RESEARCH PAPERS
FACULTY OF MATERIALS SCIENCE AND TECHNOLOGY IN TRNAVA
SLOVAK UNIVERSITY OF TECHNOLOGY IN BRATISLAVA

10.1515/rput-2015-0012 2015, Volume 23, Number 36

COMPREHENSIVE STUDY OF DUCKWEED CULTIVATION AND GROWTH CONDITIONS UNDER CONTROLLED EUTROPHICATION

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Abstract

The paper discussed the issue of eutrophication. The most conspicuous effect of eutrophication is the creation of dense blooms of noxious, foul-smelling phytoplankton that reduce water clarity and harm water quality. Nutrient concentration, temperature and pH of the water largely influence the growth rate and composition of duckweed in general, but it can be said that the temperature and solar irradiation are the most important factors. In order to compare the rate of biomass increase of duckweed biomass in natural conditions and in a laboratory grown sample was analysed by spectrophotometric methods in UV/VIS region (Spectrophotometer GENESYS^{TM}) for the selected nutrients such as ammonium, ammonium nitrogen, nitrite, nitrate, and phosphate.

Key words

duckweed, Lemna minor, cultivation, eutrophication, pollution

INTRODUCTION

After the initial debate on which nutrient is primarily responsible for limiting productivity in lakes and rivers, known as the limiting nutrient controversy, freshwater scientists have largely concentrated on P as a key element in controlling eutrophication (1). Many rivers and lakes have experienced anthropogenic changes in morphology, such as building of weirs, dams and bank engineering structures that can cause changes in water flow. These modifications can create large zones of ‘dead’ water where phytoplankton as well as other water plants have been observed. Changes in runoff regime, water temperature and associated physico-chemical alterations, as a result of climate change, climate instability, further land use change and modifications to the hydrologic cycle are also expected to exacerbate eutrophication (1).

Duckweed is the common name given to the simplest and smallest flowering plant that grows ubiquitously on fresh or polluted water throughout the world. They have been botanical

curiosities with an inordinate amount of research aimed largely at understanding the plant or biochemical mechanisms. Duckweeds have great application in genetic or biochemical research. This has been more or less in the same way that drosophila (fruit flies) and breadmould have been used as inexpensive medium for genetic, morphological, and physiological and biochemical research. Duckweeds are small, fragile, free floating aquatic plants. However, at times they grow on mud or water that is only millimetres deep to water depths of 3 metres. Their vegetative reproduction can be rapid when nutrient densities are optimum. They grow slowly where nutrient deficiencies occur or major imbalances in nutrients are apparent. They are opportunistic in using flushes of nutrients and can put on growth spurts during such periods.

Duckweeds belong to four genera; *Lemna, Spirodela, Wolfia* and *Wolffiella*. About 40 species are known worldwide. All of the species have flattened minute, leaflike oval to round "fronds" from about 1 mm to less than 1 cm across. Some species develop root-like structures in open water which either stabilise the plant or assist it to obtain nutrients where these are in dilute concentrations (2).

Fig. 1 *Lemna minor (3), (4)*

Each plant consists of a flattened oval rounded green leaves with serrated leathery consistency. Duckweed prefers cooler temperature and a pH below 4 (3). Its simple cultivation under laboratory conditions, simple structure, asexual reproduction and short playing time are the main reasons why the organism is also used in toxicity tests. It is also used for the toxic effects of samples of different types of waste water and sludge samples from river sediments, that is, the sample is usually turbid with large amounts of suspended matter (5).

The growth rate and the chemical composition of the duckweed are highly dependent on the concentration of minerals in the water, pH, temperature, as well as solar radiation. The water content in the body duckweed is between 86 % and 97 %. The solid fraction from natural colonies growing duckweed poor in nutrients to the water is between 15 to 25%; and the protein content is from 15 % to 30 %. Duckweed grown in ideal conditions can have a fibre 5 – 15 % and a protein content of 35 – 45 %, depending on its type (6). Some cells of *Lemna minor* (Figure 1) are likely to contain calcium oxalate. Compared with most plants, it contains a greater amount of essential amino acids and trace minerals that predispose duckweed as a supplementary feed for animals. In Thailand, even also used as food for humans (2).

Part of the objective was therefore to determine the content of ammonium ions NH_4^+, NO_3^- nitrate, nitrite NO_2^- and PO_4^{3-} phosphates in water samples from receiving waters. For the quantitative evaluation of the calibration line were used methods in (7).

MATERIALS AND METHODS

Nutrients in water samples were determined by the UV-VIS spectrophotometric method at *Spectrophotometer GENESYS^{TM}* by STN ISO or EN standards. Analysis of surface water from the site of collection of natural duckweed was performed always up to two days after collection. Terrain variables - temperature in °C and pH were determined on the spot. Natural *Lemna minor* as well as water simple from receiving waters were collected in the period of March 2012 - September 2013 of the seepage channel "NPR Trnavské rybníky" (GPS coordinates 48° 21'27.16 "N, 17° 33'30" E).

Ammonium ions NH_4^+

Ammonium ions were determined in the water samples collected during each collection duckweed according to STN ISO 7150-1 (75 7451) Water quality. Determination of ammonium. Part 1: Manual spectrometric method. For quantitative evaluation calibration line by (7) was used.

