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RESEARCH ARTICLE

Facilitated decrease of anions and cations in influent and effluent of sewage treatment plant by vetiver grass (*Chrysopogon zizanioides*): the uptake of nitrate, nitrite, ammonium, and phosphate

Mohsen Gholipour[1,2] · Pooyan Mehrabanjoubani[3] · Ahmad Abdolzadeh[4] · Mostafa Raghimi[5] · Seyedmohammad Seyedkhademi[6] · Ehsan Karimi[7] · Hamid Reza Sadeghipour[4]

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Abstract

The ability of vetiver grass (*Chrysopogon zizanioides* L.) for the reduction of anions and cations especially inorganic nitrogen compounds from the influent and effluent of sewages was investigated. Vetiver grass was grown hydroponically in influent (IN) and four different effluent (EF) sewages including control, 125 (EF125), 250 (EF250), and 500 (EF500) mg L^{-1} $Ca(NO_3)_2$. During 18 days, phosphate concentration gradually declined in both influent and all effluent treatments. Unlike effluent treatments, the amount of ammonium in influent was greater than the standard (39.52 mg L^{-1}) and decreased severely down to 4.85 mg L^{-1} at the end of the experiment. After just 48 h, the concentration of nitrate in EF treatment reached 2.25 mg L^{-1} that is lower than the standard. The decrease of nitrate to concentrations less than the standard was also observed at days 8, 11, and 18 in EF125, EF250, and EF500 treatments, respectively, and about 90% of nitrate had been removed from 500 mg L^{-1} $Ca(NO_3)_2$ treatment. Other ions such as Cl^-, Ca^{2+}, and K^+ decreased in influent and all effluent sewages due to phytoremediation process. Accordingly, phytoremediation by vetiver grass could decrease concentrations of nitrate, ammonium, phosphate, chloride, and calcium in influent and all effluent sewages. Increasing the concentration of nitrate resulted in the increase in its uptake rate. In addition, a positive correlation was shown between the uptake rate of nitrate by vetiver grass and the duration of cultivation of this plant in nitrate-containing medium.

Keywords Phytoremediation · Influent and effluent sewages · Inorganic nitrogen compounds

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✉ Pooyan Mehrabanjoubani
p.mehraban@sanru.ac.ir

[1] Specific Service Center for Processing of Native Medicinal Plants, Academic Center of Education Culture and Research (ACECR), Golestan Branch, Gorgan, Iran

[2] Department of Geology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

[3] Department of Basic Science, Faculty of Animal Science and Fisheries, Sari Agricultural Sciences and Natural Resources University, P.O. Box 587, Sari, Iran

[4] Department of Biology, Faculty of Sciences, Golestan University, Gorgan, Iran

[5] Department of Geology, Faculty of Sciences, Golestan University, Gorgan, Iran

[6] Department of Analytical Chemistry, Duisburg-Essen University, Duisburg, Germany

[7] Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran

Introduction

The urban drinking water is more susceptible to pollution by domestic sewage and industrial wastewaters. Urban and domestic sewage are composed of organic (nitrogen, phosphorus, and carbon) and inorganic (anionic and cationic) compounds (Hamilton and Helsel 1995; Sarma and Tay 2018). The reduced quality of potable water is due to the increased levels of nitrogen-containing compounds (such as ammonium, nitrite, and nitrate) beyond the allowed standards. Due to harmful effects of nitrate to human health, removal of nitrate from drinking water sources is a major challenge (Tugaoen et al. 2017). Nitrate is a stable and dynamic anion in water due to the presence of dissolved oxygen (Connolly and Paull 2001) and lack of interaction with the water matrix (Terblanche 1991). It is also the main and most common pollutant of groundwater. Generally, nitrogen fertilizers, sullage of domestic sewage, and wastewater from food industries are considered as the source of nitrate in the environment (Pacheco et al. 2001). High levels of nitrate in the drinking water may increase the risk of some diseases such as methemoglobinemia in infants and children, gastric cancer, and neoplastic changes in the stomach (Van Maanen et al. 1996; Johnson 2019). The standard concentration of nitrate in the drinking water is 45 mg L^{-1} (WHO 2004). At the present time, millions of people in the world should use drinking water with the nitrate content over the limit of standards (Kumar and Puri 2012). Due to the environmentally hazardous effects of low-quality groundwater, the use of untreated sewage for irrigation of farms, forests, and green spaces is under severe control and legislation. There are several refinement methods for the removal of water contaminants from wastewater before reuse in various urban, industrial, and agricultural applications. In general, the wastewater treatment plant separates most of the suspended solid materials by sedimentation, oxidizes carbonaceous, and nitrogenous biological materials by aeration and decreases microbial flora of water by chemical oxidation (Zabava et al. 2017; Macêdo et al. 2019). Despite this, the majority of the dissolved materials such as nitrate and calcium remain unchanged. The separation of dissolved materials in wastewater by methods such as reverse osmosis could raise the treatment costs intensively.

Bioremediation is a valuable environmental cleanup technique as it is an environmentally friendly and economically cost-effective approach. Phytoremediation, i.e., the use of special plant species for removing wastewater contaminants, is one of the bioremediation applications (Prashanthi et al. 2017). It is a technology for transfer, decomposition, and immobilization of water and soil contaminants which employ plants activity and their associated microbial community. These plants can function as a filter or trap and absorb and stabilize nitrogen compounds, phosphates, and heavy metals. These plants also have the ability to increase the dissolved oxygen and decrease the turbidity of water (Abdel-Shafy and Mansour 2018).

Vetiver grass (*Chrysopogon zizanioides* (L.) Nash ex Small) is a perennial bunchgrass that can grow up to 150-cm height and has a deep and massive root system. This species has been employed for many different purposes as diverse as control of soil erosion, animal feed, and oil extraction (Gupta et al. 2012). This plant can successfully withstand a wide range of adverse conditions such as high levels of toxic elements, saline, and acidic conditions and the excess of nitrogen compounds, phosphate, and geochemical materials (Chen et al. 2004). Vetiver grass has effectively been used to treat wastewater and reduce contaminants in mine tailings and landfills (Mudhiriza et al. 2015; Sinha et al. 2013; Percy and Truong 2003). Nitrate removal from high nitrate synthetic wastewater by vetiver grass has been reported (Almeida et al. 2017). This plant is able to decrease the contents of nutrients in domestic wastewater and contaminated surface water efficiently (Akbarzadeh et al. 2015).

