LEMNA MINOR L. AND *PISTIA STRATIOTES* L. IN THE ACCUMULATION OF TOTAL PHOSPHORUS FROM THE WATER

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Abstract
In this study we wanted to evaluate the absorption capacity of total phosphorus (TP) from water by two aquatic plants (*Lemna minor* L. and *Pistia stratiotes* L.) belonging to the same family (Araceae) which have a huge potential for remediation the water loaded with organic and inorganic substances (total phosphorus, total nitrogen, heavy metals, etc.). Four experimental variants with initial concentrations of total phosphorus in water of 4, 6, 8 and 10 mg/L (TP 4, TP 6, TP 8 and TP 10) at an ambient temperature of 25° C were used to achieve these things. The total amount of phosphorus was quantified at the initial moment and after 72 hours both in water (expressed in mg/L), and in plants (expressed in g/kg of dry matter), respectively. The best results were given by the *Lemna minor* L. plant at an initial phosphorus concentration in water of 6 mg/L.

Introduction
Phosphorus is an essential macronutrient for the harmonious growth and development of terrestrial and aquatic plants [1], but in large quantities, it harms the environment, especially water, because it is largely responsible for the occurrence of eutrophication, as reported by the OECD (World Economic Cooperation and Development Organization), which attributes over 80% of total phosphorus as the main cause of the appearance of eutrophication in water bodies [2]. Eutrophication has emerged as a global problem that produces a multitude of negative effects, such as the overgrowth of algae that leads to hypoxia, which in turn results in the killing of fish [3] and other aquatic organisms dependent on oxygen dissolved in water, which causes a major imbalance in aquatic ecosystems. Because of these negative effects caused by the excess of total phosphorus in the water, it is imperative to find a solution that is as efficient and sustainable as possible in eliminating it, and a solution could be the use of aquatic plants. The use of plants in naturally eliminating contaminants from a water source is called phytoremediation. This process uses the plant's metabolic system to remove nutrients and contaminants from the surrounding area and store them in their biomass [4].
Lemna minor L. and *Pistia stratiotes* L. are floating aquatic plants belonging to the Araceae family and which many authors report as being able to accumulate significant quantities of organic and inorganic substances from the aquatic environment, such as heavy metals (Cd, Pb, With etc.) [5], total nitrogen or phosphorus [6, 7]. These species are found almost all over the globe [4], but mainly in tropical [8], subtropical [4] and temperate [8] regions and develops in almost all types of freshwater (clean, polluted, muddy, stagnant) [9], having very high growth rates. *Pistia stratiotes* L. doubling its population within a few weeks [4], and *Lemna minor* L. being able to double its mass within a week [9], which is a major advantage because the need to add new plants to a remediation pond decreases [4]. These considerations mentioned above, make the two species of plants as ideal candidates in studies of phytoremediation of waters loaded with organic substances.

Experimental

Plant material

The *Lemna minor* L. and *Pistia stratiotes* L. plants were harvested from a natural pond and transferred to the laboratory, where they were thoroughly washed with distilled water to remove mud and impurities. Plants were acclimatized for 7 days to laboratory conditions, at a temperature range from 19˚ to 25˚ C, in tap water.

Experimental conditions

Approximately 10 grams of plant material were placed in 10 cm diameter transparent plastic containers, containing 250 ml of tap water enriched with total phosphorus (TP) up to concentrations of 4, 6, 8 and 10 mg/L (TP 4, TP 6, TP 8 and TP 10 variants). The experimental duration was 72 hours at 25° C (± 0.5° C), with a day/night cycle of 16/8, and the total amount of phosphorus in water, expressed in a mg/L, and in plants, expressed in a g/kg of dry matter (d.m.), was quantified at the initial moment and after 72 hours, during which the plants were in the water at preset temperature.

Methods of analysis

Plants. About 5 g of wet vegetal material was mixed for 5-10 minutes with 0.5-1 g of animal charcoal. The sample was transferred into an Erlenmeyer flask and 50 mL of 2% CH_3COOH was added, incubated for 10-15 minutes then it was filtered. 1 mL of the acetic acid extract was transferred in a 25 mL solution containing 3 mL of molybdenum reagent and 1 mL of ascorbic acid then the sample was incubated in the dark for 15-20 minutes. The detection of total phosphorus was spectrophotometrically analyzed at 600 nm.
Water. The determination of total phosphorus in water was performed using the Specord PC 205 spectrophotometer, Analytic Jena, according to SR EN ISO 6878: 2005.

Results and discussion

Water purrification

The plants were grown in presence of four phosphorus concentrations, chosen based on the most commonly TP concentration values detected in untreated wastewater.
Figure 1 shows the decrease of the total amount of phosphorus in water after 72 hours at an ambient temperature of 25° C, for *Lemna minor* L. and *Pistia stratiotes* L. This temperature was chosen because most terrestrial and aquatic plants prefer a medium with relatively high temperature for cellular processes, especially for photosynthesis [10].

