CAPACITY OF *LEMNA MINOR* FOR NITROGEN BIOACCUMULATION FROM WASTEWATER OF A RECIRCULATING AQUACULTURE SYSTEM

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Abstract

In order to capitalize on the nitrogen resulting from feed residues and detritus of fish reared in a recirculating system, the technological drainage water was inoculated, in 3 aquariums, with 50 plants belonging to the Lemna minor species. After 31 days, the amount of nitrogen (as nitrate, nitrite, ammonium and ammonia) in the water was quantified. The difference between the initial and final amount of nitrogen compounds is the degree of bioaccumulation of nitrogen in the tissues of Lemna minor. The average absorption reached the value of 70.88% nitrogen accumulated from the aquatic environment. Both the dry mass increased from 4.4 ± 0.1% to 5.1 ± 0.1% and the plant protein content from 39.1 ± 0.4% to 43.6 ± 0.3 %. The plant can be used successfully both to improve the aquatic environment and to transform nutrients from water (nitrogen) into compounds that can be reintroduced into the technological chain (proteins that can be used in fish feed).

Key words: phytoremediation, nutrient accumulation, wastewater, recirculating system

INTRODUCTION

Aquatic plants can be used both to treat polluted water and as an alternative method of converting nutrients from wastewater into useful compounds [4].

Because it has an accelerated growth rate even in unfavourable conditions of the environment, duckweed is successfully used for the accumulation of nutrients and minerals in eutrophic wastewater [8], [15], [16].

Belonging to the Lemnaceae family of monocotyledonous plants, duckweed is a small macrophyte species that floats on the surface of the water, without stems. The body of the plant is represented by a rudimentary organ called thallus, which contains chloroplasts and is capable of photosynthesis. The tissue of the plant is provided with intercellular spaces filled with air, called aerenchyma, due to which the plants float on water surfaces. Duckweed may be rootless or may have one, two simple roots capable of photosynthesis, with the role of absorbing nutrients from water and stabilizing the plant [6].

Based on its wastewater treatment potential, it is assumed that *L. minor* can also be used as a cost-effective and technically feasible option for leachate treatment. Therefore, this study was designed to investigate the growth of duckweed and its effectiveness in removing nutrients (N) from wastewater of a recirculating aquaculture system.

Duckweed has the potential to treat agricultural wastewater by including it in the treatment system, due to its high growth rate, easy harvesting and excellent ability to absorb mineral nutrients.

Due to its high protein content, which can reach up to 45% of the total dry mass of the plant, *L. minor* can be used as a food source for both humans and animals [11].

The increase in world population and the improvement of living standards have led to an increase in the consumption of meat and fish products. It is estimated that global animal production will increase by 13% between 2021 and 2030. In this context, proteins play a key role in animal feed as a source of nitrogen and essential amino acids. Therefore, there is an urgent need to identify

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alternative sources of protein-rich feed components, by capitalizing on agricultural areas or water surface [10], [14].

L. minor has been shown to grow on the surface of wastewater and subsequently produce a high-protein feed component suitable for use in feeding farm animals. *L. minor* can be a solution for protein deficiency, due to the ability of this plant to take nutrients from water and turn them into macronutrients. Unlike crops rich in proteins and starches but which have a large amount of fibre, this plant can be fully exploited due to the lack of supporting tissues.

Nitrogen is fixed in plant tissue through assimilation by leaves and roots, in the form of proteins and essential amino acids. The main forms of nitrogen used by duckweed are nitrate and ammonium. The absorption of ammonium is 3 to 11 times higher than that of nitrates [3]. This makes duckweed effectively used in a wide range of wastewater treatments, as ammonium is an element that is found in significant quantities in domestic and agricultural wastewater [12].

In the present experiment, an attempt was made to capitalize on nitrogen, accumulated in the water of a recirculating system in which fish were reared. Feed residues and debris from the aquatic environment were used by *Lemna minor*, a small floating plant, for the bioaccumulation of nitrogen compounds.

