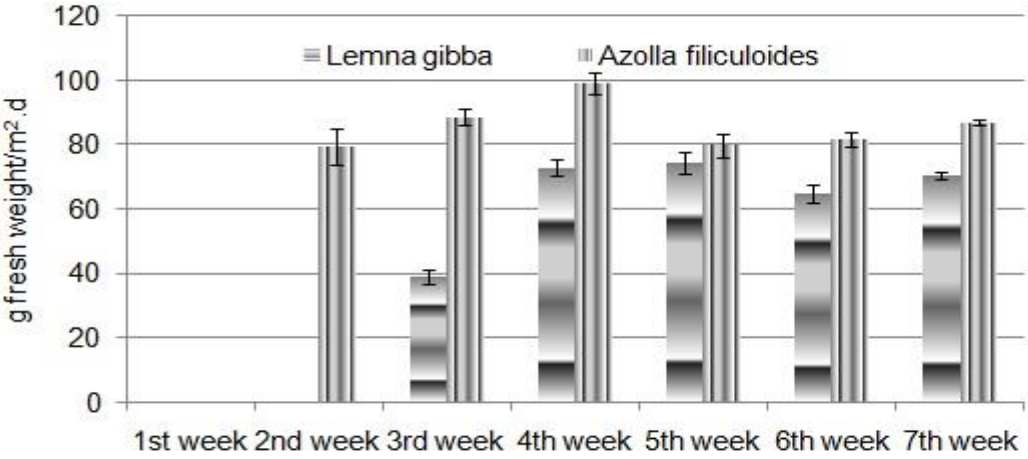

Figure 1: Fresh biomass production in g fresh weight/m^2.day during the experiment

On dry matter basis, the Azolla has significant higher production rates with a range of 4.8-5.9 g dw/m^2.d (Figure 2) and average value of 5.4 g dw/m^2.d. The corresponding dry weight production range of duckweed was 2.1-5.3 g dw/m^2.d (Figure 2) and an average value of 4.0 g dw/m^2.d. These results are in agreement with the report that the Azolla has higher growth rate than duckweed at low temperature and solar irradiation [11]. At low temperature and solar irradiation, Muradov [11] reported a higher growth rate of Azolla sp. (3.3 g dw/m^2.d) comparing to duckweed sp. (2.3 g dw/m^2.d). The high temperature of Summer (14-35 C°) has negative impacts on the growth performance of *Azolla* grown on wastewater from fish farm [12] and it was recommended to utilize the fern to remove ammonia nitrogen from fish farm effluent in Spring and early Summer. *Azolla filiculoides* achieved maximum dry weight production (growth rate) at 22 °C with protein content of 25.9% [13]. The results of Azolla production in this study is higher than the daily growth rate of *Azolla* grown either on municipal wastewater (3.4 g dw/m^2.d) or on mineral synthetic media (2.8-4.6 g dw/m^2) [14].

