Ex-situ Biological Removal of Nitrate from Groundwater with Sterile and Non-sterile Porous media (Lab Experiment and Simulation)

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Abstract

Nitrate is potentially harmful in an aquatic environment however it can be converted into other forms by microbial activities. In this research, we tested the efficiency of nitrate removal by using a denitrification bed containing two porous media; sterile soil (SS) and non-sterile soil (NSS). A Marriott column which is making constant water flux and a Plexiglas column filled with a clay loam soil, respectively. Potassium nitrate used to make different nitrate concentrations including 24, 50 and 100 (mg l^{-1}). The nitrate was injected up to ten pore volumes (PV) .The solute transfer parameters were estimated by using soil hydraulic parameters at HYDRUS model. As result, the growth of bacteria and the concentration of pollutants in the output of the column decreased in SS media. In the NSS with a concentration of 100 mg/ l nitrate, the number of bacteria increased to the highest, 80000 bacterial number. The presence of rival anions in the solution has a negative impact on removal of nitrate. In NSS with nitrate concentration of 24 (mg l^{-1}), the removal nitrate efficiency nearly 99.5 (%). Sensitivity analyses demonstrated that saturated hydraulic conductivity had the greatest effect on the variations of nitrate concentration. Besides, the correlation coefficient between simulated and observed values varied between 0.90 and 0.99. Overall, the NSS with an inlet nitrate concentration of 24 (mg l^{-1}), the highest nitrate removal efficiency was obtained. The total amount of bacteria of NSS at the end of the experiment has been increased because of the bacteria growth.

Keywords: Bacteria, HYDRUS, Nitrate, Non-sterile soil, Sterile soil.

Introduction

Nitrogen is one of the essential nutrients that found in a widely various forms in soil. Typical forms of inorganic nitrogen include nitrate (NO_3), nitrite (NO_2), nitrogen gas (N_2), ammonium (NH_4) and etc. Nitrate is the main form of nitrogen and the common contamination of the groundwater because of its high solubility and not being retained by soil because of low sorption of nitrate, therefore as result, make the water unacceptable for use (Ghanbari et al. 2014; Wang and Wang 2013). It is directly or indirectly produced by decomposition and biochemical changes of various organic compounds in soil. Nitrate has negative charge and is easily transported into the surface water by rainwater (Jafari et al. 2015). Povilaitis and Matikiene (2020) conducted a study to investigate nitrate removal from the drainage water. The performance of three pilot-scale bioreactors, one only filled with woodchips and the others respectively amended 10 (v/v) with activated carbon and flaxseed cake, were tested under field conditions for nitrate removal from tile drainage water in Lithuania. The results showed that the average nitrate removal efficiency in the bioreactor with no additives was 40.3 (%) and 44.1 (%) in the bioreactor with activated carbon additives.

Purification of polluted groundwater is one of the most important issues that directly related to human health for many consumers of groundwater as the drinking water. The most important methods of nitrate removal from aquatic environments include ion exchange, biological or chemical denitrification, inorganic nitrogen transformed to organic compounds by plants, reverse osmosis, electro dialysis, and catalytic denitrification (Luk and Au-Yeung 2002). Among these methods, only biological removal methods include biological denitrification, transformed inorganic nitrogen to organic compounds and ion modification applied to remove nitrate from underground drainages in farms and basins scales. The maximum removal efficiency of nitrate obtained by ion exchange 90 (%), reverse osmosis 97 (%), electro dialysis 65.9%), chemical denitrification 70 (%) and biological denitrification 100 (%) (Ruppenthal 2007).

There are two types of biological denitrification including autotrophic denitrification and heterotrophic denitrification (Wang and Chu 2016). Autotrophic denitrifies consume hydrogen, iron, and sulfur as sources of energy and inorganic carbon sources such as carbon dioxide and bicarbonates as carbon sources (Karanasios et al. 2010). Most denitrifying bacteria are heterotrophic; these bacteria are a heterophytic type that requires an energy source such as organic carbon, carbon dioxide or sulfur. These nitrate-degrading bacteria are a type of bacteria that use nitrate or nitrite as the electron acceptor to oxidize inorganic compounds in the absence of oxygen and produce nitrogen gases (Della Roca et al., 2007). Denitrification process requires several factors; oxygen deficiency, nitrate as an electron acceptor, and unstable carbon (C) as a source of energy for denitrifications. Dong et al. (2020) introduced a new ion exchange adsorbent, NSR-NanoZr, for simultaneous selective removal of nitrate, phosphate and fluoride. Nabi Bidhendi et al. (2006) at biological nitrate removal by using acetic acid as the carbon source, retention time of 2 (h) was determined to be optimum, in which 77 (%) nitrate removal was achieved. The breakthrough point occurs when the ion concentration in the output flow reaches 3 to 5 (%) of its concentration in

the input flow and the saturation point also occurs when the ion concentration reaches a constant value in the output flow although the column (Singh and Datta 2004). Also, the breakthrough curve shows how the available ions in solution are loaded onto the absorber column bed, in which changes in input and output concentrations (Chavoshi et al. 2015).

Using models to predict the movement of solids and pollutants in the soil can save time and cost but the accuracy of the model firstly must be examined and evaluated under controlled conditions. Inverse modeling as one of the indirect methods that is mostly used in engineering work. Also, inverse modeling is capable to estimating hydraulic and solute transport properties. HYDRUS inverse model is one of the advanced models with ability of simulating one-dimensional movement of water, solute, heat and water uptake by root in the saturated and unsaturated conditions of the soil. The model was developed by Simunek et al. (2008) at the US Soil Laboratory. In order to investigate nitrate transport in the soils under sugarcane cultivation, Derakhshan nejad et al. (2010) used the HYDRUS-1D model. Their results showed that though this model was able to well estimate the nitrate transport process in the soil. The optimized (fitted) values using in model were less than those determined in the field experiments. Asadi and Feli (2013) used HYDRUS-1D software to investigate the nitrate transport in a sandy loam soil treated with zeolite. The simulation results showed that this model has a good estimation of nitrate transport in the soil. The correlation coefficient amplitude between simulated and laboratory values was calculated to be 0.94 to 0.97.

