Phytoremediation of nitrate contaminated water using ornamental plants

S. Shyamala, N. Arul Manikandan, Kannan Pakshirajan, Van Tai Tang, Eldon R. Rene, Hung-Suck Park and Shishir Kumar Behera

ABSTRACT

This work aims at evaluating the potential of two ornamental plant species, i.e., money plant (*Epiprennum aureum*) and arrowhead plant (*Syngonium podophyllum*), to treat nitrate containing wastewater. Statistically designed experiments were performed to ascertain the effect of initial nitrate concentration (40–120 mg/L), growth period (1–12 days) and plant density (20–80 g/L) on nitrate removal. Based on the results of analysis of variance (ANOVA), it was observed that the individual effects ($F = 78.04$ and $P = 0.013$) of process parameters influenced the nitrate removal efficiency by money plant stronger than the 2-way ($F = 0.2$ and $P = 0.89$) and 3-way interaction effects ($F = 0.46$ and $P = 0.569$). In the case of the arrowhead plant, the individual effects significantly affected the nitrate removal efficiency than the 2-way and 3-way interaction effects. Low nitrate concentrations (40 mg/L) and high plant density (80 g/L), showed ~88% nitrate removal by arrowhead plant, during a growth period of 6 d. On the contrary, under similar conditions, the money plant showed a nitrate removal efficiency of ~93% during a growth period of 12 d. Concerning the removal kinetics, an increase in the growth period increased the nitrate removal rate for both the plants.

Key words | arrowhead plant, growth period, initial nitrate concentration, money plant, plant density, phytoremediation

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INTRODUCTION

As a consequence of rapid urbanization, industrialization and the excessive use of fertilizers, the levels of nitrate in ground and surface water has increased several-fold in both developing and rapidly developing countries (Weldeslassie *et al.* 2018). The presence of high nitrate concentrations in drinking water results in health issues such as blue baby syndrome (methemoglobinemia), stomach cancer, high blood pressure, thyroiditis, cytogenetic

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malfunction and several other defects in newborn babies (Sharma *et al.* 2017; Soomro *et al.* 2017; Nieder *et al.* 2018). According to the World Health Organization (WHO), the maximum level of nitrate in drinking water has been fixed at ~11.0 mg N/L. The maximum nitrogen level stipulated by US Environmental Protection Agency (USEPA) and China Environmental Agency is 10 mg N/L in drinking water (Liu *et al.* 2015; Pennino *et al.* 2017). According to the prevailing Indian situation, Rai (2003) reported that ~118 million people are drinking water with nitrate levels in the range of 45–100 mg N/L and more than 108 million people with levels >100 mg N/L (e.g., the Bureau of Indian Standards desirable limit is <45 mg N/L).

The main anthropogenic source for the release of nitrate into groundwater and surface water includes a number of issues such as improper agricultural practices, excessive use of synthetic fertilizers, fossil fuel combustion, among others (Chen *et al.* 2017; Wollheim *et al.* 2017). Quintessentially, in the developing nations, groundwater is the real source of drinking water as the risk of microbial contamination is much less compared to other drinking water sources (Fout *et al.* 2017; Li *et al.* 2018). Therefore, the removal of nitrate from both groundwater and surface water is essential before it is used for human consumption.

Reverse osmosis, adsorption, ion exchange, chemical denitrification, photocatalytic reduction and electro-dialysis are conventional physico-chemical processes for the remediation of water containing excessive levels of nitrate (Bulgariu *et al.* 2012; Arun *et al.* 2017; Challagulla *et al.* 2017; Tyagi *et al.* 2018). Some of these physico-chemical processes are rather expensive, energy intensive, and they also generate secondary pollutants during their operation (Sinha *et al.* 2017; Wang *et al.* 2018). Nevertheless, for the treatment of low nitrate concentrations in ground and surface waters, biological processes are often considered as the best suitable technological option from a techno-economic viewpoint.

In this line of continuous research that targets biotechniques for water treatment, phytoremediation is considered to be a versatile and sustainable natural treatment technique. Phytoremediation is an inexpensive, non-invasive and versatile treatment technology for removing nutrients and trace contaminants from wastewater and surface water sources (Sinha *et al.* 2018). The inherent capacity of certain (native) plant species in sequestering nitrate from water for their growth is used in this technique for the removal of nitrate from contaminated water environments (Li *et al.* 2016). More specifically, macrophytes are extensively employed in the field of phytoremediation because of their ability to sequester high amounts of pollutant, in a shorter span of time, thereby demonstrating high pollutant removal rates (Bartucca *et al.* 2016). Besides, these plants promote the growth of microbial communities around their root systems, thus having a cumulative (synergistic) effect in removing the pollutants from water (Lingua *et al.* 2015; Xu *et al.* 2018).

According to the literature, a variety of macrophytes has been employed for the removal of pollutants present in water (Liu *et al.* 2017; Sinha *et al.* 2018). These include N, P and K, heavy metals, organic and inorganic forms of nitrogen present in water bodies, micropollutants, fluoride and other trace metallic species (Pavlineri *et al.* 2017). For example, *Pistia stratiotes* and *Spirodela polyrhiza* were used to remove fluoride and heavy metal ions from water (Volf *et al.* 2015; Karmakar *et al.* 2016). *Vallisneria natans* showed good potential for the treatment of wastewater contaminated with excessive levels of arsenic (Chen *et al.* 2015). In another recent study, the Italian ryegrass grown in a vegetated floating system was tested for its potential to treat nitrate-contaminated water (Bartucca *et al.* 2016). Rye-grass (*Lolium perenne*) has proven to be effective in removing inorganic nitrogen from water, in both hydroponic systems and in sand filters (Liu *et al.* 2017; Escobar-Alvarado *et al.* 2018; Radziemska *et al.* 2019). However, due to their aesthetic looks, commercial value and easy adaptability to diverse environments, ornamental plants seem to be a promising alternative to fast-growing plants/weeds for treating nitrogen-contaminated surface/groundwater in households. In this study, as a proof-of-concept, and to demonstrate the application of ornamental plants for the treatment of nitrate-containing water, laboratory-scale experiments were performed with the following objectives: (i) to test the nitrate removal efficiency by two ornamental plants, i.e., money plant and arrowhead plant; (ii) to elucidate the main and interaction effects of process factors such as initial nitrate concentration, growth period and plant density on the nitrate removal efficiency; and (iii) to perform a kinetic analysis on the nitrate uptake capacity by the two plants.

