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The -Effect of Zeolite and Nitrifying Bacteria on Remediation of Nitrogenous Wastewater Substances Derived from Carp Breeding Farm

Motesharezadeh, B.[1*], Arasteh, A.[1], Pourbabaee, A. A.[1], and Rafiee, Gh. R.[2]

[1]Department of Soil Science Eng., University College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran

[2] Department of Fishery and Environment, University College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran

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ABSTRACT: Limitation of water resources and necessity in profitable production, and consequently productivity enhancement has taken crop production by means of unconventional water into consideration. In this study, the effect of different levels of zeolite and nitrifying bacteria were investigated on nitrogenous compounds absorption in a closed system of carp breeding with alfalfa. Treatments of zeolites at three levels (with a weight percent of 0, 5, and 10%) and bacterial treatments including two levels (without bacteria and with nitrifying bacteria) were used for six weeks in such way that produced effluent of the carp breeding from an experiment unit were entered to alfalfa culturing medium; so a closed cycle was established. The results of this study showed that use of zeolite and nitrifying bacteria significantly decreased ammonia values in comparison to the control in effluent, in addition, nitrate amount in treatments containing zeolite and bacteria were significantly ($P > 0.05$) more than the control treatments. Dry weights of alfalfa in the treatments containing zeolite and bacteria were more than the control treatments; additionally, use of bacteria was also significant on dry weight of alfalfa ($P > 0.05$).This study showed that application of zeolite and nitrifying bacteria to soil, conversion of ammonia to nitrate happens during nitrification that improves water quality of aquaculture and causes more uptake of nitrate by plant that reduces water and soil pollution by this element.

Key words: Phytoremediation, Recirculating aquaculture system, Effluent of fish breeding, Nitrogen, Soil pollution

INTRODUCTION

Considering to the growing demand for fish breeding, existing water resources are not enough (Vannuccini, 2003). Reusing wastewater, constructing offish breeding farms with appropriate intervals, constructing precipitation ponds after outputs, and removing waste are known as suitable solutions in order to maintain the stable production that are along with identification of the substances and load of pollutants in wastewater, it can proceed to remove or reduce its concentration, then reuse and try to employ other economic ways. One of the major problems in developing aquaculture is some compounds which are produced from metabolism of nutrients in the gastrointestinal tract of aquatic creatures, and then their excretion to the aquatic environment that harms fish and other aquatic organisms to survive. The most important substance among them is a highly toxic substance called ammonia which a large amount of input water requires to remove it from the nurturing environment. A maximum concentration of NH_3 (aq), 21 μgN/ L was proposed as a threshold value for most marine and fresh water aquaculture species (Eddy, 2005).Due to the fact that water resources are limited, an abundant supply of water in order to refine aquatic environment in many countries is not possible (Vannuccini, 2003). Moreover, output effluent from such farms is considered as serious threat for polluting the environment (Quillere *et al*., 1993). The main effects of effluent entering into the river that can be mentioned are increasing in concentration of solids, suspended materials, and dissolved organic matter, decreasing in dissolved oxygen levels, occurring nasty smells caused by anaerobic oxidation, migrating the mortality aquatic animals, entering large amounts of nutrients such as nitrogen and phosphate compounds followed by eutrophication, preventing sunlight penetration to perform the photosynthesis process, and entering pathogens into the water (Taghavi *et al*., 2006). In the last two decades, the most serious consideration has