This study was conducted to find the following objectives: I-Finding the bacterial growth effects on the nitrate amount in the groundwater in sterile soil (SS), II- the amount of nitrate reduction and bacterial growth in a non-sterile soil (NSS), III-simulating the dispersivity and absorption coefficients by inverse solution method using HYDRUS-1D model.

Materials and Methods

In this laboratory study, a column was used as a soil bed, also Marriott column was used to produce saturated and uniform water flow and constant head. The required soil collected from 0 to 45 (cm) depth of a farm located in Shahrekord University, Iran. The background solution collected from the Shahrekord aquifer. Some physical and chemical characteristics of the soil and the water are presented in Tables 1 and 2, respectively.

Table 1 Initial soil characteristics

Soil Texture	pH	pb (g cm^{-3})	EC (dS m^{-1})	NO_3^-	SO_4^{2-} (mg l^{-1})	Br	OM (%)	TN
Clay Loam	7.31	1.38	0.33	2.50	0.05	0	0.28	49

pH: Acidity; EC: Electrical Conductivity; OM: Organic Matter; TN: Total nitration; pb: Bulk density

Table 2 Chemical characteristics of water

pH	EC (dS m^{-1})		NO_3^-	SO_4^{2-}	Br$^-$ (mg l^{-1})	NO$^-$	NH_3
7.60	0.32		24	10.70	46	0.05	0.03

Before any sampling being carried out from the flow exiting of the bed, the test column was put under the flow for one week to achieve the column contents stabilization and stable flow. Samples were collected interval in transparent plastic bottles and kept at a temperature below 4 (C°) until being analyzed. Nitrate and sulfate were measured by a DR 5000, HACH spectrophotometer (Paul Chen et al. 2003).

Experimental setup

Each Plexiglas column was designed in order to provide a physical model. The Marriott column that is retaining constant water flux in 100 (cm) height and 25 (cm) diameters to supply and the Plexiglas column as soil column with 50 (cm) and 10 (cm) in order to height and diameter were selected but only the 40 (cm) of soil column were used as bed and simulated. A shut-off valve was installed on the column inlet and outlet (Fig 1).
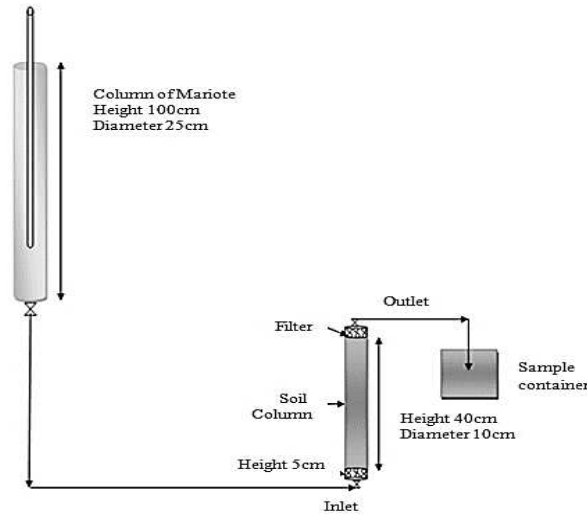
Fig. 1 Schematic of physical model of study columns

In order to fill the soil column, topside of the soil column was first blocked with a Plexiglas cap and a hole was created to set the inlet flow pipe. The 5 (cm) of soil column height at the top and end of column was filled with gravel and sand as filter layers to unify the entrance flow and also, to prevent from rinsing its inside contents. The prepared column was put on metal stand. The two columns were accomplished until the saturation soil condition and the inflow into the soil column was considered to be upward (Healy et al. 2012). The input flow simulation into the soil column was done as follow: By first, the flow from the using 49-liter Marriott column that contains nitrate solution was transported to the bottom of the soil bed. Besides, continuous sampling was done from the entering and exiting flow. Considering that the water velocity in the groundwater flow is between 0.1 and 0.5 (m day^{-1}) (Al- Tabbaa et al. 2000), the flow rate was constant about 0.34 (m day^{-1}) to create a uniform flow in the column. The exiting volumetric flow rate (ml h^{-1}) from the soil column was also measured by determining the amount of time needed to collect a specific pore volume (PV) of the output flow until 10 PVs. Each pore volume (PV) was estimated roughly 1523 (mL).

The steps of experiment

Experiments were carried out in 6 steps. From the first to third step, water contained 24, 50 and 100 (mg l^{-1}) of nitrate concentrations in the sterilized soil column (by sterilization we mean the complete destruction of the entire soil microbial content, this aim was done by using autoclave) until it reached to its saturation threshold. From step 4 to 6, water source with 24, 50 and 100 (mg l^{-1}) nitrate concentrations from 0.35, 0.5 and 0.7 (g) of potassium nitrate salt was applied and the heterotrophic bacteria fed by ethanol + methanol + acetate + glucose as carbon resource.

Nitrate removal efficiency

The nitrate removal efficiency was calculated by using the following equation (Zhou et al. 2015):

$$R \% = \frac{c_i - c_{ef}}{c_i} \times 100 \tag{1}$$

Where C_i and C_{ef} are inlet and outlet nitrate concentrations (mg l^{-1}), respectively.
The nitrate removal rate (mg l^{-1}h^{-1}) was calculated by using the following equation (Ghane et al. 2015):

$$R_{NO_3} = \frac{-\Delta C}{HRT} \tag{2}$$

Where R_{NO_3} and $-\Delta C$ are the drop rate and the difference of nitrate concentration (mg l^{-1}) and HRT is actual hydraulic retention time calculated by the following relationship (Ghane et al. 2015):

$$HRT = \frac{L - n_e}{q} \tag{3}$$

Where L, n_e and q are the column length (m), the effective porosity (dimensionless), and the hydraulic loading rate (m h^{-1}), respectively.

The hydraulic loading rate (m h^{-1}) was calculated using the relationship proposed by Lin et al. (2008):

$$q = \frac{Q}{A} \tag{4}$$

Where Q and A are the output flow rate (m³ h⁻¹) and the flow cross-section (m ²), respectively:
The most widely used adsorption isotherms are Langmuir and Freundlich; the last isotherm was outperformed for the former similar researches.

$$C_E = \frac{x}{m} = K_F\, C_E^{\beta} \tag{5}$$

Where C_E: Equilibrium concentration of the substance in solution (mg cm⁻³), x: the amount of absorbed solution (mg l⁻¹), β: exponential coefficient of Freundlich isotherm, and m: absorbent mass (g).