MATERIAL AND METHOD

The plants were harvested from the natural environment, on the surface of Lake Brateș, in Galați County. These were kept for 5 minutes in 1% hydrogen peroxide solution to remove organisms (bacteria, algae, invertebrates) that could interfere with the bioaccumulation process.

The plant material was accommodated for 3 days in aquariums with wastewater from the recirculating aquaculture system. To ensure genetic uniformity, all plant materials used to mount the experiment come from a single *L. minor* colony.

The water used as a culture medium was represented by the technological water with metabolic residues from the recirculating system, belonging to ICDEAPA Galati, used to grow carp.

The experiment was mounted in 4 glass aquariums with dimensions 100×38×34 cm. An aquarium was considered as control, in which only wastewater was added. In experimental aquariums, 50 *L. minor* leaves were used for each aquarium. The volume of water used (38 litres) was 10 cm high, enough to allow the plant's roots to extract nutrients. The water lost by evaporation was replaced with dechlorinated drinking water so as not to alter the dilution of the compounds with nitrogen.

The experiment was carried out in the inner section of the recirculating aquaculture system, with strong indirect natural light, for 31 days.

The analysis of the water used in the experiment, both at the beginning and at the end, were performed using the portable multiparameter with individual probes for physico-chemical parameters from HACH, and for determining nitrogen compounds was used the portable spectrophotometer HACH DR1900 with specific kits for nitrites, nitrogen, ammonium and ammonia.

Wet plant samples were washed with deionized water and dried in an oven at 105°C for about 12 hours to determine the dry weight. The dried vegetable mass was ground and homogenized before being subjected to biochemical analyses.

The biochemical profile of *Lemna minor* was performed using standard analyses for each macronutrient, as follows:

- moisture was determined by official standard analysis methods from AOAC (1990) [1].
- total ash was determined by kiln calcination described by AOAC (1990) [1].
- crude protein content of the samples was determined using the Kjeldahl AOAC method (17th edition), which involved protein digestion and distillation, where F (conversion factor) is equivalent to 6.25 [2].
- total fats were determined using VELP Scientifica SER 148 Solvent Extractor, with modified AOAC ether extraction methods (edition 17) [2].

- both insoluble and soluble fibres were determined using FIBERBAGS SYSTEM from GERHARDT.

The relative growth rate of *L. minor* was calculated using the following equation [13]:

$R = (\ln W_2 - \ln W_1)/(T_2 - T_1)$, where:

- R is the relative growth rate measured as g/(g×day),

- W_1 and W_2 r represents the initial and final weights,

- the difference $T_2 - T_1$ represents the duration of the experiment.

The leaf area was measured using the ImageJ image processing program version 1.53c, pictures were taken of the leaves from the same height both at the beginning and at the end of the experiment.

The wastewater and plants in each aquarium were gently stirred for about 5 minutes each day to ensure homogeneity of the culture medium.

All analyses were performed in triplicate. Statistical analysis was performed using Excel tools. Mean values are reported along with standard deviations. Statistical interpretation of the data was performed according to a significance threshold of $P < 0.05$.

RESULTS AND DISCUSSIONS

The physico-chemical parameters of the water rich in residues, collected at the evacuation of the premises from the recirculated system, are presented in table 1. Due to feed residues and accumulated detritus, nitrogen compounds are found in quantities exceeding the maximum values allowed by the literature. The quantity of nitrates identified at the analysis of the evacuation water of the premises is up to 5 times over the maximum allowed limits, according to the provisions of MMGA Order no. 161/2006.

Also, in table 1 were recorded the values of nitrogen compounds at the end of the experiment.

It can be seen that of the amount of nitrogen compounds removed, only a part is taken up by duckweed. The rest, although in small proportions, remains in the growth medium or is removed by processes such as sedimentation of particles containing nitrogen or by conversion to gaseous forms, such as volatilization or denitrification.

