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RESEARCH

Strategy to remove ammonium compounds released from fish feed in aquaculture using natural filtration materials

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Abstract The effects of addition of adsorbent mixture at two different stages in aquaculture system were investigated for the first time in this study. In the first stage, in order to determine the effects of natural adsorbents in an environment without feed, a trial consisting of four treatment groups with three replications was conducted. In this trial, individual and combined effects of two natural adsorbents on water parameters in absence of feed were assessed for five days. In the second stage for 9 experimental days, a total of 18 aquaria consisting of three treatment groups with three replications for two different fish feeds were used. Of these aquaria, the first six received only two types of feeds containing 33 and 40% protein, designated as two control groups (C1 and C2). Other two groups (T1 and T2) were prepared by adding a leonardite: zeolite mixture (at 2:1 ratio) to next six replicates simultaneously. The last six replicates received a leonardite: zeolite mixture (at 2:1 ratio) after the 8th day of the study, and formed the last two treatment groups (T3 and T4). In this study, mean pH values varied between 7.01 and 7.82 and ammonia values were found to be maximum of 94.96% and minimum of 38.73% lower compared to the control group when 3 g adsorbent mixture (2L: 1Z) was used to balance pH and ammonium (NH_4^+) values in an aquatic environment containing 0.5 l freshwater and 0.5 g fish feed with 33–40% protein contents. It was demonstrated that the combined use of zeolite and leonardite had positive effects on ammonium removal and providing optimum pH levels for aquaculture. The combined use of these two adsorbents helped balance the pH-reducing effect of leonardite with zeolite, and the pH-reducing effect of leonardite contributed to the NH_4^+ adsorption efficiency of zeolite.

Keywords Leonardite · Zeolite · Ammonia toxicity · Adsorbent · Freshwater · Aquaculture

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Introduction

Water is a natural resource that is essential for all living creatures. In aquaculture, it is necessary to provide and maintain suitable water quality for target species. Sevaral studies have been conducted to develop the most effective methods ideal for aquaculture, and efforts are continuing on this direction. Maintaining good water quality not only directly affects the success of aquaculture but also contributes to the conservation of natural water resources and the environment by improving the quality of wastewater. In aquaculture, some physical and chemical characteristics of water, such as temperature, pH, dissolved oxygen, and nitrogenous compounds must be maintained at levels specific to the species under culture

(Öz et al., 2017; Munguti et al., 2021; Basyuni et al., 2023; Şahin et al., 2023).

The physico-chemical characteristics of water vary greatly depending on many factors. For example, water temperature can affect dissolved oxygen values. Moreover, the pH of water affect the ammonium/ammonia ratio. Since the degree of ammonia toxicity is related to pH, monitoring pH and ammonia is essential in an aquatic environment. The higher the pH level, the greater the toxicity of ammonia is (Ogbonna & Chinomso, 2010; Shalaby et al., 2021; Vajargah & Yalsuyi, 2022).

Furthermore, the physico-chemical parameters of water are also influenced by other compounds present therein. For example, metabolic wastes of fish, plants, and invertebrates, compounds from unconsumed feed, or substrate materials in contact with water may have an impact on the physico-chemical properties of water. Additionally, the quality of feed used in aquaculture systems and the feeding methods are the most critical factors affecting water quality (Ogbonna & Chinomso, 2010; Sirakov et al., 2015; Kibria et al., 1997).

By taking advantage of these dynamic properties of water, different approaches can be applied to maintain water parameters in accordance with the biological requirements of the species under culture. These approaches are categorized as physical (mechanical), chemical, and biological methods. Applications such as aeration, replacement, heating/cooling of water or use of physical (mechanical)/ chemical/ biological filtration materials are the most commonly used techniques (Lebedynets et al., 2004; Prema Latha & Lipton, 2007; Wang et al., 2015; Basyuni et al., 2023).