MATERIALS AND METHODS

Growth conditions of plants

Money plants (*Epiprennum aureum*) and arrowhead plants (*Syngonium podophyllum*) were collected from a local nursery, at the GMRIT campus, India. These two plants were selected based on their wide occurrence as well as their ability to grow under harsh conditions, with minimal nutrient requirements. Prior to the regular experiments, all the selected plants were allowed to grow only with tap water for a total period of 2 weeks. As per the experimental requirement, in order to study the effect of plant density, varying sizes of well-grown and healthy plants were chosen. Prior to use, these plants were rinsed thoroughly in tap water in order to eliminate sediments and other dirt elements attached to them. Finally, the washed plants were placed in clean, transparent cylindrical plastic containers for regular experiments (Figure 1). Different experimental setups were used for money plants and arrowhead plants, respectively. To each of these containers was added 2 L of 50% (*v/v*) Hoagland's solution (HS) of composition (in mM): KNO_3 (3.0), $Ca(NO_3)_2$ (2.4), $MgSO_4$ (1.0), KH_2PO_4 (1.0) and NaCl (0.5), and Fe-EDTA (44.8), H_3BO_3 (23.1), $MnCl_2$ (4.6), $ZnSO_4$ (0.38), $CuSO_4$ (0.16) and H_2MoO_4 (0.052) complex prepared using tap water (Sinha *et al.* 2017).

The plants were grown under ambient environmental conditions, with a temperature range of 25–30 °C. All the chemicals used in the study were obtained from Desai Chemical Company (Visakhapatnam, India) and Lotus Granges India Limited (Visakhapatnam, India), respectively. All the chemicals used in this study were of analytical grade.

Statistical design of experiments

The factorial design of experiments is a statistically significant technique used widely to infer maximum information by performing fewer actual experiments (Sinha *et al.* 2017). In this design, the effects of various processes or operational factors on a specific response variable, at different levels, can be measured simultaneously. Both the individual and interaction effects induced by each factor can be ascertained in a statistically significant manner (Hazarika *et al.* 2015). A 2^3 full-factorial experimental design, comprising ten runs, i.e., eight actual runs and two additional runs, was used in this study. The initial nitrate concentration (X_1), growth period or time (X_2) and plant density (X_3) were chosen as the three important factors that affect the nitrate removal efficiency in plants. Table 1 presents the three different factors, along with the three different levels chosen in the present study. The low (−1), medium (0)

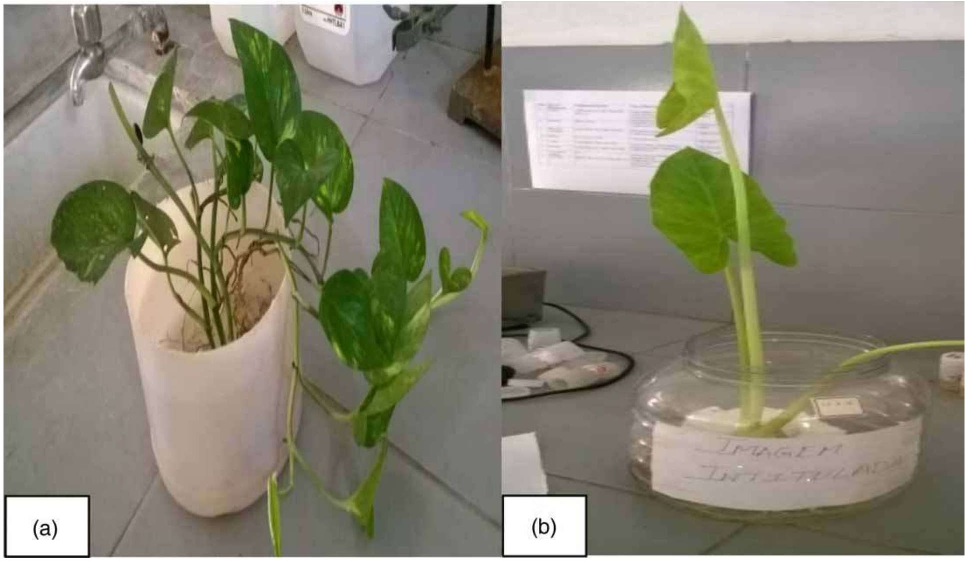

Figure 1 | Photograph of the experimental setup used in the study: (a) money plant and (b) arrowhead plant.

Table 1 | Experimental factors and levels investigated in this work

	Factors			
	Initial nitrate concentration (mg/L)	**Growth period (d)**		**Plant density (g/L)**
Levels	**Money plant and arrowhead plant**	**Money plant**	**Arrowhead plant**	**Money plant and arrowhead plant**
Low	40	3	1	20
Centre	80	7.5	3.5	50
High	120	12	6	80

and high (+1) levels of each factor were chosen based on previous literature reports (Khajuria & Kanae 2013; Sinha *et al.* 2017) and based on preliminary experiments conducted prior to this study. The responses of nitrate removal by money plant and arrowhead plant were expressed in terms of the nitrate removal efficiency (%). Thereafter, a linear polynomial model was proposed to correlate the individual and interaction effects of these factors with the response variable. The equation takes the form as shown in Equation (1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 \cdot X_2 + \beta_{13} X_1 \cdot X_3 + \beta_{23} X_2 \cdot X_3 + \beta_{123} X_1 \cdot X_2 \cdot X_3 \quad (1)$$

where Y is the nitrate removal efficiency as observed in two different plants, β_0 is a constant term, β_1, β_2 and β_3 are the coefficients that describe the individual effects of initial nitrate concentration (X_1), growth period (X_2) and plant density (X_3), respectively. β_{12}, β_{13} and β_{23} are the coefficients that reveal information on the interaction effects between initial nitrate concentration × growth period, initial nitrate concentration × plant density and growth period × plant density, respectively. The coefficient β_{123} indicates the interaction effect of initial nitrate concentration × growth period × plant density, while X_1, X_2 and X_3 are the independent variables. The difference observed in the response of any factor amid its variation from low to high levels is defined as the effect and the value of the effect is divided by two to obtain the regression model coefficients. Thereafter, the regression model coefficients are divided by the standard error coefficient to get the value of standardized effects (T) (Villa-Gomez *et al.* 2015).

The statistical significance of any factors in the regression model equation was determined based on its probability (P) value (Sinha *et al.* 2017). The coefficient values of the individual and interaction effects of each factor observed for a specific response showed its effect on a particular experimental condition. The individual and interaction effects of the factors which cross the reference line (vertical line at 4.30) in a Pareto plot (Figure 2(a) and 2(b)) are considered to be potentially important at the 95% significance level (Roy *et al.* 2015). In the present study, Minitab$^{\text{TM}}$ Release 16, 2006 software was used to analyse the experimental results and the same software was also used to determine the statistical indices, namely, the regression coefficient, the probability value (P) and Fischer's value (F).

Experimental methodology

1.631 g of potassium nitrate was dissolved in 1 L of double distilled water to prepare a nitrate stock solution of 1,000 mg/L (Sinha *et al.* 2017). Thereafter, the stock solution was stored under refrigerated conditions. The desired concentration of nitrate for each experimental run (Table 2) was obtained by diluting the stock solution with 50% (*v/v*) Hoagland's solution. Control experiments in the present study refer to the experiments that were performed in the absence of plants.

