Efficiency of *Cyperus alternifolius*, *Typha latifolia*, and *Juncus inflexus* in the removal of nitrate from surface water

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**ABSTRACT**

Nitrate is one of the most toxic and pathogenic substances in drinking water. Nitrate levels have increased significantly in groundwater and surface water because of the high usage of nitrogenous fertilizers, which have lethal effects on human health. Therefore, the removal of this toxic material from surface water is absolutely essential. The goal of this paper is to evaluate the efficiency of three aquatic plant species, called *Cyperus alternifolius*, *Typha latifolia*, and *Juncus inflexus*, in the removal of nitrate from surface water. This experiment has been carried out in the laboratory scale using a hydroponics method. To do this, the water samples containing nitrate were collected and evaluated before and after the treatment to see the effects of these three plants on both the nitrate removal and the pH variation of water. The results show that all the plants can remove excessive levels of nitrate from the surface water. The comparison of the results has revealed that *T. latifolia* plant has higher ability to remove nitrate from water than *C. alternifolius* and *J. inflexus*. It was observed that the nitrate removal efficiency of *T. latifolia* was 95%, *J. inflexus* was 85%, and *C. alternifolius* was 70% after 10 days. Moreover, it was observed that the pH values of the treated water have been affected by these plants. The results of this study can be employed for further improvement of surface water quality, thereby lowering water purification time and costs as well as decreasing the nitrate-related diseases significantly.

**Key words** | *Cyperus alternifolius*, *Juncus inflexus*, nitrate, phytoremediation, surface water, *Typha latifolia*

**HIGHLIGHTS**

- *Typha latifolia*, *Cyperus alternifolius*, and *Juncus inflexus* plants can remove the excessive levels of nitrate from the surface water.
- *C. alternifolius* plant slightly decreases the pH value of water after 10 days.
- *J. inflexus* and *T. latifolia* plants increase the pH value of water.
INTRODUCTION

The surface and groundwater contaminated by nitrate (NO$_3^-$) have significantly increased in most countries (Weldeslassie et al. 2018). The levels of nitrate in drinking water also increase alarmingly in different parts of the world causing serious diseases such as thyroiditis, high blood pressure, stomach cancer, cytogenetic malfunction, blue baby syndrome (methemoglobinemia), and many other illnesses in children and newborn babies (Sharma et al. 2017; Soomro et al. 2017; Nieder et al. 2018). Based on the reports released by the World Health Organization (WHO) in 2004, it was revealed that 30% of the 2,000 water sources in the world had more than 24 mg L$^{-1}$ nitrate. Moreover, the Environmental Protection Agency (EPA) reported that about 250,000 water supply sources had the highest nitrate contamination in 1990 (Liu et al. 2015; Pennino et al. 2017).

There are many reasons for diffusing nitrate into the ground and surface water such as inappropriate agricultural operations, changing the patterns of land-using, extreme utilization of nitrogenous fertilizers, fossil fuel combustion, and increasing the domestic wastewater recycling (Chen et al. 2017; Wollheim et al. 2017). The developing countries use groundwater as the main source of drinking water because the risks of microbial contamination are much less in comparison with other sources of drinking water (Fout et al. 2017; Li et al. 2018). Thus, nitrate elimination from both surface water and groundwater is necessary before it can be consumed by human. There are many physico-chemical processes such as ion exchange, adsorption, reverse osmosis, electro-dialysis, photocatalytic reduction, and chemical denitrification applied routinely for removing nitrate from water with high levels of nitrate (Bulgariu et al. 2012; Arun et al. 2017; Challagulla et al. 2017; Tyagi et al. 2018). The aforementioned processes can be very expensive, high energy demanding, and also produce secondary pollutants during their treatment (Sinha et al. 2017; Wang et al. 2018). However, from a techno-economic perspective, the biological processes are mainly regarded as the optimum strategy to purify ground and surface waters containing low nitrate concentrations.

