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Biorefinery of phosphorus from eutrophic water: A circular economy approach

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Kisvárdai Bessenyei György Highschool

„Stockholm Junior Water Prize – Hungarian competition, 2024"

Stockholmi Ifjúsági Víz Díj
magyar verseny

"Az ifjúság a víz jövője"

GWP Magyarország Alapítvány

SIWI STOCKHOLM JUNIOR WATER PRIZE

Kisvárda

2024

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Abstract

Phosphorus is a vital nutrient for plant growth, playing a crucial role in various physiological processes, including energy transfer, nucleic acid synthesis, and root development. As an essential component of DNA, RNA, and ATP, phosphorus is indispensable for the overall health and productivity of plants. Despite its significance, phosphorus is considered a non-renewable resource with limited global reserves. The primary source of phosphorus is phosphate rock, and the extraction and production processes are energy-intensive. The finite nature of phosphorus resources raises concerns about future availability and sustainability, emphasizing the need for efficient phosphorus management. Thus, the present study, aims at benefit from the phosphorus present in eutrophic water through two aquatic plants (i.e., *Pistia stratiotes* and duckweed). This study investigates the feasibility of growing these aquatic plants on different water types. The focus is on observing the growth patterns, leaf protein production, and the generation of brown juice as potential by-products containing high phosphorus content and a possible plant growth biostimulant. The study aims to provide insights into sustainable phosphorus utilization and recovery from eutrophic water within the context of a circular economy.

1. Introduction and Literature Review

Phosphorus is a highly reactive non-metal element essential for life, playing key roles in proteins and nucleotides, e.g., DNA, RNA, and ATP. It is commonly found in fertilizers, detergents, and matches, and is crucial for plant growth and metabolism. In case of lack of phosphorus, the plants become dark green; the edge of the leaves turns yellow, brown and finally dry up. The phosphorus also contributes to the development of cell division (Berendy, 2009). High phosphorus concentration cause blossom and seed formation in an early stage, which can influence the eutrophic process.

Eutrophication is also a significant problem of our Earth. The process affects stagnant waters rich in nutrients (mainly phosphorus and nitrogen). The plants growing on the surface block the path of light to the bottom of the water association, thus the intensity of photosynthesis is significantly reduced. Therefore, the oxygen content of the nutritious waters gradually decreases, which at the same time entails a decrease in biodiversity. Also, anaerobic and toxic species can spread, further worsening the water quality (Harper, 1992).

The excessive amount of nitrogen can drain into soil water and raise its nitrate level or enhance eutrophication. To prevent such negative impacts, some countries such as Denmark has limited the periods of application (Thomsen et al., 2003).

In the wild, duckweed (*Lemna minor*) is an important feed source for fish and waterfowl. Duckweed provides a habitat for certain types of frogs and fish. It also maintains the habitat by providing enough shade to keep the growth of oxygen-robbing algae down.

Pistia stratiotes is a floating aquatic plant. Its leaves spread in a rosette on the water surface, which inhibits algae growth. This spongy, rounded plant has conspicuously indented main veins. It grows in still waters of ponds, ditches, swamps, and slow-moving streams and features

Brown juice can be utilized as a plant growth biostimulant due to its high content of macro- (e.g., K, P, Ca, and Mg) and microelements (e.g., Zn, Fe, and Cu) and bioactive metabolites (Barna et al., 2023). The effects of brown juice on plant growth were tested using several plant species, i.e. for the growth of cock’s comb (*Celosia argentea* var. plumose ’Arrabona’) (Bákonyi et al., 2020), sweet basils (*Ocimum basilicum*) (Kisvarga et al., 2020), and French Marigold (*Tagetes patula* L.) (Barna et al., 2021).

It is shown by literatures above that investigation dealing with reusing of brown juice have already been started. However, the effect of brown juice as biostimulator and its cleaning/treatment had not been examined yet.