Table 1 Evolution of physico-chemical parameters and nitrogen compounds during the experiment

Physico-chemical determinations / U.M.	Average temperature	Average O_2 dissolved	Average saturation	Average pH	Nitrites NO_2-	Nitrates NO_3-	Ammonium NH_4+	Ammonia NH_3
	ºC	mgO2/l	%	upH	mgN/l	mgN/l	mgN/l	mgN/l
Wastewater - start of experiment	26,3±0,1	8,02 ±0,01	72,0 ±0,5	8,15 ±0,02	0,276 ±0,002	20,8 ±0,28	4,42 ±0,05	0,398 ±0,007
Control aquarium - end of experiment	25,8±0,3	7,99 ±0,02	71,6 ±0,6	8,19 ±0,02	0,271 ±0,002	20,5 ±0,26	4,37 ±0,04	0,382 ±0,007
Aquarium 1 - end of experiment	25,8±0,2	8,01 ±0,01	71,5 ±0,5	8,01 ±0,01	0,071 ±0,001	6,45 ±0,15	0,39 ±0,05	0,112 ±0,004
Aquarium 2 - end of experiment	25,8±0,2	7,92 ±0,02	70,2 ±0,5	8,05 ±0,02	0,073 ±0,001	6,80 ±0,17	0,35 ±0,07	0,109 ±0,008
Aquarium 3 - end of experiment	25,8±0,1	7,95 ±0,02	71,0 ±0,6	8,06 ±0,01	0,069 ±0,001	6,73 ±0,15	0,22 ±0,08	0,133 ±0,010
Max. val. allowed by Ord. 161/2006 for the second quality class	-	7	-	6,5-8,5	-	3	-	-
Max. allowed val. By the literature	-	4-14	-		0,2	4-5	2	0,2

Average ± standard deviation

The percentages of nitrogen compounds that have been removed from wastewater by duckweed are found in Table 2.

The best degree of absorption was reached in aquarium 1 (71.47%), with an average absorption of 70.88%.

In the experiment conducted by Xumeng et al, in 18 days, *Lemna minor* removed from the growing medium 75% of the amount of nitrates, compared to 66.54% in the current experiment and 100% of the amount of ammonium, compared to 91.63% obtained in current experiment [8]. Indeed, ammonium has been absorbed from the environment to a large extent.

Table 2 Degree of elimination from the environment of nitrogen compounds present in wastewater, by the *Lemna minor*, after 31 days

Percent %	Aquarium 1	Aquarium 2	Aquarium 3
Nitrites NO_2-	72,46±0,21	71,74±0,23	73,19±0,20
Nitrates NO_3-	67,55±0,18	65,87±0,15	66,20±0,11
Ammonium NH_4+	90,05±0,11	90,95±0,12	93,89±0,07
Ammonia NH_3	67,84±0,20	68,59±0,23	62,56±0,18
Total nitrogen	71,47±0,17	70,24±0,14	70,94±0,12

Average ± standard deviation

Further research into the toxicity of nitrogen compounds present in the aquatic environment to commercially viable species (fish, crayfish, mussels, etc.) is needed to establish a circular system in which duckweed is not only used to improve residual water, but also included in feed diets for the same animals [5].

From a biochemical point of view, an increase in the amount of protein can be observed, justified by the accumulation of nitrogen from the culture medium (Table 3).

The amount of protein increased on average by 4.46% at the end of the experiment in the plants from the 3 aquariums, compared to the plants at the beginning of the experiment.

Ge X. et al obtained a higher percentage of lipids (8.7% ± 0.6) and a comparative percentage of ash (17.7% ± 0.1) [8].

The high ash content indicates an excellent ability of *L. minor* to recover minerals.

Table 3 Biochemical composition of *Lemna minor*, at the beginning and at the end of the experiment

Chemical composition (% dry matter)	Plants at the start of the experiment	Aquarium 1	Aquarium 2	Aquarium 3
Dry matter	4,4±0,1	5,2±0,1	5±0,1	5,1±0,1
Crude protein	39,1±0,4	43,6±0,3	43,6±0,3	43,5±0,4
Crude fat	5,3±0,1	4,6±0,3	4,9±0,2	4,9±0,3
Acid detergent fibre	17,4±0,2	16,1±0,2	16,4±0,2	16,1±0,2
Non-fibre carbohydrate	19,6±0,2	16,6±0,2	17,2±0,1	16,9±0,2
Ash	18,1±0,1	17,6±0,1	17,6±0,1	17,6±0,1

Average ± standard deviation

Lemna minor can double its biomass in 2 days, if the climatic conditions and the nutritional profile of the growing environment are within the optimal range [7].