Considering the bio-technique as a method for water treatment, phytoremediation can be regarded as a sustainable natural remediation method. Phytoremediation is an economical, environmentally friendly, and multi-purpose treatment approach used for eliminating contaminants and impurities from surface water and wastewater (Sinha et al. 2018). The intrinsic capability of some plant species in absorbing nitrate from water for their growth can be applied as a technique for eliminating nitrate from ground and surface water sources (Li et al. 2016). Moreover, macrophytes are widely used in the field of phytoremediation because of their ability to absorb high amounts of pollutant in a short period of time, thus showing high rates of pollutant elimination (Bartucca et al. 2016). In addition, these plants enhance the growth of microbial communities in the vicinity of their root areas; thereby, a cumulative (synergistic) condition is created to eliminate the pollutants from water (Lingua et al. 2015; Xu et al. 2018). Based on the literature, various macrophytes have been used to remove pollutants from water sources (Li et al. 2018; Sinha et al. 2018). The pollutants can consist of K, N, and P, heavy metals, fluoride, micropollutants, and inorganic or organic forms of nitrogen available in water sources (Pavlineri et al. 2017). For instance, Vallisneria natans has been used to eliminate the excessive levels of arsenic from wastewater (Chen et al. 2015). Both Spirodela polyrhiza and Pistia stratiotes plants presented the reasonable potential for the purification of water contaminated with high levels of fluoride and heavy metal ions (Volf et al. 2015; Karmakar et al. 2016). Bartucca et al. (2016) has grown ryegrass (Lolium perenne) in a vegetated floating system to evaluate its potential for treating water contaminated with nitrate. It was proven that the Italian ryegrass has high efficiency in treating inorganic nitrogen-containing water, in both sand filters and hydroponic systems (Escobar-Alvarado et al. 2018; Liu et al. 2018; Radziemska et al. 2019). Samimi Loghmani et al. (2011) investigated the capability of Typha latifolia plant to remove nitrogen and phosphorus from the treated municipal wastewater. They concluded that T. latifolia plant has a significant effect on the reduction of organic nitrogen, ammonium, nitrate, total nitrogen, and dissolved phosphorus (Samimi Loghmani et al. 2015). Shyamala et al. (2019) evaluated the potential of two ornamental plant species called arrowhead plant
(Syngonium podophyllum) and money plant (Epipremnum aureum) in treating wastewater containing nitrate. They concluded that each process variable, such as the plant density, the growth period, and the initial nitrate concentration, has a significant effect on the nitrate removal process. Of all these variables, the growth period has the most considerable effect on nitrate removing using both arrowhead plant and money plant (Shyamala et al. 2019). Therefore, the identification of the easily accessible plants with high capability in removing nitrate can be of great importance.

Nitrate can be considered as the main source of ground-water contaminant (Goulding 2000) that is also regularly reported in Iran (Jalali & Kolahchi 2005). The average annual application rate of nitrate fertilizers is currently over 200 kg ha⁻¹ in Iran (Jalali 2005). A survey conducted in well waters of western Iran showed that nitrate concentration is varied from 7 to 122 mg L⁻¹ with an average of 41 mg L⁻¹. Nitrate concentrations in 58% of samples were in the range of 25–50 mg L⁻¹, 18% of samples were in the range of 51–75 mg L⁻¹, and 6% of samples had concentrations above 75 mg L⁻¹. The survey shows that the concentration of nitrate in 24% of samples is above the recommended guidelines of the WHO (50 mg L⁻¹ NO₃⁻) (Jalali & Kolahchi 2005).

Therefore, it is rational to consider that we can achieve higher denitrification efficiency by using aquatic plants. Most previous studies reported on the remediation efficiency of nitrate-contaminated water have used single or two plant species. However, the removal mechanisms of water nutrients using combinations of different aquatic plant species and the subsequent remediation of polluted water need to be more thoroughly explored. The main objective of this study is to investigate the potential efficiency of three plants called Cyperus alternifolius, Typha latifolia, and Juncus inflexus for the treatment of nitrate-containing water. C. alternifolius is a herbaceous plant with 30–90 cm height and it reaches up to 150 cm in places where the environmental conditions are suitable. T. latifolia is a perennial herbaceous plant with underground stems from the Typhaceae family. J. inflexus is another perennial plant that has smooth and hairless leaves. To examine the efficiency of these three plants in removing nitrate from water, a laboratory-scale experiment has been conducted under uniform controlled environmental conditions. The paper also presents the effect of these plants on the variation of pH and temperature of water.

