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Effectiveness of aquatic plants in reducing water nitrates

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ABSTRACT

Due to the lack of fresh water, the purification of polluted water is of utmost importance and places a significant financial burden on countries. The effect of *Myriophyllum spicatum* and *Lemna gibba* on nitrate absorption in an area of the Tigris River has been investigated in the light of the low cost and ease of application of aquatic plants in purifying water pollution. Aquarium experiments were designed with three treatments, three replicates, and a closed flow. In the aquarium, the biological requirements for the growth of the studied plants were met. The residence time was determined to be 36 days, and nitrate changes were recorded every three days, 12 times. Except for periods 3 and 4, there were significant differences in the rate of nitrate absorption between the studied plants in all treatments ($P < 0.05$). At the end of the 12th period, *M. spicatum* and *L. gibba* had contaminant removal efficiency of 83.31% and 86.27%, respectively. *L. gibba* ability to utilize nitrate as a nutrient was demonstrated by its significantly increased dry weight at the end of the experiment ($P < 0.05$). In the current study, the significant difference between the average levels of nitrate in the samples and the control sample indicates the presence of a factor other than bacterial decomposition, namely the presence of plants. According to the findings, these macrophytes are viable for reducing nitrate and organic matter loads in polluted waters. Controlling these macrophytes, so that the nutrients in their tissues do not return to the environment during decomposition is necessary to improve water quality and maintain the achieved quality.

Keywords: Nitrate, *Myriophyllum spicatum*, *Lemna gibba*, Contaminant removal.
Article type: Research Article.

INTRODUCTION

The quality of water and soil resources has always determined the development of cities and cultures. This development has gradually polluted and even depleted these invaluable resources (Ciji & Akhtar 2020). The primary water sources are oceans, lakes, rivers, and shallow groundwater. In developing nations, water quality problems are frequently severe and receive less attention (Omidi & Shariati 2021). Due to the excessive exploitation of underground water in many nations, the depth of the groundwater table has decreased, resulting in an increase in a load of pollutants and the destruction of the environment (Manikandan *et al*. 2019). Sources of water pollution include untreated sewage, discharge of chemicals, seepage of oil, accumulation of waste in pits, and leaching of chemicals from agricultural lands (Ameri Siahouei *et al.* 2020). These sources contribute to an increase in nitrogen and phosphorus ions in water, which play a crucial role in water pollution (Mustafa & Hayder