Model Theory and Simulation

Generally, the displacement and distribution of solute in soil matrix are flowed through three mechanisms: mass flow, diffusion and hydrodynamic dispersion. To Consider the effects of these mechanisms on movement of ions and solutes in the soil, the convection-diffusion equation (CDE) used in a one-dimensional homogeneous porous medium under constant flow condition (Abbasi 2015):

$$\frac{\partial C}{\partial t} = -v\,\frac{\partial C}{\partial Z} + D\,\frac{\partial^2 C}{\partial Z^2} - \mu'_w \theta C \tag{6}$$

Where D: diffusion coefficient (mg² m⁻¹), C: concentration of solute or intended ion (mg l⁻³), V: true average water velocity (m h⁻¹), Z: distance (m), and t: time (h), μ´w: first-order rate constant s, Θ water content (l³ l⁻³).
In this model, the modified Richards equation is solved numerically to study the movement of water in the soil, which is expressed as the following equation (Mualem 1976):

$$\frac{\partial \theta}{\partial t} = \frac{\partial}{\partial Z}\left[K_\theta\left(\frac{\partial h}{\partial z} + \cos\beta\right)\right] - S \tag{7}$$

Where θ: volumetric moisture content (l³ l⁻³), t: time (h), K(θ): unsaturated hydraulic conductivity (m h⁻¹), h: Matric suction (m), β: the angle between the flow path and the vertical axis (for vertical movement of water in the soil, β = 0 for horizontal motion, β = 90 and for other routes 0< β <90), S: water absorbed by the root (m³ m⁻³h⁻¹) and z is the distance (m). In the Richards equation, various equations have been defined to describe the hydraulic properties of the soil such as water retention curve and soil's water conductivity. The most common equation is the van Genuchten– Mualem relationship:

$$\theta(h) = \begin{Bmatrix} \theta_r + \dfrac{\theta_s - \theta_r}{[1 + \alpha\, h^n]^m} \\ m = 1 - \dfrac{1}{n} \end{Bmatrix} \quad n > 1 \tag{8}$$

$$K(S_e) = K_s\, S_e^1 \left[1 - \left(1 - S_e^{\left(\frac{1}{m}\right)} \right)^m \right]^2 \tag{9}$$

Where θ_s: saturation moisture content, θ_r: residual moisture content, n: water retention curve shape parameter; this parameter is larger in coarse-textured soils in which soil moisture retention curve has a steep gradient compared to fine textured soils (Singh and Data 2004), Ks: Saturated hydraulic conductivity (m h⁻¹), α: inverse of air entrance point (m⁻¹) (which is highly dependent on the texture and structure of the soil, and has the least amount in soils with fine pores), Se: relative or effective degree of saturation, and l: the parameter of pore connectivity, which is considered to be 0.5 for most soils. Model parameters such as soil hydraulic properties including soil moisture curve, soil saturated hydraulic (K_s), residual moisture content (θ_r) and residual saturated moisture content (θ_s) in the Van Genuchten-Moeller model by using soil mechanical analysis data and bulk density measurements were predicted by the RETC model. In this study, volumetric moisture content and porosity were measured as baseline data while simultaneously measuring moisture content and bromide (as a tracer) content in soil and groundwater used as a water source, and modeled. Due to the fact that the water flow was saturated and steady, only the boundary conditions were defined at the beginning and end of the soil column. The upper and lower boundary conditions were constant water flux and free drainage, respectively. The initial soil condition was the nitrate concentration of soil bed. The parameters of the adsorption isotherms of Freundlich and dispersivity of nitrate ions in MIM model were estimated by HYDRUS model in inverse solution.

Sensitivity Analysis
The main parameters were identified by changing their value and the results of the model underwent significant changes, all statistical analyzes were performed by SPSS 22 software using paired test at 95 (%) probability level

Results and discussion

Effect of competitor anions

The results show that the presence of competing anions such as sulfide and sulfate in the solution has a negative effect on reduceing of the absorption and removal of nitrate by natural soil with initial concentrations of 24, 50 and 100 (mg l^{-1}) (Fig 2). Also, the maximum and minimum of pH value that nitrate adsorbed high are 7.2 and 8.03 based on standard value (Shirvani Ichi et al. 2021). In this study, the pHof soil and water are be in the standard range (Tables 1 and 2). Divband Hafshjani (2016) concluded carbonate and sulfate had the greatest effect on removal of nitrate by biochar and vermicompost of sugarcane bagasse. In the other studies, reported carbonate and sulfate (Islam and Patel 2010) and carbonate and chloride (Halajinia et al. 2013) had the most and least effects on reducing the nitrate removal, respectively. The number of total bacteria is shown in the (Table 3). Biological degradation (BD) of nitrate acts as an electron receptor terminal in the absence of oxygen and naturally uses certain bacteria. The total amount of bacteria of NSS at the end of the experiment has been increased because of the bacteria growth (Table 3).

Table 3 The number of bacteria during experiment

Soil type	Inlet water nitrate concentration (mg l^{-1})	Total bacteria (CFUg^{-1})*1000
SS*	24٬50٬100	0
NSS	0	12
NSS	24	66
NSS	50	74
NSS	100	80

*SS: sterile soil and NSS: non-sterile soil

Efficiency and removal rate of nitrate

As shown in Fig. 2, in SS, the rate of nitrate releasing is very low. The content of bacteria inside SS is zero and the soil matrix act as an adsorbent and the nitrate pollutants are adsorbed on the soil surfaces and after a certain period of time, mentioned active surfaces became saturated with pollutants, the amount of nitrate in the column outlet was increased until became equal with the initial concentration of the column inlet (Fig. 3). Also, with increasing nitrate content, the rate of adsorption decreases because of the effect of competing anions with nitrate. If the soil is not sterile and the bacterial content is not zero, over time, the bacteria grow and the contaminants in the water are used by the bacterial as feed, and as result, the growth of bacterial increase, the pollutant concentration at the outlet of the column decreases. These results are consistent with findings of Tangsir et al. (2018) and Rajta et al. (2021).