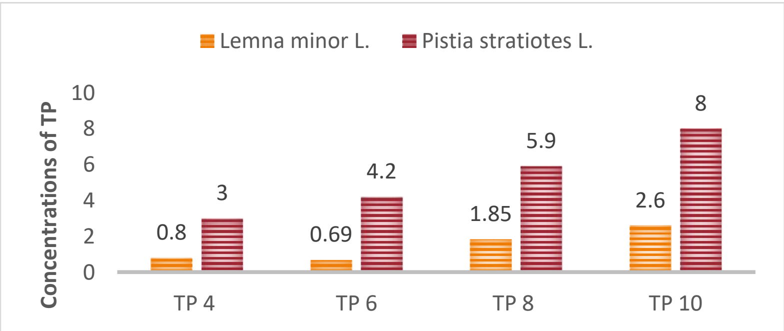

Figure 1. The decrease of the initial total phosphorus concentration in the water after 72 hours. The data represents the arithmetic average value of three replicates.

From figure 1 we can see the quite large difference between the two aquatic plants in terms of purification of total phosphorus-loaded water. For both species of plants the best results were in the case of the experimental variant with 6 mg/L initial TP in water, namely: for the *Lemna minor* L. plant a percentage decrease of 88.5% from 6 mg/L to 0.69 mg/L TP is observed, and for the *Pistia stratiotes* L. species the percentage decrease is 30% from 6 mg/L to 4.2 mg/L TP. Comparing the treatment capacity of total phosphorus-loaded water, of the two aquatic plants, we can observe a markedly higher difference in favor of the species *Lemna minor* L. in all experimental variants.

Absorption of total phosphorus by Lemna minor L. and Pistia stratiotes L.

Table 1 shows the TP bioaccumulation by the two plant species. Comparing the initial quantities of phosphorus detected in plants, a higher affinity for phosphorus of the species *Lemna minor* L. is observed to the detriment of the species *Pistia stratiotes* L., these having in their body from the beginning 3.6 respectively 2.3 g/kg d.m.

In the case of *Lemna minor* L. the amount of phosphorus accumulated in its body is directly proportional to the amount of TP in the water, but the differences between the experimental variants TP 6, TP 8 and TP 10 are very small, which leads to the conclusion that, the value of 15.5 g kg d.m. is the maximum amount of TP that can be accumulated by the body of the plant, regardless of whether the initial phosphorus dose is increased.

In the case of the first three experimental variants (TP 4, TP 6 and TP 8) for the species *Pistia stratiotes* L. the amount accumulated in the body of the plant is the same as in the case of the first species directly proportional to the initial amount of phosphorus in the water reaching a maximum of 9.0 g/kg d.m. For the experimental version TP 10, we observe a sudden decrease in the amount of phosphorus in the plant, compared to the previous version (TP 8= 9.0 g/kg d.m. and TP 10= 4.9 g/kg d.m.), which means that the plant reaches maximum saturation, and the bioaccumulation processes are stopped, the plant begins to gradually remove the TP from its body back into the water.

For the most efficient purification of the water loaded with TP, it is recommended to use the species *Lemna minor* L., to the detriment of the species *Pistia stratiotes* L., at initial quantities of TP in water of maximum 6 mg/L, for approximately 72 hours after which the plants can be renewed.

Because the plants present considerable amounts of TP in their bodies, after harvesting they can be used as a nutritional supplement for animal feed [11] or as a fertilizer for phosphorus-deficient soil.

Table 1. Total phosphorus accumulation in plants after 72 hours, expressed in g/kg d.m. The data represents the arithmetic average value of three replicates.

Experimental variants	*Lemna minor* L.		*Pistia stratiotes* L.	
	Initial	72 h	Initial	72 h
TP 4	3.6	10.7	2.3	5.7
TP 6	3.6	14.6	2.3	7.2
TP 8	3.6	15.0	2.3	9.0
TP 10	3.6	15.5	2.3	4.9

The data presented above are also supported by the data from the literature where Sudiatro et al. in 2019 [7], studied four different species of aquatic plants, among which *Lemna sp.* and *Pistia stratiotes* L. and concluded that regarding the removal of TP from wastewater from the agri-food industry, *Lemna sp.* it is the most efficient with a percentage of phosphorus removal of 36.15% by it. Other authors reported purification yields similar to those obtained in our experimental sets (45.5%-73.3%) after 96 hours of treatment with *Lemna gibba* L. in the presence of 8 mg/L TP, at a temperature range between 10 and 32° C [12].

Conclusion

This study demonstrated the ability of *Lemna minor* L. and *Pistia stratiotes* L. plants to accumulate TP from synthetic water. The degree of bioaccumulation of phosphorus by plants was influenced on the one hand by the studied species and on the other hand by the initial amount of phosphorus present in the water. Therefore, both species showed the best results in the experimental version TP 6, but the differences between the two species were significant, namely: the percentage decrease of TP in the species *Lemna minor* L. was 88.5%, and in the case of *Pistia stratiotes* L. was only 30%.
Regarding the bioaccumulation of organic substances by the plants in their body, there are notable differences between the two species. The degree of saturation for the *Lemna minor* L. species is around 15 g/kg d.m., while the *Pistia stratiotes* L. species reaches saturation around 9 g/kg d.m.
From the ones presented above, a general conclusion can be drawn, namely, that of the two species of floating aquatic plants, the species *Lemna minor* L. is far superior to the other, and is much better suited to the treatment of water loaded with TP.

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