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2021). Plankton and algae growth, an increase in organic load, the demise of fish, and an increase in water turbidity are all results of water pollution (Priya *et al.* 2022). The increase of nitrogenous pollutants in water threatens human health, and the consumption of water with a high concentration of nitrates causes methemoglobinemia in infants and increases carcinogenic nitrosamine compounds in adults (Dey *et al.* 2022). To protect freshwater resources, prevent environmental pollution, and maintain public health, collecting and purifying polluted water before reusing it for non-domestic purposes, especially in Iraq, where drinking water is scarce (Thapa *et al.* 2018). It is essential to choose the appropriate technology based on each region's climatic, economic, and social conditions so that the desired method can be implemented (Wickramasinghe & Jayawardana 2018). In this situation, simple and inexpensive methods for purifying polluted water are of utmost importance. Among the simple and inexpensive methods for purifying polluted water are natural methods (Valenca *et al.* 2021). Due to the low initial cost, low energy consumption, straightforward management, and lack of need for specialized operating personnel, these methods are favored by researchers attempting to develop more suitable and cost-effective alternatives. Some aquatic plants are capable of removing inorganic and organic substances from water (Li *et al.* 2019). These plants can absorb dissolved substances and incorporate them into their structure. In addition, these plants can be safely removed and utilized as a fertilizer or for producing fibers or biogas. In agriculture, nitrate, ammonium compounds, phosphate, and organic carbon frequently require nutrients. Fertilizer production requires energy, so its price continues to rise. It is important to note that the cost of imported chemical fertilizers is prohibitive for rural farmers (Mahmud *et al.* 2020). Therefore, aquatic plants are a dependable local source of inexpensive organic fertilizer and soil conditioner for farmers (Luo *et al.* 2020). *Myriophyllum spicatum* and *Lemna gibba* can be included among the aquatic plants mentioned (Zhang *et al.* 2018). Nitrogen is one of the most abundant chemical elements in the earth's atmosphere and is vital to life. Approximately 80% of the atmosphere is composed of nitrogen molecules, which are the most abundant in the air. It is the fourth most important element in plant tissues, after carbon, oxygen, and hydrogen, and is comprised primarily of amino acids and nucleotides (Isaza *et al.* 2020). The forms of nitrogen found in soil and water are ammonium nitrate and nitrite. Human activities have increased the amount of nitrate-bound nitrogen in the environment (Mushtaq *et al.* 2020). The increase in nitrogen flow has adverse effects on aquatic ecosystems, including acidification, the spread of algae, and a reduction in the growth and survival of aquatic animals (Wang *et al.* 2020). Nitrate is a combination of nitrogen and three oxygen atoms. It is one of several forms of nitrogen naturally converted to other forms in soils and waters by bacteria (Yun *et al.* 2019). Nitrate can also be released directly into surface water from industrial, residential, and agricultural locations (Isiuku & Enyoh 2020). Nitrate is utilized as a plant fertilizer, since plants readily absorb it. Nitrate and nitrite compounds are highly water-soluble and move rapidly through the environment (Jungers *et al.* 2019). Nitrate can, therefore, quickly enter surface waters via precipitation, agricultural, domestic, and industrial waters. Nitrate is not the only factor contributing to the growth of algal colonies; ammonium, phosphorus, silica, water movement, and the amount of available light also play a role. For this reason, algal growth can occur at concentrations below the standard (Szalińska *et al.* 2018). Physical, biological, and chemical processes remove nitrates from water. To remove nitrate from water, ion exchange, reverse osmosis, biological denitrification, and chemical reduction have been utilized (Zuo *et al.* 2020). Reverse osmosis and ion exchange processes cannot selectively remove nitrates and require continuous environmental regeneration. These two processes do not alter the chemical structure of nitrate, resulting in polluted wastewater. Biological denitrification is typically performed in wastewater and is undesirable in water treatment due to the requirement for organic substrate and high system maintenance (Gomez Isaza *et al.* 2020). Therefore, it is necessary to investigate the effect of aquatic plants on nitrate reduction in polluted waters. Given the numerous advantages of using aquatic plants to purify polluted river water, the current study should be conducted. This study was conducted to determine the feasibility and investigate the removal of nitrate from agricultural wastewater by *M. spicatum* and *L. gibba*, as well as to compare the efficacy of these plants in removing nitrate from wastewater compared to the absence of plants. The effect of residence time on the amount of nitrate absorption by the studied plants is one of the innovations.