**MATERIALS AND METHODS**

This study was conducted to evaluate the potential of C. alternifolius, T. latifolia, and J. inflexus plants for reducing or removing nitrate from water in the laboratory scale. The pictures of these tested plants are shown in Figure 1(a)–1(c). The identification keys of these three plants are also listed in Table 1. To do the laboratory-scale experiments, two stages were implemented; the first stage involved the reproduction and adaptation of plant species, and the second stage was making an appropriate solution for the experiment. For the first stage, C. alternifolius specimens were obtained from a greenhouse in Bushehr, Iran, which were propagated by inverted (upside down) cuttings after 1 month. To reproduce the specimens of T. latifolia and J. inflexus plants, the mechanism of moving the rhizome parts to the aquatic environment has been used.

To enhance the diagnosis of the nitrate effect on plants, they were hydroponically (the process of growing plants in a nutrient-rich solution in the place of soil) cultured at a temperature of 2–3 °C for 1 month. The plants were indirectly exposed to the sunlight for an average of 2 hours per day. This method of adaptation reduced the interaction between soil and nitrate/plant. It also adapted the plants to the environmental conditions of the study. Moreover, the solutions were made by distilled water to reduce the effect of ions and minerals of the water on the experimental results, thereby only the effects of the tested plants on the nitrate removal of the water are investigated. For the second stage, 0.56 g nickel nitrate has been added to 15 L water to provide a solution with a concentration of 30 ppm nitrate. Among three salts (i.e. nickel nitrate, sodium nitrate, and silver nitrate), the nickel nitrate has been used for contaminating the water sample because it has low toxicity and a high solubility in water compared with the other two salts.

The experimental study was designed and conducted as illustrated in Figures 2 and 3. Four aquariums with dimensions of 40 cm length, 25 cm width, and 45 cm height have been used to keep the plants. Two fluorescent lamps were used to supply the required lighting. About 15 L distilled water was poured into each aquarium and then the required nutrients were added to the culture medium. The concentrations of 30 mg L⁻¹ nitrate have been considered as the initial nitrate concentration of the aquariums obtained by...
adding 56 g nickel nitrate. For each concentration, two aquariums were considered in which one aquarium had zero concentration used as a blank treatment.

All stages of work and tests were carried out in the laboratory conditions at an ambient temperature of 25 °C. An aquarium pump was used for continuous aeration of plants with the air hose placed inside each aquarium. The water sampling was carried out in three steps from the aquariums to maintain the plants, using 1 L containers for specified volumes. The required parameters were determined before the actual tests. After 10 days, the water samples were collected from the aquariums.

The desired variables of the study such as nitrate, concentration, pH, and temperature were assessed before the tests and determining the variables from both before and after the treatment, the efficiency of nitrate removal was determined. Lastly, we compared the removal of nitrate for the three aforementioned plants. In this study, the nitrate, pH, and temperature parameters were measured in water samples. The test time was 10 days and the parameters (such as nitrate, pH, and temperature) were measured daily.

### SAMPLING AND ANALYSIS OF WATER SAMPLES

In this test, a 50 ml water sample was taken from the subsurface of plants (a total of 4 water samples were taken). At each stage, the main controlling parameters in the system were examined. Ambient temperature and water pH were measured daily to maintain environmental conditions. Ambient temperature during the experiments was controlled based on the desired thermal level of plants, i.e. 23–25 °C. According to the physiology of the plant, neutral pH was also controlled in their tolerance range. To analyze the water
sample, 20 ml water has been daily taken from each aquarium of C. alternifolius, T. latifolia, and J. inflexus plants. After sampling, the value of pH, temperature, and the amount of nitrate removal from the water were measured.

