Lemna minor, A SOLUTION FOR THE DECONTAMINATION OF POLLUTED WATERS BY NITRATES

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Abstract. *This paper presents some experiments on the efficiency of the duckweed (Lemna minor L.) applied for the purpose of removing nitrates from wastewater from a pig farm, so as to reach the behaviour of clean water enriched with standard nutrients. The efficiency was tested by measuring the biomass, protein and nitrate contents after each interval of twenty days. The maximum biomass and protein production was obtained by algae development on wastewater (43.6 mg/L; 52% respectively) compared with those obtained on clean water (6.6 mg/L; 48% respectively). The maximum nitrate removal efficiency was obtained after 100 h on wastewater (89.72%) and after 120 h on clean water (85.14%).*

Key words: biomass, *Lemna minor*, nitrate, protein

1. Introduction

The environmental pollution, which can affect all environmental compartments (water, air, soil) has as result not only fauna and flora quality degradation followed even by extinction of species, but also a severe impact on human health. The phenomenon of pollution can be generated by chemicals, various forms of energy (light, heat) and noise that cause imbalances, instability, disorder, harm or discomfort to the environment, ecosystems and humans.

In this context water quality has become an increasing, environmental and social constraint for modern society. The relationship between water quality and quantity, on one hand, and health state of human beings, on the other hand, represents nowadays an acute a public health issue worldwide. Nitrates and nitrites are included among the major pollutants of water. The toxic effects of nitrates to babies are due to their endogenous conversion to nitrite, which is implicated in the occurrence of methemoglobinemia (inability to transport oxygen to tissues). Methemoglobinemia is also known as "blue baby syndrome" because its first manifestation is a bluish colour of the infant's skin. Babies of 0-1 years, fed artificially is the foremost group exposed to diseases risk, especially in rural areas where well water is used for cooking. Other negative effects are reported as well, due to large quantities of nitrates in water: gastric cancer [1], central nervous

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system defects and some other cancers [2, 3]. Also, nitrate can interfere with iodine retention by thyroid, resulting in thyroid hypertrophy [4]. There is a positive association between nitrates in drinking water and non-Hodgkin lymphoma and colorectal cancer [5].

An European Commission Report [6] reveals a number of regions where nitrate levels are worrying. The European Commission (EC) Scientific Committee on Food (SCF) prescribed in 1995 the Acceptable Daily Intake (ADI) of nitrate and nitrite ions according to the body weight of a person. ADI for the nitrate ion is 3.65 mg/kg body weight (equivalent to 219 mg/day for a 60 kg person) and for nitrite ion is 0.06 mg/kg body weight (equivalent to 3.6 mg/day for a 60 kg person). SCF recommended a drastic reduction of the exposure to nitrate via food and urged the adoption of good agricultural practices so as to ensure nitrate levels as low as reasonably attainable [6]. In terms of contribution to the total human nitrate intake, it was found that fruit and vegetables account for 70%, drinking water 21%, while meat and meat products about 6% [7].

Currently, in many parts of Romania, nitrate concentrations in water are close to levels unacceptable under current legislation of the European Union such as Nitrate Directive [8] and Drinking Water Directive [9, 10]. The Maximum Allowable Concentration of nitrates in drinking water is 50 mg/L, but pediatricians recommend for children a maximum level of 10 mg/L.

Today, numerous efforts are involved in helping nature to recover after poluution or prevent this phenomenon. This means to find the most opportune and sustainable solutions and the best way to apply them in an eco-efficient manner. Literature in the field of environmental remediation shows that algae are veritable indicators of water pollution. Moreover, algae can be used for water treatment due to their high growth rate and availability to remove and bioaccumulate various chemical contaminants from aquatic environments.

Worldwide, farmers utilize natural aquatic plants of various species for different purposes such as animal food, green manure, human food or biofuels. Among the most common aquatic plants used for these purposes are: water hyacinth (*Eichhornia crassipes*), water lettuce or Nile lettuce (*Pistia strationes*), watercress (*Nasturtium officinale*), duckweed (*Lemna minor*), *Azolla* and others. There are four types of duckweed: little duckweed (*Lemna minor*), great duckweed (*Lemna polyrrhiza*), thick duckweed (*Lemna gibba*) and cross duckweed (*Lemna triscula*).