*Corresponding author E-mail: moteshare@ut.ac.ir

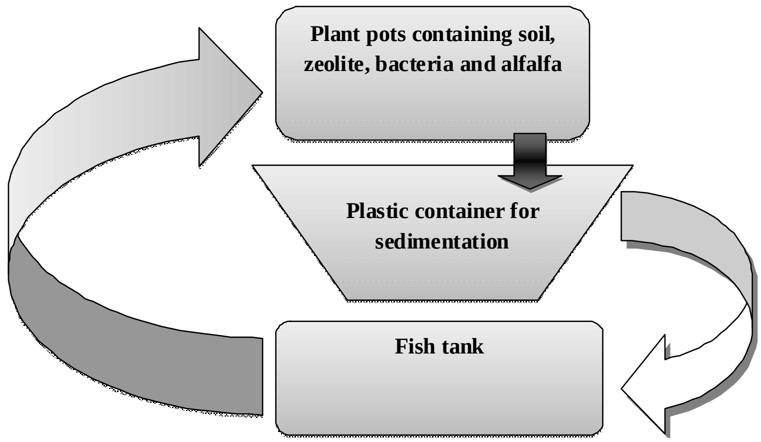

Fig. 1. A schematic view of water flow direction in an experiment unit

been to the qualitative biological control of water through the conversion of ammonia to nitrite and nitrate during bacterial process of ammonia oxidation, whereas nowadays the most attention is paid on the nitrate accumulation derived from this process in these systems (Van Rijn, 1996). In a study, the researchers designed an aquaculture system of lettuce and potato with thin layer of nutrient in a perlite bed on the top of cylindrical ponds of Tilapia fish (*Oreochromis aureus*). They succeeded in producing 6.8 kg/m^2 potato and 236 g lettuce per each head of lettuce (Burgoon & Baum, 1984). In a research, recirculating grow system of cucumbers with thin layer of nutrient was compared to another system of sand-bed with the area of 2 m^2 (aquatic volume was equal to 7.2 m^3).At the end of the experiment, the researcher achieved 0.7 kg cucumber per each plant in a sandy bed system. Besides, the results of their study indicated that the use of cucumber led to remove 11% nitrate and 30% phosphate in every water transmission through the plant container with sand bed (Wren, 1984). Scholars evaluated a re-circulating system of simultaneous breeding of red Tilapia and culturing lettuce, and finally they reported that the Electrical Conductivity of water among all weight groups of red Tilapia increased from 0.16mmhos/cm to 0.5mmhos/cm which indicated that TDS increased owing to low rates of daily water transmission and mineralization of organic matter by microorganisms. The pH values decreased in all treatments that was due to acid production through bacterial activity in decomposition process of organic matter; and eventually, nitrite concentration increased from 3.75mg/L to 9.77 mg/L. Considering to the limitation of unconventional water resources, and also economic crop production per unit area, the current study was carried out in order to investigate the effect of different levels of zeolite and nitrifying bacteria on quality of produced waste water in a closed aquaculture system and its effect on Alfalfa growth.

MATERIALS & METHODS

Each experiment unit included three parts. The first part was comprised of a pan with 35 liters volume, and a height of 30 cm for breeding the common crap fish. In this part, in each experiment unit, six fish were chosen so that initial weights of all units were the same as each other, and this amount was equal to 123±3. The water containing fish excretory material was transferred to the second part by means of a water pump. The second part of experiment contained three one-kilogram pots, which had been put next to the each other by yonolit in such a way that running water on its surface was totally poured in to the pots and there was no any water out. In each pot the treatments of zeolite and bacterial were applied, so that three zeolite levels (0, 5, and 10 weight percent) and two levels of bacteria (without bacteria and containing nitrifying bacteria) in three replications were added to each pot containing soil. After transferring water from the first part to the second part, amount of ammonia was converted to ammonium and absorbed by the soil exchange surfaces and zeolite, a portion of it converted to nitrate during the nitrification process and was absorbed by the plant, and the remaining was entered into the part three from the bottom of the pot along with drained water. In the third part of experiment unit, there was a piece of glass wool; so, in spite of the small window that was slightly above the bottom of the container, glass wool played the role as a major factor in depositing the output materials from the pots, and also trapping washed bacteria from the second part. In the final step, the water which its ammonia had been reduced during nitrification and absorption process, was transferred from the third part to first part (containing fish), and this cycle adjusted using a timer with respect to pots capacity and soil field capacity, and the progression trend of water quality parameters of fish farming had been measured for six weeks (Fig. 1).