Among these techniques, use of natural adsorbents to control nitrogenous compounds, which exert toxic effects on aquatic organisms exposed to high concentrations, has several advantages (Öz et al., 2010; Farhangi et al., 2014; Abdel Rahim, 2017; Sava et al., 2019; Şahin et al., 2019; Öz et al., 2021; Shalaby et al., 2021; Öz et al., 2022; Şahin, 2023). These advantages include their natural origin, effectiveness, non-toxicity, economic availability, easy to process, reusability, and a prolonged lifespan (Ghiasi & Jasour, 2012; Lebedynets et al., 2004; Öz et al., 2010; Sava et al., 2019). However, the effectiveness of these natural minerals is affected by several factors, including their region of mining originand the environment where they will be used. Water, aquatic organisms, nutrient components, and adsorbent substances in aquaculture system can alter the aquatic balance due to their interactive effects. Therefore, it is of utmost importance to conduct the research on the use of adsorbent materials in aquaculture environments and demonstrate their properties suitable for the environment where they will be utilised.

There exists the only study on removal of ammonium compounds using a mixture of zeolite and leonardite in aquaculture systems (Şahin, 2022). In the current study, unlike the previous study (Şahin, 2022), the individual and combined effects of adsorbent substances, as well as their use in two different culture environments provided with feed and without feed, and different addition stages were studied. It was aimed to determine the possible combined effetcs of zeolite, which has been reported to be used in many fields as a natural adsorbent, with leonardite, for which there is no sufficient research yet on its use in aquaculture, and to reduce the ammonia concentrations in aquatic environment.

Materials and Methods

Crude materials used in the study

Leonardite is a natural organic material formed through a decomposition process over a billion years. It is a concentrated form of humic and fulvic acids used in agricultural production and found in soft, coal-like deposits formed at shallow depths (Fig. 1a) (Moreno et al., 2017; Ratanaprommanee et al., 2016).

Zeolite is a microporous crystalline hydrated aluminosilicate used in various applications due to their unique physico-chemical features, such as ionic exchange and adsorption–desorption properties (Fig. 1b) (Ghasemi et al., 2018; Moreno et al., 2017; Shalaby et al., 2021).

Experimental design

This study was conducted in two stages at Sinop University, Faculty of Fisheries. In the first stage, a trial setup consisting of four treatment groups with three replications was conducted to determine the effects of natural adsorbents in absence of feed. After filling all aquaria with 500 ml of tap water, Group 1 without any adsorbent was designated

Fig. 1 Leonardite (**a**) and zeolite (**b**)

as the control group (C). LZ, L, and Z treatment groups were made by adding 3 g of leonardite: zeolite mixture (2:1, L:Z), 3 g of leonardite, and 3 g of zeolite, respectively. In the first stage, the effects of natural adsorbents individually and in combination on water quality parameters in an environment without feed was evaluated for five days.

In the second experiment, commercial fish feeds having two different protein contents (33 and 40%) were used at a rate of 0.5 g feed per 500 ml tap water (Kibria et al., 1997). The experiment was conducted in three treatment groups with three replications for each feed in a total of 18 aquaria. At the beginning of the experiment, feeds containing protein at two different levels were added to all aquaria. Two different control groups (C1 and C2) were kept by adding only feeds containing 33 and 40% protein levels to the first six replicates aquaria. T1 and T2 groups were formed with the other six replicates by adding a 2:1 ratio leonardite: zeolite mixture in addition to the feed added at the beginning of the experiment (Şahin, 2022). The last six replicates were used to assign as the T3 and T4 groups, which received a 2:1 ratio leonardite: zeolite mixture after the 8th day of the experiment. Water quality parameters were measured daily for the first eight days. After these water parameter analyses, the adsorbent mix was added to the T3 and T4 groups, and the study was completed after assessing the water parameters once again on the 9th day.

The adsorbents used in this study were obtained from two commercial companies in powder form (100 μ). Before use, they were sieved, washed with tap water, and dried at 105 °C (Wang et al., 2015).

Zeolite sample was obtained from Rota Mining Corporation (Manisa, Türkiye), whereas leonardite was provided by Kütahya Chemistry (Kütahya, Türkiye).

Determination of water parameters

Temperature, pH, and ammonium nitrogen (NH_4^+-N) values were determined using a multiparameter quipment (YSI Professional Plus). Ammonia (NH_3) and total ammonia nitrogen (TAN) values were calculated using these values (Chow et al., 1997; EPA, 1999; Emerson et al., 1975; Jorgensen, 2002). It has been depicted that the Nessler method and conventional electrode method yielded similar results (Prajapati, 2014). Ammonia and TAN values were calculated using the following equations (Purwono et al., 2017):

$$pK(NH_3) = 2726.3273 + {}^\circ C + 0.0963 \tag{1}$$

$$NH_3 - N = 10(pH - pK(NH_3)) \times NH_4^+ - N \tag{2}$$

$$TAN = NH_3 - N + NH_4^+ - N \quad (3)$$

SEM and XRF analyses were conducted at the Central Research Laboratory, Kastamonu University, Türkiye. The pH was computed according to Tokat (2019). The SiO_2/Al_2O_3 ratio was calculated as per the method of Chuan-hsia (2000).