In the last years the duckweed (*Lemna minor*) began to become more and more attractive for application in environmental bioremediation, due to its capabilities to extract inorganic persistent chemicals such as heavy metals from polluted waters, providing biologically based wastewater treatment alternatives. For example duckweed was successfully applied in phytoremediation processes of wastewaters and for improving pond water quality [11-17].

Since pollution with heavy metals is also a problem for many countries *Lemna minor* was tested in different studies to evaluate its capacity to remove heavy metals such Zn, Mn, Cu, Cd and Pb. It was shown that *Lemna minor* reduced the concentrations of Zn, Mn, Cu and Cd from the wastewater, but the removal of Pb was negligible [16]. In the same time other studies have shown that in appropriate conditions Cd, Hg, Zn, Mn, Pb and Ag can be removed in high proportions ranged between 93.3% and 100% [18]. pH value plays an important role in the removal of heavy metals from aqueous effluents. So, using thick duckweed (*Lemna gibba*) type at pH = 7 the removal of the two metals was around 98%. These results, thus suggest that *L. gibba* can be a suitable candidate for removal of heavy metals from polluted water bodies [19]. Some studies have been conducted for assessing the oils removal from the water by using duckweed [18, 20]. Other studies have been done on the ability to remove nitrates from water by duckweed. In adequate conditions nitrates were assimilated by duckweed virtually completely [15, 16, 21 - 26].

In addition, this plant has attracted the attention of scientists due to possible higher potential to be a nutritional resource for animals. Duckweed grows in aquatic environments with relatively high levels of nitrogen, phosphorus and potassium, minerals concentrating and synthesizing proteins [12, 27 - 32].

In medicine the duckweed is used in homeopathic treatments because the leaves have an antipruritic effect, antiscorbutic, astringent, cleansing, diuretic, febrifuge and soporific. It is recommended for colds, measles, edema, arthritis or urinary problems. It is used also in skin diseases and juice of plant is helpful in ophthalmology [33]. In some Asian countries it is used in human food (e.g. Brunei, Laos, Thailand).

This paper aims to evaluate the potential of duckweed species that grows in Romania in the remediation of water effluents contaminated with nitrates and nitrites.

2. Experimental part

2.1. Materials

The biological material used was the aquatic plant *Lemna minor*. This was obtained from Bucharest Botanical Garden.

The analyses were performed using a spectrophotometer Metertek SP830 Plus, a Metler Tolledo pH meter, a centrifugal homogenizer type, Millipore distillation plant, bathroom thermostat, stirring mixer. The nitrites and nitrates solutions were prepared using distilled water. Chemically pure reagents from Merck or Sigma were used.

2.2. Methods

The experimental plan was built to study the potential of duckweed to remove nitrite and nitrates at different concentrations in water. The experiments were performed in a system consisting of four glass tanks with volumes of 20 liters each, with aeration and agitation systems if needed. Culture tanks are equipped with valves at the bottom side for water sampling. The main factors influencing significantly the development of duckweed are: incidence of light, pH and temperature of the water. In this context, considering literature data, the culture tanks were maintained at an average temperature of 25 ± 1^{0}C (considered for the period spring - autumn) in all carried out experiments, with light source for 16 hours a day and at a pH between 7.5 and 8.0.

Two types of experiments were performed to assess the effectiveness of duckweed in the purification of nitrate-rich waters were performed. In one category of experiments, the water was prepared with a nitrogen content of 325 ppm (maximum limit is 50 ppm) and the mineral element nutrients as 88.42 ppm K_2O and 168.2 ppm P_2O_5. In the second category, experiments were performed using polluted water after crossing the Siret River near a pig farm, before reaching the wastewater treatment plant. From this polluted wastewater samples of 20 L were taken during 7 days. Samples were taken in the last two weeks of May and then in June, 2015. The average temperature for this period was 25^{0}C. Water samples were taken at about 2 m at the point of discharge of wastewater from pig farm in Siret. Sampling of wastewater was done by dipping each sample bottle at approximately 30 cm below surface. Each bottle was geared with the mouth in the direction of water flow. The samples were evaluated regarding the content of nitrates, then homogenized and divided into the four experimental tanks. Each experiment was started by cultivation of 20 plants per experimental tank.