2. Objective

In Europe, the European Environment Agency (EEA) reports that diffuse agricultural sources are the primary contributors to phosphorus pollution in rivers and lakes, with phosphorus leaching from agricultural soils being a major concern (EEA, 2020). For instance, agricultural phosphorus losses to surface waters in the United States ranged from 1 to 3 kg P per hectare per year, with higher losses occurring in areas with high phosphorus inputs and erodible soils (Sharpley et al., 1994). Hence, the objectives of this study were to:

- investigate the potential for phytomining phosphorus from aquatic environments employing two promising aquatic plants (*Pistia stratiotes* and *Lemna minor*),
- employ a novel microwave coagulation technique to extract accumulated phosphorus from plant tissues, yielding liquid brown juice suitable for use as a biostimulant for plant growth,
- utilize the harvested plant materials as initial resources for producing leaf protein intended for human and animal consumption.

3. Method and Material

3.1. Experimental layout and location

Pot experiment organized in the Completely Randomized design was conducted under greenhouse conditions in the Biodrome (Biological Plant Breeding and Experimental Greenhouse) at the Department of Applied Plant Biology, University of Debrecen, Debrecen, Hungary.

3.2 Applied treatments and growth conditions

The trial consisted of 5 treatments per plant species. Each 12-L polyethylene pot was filled with 8 liters of various water types: tap water, phosphorus concentrations of 5 mg/L and 50 mg/L, ½ strength Hoagland solution, and wastewater. Phosphorus treatments utilized KH_2PO_4. The composition of the ½ strength Hoagland solution utilized in this study is outlined in Table 1. Wastewater was obtained from biodomes following the cultivation of green pepper plants. Prior to application, the wastewater underwent filtration to eliminate plant debris and growth medium particles. For duckweed experiment, 19.2 g/ pot duckweed fresh weight were used, while for *Pistia stratiotes* experiment, 20.5 g/pot *Pistia stratiotes* fresh weight were used. The experiment extended over 6 weeks, with pots being monitored every three days to record parameters such as phosphorus content, water temperature, pH, electrical conductivity (EC), and water volume (maintained at 8 L by refilling with tap water). Greenhouse temperature and relative humidity were also recorded at 3-day intervals.

Table 1: Composition of ½ strength Hoagland solution	
Chemical	mL/ 8 L
1M KNO_3	160
1M $Ca(NO_3)2.4H_2O$	160
1M KH_2PO_4	32
1M $MgSO_4.7H2O$	64
Microelements solution	32
Preparation of microelements solution	
Chemical	g/ L
H_3BO_3	2,86
$MnSO_4 \cdot H_2O$	1,54
$ZnSO_4 \cdot 7H_2O$	0,22
$CuSO_4 \cdot 5H_2O$	0,08
Na_2MoO_4	0,02
Fe-EDTA	6 mL/L

3.3 Water chemical properties

Phosphorus content, water pH, EC, and temperature were measured before experiment and each three days during the experimental period. The EC was determined using EC-meter (Thermo Scientific, Orion Model 209A+type, Germany). Water pH was measured using pH-meter (SevenEasy pH, Mettler-Toledo GmbH, Schwerzenbach, Switzerland). Water temperature was recorded using Durac digital thermometer (VWR, Budapest, Hungary). Phosphorus contents of water and brown juice were measured by the ascorbic acid method as outlined by Sparks et al (1996) and expressed as mg/ L. Phosphorus contents in plant tissues, leaf protein, and fiber fractions were determined using the ascorbic acid method (Sparks et al., 1996) after digestion by the mixture of HNO_3 and H_2O_2.

3.4 Plant harvest

Upon conclusion of the experimental duration, plants were carefully removed from their respective pots and rinsed with distilled water to eliminate any surface debris. Subsequently, they were placed on absorbent paper towels. The fresh weight of each plant was then measured, after which the samples were transferred to a -20 °C freezer for freeze-drying utilizing a lyophilizer (Alpha 1–4 LSC basic, Martin Christ Ltd., Germany) to determine their dry weight.