Gorgan's wastewater consumes for agricultural crops after treatment at Gorgan treatment plant and may pollute rivers. Also, the lands of this area are agricultural, and due to the excessive use of nitrogen and phosphate fertilizers, some of these fertilizers leached and enter the rivers, which exacerbate the pollution caused by urban sewage. The overall objective of this study was to estimate the contamination of Gorgan's urban wastewater, including heavy metals, nitrogen compounds, and others, and to evaluate the refinement of these pollutants by Gorgan treatment plant and by the use of vetiver grass.

Materials and methods

The influent (IN) and effluent (EF) water of Gorgan's wastewater treatment plant were used in this experiment. In this plant, water refinery is achieved through aerobic digestion with active sludge system in which development of biological floc is used to oxidize organic carbonaceous and nitrogenous compounds. The experiments were conducted at the field of sewage treatment around Gorgan city, located in the north of Iran (latitude, 36° 50′ 33″ N; longitude, 54° 26′ 36″ E, above sea level 135 m during summer). The physicochemical parameters including pH; temperature (T); dissolved oxygen (DO); total dissolved solids (TDS); electrical conductivity (EC); turbidity; and concentrations of anions (NO_3^-, NO_2^-, NH_4^+, and PO_4^-), cations (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, and SO_4^{2-}), and heavy metals (Pb, Cd, Cr, Co, Cu, Zn, Al, Fe, Mn, As, and Ni) were measured in influent and effluent water of Gorgan's wastewater treatment plant. The ability of vetiver grass for uptake and decrease of sewage pollutants from influent and effluent and deliberately nitrate-contaminated effluent waters was investigated through hydroponic culture of this plant. Vetiver plants (*Chrysopogon zizanioides* cv. Sunshine) were propagated through separation of their tillers and cultivated in the field. Healthy vetiver grass of uniform size (about 70 leaves) was hydroponically transplanted into 90-L black plastic containers filled with influent (IN), effluent (EF), and three deliberately nitrate-contaminated waters by adding 125 (EF125), 250 (EF250), and 500 (EF500) mg L^{-1} nitrate as $Ca(NO_3)_2$ to the effluent water (EF) which already contained 68.8 mg L^{-1} NO_3. The averages for the annual rainfall, relative humidity, and temperature of Gorgan city are 622 mm, 69%, and 17.75 °C, respectively. Meteorological conditions of this area according to Koppen climate classification is Mediterranean climate and based on Emberger climatic type is sub-humid temperate. The agro-climatic characteristics of Gorgan city allow easy growth of vetiver grass in this area. To monitor changes in water quality through decrease of pollutants and calculate their uptake by vetiver grass, water samples were collected from the containers at 0, 1, 2, 3, 4, 8, 11, 15, and 18 days after transplanting plants, and the physicochemical parameters were determined. The concentrations of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, and SO_4^{2-} were also measured only at 1, 8, and 18 days after transplanting. Furthermore, estimates were made for the uptake rate (mg g^{-1} root dry mass d^{-1}), of the NO_3^-, NO_2^-, NH_4^+, and PO_4^- by vetiver grass.

Physical parameters for wastewater samples were determined onsite using a multi-parameter ion-specific meter (HACH 2100Q). NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-} were measured by a spectrophotometer HACH DR5000 using standard methods. NO_2^- and NO_3^- were analyzed using the ferrous sulfate and the cadmium reduction methods, respectively (HACH 2008). PO_4^{3-} was determined using the molybdovanadate method (HACH 2008). The concentrations of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, and SO_4^{2-} in samples were determined by ion chromatography (Metrohm 850 Professional IC). Turbidity was measured at 860 nm using the method of Reineck and Singh (2012).

For interpretation, the types and geochemical evolution of water samples from the charts were developed by Durov (Lloyd and Heathcote 1985). Durov diagram was drawn by plotting the major ions as percentages of milli-equivalents.

Statistical analyses of data were performed by SAS statistical software. Analyses of variance (ANOVA) were used for analysis of all data, and Duncan's multiple range tests were carried out for comparisons of means.

Results

Physicochemical and element parameters of influent and effluent sewages before phytoremediation

The influent water had high EC and TDS, but the DO was low in compare to WHO standard for sewage water. In addition, the concentrations of Ca^{2+}, HCO_3^-, and Cl^- were higher than the standard level in the influent water, but the concentrations of Na^+ and K^+ were lower. Also, the heavy metal (including Pb, Cd, Cr, Co, Cu, Zn, Al, Fe, Mn, As, and Ni) contents in the influent water were much less than the WHO standard limit.

Comparison of the physical and chemical properties of the influent and effluent waters from the sewage treatment plant before starting phytoremediation indicated that TDS, EC, and turbidity in the influent were more than effluent, while DO was much greater in the effluent water (Table 1). The concentrations of NH_4^+, Cl^-, and HCO_3^- decreased, and NO_3^- increased after wastewater processing by the sewage treatment plant. Thus, the influent had higher concentrations of NH_4^+, Cl^-, and HCO_3^-, than effluent water; however, the concentration of NO_3^- in the effluent water was greater than the incoming wastewater (Fig. 1), and the heavy metal contents decreased markedly in the effluent water, following processing by treatment plant (Table 2). The decrease of Ni content in effluent (about 93%) was higher than other heavy metals.