Texture class of the used soil was Loam with 45% sand, 32% silt, and 23% clay. Moreover, alfalfa was cultured in the mentioned soil. Some characteristics of the used soil are provided in Table 1. In this study, used variety of alfalfa was Hamedani, which is the most commercial variety in Iran (Mazaheri-Laghab, 2008). Time length and growth period of alfalfa totally lasted six weeks.

The amount of oxygen in water was measured by using Winkler method (Bertha *et al.*, 1992).Used bacteria in this study consisted two parts; the first part was nitrite bacteria, which included Nitrosomonas and Nitrococcus; and the second part, was nitrifying bacteria that Nitrobacter was used for this part. Used zeolite in this experiment was in the form of Klinoptilolith, which was used in three treatments including control, 5, and 10 weight percent. The zeolite was initially heated in oven in order to evaporate its water. Electrical conductivity values of water were measured in a two-day time interval and two times in each day, once before morning feeding and once before evening feeding from water samples, which were taken from the middle column of water. The amount of oxygen was measured by Winkler method (Bertha *et al*., 1992). To determine the amount of ammonia, water samples were taken from the middle column of pond once a week before feeding in the morning shift. The samples were measured by spectrophotometer. Nessler method was chosen to measure ammonia concentration (APHA, 1998).

Alfalfa organic nitrogen was measured by using Kjeldahl Method (Radojevice & Boshkin, 1999).To determine the nitrate concentration, every two weeks before morning feeding time, water samples were taken from the middle column of the pool. In laboratory, the samples were recorded with two wavelengths of 220 and 275 nm by spectrophotometer after cleaning by a filtering paper of0.45 microns and during preparation steps. In order to measure nitrate, spectrophotometric method with ultra-ray was chosen (APHA,1998).

This project was conducted in a factorial experiment design as randomized complete block design with three replications. The first factor was zeolite, which was considered in three weight percent levels including control, 5, and 10. The second factor was bacteria, which was exerted in two levels; without bacteria and with nitrifying bacteria in the experiment units. Analysis of variance was performed by using SPSS software. Mean comparison by means of LSD method was done at level of5%, and plotted diagrams have been presented by Microsoft Excel software.

RESULTS & DISCUSSION

The results related to some physicochemical characteristics analysis of the used soil in this study are presented in Table 1. According to the table, soil texture was loam that is suitable for culturing alfalfa; in addition to, it has appropriate drainage conditions, which is compatible with the aims of study. The obtained results related to analysis of variance of measured characteristics are listed in Table 2. In this regard, the effect of zeolite on all studied traits was significant at the level of 1%; furthermore, the effect of bacteria on characteristics of the plant nitrogen and nitrate at the level of 1%, and also, on dry weight of the plant at the level of 5% were significant (Table 2).

Table 3 shows the measured ammonia of the water during six weeks of research. In this table, it is obvious that the amount of ammonia in water in the beginning of experiment decreased by adding zeolite to the soil. Rafiee and Saad (2006) in a study investigated integrated systems of farming and production (fish and

Table 1. Some Physical and chemical properties of the soil used in the experiment

Characteristics	Value	Characteristics	Value	Characteristics	Value
Sand (%)	45	pH	8.4	CEC($cmol_c$/kg)	19.11
Silt (%)	32	EC(dS/m)	1.15	Mn (mg/kg)	13.9
Clay (%)	23	Total N (%)	0.095	Zn (mg/kg)	3.21
Soil texture	Loam	Available P (mg/kg)	43.5	Fe (mg/kg)	7.42
OC (%)	0.87	Available K (mg/kg)	330	Cu (mg/kg)	1.22

Table 2. Analysis of variance for the used characteristics

Source of variation	DF	F			
		Ammonia	Nitrate	Dry matter of plant	Nitrogen of plant
Zeolite	2	18.820^{**}	25.211^{**}	26.713^{**}	76.175^{**}
Bacteria	1	0.652^{ns}	10.673^{**}	7.320^{*}	12.469^{**}
Zeolite×Bacteria	2	0.059^{ns}	1.663^{ns}	1.035^{ns}	7.408^{**}

