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## Utilization of duckweed (DW) in nutrient removal from agricultural waste water and producing alternative economic animal fodder

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### ABSTRACT

Agriculture wastewater can be regarded as a water resource with increasing importance in countries with arid climate conditions and water scarcity like Egypt. Therefore, purification and reuse is an attractive solution which serves a dual purpose: low-cost sanitation and reuse of the available resources. Duckweed are small, floating aquatic plants belonging to the family Lemnaceae. Most common duckweed species reproduce asexually, i.e. without flowers or seeds. Under favorable conditions duckweed can reproduce faster than any other higher land plant (Lemna can double its weight in one day). In this study, DW was used for agricultural drain wastewater purification and for producing an economic animal fodder rich in protein content. The achieved values of nutrient (Phosphorus and Nitrogen) removal from raw water in the effluent of duckweed pond (DWP) operated at 10 days hydraulic detention times (HDT) were 76.9% and 68.3% respectively on average. Whereas, the investigated fresh and dry weight yield were about 745.8 and 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%. The contents of protein and phosphorus of such dry matter were 28.1 % and 0.83 %. The total phosphorus in the dry matter of the duckweed was 0.83 % on average. The results investigated that duckweed is rich in protein and highly digestible; from that perspective it is interesting as fish and cattle fodder. So duckweed can supplement inexpensive feeds like broken rice, rice bran and cassava as an alternative feed ingredient for poultry and fish. Even a partial replacement of livestock feeds with duckweed can save farmers money.

**Key words:** duckweed (DW), agriculture wastewater, nutrient removal, alternative animal feed.

### INTRODUCTION

An adequate protection of the environment is a fundamental right for all living creatures. The disposal of untreated sewage is a serious environmental and public health risk, leading to water source and soil pollution and consequently affects man, animal and plants. The fresh water resources for Egypt are limited and diminishing and scarcity of such resources is underway in few years. In view of the growing needs of the country without corresponding increase in the permissible share for Egypt in the Nile water (55 billion m<sup>3</sup>/year), it is imperative to rationalize and preserve the current resources. The use of reclaimed wastewater for irrigation provides the answer to the problem, which has been adopted by most countries [1-5].

A stabilization pond (SP) is a shallow earth basin where wastewater is treated by biological processes. SP has a great reputation for its low construction investment, less operation costs, and therefore has found common application in raw municipal wastewater treatment, with the main goals to remove organic material and suspended solids. SP can also be used as post-treatment system for municipal wastewater.

Because of the main shortcomings of SP such as very long HRT, low biomass concentration and large land area requirements for pond construction are its further utilization is limited. It is estimated that ponds are suitable for post-treatment only if land is relatively cheap (<\$15/m<sup>2</sup>). Besides, there has been a great need for the improvement of pond performance for organic matter removal. An attached-growth pond system developed by Zhao and Wang (1996) may partially overcome these drawbacks and provide a potential post-treatment system.

The use of aquatic macrophytes, such as water hyacinth, duckweed, water Lettuce etc., in wastewater treatment has attracted global attention in recent years [5- 9].

Duckweed species have shown characteristics that make duckweed-based systems very attractive, not only for wastewater treatment but also for nutrient recovery. The reason for this is the rapid multiplication of duck-weeds and the high protein content of its biomass which is about 30-49% of dry weight [10-11].