Changes of some physicochemical parameters of sewage during phytoremediation

Except for influent water treated plants whose older leaves showed necrosis, the morphology of plants did not change in the other treatments. Ranges for the pH and temperature in all treatments were about 6.96–7.34 and 25.5–28.3 °C, respectively. Phytoremediation by vetiver grass slightly decreased the pH of both influent and effluent sewage water in all treatments (Table 3). Furthermore, all treatments had the highest concentrations of Cl^- (256.3 mg L^{-1}) and turbidity (53.3 NTU) and the lowest DO (0.17 mg L^{-1}) in the influent water before the start of phytoremediation. The Na^+ is not essential elements in plants, and their levels in all treatments were about 44–56.6 mg L^{-1} and did not change significantly with the experimental treatments. The concentration of Mg^{2+} and SO_4^{2-} in all treatments ranged from 41.1 to 51.6 and 110.18 to 142.9 mg L^{-1}, respectively, which is much more than the plant demands for these elements. The decrease of Mg^{2+}, HCO_3^-, and SO_4^{2-} concentrations in influent and all effluent sewage waters was not significant during 18 days of the phytoremediation process. The concentration of K^+ in all waters was much lower than the plant requirement and reduced significantly in all treatments due to the growth of vetiver grass. Concentration of Cl^- in all waters was much higher than that of plant requirement but decreased with plant cultivation in all waters. The Ca^{2+} concentration declined under all phytoremediation treatments, and this decline could be attributed to the addition of calcium nitrate to the effluent sewage. The EC and TDS decreased during 18 days growth of vetiver grass in all treatments. The highest TDS (1048 mg L^{-1}) and EC (2150 μmho cm^{-1}) were observed in the EF500 treatment and decreased drastically by phytoremediation. DO and TU were also lower in all sewage waters compared to those

Table 1 Physical parameters in the influent and effluent of sewage treatment plant before the start of phytoremediation by vetiver grass

	pH	T C°	DO mg L^{-1}	TU NTU	TDS mg L^{-1}	EC μmho cm^{-1}
Influent	7.08 ± 0.02	25.5 ± 0.21	0.4 ± 0.70	53.3 ± 0.97	833 ± 12.3	1667 ± 24.0
Effluent	7.11 ± 0.08	26.1 ± 1.10	4.88 ± 2.50	27.5 ± 11.9	725 ± 4.58	1465 ± 7.21

Values are means + SE of three replicates

untreated ones which had observed phytoremediation for 8 or 18 days.

The average concentrations of Mg^{2+}, Cl^{-}, and SO_4^{2-} in all the influent and effluent treatments were lower than the allowed standard for effluent discharge (Table 3); however, Ca^{2+} concentration was greater than the standard for the effluent discharge due to calcium nitrate treatments (Table 3).

Changes of nitrogen compounds and PO_4 during phytoremediation

Before phytoremediation process, the nitrate concentration of influent sewage was less than the standard effluent discharge, while nitrate concentration in all effluent treatments was more than the standard value for discharge to surface water (50 mg) (Fig. 2). Phytoremediation for just 48 h, resulted in declined nitrate concentration in the EF treatment (2.25 mg L^{-1}) that is lower than the standard value. The concentration of nitrate in both influent and all effluent treatments reduced gradually during the phytoremediation process. Decrease in nitrate concentration lower than the standard value following phytoremediation by vetiver grass occurred at days 8, 11, and 18 in EF125, EF250, and EF500 treatments. At the end of experiment, nitrate concentrations of influent and all effluent treatments were much less than the standard values for the effluent discharge (around 10 mg L^{-1}).

Nitrite concentration initially increased in influent and all effluent treatments due to chemical and/or microbial conversion of nitrate to nitrite during denitrification; however, it decreased to the undetectable amount at the end of phytoremediation process. To test the role of microorganisms in this phenomenon, the frequency of nitrate reducing bacteria was measured after 1, 4, and 10 days. The results showed that nitrate-reducing bacteria increased by day 4, but by day 10, their numbers were low (Supplementary 1).

Before starting the experiment, the concentration of ammonium in all effluent treatments was less than the standard value for discharge to surface water (2.5 mg L^{-1}) and did not change significantly during the phytoremediation process (Fig. 2). However, the ammonium concentration of influent treatment was much greater than the standard value (about 39 mg L^{-1}).

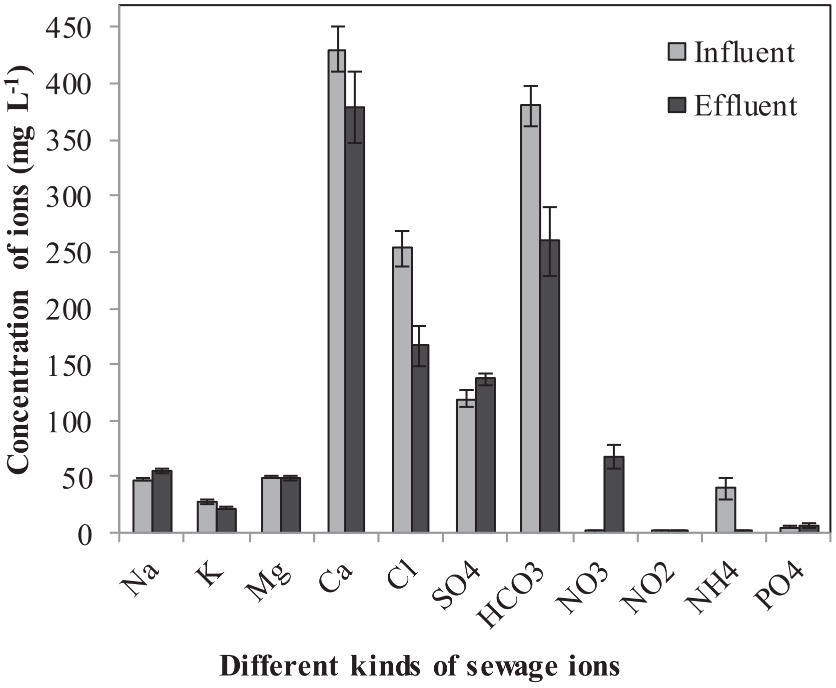

Fig. 1 Comparison of major cations and anions in the influent and effluent water of sewage treatment plant before the start of phytoremediation. Vertical bars represent standard error of three replicates

