



Comparative evaluation of natural water conditioners for their potential use in freshwater aquaculture

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Abstract

In this study, the use of natural adsorbents, such as zeolite (clinoptilolite), leonardite, and duckweed (*Lemna minor*, L. 1753) was investigated for the regulation of optimum water qualities in freshwater aquaculture. The study was carried out in 3 experiments in triplicate. In the first experiment, aquarium fish feed (35% protein) was used as the ammonia source at 3 different rates (0.2, 0.4, and 0.6 g feed per 500 mL of tap water). In the second experiment, clinoptilolite (C) and leonardite (L) mixture (C:L = 1:2) were added to balance excessive ammonia. In the third experiment, duckweed (1.5 g/500 mL) was added to aquaria (10 cm in diameter) in a way to cover the surface area, and the ammonium adsorption of duckweed at low NH_4^+ -N concentrations was determined with 9 measurements. In this study, NH_3 values reached their peaks (0.19 mg/L) at the end of 1st experiment, in which ammonia values originating from the unconsumed feed were determined. In the 2nd experiment, NH_3 values began to decline (0.06 mg/L) with the addition of natural adsorbents (zeolite + leonardite) and were decreased to 0.003 mg/L with the addition of duckweed in experiment III, where natural adsorbents started to reach saturation. When the data obtained at the end of this study were evaluated, it was determined that all 3 natural materials had a positive effect on water parameters in aquaculture systems. As a result, it was determined that in high concentrations of zeolite + leonardite mixture and in low concentration of duckweed there had been a good removal efficiency.

Keywords Clinoptilolite · Leonardite · *Lemna minor* · Ammonium · Aquaculture

Introduction

Water quality is a critical factor when culturing any aquatic organism. Optimal water quality varies by species and must be monitored to ensure growth and survival. Water quality parameters that are commonly monitored in the aquaculture industry include temperature, dissolved oxygen, pH, ammonium as 18–28 °C, 5–8 mg/L, 6.5–8.5 and 1–5 mg/L, respectively (Jorgensen 2002; Iskander et al. 2014; Ismadji et al. 2016). Removal of excessive quantities of nitrogenous compounds in aquaculture systems is an obligatory process. There are physical, chemical, and biological treatments employed to achieve this. For this purpose, adsorbent substances, such as bentonite, diatomite, zeolite, etc., as well as aquatic plants, such as water lettuce and duckweed are

used. While natural adsorbents, such as zeolite are effective at high ammonium concentrations (Ergün et al. 2008; Öz et al. 2016, 2017; Tekeşoğlu and Ergün 2020), plants such as water lettuce and duckweed are effective in the removal of low ammonium concentrations of ammonia (Şahin et al. 2009).

Zeolites are hydrated alumina silicates of alkali and alkaline earth metals with a crystal structure and are in the framework silicates group. Natural zeolites are inexpensive materials that can be found widely in nature and have high cation exchange capability, molecular sieve properties, and special design for adsorption and catalysis (Mazeikiene et al. 2008).

Leonardite is the highly oxidized form of lignite during coalification and contains humic acid ranging from 35 to 85%. Leonardite is also defined as a complete organic matter that has not reached the phase of coal and contains carbon, macro- and micronutrients apart from high levels of humic acids. It has important economic value due to its high humic acid content. It is black-brown in color with the appearance of consolidated soil and is soft enough to

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be crumbled easily by hand. The pH value of leonardite varies between 3 and 5, and its solubility in water is low (Ratanaprommanee et al. 2016). Due to the plant nutrients, low toxic element, and high humic acid contents of leonardite, most previous works investigated its use as a fertilizer, effect on plant yield, fertilizer value, organic matter content, and humic substance content. As a result of its use in conventional and organic agriculture, beneficial results have been obtained in terms of improving soil and roots, and supplementation of nutritional elements to plants (Engin and Cöcen 2012).

The use of aquatic macrophytes, such as water hyacinth, duckweed, water lettuce, etc., in wastewater treatment has attracted global attention in recent years. These plants can be applied on the surface of stabilization ponds, and may contribute to nutrient recovery from wastewater (Caicedo et al. 2000). Duckweed has the great capacity in absorbing the nutrients and their high nutrient removal efficiency can be used to clean wastewater in an effective, cheap, and simple way (Gürtekin and Güneşdağ 2008).