Plant development has been monitored during 5 weeks for each category of experiments and every 20 hours, it was taken samples and determined the development of biomass, protein and nitrate content. All experiments were performed in parallel samples in the four tanks, and the results were expressed as their average.

Determination of nitrates was made by iodometric method using a pH meter/Ionometru Mettler Tolledo 355 with ion-selective electrode for nitrate ions. Determination of proteins was made by Lowry method [34].

3. Results and discussions

First of all we determined concentrations of nitrates, nitrates and pH in polluted waters from Siret River. The results are presented in Table 1. The analysis of the data presented in Table 1 shows that the values obtained show quite high variability, between 175.7 and 541.8 ppm for nitrate and 0.34 and 3.45 ppm for nitrite. Also, pH value fluctuated between 7.2 and 8.4. The average values of the homogenized sample of wastewater were 325.5 ppm nitrate, 1.93 ppm nitrite and 7.9 pH.

In this context, homogenized sample of polluted water has been corrected in order to have concentrations of 325 ppm nitrate, 2 ppm nitrite and pH of 8.0. These concentrations were achieved in distilled water sample solutions. In addition, because *Lemna minor* plants development requires a number of nutrients such as nitrogen, phosphorus and potassium as was mentioned before, potassium and phosphorus were added in water samples in concentrations appropriate for the algae to grow properly.

Table 1. Values of nitrate, nitrite and pH in polluted waters

Week	*Nitrate, ppm*	*Nitrite, ppm*	*pH*
1	406.2	1.02	7.5
2	321.2	0.68	7.8
3	283.7	3.41	8.2
4	227.5	2.75	8.3
5	322.4	1.88	7.9
6	541.8	0.34	7.2
7	175.7	3.45	8.4
Average	**325.5**	**1.93**	**7.9**

For analyzes, samples of *Lemna minor* plants were taken randomly at every 20 hours. They were placed on filter paper to remove excess water, then weighed and analyzed. The data obtained were extrapolated to the entire mass of algae in the respective tank. In calculating biomass per week it was taken into account the values of samples collected during the previous weeks. The comparative evolution of average weights of biomass produced is shown in Table 2 and Fig. 1.

Table 2. *L. minor* biomass production on the two categories of substrates

Hours	*Clean water*		*Polluted water*	
	biomass, mg	±	*biomass, mg*	±
0	22.00	0.022	82.00	0.071
20	28.52	0.089	154.23	1.452
40	35.42	0.127	302.10	3.211
60	55.40	2.231	543.12	7.456
80	67.80	4.562	725.31	9.256
100	92.55	7.282	801.30	11.222
120	121.32	8.351	872.08	12.315

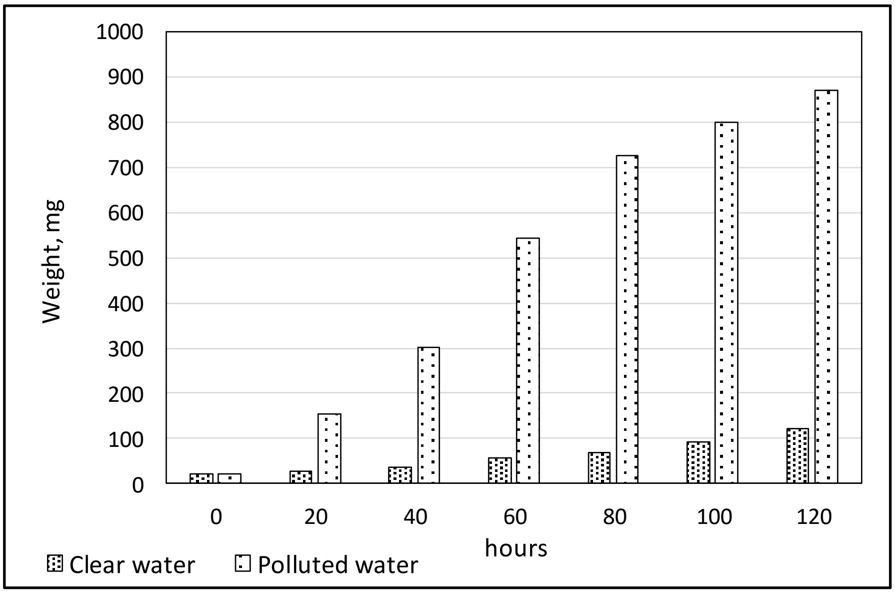

Fig. 1. The average biomass *L. minor* production on the two categories of substrates