Analytical method and kinetics

In order to determine the nitrate removal efficiency by the plants, samples were collected at different time intervals as shown in Table 2. For this analysis, 2 mL of working solution was collected in a beaker and 0.1 mL of concentrated HCl was added. The samples were agitated thoroughly for 5 min and allowed to remain in suspension for 10 min. Thereafter, the samples were examined using a UV-Vis spectrophotometer at a wavelength (λ_{max}) of 220 nm. The nitrate removal efficiency was calculated as follows:

$$\textit{Nitrate removal efficiency}\ (\%) = \frac{C_0 - C_e}{C_0} \times 100 \quad (2)$$

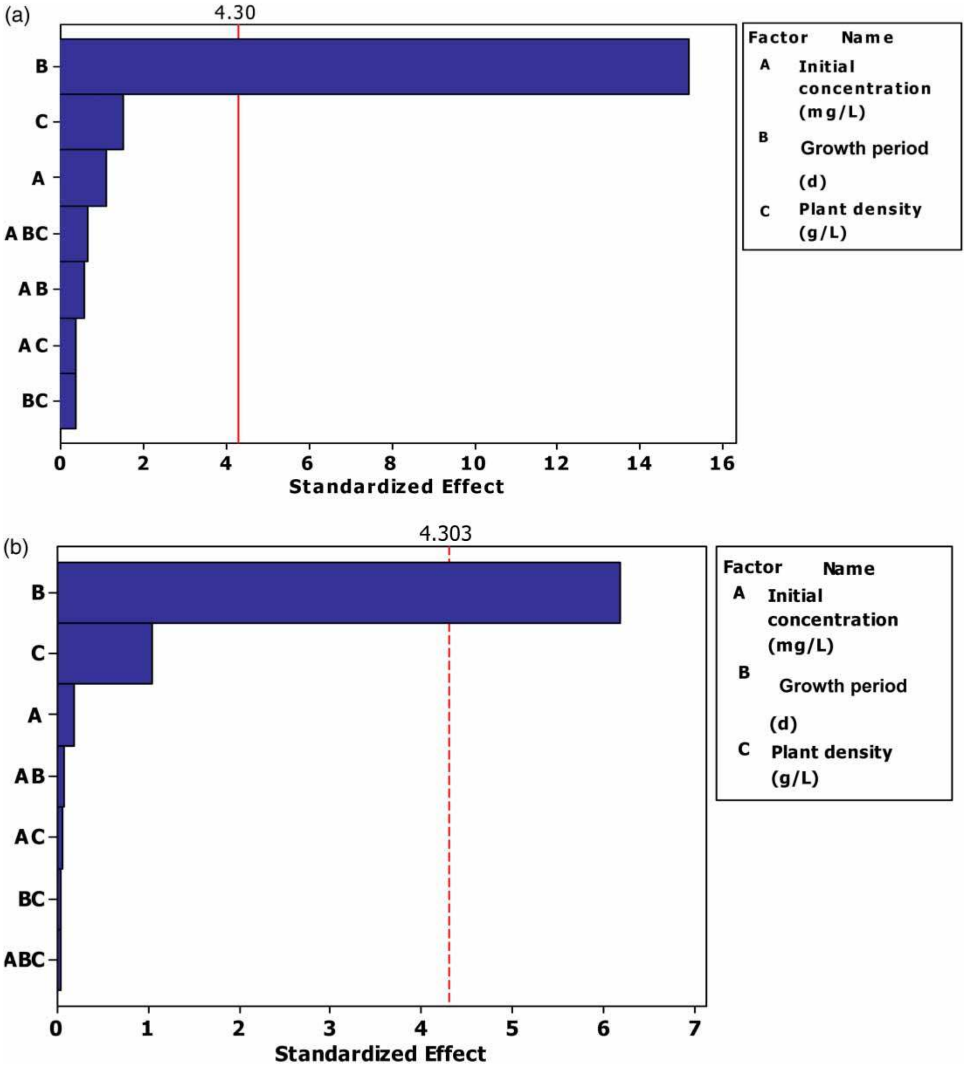

Figure 2 | Pareto chart showing the standardized effects of various factors on nitrate removal efficiency (%) by: (a) money plant and (b) arrowhead plant.

where C_0 is the initial nitrate concentration (mg/L) and C_e is the equilibrium nitrate concentration (mg/L).

The kinetics of nitrate uptake by money plant and arrowhead plant was ascertained by fitting the nitrate removal results obtained in each experimental run at different intervals of time to different kinetic models reported in the literature. However, only the Lagergren pseudo first-order gave an accurate fit. The pseudo first-order rate expression can be defined as shown in Equation (3) (Sinha *et al.* 2017):

$$\ln\,(C_e - C_t) = -kt + \ln C_e \tag{3}$$

where C_e and C_t, both expressed in mg/g, are the uptake capacities at equilibrium and at time t (min), respectively, and k (min^{-1}) is the pseudo first-order rate constant. The values of k for both the ornamental plants were calculated from the slope of a linear plot of ln $(C_e - C_t)$ versus t.

RESULTS AND DISCUSSION

Model fitting and statistical analysis

Table 2 presents the nitrate removal efficiency observed at three levels of various factors tested for money plant and

Table 2 | Full factorial design for determining the nitrate removal by money plant and arrowhead plant

	Factors				**Nitrate removal efficiency (%)**	
Run no.	**Initial nitrate concentration (mg/L), X_1**	**Money plant Growth period (d) (d), X_2**	**Arrowhead plant Growth period (d) (d), X_2**	**Plant density (g/L), X_3**	**Money plant**	**Arrowhead plant**
1	40	1	3	20	9	15
2	120	1	3	20	5	7
3	40	6	12	20	79	77
4	120	6	12	20	76	71
5	40	1	3	80	13	24
6	120	1	3	80	12	18
7	40	6	12	80	93	88
8	120	6	12	80	80	82
9	80	3.5	7.5	50	37	32
10	80	3.5	7.5	50	40	34

arrowhead plant, respectively. The highest nitrate removal efficiency for both the plants occurred when experiments were performed under the conditions described for run no. 7 where a lower nitrate concentration, a higher growth period and a higher plant density were used. Under this condition, the maximum nitrate removal efficiency was found to be 93% and 88% for money and arrowhead plants, respectively.

The lowest value of nitrate removal efficiency, i.e., 5% and 7% was obtained in run no. 2 for both money and arrowhead plants, respectively. The increase of the plant growth period and plant density showed a steady increase in nitrate removal efficiency for all the experimental runs. These observations were similar to those reported previously by Bartucca *et al.* (2016) and Lingua *et al.* (2015). On the contrary, increasing the initial nitrate concentration from low to high levels decreased the nitrate removal efficiency (Iamchaturapatr *et al.* 2007; Ayyasamy *et al.* 2009).