**, * and ns are significant at 1% and 5% levels and non-significant, respectively.

Table 3. The amount of water ammonia in treatments (T) during six weeks

T	Week 1 mg/L	Week 2 mg/L	Week 3 mg/L	Week 4 mg/L	Week 5 mg/L	Week 6 mg/L
Z_0B_0	0.026	0.027	0.030	0.030	0.031	0.031
Z_0B_1	0.027	0.026	0.026	0.033	0.032	0.030
Z_5B_0	0.022	0.024	0.026	0.031	0.030	0.028
Z_5B_1	0.022	0.023	0.025	0.031	0.029	0.026
$Z_{10}B_0$	0.020	0.022	0.024	0.025	0.024	0.024
$Z_{10}B_1$	0.020	0.021	0.023	0.026	0.022	0.020

Z_0= No zeolite (Control); Z_5= 5% zeolite; Z_{10}= 10% zeolite; B_0= No bacteria; B_1= With bacteria

Table 4. The amount of water nitrate in treatments (T) during experiment period

T	First week mg/L	Week 2 mg/L	Week 4 mg/L	Week 6 mg/L
Z_0B_0	9.62	10.57	11.84	13.03
Z_0B_1	11.00	10.95	12.93	14.23
Z_5B_0	12.31	11.69	13.09	14.40
Z_5B_1	12.90	12.26	13.73	15.10
$Z_{10}B_0$	12.56	11.85	13.27	14.60
$Z_{10}B_1$	12.88	12.11	13.56	14.47

Z_0= No zeolite; Z_5= 5% zeolite; Z_{10}= 10% zeolite; B_0= No bacteria; B_1= With bacteria

Table 5. The concentration of plant nitrogen (Mean ± SD) in treatments (T) at the end of period

T	N of plant (%)
Z_0B_0	0.93 ± 0.05^c
Z_0B_1	1.18 ± 0.03^b
Z_5B_0	0.99 ± 0.06^c
Z_5B_1	1.12 ± 0.13^b
$Z_{10}B_0$	1.29 ± 0.05^a
$Z_{10}B_1$	1.33 ± 0.25^a

Z0= No zeolite; Z5= 5% zeolite; Z10= 10% zeolite; B0= No bacteria; B1= with bacteria

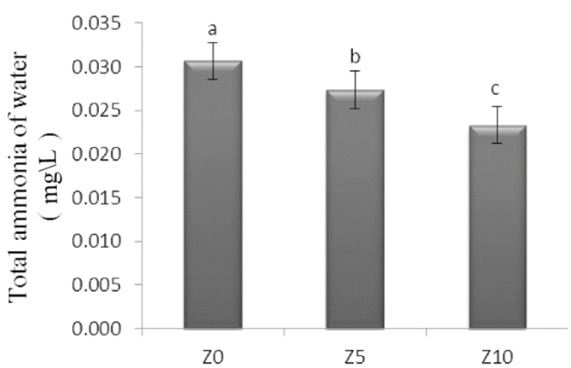

Fig. 2. Water ammonia at different levels of zeolite

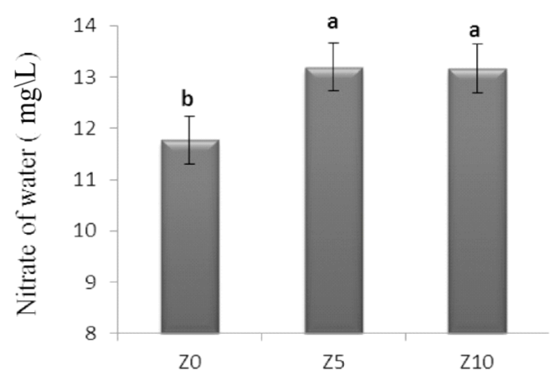

Fig. 3. Mean comparison of nitrate in different levels of zeolite

lettuce production); they showed that adding 10 g zeolite to the system can reduce the amount of ammonia. With respect to the high level of exchange capacity of zeolite (high cation-exchange capacity; CEC) and the possibility of ions absorption over this mineral, it is expected that applying zeolite would have an important role in reducing the concentration of nitrogen compounds. Ammonia alteration to ammonium in the aquatic environment and further ammonium absorption on the exchange surfaces of zeolite and soil than the control conditions caused this difference. Fig. 2 illustrates the mean comparison of ammonia at differ-