MATERIALS AND METHODS

Throughout the year, the amount of nitrates in agricultural wastewater was measured in the most significant agricultural wastewater overflow beside the Tigris River. Due to the similarity of the testing procedure to natural conditions, the average agricultural effluent of this region (228.4 mg L^{-1}) was utilized. The aquarium provided the environmental conditions and physical requirements necessary for the growth and reproduction of the studied plants. Each aquarium bottom was covered with coarse sand to prevent plant root rot and the growth of harmful anaerobic microorganisms. Then, clay containers were prepared, and their bottoms and walls were perforated to allow for the exchange of water, soil, and oxygen, as well as space for roots to reach food, in order to protect, stabilize, and facilitate the mobility and movement of plants. Inside each container, sand with a diameter of 2 to 5 mm was placed, followed by sand and soil, aquatic plant leaves, and tree leaves. The final layer was 10 cm of sand, and the plants were then cultivated. Air pumps were also used to circulate water. The aquariums were placed at the same distance and accuracy so that they would receive the same amount of light and temperature, and they were covered with glass to prevent the entry of foreign objects brought in by the wind, etc. In the first portion of the study, plants were grown separately in aquariums, and then, based on the average density of river plants, two plants were considered for each 0.6 m^2 aquarium. Changes in temperature between 16 and 30 ^{o}C were deemed comparable to natural conditions. After a 30-day growth period and ensuring proper plant growth, the aquarium were gradually emptied of all water and then refilled with the original agricultural effluent. Each aquarium has an insulating cap to prevent water from transpiring and dust from entering, glass to allow light to pass through, an air pump to create flow and supply oxygen, sewage, a mixed layer of plants, soil, and a sand layer of the riverbed. The control aquarium contained all the components of the main aquariums but no plant samples to eliminate the influence of other variables. The difference in nitrate reduction rate between the aquariums containing plants and the control sample can be attributed solely to the presence of the studied plants. Like other aquariums, the initial nitrate concentration in the control aquarium is 228.4 mg L^{-1}. Beginning with two water samples from each aquarium, the amount of nitrate was determined using spectrophotometry, and the rate of nitrate reduction in each treatment was recorded. Using a spectrophotometer model S-2150UVE, the nitrate concentration was measured and recorded every three days, 12 times. The contaminant removal efficiency was calculated by dividing the amount of nitrate absorption or reduction by each plant during each time period by the initial concentration of nitrate. To determine the fresh weight of plants, sieved plant samples were placed for three minutes between two drying papers and then weighed. To determine the dry weight of the plants, plant samples were washed with deionized water, dried in a 70 °C oven for 24 h, and then weighed. The index of plant biomass production is derived from the difference in the plant's net fresh weight between the study periods. Most nitrogen in the plant is in the form of organic compounds, such as protein, while only a small amount is in the form of ammonium and nitrate. Therefore, total nitrogen measurement is the most suitable method for expressing this element's status in a plant. Total Kjeldahl Nitrogen (TKN) is recently best method for measuring total nitrogen in soil and plants. Powdered samples of oven-dried plant material used to determine biomass production index were used to determine total nitrogen. A one-way analysis of variance and Duncan's multiple range test were utilized to compare the water nitrate concentration in different periods and variations in the average dry weight and total nitrogen of the examined plants. In this regard, version 19 of the SPSS software was used for data analysis.

RESULTS AND DISCUSSION

Table 1 shows the mean nitrate concentrations for the three investigated conditions. According to the table, it is evident that the mean nitrate concentration is always higher than the other two conditions in the aquarium, as some nitrate has been absorbed by plants 1 and 2 in the larger aquariums. According to Table 1, it is evident that plants absorb an increasing amount of nitrate over time. The control sample is devoid of plants and has the same initial nitrate concentration as the other samples. Small-scale bacterial decomposition of nitrate is responsible for the sporadic decrease of nitrate in the control sample over different periods. In this study, the significant difference between the mean levels of nitrate in the samples and the control sample indicates the presence of a factor other than bacterial decomposition, i.e., the presence of plants. It is necessary to explain that Duncan's test was used to obtain the results listed in Table 1 and to extract the contaminant removal efficiency used to draw Fig. 1 which depicts the contaminant removal efficiency for each sample and control. According to Fig. 1, it is clear that the control aquarium absorbs significantly less nitrate than the other two aquariums. In addition, *L. gibba* exhibits a greater contaminant removal efficiency than *M. spicatum*, excluding periods 3 and 4. The decreasing slope of the graph shown in Fig. 1 shows that the aforementioned plants are more capable of absorbing nitrate during the initial periods. Other studies suggest that vegetated systems reduce nitrates more than non-vegetated systems (Eribo *et al.* 2019; Töre & Özkoç, 2022). Plants transform sediments into an ideal environment for the growth of nitrogen-consuming microbial communities, and by providing more support for bacteria, they increase microbial nitrate absorption in the rhizosphere. One-way analysis of variance was used to investigate the difference between the original aquarium and the control aquarium, as well as the significance level of difference. The analysis of variance results in Table 2 shows that, except in periods 3 and 4, there is a significant difference in nitrate absorption between two plant samples and a control sample in all treatments (P < 0.05). To determine the significance level between the two investigated plants and the control aquarium, it is necessary to employ Duncan's multiple range test.