Duckweed ponds (DWP) are a modified type of stabilization ponds, covered with a floating mat of plants. Duckweed are small, floating aquatic plants belonging to the family *Lemnaceae*. *Lemnaceae* includes 5 genera: *Wolffia*, *Landoltia*, *Spirodela*, *Lemna* and *Wolffiella*. The first 4 genera all grow well on wastewater. Most common duckweed species reproduce asexually, i.e. without flowers or seeds. Under favorable conditions duckweed can reproduce faster than any other higher land plant (*Lemna* can double its weight in one day). The treatment process in duckweed ponds is based on settling of suspended solids and on bacterial activity. Since the floating duckweed mat prevents mixing of the pond by wind action, excellent conditions for settling prevail. Moreover, the duckweed mat reduces solar radiation penetration, suppressing algae growth, yielding a very clear effluent. The bacterial activity is either aerobic or anaerobic, depending on the organic load and oxygen input. The duckweed plants in principal could also contribute to the treatment process by direct assimilation of simple organic compounds such as simple carbohydrates and various amino-acids. Nutrients are removed from the wastewater by several processes, including volatilization of NH<sub>3</sub> and sedimentation of suspended solids with organic nitrogen. Parts of the nutrients are removed by conversion into plant proteins and by harvesting the biomass [12]. Duckweed is small green freshwater plants with fronds from 1 to 12 mm in diameter. They are the smallest and simplest flowering plant and have one of the fastest production rates with doubling time of 2 to 3 days only. This is because all the plant consists of metabolic active cells with very little structural fiber. Some of the specific properties of duckweed are that the plants have the capability of converting degradable pollutants directly into protein rich fodder. The plants can also be used for agricultural fertilization while the effluent is suitable for irrigation. The use of aquatic plants is a constructive approach for ammonia removal from wastewater. Instead of releasing the nitrogen to the atmosphere, it is trapped by the aquatic plants to produce protein rich biomass. The direct conversion of ammonia into plant protein in duckweed pond is a relatively high energy efficient process compared to other alternative methods [5&13-16].

Duckweed has high productivity, large nutrient uptake, easy handling and harvesting, extended growing period, no mosquito problem, high protein content and low fiber content. Under adequate operating conditions the duckweed pond provides quality secondary effluent of accepted quality that meets the irrigation reuse criteria. The annual yield of duckweed is about 55 ton / ha, dry matter [17].

[18] reported that microbial hydrolysis of the more complex organic N and P into NH<sub>4</sub><sup>+</sup> and ortho PO<sub>4</sub><sup>3-</sup> is the limiting step for enhancing biomass production, when using full-scale duckweed-covered sewage lagoon. He added, it is important to provide adequate pre-treatment for sewage to release organically bound N and P. It was mentioned that organic carbon represented in COD affect the efficiency of duckweed pond.

Degradation of organic material was enhanced by duckweed through both additional oxygen supply and additional surface for bacterial growth. The structure of attached bacterial communities and the way oxygen was supplied appear important, because the influence of the living duckweed community could not be simulated satisfactory by artificial surfaces for bacterial growth, by oxygen pumps or by a combination of both[19]. The nitrogen in anaerobic effluent is present mainly as ammonia (NH<sub>4</sub><sup>+</sup>). This is an advantage because duckweed has a preferential up take of ammonium over other sources of nitrogen [20]. Therefore, duckweed can accumulate considerable amounts of nutrients that can be removed by simple and low cost harvesting technologies. The harvesting duckweed may be used as a valuable fish or animal feed [11]. It has been shown that post-treatment of anaerobic effluent in a series of shallow stabilization ponds can reduce the concentration of both helminthes eggs and fecal coliforms below the guideline concentrations of the WHO for unlimited irrigation [21-22]. Duckweed covered systems removed 120-590 mg N/m<sup>2</sup>/d and 14-74 mg P/m<sup>2</sup>/d in three days. Duckweed (*Lemna gibba*). Was directly responsible for 30-47% of the total N-loss by uptake of ammonium and, probably dependant on the initial P-concentrations, for up to 52% of the total P-loss. Duckweed plants have a relatively high nutritional value because the entire plant body consists of metabolically active non-structural tissue [19].

*Lemna gibba* can be used to treat wastewater containing high total ammonia concentration as long as certain pH levels have not exceeded 8.7 and maximum NH<sub>3</sub> concentration levels (8mg N/l), above which duckweed died. He added that at pH of 7.8 a substantial production of 55 kg DW. / ha/d was achieved. Thus wastewater treatment using *Lemna gibba* become impossible at pH levels above approximately 9.8 depending on the temperature [23].

Performance of the duckweed species, *Lemna gibba*; as purifier of domestic wastewater. He found that, the quality of secondary effluents meets irrigation reuse criteria. Also he stated that the annual yield of duckweed, harvested two to three times a week, is about 55 ton / ha, with protein content 30%. Hence by cultivating duckweeds the ammonia in ponds for domestic wastewater treatment is converted into valuable protein rich biomass which subsequently can be used for animal feed or agricultural fertilization [10].