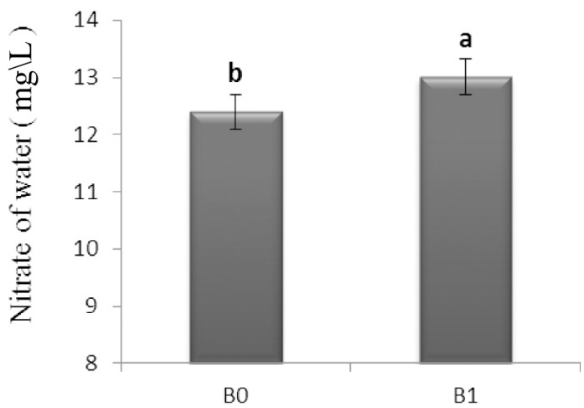

Fig. 4. Mean comparison of nitrate in different levels of bacteria

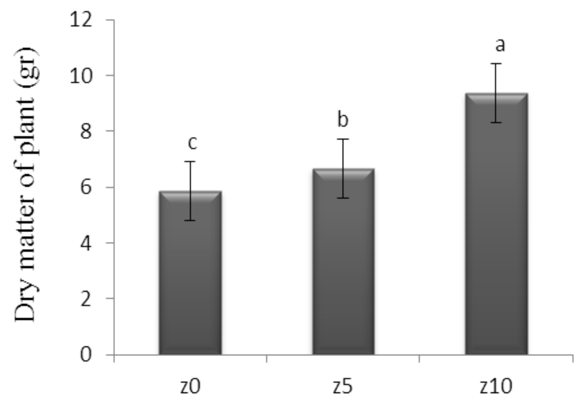

Fig. 5. Mean comparison of dry weight of the plant in different levels of zeolite

(Z_0 = without zeolite; Z_5 = 5% zeolite; Z_{10} = 10% zeolite)

Table 6. Temperature and oxygen (Mean ±SD) of water in treatments (T)

	Parameter	
T	**Water temperature (ºC)**	**Oxygen of water (mg/L)**
Z_0B_0	22.5±1.5	8.33±0.22
Z_0B_1	22.4±1.5	8.38±0.14
Z_5B_0	22.4±1.5	8.33±0.18
Z_5B_1	22.5±1.5	8.40±0.20
$Z_{10}B_0$	22.3±1.5	8.32±0.23
$Z_{10}B_1$	22.5±1.5	8.38±0.19

Z0= No zeolite; Z5= 5% zeolite; Z10= 10% zeolite; B0= No bacteria; B1= With bacteria

Table 7. Water pH during experiment period

T	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
$\mathbf{Z_0B_0}$	7.68	7.95	8.09	7.97	8.25	8.27
$\mathbf{Z_0B_1}$	7.73	7.87	8.05	7.99	8.29	8.20
$\mathbf{Z_5B_0}$	7,81	7.83	8.09	8.07	8.30	8.34
$\mathbf{Z_5B_1}$	7.73	7.75	8.04	7.96	8.27	8.31
$\mathbf{Z_{10}B_0}$	7.77	7.69	7.96	7.87	8.05	8.31
$\mathbf{Z_{10}B_1}$	7.72	7.84	8.08	7.89	8.27	8.28

Z0= No zeolite; Z5= 5% zeolite; Z10= 10% zeolite; B0= No bacteria; B1= With bacteria

ent zeolite levels by using LSD test. Based on this figure, the amount of ammonia at the levels that contain zeolite is less than the control treatment.