Table 2 Comparison of heavy metal contents in the influent and effluent of sewage treatment plant with WHO standards

	Pb µg L^{-1}	Cd	Cr	Co	Cu	Zn	Al	Fe	Mn	As	Ni
WHO Standard	1000	100	2000	1000	1000	2000	5000	3000	1000	100	1000
Influent	163 ± 15	15 ± 0.1	65.3 ± 8.9	12.7 ± 0.5	60.6 ± 11	277 ± 114	772 ± 40	105 ± 32	92.0 ± 49	40.0 ± 1.1	207 ± 2.4
Effluent	122 ± 15	7.0 ± 0.6	38.9 ± 23	8.5 ± 0.3	21.2 ± 6.9	157 ± 40	367 ± 29	65.1 ± 5.6	54.5 ± 26	12.6 ± 0.9	12.1 ± 0.7
percentage decrease	25%	54%	40%	33%	65%	44%	52%	38%	41%	59%	93%

Values are means + SE in 8 months samples

The concentration of ammonium in the influent sewage increased to 53.18 mg L^{-1} during first 3 days of phytoremediation, and then, a decreasing trend was observed until the end of the experiment so that it dropped to 4.85 mg L^{-1} until the 18th day.

The phosphate concentration of all influent and effluent treatments was around the standard value for discharge to surface water (6 mg L^{-1}) (Fig. 2); these decreased markedly during the phytoremediation process.

Nitrate uptake by vetiver grass varied based on the nitrate treatments of the effluent water (Fig. 3). It was greater with increased nitrate concentration of the sewage water. Nitrate concentration in the effluent treatments of 0, 125, 250, and 500 mg L^{-1} of Ca(NO3)$_2$ in the first 4 days decreased by 96, 67, 28, and 28%, respectively. From 4 to 8 days of phytoremediation, nitrate decreased by about 85, 49, and 30% in 125, 250, and 500 mg L^{-1} $Ca(NO_3)_2$ treatments, respectively. Between 8th to 13th day of phytoremediation, the

Table 3 Changes in physical and chemical parameters of influent and effluent during 18 days phytoremediation by vetiver grass

Factors Treatments	Days	Na mg L^{-1}	K	Mg	HCO_3^-	Ca	Cl	SO_4	TDS	EC µmho cm^{-1}	pH	T	DO mg L^{-1}	TU NTU
Standard		200		50	250	250	250	200						
Influent														
Control	1	47.6	28	49.8	380	430.6	253.2	118.9	833	1667	7.08	25.5	0.17	53.3
	8	45.1	24	48.8	365	428.1	199.1	112.7	818	1644	7.34	27.9	0.21	14.7
	18	44	17	45.4	359	429.2	126	110.1	582*	1173	7	28	0.3	16.3
Change[a]		7%	40%*	9%	6%	1%	51%*	8%						
Effluent														
Control	1	55.4	21.9	49	260	378.8	167.1	137.2	725	1465	7.11	26.1	4.88	27.5
	8	54.4	20.4	48.9	255	314.9	160.6	134.8	734	1490	7	27.3	0.76	16
	18	50	15.5	44	251	284	108	124.4	754	1514	6.97	28.3	0.94	22.7
Change		9%	29%*	10%	4%	25%*	25%*	9%						
+125 mg L^{-1} $Ca(NO_3)_2$	1	56.6	23.7	50.9	330	496.5	176.1	142.9	798	1607	7.14	26.5	3.02	31.1
	8	53.4	21.8	50	327	450	175.6	132.4	788	1587	7.02	27.3	0.54	10.4
	18	51.6	16.8	43	315	304	122	128.2	687*	1586*	6.93	28	0.32	23
Change		9%	29%*	15%	4%	39%*	31%*	10%						
+250 mg L^{-1} $Ca(NO_3)_2$	1	56.2	20.1	51.2	402	626.2	173.6	137.6	899	1800	7.11	25.9	4.62	25.4
	8	54.4	18.7	49.8	394	526	142.5	134.8	857	1721	7.02	26.7	0.6	6.13
	18	50.4	14.2	41	387	414	94.6	125.3	658*	1335*	6.93	27.2	0.27	16.3
Change		10%	29%*	20%	6%	34%*	45%*	9%						
+500 mg L^{-1} $Ca(NO_3)_2$	1	56.6	21.5	51.6	430	847.9	175.1	138.8	1084	2150	7.1	26.1	2.71	41.4
	8	50.9	20.3	47.1	425	672	159.6	126.5	1033	2006	7	26.9	1.04	4.38
	18	48.6	14.3	41	418	484	95.6	121.1	631*	1279*	6.95	27.1	1.13	7.18
Change		14%	33%*	21%	3%	43%*	46%*	13%						

[a] The percentage of decrease in anions and cations concentration due to phytoremediation by vetiver grass during 18 days

*Average values of triplicate samples are significantly different ($P < 0.01$; Duncan test) when compared to the beginning of the phytoremediation process