Statistical analysis

Means of all the parameters assessed were compared with one-way analysis of variance (One-way ANOVA), and differences in groups were determined with Tukey's HDS test. The confidence interval was set to 95%. All data are presented as mean ± standard error (SE). Minitab Statistical Software for Windows (release 17) was used for statistical analyses.

Results

In this study, the effects of two natural adsorbents, zeolite and leonardite on the removal of ammonia which was produced from fish feeds with two different protein contents were examined in freshwater environment. The effects of addition of adsorbent mixtures at two different stages in aquaculture were investigated for the first time in this study.

Morphology and crystalline structure of zeolite and leonardite

The XRF (X-ray fluorescence spectrometry) and BET (Surface area measurement instrument- multipoint) analyses of leonardite and zeolite tested in the study are presented in Table 1. Analyses were carried out at Kastamonu University Central Research Laboratory. XRF analyses were performed using a Spectro brand Xepos II model device, and BET analyzes were conducted with a Quantachrome brand Nova Touch LX4 model instrument. SEM (Scanning Electron Microscopy) analyzes were performed using the FEI brand Quanta FEG 250 model device available at Kastamonu University Central Research Laboratory.

The pH was calculated according to Tokat (2019). The pH values of zeolite and leonardite were 8.81 ± 0.02 and 4.31 ± 0.01, respectively.

The zeolite and leonardite used in the study were characterised by SEM imaging and EDS analysis (Fig. 2).

Effects of zeolite and leonardite on water parameters

Water temperature, pH, and ammonium values at the beginning of the study were 23.5 ± 0.01℃, 8.15 ± 0.02, and 0.2 ± 0.01 mg/l, respectively. There

Table 1 Major and trace element contents of zeolite and leonardite determined by XRF analysis

The major oxides of the zeolite* and leonardite**											
Oxides	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	CaO	MgO	MnO	P_2O_5	K_2O	Na_2O	SiO_2/Al_2O_3
*Mass %	78.41	13.83	1.41	1.05	3.88	1.65	0.29	0.58	2.37	1.04	5.67
**Mass %	13.68	7.07	1.24	1.25	0.32	0.11	0.027	0.054	0.045	< 0.014	1.93

Important elements of zeolite* and leonardite** having role in aquaculture		
Elements	ppm*	ppm**
Na	7730	< 101
Mg	9927	643
Al	73200	37420
Si	366500	63960
K	19690	3765
Ca	27770	2306
Mn	222.3	21.1
Fe	9889	8662
P	254	237
Se	0.4	3.5
Spesific Surface Area (BET, m^2/g)	34.3162	12.253