To measure the amount of nitrate removal, firstly, 10 ml water sample with special powder (NitraVer X), used for measuring nitrate, were poured inside the tube, and then, it was shaken vigorously to mix well. Then, the spectrophotometer was set at the wavelength of 500 nm as well as at the time of 1 min. After the time is up, we waited more than 5 min for the reaction to stop, and then, we read the number of nitrate. In the next step, we used the distilled water as a benchmark (blank) and read the nitrate value. To obtain the value of nitrate, we used Equation (1).

$$\text{NO}_3 (\text{mg L}^{-1}) \times 4.4 = \text{NO}_3 (\text{mg L}^{-1})$$  \hspace{1cm} (1)

**RESULTS AND DISCUSSION**

This study investigated the efficiency of nitrate removal, using three plants called T. latifolia, C. alternifolius, and J. inflexus, from surface water. We obtained a 95% efficiency for nitrate removal using T. latifolia. Considering the high nitrate levels of the water samples at the start of the experiment, our results demonstrate an 85% efficiency for J. inflexus for removing nitrate from the nitrate-contaminated water up until the tenth day of experiments. In addition, there was a significant decrease in the nitrate concentration of water samples using C. alternifolius plant. Based on the findings of this study, these plants are being developed in Iran to minimize the nitrate content in the surface water.

The reduction of nitrate levels can be attributed to the process of denitrification that can be occurred in the groundwater/root interface. Denitrification has a significant effect on the bioremediation of nitrates leaching into the soil.

The process of denitrification is a stepwise reduction as bellow:

Nitrate ($\text{NO}_3^-$) $\rightarrow$ Nitrite ($\text{NO}_2^-$) $\rightarrow$ Nitric oxide NO
$\rightarrow$ Nitrous Oxide ($\text{N}_2\text{O}$) $\rightarrow$ Nitrogen ($\text{N}_2$)

The reduction of nitrate to nitrogen gas is conducted by enzymes. The main process of nitrate assimilation is located in leaves, while roots serve as the place for nitrate deposition (Melvani *et al.* 2006).

In this study, the blank plant indicates the laboratory condition, in which the comparisons of different factors of the tested plants are performed. The nearly constant values of different factors of the blank plant indicate the controllability of variables in the test results (especially the amount of the residual nitrate); thereby, the results become more validated. The statistics indices of the blank plant are listed in Table 2 and various factors of blank plant in different days are shown in Figure 4.

**pH changes in the water of the tested plants**

To study the pH changes in the water of the tested plants, the pH statistical indices were calculated on different days. The results are shown in Figure 5. In all the plants, water pH had clearly changed. The variation of pH water was significant in both T. latifolia and J. inflexus plants because of their features and basic secretion from their roots. In C. alternifolius plant, the highest change of pH water occurred on the fourth day after which there was a slight

| Table 2 | Statistical indices of different factors in blank plant |
|---------|---------------------------------------------------------|
| Factor  | Frequency | Average | Standard deviation |
| Nitrate (mg L$^{-1}$) | 27 | 30.00 | 0 |
| pH | 9 | 7.2356 | 0.02407 |
| $T$ (°C) | 9 | 24.5 | 0.2201 |

**Figure 4** Average nitrate and pH of the blank plant in different days.
variation in pH water. The figure illustrates that *C. alternifolius* slightly decreased the pH value of water after 10 days. However, *J. inflexus* and *T. latifolia* increased the pH value of water from 6.84 and 6.9 to 7.2 and 7.3, respectively.

**Nitrate levels in different plants and days**

To compare the amount of residual nitrate in the tested plants and different days accompanying their interaction, the two-way ANOVA analysis with interaction has been used. The descriptive statistical indices and the analytical results of this test are shown in Tables 3 and 4, respectively. Figures 6 and 8 are also drawn for the graphical observation of the results. Table 4 shows that there is a significant difference in the amount of residual nitrate between different plants and also between different days at the significance level of 0.01 ($P < 0.01$). Also, a significant interaction was observed between plants and different days at the level of 0.01 ($P < 0.01$). Based on the experimental results, for *C. alternifolius* and *J. inflexus* plants, the highest nitrate removal occurred on the fifth day and for *T. latifolia* plant, the highest nitrate removal happened on the second day.