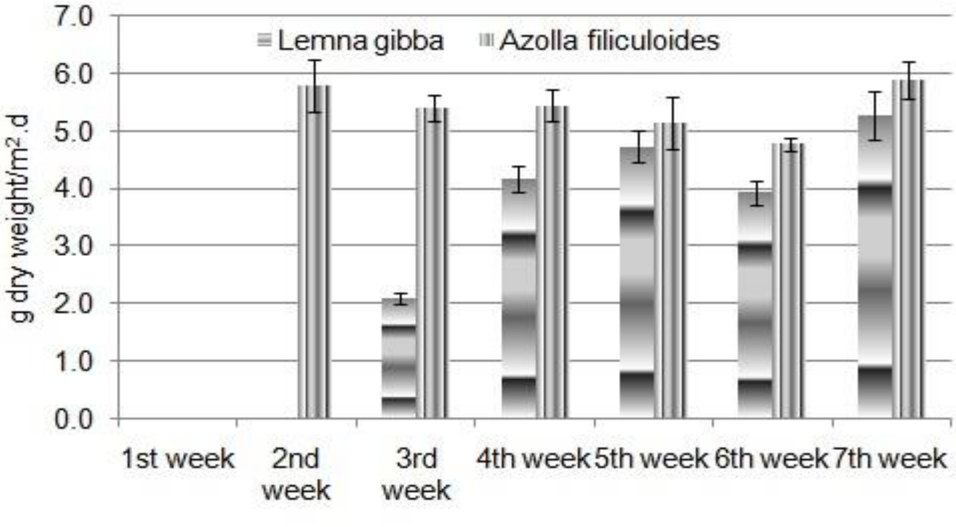

Figure 2: Dry biomass production in g dry weight/m^2.day during the experiment

Also the results of Azolla (4.8-5.9 g dw/m^2.d) in this study are better than the reported average production rate (3.7) of Azolla grown on 2.5% anaerobic digested swine wastewater [11] and dry matter production (2.9 g dw/m^2.d) of Azolla grown on mineral nutrients [15] but less than the reported value of

Azolla cultured on sewage effluent (6.3-13.2 g dw/m^2.d) and Azolla production (11.3 g/m^2.d) grown on mineral media [16 and 17]. On the other hands, Wang and Freemark [18] reported a value of 6.25 g dw/m^2.day for *Azolla* which is little higher than the results of this study.

Maximum growth rate of duckweed (5.3 g dw/m^2.d) in this study is similar to the maximum growth rate (5.4 g dw/m^2.d) of duckweed grown in 10% dilution anaerobic digested swine wastewater [11] and comparable to the results of *Lemna gibba* (3.3 g dw/m^2.d) grown on pre-treated sewage during the winter season [4].

The data depicted in Table (2) shows significant higher values for total and net production rates of *Azolla* comparing to the total and net production rates of duckweed. The calculated average monthly production of *Lemna* and *Azolla* in dry matter is 0.9 and 1.3 ton/ha, respectively. Recently, price of animal feed and animal feed ingredients increase expensively to a level threatening poultry and animal industry. The price of corn and soybean is around LE 3500 while the price of wheat bran as low quality protein ingredient is around LE 2500. Therefore the minimum estimated values of the monthly biomass production is 2250 LE and 3250 LE for both duckweed and water fern respectively.

Table 2: Total and net biomass production

	Lemna gibba		*Azolla filiculoides*	
Item	Total	Net	Total	Net
Fresh weight, g/m^2.d	69± 0.1^a	44.8± 0.1^a	95±1.8^b	74.7±1.8^b
Fresh weight, ton/ha.d	0.69±0.001^a	0.45±0.001^a	0.95±0.018^b	0.75±0.018^b
Dry weight, kg/ha.d	43.4±0.5^a	30.4±0.5^a	57.9±1.0^b	44.4±1.0^b
Protein, kg/ha.d	11.6±0.3^a	8.1±0.3^a	15.1±0.3^b	11.9±0.3^b

Different superscripts letters of parametric values in the two parallel columns indicates significant difference ($p<0.05$).

Characteristics of biomass

The characteristics of the initial biomass of duckweed and Azolla show average dry matter contents of 5.28% and 6.67% for both respectively. The protein and phosphorous content of the biomass were 26.3% and 0.24% for duckweed and 23% and 0.26% for Azolla. The quality of weekly harvest from the ponds is depicted in Table (3). The data show no significant differences between the two plants except for phosphorus which is significantly high in duckweed biomass. In general, both duckweed and Azolla biomass have good results for protein content with average values of 25.6% and 26.9% for Azolla and duckweed respectively. The protein content of Azolla in this study, is similar to the average protein content (25.8%) of *Azolla* collected from natural ponds [19] and higher than the average protein content (21.4%) of *Azolla* harvested from fish pond [20]. In this research, the protein and P content of *Azolla* is lower than reported [14] values of P (0.68%) and protein (35.7%) of *Azolla* grown on sewage while it is higher than the protein (15.6%) and comparable to the P content (0.62%) of *Azolla* grown on sewage [21].

The protein content of the duckweed in this study is better than the reported value of *Lemna gibba* (19.8%-25.7%) grown on pre-treated sewage [4]. The TP content of duckweed and water fern in this study is lower than the reported range (0.68-0.9%) of *Lemna gibba* grown on sewage [4].