*Zeolite, **Leonardite

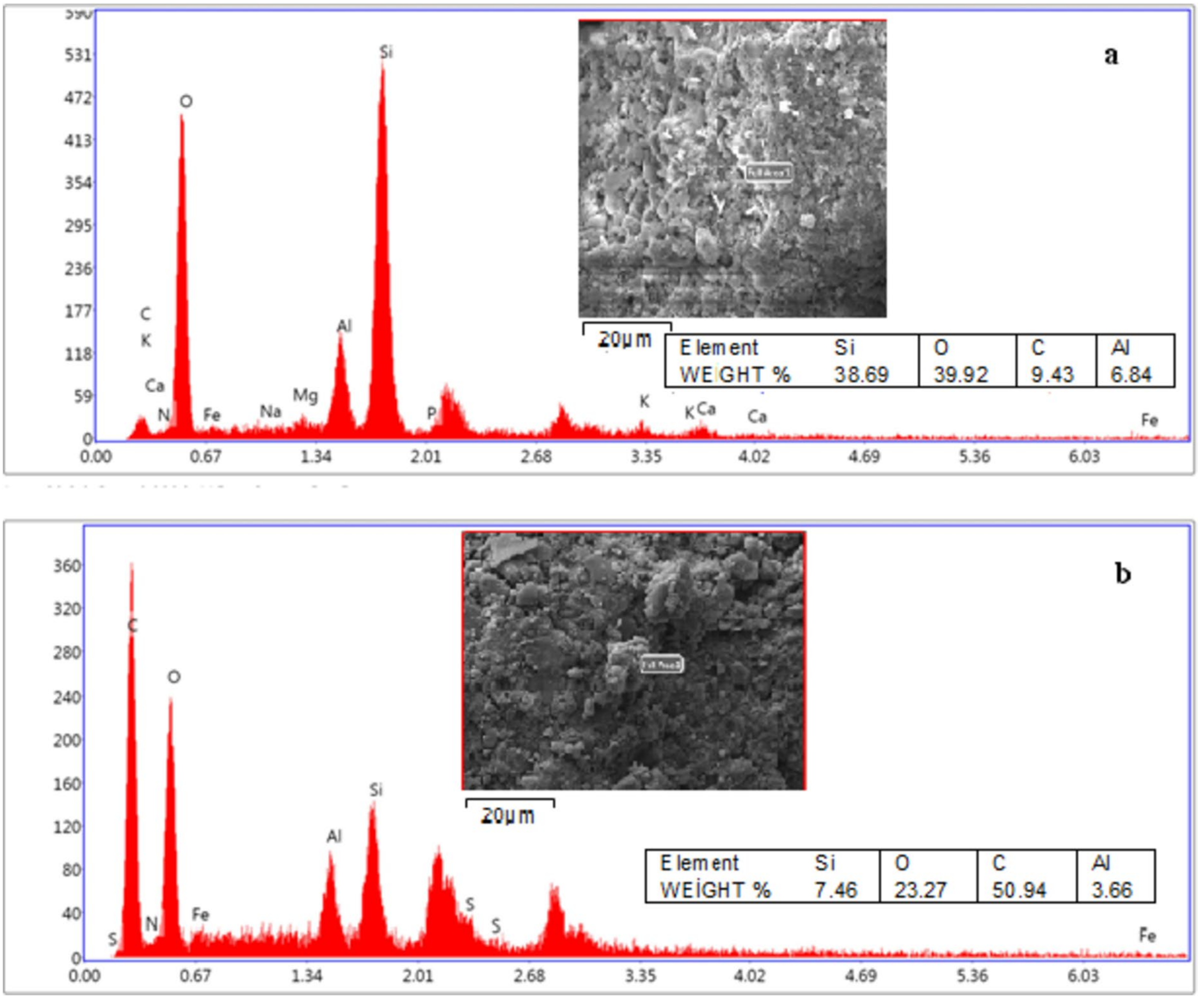

Fig. 2 SEM images and EDS analyses of zeolite (**a**) and leonardite (**b**)

were no differences in the values of these parameters among different groups ($P > 0.05$).

Table 2 displays the water parameters determined at the end of the first stage. At the end of this trial, the differences between pH, TAN, and NH_4^+ values were statistically significant between experimental groups ($P < 0.05$). However, there was no significant difference in terms of water temperature values ($P > 0.05$).

Table 2 Temperature, NH_4^+-N, TAN, and pH values in experimental groups of the first stage of the study for five days

hba	Water temperature (°C)	TAN (mg/l)	NH_4^+-N (mg/l)	pH
LZ	21.43 ± 0.18	0.32 ± 0.01^{c}	0.32 ± 0.01^{ab}	7.52 ± 0.04^{b}
L	21.39 ± 0.19	0.21 ± 0.01^{d}	0.21 ± 0.01^{c}	7.35 ± 0.04^{ca}
Z	21.51 ± 0.21	0.49 ± 0.03^{a}	0.36 ± 0.02^{ab}	8.77 ± 0.01^{a}
C	21.55 ± 0.20	0.39 ± 0.01^{b}	0.29 ± 0.03^{b}	8.65 ± 0.02^{a}

* LZ (2 g L:1 g Z), L (3 g), Z (3 g), C (control). Different superscript letters in a column indicate significant differences between groups ($P < 0.05$); L: leonardite, Z: zeolite

Water parameters determined at the end of second phase of the study are provided in Table 3. Similar to the first stage, NH_4^+, TAN, and pH values differed between groups ($P < 0.05$), while water temperature values were similar ($P > 0.05$).