Table 1. Comparison of mean nitrate in the studied periods

Period	Day	Mean (±SD*) Nitrate (mg L^{-1})		
		Control	*Myriophyllum spicatum*	*Lemna gibba*
1	3	223.43 ± 0.57	217.64 ± 4.16	206.72 ± 6.34
2	6	218.71 ± 1.14	189.43 ± 18.41	176.16 ± 10.57
3	9	211.28 ± 0.76	148.57 ± 23.76	162.23 ± 16.95
4	12	205.19 ± 2.14	125.62 ± 31.63	135.14 ± 33.72
5	15	201.45 ± 1.27	119.62 ± 25.18	103.41 ± 38.24
6	18	196.14 ± 1.64	103.17 ± 16.34	84.83 ± 52.49
7	21	192.84 ± 1.07	84.25 ± 21.55	68.19 ± 43.18
8	24	186.37 ± 2.43	71.84 ± 11.73	57.52 ± 26.51
9	27	183.56 ± 2.79	58.72 ± 19.21	48.19 ± 17.83
10	30	179.18 ± 2.13	49.18 ± 26.17	40.27 ± 12.09
11	33	176.27 ± 1.81	42.41 ± 32.74	34.65 ± 11.34
12	36	174.86 ± 2.26	38.13 ± 14.23	31.36 ± 16.87

*SD = Standard deviation.

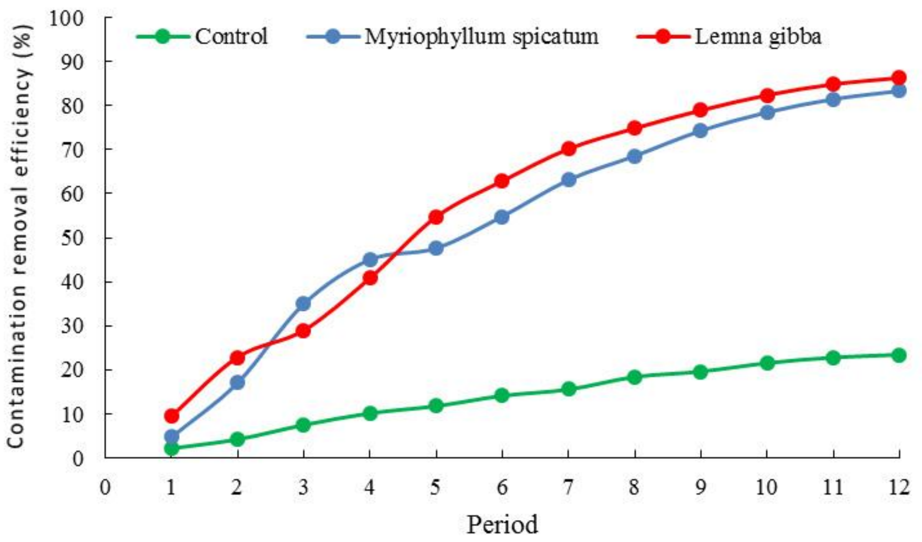

Fig 1. Comparison of nitrate removal percentage of studied plants and control sample in different periods.

Table 2. Analysis of variance to compare nitrate concentration in different periods.

Period	Day	F-value	P-value
1	3	67.52	<0.001
2	6	46.12	<0.001
3	9	6.54	0.073
4	12	1.78	0.146
5	15	10.57	0.029
6	18	23.61	0.004
7	21	49.27	<0.001
8	24	135.42	<0.001
9	27	214.75	<0.001
10	30	482.18	<0.001
11	33	614.27	<0.001
12	36	702.19	<0.001

Additionally, only in periods 1 and 2 the results of the two tested plants differ considerably. Table 3 displays changes in the mean dry weight of plants from the beginning to the end of the experiment. Analysis of variance exhibited in Table 3 reveals that the *M. spicatum* and *L. gibba* dry weights at the end of the experiment (after 36 days) were greater than their initial dry weights. In contrast, this weight change is significant only for *L. gibba* ($p < 0.05$). Although the nitrogen concentration of *L. gibba* was greater than *M. spicatum* after the experiments, there was no significant difference between them ($P > 0.05$).