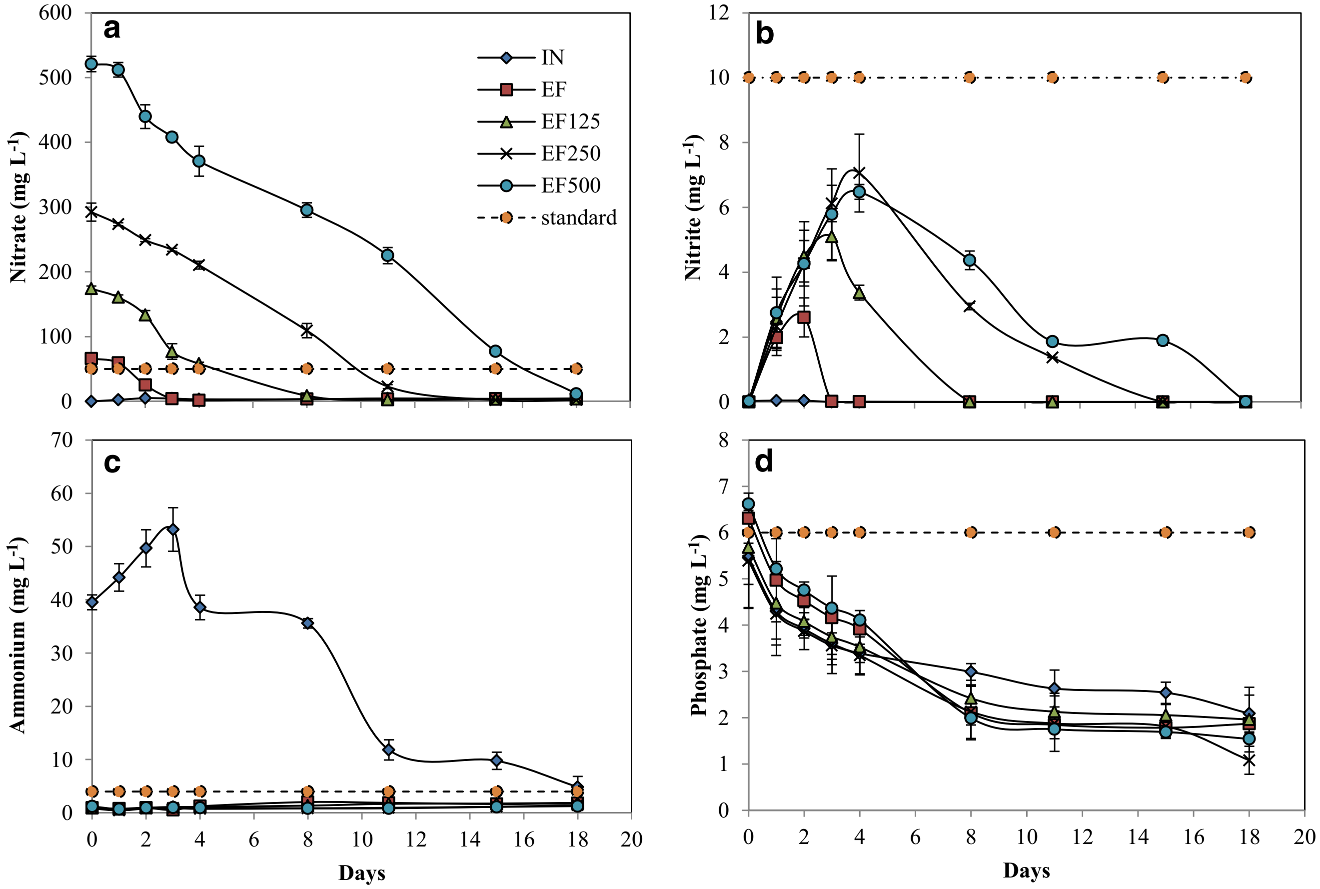

Fig. 2 Comparison of nitrate, nitrite, ammonium, and phosphate in the influent and effluent of sewage treatment plant during 18 days phytoremediation. *IN* influent, *EF* effluent control, *EF125* effluent+125 mg L^{-1} $Ca(NO_3)_2$, *EF250* effluent+250 mg L^{-1} $Ca(NO_3)_2$, *EF500* effluent+500 mg L^{-1} $Ca(NO_3)_2$. Vertical bars represent standard error from three replications

relative decrease in nitrate concentration reached to 79 and 59% in 250 and 500 mg L^{-1} $Ca(NO_3)_2$ treatments, respectively, and finally, up to 18th day, about 90% of nitrate had been removed from 500 mg L^{-1} $Ca(NO_3)_2$ treatment.

The nitrate uptake by the vetiver grass in controlled (EF) condition started at the second day, achieved 0.2 mg L^{-1}per day, and did not significantly change until the eighteenth day. With the increase of nitrate concentration in EF125 treatment, its uptake by vetiver grass increased and reached to 0.4 mg L^{-1} at day 4 and then remained unchanged until the end of treatment. Uptake of nitrate in both EF250 and EF500 started from the fourth day and continuously increased until the end of the treatments. The ammonium uptake rate increased, as its concentration rised in sewage water. The phosphate uptake rate increased during phytoremediation in effluent water. Efflents without the addition of $Ca(NO_3)_2$ and 500 mg L^{-1} $Ca(NO_3)_2$ treatment indicated higher phosphate uptake rate compared to other treatments.

In the influent, water ammonuim uptake rate increased during phytoremedation process (Fig. 3).

Changes in the order of cations and anions concentrations in sewage water by phytoremediation

Due to the higher concentration of ammonium than Na and K before starting the experiment, the order of cation concentration in the influent sewage was found to be Ca > Mg > NH_4 > Na > K (Supplementary 2). During phytoremediation, the ammonium concentration in the influent decreased to amounts lower than potassium (K > NH_4) that indicated high potential of vetiver grass for ammonium uptake. The order of anion concentration in the influent at the beginning of the experiment was Cl > HCO_3 > SO_4> > PO_4 > NO_2 which altered during phytoremediation by vetiver grass, so that Cl concentration reduced to levels lower than bicarbonate (HCO_3 > Cl).