Table 3: Quality of *Lemna gibba* and *Azolla filiculoides* biomass

Parameter	Unit	*Lemna gibba*	*Azolla filiculoides*
Dry matter	%	6.2±0.81^a	6.3±0.66^a
Protein content	% of dry matter	26.9±3.7^a	25.6±3.0^a
Organic nitrogen	% of dry matter	4.30±0.59^a	4.10±0.48^a
Total phosphorous	% of dry matter	0.58±0.03^a	0.48±0.03^b

Different superscripts letters of parametric values in the two parallel columns indicates significant difference ($p<0.05$).

Sediment analysis

The results of sediment analyses show significant higher sediment accumulation (3.13 g/m^2.d) in duckweed ponds comparing to the Azolla (2.53 g/m^2.d). This is mostly attributed to the high rate of die off in the duckweed biomass during the initial start of the experiment and to the more proliferation of algae and periphyton in duckweed ponds. The percentage of TN in the sediment was 1.51±0.12 in duckweed ponds and 1.12±0.07 in *Azolla* ponds which are comparable to the published result (1.3-2.0%) of duckweed ponds receiving pre-treated sewage [4].

Nutrients mass balance

The data depicted in Figures (3 and 4) show that the nitrogen recovery recorded significant higher value for the *Azolla* (89%) comparing to the duckweed (67.3%) which represent 0.24 and 0.18 gN/m^2.d for both, respectively. Both plants provide similar total N removal of 0.25 gN/m^2.d which are comparable with the nitrogen uptake rate of *Azolla* grown on the effluent of wastewater stabilization pond (0.19 gN/m^2.d) and *Azolla* (0.16-0.25 gN/m^2.d) grown in mineral media [14]. The nitrogen uptake rate of Azolla in this study is better than the value of *Azolla* (0.16 and 0.25 gN/m^2.d) grown in swine wastewater [14] as well as the reported value of *Lemna gibba* (0.12-0.21 gN/m^2.d) grown on domestic sewage [17]. The results of nitrogen uptake rates of *Azolla* and duckweed in this research is lower than the nitrogen uptake rate of *Lemna gibba* (0.44 gN/m^2.d) grown on sewage during the warm seasons but higher than the maximum value (0.15) during the winter [4] season. The current result is also lower than the nitrogen uptake rate of *Spirodela punctata* (2.03 gN/m^2.d) grown on anaerobic digested swine wastewater [22].

Contribution of N in sediment and final effluent was significantly high in duckweed ponds (9.4% and 17.2%) comparing to the *Azolla* (6% and 10%). This is mostly attributed to the high concentration of algae and periphyton in the duckweed ponds and significant high growth rate of *Azolla*. Finally the unaccounted for of nitrogen was 6.1% in duckweed ponds comparing to - 6.2% in the *Azolla*. The negative value of *Azolla* is attributed to the ability of the *Azolla* to fix nitrogen from the air. The minimum estimated nitrogen fixation rate of the Azolla in this experiment is 20 mgN/m^2.d. The water fern grown in water with limited nitrogen (0.3 mg N/L) concentration [23 and 17] has the ability to fix nitrogen and produce biomass with considerable protein content.