## MATERIALS AND METHODS

### Raw water

Surface water samples were collected from agriculture drain pour in Ibrahemia canal at Benisuif district. Samples were collected as a weekly basis from the points of entry (influent) and exit point after DWP (effluent) to make a continues feeding of the DWP with a hydraulic detention time of 10 days.

After they were collected, the samples were transferred to the analytical laboratory in the Environmental Research Division, Water Pollution Research Department. The analysed parameters included total Kjeldahl nitrogen (TKN), ammonia nitrogen (N-NH<sub>3</sub>), nitrite (N-NO<sub>2</sub>), nitrate (N-NO<sub>3</sub>), total phosphorus (TP), pH, chemical oxygen demand (COD), Biological oxygen demand (BOD), total suspended solid (TSS) and other important parameters using the Standard Methods for Examination of Water and Wastewater [24]. To determine mean values and statistical inference was used to evaluate the results.

### Duckweed pond

A Perspex pond with 10,000 Cm<sup>2</sup> surface area and 50 Cm depth effective volume 500 liters was used. The duckweed species, *Lemna gibba* and *Lemna minor* are both naturally found in Egypt. The growth rate of *Lemna gibba* exceeds that of *Lemna minor* [5,12 and 23 ].While there is no difference in protein content and amino acids profile. *Lemna gibba* was chosen as duckweed species in this study. The drain water effluent was fed to the duckweed pond, operating at hydraulic retention time of 10 days. Table (1) shows the dimensions of the duckweed pond. The duckweed pond was inoculated with *Lemna gibba*, obtained from a local drain at 600 grams fresh duckweed per m<sup>2</sup>. The duck weed biomass was harvested once a week. The thickness of the residual lemna after harvesting was maintained at 600g/m<sup>2</sup> (One Layer). The harvested biomass was drained; weighed and dried in an oven at 70 °C, the dry matter content was calculated.

Table (1) Dimensions of the Duckweed Pond

Dimensions	
Length	100 Cm
Width	100 Cm
Depth	50 Cm
Volume	500 L

### Duckweed analysis

The plant growth rate and yield were monitored once per week in the pond. The thickness of the residual cover after harvesting was maintained at 800 g/m<sup>2</sup> (one layer). The harvested biomass was drained, weighed and dried in an oven at 70°C. The dry matter content was calculated. The dry matter was powdered in a tissue grinder and 0.2 g was used for organic N analysis. The protein content was calculated based on: protein (g/g) = organic N (g/g) x 6.25 [12 and 25]. 0.1g from the powder was taken and burned at 550°C for one hour. The ash was analyzed for phosphorus content using the per-sulphate digestion method [26].

## RESULTS AND DISCUSSION

### Performance of the Duckweed Pond (DWP).

The raw water was fed continuously to a duckweed pond inoculated with *LemnaGibba*, obtained from a local agriculture drain, at 600 grams fresh duckweed per m<sup>2</sup> and HRT of 10 days.

In this study, Table 2 shows the removal ratios from the duckweed pond operated at 10 days HRT resulted in COD removal range between 45.9% and 66.3% with an average value of 58.7%, BOD removal range between 50.4% and 71.9% with an average value of 63.1%, and TSS removal range between 31.1% and 69.6% with average value of 55.7%, respectively (Fig.3). [15]observed COD and BOD removal values for lemna gibba covered mini-ponds of about 63% and 92%, respectively at 20 days total HRT.

The achieved results indicated that the photosynthetic activity of the duckweed raises the pH from 7.3 in the raw water to 8.2 in the DWP effluent.