Nitrate NO_3^-

Nitrate was determined according to STN ISO 7890-3 (75 7455) Spectrophotometric determination of nitrate NO_3^- sodium salicylate from each water sample collected during the duckweed collection. Manual spectrometric method. For quantitative evaluation calibration line by (7) was used.

Nitrite NO_2^-

Nitrite was determined according to EN 26777 (75 7438): The spectrophotometric determination of nitrite NO_2^- sulfanilic acid and α-naphthylamine in each of the water samples taken during collection duckweed. Manual spectrometric method. For quantitative evaluation calibration line by (7) was used.

Phosphates PO_4^{3-}

Phosphate content was determined according to ISO 6878 (75 7465): Spectrophotometric determination of phosphate PO_4^{3-} by ammonium molybdate of each water sample collected during collection of duckweed. Manual spectrometric method. For quantitative evaluation, calibration line by (7) was used.

RESULT AND DISCUSSION

In order to compare the rate of biomass increase of duckweed in natural conditions and in a laboratory grown sample was analysed for selected nutrients such as ammonium, ammonium nitrogen, nitrite, nitrate and phosphate.

Table 1 show the detected concentration of nutrients for the period from March 2012 to September 2013, with the exception of the growing season. The average temperature during the collection period was around 20 °C and pH 6.5. The content of ammonium and ammonia nitrogen was about 0.43 mg L^{-1}, nitrate content was very moving around 0.55 mg L^{-1}, nitrites were relatively low around 0.0034 mg L^{-1} because they are unstable and surface water passes quickly to nitrates. Of all monitored ions, phosphates were highest at around 1.09 mg L^{-1}.

THE CALCULATED VALUES OF THE CONCENTRATION OF NUTRIENTS IN SAMPLING WATER

Table 1

Number sampling	NH_4^+ [mg L^{-1}]	N-NH_4^+ [mg l^{-1}]	NO_3^- [mg L^{-1}]	NO_2^- [mg L^{-1}]	PO_4^{3-} [mg L^{-1}]	pH	Temperature [°C]
1	0.2321	0.1802	0.3001	0.0037	1.2336	6.5	18
2	0.4045	0.3141	0.2229	0.0011	0.8388	6.5	18
3	0.4558	0.4540	0.8654	0.0039	0.9651	6.8	19
4	0.3976	0.3489	0.6541	0.0031	1.0286	6.5	20
5	0.4017	0.7639	0.7672	0.0056	1.7650	6.0	20
6	0.4378	0.3539	0.5873	0.0039	1.0498	6.5	24
7	0.4876	0.7641	0.5409	0.0032	0.9761	6.7	24
8	0.4945	0.2998	0.6019	0.0043	0.6541	6.7	20
9	0.5339	0.3409	0.5382	0.0029	0.7651	6.5	18
10	0.5086	0.4146	0.5004	0.0019	1.6764	6.5	18
Average	**0.4354**	**0.4234**	**0.5578**	**0.0034**	**1.0953**	**6.5**	**20**

As mentioned above, the nutrient concentration, water temperature and pH are greatly influenced by the growth rate and composition of duckweed in general, but it can be said that the temperature and solar irradiation are the most important factors (1). Table 1 shows optimal pH for cultivation duckweed can be in range in 6.5 – 7. The optimal temperature is from 25 °C to 30 °C, average temperature of the water was about below 20 °C. Differences between the measurements may be caused by weather conditions. To monitor the increase of biomass in natural conditions, the tracking area of three 20 x 20 cm was determined, in which strong healthy plants with the number of petals 6, 5 and 4 were placed. In a similar laboratory experiment, the biomass of duckweed with the number of petals 10, 6, and 4 of Hoagland's culture medium was examined. The experiment was conducted for the time of 10 days, during which there was the increase of natural biomass in average of 10 new leaves, or 2.9-fold increase in the total biomass, and in the case of biomass in the room by in average of 15.7 times. The largest increase and an average of 15 new leaves were observed after the first two days of laboratory culture.

For the concentrations of the components Hoagland's medium, see Table 2. In addition, the culture medium contains the optimal ratio of elements such as sulfur, magnesium, bromine, chlorine, zinc, sodium, copper and iron, which help the growth of duckweed. As apparent from the measurement results, the presence of nutrients N and P has the greatest impact on eutrophication.

COMPOSITION OF HOAGLAND CULTIVATION MEDIA Table 2

Element	Concentration [g L^{-1}]	Element	Concentration [g L^{-1}]
NO_3^-	87.590	**B**	0.500
PO_4^{3-}	122.880	**Mn**	0.460
S	24.012	**Cl**	0.333
Mg	32.000	**Na**	0.009
Ca	40.000	**Mo**	0.040
Fe	0.100	**Cu**	0.023
Zn	0.050	**K**	90.000

CONCLUSION

The difference between the rates of increase in biomass in laboratory culture is due to the better conditions for its growth. The only method that has proven success in reducing the eutrophication of lakes and rivers is reducing the input of phosphorus. However the technologies for wastewater treatment to reduce P and N, must be also taken into consideration. The first step should be the reduced use of fertilizers, handling manure, soil conservation practices, and restoration of wetlands.

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doc. RNDr. Iveta Marková, PhD.