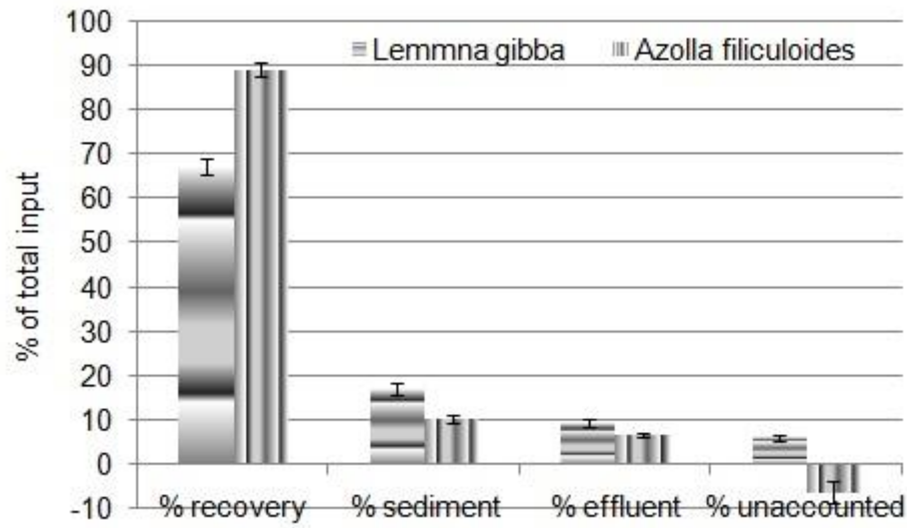

Figure 3: Nitrogen mass balance in *Lemna gibba* and *Azolla filiculoides* ponds

Duckweed ponds recorded 62.5%, 10.5%, 14.9% and 12.1% for P recovery, P in sediment, P in the final effluent and unaccounted for P. The corresponding values of the *Azolla* ponds are 67.5%, 6.8%, 16.7% and 9.0%, respectively. On daily basis the Azolla and duckweed ponds remove 34.3 and 34.7 mgP/m^2.d, respectively. The corresponding recovery ranges of the Azolla and the duckweed are 25.5-28 mgP/m^2.d and 24.1-26.8 mgP/m^2.d, respectively. The range of P recovery of the Azolla in this study is higher than the maximum P uptake rate (21.4 mg P/m^2.d) of *Azolla* [24]. The range of P uptake rate of duckweed in this study is comparable to the P recovery range (27-32 mgP/m^2.d) of *Lemna gibba* grown on sewage during the winter [4]. The unaccounted for is mostly attributed to the adsorption of phosphorus to the inner surface of the plastic containers.

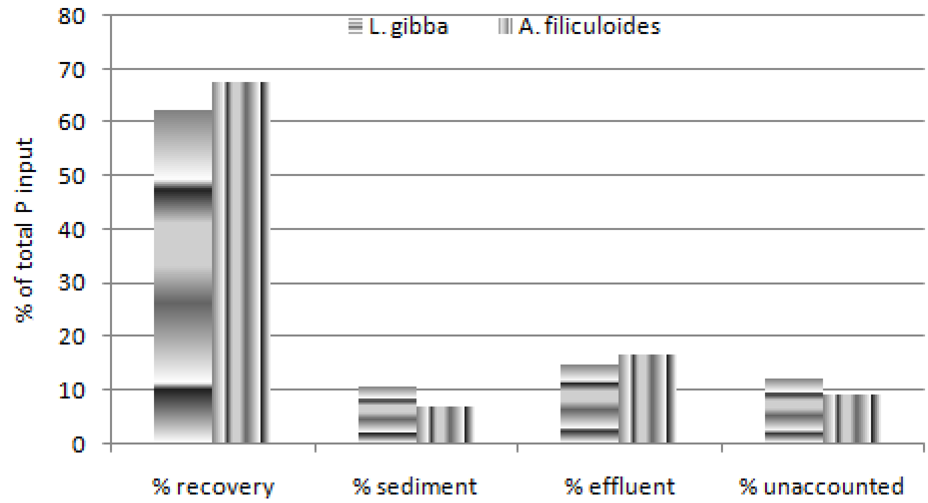

Figure 4: Phosphorous mass balance in *Lemna gibba* and *Azolla filiculoides* ponds

CONCLUSIONS

Application of duckweed and Azolla for nutrients removal and biomass production using municipal sewage at 15-26 ^{o}C is feasible with significant higher production of Azolla. The duckweed and Azolla can be used interchangeably in Summer (duckweed) and Winter (Azolla) to get temperature range suitable for each plant. The duckweed and Azolla has protein content of 26.9% and 25.6%, respectively which makes them competitive protein source for fish, poultry and animal feed industry in Egypt. Monthly productions of duckweed and *Azolla* are 0.9 and 1.3 ton/ha, respectively with estimated value of LE 2250 and 3250 (US$ = LE 7.8) which represents cost recovery of sewage treatment in rural Egypt.

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