3.5 Leaf protein isolation and brown juice production

Only plants cultivated in wastewater were selected for further processing towards leaf protein isolation and the production of brown juice, as the plant materials from the other treatments were relatively small in size (Figure 1). The fresh biomass underwent mechanical pressing to extract green juice and pressed cake (fiber) fractions using a twin-gear juicer machine (Angel Juicer 5500, Eujuicers.com Ltd., Czech Republic). Subsequently, the green juice was subjected to coagulation using a microwave technique at 80 °C. The resulting coagulum was then separated using a 35µm filter mesh, with the brown juice being collected and stored at -20 °C for subsequent analysis. The fresh and dry weights of the fiber and leaf protein fractions were recorded, along with the volume and weight of the brown juice. The buffer-soluble protein (Bradford, 1976) and phosphorus (Sparks et al., 1996) contents in each fraction were also measured.

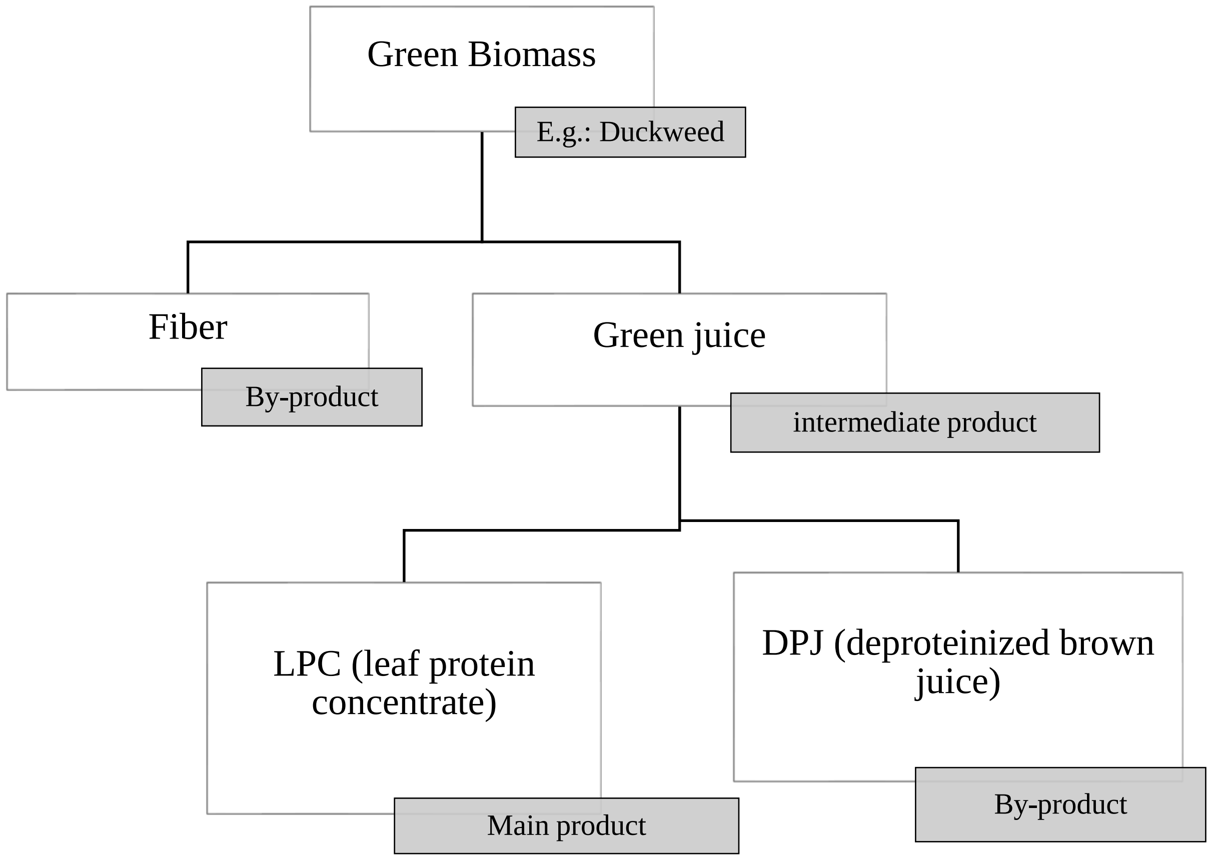

Figure 1. Scheme of the whole process of leaf protein isolation

3.6 Statistical analysis

For the statistical analysis Microsoft Office Excel program was used. The data were averaged with the help of SigmaPlot program. Means were compared by the Tukey's Test.

4. Results and conclusions

4.1 Yields of different fractions of aquatic plants

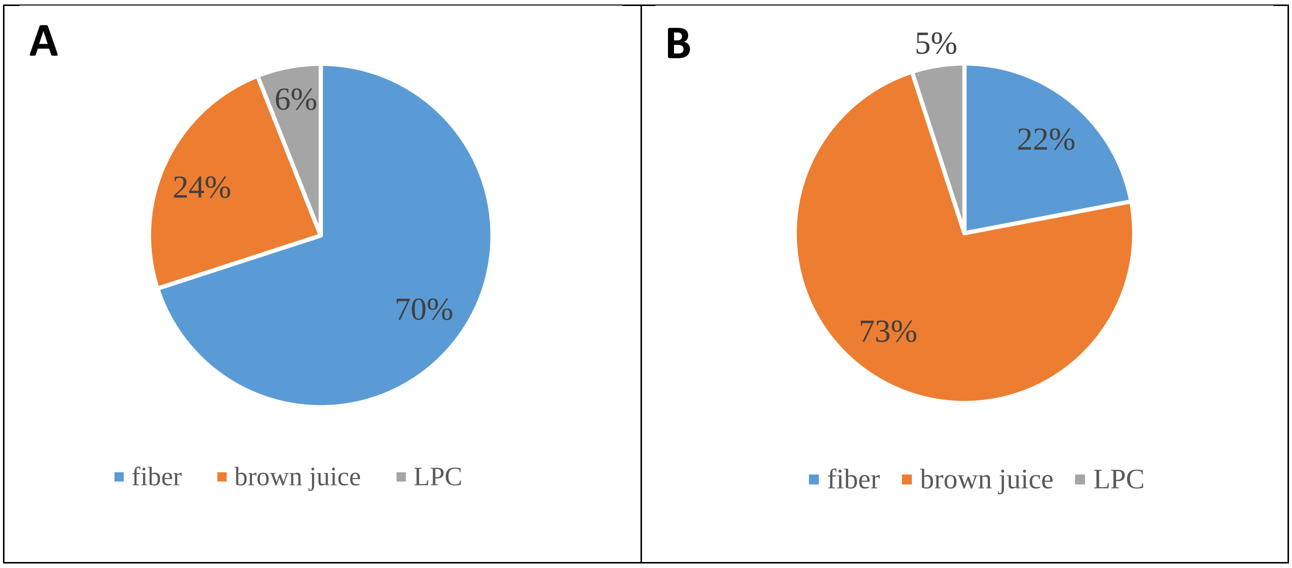

Figure 2.: Yields of different fractions obtained by biorefinery process of fresh biomass of: A) *Pistia stratiotes* and B) duckweed (*Lemna minor*)grown on wastewater

Processing fresh biomass of *Pistia stratiotes* yielded 6% leaf protein concentrate (LPC), 70% fiber, and 24% brown juice (Figure 1A). Fiber, leaf protein, and brown juice fractions represented 22%, 5%, and 73%, respectively (Figure 2B).