NH_4^+-N values on the last day of the experiment (9th measurement) were found as 14.69 ± 0.94, 14.77 ± 0.33, 0.74 ± 0.06, 0.99 ± 0.17, 9.00 ± 0.26, and 8.04 ± 0.25 in C1, C2, T1, T2, T3, and T4 groups, respectively. At the end of experiment, ammonium reduction rates in all groups were determined to be 94.96, 93.3, 38.73, and 45.57% in T1, T2, T3, and T4, respectively. NH_3 values are shown in Fig. 3.

Discussion

Ammonia, which contains nitrogenous compounds, is directly or indirectly toxic to many aquatic animals when it exceeds certain threshold levels. Ammonia and other toxic metabolites in water can be ameliorated by filtration methods (Mechanical, chemical and biological filtrations) (Prema Latha & Lipton, 2007; El-Gendy et al., 2015; Shalaby et al., 2021).

TAN consists of toxic (un-ionized) NH_3 and non-toxic (ionised) NH_4^+. Only a small portion of TAN exists as toxic ammonia, which is in equilibrium with non-toxic ionised ammonium. When ammonia

Table 3 Temperature, NH_4^+-N, TAN, and pH values in experimental groups of the second stage of the study for nine days

Experimental groups*	Water temperature (°C)	TAN (mg/l)	NH_4^+-N (mg/l)	pH
C1	20.47 ± 0.21	5.37 ± 1.15^a	5.17 ± 1.11^a	7.79 ± 0.08^a
C2	20.44 ± 0.20	5.23 ± 1.02^a	5.02 ± 0.97^a	7.82 ± 0.07^a
T1	20.44 ± 0.21	0.76 ± 0.05^b	0.75 ± 0.05^b	7.01 ± 0.06^b
T2	20.45 ± 0.21	0.77 ± 0.04^b	0.76 ± 0.04^b	7.07 ± 0.05^b
T3	20.45 ± 0.20	5.36 ± 0.99^a	5.22 ± 0.98^a	7.71 ± 0.07^a
T4	20.46 ± 0.20	4.40 ± 0.79^a	4.31 ± 0.77^a	7.66 ± 0.07^a

*C1: Group received 33% crude protein feed, C2: Group received 40% crude protein feed, T1: Group received 33% crude protein feed + 3 g of 2:1 L:Z adsorbent, T2: Group received 40% crude protein feed + 3 g of 2:1 L:Z adsorbent, T3: Group received 33% crude protein feed and 3 g of 2:1 L:Z adsorbent after the 8th day of the experiment, T4: Group received 40% crude protein feed and 3 g of 2:1 L:Z adsorbent after the 8th day of the experiment. Different superscript letters in a column indicate significant differences between groups ($P < 0.05$)

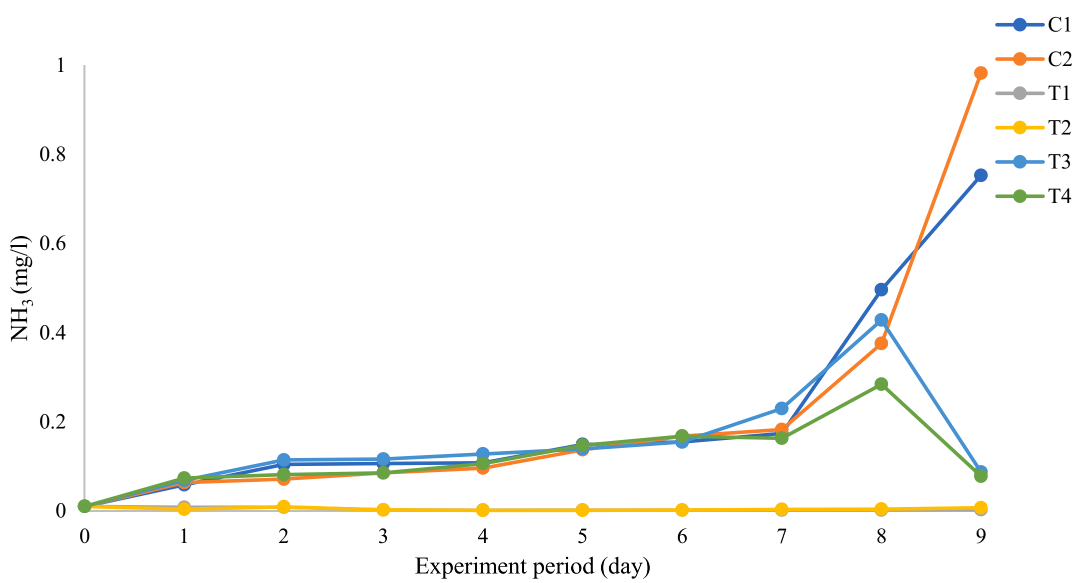

Fig. 3 Changes in ammonia (NH_3) concentrations (mg/l) throughout the experimental period of 9 days