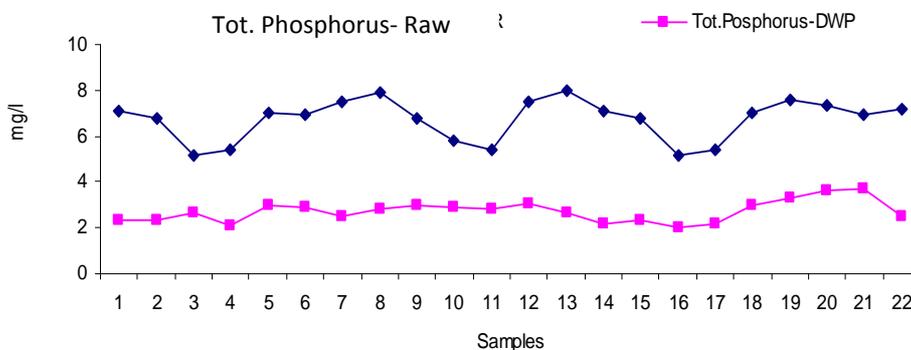
### Nutrients Removal

Nutrient removal was significant in the duckweed pond, particularly for nitrogen and phosphorus. The removal efficiencies were varied from 62.6% to 88.0% with an average of 76.9% for TKN and NH<sub>3</sub>-N removal. While, those for total phosphorus removal were varied from 43.4% to 72% with an average efficiency of 68.3%. Although the concentration of nutrients varied greatly, primarily because of the raw wastewater variations and rain fluctuations that occurred during the experimental period, the efficiency remained high. The median values and standard deviation obtained during the experimental period can be observed in Table 2 and Figs. (1&2).

The recovery of nutrients from the pond might have caused a deficiency in these nutrients, which might affect the fecal coliform removal. [5, 12, 27 and 28]. reported that *Lemnagibba* might serve a dual benefits as an effective environmental reservoir for nutrient removal and producing a protein rich plant with significant fast growth rate that can double its mass within 16 to 48 hrs only. So, it can be suitable for use in animal and fish diets.

**Table (2): Physico-chemical characteristics of raw water and DWP effluent**

Parameters	Unit	Raw water			DWP Effluent			% Removal		
		min.	max.	average	min.	max.	average	min.	max.	average
Temperature	°C	22.0	27.0	24.3	21.5	25.5	23.2	--	--	--
pH		6.8	7.4	7.3	7.2	8.8	8.2	--	--	--
Tot.COD	mg/l	104.0	195.0	144.5	42.0	81.0	58.0	45.9	66.3	58.7
Tot.BOD	mg/l	65.0	137.0	92.4	23.0	52.0	33.6	50.4	71.9	63.1
Ammonia	mg/l	21.0	39.5	30.9	6.0	13.0	8.6	59.8	85.3	74.6
Nitrite	mg/l	0.001	0.050	0.009	0.065	0.170	0.121	--	--	--
Nitrate	mg/l	0.009	0.129	0.040	0.320	0.750	0.500	--	--	--
TKN	mg/l	27.0	55.0	38.2	87.0	15.0	11.0	62.6	88.0	76.9
Tot.Phosphorus	mg/l	6.2	10.1	7.8	2.3	4.7	2.5	43.4	72.0	68.3
TSS	mg/l	37.0	109.0	83.7	21.0	54.0	36.9	31.4	69.6	55.7
VFA	mg/l	13.0	24.0	19.8	7.2	12.0	8.9	30.3	59.1	48.9