Based on the concentration of ions in the sewage during the experiment, the order of cation concentration in all effluent treatments (EF, EF125, EF250 and EF500) was as follow: Ca > Mg > Na > K > NH_4 (Supplementary 2). Changes in the order of anion concentration in effluent treatments showed that its anion concentration order which had been Cl > HCO_3 > SO_4 > NO_3 > PO_4 at the beginning of the experiment changed after 8 days, so that nitrate concentration declined drastically

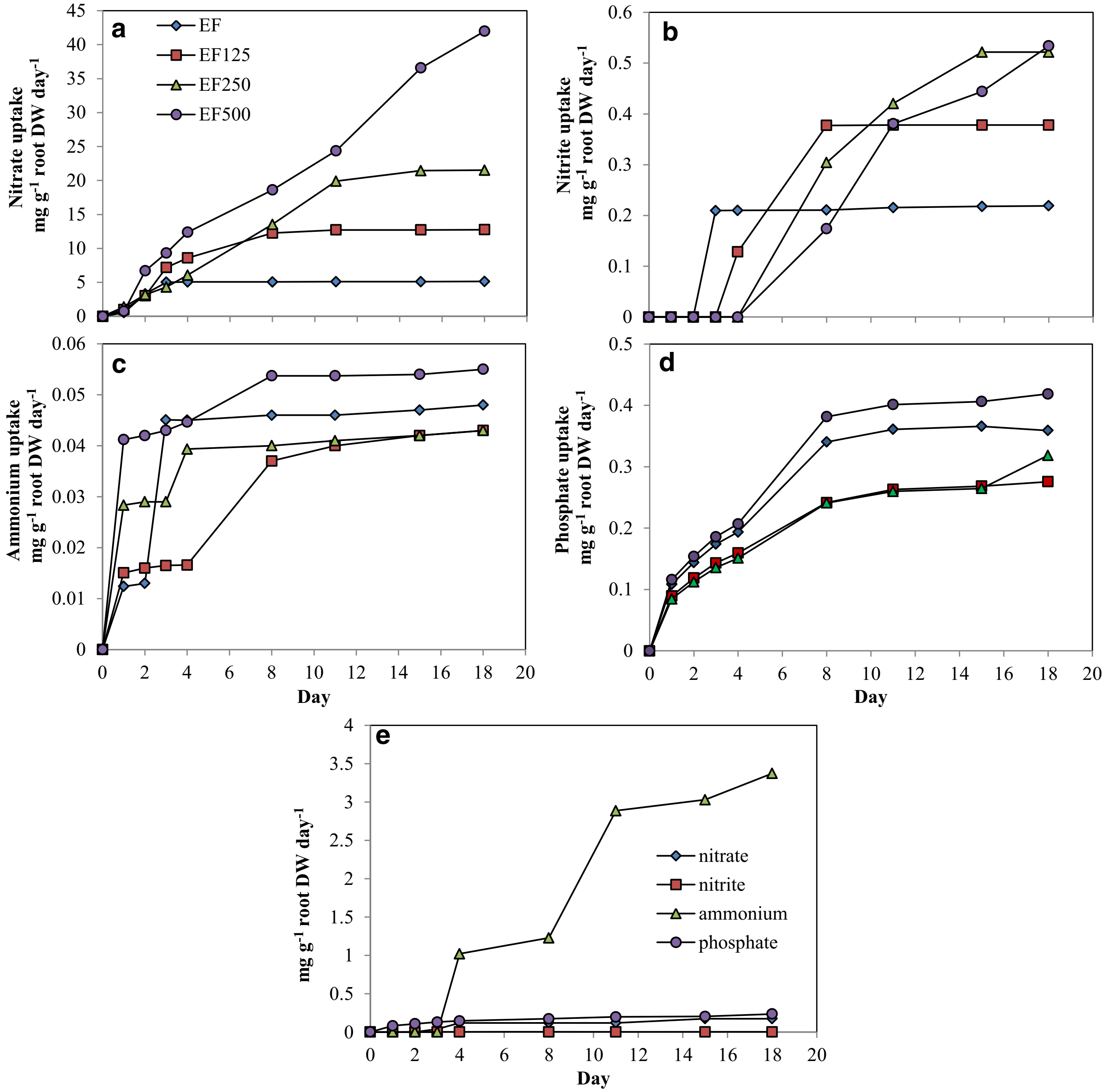

Fig. 3 Changes in the uptake rate of nitrate (**a**), nitrite (**b**), ammonium (**c**), and phosphate (**d**) in all effluent treatments and influent water (**e**) of sewage treatment plant during 18 days of phytoremediation. *IN* influent, *EF* effluent control, EF125 effluent+125 mg L^{-1} $Ca(NO_3)_2$, *EF250* effluent+250 mg L^{-1} $Ca(NO_3)_2$, *EF500* effluent+500 mg L^{-1} $Ca(NO_3)_2$

and phosphate was replaced by nitrate resulted in new order of anion concentration as follows: $Cl > HCO_3 > SO_4 > PO_4 > NO_3 > NO_2$. This situation persisted until the end of the phytoremediation process, except the replacement of Cl by HCO_3 in the order ($HCO_3 > Cl$). Trend of changes in the order of anions in the EF125 was also similar to EF. Due to higher contents of NO_3 than SO_4 in EF250 at the beginning of the phytoremediation, the order of anion concentration was found to be $HCO_3 > Cl > NO_3 > SO_4 > PO_4 > NO_2$. Phytoremediation by 8 days resulted in higher sulfate concentration than nitrate ($SO_4 > NO_3$). The decrease in nitrate concentration continued until the end of the experiment and finally led to much lower concentration than phosphate ($PO_4 > NO_3$) at day 18. At the beginning of the phytoremediation, the nitrate concentration in EF500 treatment was much higher than other anions, and the order of anion sequence was as follows: $HCO_3 > NO_3 > Cl > SO_4 > PO_4 > NO_2$. Nitrate concentration decreased drastically by vetiver grass and after 8 days, and chlorine concentration was higher than nitrate ($Cl > NO_3$). At the end of phytoremediation (18 days), nitrate concentration in EF500 was less than the phosphate, and the order of anion concentration was as follows: $HCO_3 > Cl > SO_4 > PO_4 > NO_3 > NO_2$. The results suggest that even if the nitrate concentration in the wastewater is significantly higher than other anions, cultivation of vetiver grass can reduce this anion to the lowest level.

Hydrochemical changes during phytoremediation

Based on the Douro diagram, dominant cation in influent and all effluents of sewage treatment plant of Gorgan was Ca. However, there was not a single dominant form of anions as HCO_3, Cl, and SO_4 subsisted together in large amounts (Fig. 4). The Douro diagram also revealed that the phytoremediation process at day 1, 8, and 18 led to significant decrease of TDS in influent and all effluents of sewage over time. Furthermore, a linear trend of pH reduction was clearly dominant in the influent and various effluent treatments during the phytoremediation process.