From the data obtained can be observed that biomass had a low increase in the first 60 hours and began to grow faster after 80 hours. Inspecting the evolution of biomass on wastewater it can be noted that the maximum rate of development is in the fourth week of culture, after which the growth rate decreases, so that in the last 40 hours a reduced development was found. By comparing data obtained by weighting biomass, it can be seen that it grows better on the polluted water from the pig farm, although the nutrients content is comparable to that considered equivalent in clean water enriched with nutrients (K, P). So, after 120 hours the amount of biomass resulting in polluted water was about 9 times greater than that obtained in distilled water enriched with nutrients. Differences between samples evaluated against the corresponding average (±) are higher as measured amount of biomass is higher.

An important fact is that after 40 hours the culture medium is cleaner. However, after the 120 hours the culture medium becomes cloudy due to the degradation of some algae plants. Besides, the abundance algae developed causes the phenomenon of aquatic eutrophication. As a result, it is necessary to extracted abundant algae developed.

As the amount of protein is low in fresh algae (values of micrograms per 100 gram fresh weight) we assessed the percentage of protein from the dry matter. The dry matter of 10 samples of the tested *Lemna minor* ranged between 81 – 85 g per kg fresh algae. For assessments it was considered the average value of dry matter for algae, namely 83 g per kg fresh algae. Thus, protein content was calculated by reference to 100 g dry weight. Comparative evolution of average concentrations of proteins is represented in Figure 2. The evolution of protein concentration has a similar behavior for the two types of water.

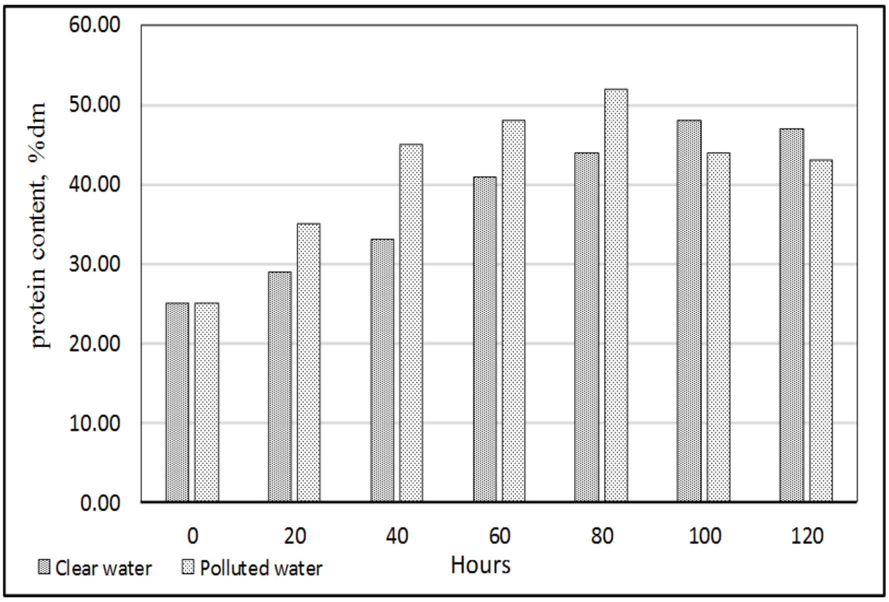

Fig. 2. Evolution of protein concentration in *Lemna minor* grown on two substrates

However, in the period up to 80 hours, the plants grown on the polluted water had clearly higher protein content, after which the values drop below the concentrations found in plants grown in clean water with added nutrients. Decreased protein concentration after 80 hours in plants grown on the polluted water may be linked to the stabilization of the protein content to a certain value, about 42% dry matter, on the entire volume of biomass. In the main time, *Lemna minor* grown in water with nutrients still produce lightweight extra amounts of protein. It should be emphasized that the quantities of protein corresponding to the biomass production, protein content is higher in plants grown on the wastewater comparative to water enriched with nutrients.