Natural zeolites, having a high affinity toward a wide range of cations (Na^+, K^+, Ca^{2+}, Mg^{2+}), are often used for separating NH_4^+ from wastewater (Lahav and Green, 1998). With regard to aquaculture, Dryden and Weatherley (1989) reported that clinoptilolite (a common natural zeolite) can be applied for continuously removing cations from water. Yousefian *et al* (2010) and Peighan (1998) in their studies showed that the application of zeolite leads to ammonia reduction. They stated that these results are due to absorption by zeolite that this situation is suitable for fish breeding. At the end of experiment, as zeolite increased, the amount of ammonia decreased; in addition, the treatments containing nitrifying bacteria showed fewer amounts than the control. The reason of this fact is, firstly occurred nitrification by bacteria, and secondly conversion of ammonia to nitrate.

Table 4 displays the alteration in nitrate values during research course. Inserting zeolite and bacteria to the soil at the beginning of the period contributed more nitrate amount in treatments than control. Moreover, there is a difference between the treatments without nitrifying bacteria and containing nitrifying bacteria in a way that in treatments, which contain bacteria, the amount of nitrate was more than the treatments without bacteria. This affair has been caused through nitrification process in the soil. At the end of experiment period the amount of nitrate in treatment which contained 10% zeolite was less than one which had 5% zeolite; according to the amount of plant nitrogen (Table 5), in this treatment, more nitrogen compounds have been absorbed by plant.

Fig. 3 displays the mean comparison of water nitrate at different zeolite levels using LSD test. Based on the figure, nitrate value in the control treatments is less than treatments which contain zeolite.

Additionally, Fig. 4 illustrates the average nitrate in the water at different levels of bacteria, and according to this figure, presence of bacteria leads to increase in nitrate mounts in water. It is worth mentioning that this increment is caused by nitrification process, which is done by bacteria. According to Lawson (1994) unionized ammonia private activity of Nitrosomanas and Nitrobacter at 10-15 mg/L and 0.1-1.0 mg/L respectively, which adversely affects the performance of the biofilter.

Table 5 shows the amount of nitrogen in the plant at the end of research period. With respect to Table 4, nitrogen values of the plant related to the treatments containing 10% of zeolite are more than other treatments.

According to a study (Ippolito *et al*., 2011), it was revealed that mixing of zeolite with soil caused to decrease in leaching of inorganic nitrogen that it can help soil to maintain nitrogen compounds. This factor can contribute to provide nitrogen compounds for plant uptake. In addition, the amount of nitrate in the last week for $Z_{10}B_1$ treatment compared to treatments containing 5% zeolite was observedin a way that it can reflect more uptake of this element by plant. Table 6 portrays a report from water oxygen and temperature.

Delos (1999), in a study showed that since the water temperature increased, consequently, ammonia toxicity increased in an aquatic environment. Besides, Naji *et al*. (2009) as a result of their research asserted that increase in water pH will enhance fish tolerance toward ammonia.

Table 7 demonstrates the status of water pH during the experiment period. Therefore, according to the table, trend of pH changes in water has been rising. Nitrification process releases proton in the environment that this proton lowers the pH of water (Rafiee & Saad, 2006). On the other hand, zeolite and systems containing zeolite tend to neutralize the system through desorption and absorption of protons (Douglas, 1985), that these process are performed with absorbing proton and releasing alkali metals on the exchange surfaces. The interaction between hydrogen ion obtained from nitrification and releasing of alkali metals into the environment causes to increase water pH during the period of the experiment. International Plant Nutrition Institute (IPNI) stated that a temperature of 23.5 degrees Celsius and a pH between 6.5 and 8.8 are identified as the optimal conditions for nitrification.

Fig. 5 depicts mean of comparison connected to the dry weight of alfalfa at different levels of zeolite by LSD test. As it is obvious, increasing zeolite in the soil caused that plant dry weight had more value than the control conditions. Rafiee and Saad (2006) reported that zeolite usage in such systems contributes to increase in plant weight. Motesharezadeh and Asgari (2013) reported that the use of zeolite up to 10 weight percent because of providing nutrients leads to increase in plant wet weight. Moreover, the effects of different levels of zeolite on the plant biomass have been investigated in some scholars' studies. They reported these effects including preventing from wasting nutrients and their gradually releasing as well as increasing soil total porosity, and better plant growth (Hojati *et al*., 2007; Rehakova *et al*., 2004).