Duckweeds are small, vascular floating macrophytes that grow rapidly and reproduce vegetatively. The ease of their culture and convenience of manipulation under aseptic laboratory conditions make them suitable experimental species for toxicity trials. The use of aquatic plants as in situ bio-monitors for water quality assessment has been a common practice for many years. Aquatic plants have also been frequently used to remove suspended solids, nutrients, heavy metals, toxic organic substances, and bacteria from acid mine drainage as well as waters in urban rain runoff, landfill, and agricultural activities (Caicedo et al. 2000). On the contrary, they have not been widely used as the test species in toxicity trials designed to assess the hazard of potential contaminants (Caicedo et al. 2000; Lewis 1995). Ammonia is known to be toxic to all higher life forms, but when absorbed at a slow rate, it is well tolerated by most organisms due to their efficient detoxifying mechanisms (Goopy et al. 2004). Decreasing the proportion of free ammonia in the solution may improve the survival of plants grown on eutrophic media. High concentrations of NH_4^+ -N inhibit the growth of duckweed, negatively affecting the biomass accumulation and the wastewater purification by duckweeds (Caicedo et al. 2000; Wang et al. 2014). Among all the species in the Lemnaceae family, *Lemna minor* is the most commonly used for toxicity testing (Selvarani et al. 2015).

This study aims to investigate the comparative efficacy of the natural adsorbents such as zeolite (clinoptilolite), leonardite, and duckweed (*Lemna minor*, L. 1753) for the maintenance of optimum water conditions (ammonium) in freshwater aquaculture.

Materials and methods

Experimental design

The study was carried out in 3 separate experiments in triplicate. In the first experiment, aquarium fish feed (35% protein) was used as the ammonia source at 3 different rates (0.2, 0.4, and 0.6 g feed per 500 mL of tap water) according to Kibria et al. (1997). The amount of ammonia resulting from the unconsumed feed in the aquaria was determined at the end of the first experiment which lasted for 7 days. In the second experiment, clinoptilolite (C) and leonardite (L) mixture (C:L = 1:2) was added to balance excessive ammonia. The characteristics of clinoptilolite and leonardite used in the study are provided in Table 1. These substances were analyzed by SEM/XRF/BFT (Fig. 1) at Kastamonu University Central Research Laboratory. The pH values were computed as per the method described by Güneş (2017) and Tokat (2019).

In the second experiment, certain water parameters were assessed by making 6 separate measurements at regular intervals after the addition of clinoptilolite and leonardite (C:L = 1:2). The third experiment was conducted after completion of the second experiment when the saturation amount was recorded in natural adsorbents. In this experiment, duckweed (1.5 g/500 mL) was added to aquaria (10 cm in diameter) in a way to cover the surface area, and the ammonium

Table 1 Chemical composition of tested water conditioners clinoptilolite and leonardite

Clinoptilolite ^a	%		
SiO ₂	78.41	SiO ₂ /Al ₂ O ₃	5.67
Al ₂ O ₃	13.83	BET surface area	34.316 m ² /g
MgO	1.646	pH	8.31
K ₂ O	2.372		
CaO	3.885		
Na ₂ O	1.042		
Fe ₂ O ₃	1.414		
P ₂ O ₅	0.058		
Leonardite ^b	%		
SiO ₂	13.68	SiO ₂ /Al ₂ O ₃	1.93
Al ₂ O ₃	7.07	BET surface area	12.253 m ² /g
MgO	0.11	pH	3.23
K ₂ O	0.454		
CaO	0.323		
Na ₂ O	<0.014		
Fe ₂ O ₃	1.238		
P ₂ O ₅	0.055		

^{a,b} Clinoptilolite and leonardite samples were provided by Rota Mining Corporation (Manisa, Turkey) and Kütahya Chemistry (Kütahya, Turkey), respectively