Because in the samples analyzed from wastewater originating from pig farm there were found amounts of nitrate which exceeded by far the minimum allowed for water to be considered potable, namely above 50 ppm, it was assessed the consumption of nitrate by *Lemna minor* from homogeneous sample consisting of wastewater provided by pig farm and from clean water to which a nitrate level of 325 ppm was added, namely the same value as the average amount from polluted water. Nitrate content continuously decreases in both types of substrate culture (Figure 3). The difference is that this content decreases faster in culture on polluted water compared to culture on clean water enriched with nutrients.

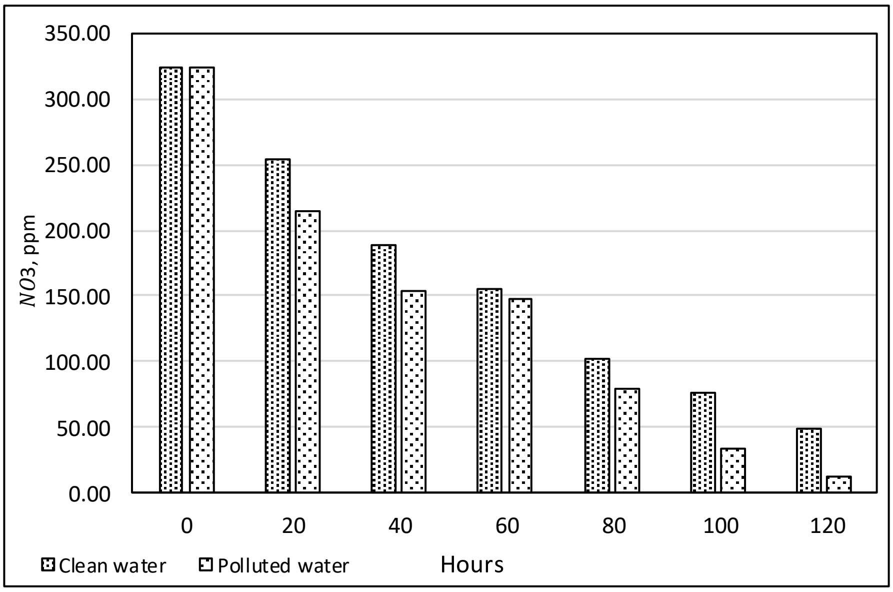

Fig. 3. Evolution of nitrate removal from culture media by *Lemna minor*

Lemna minor consumes higher concentrations of nitrates from waste water than from fresh water enriched with nutrients. It has also been observed that differences between maximum and minimum values decrease so, at lower concentrations of nitrates in experimental cultures, the values between parallel samples are very close.

After 100 hours of development of algae on the polluted water nitrate content falls below the maximum permissible limit. In the case of cultures on clean water enriched with nutrients, nitrate concentration below the allowed limit is obtained only after 120 hours. This behavior suggests that excess nutrients from polluted water not only contributes to the development more abundant of the plant but allows a higher nitrate assimilation by this.

Conclusions

Lemna minor, popularly known as duckweed is a plant that often creates problems by abundance developing on tranquil waters, suffocating aquatic environment, but it can become from an undesired plant, an useful one through the special properties its own. Duckweed is able to use a number of compounds of nitrogen in order to synthesize the protein and other nutritional compounds.

Lemna minor plants can be used on one hand for purifying nitrogen-rich wastewater or other pollutants as well as feed for different animals.

The use of *Lemna minor* combined possibly with other treatments can help to solve three of our day’s problems: extracting nutrients from wastewater, wastewater purification in order to be used again and using plants obtained for different purposes (fertilizers, animal feed, for obtaining biofuels or green power).

Our results lead to the conclusion that, in order to depollute different wastewater it is imperative to study on a part their composition, and on the other mode of development of duckweed so it can be harvested in the optimum moment, before beginning its degradation and plant death.

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