Figure 6 shows that the highest amount of residual nitrate belong to *C. alternifolius* and *J. inflexus* plants, and there is no significant difference between these two plants at the significance level of 0.05. In contrast, *T. latifolia* plant has the lowest amount of residual nitrate and there is a significant difference between *T. latifolia* plant and the other two plants.
at the significance level of 0.05. This indicates the higher potential of *T. latifolia* plant for removing nitrate from water compared with *C. alternifolius* and *J. inflexus* plants.

![Figure 6] Average nitrate in different plants.

![Figure 7] Evaluation of the homogeneity of different days according to the amount of residual nitrate.

Based on the results of the Duncan test for examining the homogeneity of the residual nitrate on different days, it was observed that there is a significant difference between all days. Moreover, a significant decreasing trend was observed from the first to the ninth day shown in Figure 7.

Figure 8 illustrates the effects of the tested plants and time on the amount of residual nitrate in the water. Until the seventh day, the two plants of *J. inflexus* and *C. alternifolius* had a relatively similar trend, but after that the slope of *J. inflexus* decreased so that the rate of nitrate removal

### Table 3: Statistical indices of the amount of residual nitrate in the water of the tested plants and different days

| Plant              | Day | Frequency | Average  | Standard deviation |
|--------------------|-----|-----------|----------|--------------------|
| *Cyperus alternifolius* | 1   | 3         | 30.0000  | 0.00000            |
|                    | 2   | 3         | 27.9667  | 1.45029            |
|                    | 3   | 3         | 24.2899  | 1.06487            |
|                    | 4   | 3         | 20.0000  | 2.00000            |
|                    | 5   | 3         | 15.8000  | 2.70555            |
|                    | 6   | 3         | 14.8333  | 1.75594            |
|                    | 7   | 3         | 12.0000  | 1.00000            |
|                    | 8   | 3         | 8.0667   | 0.11547            |
|                    | 9   | 3         | 3.8667   | 0.80829            |
| **Total**          | 27  |           | 17.4248  | 8.63411            |
| *Typha latifolia*   | 1   | 3         | 30.0000  | 0.00000            |
|                    | 2   | 3         | 24.7667  | 1.66233            |
|                    | 3   | 3         | 17.2667  | 0.95044            |
|                    | 4   | 3         | 15.3333  | 1.22202            |
|                    | 5   | 3         | 11.3333  | 0.41633            |
|                    | 6   | 3         | 6.1157   | 0.87655            |
|                    | 7   | 3         | 5.2000   | 0.75498            |
|                    | 8   | 3         | 2.9000   | 0.55678            |
|                    | 9   | 3         | 3.1732   | 0.30000            |
| **Total**          | 27  |           | 12.8988  | 9.39709            |
| *Juncus inflexus*   | 1   | 3         | 30.0000  | 0.00000            |
|                    | 2   | 3         | 25.5667  | 0.45092            |
|                    | 3   | 3         | 22.4056  | 1.26166            |
|                    | 4   | 3         | 18.7333  | 0.65064            |
|                    | 5   | 3         | 15.4245  | 0.99260            |
|                    | 6   | 3         | 13.4688  | 1.30812            |
|                    | 7   | 3         | 12.0667  | 1.90088            |
|                    | 8   | 3         | 12.1667  | 1.25033            |
|                    | 9   | 3         | 11.1335  | 0.83565            |
| **Total**          | 27  |           | 17.8935  | 9.45632            |

### Table 4: Comparison of the amount of residual nitrate in the tested plants and different days with their interaction

| The origin of the changes | SS     | df | MS    | F      | Sig. |
|---------------------------|--------|----|-------|--------|------|
| Modified model            | 5,685.318 | 26 | 218.666 | 156.654 | 0.000 |
| Intercept                 | 20,916.769 | 1  | 20,916.769 | 14,984.918 | 0.000 |
| Plant                     | 410.054 | 2  | 205.027 | 146.883 | 0.000 |
| Day                       | 5,046.233 | 8 | 630.779 | 451.895 | 0.000 |
| Plant × Day               | 229.031 | 16 | 14.314 | 10.255 | 0.000 |
| Error                     | 75.376 | 54 | 1.396 |        |      |
| Total                     | 26,677.464 | 81 |       |        |      |
| **Total modified**        | 5,760.694 | 80 |       |        |      |
from water was greatly reduced and the variation of the remaining nitrate in the water became very limited.