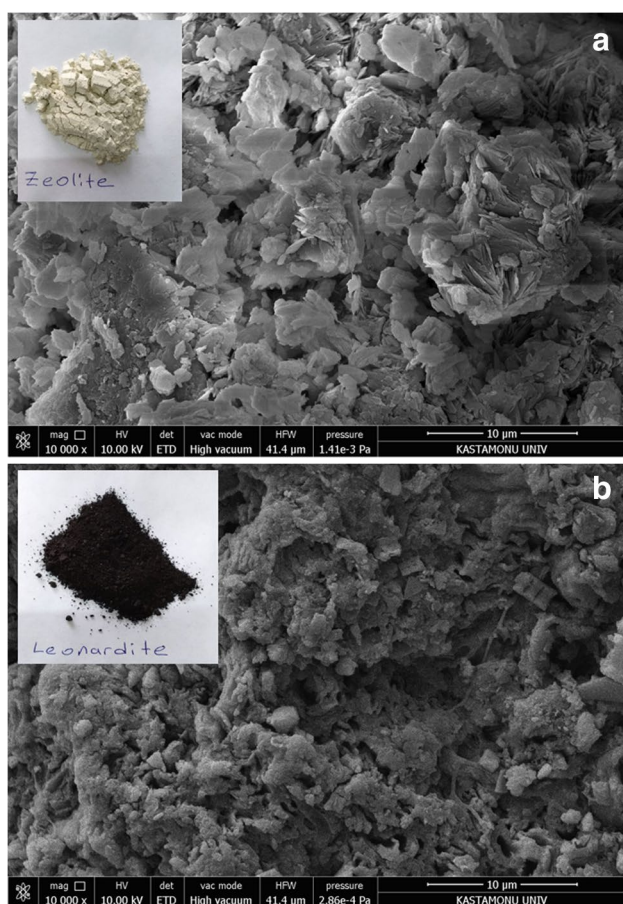
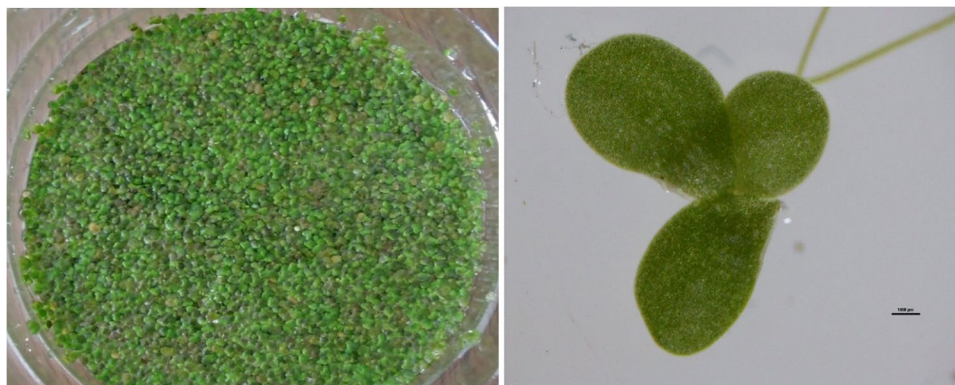


Fig. 1 SEM analysis of clinoptilolite (a) and leonardite (b)

adsorption of duckweed at low NH_4^+ -N concentrations was determined with 9 measurements. Duckweed was visualized with NIKON SMZ 745 T microscope (Fig. 2).

In addition, the adaptation of duckweed to the environment was evaluated. In the experiment, aquaria (containing 500-mL water) were used and no ventilation or heating mechanism was employed. The photoperiod was maintained as 12 h light and 12 h dark.

Fig. 2 *Lemna minor* (duckweed) used for the study



Water parameters (temperature, dissolved oxygen, pH, and NH_4^+) were measured with YSI Professional Plus Series instrument. In each replication, a total of 3 g natural water-regulating materials (1 g of clinoptilolite and 2 g of leonardite) was used per 500 mL of water.

The duckweed used in the experiment was collected from a natural water body with a scoop net and washed thoroughly and gently using tap water to remove plant and insect remains. Before starting the experiment, the duckweed was acclimatized for a week by keeping in an environment with conditions (T: 17.2 °C, DO: 0.43 mg/L, pH: 7.75, NH_4^+ : 0.3 ± 0.01 mg/L) close to its natural requirements. All experimental plans of the study are demonstrated in Fig. 3.

Data evaluation

The levels of NH_3 and TAN were calculated using NH_4^+ , water temperature, and pH data (EPA 1999; Emerson et al. 1975; Chow et al. 1997). The ammonium concentration was calculated as given below:

The dissociation constant of ammonium ion (K_a) was expressed as:

$$K_a = [\text{NH}_3][\text{H}^+][\text{NH}_4^+] \quad (1)$$

Equation (1) can be further arranged as

$$[\text{NH}_3][\text{NH}_4^+] = [\text{H}^+] \quad (2)$$

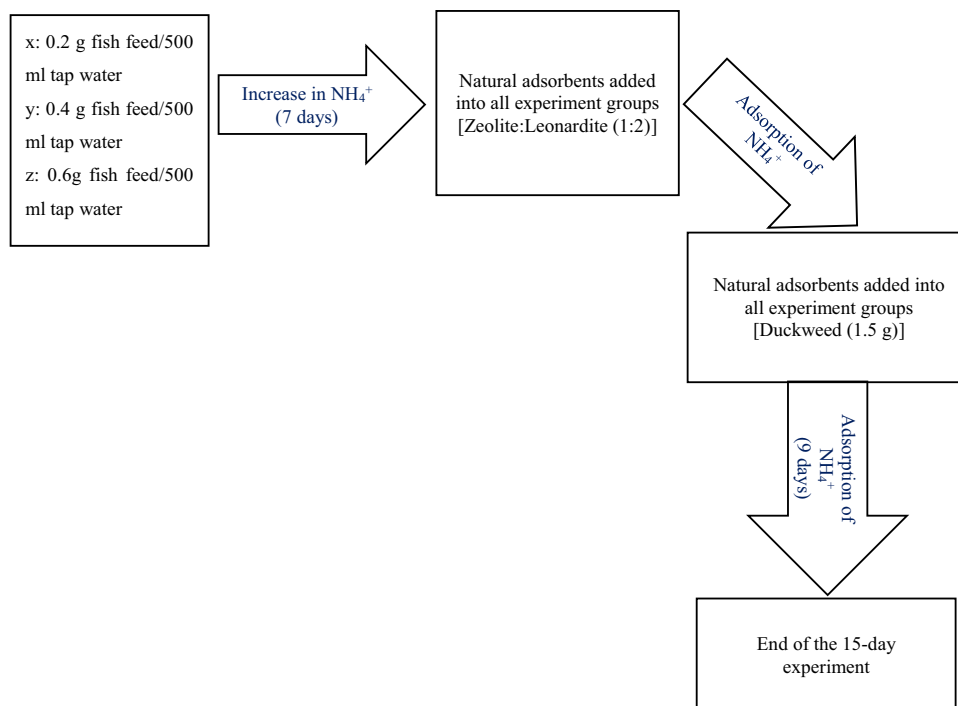
Thus, the relationship between ammonia and ammonium concentrations is described by:

$$\log_{10}[\text{NH}_3][\text{NH}_4^+] = \text{pH} - \text{p}K_a \quad (3)$$

$\text{p}K_a$ varies according to solution temperature. This temperature dependence is given by Emerson et al. (1975) as follows:

$$\text{p}K_a = 0.09018 + 2729.92(273.2 + T) \quad (4)$$

where, T is the solution temperature in °C. Also:

Fig. 3 Experimental design

$$[\text{NH}_4^+] = [\text{NH}_3]T - [\text{NH}_3] \quad (5)$$

$[\text{NH}_3]T$ is the total concentration of ammonia forms. Rearrangement of this equation yields:

$$\log_{10}[\text{NH}_3][\text{NH}_3]T - [\text{NH}_3] = \text{pH} - [0.09018 + 2729.92(273.2 + T)] \quad (6)$$

The removal efficiency (%) for the three trial groups was calculated using the following equation (Yunnen et al. 2016; Tang et al. 2020):

$$\text{Removal efficiency}(\%) = \frac{C_0 - C_t}{C_0} * 100 \quad (7)$$

where, C_0 is the initial ammonium concentration (mg/l) and C_t is NH_4^+ concentrations after time t (mg/L), and t is time of process (mg/L).

Statistical analyses

The statistical analyses were conducted using Minitab Release software (version 17) for Windows. All data were expressed as mean $\pm$ standard error (SE). Data belonging to different experimental groups for each sampling period were compared by one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test for multiple comparisons with the level of significance at $P < 0.05$.