dissolves in water, it partially ionises depending on pH and temperature. Ionised ammonia is called ammonium and non-toxic to fish. As pH and temperature decrease, ionisation and ammonium increase, and this reduces toxicity (Chow et al., 1997; Ogbonna & Chinomso, 2010).

The results obtained in this study were discussed under three headings.

1) Analytical values of zeolite and leonardite and their potential use in aquaculture
2) Effects of adsorbent substances on water parameters in absence of feed
3) Ammonium contents resulting from two different feeds and the effects of adsorbents on them

Analytical values of zeolite and leonardite and their potential use in aquaculture The analytical values obtained in this study (macro and micronutrients, elements, pH, surface area, Si/Al ratio, etc.) were found to be similar to the previous studies conducted with these adsorbents (Yolcu et al., 2011; Zendelska & Golomeova, 2014; Zengin, 2013; Ratanaprommanee et al., 2016; Rittirat et al., 2018; Şahin, 2023; Velarde et al., 2023). Any differences in the valuses between studies may be due to different conditions, the method used or region from where the adsorbents were mined (Ratanaprommanee et al., 2016).

Calcium, sodium, chloride, potassium, chlorine, sulfur, phosphorus, and magnesium are common dietary macrominerals for animals. These minerals support the development and integrity of bones and regulate osmotic balance. Iron, copper, chromium, iodine, manganese, zinc, and selenium, on the other hand, are common microminerals. Enzyme and endocrine systems require trace minerals in small amounts (Hardy & Kaushik, 2021). Nutrients, such as Na, Ca, Mg, P, and Se present in the structure of zeolite and leonardite are elements that are necessary for the metabolic activities of aquatic animals. Ratanaprommanaee et al. (2016) found that large amounts of macronutrients (N, P, and K) and micronutrients, especially trace elements, necessary for plant growth, are in the molecular structure of leonardite, but they also reported that it can reduce pH values. Similarly, Rittirat et al. (2018) determined that pH of leonardite in water was 2.85 and it affected the ion exchange capacity depending on this low pH property. Since pH values suitable for aquaculture should be in the range of 7.5–8.5, it is thought that pH values can be maintained in the suitable range using a mixture of zeolite and leonardite, similar to the observation of Szatanik-Kloc et al. (2021). Suitable pH values according to the doses used in this study [500 ml tap water, 3 g adsorbent mixture (2L: 1Z), and 0.5 g fish feed] were obtained, and these doses were determined to be the recommended values.

EDS spectra of the adsorbents showed that zeolite and leonardite are structurally different. Leonardite contains a high carbon content, similar to the structure of activated carbon (Deshmukh et al., 2018; Jitjamnong et al., 2020). It is thought that the decrease in ammonia concentration by leonardite is due to its carbon content, which has a structure similar to the activated carbon. Zeolite, on the other hand, has an effective property of ammonium removal due to its structure, Al/Si ratio and cation content. In addition, in term of pH, leonardite is acidic and zeolite is slightly basic, and their combined use can balance the pH value.

Effects of adsorbent substances on water parameters in absence of feed In the 5-day trial at the first phase of the study, NH_4^+ values in LZ, L, Z, and C groups were found to be 0.32 ± 0.01, 0.21 ± 0.01, 0.36 ± 0.02 and 0.29 ± 0.03 mg/l, respectively. It was observed that especially, the application of leonardite effectively reduces pH level in freshwater. Moreno et al. (2017) and Sanli et al. (2013) found that the mixture of zeolite and leonardite increases the P and K levels, as well as inorganic N forms (nitrate and ammonium) in the soil. In this study, NH_4^+ values varied between 0.21 and 0.36 mg/l. This could be due to the structural features of natural adsorbents. However, it is at very low values and within the suitable limits for aquaculture.