Discussion

The influent wastewater indictaed high concentration of Ca^{2+}, HCO_3^-, and Cl^- and the low K^+/Cl^- ratio due to the high use of detergents in the homes, but its heavy metal contents is not high. Therefore, this study focused on non-heavy elements of wastewater.

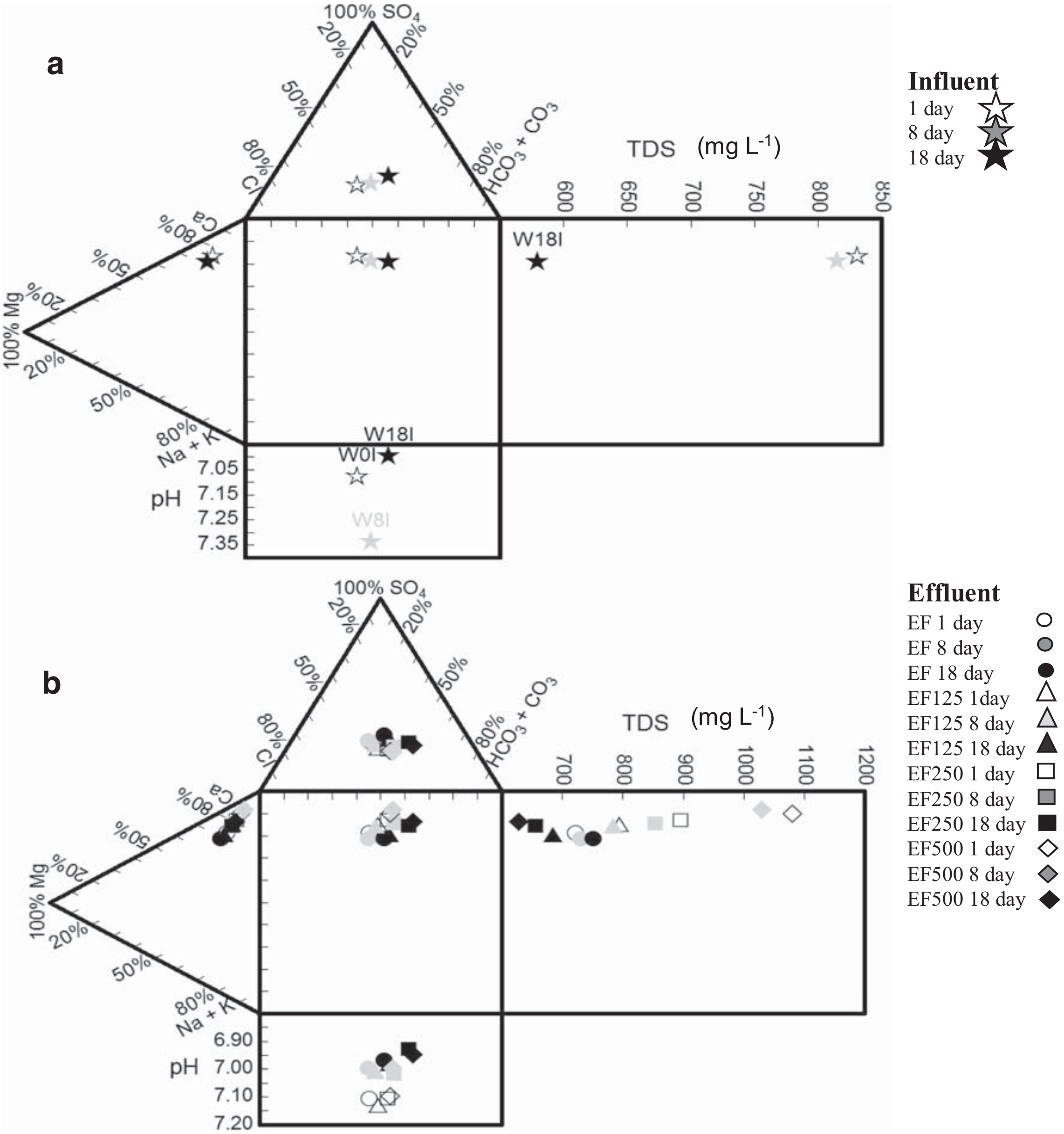

Fig. 4 Graphical representation of influent (**a**) and effluent (**b**) by Durov diagram

Wastewater processing by the treatment plant resulted in reduction EC and TDS in the effluent water but increased DO. Also, concentrations of NH_4^+, Cl^-, and HCO_3^- and all heavy metals decreased while NO_3^- increased. Removal of some organic nitrogen, organic phosphorus, and heavy metals associated with solids has been reported during primary sedimentation wastewater treatment (Sonune and Ghate 2004). Gorgan wastewater treatment plant has no equipment to reduce the water nitrate concentration. On the other hand, decomposition of organic nitrogen in wastewater by ammonification and nitrification processes increases the concentration of nitrate in water (Pan et al. 2018; Mook et al. 2012). Vetiver grass decreased the concentration of anions and cations including Ca, K, Cl, and HCO_3 in the influent sewage and in the case of Cl even it achieved the optimal level of sewage discharge.

The ammonium and nitrate were the major inorganic nitrogen forms in the influent sewage. The ammonium concentration increased initialy, probably due to microbial conversion of organic nitrogen to ammonia. Absorbtion of both ammonium and nitrate by vetiver grass grown in the influent water led to drastic reduction of their concentrations. Vetiver grass due to its nitrate reductase activity has a high ability for the rapid uptake of trinitrotoluene in the soil following urea application (Das et al. 2015). During 22 days, vetiver grass removed trinitrotoluene by about 80%, and translocated this substance from root to shoot by about 37%. The enhanced expression of nitrate reductase gene after exposure to trinitrotoluene has also been reported in *Arabidopsis* (Landa et al. 2010). The nitrite concentration of the influent sewage was negligible; however, it increased during the first 5 days of experiment probably due to microbial conversion ofammonium to nitrite or nitrate to nitrate. Also,under certian environmental conditions such as high amount of some nutrients like cholride, photo-induced and the microbial convertion of nitrate to nitrite could also occured in water (WHO 2003; Beschkov et al. 2004; McIlvin and Altabet 2005; Ryabenko et al. 2009). Increase in the concentration of nitrate (in effluent treated by $Ca(NO_3)_2$) induced the conversation of nitrate to nitrite. However, it decreased gradually until the end of the experiment possibly due to vetiver grass absorbtion and/or microbial activity.