4.2 Dry matter content of plants

Except for the tap water treatment, duckweed plants showed higher dry matter content than *Pistia stratiotes* (Figure 3). Both treatments of 5 and 50 mg/L phosphorus displayed the highest dry matter content of duckweed. Regarding *Pistia stratiotes*, tap water treatment exhibited the highest dry matter content, followed by 5 and 50 mg/L phosphorus. These results revealed that the addition of phosphorus in the growth medium enhanced the dry matter content in both aquatic plants.

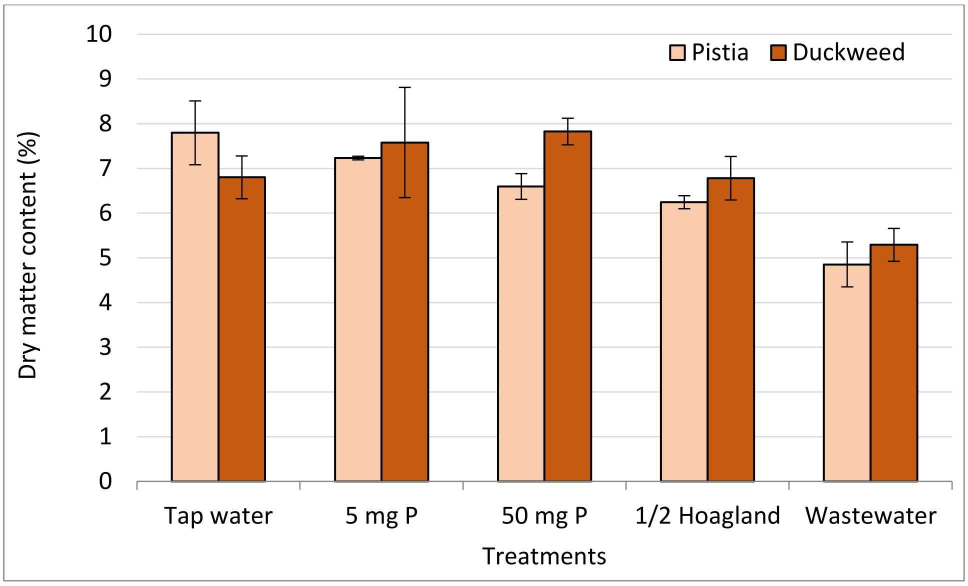

Figure 3: Dry matter content (%) of *Pistia stratiotes* and duckweed (*Lemna minor*) grown on different water types.

4.3 Phosphorus content in different plant fractions

4.3.1 Changes in phosphorus content in water medium during the experiment

Phosphorus content in different water types markedly varied during the experimental period (Table 2). The content of phosphorus at the end of the experiment in the treatment of 5 and 50 mg/L phosphorus dropped to 0.1 and 12.2 mg/L, respectively, (for *Pistia stratiotes*) and to 0.3 and 15.6 mg/L, respectively, (for duckweed). Similarly, the initial concentration of phosphorus in the treatments of ½ strength Hoagland solution and wastewater were 31.4 and 76.3 mg/L, respectively, and reduced to 0.3 and 53.6 mg/ L, respectively, (for *Pistia stratiotes*) and to 1.3 and 50.8 mg/ L, respectively, (for duckweed). These results prove the significance of these two aquatic plants to uptake substantial amounts of phosphorus in different water bodies, supporting the phytomining approach. Also, these findings revealed the fact that phosphorus uptake from water bodies by the aquatic plants is better when other nutrients are present such as nitrogen, potassium and other micronutrients.