Fig. 6 also depicts mean comparison of alfalfa dry weight for different levels of bacteria.

Turan (2006) evaluated the effect of different amounts of zeolite on quantitative and qualitative characteristics of alfalfa in greenhouse conditions; so concluded that the plant height and total dry weight were significantly affected by zeolite application. Ranjbarchoobe *et al* (2004) studied the effect of different irrigation levels and natural zeolite application on the yield of tobacco; finally they

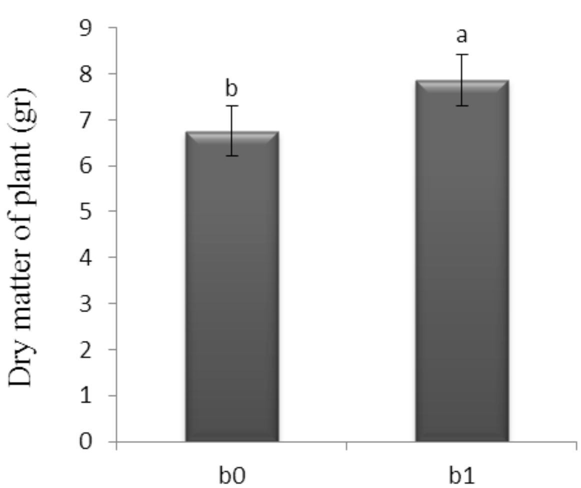

Fig. 6. Mean comparison of dry weight of alfalfa in different levels of bacteria

(B_0 = without bacteria; B_1 = containing bacteria) reported that the effect of zeolite on some plant properties such as plant dry weight was significant at the level of 1%; in general, they concluded that zeolite application was effective for quantitative and qualitative promotion of tobacco yield. Motesharezadeh and Asgari (2013) declared that application of zeolite up to 10% contributes to dry weight increment and productivity of plants; this issue can be stemmed from increase in nutrient storage capacity, especially nitrogen, phosphorus, and potassium for further usage of the plants.

CONCLUSIONS

This study, using phytoremediation, aimed to assess the efficiency of a combined system of plant culturing and fish breeding with a water re-circulating approach in order to reduce nitrogen compounds released from gastrointestinal metabolism of carp breeding in aquatic environment and absorption of these compounds through the soil and plant. The results showed that treatments containing zeolite and bacteria were more successful to reduce existing ammonia in the effluent of fish breeding system. This issue may be due to the conversion of ammonia to ammonium, ammonium uptake by plants, and soil and zeolite exchange surfaces as well as conversion of ammonia to nitrate during nitrification process, and uptake of these compounds by plants, as a result nitrogen in alfalfa plant showed more values. The amount of nitrate in the water for treatments containing zeolite was significantly higher than control treatments that these differences are the result of nitrification process. Dry weight of alfalfa plant in the treatments containing zeolite and bacteria were significantly more than control treatment as well; improving the conditions of culturing medium by zeolite, and providing nitrogen compounds by bacteria compared to without bacteria conditions are the reasons behind this increment. Monitoring water pH during the experiment period revealed an increasing trend. In other words, this parameter was balanced by two factors including hydrogen ion production through nitrification, and subsequently its absorption by plant cultivation bed, and release of alkali metals in the aquatic environment; eventually, the mentioned balance was going to move up and pH values were rising up. Ultimately, considering the significant effect of zeolite on reducing levels of nitrate compounds and water quality as well as supplying required nitrogen for crop production, it is recommended that besides recognizing various aspects of this work and optimization, many attempts would be made to develop the integrated manufacturing systems in order to increase water use efficiency and crop production using inorganic and biological treatment (zeolite and bacteria).

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