The individual (main) and interaction effects of all the factors and the coefficients of regression, total sum of squares (TSS), probability value (P) and Fischer's value (F) for each response (i.e., the nitrate removal by money plant and arrowhead plant) are shown in Table 3. The coefficients (β) shown in Table 4 can be substituted in the regression model Equations (4) and (5), and the polynomial equations developed in the present study can be used further for evaluating the nitrate removal efficiency under different values of process parameters. The polynomial equations are as follows:

$$\begin{aligned}&\textit{Nitrate removal efficiency in money plant}\\&\quad = -12.7376 - 0.083\,X_1 + 7.12\,X_2 - 0.045\,X_3\\&\qquad + 0.0073\,X_1X_2 + 0.0015\,X_1\,X_3 + 0.031\,X_2\,X_3 \end{aligned} \tag{4}$$

$$\begin{aligned}&\textit{Nitrate removal efficiency in arrowhead plant}\\&\quad = 1.26 - 0.114\,X_1 + 12\,X_2 - 0.132\,X_3\\&\qquad + 0.01\,X_1\,X_2 + 0.0004\,X_1\,X_3 + 0.009\,X_2\,X_3 \end{aligned} \tag{5}$$

Based on the results shown in Figure 2(a) and 2(b), it can be ascertained that the plant growth period posed the maximum effect on nitrate removal efficiency, for both the ornamental plants. Figure 3 depicts the degree of fitness of the proposed model with the experimental results. The proposed model for estimating the nitrate removal efficiency by both the ornamental plants had R^2 values >0.95.

Student's *t*-test and analysis of variance (ANOVA)

The Student's *t*-test and analysis of variance (ANOVA) was carried out to get a better insight into the experimental results. In general, regression models are said to be statistically significant if the Fischer's 'F' value is high and the probability 'P' value is low. Furthermore, the Student's

Table 3 | ANOVA for nitrate removal by (a) money plant and (b) arrowhead plant

	DF	Seq SS	Adj MS	*F*	*P*
(a) Source					
Main effects	3	10,596.2	3,532.1	78.04	0.013
X_1	1	55.5	55.5	1.23	0.383
X_2	1	10,434.5	10,434.5	230.53	0.004
X_3	1	106.3	106.3	2.35	0.265
2-way interactions	3	26.9	9	0.2	0.89
$X_1 \times X_2$	1	14.7	14.7	0.32	0.626
$X_1 \times X_3$	1	6.3	6.3	0.14	0.746
$X_2 \times X_3$	1	6	6	0.13	0.751
3-way interactions	1	20.6	20.6	0.46	0.569
$X_1 \times X_2 \times X_3$	1	20.6	20.6	0.46	0.569
Error	1	4.2	4.2	4.2	
Total	9	10,734.3			
(b) Source					
Main effects	3	8,316.27	2,772.09	13.11	0.072
X_1	1	6.93	6.93	0.03	0.873
X_2	1	8,083.56	8,083.56	38.22	0.025
X_3	1	225.78	225.78	1.07	0.41
2-way interactions	3	1.86	0.62	1	1
$X_1 \times X_2$	1	1.05	1.05	0	1.95
$X_1 \times X_3$	1	0.45	0.45	0	0.967
$X_2 \times X_3$	1	0.36	0.36	0	0.971
3-way interactions	1	0.36	0.36	0	0.971
$X_1 \times X_2 \times X_3$	1	0.36	0.36	0	0.971
Error	1	2.51	2.51		
Total	9	8,741.45			

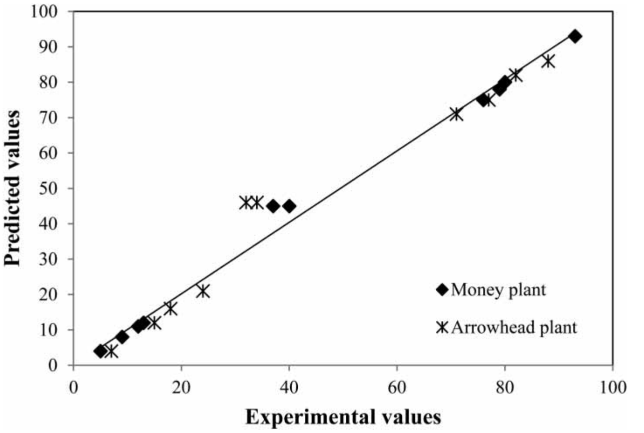

Figure 3 | Linear plot showing the distribution of experimental and predicted values of nitrate removal efficiency by money plant and arrowhead plant.

t-test was conducted to identify the 'course of the effect (positive or negative)', posed by each individual factor and its significance on the nitrate removal efficiency. The Student's *t*-test results were additionally interpreted for their interaction effects and their statistical index (*t*, *F* and *P*) values are shown in Table 4 for money plant and arrowhead plant, respectively.

The corresponding coefficient terms can be considered to be highly significant when the magnitude of '*t*' is larger and smaller for '*P*'. Generally, the Student's *t*-test and ANOVA conducted in the present study show that the plant growth period had a positive effect on the nitrate removal efficiency, while there were no significant effects by other factors in both the plants. The growth period showed the highest positive effect ($t = 15.18$ and

Table 4 | Student's *t*-test of the model coefficients for nitrate removal by money plant and arrowhead plants

	Nitrate removal efficiency (%)							
	Money plant				Arrowhead plant			
Term	Effect	Coefficient	*t*-value	*P*-value	Effect	Coefficient	*t*-value	*P*-value
Constant		−12.74	20.88	0.002		1.26	9.62	0.011
X_1	−0.166	−0.083	−1.11	0.383	−0.228	−0.114	−0.18	0.873
X_2	14.24	7.12	15.18	0.004	24	12	6.18	0.025
X_3	−0.09	−0.045	1.53	0.265	0.264	0.132	1.03	0.41
$X_1 \times X_2$	0.0146	0.0073	−0.57	0.626	0.02	0.01	0.07	0.95
$X_1 \times X_3$	0.003	0.0015	−0.37	0.746	0.0008	0.0004	0.05	0.967
$X_2 \times X_3$	0.062	0.031	0.36	0.751	0.018	0.009	0.04	0.971

$P = 0.004$) for money plant. Similarly, the growth period has shown the highest positive effect ($t = 6.18$ and $P = 0.025$) for arrowhead plant. Thus, it was observed that the individual effects had greater influence on the nitrate removal efficiency when compared to the interaction effects (Table 3).