Results

Results of experiment I

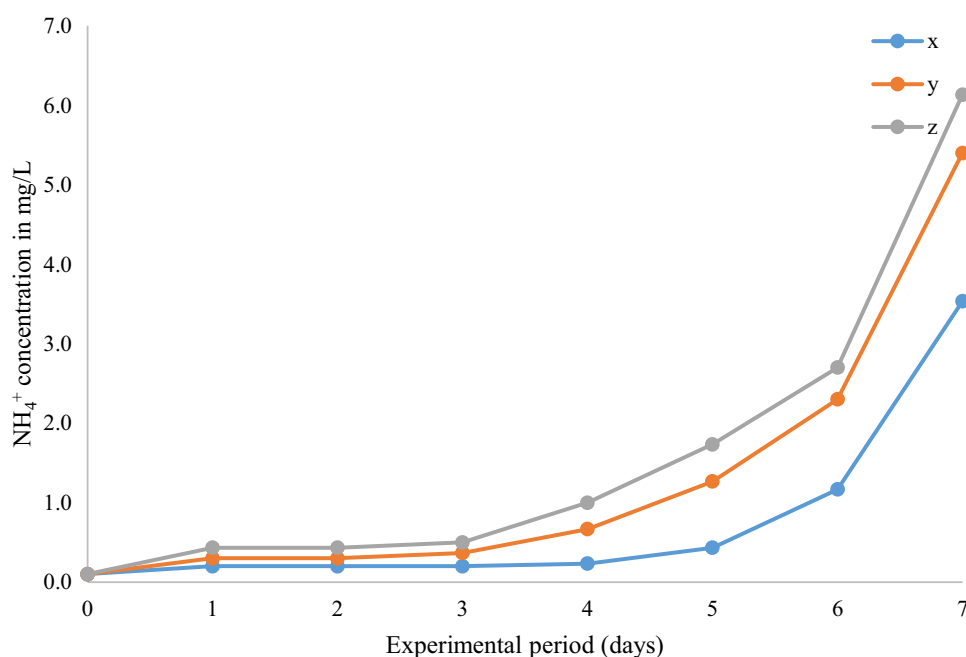
At the beginning of the study, water temperature, dissolved oxygen, pH, and ammonium values were determined as 19.3 ± 0.03 °C, 0.45 ± 0.01 mg/L, 8.62 ± 0.01 , 0.1 ± 0.01 mg/L, respectively, and the difference between groups was statistically insignificant ($p > 0.05$). In the experiment, the control blank created at the beginning was used as the control group. Water temperature, dissolved oxygen, pH, and ammonium values were determined as 20.1 ± 0.02 °C, 0.49 ± 0.01 mg/L, 8.51 ± 0.01 , 0.1 ± 0.01 mg/L, respectively in the control blank.

Three different concentrations [x (0.2 g), y (0.4 g), and z (0.6 g)] of aquarium fish feed, containing 35% protein, were added into water. At the end of the 1st experiment, the mean water temperature was determined as 20.57, 20.97, and 20.46 °C, pH was determined as 7.51, 7.47, and 7.48, and dissolved oxygen was determined as 0.32, 0.31, and 0.28 mg/L for the groups of x, y, and z, respectively ($P > 0.05$). Furthermore, the increase in NH_4^+ level caused by three different concentrations of the feed is shown in Fig. 4.

Results of experiment II

The water parameter values obtained in the 2nd experiment as a result of six measurements taken at regular intervals

Fig. 4 Temporal changes in Ammonium ion (NH_4^+) concentration (mg/L) during experiment I



are presented in Table 2. At the end of the 2nd experiment, it was observed that NH_4^+ , TAN, dissolved oxygen, and pH values differed between groups ($P < 0.05$) but no statistical significance was determined in case of temperature ($P > 0.05$). It was observed that TAN removal efficiency (Fig. 5) was significantly different in group z ($P < 0.05$).

The experiment demonstrated that natural zeolite and leonardite positively influenced the water quality parameters by reducing the increased TAN and NH_3 levels. The removal efficiency of the adsorbents increased as the feed and the amount of TAN originated from feed increased.

Results of experiment III

At the end of the 2nd experiment, 1.5 g of duckweed per 500 mL was added onto the water in a way to cover the surface of water. In the 3rd phase, the effects of duckweed on ammonia removal and other water parameters were determined. The data obtained are presented in Table 3. The TAN

removal efficiency (Fig. 6) was significantly higher in group z ($P < 0.05$) than in the other groups.

Fishes produce nitrogenous wastes as a consequence of the catabolism of amino acids. The assimilation of nitrogen is important for both fish health and profitability of the production (Oluwaseyi 2016). Ammonia exists in water in two different forms, (i) unionized ammonia (NH_3) and (ii) ionized ammonium (NH_4^+). It is well known that ammonia (NH_3) is highly toxic to fish (Prema Latha and Lipton 2007). For many fish species, it has been reported that the toxicity of unionized ammonia begins at 0.05 mg/L, and it causes death at 2 mg/L (Floyd et al. 2015). In the present study, NH_3 values reached their peaks at the end of the 1st experiment, in which the ammonia values originating from the unconsumed feed were determined. In the 2nd experiment, NH_3 values began to decline with the addition of natural adsorbents (zeolite + leonardite) and were decreased to 0.003 mg/L (Fig. 7) with the addition of duckweed in experiment III, where natural adsorbents started to reach saturation.