Duckweed follows, however, an exponential growth rate [17].

In the first 6-7 days, the plant had a slower growth rate. After day 7 and until day 22, the growth was exponential, and from day 22 until the end of the experiment (day 31) the plant growth was linear. This evolution of foliar development can be found in figure 1.

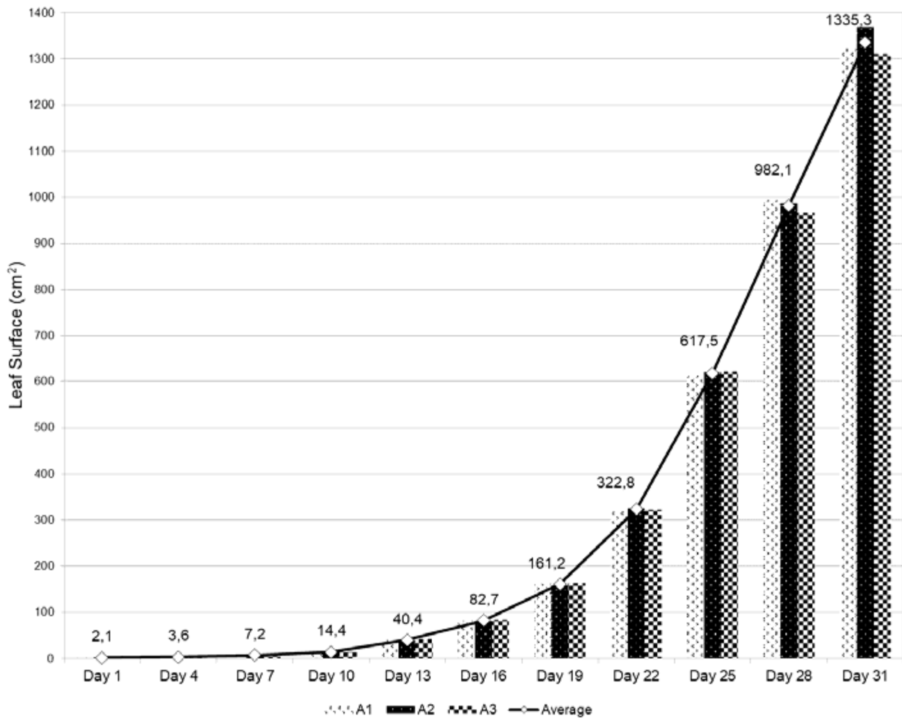

Figure 1 Leaf surface development of the species *Lemna minor*, after 31 days

By calculating the relative growth rate, it is possible to understand the well-being of the plant in the environmental conditions of the experiment.

The values obtained during the experiment are presented in table 4. The average relative growth rate of *Lemna minor* is 0.208 g/(g×day) in the 3 aquariums, in which duckweed was grown in wastewater from a recirculating aquaculture system. This value is lower than that obtained by Hodgson, who in an optimal culture environment reached a plant growth rate of 0.39 g/(g×day) [9].

This low rate obtained in the present experiment can be explained by an incipient inhibition of plants by high concentrations of nitrogen compounds.

Table 4 Relative growth rate of the plant throughout the experiment

	U.M.	Aquarium 1	Aquarium 2	Aquarium 3
Relative growth rate	g/(g×day)	0,209	0,209	0,207

CONCLUSIONS

- Lemna minor is a species of floating plant that can be used successfully to extract nitrogen from wastewater, from a recirculating system in which fish is reared.
- Due to the high protein content in the dry mass of the plant, it can be an important component in feed diets for different species of fish.

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