Table 4 shows the efficiency (R), removal rate of nitrate (R$_{NO3}$-) and hydraulic retention time (HRT) in the both SS and NSS conditions. The removal efficiency amount is zero in the soils so due to the absence of any bacteria in the soil profile. In fact, no bacterial activity has taken place. In the NSS with an inlet nitrate concentration of 24 (mg l^{-1}), the highest nitrate removal efficiency was obtained.

Table 4 Hydraulic parameters of the treatments

Concentration	Treatment	R	R$_{NO3}$- (mg l^{-1}h^{-1})	HRT (h)
24	NSS	99.5	14.93	0.17
24	SS	0	0	0.17
50	NSS	79.2	24.75	0.17
50	SS	0	0	0.17
100	NSS	58.4	36.5	0.17
100	SS	0	0	0.17

SS: sterile soil, NSS: non-sterile soil, R: Removal efficiency; R$_{NO3}$-: removal rate of nitrate; HRT: hydraulic retention time

These results are similar to the results that reported by several researchers. Hashemi et al. (2011) at the study of the efficacy of nitrate removal in denitrified substrates made of barley straw observed the output nitrate is reduced to less than the input nitrate especially in media with new carbon source. Cameron et al. (2012) illustrated in beds containing Bagasse, nitrate removal efficiency remained above 80 (%), due to high emission of organic carbon at

the beginning phase and while in beds without bagasse it has decreased to 27.81 (%) .The average elimination efficiency of nitrate in in beds containing bagasse and no bagasse, was estimated to be 9.36 and 46.82 (%), respectively (Tangsir et al., 2017). Also based on the result (Table 4), by increasing NO_3^- concentration of input solution, the removal rate of nitrate (R_{NO3}^-) increased from 14.94 (mg $l^{-1}h^{-1}$) 24-NSS treatment to the maximum value 36.5 (mg $l^{-1}h^{-1}$) of R_{NO3}^- in 100-NSS treatment, in additional, hydraulic retention time (HRT) are constant in all treatments that meant the HRT is independent to nitrate concentration (Table 4).

Petrovic and Simonic (2015) reported the highest removal efficiency of achieved at 50 (mg l^{-1}) of initial nitrate concentration under different carbon sources between 93-99 (%). Wang and Wang (2013) reported the complete removal of 50 (mg l^{-1}) of nitrate-nitrogen was achieved in a 23-day-old reactor with 2.1 (h) of hydraulic retention in HRT without inoculating with any external microorganisms and accumulation on nitrite and nitrate residue was detected when HRT was lower than 2.1 (h). Also, nitrate removal efficiency reducing to about 75 (%) when HRT was lower to 1.4 (h). Based on the statistical analysis, the correlation coefficients (Paired Samples Correlation) were compared between the concentrations of 24, 50 and 100 (mg l^{-1}) in the both SS and NSS conditions was significant more than 0.91 except 24-NSS and 100-NSS with 0.77 correlation coefficient.

Determination of adsorption isotherms
To investigation of adsorption isotherms, the results showed that the soil nitrate adsorption has followed the Freundlich's adsorption model, because its R^2 value is greater than the Langmuir's linear adsorption isotherm. This is consistent with the results from studies conducted by Sayyad et al. (2009). The results are shown in (Table 5).

Table 5 Freundlich adsorption isotherm coefficients and longitudinal dispersivity coefficients by CDE model

Treatment	Model type	k_d (mg /l)	β	Dl (cm)	$Sink_1$ (μ'_w)
24NSS	CDE	0.52	0.51	5	0.41
24SS	CDE	11	1.61	4.09	0.00004
50NSS	CDE	4.71	0.1	5	0.2
50SS	CDE	13.2	1.61	4.09	0.00042
100NSS	CDE	7.9	1.71	4.09	0.00016
100SS	CDE	3.15	1.6	4.09	0.11

SS: sterile soil, NSS: non-sterile soil $Sink_1$ (First-order rate constant for dissolved phase, (μ'_w), [h^{-1}], representing the chain reaction)

After the hydraulic parameter (Ks), Freundlich equilibrium constant and dispersivity that were estimated from the various hydraulic and solutes transport parameters, the main parameters that changed significantly (Table 6). Among the hydraulic parameters, the Ks (cm h^{-1}) were the highest sensitive parameter to its change. The result is similar Ansari Samani et al. (2019) reported Ks (cm h^{-1}) with 2.64 has the highest sensitive coefficient of observation and simulation bromide in HYDRUS-1D. Nazem et al. (2021) showed Ks of soil has the biggest effect on nitrate transport in soil matrix toward groundwater. Besides, the RMSE coefficient with a value 0f 0.04 was the lowest and ME coefficient with the value of -0.0001 had the most parameter variation of simulation of bromide in HYDRUS-1D (Ansari Samani et al. 2019). Additionally, among the parameters affecting solutes transport, the Freundlich equilibrium constant was the most sensitive one.

Table 6 Optimal parameters and sensitivity of input parameters in HYDRUS-1D model