Individual (main) effects plot

The individual effects or the main effects plot for money plant and arrowhead plant are shown in Figure 4(a) and 4(b), respectively. The individual effects demonstrate the relative quality of the effects of different factors in an

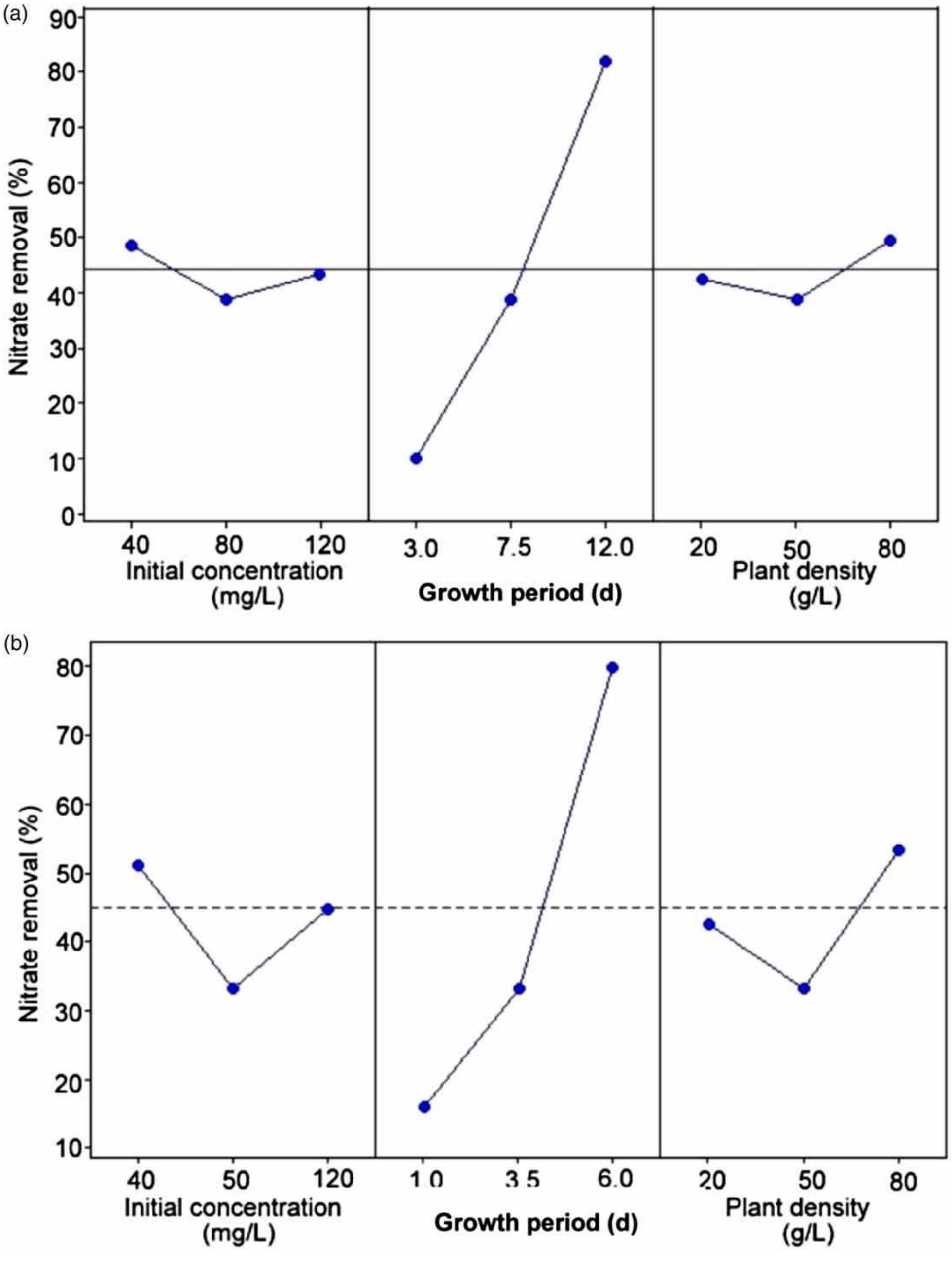

Figure 4 | Individual (main) effects plot for nitrate removal efficiency (%) by: (a) money plant and (b) arrowhead plant.

experimental design. A factor is said to affect the process individually without the interaction of other factors when the mean responses of the individual factor exceed the other levels of the same factor (Singh *et al.* 2016). Further, the sign associated with the individual effects of the factors clearly depicts its course of action in removing nitrate from the water.

The increase in the nitrate removal profiles with an increase in the growth period and plant density at different levels reveals that both these factors positively affected the nitrate removal efficiency in money plant (Figure 4(a)). For money plant, an increase in the growth period from low to high level resulted in an increase in nitrate removal efficiency from 10% to 92%. Similarly, in the case of arrowhead plant, the nitrate removal efficiency increased from 18% to 80%. Enhancement in nitrate removal efficiency along with an increase in the growth period may be correlated with the fact that longer retention of the pollutants in the system will result in longer contact time between the plant surface and the pollutants (Xu & Shen 2011; Shu *et al.* 2017).

However, it was observed that the nitrate removal efficiency always decreased with an increase in the initial nitrate concentration, irrespective of the growth period and plant density factors being at any level. For instance, in the case of money plant, an increase in the initial nitrate concentration from low level to high level resulted in a decrease in the nitrate removal efficiency from 49% to 44%. Similarly, for the arrowhead plant, the nitrate removal efficiency decreased from 52% to 45%. This was also supported by the negative Student's *t*-test value ($t = -1.11$) observed in the present experiment. In conventional phytoremediation systems, the uptake capacity of plants reduces with an increase in initial nitrate concentration due to changes in the osmotic pressure (Ayyasamy *et al.* 2009; Ng & Chan 2017). Moreover, high nitrate concentration reduces the regeneration rate of active sites in the plant tissues which would eventually end up in slow metabolic nitrate uptake (Saber *et al.* 2018). On the other hand, at low nitrate concentrations, the plant accumulation capacity is usually high. Similar results were also observed in the case of arrowhead plant, i.e., the growth period and plant density showed a positive effect on the nitrate removal efficiency, while the initial nitrate concentration had a negative effect. This observation was also supported by the negative Student's *t*-test values ($t = -0.18$) for arrowhead plant. An increase in the plant density from low to high levels, both in money plant as well as the arrowhead plant, resulted in an increase in the nitrate removal efficiency from 44% to 50% and 43% and 55%, respectively. In the case of arrowhead plant, plant density was found to play a significant role in nitrate removal. It is because of the plant's wider leaves, with a well-developed root system and high growth rate (Sinha *et al.* 2017). Thus, an increase in the growth period in both the cases resulted in an increase in nitrate removal efficiency. However, in both the plants, nitrate removal efficiency was found to decrease with an increase in initial nitrate concentration, at some growth period and plant density conditions.

Interaction effects plot

The mean responses of any two factors, in all possible combinations, are usually plotted in the form of interaction effects plot. Non-parallel lines in the interaction effect plot indicate there is an interaction between the two tested factors. Nevertheless, the appearance of parallel lines in the plot shows that there is no interaction between the two factors (Singh *et al.* 2016). Figure 5(a) and 5(b) show the interaction effect plots for money plant and arrowhead plant, respectively. In the case of money plant (Figure 5(a)), positive interaction between the growth period × plant density was observed. On the contrary, a negative interaction effect was observed between the growth period × initial nitrate concentration and the plant density × initial nitrate concentration. An increase in the growth period from 3 to 12 d increased the nitrate removal efficiency by 72% (from 11 to 83%), at a plant density value of 80 g/L. Increasing the initial nitrate concentration from 40 to 120 g/L caused the nitrate removal efficiency to reduce by 5% (from 82 to 77%), at a growth period value of 12 d. Likewise, an increase in the initial nitrate concentration from low to high level decreased the nitrate removal efficiency value by 3% (from 45 to 42%), at a plant density value of 80 g/L.