Table 2 Values of selected water quality parameters (Temperature, TAN, NH_4^+ , pH, and DO (dissolved O_2)) in treatment groups of experiment II (mean $\pm$ SE)

Experimental groups*	Water parameters				
	Temperature ($^{\circ}\text{C}$)	TAN (mg/L)	NH_4^+ (mg/L)	pH	DO (mg/L)
x	19.71 $\pm$ 0.22	2.61 $\pm$ 0.12 ^a	2.58 $\pm$ 0.12 ^a	7.51 $\pm$ 0.03 ^a	0.35 $\pm$ 0.01 ^a
y	19.86 $\pm$ 0.22	3.84 $\pm$ 0.11 ^a	3.81 $\pm$ 0.10 ^a	7.44 $\pm$ 0.03 ^{ab}	0.29 $\pm$ 0.01 ^b
z	20.22 $\pm$ 0.22	4.46 $\pm$ 0.41 ^b	4.42 $\pm$ 0.41 ^b	7.39 $\pm$ 0.02 ^b	0.26 $\pm$ 0.02 ^b

* x: 0.2 g fish feed, y: 0.4 g fish feed, z: 0.6 g fish feed. Different superscript letters in a column indicate significant differences between groups ($P < 0.05$)

Fig. 5 Temporal changes in TAN removal efficiency (%) between treatment groups x, y, and z according to 6 different measurements

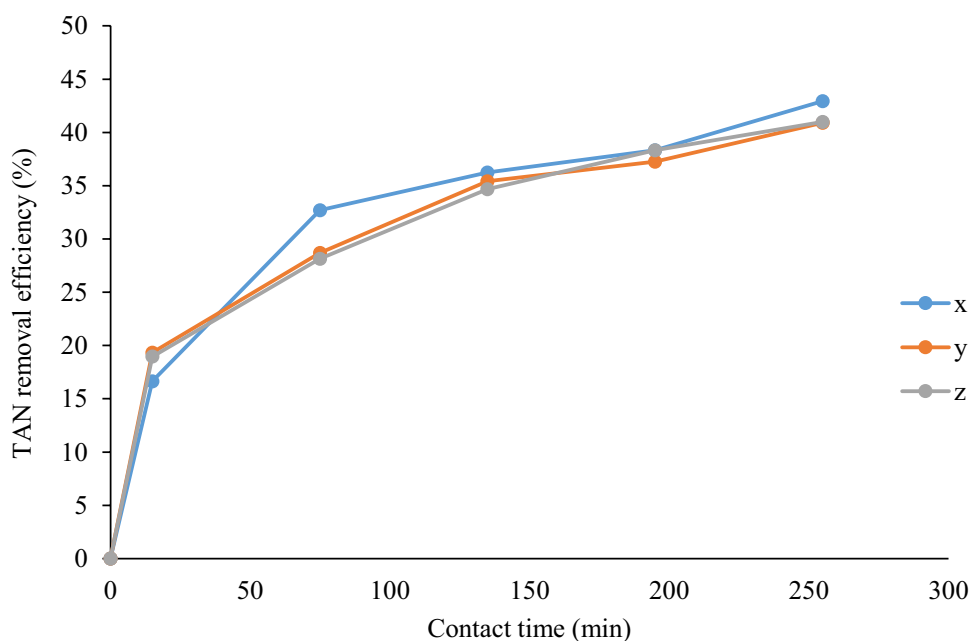
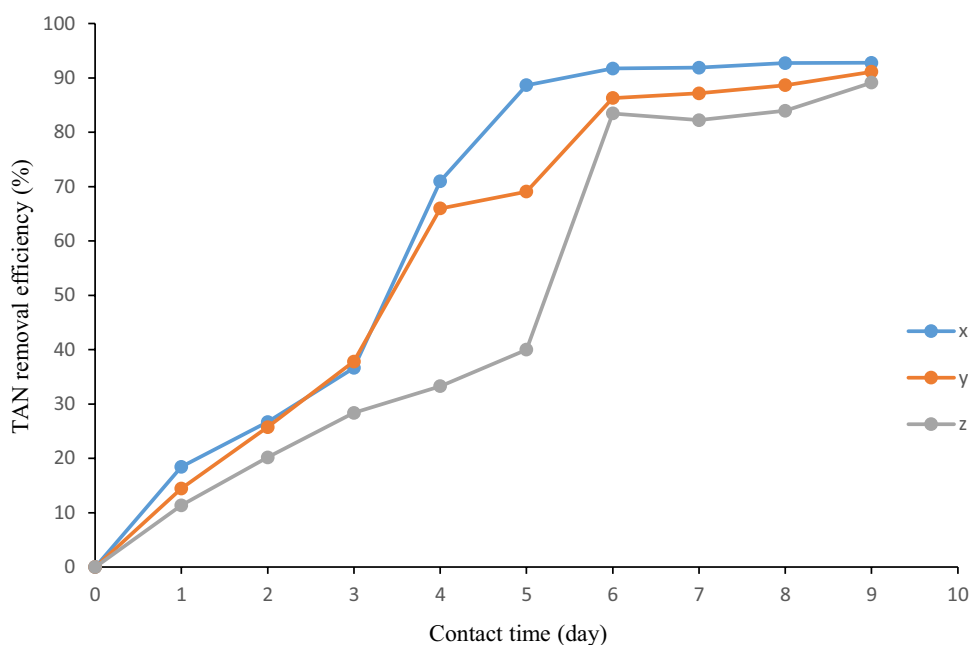