Table 2: Variations of phosphorus content in the growth medium (different water types) of two aquatic plants (*Pistia stratiotes* and duckweed) during the experimental period

Treatment	Nov. 30	Dec. 3	Dec. 6	Dec. 9	Dec. 12	Dec. 15	Dec. 18	Dec. 21	Dec. 24	Dec. 27	Dec. 30	Jan. 2	Jan. 5	Jan. 8	Jan. 11	Jan. 19
								Pistia stratiotes								
Tap water	0.0±0.00	0.2±0.02	0.1±0.01	0.1±0.00	0.0±0.00	0.0±0.00	0.0±0.01	0.1±0.12	0.0±0.04	0.1±0.08	0.1±0.04	0.0±0.05	0.0±0.05	0.0±0.00	0.0±0.00	0.1±0.01
5 mg P	5.0±0.08	3.8±0.10	2.4±0.08	1.8±0.08	0.4±0.04	0.5±0.05	0.6±0.07	0.6±0.00	0.6±0.08	0.4±0.08	0.2±0.06	0.4±0.09	0.6±0.08	0.0±0.00	0.0±0.00	0.1±0.08
50 mg P	50.0±0.46	37.2±1.00	35.4±1.20	23.3±0.34	18.7±0.27	12.7±0.21	12.1±0.13	12.0±0.22	11.3±0.15	11.3±0.23	10.2±0.01	11.7±0.22	11.9±0.04	12.3±0.04	12.1±0.04	12.2±0.12
1/2 Hoagland	31.4±0.52	16.7±0.15	6.4±0.08	3.9±0.05	3.6±0.28	2.9±0.01	2.8±0.11	2.5±0.06	1.5±0.00	1.3±0.04	1.2±0.02	1.2±0.09	1.0±0.08	0.0±0.00	0.0±0.00	0.3±0.11
Wastewater	76.3±0.26	69.3±0.46	66.1±0.10	61.2±0.20	60.2±0.05	59.2±0.18	58.7±0.10	55.9±0.06	52.8±0.19	51.9±0.08	50.4±0.08	51.4±0.04	52.2±0.11	52.9±0.04	53.8±0.05	53.6±0.00
								Duckweed								
Tap water	0.0±0.00	0.1±0.00	0.1±0.06	0.2±0.00	0.0±0.00	0.2±0.02	0.0±0.03	0.0±0.01	0.0±0.01	0.2±0.09	0.1±0.06	0.1±0.09	0.0±0.05	0.0±0.00	0.0±0.00	0.0±0.03
5 mg P	4.3±0.08	3.8±0.03	3.1±0.16	2.7±0.01	1.5±0.06	1.3±0.06	1.5±0.12	1.6±0.01	1.4±0.04	1.4±0.04	1.6±0.12	1.5±0.08	1.3±0.15	0.0±0.00	0.8±0.11	0.3±0.11
50 mg P	48.6±0.46	23.1±0.61	22.1±0.27	20.3±0.16	20.3±0.11	19.8±0.30	18.1±0.19	17.9±0.11	12.0±0.57	10.6±0.11	8.5±0.27	10.5±0.08	12.9±0.34	13.6±0.03	13.9±0.18	15.6±0.27
1/2 Hoagland	31.4±0.52	14.1±1.22	4.4±0.19	1.9±0.09	1.9±0.00	1.8±0.10	1.6±0.20	1.6±0.05	1.2±0.00	1.2±0.15	1.1±0.10	1.3±0.05	0.6±0.08	0.9±0.04	1.2±0.05	1.3±0.04
Wastewater	76.3±0.26	66.6±1.19	63.4±0.27	62.1±0.35	60.5±0.41	59.9±0.18	59.6±0.34	58.8±0.29	59.6±0.11	59.8±0.76	61.7±0.87	65.1±0.72	61.3±0.14	52.9±0.67	55.5±0.52	50.8±0.30

4.3.2 Phosphorus content in plant tissues and plants fractions

Results presented in Figure 4 show that duckweed had higher phosphorus content in all fractions compared to *Pistia stratiotes*. The highest phosphorus content corresponded to the treatment of 50 mg/ L phosphorus, while ½ strength Hoagland and wastewater showed almost the same content. After fractionation process and coagulation of leaf protein, significant phosphours content was detected in the three fractions; hwoever, LPC displayed the highest content, followed by fiber and brown juice. Nevertheless, the phosphorus concentration on the brown juice makes it a promising plant growth biostimulant. This result supported strongly our hypotheses and the possible use of these two plants for phytomining of phosphours from water bodies.