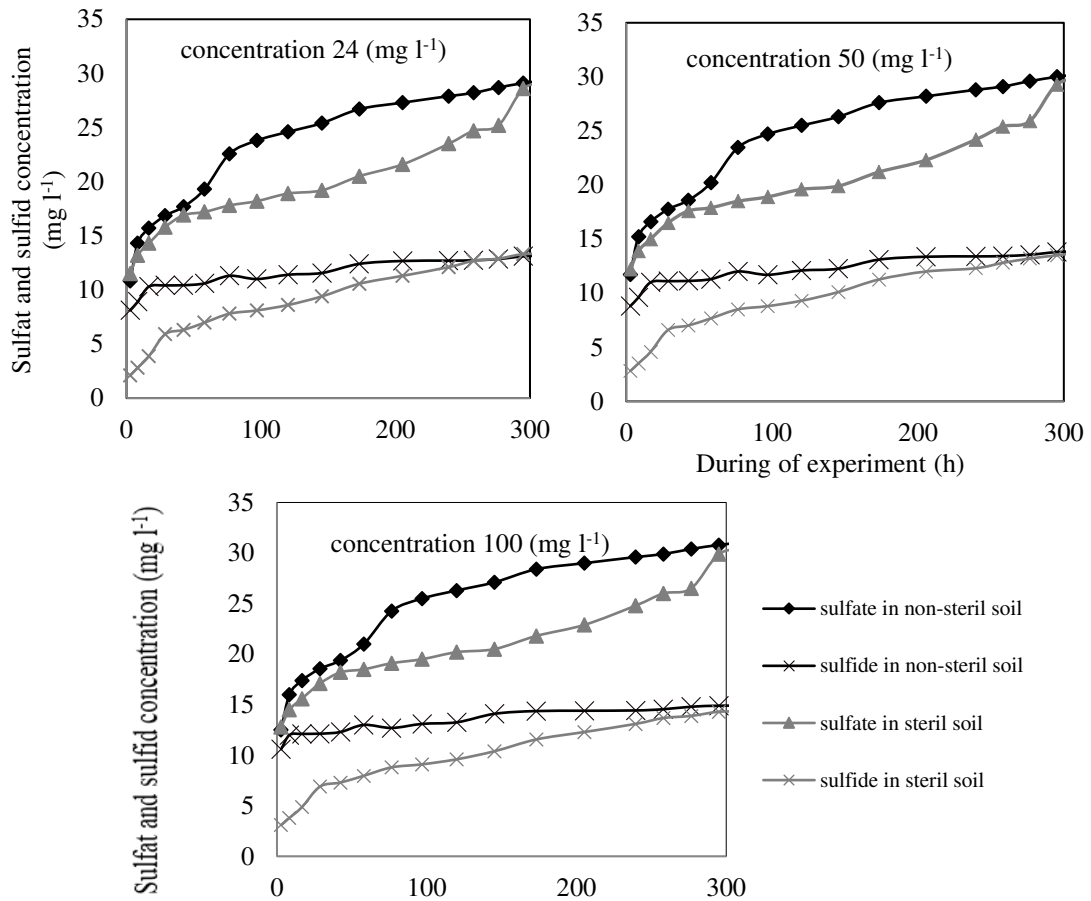| D (cm)	Ks (cm h^{-1})	l	n	$\dot{\alpha}$ (cm^{-1})	θr	θs	R^2	RMSE	ME
				Optimal parameters					
4.09	1.04	0.50	1.41	0.02	0.07	0.44	0.89	0.84	0.32
				Sensitivity coefficient					
-	2.65	0.0001	-	0.0005	0.0027	0.0003	-	-	-
				Rate of sensitivity					
-	High	Low	-	Low	Low	Low	-	-	-