A similar trend as observed in money plant was also observed in the case of arrowhead plant. From Figure 5(b), it is evident that an increase in the growth period from 1 to 6 d increased the nitrate removal efficiency by 63%

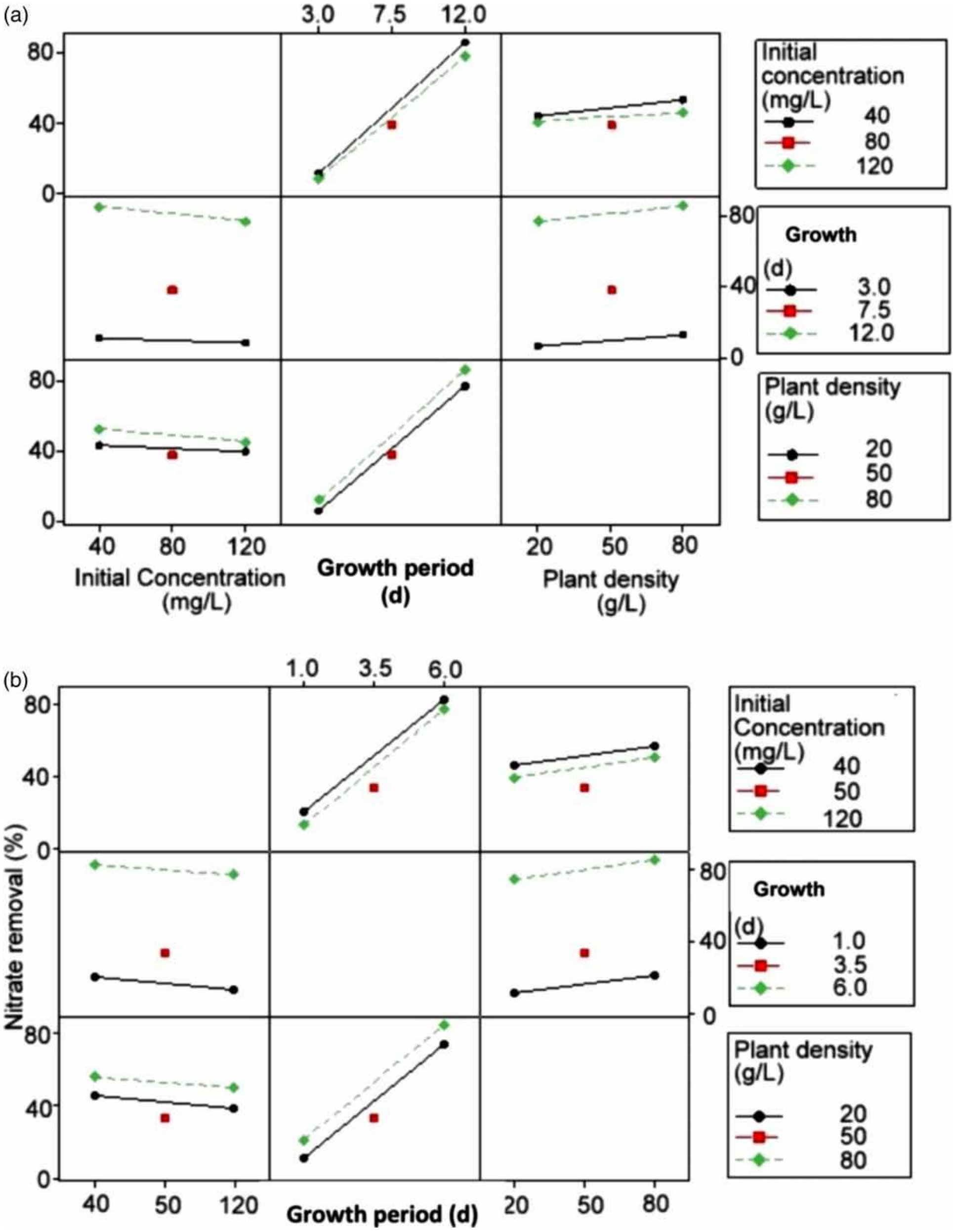

Figure 5 | Interaction effect plot for nitrate removal efficiency (%) by: (a) money plant and (b) arrowhead plant.

(from 20 to 83%), at an initial nitrate concentration of 40 g/L. Increasing the initial nitrate concentration from 40 to 120 g/L decreased the nitrate removal efficiency by 3% (from 82 to 79%), at a growth period of 6 d. Likewise, an increase in the initial nitrate concentration from low to a high level slightly reduced the nitrate removal efficiency by 2% (from 45 to 43%) at a plant density value of 80 g/L. The cumulative effects of different factors on the nitrate removal efficiency can be visualized in the form of 3D surface plots (Supplementary material, Figures S1 and S2), for both money plant and arrowhead plant, respectively.

Kinetic modelling of nitrate removal by two plants

The values of the estimated kinetic model parameters, obtained using actively growing money and arrowhead

Table 5 | Estimated pseudo first-order kinetic parameters of nitrate uptake by (a) money plant and (b) arrowhead plant

Factors				Response
Run No.	Initial nitrate concentration (mg/L)	Plant density (g/L)	Growth period (d)	Kinetic constant (d^{-1})
(a) Money plant				
1	40	20	3	0.031
2	120	20	3	0.017
3	40	80	3	0.046
4	120	80	3	0.042
5	40	20	12	0.13
6	120	20	12	0.118
7	40	80	12	0.221
8	120	80	12	0.134
(b) Arrowhead plant				
1	40	20	1	0.162
2	120	20	1	0.072
3	40	80	1	0.278
4	120	80	1	0.199
5	40	20	6	0.244
6	120	20	6	0.208
7	40	80	6	0.353
8	120	80	6	0.289

plants, are presented in Table 5. The results reveal that nitrate removal by both the ornamental plants followed the pseudo first-order kinetics well with an R^2 value close to 1, which is in agreement with the literature report on pollutant removal in various other plant species (Li *et al.* 2002; Lv *et al.* 2016). For both the plants, the maximum estimated nitrate removal rate (Table 5(a) and 5(b)) obtained in run 7 using this model also matched well with the maximum nitrate removal efficiency (84.3%) obtained in run no. 7 (Table 1). From Table 5, it can be observed that at similar initial nitrate concentration and plant density, the nitrate removal rate constant ($k = 0.353\ d^{-1}$) for arrowhead plant was superior to money plant ($k = 0.221\ d^{-1}$), irrespective of the different growth period values (Shyamala *et al.* 2017). The maximum nitrate removal rate observed in run no. 7 may be attributed to the higher metabolic rate of these plants, which in turn, can be attributed to the higher plant density, i.e., the presence of a greater number of active sites in the plants to draw nitrate from the system (Saber *et al.* 2018). Further, low concentration of nitrate in this run would have kept the regeneration rate of these active sites intact, thereby leading to an unhindered nitrate uptake. The results obtained from this study clearly show that both these ornamental plants may be used in constructed wetland systems as a cheap, natural, environmentally and technically viable treatment technique for nitrate removal from water.