**Figure (1) variation of phosphorus in raw water and DWP effluent**

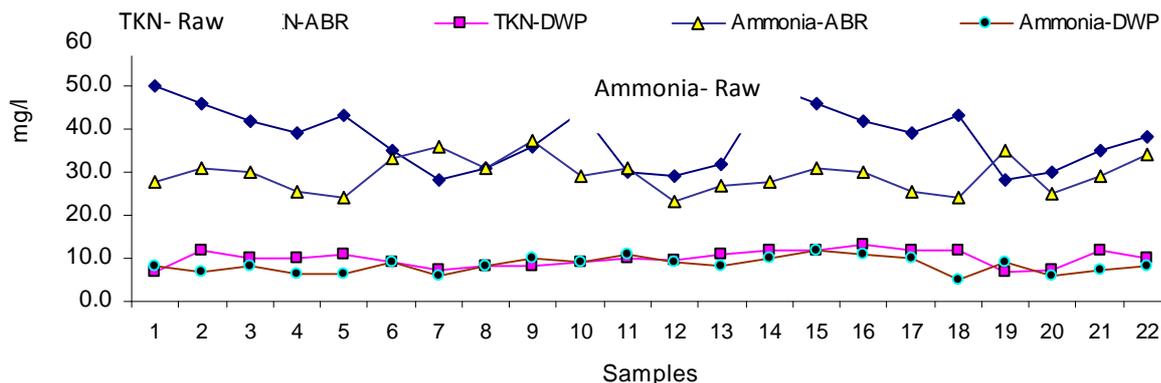


Figure (2) variation of TKN and Ammonia in raw water and DWP effluent

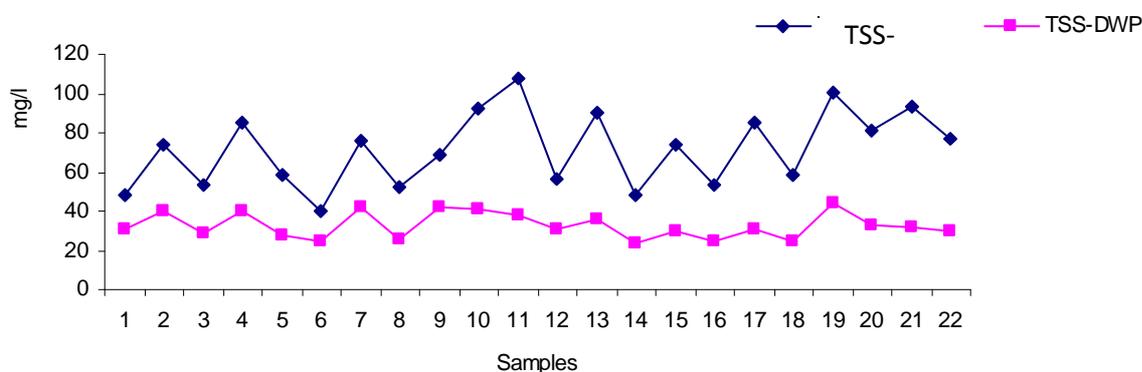


Figure (3) variation of TSS in raw wastewater and DWP effluent

**Using DW as animal fodder**

The environmental benefits from DW that its biomass generated during treatment may contain high nutritional value with high productivity. For over 30 years, researchers have demonstrated the potential use of duckweed in feed for farmed animals. Therefore, because of the substantial growth rate and high protein content, Duckweed production rate is recorded in table (3). Duckweed harvesting was carried out every 6 days. Fresh and dry weight yield were about 745.8 and 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%. The contents of protein and phosphorus of such dry matter were 28.1 % and 0.83 %. The total phosphorus in the dry matter of the duckweed was 0.83 % on average as shown in table 3.

Table (3): Duckweed production and nutrient content in duckweed pond of HDT of 10 days

Parameters	Unit	Min	Max	Average
Fresh yield	Kg/ha/d	1350	2280	745.8
Dry yield	Kg/ha/d	83.7	140.3	108
Dry matter	%dry matter	5.5	7.2	6.1
Protein content	%protein content	24.2	33.7	28.1
Phosphorus content	% P. content	0.59	0.99	0.83

The results investigated that duckweed is rich in protein and highly digestible; from that perspective it is interesting as fish and cattle fodder. So duckweed can supplement inexpensive feeds like broken rice, rice bran and cassava as an alternative feed ingredient for poultry and fish. Even a partial replacement of livestock feeds with duckweed can save farmers money.

Duckweed has the potential to be a competitive source of plant protein for livestock and fish. Low dry matter content and difficulty with harvesting and drying are challenges for those considering producing duckweed.

## CONCLUSION

The duckweed ponds revealed, under the presented conditions, a great potential for polishing and valorisation of nutrient loads in wastewater. The observed nitrogen and phosphorus removal rates were one of the highest reported in the literature.

Duckweed has the potential to be a competitive source of plant protein for livestock and fish. The biomass produced during the treatment showed a high protein content and a fast growth rate. The results investigated that duckweed is rich in protein and highly digestible; from that perspective it is interesting as fish and cattle fodder. Duckweed can supplement inexpensive feeds for poultry and fish.

Thus, this technology should be better exploited to improve the sustainability and reuse of agriculture wastewater and producing an alternative economic animal fodder.

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