Fig.2 Sulfate and sulfide concentration vs. time in sterile soil (SS) and non-sterile soil (NSS)
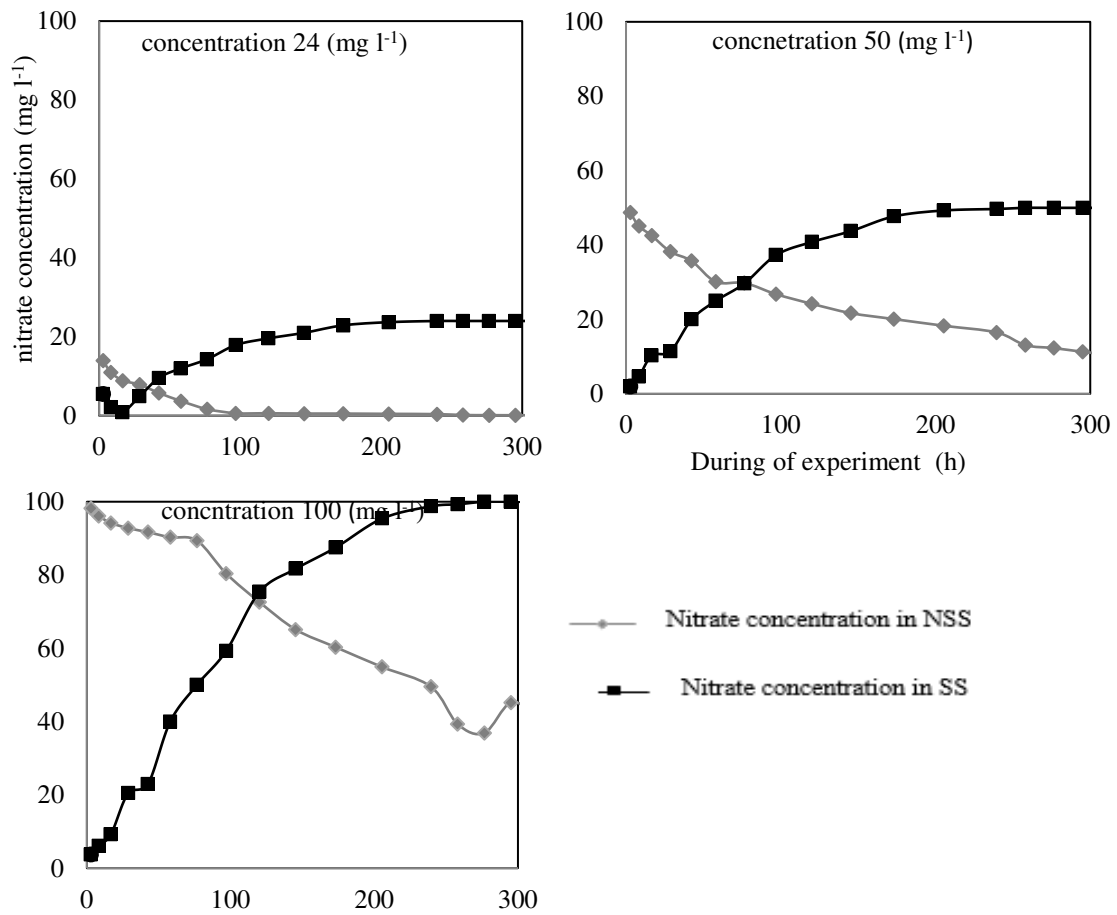
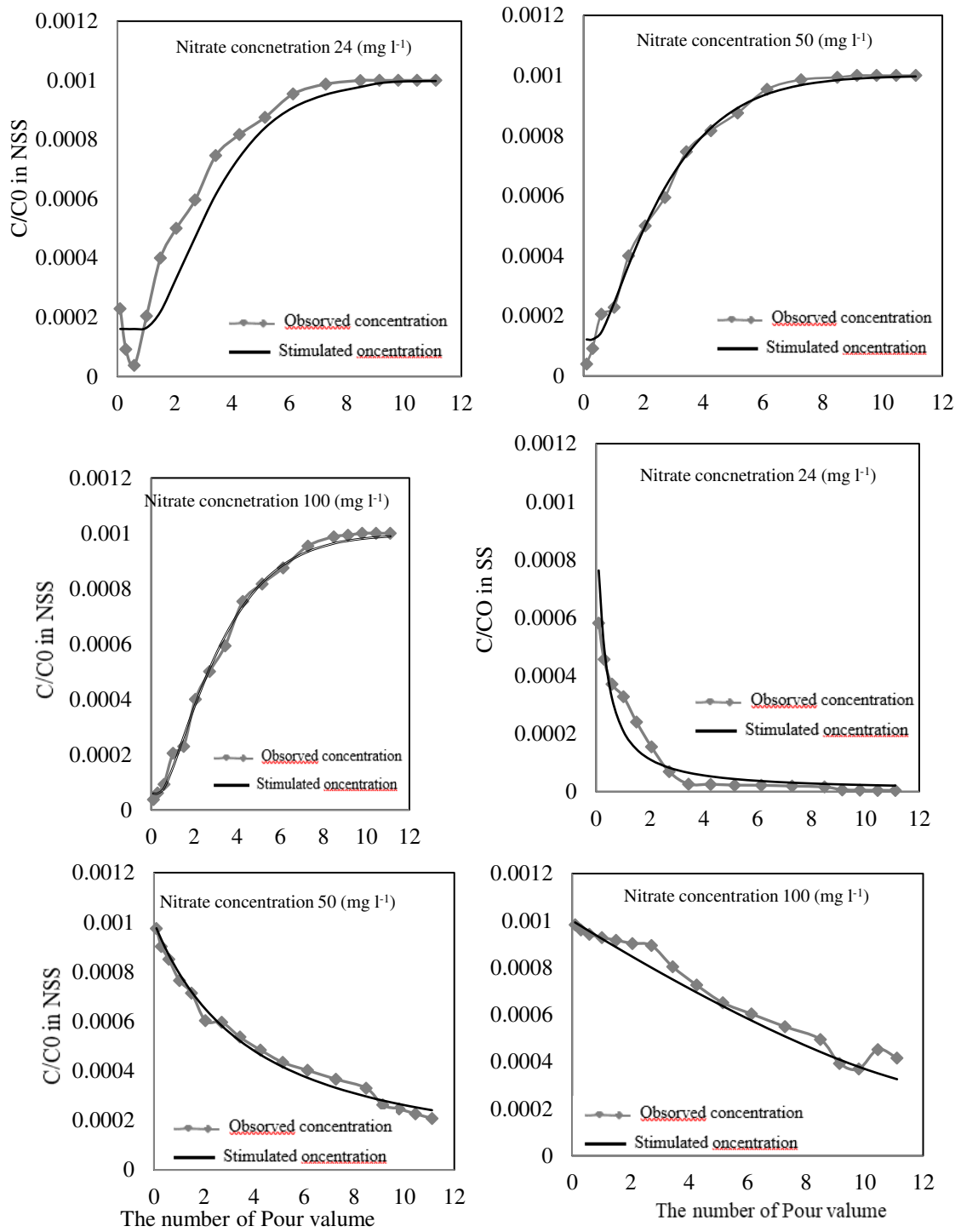**Fig. 3** Nitrate concentration vs. time in sterile soil (SS) and non-sterile soil (NSS)

Fig.4 The observed and simulated breakthrough curve in different nitrate concentration in NSS and

Simulation and analysis of breakthrough curve sensitivity by HYDRUS-1D

As shown in Fig 4, the fitted breakthrough curves obtained with the HYDRUS-1D model showed good agreement with the values observed in the laboratory. As seen in Fig 4, in the SS with three different nitrate concentrations, the output nitrate concentration has an ascending trend; therefore, at the end of each test, the output concentration has become approximately equal to the input concentration. Whereas in figures related to the NSS, they have a descending trend and the nitrate concentration has decreased in the outlet (Fig 4). Investigation of breakthrough curves in the following figures showed that the highest nitrate concentrations in all figures occurred in pore volume of 11 and 0.1 in the SS and NSS conditions, respectively. In fact, at the beginning of the injection, as well as at the end of the experiment related to this concentration.

In recent study, HYDRUS-2D model was be effective in simulating the ponding water depth (RMSE =0.717) and nitrogen concentration in ponding water. Albeit, the simulating NH_4^+ -N concentration with increasing of soil depth did not agree well (Sun et al. 2022).

Statistical comparison of accurate evaluation the HYDRUS-1D

By using RMSE, SSQ, ME and R^2 statistical methods between the simulated and measured values, the accuracy of the HYDRUS-1D model in fitting breakthrough curve laboratory observations to achieve the CDE model was investigated (Table 7).

Considering the R^2 values shown in Table 7, the R^2 values actually higher than 90 (%) and the lower error rates, the HYDRUS-1D is the better model that has been able to simulate the concentration of inlet and outlet nitrates. The higher accuracy of the fitted values for nitrate ions with a concentration of 100 (mgl⁻¹) was seen. Also, the correlation coefficient (R^2) in this treatment is also the highest. This is confirmed by previous studies conducted by Azadifar et al. (2016); Asadi and Feli (2013) and Moradzadeh et al. (2013).

The biggest and lowest of SSQ were obtained in NSS and SS treatments. The better stimulation by HYDRUS-1D with the high accuracy was seen in treatments with 100 (mg l⁻¹) nitrate concentration, besides the highest R^2 was in this nitrate concentration (Table 7). Ahmadi-moghadam and Tabatabaei (2021) reported the highest amount of correlation coefficient was 0.97 at the concentration of 1300 (mg l⁻¹) and MIM model. In the estimation of the dispersion coefficient (D), minimum error was o and 3.5 % at CDE and MIM model.