In contrast, the amount of nitrate remaining in the water of *C. alternifolius* plant, with a significant difference, is always higher than *T. latifolia* plant; however, the rate of nitrate removal increases gradually so that the amount of nitrate remaining in the water of the two plants is almost the same after 9 days. This indicates the existence of an interaction between the two variables of plant type and day, which based on the performed test in Table 4; this interaction was found at the significant level of 0.01.

**The relationship between nitrate and pH in the tested plants**

To investigate the correlation coefficients of residual nitrate and pH in the tested plants, a Pearson correlation coefficient has been used. In Table 5, the positive and negative correlation coefficients, respectively, indicate that there are direct and inverse relations between the factors. Also, the evaluation of the intensity of the calculated correlation coefficients is taken place based on Table 6. This interpretation is also applicable to the negative values of the correlation coefficients. According to Table 5, there is a significant
reverse relationship between the amount of residual nitrate and pH in *T. latifolia* and *J. inflexus* plants at the level of 0.01.

While this relationship in *C. alternifolius* plant is direct and significantly at the level of 0.01. Figure 9 depicts the distribution of aquatic plant species based on the amount of nitrate and pH variables in the tested plants.

### CONCLUSION

This experimental study evaluated the efficiency of three plants called *T. latifolia*, *C. alternifolius*, and *J. inflexus* on the removal of nitrate from the water. Nitrite concentration

| Plant                  | Frequency | Correlation Coefficient | P-value |
|------------------------|-----------|-------------------------|---------|
| *Cyperus alternifolius*| 27        | 0.776                   | 0.000   |
| *Typha latifolia*      | 27        | −0.591                  | 0.001   |
| *Juncus inflexus*      | 27        | −0.879                  | 0.000   |

Table 6 The basis for evaluating the intensity of correlation coefficient

| Correlation coefficient | Intensity of correlation coefficient |
|-------------------------|-------------------------------------|
| 0.0–0.19                | Very low                            |
| 0.2–0.39                | Low                                 |
| 0.4–0.69                | Medium                              |
| 0.7–0.89                | High                                |
| 0.9–1.00                | Very high                           |

Figure 9 Distribution of aquatic plant species based on the amount of nitrate and pH variables (a) *Cyperus alternifolius*, (b) *Typha latifolia*, and (c) *Juncus inflexus*. 
was approximately 30 mg L\(^{-1}\) in all water samples. The following results were obtained:

Nitrate removal rates by \textit{T. latifolia}, \textit{C. alternifolius}, and \textit{J. infflexus} plants were 87.1, 82.5, and 82.1\%, respectively. Moreover, pH water is affected by the plants. Both \textit{T. latifolia} and \textit{J. infflexus} increase the pH water after 10 days. However, \textit{C. alternifolius} has a slight effect on the pH variation of water.

Among the three plants, \textit{C. alternifolius} plant had the highest growth of shoots, roots, and stems resulting from the supply of plant with the required nutrients, indicating the role of other elements as a limiting factor in plant growth. It can also be concluded that in each aquarium, the amount of nitrate removal from water is directly related to the weight of plant roots. The nitrate removal from water increases as the weights of roots or rhizomes of the plants increase.

Overall, the results demonstrated that biological methods could be a suitable method for removing nitrate from surface water. Moreover, because of the efficiency of phytoremediation compared with the other methods of nitrate removal from water, it can be concluded that phytoremediation is a cost-effective, environmentally friendly and acceptable process for many countries. With the increase of studies in this field, a new step can be taken in the direction of solving the problem of water shortage and enhancing both the quantity and quality of water purification. Finally, we recommend conducting research on the effect of these aquatic plants on other parameters such as water hardness, light radiation, electrical conductivity of water, and removal of heavy metals.

**DECLARATION OF INTEREST STATEMENT**

There is no conflict of interest regarding the submitted manuscript.

**DATA AVAILABILITY STATEMENT**

All relevant data are included in the paper or its Supplementary Information.

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