Table 3 Values of selected water quality parameters (Temperature, TAN, NH_4^+ , pH, and DO (dissolved O_2)) in treatment groups of experiment III (mean $\pm$ SE)

Experimental groups*	Water parameters				
	Temperature ($^{\circ}\text{C}$)	TAN (mg/L)	NH_4^+ (mg/L)	pH	DO (mg/L)
x	19.73 ± 0.19	1.37 ± 0.20^a	1.04 ± 0.19^a	7.59 ± 0.02^a	0.38 ± 0.01
y	19.88 ± 0.21	1.90 ± 0.24^a	1.50 ± 0.23^a	7.53 ± 0.02^{ab}	0.37 ± 0.01
z	20.21 ± 0.25	2.97 ± 0.38^b	2.48 ± 0.39^b	7.51 ± 0.02^b	0.36 ± 0.01

* x: 0.2 g fish feed, y: 0.4 g fish feed, z: 0.6 g fish feed, Different superscript letters in a column indicate significant differences between groups ($P < 0.05$)

Fig. 6 Temporal changes in TAN removal efficiency (%) between treatment groups x, y, and z according to 9 different measurements



Discussion

In fish culture systems, it is known that the main wastes are excessive fish feed and feces. It has been reported by various studies that amount of wastes resulting from feces and unconsumed feed in intensive culture is about 10–30% (Daudaa et al. 2019; Kibria et al. 1997). In this study, zeolite and leonardite at high ammonium concentrations as well as duckweed at low ammonium concentrations were used to balance high ammonium levels in the water. At the end of the 1st experiment, NH_4^+ values which resulted from the feed containing 35% protein for 7 days at mean temperature of 20.3 ± 0.22 °C and pH of 7.95 ± 0.03 reached 3.53, 5.40, and 6.13 mg/L in the experimental groups x, y, and z, respectively. Similarly, Kibria et al. (1997) determined that the amount of NH_4^+ was approximately over 20 mg/L at 20 °C and 7.0 pH when a feed containing 45% protein was used for 7 days.

The majority of nitrogen in anaerobic wastewater exists in the form of ammonium (NH_4^+). This is an advantage because duckweed has a preferential ammonium uptake over other nitrogen forms (Porath and Pollock 1982). The same phenomenon is also true for natural adsorbent such as zeolite. The clinoptilolite type zeolite used in the present study can also effectively adsorb the NH_4^+ in water. The clinoptilolite type zeolite used in this study can also adsorb NH_4^+ in water effectively, and this study was conducted by Öz et al. (2016), and Şahin et al. (2019) show similarities with their studies. In their studies, Chammui et al. (2014) and Terdputtakun et al. (2017) determined that leonardite is an advantageous substance in the removal of undesired

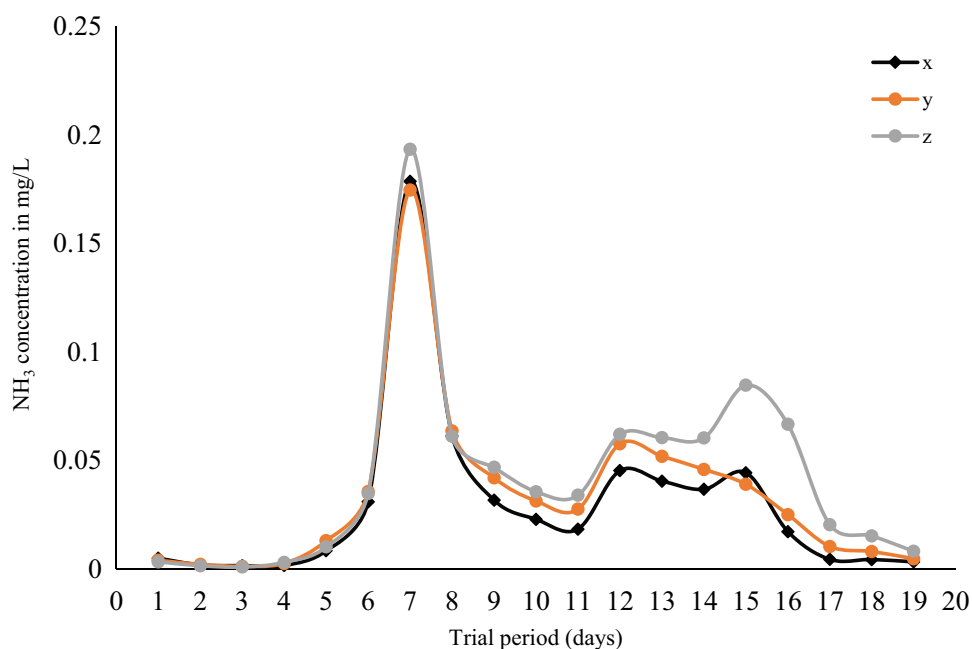
compounds (Ammonia, Cd, Zn) in aquatic environments. Moreover, a similar study conducted by Zengin (2013) reported that the use of mixture of clinoptilolite and leonardite is effective on removal zinc.