Table7 Statistical comparison of different treatments

Concentration	Treatment	R^2	SSQ	ME	RMSE
24	NSS	0.90	0.12	0.058	0.086
	SS	0.98	0.016	-0.0032	0.032
50	NSS	0.98	0.012	-0.0034	0.026
	SS	0.99	0.0072	-0.0037	0.021
100	NSS	0.97	0.033	0.0091	0.045
	SS	0.99	0.0038	0.0037	0.015

R2: Coefficient of Determination; RMSE: Root Mean Square Error; ME: Maximum Error; SSQ: Summation Square Error

Conclusion

The purpose of this study was to investigate Finding the bacterial growth effects of sterile and non-sterile soils performance on nitrate removal. The HYDRUS-1D model was calibrated and validated using the collected data. Using inverse modeling and soil hydraulic parameters, the solutes transport parameters were estimated. The results of this study showed that the correlation coefficient between values simulated by the HYDRUS-1D model and observed values varied between 0.90 and 0.99, and the highest concentration was dedicated to nitrate concentration of 100 (mg l⁻¹) in the sterile soil. The lowest ME amount was associated to the concentration of 24 (mg l⁻¹) in the sterile soil with a correlation coefficient of 0.98. In fact, due to the lack of bacteria, the inlet nitrate concentration was nearly equal to the output nitrite concentration at the end of the experiment in the sterile soil, and the software was able to do breakthrough curve fitting well. The higher the input nitrate concentration, the greater the total bacteria content, indicating the growth of denitrifying bacteria. Fitting of linear adsorption isotherm, the Langmuir and Freundlich models to the soil nitrate adsorption data showed that the Freundlich model described the adsorption process better than other models. The significant coefficient at concentrations of 100, 50 and 24 (mg l⁻¹) in the both sterile and non-sterile conditions is less than the 5 (%) confidence level. In fact, there is a significant

difference between these two conditions. It can be concluded that there is a significant difference between the mean concentrations that are compared in Pair wise conditions.

References

Abbasi F (2015) Advanced Soil Physics. Tehran University Publishers. 3rd edition (In persion).

Ahmadi-moghadam Z, Tabatabaei SH (2021) Evaluation of CDE and MIM models to simulate TCE transport in a arbonate porous media using inverse solution method. Amirkabir J Civil Eng 53:383-394. http://doi.org/10.22060/CEEJ.2018.13256.5356 (In Persian).

Al-Tabbaa A, Ayotamuno JM, Martin RJ (2000) One-dimensional solute transport in stratified sands at short travel distances. J Hazar Mater 73: 1–15. http;//doi.org/10.1016/S0304-3894(99) 00169-7

Ansari samani F, Tabatabaei SH, Abbasi F, Alaiee E (2019) Hydraulic Parameters Sensitivity Analysis of Porous Media at Inverse Solution of Bromide Transport. J of Water and Soil Sci 23 (3): 355-366 (In Persian).

Asadi A, Feli Z (2013). Effect of irrigation with municipal wastewater and potassium zeolite. J Hydro 42:99-111.

Azadifar A, Mohammadi A, Moazed H, Firouzi A (2017) Investigation of the emission of nitrate ions in sandy soils using HYDRUS-D model. Water and Soil Res J 47 (4):774-765. http://doi.org/ 10.22059/IJSWR. 2016.59983

Cameron SG, Schipper LA (2012) Hydraulic properties, hydraulic efficiency and nitrate removal of organic carbon media for use in denitrification beds. Eco Eng J 41: 1-7. http;//doi.org/10.1016/j.ecoleng.2011.11.004

Chavoshi E, Afyuni M, Hajabbasi MA (2015) Simulation of fluoride transport in a calcareous soil using HYDRUS-1D. Water and Soil Sci. J 19:205-215. http;//doi.org/10.18869/acadpub.jstnar.19.72.18

Della Rocca C, Belgiorno V, Meric S (2007) Overview of in-situ applicable nitrate removal processes. Desal J 204:46–62. http;//doi.org/10.1016/j.desal.2006.04.023

Derakhshannejad Z, Sayyad GH, Jafarnejadi A.R (2010) Simulation of nitrate transportation in a soil under sugarcane cultivation using HYDRUS-1D solute transport model. First National Conference on Sustainable Agriculture and Healthy Product Production. Research Center for Agriculture and Natural Resources of Isfahan. 9-10 November. Isfahan.

Divband Hafshjani L (2016) Investigating the impact of vermicompost and biochar bagas sugarcane on preventing nitrate flushing in soil. PhD thesis. Shahid Chamran University of Ahvaz.

Dong H, Shepsko CS, German M, SenGupt AK (2020) Hybrid nitrate selective resin (NSR-NanoZr) for simultaneous selective removal of nitrate and phosphate (or fluoride) from impaired water sources. J Env Chem Eng 8: 2213-3437. https://doi.org/10.1016/j.jece.2020.103846

Ghanbari F, Moradi M, Mohseni-Bandpei A, Gohari F, Mirtaleb Abkenar T, Aghayani E (2014) Simultaneous application of iron and aluminum anodes for nitrate removal: a comprehensive parametric study. Int J Environ Sci Technol 11:1653-1660. http://doi.org/ 10.1007/s13762-014-0587-y

Ghane E, Fausey NR, Brown LC (2015). Modeling nitrate removal in a denitrification bed. Water Res J 71: 294-305. http://doi.org/10.1016/j.watres.2014.10.039

Halajinia A, Oustan S, Najafi. N, Khataee A, Lakzian A (2013) Adsorption–desorption characteristics of nitrate, phosphate and sulfate on Mg–Al layered double hydroxide. Appli Clay SciJ 80: 305-312. http://doi.org/10.1016/j.clay.2012.09.007

Hashemi SA, Heidarpour M, Mustafa ZadehFard B (2011) Study of nitrate removal in two positions of biological filters in underground drainage systems. Irri Sci & Eng 34: 81-71 (In Persian).