Future perspectives

Since the money plant used in the present study offered a greater nitrate removal efficiency and the arrowhead plant presented a maximum nitrate uptake rate, further investigation by combining both these plants in a single system may result in the development of a faster and efficient nitrate removal system. Moreover, it would be fascinating to carry out optimization studies by involving various other process parameters like pH, dissolved oxygen and wastewater volume, etc. However, in order to realize the full potential of the ornamental plants used in this study (money plant and arrowhead plant) for treatment of nitrate-containing wastewater, a detailed investigation by adopting the pilot-scale wetland system is needed instead of the lab-scale hydroponic system. As well, investigation on the effect of co-ions on nitrate removal and mimicking the current system with that of the real-world scenario by subjecting it to shock loads of different volumes of wastewater is warranted. A cost–benefit analysis on such a system would unleash the use of ornamental plants in phytoremediation. Thus, the results of this study would be valuable to establish the use of these ornamental plants for nitrate removal from water in field-scale systems.

CONCLUSIONS

The individual (main) effect of the process variables, i.e., the initial nitrate concentration, growth period, plant density, was found to play a major role in affecting the nitrate removal process. Among the individual effects, growth period appeared to have a significant influence on nitrate removal for both money plant ($t = 15.18$, $P = 0.004$) and arrowhead plant ($t = 6.18$, $P = 0.025$), respectively. At their highest growth period, the arrowhead plant demonstrated better nitrate removal kinetics when compared to money plant. For example, at an initial nitrate concentration of 40 mg/L and a plant density of 20 g/L, the rate constant for nitrate removal was found to be $0.13\,d^{-1}$ and $0.244\,d^{-1}$, respectively, at the growth periods of 12 and 6 d for money plant and arrowhead plant.

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SUPPLEMENTARY DATA

The Supplementary Data for this paper is available online at http://dx.doi.org/10.2166/aqua.2019.111.

REFERENCES

Arun, S., Manikandan, N. A., Pakshirajan, K., Pugazhenthi, G. & Syiem, M. B. 2017 Cu (II) removal by *Nostoc muscorum* and its effect on biomass growth and nitrate uptake: a photobioreactor study. *Int. Biodeterior. Biodegradation* **119**, 111–117.

Ayyasamy, P. M., Rajakumar, S., Sathishkumar, M., Swaminathan, K., Shanthi, K., Lakshmanaperumalsamy, P. & Lee, S. 2009 Nitrate removal from synthetic medium and groundwater with aquatic macrophytes. *Desalination* **242** (1–3), 286–296.

Bartucca, M. L., Mimmo, T., Cesco, S. & Del Buono, D. 2016 Nitrate removal from polluted water by using a vegetated floating system. *Sci. Total Environ.* **542**, 803–808.

Bulgariu, L., Ceica, A., Lazar, L., Creţescu, I. & Balasanian, I. 2012 Equilibrium and kinetics study of nitrate removal from water by Purolite a520-E resin. *Environ. Eng. Manage. J. (EEMJ)* **11** (1), 37–45.

Challagulla, S., Tarafder, K., Ganesan, R. & Roy, S. 2017 All that glitters is not gold: a probe into photocatalytic nitrate reduction mechanism over noble metal doped and undoped TiO_2. *J. Phys. Chem. C* **121** (49), 27406–27416.

Chen, G., Liu, X., Brookes, P. C. & Xu, J. 2015 Opportunities for phytoremediation and bioindication of arsenic contaminated water using a submerged aquatic plant: *Vallisneria natans* (lour.) Hara. *Int. J. Phytoremed.* **17** (3), 249–255.

Chen, J., Wu, H., Qian, H. & Gao, Y. 2017 Assessing nitrate and fluoride contaminants in drinking water and their health risk of rural residents living in a semiarid region of northwest China. *Expos. Health* **9** (3), 183–195.

Escobar-Alvarado, L. F., Vaca-Mier, M., López-Callejas, R. & Rojas-Valencia, M. N. 2018 Efficiency of *Opuntia ficus* in the phytoremediation of a soil contaminated with used motor oil and lead, compared to that of *Lolium perenne* and *Aloe barbadensis*. *Int. J. Phytorem.* **20** (2), 184–189.

Fout, G. S., Borchardt, M. A., Kieke, B. A. & Karim, M. R. 2017 Human virus and microbial indicator occurrence in public-supply groundwater systems: meta-analysis of 12 international studies. *Hydrogeol. J.* **25** (4), 903–919.

Hazarika, J., Pakshirajan, K., Sinharoy, A. & Syiem, M. B. 2015 Bioremoval of Cu (II), Zn (II), Pb (II) and Cd (II) by *Nostoc muscorum* isolated from a coal mining site. *J. Appl. Phycol.* **27** (4), 1525–1534.

Iamchaturapatr, J., Yi, S. W. & Rhee, J. S. 2007 Nutrient removals by 21 aquatic plants for vertical free surface-flow (VFS) constructed wetland. *Ecol. Eng.* **29** (3), 287–293.

Karmakar, S., Mukherjee, J. & Mukherjee, S. 2016 Removal of fluoride contamination in water by three aquatic plants. *Int. J. Phytoremed.* **18** (3), 222–227.

Khajuria, A. & Kanae, S. 2013 Potential and use of nitrate in agricultural purposes. *J. Water Resour. Prot* **5** (05), 529.

Li, H., Sheng, G., Sheng, W. & Xu, O. 2002 Uptake of trifluralin and lindane from water by ryegrass. *Chemosphere* **48** (3), 335–341.

Li, K., Liu, L., Yang, H., Zhang, C., Xie, H. & Li, C. 2016 Phytoremediation potential of three species of macrophytes for nitrate in contaminated water. *Am. J. Plant Sci.* **7** (08), 1259.

Li, Q., Yang, J., Fan, W., Zhou, D., Wang, X., Zhang, L., Huo, M. & Crittenden, J. C. 2018 Different transport behaviors of *Bacillus subtilis* cells and spores in saturated porous media: implications for contamination risks associated with bacterial sporulation in aquifer. *Colloids Surf. B. Biointerfaces* **162**, 35–42.

Lingua, G., Copetta, A., Musso, D., Aimo, S., Ranzenigo, A., Buico, A., Gianotti, V., Osella, D. & Berta, G. 2015 Effect of arbuscular mycorrhizal and bacterial inocula on nitrate concentration in mesocosms simulating a wastewater treatment system relying on phytodepuration. *Environ. Sci. Pollut. Res.* **22** (23), 18616–18625.