Table 3. The results of variance analysis of the changes observed in the experiment plants.

Plant	Initial dry weight	Final dry weight	Final nitrogen content	F-value	P-value
Myriophyllum spicatum	5.19	7.63	5.34	24.61	0.007
Lemna gibba	8.27	14.35	7.84	3.49	0.127

In aquariums, macrophytes exhibited significant visual growth. The *L. gibba* dry weight upraised at the end of the experiment. This more remarkable ability of *L. gibba* to elevate biomass compared to *M. spicatum* may be attributable to the physiological characteristics of *L. gibba* and its efficiency in nitrate utilization. According to previous study, the release of oxygen required for the decomposition of organic matter from the roots of plants may be responsible for the increased decomposition of organic matter in plant beds (Ergönül *et al.* 2021). In addition, the oxygen concentration in dense systems of aquatic plants, such as *L. gibba*, was lower than in systems with submerged aquatic plants, since submerged plants release oxygen due to an imbalanced photosynthesis and respiration process in the water (Kumar *et al.* 2022). Based on the study results and the high efficiency of both plants, removing nitrates from contaminated water is recommended. Through canals, the drainage of several hectares of agricultural land flows directly into the Tigris River. According to the findings of the present study, artificial ponds or wetlands containing the abovementioned plants should be created, hence, the appropriate retention time in these areas based on the effluent discharge, can significantly reduce the pollutants entering the Tigris River, particularly nitrate. In general, rivers devoid of vegetation are effective at removing nutrients, and their efficacy has been demonstrated by numerous studies (Egbi *et al.* 2020; Kareem & Kadhim 2021). In general, however, rivers with vegetation are more effective at removing nutrients than rivers without vegetation (Sathishkumar *et al.* 2020). In all rivers, microbial processes play a significant role in nutrient removal. The roots of plants provide a suitable habitat for microbial activities and cause an increase in microbial populations in rivers with vegetation compared to rivers without vegetation (Wang *et al.* 2020). In addition to absorbing nitrogen, aquatic plants emit the oxygen required for bacteria to convert ammonium oxide to nitrate through photosynthesis in aquatic environments. In addition, plant respiration can reduce the amount of oxygen in rivers, activate denitrification, and convert nitrate to nitrogen gas. The mechanisms for nitrate removal in rivers include plant uptake and denitrification (Zhu *et al.* 2020). The results of this study and other studies suggest the more significant reduction of nitrate in systems with plants compared to systems without plants, indicating that aquatic plants have a more significant effect on nitrate reduction (Chen *et al.* 2020; Gecheva *et al.* 2022). Due to the presence of nutrients such as nitrate and phosphate, the plants used in this method can also produce fertilizers (Misra & Misra 2019). Further research on the behavior of these species in a system with a higher load of organic nutrients and a shorter sampling interval can help confirming the results of this study and identifying the optimal parameters for their use in phytoremediation. Among the present study's limitations was that it was conducted in an aquarium with only two types of plants. Another limitation of this study was the investigation of nitrate concentration at only one point of the Tigris River. For future studies, conducting experiments with larger dimensions, comparing them with software results, and as a result, evaluating the precision of each technique, as well as the evaluation of other aquatic plants are recommended.

CONCLUSION

According to the findings, these macrophytes are viable for reducing nitrate and organic matter loads in polluted waters. Notably, the requirement to improve water quality and maintain the quality achieved is the control of these macrophytes and preventing the nutrients in their tissue from returning to the environment during decomposition. Young species are recommended for use in water remediation projects that employ plant remediation techniques. Collecting these plants from the desired location in the subsequent step helps to reduce a load of these pollutants, particularly phosphorus and nitrogen.

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