Healy MG, Ibrahim TG, Lanigan GJ, Serrenho AJ, Fenton O (2012) Nitrate removal rate, efficiency and pollution swapping potential of different organic carbon media in laboratory denitrification bioreactors. Ecol Eng 40: 198-209. http://doi.org/10.1016/j.ecoleng.2011.12.010

Islam M, Patel R (2010) Synthesis and physicochemical characterization of Zn/Al chloride layered double hydroxide and evaluation of its nitrate removal efficiency. Desal J 256: 120-128. http://doi.org/ 10.1016/j.desal.2010.02.003

Jafari S J, Moussavi G, Yaghmaeian K (2015) High-rate biological denitrification in the cyclic rotating-bed biological reactor: Effect of COD / NO_3^- nitrate concentration and salinity and the phylogenetic analysis of denitrifiers. Bioresour Tech J 197: 482-488. http://doi.org/ 10.1016/j.biortech.2015.08.047

Karanasios K, Vasiliadou I, Pavlou S, Vayenas D (2010) Hydrogenotrophic denitrification of potable water: a review. J of hazard materials 180 (1):20-37. http://doi.org/ 10.1016/j.hazmat.2010.04.090

Lin YF, Jing S R, Lee DY, Chang YF, Shih KC (2008). Nitrate removal from groundwater using constructed wetlands under various hydraulic loading rates. Bioresour Technol J 99:7504-7513. http://doi.org/ 10.1016/j.biortech.2008.02.017

Luk G, Au-Yeung W (2002) Experimental investigation on the chemical reduction of nitrate from groundwater. Advan Environ Res J 6:441-453.

Moradzadeh M, Moazed H, Sayyad G (2013) Simulation of nitrate ion leaching in a sandy loam soil treated with zeolite using HYDRUS-1D model. Water and Soil Sci J 23: 95-107. http://doi.org/10.22069/JWSC.2018.12435.2706

Mualem Y (1976) A new model for prediction of the hydraulic conductivity of unsaturated porous media. Water Resour Res J 12: 513–522. http://doi.org/ 10.1029/WR012i003p00513

Nabi Bidhendi Gh, Nasrabadi T, Sharif Vaghefi H.R, Hoveidi H (2006) Biological nitrate removal from water resources, Int J Environ Sci Technol 3: 281-287. ISSN: 1735-1472.

Nazem Z, Tabatabaei SH, Hosseinpur A, Najafi P, Ghobadinia M (2021) Effect of drip and surface irrigation systems on contaminant transport in a soil affected by cracking pathway. J Water and Soil Conser 28:219-236. http://doi.org/10.22069/JWSC.2021.18889.3435 (In Persian).

Paul Chen J, Yoon JT, Yiacoumi S (2003) Effects of chemical and physical properties of influent on copper sorption onto activated carbon fixed-bed columns. Carbon J 41: 1635–1644. http://doi.org/10.1016/s0008-6223 (03)00117-9

Petrovic A, Simonic M. (2015) The effect of carbon source on nitrate and ammonium removal from drinking water by immobilized Chlorella sorokiniana. Int J Environ Sci Technol 12:3175-3188. http://doi.org/10.1007/s13762-014-0747-0

Povilaitis A, Matikiene J (2020) Nitrate removal from tile drainage water:The performance of denitrifying woodchip bioreactors amended with activated carbon and flaxseed cake. Agri Water Manage J 229 (C). http://doi.org/10.1016/j.agwat.2019.105937

Rajta A, Setia H, Skukla S, Bhatia R (2021) Heterotrophic aerobic denitrification by novel bacterium Georgenia daeguensis ARB2 for treatment for nitrate contaminated waters. Int J Environ Sci Technol. http://doi.org/10.1007/s13762-021-03253-x

Ruppenthal S (2007) Nitrate removal solutions for drinking water: how a flexible approach can deliver reliable results. Ame Water Works Asso J 99: 1-28. http://doi.org/10.1002/j.1551-8833. 2007.tb07951.x

Sayyad G, Afyuni M, Mousavi SF, Abbaspour. KC, Richards BK, Schulin R (2009) Simulation of cadmium motion Copper, lead and zinc in an intact soil under cultivation of wheat and safflower using the solvent transfer model HYDRUS-1D. Iran J Agri Sci 39:187-200

Shirvani Ichi H, Tabatabaei SH, Ghobadinia M, Nourmahnad N (2021) Use of sand-zeolite-geotextile in order to reduce the nitrate in Shahrekord University wastewater and compare its quality with standards. J Water and Soil Conser 28: 23-42. http://doi.org/10.22069/JWSC.2021.19350.3482.3WG (In Persian).

Simunek J, Van Genuchten MTh, Sejna M (2008) Development and applications of the HYDRUS and STANMOD software packages and related codes. Vadose Zone J 7: 587-600. http://doi.org/10.2136/vzj2007.0077

Singh RM, Datta B (2004). Groundwater pollution source identification and simultaneous parameter estimation using pattern matching by artificial neural network. Environ. Forensics J 5(3):143-153. http://doi.org/10.1080/15275920490495873

Sun X, Tong J, Liu Cong, Ma Yanbao (2022) using HYDRUS-2D model to simulate the water flow and nitrogen transport in a paddy field with traditional flooded irrigation. Environ Sci Pollut Res J. http://doi.org/ 10.1007/s11356-021-18457-4

Tangsir S, Moazed H, Naseri A A, Garmdareh SEH, Broumand-nasab S, Bhatnagar A (2017). Investigation on the performance of sugarcane bagasse as a new carbon source in two hydraulic dimensions of denitrification beds. J of Clean Prod 140:1176-1181. http://doi.org/10.1016/j.jclepro.2016.10.044

Wang J, Chu.L. (2016) Biological nitrate removal from water and wastewater by solid-phase denitrification process. Biotechno Advan J 34:1103-1112. http://doi.org/ 10.1016/j.biotechadv.2016.07.001

Wang XM, Wang JL (2013) Nitrate removal from groundwater using solid-phase denitrification process without inoculating with external microorganisms. Int J Environ Sci Technol 10:955-960. http://doi.org/10.1007/s13762-013-0236-x

Zhou L, Meng Q, Ren L, Liu W, Zhang X, Huo Y, Zhou Z (2015) Effects of nitrate addition on rumen fermentation, bacterial biodiversity and abundance. Asian-Aus J Animal Sci 28: 1433-1441. http://doi.org/ 10.5713/ajas.15.0091

Statements and Declarations