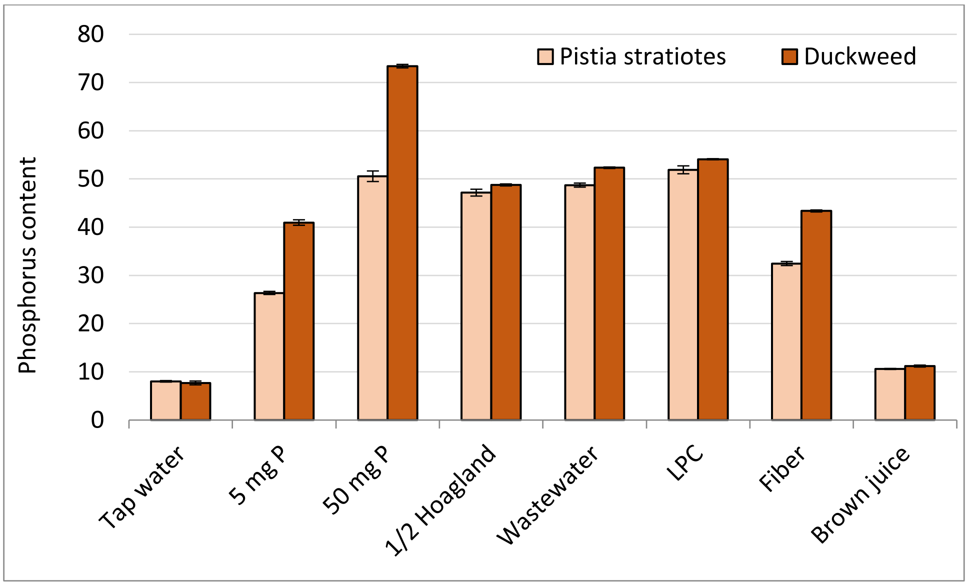

Figure 4: Phosphorus content in plant tissues and LPC and fiber fractions (mg/kg) and brown juice (mg/L) of the two aquatic plants (i.e., *Pistia stratiotes* and duckweed) after growing for 6 weeks on different water types. LPC, fiber, and brown juice fractions were obtained by green biorefinery process on the obtained plant fres biomass of the treatment of wastewater only.

4.4 Buffer-soluble protein contents

4.4.1 Protein content of the two aquatic plants grown on different water types

Results of buffer-soluble protein content of the two aquatic plants after 6 weeks growing on different water types are depicted in Figure 5. Overall, duckweed displayed higher protein content than *Pistia stratiotes* in all treatments, except for the treatemnt of wastewater where *Pistia stratiotes* recorded markedly higher content. Also, the treatment of 50 mg/ L phosphorus revealed the lowest proetin content among all the treatments in both plants. Both 5 mg/ L phosphorus and ½ strength Hoagland treatments exhibited almost the same protein content in both plants.