In this experiment, pH values in LZ, L, Z, and C groups were determined as 7.52 ± 0.04, 7.35 ± 0.04, 8.77 ± 0.01, and 8.65 ± 0.02, respectively. The differences in results indicated that leonardite and zeolite had an influence on pH values, similar to the observations by Ratanaprommanaee et al. (2016) and Öz and Aral (2023).

Ammonium contents resulting from two different feeds and the effects of adsorbents on them At the end of the second phase of 9-day trial, the mean ammonium values in groups C1 and C2, which contained 0.5 l of freshwater and 0.5 g of fish feed, were found to be 14.69 ± 0.94 and 14.77 ± 0.33 mg/l, respectively. Feeds with two different protein contents used in the experiment (33 and 40%) caused similar levels of ammonium release to the water. Similar to the observations of Şahin (2022) and Şahin (2023), ammonium release is thought to be related to the nutrient contents of feed and structure of the nutrients released from them rather than the protein content in the feed. Likewise, Hua and Bureau (2006) reported that the nutrients released from different types of fish feeds are expected to vary.

Similar to all aquatic organisms, optimum water conditions must be provided to fish also for their growth and reproduction. This optimum aquatic environment can be achieved by maintaining the desiarble water quality parameters. Ammonia level is a very critical parameter in fish farming. Routine monitoring of important parameters is necessary due to the dynamic nature of water quality (Chow et al., 1997). For many fish species, unionised ammonia (NH_3) toxicity begins even at a very low level of 0.05 mg/l and leads to mortality at 2 mg/l (Floyd et al., 2022). When the mean NH_3 values recorded in this study were examined, it was evident that the levels in the adsorbent groups were lower than in the control groups. Ammonium reduction rates in T1, T2, T3, and T4 groups were found to be 94.96, 93.3, 38.73, and 45.57%, respectively. The use of adsorbent together with feed at the beginning (T1 and T2 groups) exhibited a very effective removal efficiency compared to the addition of adsorbent at later stages (T3 and T4 groups), i.e., when ammonia values already increased. In the line with these results, it was reported that the use of a clinoptilolite-leonardite mixture was effective in removal of zinc and ammonium (Zengin, 2013; Şahin, 2022, 2023). Furthermore, Şahin et al. (2019) tested different doses and particle sizes of zeolite and found that its favourable efficiency (44.1%) was achieved when 0.075 mm zeolite was applied at 15 g/l. Our results demonstrated that zeolite/leonardite could be used effectively for pH balancing and ammonia removal in aquaculture.

In this study, it was demonstrated that the combined use of zeolite and leonardite had positive effects on ammonium removal and maintaining optimum pH levels for aquaculture. Zeolite is slightly basic due to its intrinsic structure, while leonardite is considerably acidic. The combined use of these two adsorbents supported balancing the pH-reducing effect of leonardite with zeolite, while the pH-reducing effect of leonardite contributed to the NH_4^+ adsorption efficiency of zeolite. Similar to this study, Kibria et al. (1997) reported that phosphorus release increased with decreasing pH, while ammonium release increased with increasing pH. Moreover, Kithome et al. (1998) found that as the pH increases (in the range of 4–7), the ammonium retention capacity of zeolite increases.

Conclusion

In this study, it was evident that the mixture of leonardite and zeolite had a positive effect on ammonium removal. In freshwater aquaculture systems, when a total of 3 g (2L: 1Z ratio) natural adsorbent mixture was used to balance NH_4^+ and pH in an environment containing 0.5 l freshwater and 0.5 g fish feed (33–40% crude protein), the mean pH values ranged between 7.01 and 7.82, and ammonia values were 94.96–38.73% less than those in the control group. Therefore, it was considered appropriate to add adsorbent mixture as an effective method that achieved the highest removal efficiency, together with the feed and when ammonium values are low. This application is also suitable for use both as filter and ground materials in a recirculatory system such as aquaria, where water exchange is very low. Addition of adsorbent mixture could be employed when ammonia concentrations are high in wastewater, and thus aquaculture discharge waters could be free from excessive amount of nutrients.

It is concluded that addition of the adsorbent mixture (zeolite: leonardite) at the start could remove approximately 93–95% ammonium compounds and it will have direct application in aquaculture environment.

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Authors' contributions Meryem Öz designed, performed the experiment and data analysis, data collection and wrote the article.

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

Declarations

Ethics approval Author has read, understood, and has complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

Competing interests The authors declare no competing interests.

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