The response of duckweed to ammonium and ammonia levels has been extensively reported (Selvarani et al. 2015; Zhang et al. 2014; Arias et al. 2016). In the present study, in line with the existing evidence, it was found that *Lemna minor* is effective in the removal of excessive ammonium in water.

Nitrogenous compounds are essential for the continuity of living organisms because nitrogen is one of the fundamental building blocks of life (Şahin et al. 2019). Among the two forms of ammonia in water (NH_3 and NH_4^+), NH_3 displays high toxicity. Common concentrations of NH_4^+ in aquaculture systems range 1–5 mg/L. In the present study, it was observed that the highest ammonia value originating from the feed reached 0.19 mg/L at the end of the 1st experiment in group z, which received the highest feed amount for 7 days. This value decreased below the critical limit for aquaculture with the addition of adsorbents in the 2nd experiment and was determined to be 0.05, 0.06, and 0.06 mg/L for the groups x, y, and z, respectively. At the end of the 3rd experiment, NH_3 values went down to 0.00 mg/L.

While there was a decrease in the oxygen concentration in group z, which received the highest amount of feed, from the beginning of the experiment to the end of the 2nd experiment, it was determined that the oxygen concentration in this group increased in the 3rd experiment after duckweed addition. Sengupta et al. (2010) also stated that there was an increase in oxygen in the *Lemna* group. Aquatic plants contribute positively to oxygen levels through photosynthesis.

Fig. 7 Temporal changes in ammonia (NH_3) concentration (mg/L) during experiment III



Similarly, Shin et al. (2011) determined that aquatic plants contributed positively to oxygen levels in the aquarium.

Kibria et al. (1997) detailed the fractional composition of nutrients as well as the factors associated with the release of nutrients from fish feed and feces. This information is highly important for the planning of nutrient management and controlling pollution. In the present study, zeolite, leonardite, and duckweed, which have the potential to be used as both filtration materials (Zengin 2013; Öz et al. 2021, Şahin et al. 2018; Selvarani et al. 2015) and feed additives (Kanyılmaz et al. 2015; Şahin et al. 2010; Turan and Turgut 2020), were used. Zengin (2013) found that leonardite and clinoptilolite mixture has a positive effect on reducing the amount of Zn from an aqueous solution, Öz et al. (2021) and Şahin et al. (2018) determined that clinoptilolite is a good filtration material in aquariums. Selvarani et al. (2015) also found that duckweed was effective in removing nitrogen compounds in the environment. Kanyılmaz et al. (2015) added zeolite, Şahin et al. (2010) added duckweed, and Turan and Turgut (2020) added leonardite in aquarium fish feed. The potential use of a low-cost, natural adsorbent mixture composed of Turkish leonardite and clinoptilolite as well as duckweed was investigated for the removal of NH_4^+ and demonstrated under different experimental conditions for aquaculture such as different concentrations of fish feed, mixing ratio (C:L, 1:2), contact time, pH (7.4–7.6), and adsorbent amount (1 g C, 2 g L, 1.5 g Duckweed).

Conclusion

In this study, it was determined that in high concentrations of zeolite + leonardite mixture and in low concentration of duckweed there had been a good removal efficiency. As a result of the present study, it was determined that remediation of nitrogenous wastes is possible using natural materials such as zeolite, leonardite, and duckweed in aquaculture. In general, when the effects of the combined use of natural adsorbents were evaluated at the end of the research, it was determined that the use of these materials has no harmful effect and the water parameters are within the optimum limits for aquaculture practices. This study will contribute to the removal of undesirable nutrient compounds with natural materials in both closed-circuit systems and other aquaculture systems.

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Author contribution Dilek Şahin designed, performed the experiment and data analysis, data collection and wrote the work.

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The author declares no competing interests.

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