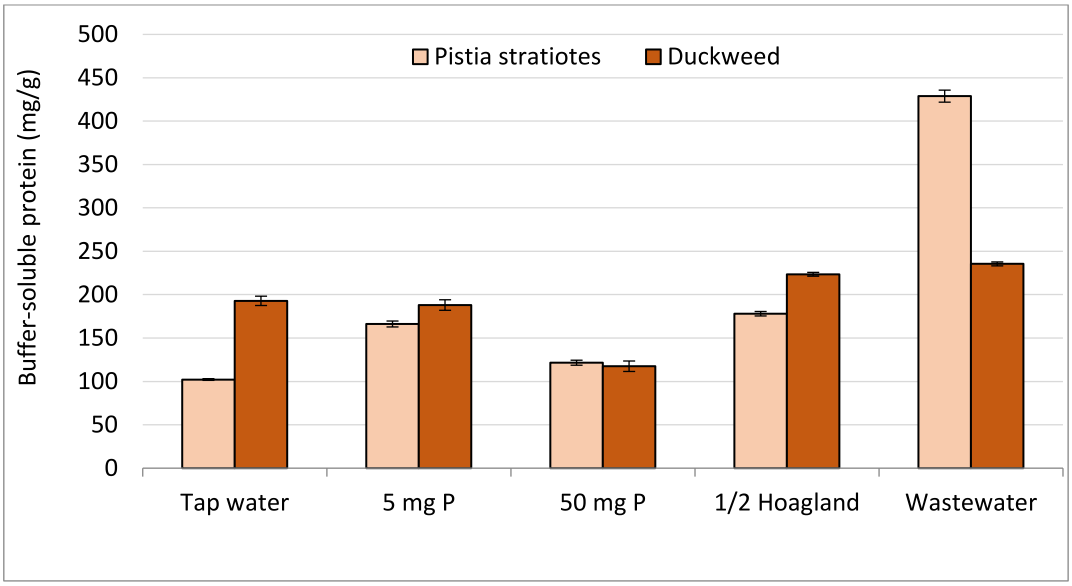

Figure 5: Buffer-soluble protein content in plant tissues (mg/g) of the two aquatic plants (i.e., *Pistia stratiotes* and duckweed) after growing for 6 weeks on different water types.

4.4.2 Protein content of different processed fractions of biomass of plants grown on wastewater treatment

LPC fraction displayed the highest protein content in both plant species with slight increase recorded for *Pistia stratiotes* (Table 3). The fiber fraction was in the second place of the highest protein content with slight increase in the case of duckweed. The lowest protein content corresponded to the brown juice fraction and the two plants showed almost the same content.

Table 3: Buffer protein content in different fractions of two aquatic plants (i.e., *Pistia stratiotes* and duckweed) grown on wastewater for 6 weeks

	Pistia stratiotes	**Duckweed**
LPC	463.5±16.02	452.5±4.23
Fiber	334.3±4.55	349.4±9.52
Brown juice	0.27±0.02	0.24±0.01

5. Summary

As for the phosphorus removing experiment both aquatic plants accumulated considerable amounts of phosphorus in their tissues. *Pistia statories* was more effective in the most case, but as for the wastewater the *Lemna minor* was observed as a better phosphorus removing plant, which can be very useful tool to prevent and mitigate the effect of eutrophication in standing water. The reduction of phosphorus was the highest for the effect of ½ Hogland treatment.

Declaration

This research was conducted at the Department of Applied Plant Biology University of Debrecen. I conducted a research related to different water types. For the experiment 2 aquatic plants, *Lemna minor* and *Pistia stratiotes* I have been studying it for over a year. After reading the literature, I joined to the research group. My task was the measuring of pH, EC, temperature, mass weight and the analysis of data. All research was led by Tarek Alshaal. I have been preparing for the competition with the help of my teachers, Dr. Koncz Gábor, Dr. Konczné Dr. Jámbrik Katalin. They help me to prepare to competitions.

Short biography

My name is Balázs Kristóf, I come from a small village named, Gyulaháza and I have been attending to Bessenyei György Grammar School and Dormitory in Kisvárda. I am in the 10th grade and I study Chemistry and Biology on an advanced level. I can fluently speak English and I have a B2 Language exam in German, as well. For my future plans I would like to study at the University of Debrecen and become a doctor later in life.

Resources

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Acknowledgement

I would like to thank my teachers, Gábor Koncz Ph.D, Katalin Jámbrik, Ph.D, my mentor, Tarek Alshaal Ph.D, assistant professor and Nóra Bákonyi, Ph.D, and Emília Kovács , for the help and preparations.