Liu, H., Tong, S., Chen, N., Liu, Y., Feng, C. & Hu, Q. 2015 Effect of electro-stimulation on activity of heterotrophic denitrifying bacteria and denitrification performance. *Bioresource Technol.* **196**, 123–128.

Liu, J., Xin, X. & Zhou, Q. 2017 Phytoremediation of contaminated soils using ornamental plants. *Environ. Rev.* **999**, 1–12.

Lv, T., Zhang, Y., Casas, M. E., Carvalho, P. N., Arias, C. A., Bester, K. & Brix, H. 2016 Phytoremediation of imazalil and tebuconazole by four emergent wetland plant species in hydroponic medium. *Chemosphere* **148**, 459–466.

Ng, Y. S. & Chan, D. J. C. 2017 Wastewater phytoremediation by *Salvinia molesta*. *J. Water Process Eng.* **15**, 107–115.

Nieder, R., Benbi, D. K. & Reichl, F. X. (eds). 2018 Reactive water-soluble forms of nitrogen and phosphorus and their impacts on environment and human health. In: *Soil Components and Human Health*. Springer, Dordrecht, The Netherlands, pp. 223–255.

Pavlineri, N., Skoulikidis, N. T. & Tsihrintzis, V. A. 2017 Constructed floating wetlands: a review of research, design, operation and management aspects, and data meta-analysis. *Chem. Eng. J.* **308**, 1120–1132.

Pennino, M. J., Compton, J. E. & Leibowitz, S. G. 2017 Trends in drinking water nitrate violations across the United States. *Environ. Sci. Technol.* **51** (22), 13450–13460.

Radziemska, M., Vaverková, M. D. & Mazur, Z. 2019 Pilot scale use of compost combined with sorbents to phytostabilize ni-contaminated soil using *Lolium perenne* L. *Waste Biomass Valori.* **10** (6), 1585–1595.

Rai, S. N. 2003 Groundwater pollution in India – an overview. In: *Groundwater Pollution* (V. P. Singh & R. N. Yadava eds). Allied Publishers Pvt. Ltd., New Delhi, India, pp. 419–436.

Roy, A. S., Hazarika, J., Manikandan, N. A., Pakshirajan, K. & Syiem, M. B. 2015 Heavy metal removal from multicomponent system by the cyanobacterium *Nostoc muscorum*: kinetics and interaction study. *Appl. Biochem. Biotechnol.* **175** (8), 3863–3874.

Saber, A., Tafazzoli, M., Mortazavian, S. & James, D. E. 2018 Investigation of kinetics and absorption isotherm models for hydroponic phytoremediation of waters contaminated with sulfate. *J. Environ. Manage.* **207** (1), 276–291.

Sharma, B., Parul, A. K., Jain, U., Yadav, J. K., Singh, R. & Mishra, R. 2017 Occurrence of multidrug resistant *Escherichia coli* in groundwater of Brij region (Uttar Pradesh) and its public health implications. *Vet. World* **10** (3), 293.

Shu, L., Jegatheesan, V., Keir, G. & Keir, M. 2017 *CESE 2017 – Abstract Book: The Tenth Annual Conference on the Challenges in Environmental Science and Engineering*. LJS Environment, Highton, Australia.

Shyamala, S., Manikandan, N. A., Pakshirajan, K., Rene, E. R. & Behera, S. K. 2017 Phytoremediation potential of two ornamental plants treating nitrate contaminated water. In: *CESE 2017 – Abstract Book: The Tenth Annual Conference on the Challenges in Environmental Science and Engineering*. L. Shu, V. Jegatheesan, G. Keir & M. Keir (eds.). LJS Environment, Highton, Australia.

Singh, M., Pakshirajan, K. & Trivedi, V. 2016 Photo-inactivation of *Escherichia coli* and *Enterococcus hirae* using methylene blue and sodium anthraquinone-2-sulphonate: effect of process parameters. *3 Biotech* **6** (2), 176.

Sinha, V., Pakshirajan, K., Manikandan, N. A. & Chaturvedi, R. 2017 Kinetics, biochemical and factorial analysis of chromium uptake in a multi-ion system by *Tradescantia pallida* (Rose) DR Hunt. *Int. J. Phytorem.* **19** (11), 1007–1016.

Sinha, V., Pakshirajan, K. & Chaturvedi, R. 2018 Chromium tolerance, bioaccumulation and localization in plants: an overview. *J. Environ. Manage.* **206**, 715–730.

Soomro, F., Rafique, T., Michalski, G., Ali, S. A., Naseem, S. & Khan, M. U. 2017 Occurrence and delineation of high nitrate contamination in the groundwater of Mithi sub-district, Thar Desert, Pakistan. *Environ. Earth Sci.* **76** (10), 355.

Tyagi, S., Rawtani, D., Khatri, N. & Tharmavaram, M. 2018 Strategies for nitrate removal from aqueous environment using nanotechnology: a review. *J. Water Process Eng.* **21**, 84–95.

Villa-Gomez, D. K., Pakshirajan, K., Maestro, R., Mushi, S. & Lens, P. N. L. 2015 Effect of process variables on the sulfate reduction process in bioreactors treating metal-containing wastewaters: factorial design and response surface analyses. *Biodegradation* **26** (4), 299–311.

Volf, I., Rakoto, N. G. & Bulgariu, L. 2015 Valorization of *Pistia stratiotes* biomass as biosorbent for lead (II) ions removal from aqueous media. *Sep. Sci. Technol.* **50** (10), 1577–1586.

Wang, Z., Jiang, Y., Awasthi, M. K., Wang, J., Yang, X., Amjad, A., Wang, Q., Lahori, A. H. & Zhang, Z. 2018 Nitrate removal by combined heterotrophic and autotrophic denitrification processes: impact of coexistent ions. *Bioresour. Technol.* **250**, 838–845.

Weldeslassie, T., Naz, H., Singh, B. & Oves, M. 2018 Chemical contaminants for soil, air and aquatic ecosystem. In: *Modern Age Environmental Problems and Their Remediation* (M. Oves, M. Z. Khan & I. M. Ismail, eds), Springer, Cham, pp. 1–22.

Wollheim, W. M., Mulukutla, G. K., Cook, C. & Carey, R. O. 2017 Aquatic nitrate retention at river network scales across flow conditions determined using nested in situ sensors. *Water Resour. Res.* **53** (11), 9740–9756.

Xu, J. & Shen, G. 2011 Growing duckweed in swine wastewater for nutrient recovery and biomass production. *Bioresour. Technol.* **102** (2), 848–853.

Xu, X. J., Lai, G. L., Chi, C. Q., Zhao, J. Y., Yan, Y. C., Nie, Y. & Wu, X. L. 2018 Purification of eutrophic water containing chlorpyrifos by aquatic plants and its effects on planktonic bacteria. *Chemosphere* **193**, 178–188.

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