Due to aerobic digestion in the active sludge system, the major nitrogen form of the effluent water was converted into nitrate. Nitrate is absorbed by plant roots and may either be accumulated in the vacuoles or reduced to produce orgnic nitrogen (Tischner 2000). During phytoremediation process, nitrate uptake from effluent water started from the first day and the uptake rate increased as nitrate concentration increased in the treatment medium. Variation in the nitrate levels of sewages affects the rate of nitrate absorption by vetiver grass. The process was initiated by exposure of plants to nitrate, leading to a continuous increase of nitrate uptake rate. There are two separate uptake systems for nitrate in plants, namely, high affinity transport system (HATS) and low affinity transport system (LATS) (Kronzucker et al. 1995). For the HATS, the energy of uptake is provided by the proton motive force, which is adequate to promote active NO_3^- uptake over a wide range of external concentrations (Crawford and Glass 1998). When plants are fed with unlimited supplies of NO_3^-, the amount of NO_3^- in the roots and shoots can reach up to 100 mM, most of which is stored within the vacuole. The LATS, however, did not show saturation kinetics rather the uptake rate increased linearly as NO_3^- concentration increased. The LATS has supposed to be diffusion (Padgett and Leonard 1994). It appears that until the fourth day, the HATS system is involved in the uptake of NO_3^- in the medium where its concentration is below 100 mg L^{-1}, and thereafter, the increase of nitrate in the treatment medium leads to the activation LATS and thereby changes the efficiency of nitrate uptake. The efficiency of nitrate uptake by vetiver grass in effluent from a domestic wastewater treatment plant was reported to be about 40–75% (Akbarzadeh et al. 2015). Other studies point to the uptake of more than 75% N, 15–58% P, 90% Zn, 75% arsenic, and 30–71% Pb from the water by vetiver grass (Liao et al. 2003). Vetiver has also the ability for uptake of organic pollutants remaining from food remnants such as coconut husk that cause bacterial and fungal growth in sullage streams of factories (Girija et al. 2011). Furthermore, that study reported declined nitrate content close to one-half and decreased phosphate content from 7.8 to 2.7 mg L^{-1}. Over a phytoremediation period of 21 days, the uptake of nitrate in *Acorus calamus* and *Phragmites australis* plants was also about 82 and 79%, respectively (Marecik et al. 2013).

Despite low phosphate content of the influent and effluent sewage, changes in the phosphate concentration were significant and decreased by about 60% during 18 days in all sewage treatments. Similarly, Akbarzadeh et al. (2015) reported 75 to 80% decrease in phosphate concentration of effluent sewage by the vetiver plant.

Similar to the influent, the amount of nitrite in the effluent was negligible. Nitrite concentration increased as nitrate increased in the effluent water due to photo-induced and/or microbial conversion (Beschkov et al. 2004; McIlvin and Altabet 2005). Nitrite uptake enhanced as nitrate concentration increased in the effluent sewages. Rawat et al. (2012) reported the phytoremediation potential of eight macrophytes for nitrite uptake from the contaminated water. All the selected species reduced nitrite concentration; among them, *Pistia stratiotes* could remove nitrite up to 72.28%.

Although ammonium was low in the effluent, it could be absorbed by vetiver plants in influent sewage from day 4 until day 18. Ammonium is the reduced form of nitrogen, and its assimilation has lower energy cost. Since increase of ammonium uptake may cause toxicity in plants, ammonium homeostasis has to be strongly regulated (Loqué and von Wirén 2004).

Total dissolved solids (TDS) were greater as $Ca(NO_3)_2$ in the effluent sewages increased, so that the figure was 34% more in EF500 compared to EF. During phytoremediation by vetiver grass, the TDS in EF250 and EF500 was significantly decreased. Significant reduction of TDS in the EF500 compared to the EF can be attributed to the higher nitrate uptake in this treatment. Similar trend was also observed for EC. Both TDS and EC in influent and all effluent treatments were severely decreased due to the absorption of abovementioned ions during phytoremediation process. Phytoremediation by vetiver grass reduced the concentrations of Ca^{2+}, K^+, and Cl^-, and this may contribute to the reduction of TDS. The decrease of Ca^{2+} by vetiver grass was pronounced as the effluent sewages received more Ca^{2+} as $Ca(NO_3)_2$. This suggests a certain capacity for Ca^{2+} uptake in vetiver grass. Douro diagram showed that all of the treated sewage waters belong to the contaminated water zone which their TDS values were decreased due to vetiver grass phytoremediation. Changing in the order of cations and anions concentrations in influent and effluent sewage by during 18 days showed that vetiver grass has certain ability for both NH_4^+-N and NO_3^--N uptake as these ions achieved to lowest concentration during 18 days phytoremediation.

Conclusion

The present study revealed that wastewater processing by Gorgan's wastewater treatment plant reduced EC, TDS, and the concentrations of all heavy metals in the effluent water but increased nitrate concentration. The vetiver grass successfully decreases TDS and removes anions and cations especially nitrate, ammonium, phosphate, and also calcium from both influent and effluent of sewage treatment plant. An increased concentration of nitrate in sewage water resulted in the increased uptake rate of this ion by the plant. Furthermore, a positive correlation was observed between the uptake rate of nitrate by vetiver grass and the duration of cultivation of vetiver grass in the nitrate-containing medium.

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