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Application and performance of bentonite-leonardite mixture for feed-derived nitrogen removal in freshwater aquaculture systems

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Abstract

Ammonia accumulation is a major constraint in freshwater aquaculture systems with limited water exchange, particularly before biological filtration becomes fully effective. Therefore, low-cost physicochemical buffering strategies are needed to control total ammonia nitrogen (TAN) under practical production conditions. To address this issue, the present study evaluated the combined effects of natural bentonite and leonardite on ammonia removal and pH regulation in aquaculture systems. The experiment was conducted in a controlled freshwater batch system without live fish, where ammonia was generated from a single addition of commercial fish feed and consisted of eight treatment groups (B: 3 g bentonite, BL1: 2.5 g bentonite + 0.5 g leonardite, BL2: 2 g B + 1 g L, BL3: 1.5 g B + 1.5 g L, BL4: 1 g B + 2 g L, BL5: 0.5 g B + 2.5 g L, L: 3 g L, C: Control (no adsorbent)), each conducted in triplicate. Commercial fish feed containing 47.5% crude protein served as the ammonia source for all experimental setups. Ammonia levels generated from uneaten feed residues in the aquaria were monitored over a 12-day period.

In this study, pH values decreased as the amount of leonardite increased. Considering the unionized ammonia (NH₃) limit values for fish farming, the limit values (0.05 mg L⁻¹) were reached in the control group on the second day of the study. This value was similar in groups BL1, BL2, BL3, and L on day 8, and in groups BL4 and BL5 on day 9. The maximum NH₄⁺ concentration in the control group reached 45.4 mg L⁻¹, whereas the highest removal performance was observed in the BL5 treatment (0.5 g bentonite + 2.5 g leonardite), achieving 87.7% removal and an apparent adsorption capacity of 6.64 mg g⁻¹. Increasing leonardite proportion resulted in progressive enhancement of ammonium removal and a concomitant decrease in pH, which shifted the NH₃/NH₄⁺ equilibrium toward the ionized form (NH₄⁺).

The findings of this study contribute to the gap in the literature by presenting the first scientifically original data, showing that the use of leonardite, an economical and natural

carbon source, together with the clay mineral bentonite has a positive effect on ammonium ion removal in freshwater aquaculture systems.

Keywords: Bentonite, Leonardite, Ammonium, Adsorption, Aquaculture

Introduction

Water quality parameters encompass the physicochemical and biological attributes of an aquatic ecosystem that are essential for maintaining the physiological and ecological functions of fish, including feeding, reproduction, metabolism, growth, and excretion. Among these parameters, variables such as water pH, alkalinity, temperature, hardness, turbidity, CO₂ (Carbon dioxide) concentration, NH₃ (unionized ammonia), NO₂ (nitrite), NO₃ (nitrate), dissolved oxygen, primary productivity, biochemical oxygen demand (BOD), and plankton abundance constitute key determinants that exert direct and measurable influences on fish health, survival, and overall production dynamics¹. The water quality must fall within specific limits that allow all living organisms to function optimally. Within these parameters, rapid fluctuations in physicochemical parameters can negatively impact fish physiology, immune response, and survival². Therefore, maintaining stable environmental conditions is essential for sustainable aquaculture production³. In aquaculture systems, ammonia accumulation commonly occurs due to high feeding rates, limited water exchange, incomplete nitrification, and microbial decomposition of organic residues^{4,5,6}.

Ammonia originates from protein metabolism excreted by fish as well as from the bacterial degradation of uneaten feed, feces, and other organic matter. In aquatic environments, ammonia exists in equilibrium between unionized ammonia (NH₃), which is highly toxic, and ionized ammonium (NH₄⁺), which is comparatively less harmful. These two forms together are referred to as total ammonia nitrogen (TAN)³. When unionized ammonia concentrations exceed approximately 0.05 mg L⁻¹, adverse physiological effects may occur in many freshwater fish species, while higher concentrations may lead to acute toxicity⁷.

Intensive freshwater aquaculture systems are increasingly challenged by nitrogen accumulation due to high feeding rates and limited water exchange^{4,5}. In such systems, total ammonia nitrogen (TAN) may rise rapidly, and the fraction of toxic un-ionized ammonia (NH₃) becomes critical depending on pH^{6,8}. Although biofiltration and nitrification represent long-term biological control strategies in recirculating aquaculture systems (RAS)⁹, physicochemical buffering remains important for immediate ammonia mitigation, particularly in static tanks and transport systems. Various materials have been investigated for ammonium removal from aquatic systems. Natural minerals such as zeolite, bentonite, and diatomite have demonstrated ammonium removal through ion-exchange mechanisms^{10,11}, while carbon-based materials such as biochar and leonardite exhibit adsorption capacity for nitrogenous compounds^{12,13}. Recently, composite adsorbents

combining clay minerals with carbonaceous materials have attracted attention because their complementary physicochemical properties may enhance adsorption performance¹⁴.

Leonardite, a by-product of lignite mining, is a particularly promising candidate. Generally, leonardite is a complex material primarily composed of carbon (55% by weight). It contains approximately 20-70% organic matter, and through the carbonization process, the organic components can be converted into activated carbon. Carbonization is a thermochemical process in which organic material is heated under limited oxygen conditions, leading to the formation of carbon-rich structures with enhanced porosity and adsorption capacity¹⁴. Leonardite is also recognized as a natural source of humic substances, which are characterized by their organic, complex structures, high molecular weight, and resistance to decomposition¹⁵. Leonardite is rich in humic substances containing carboxylic ($-\text{COOH}$) and phenolic ($-\text{OH}$) functional groups. These groups act as binding sites for ammonium ions (NH_4^+) through electrostatic attraction and surface complexation mechanisms. Additionally, the porous carbon matrix enhances diffusion and physical adsorption. Thus, humic acid fractions in leonardite serve as the primary nitrogen-binding sink¹⁶.

Bentonite is a hydrous aluminum silicate, a common group of clay minerals reported as low-cost materials. However, the low adsorption capacity of bentonite is the major problem for its possible application for denitrification; therefore, it is important to modify raw bentonite to determine whether its adsorption rate can be improved to suit a potential application for ammonia removal¹⁷. Bentonite consists primarily of montmorillonite, a layered aluminosilicate clay with high cation exchange capacity (CEC). Negative structural charges within its interlayer spaces allow exchangeable cations (Na^+ etc.) to be replaced by ammonium ions (NH_4^+). Thus, the montmorillonite structure acts as the primary ammonium sink via ion exchange mechanisms¹¹.

Bentonite is widely available, low-cost, and possesses high cation exchange capacity (CEC), making it suitable for ammonium ion retention through interlayer exchange mechanisms. Leonardite, as a carbon-rich humic material, contains functional groups capable of surface complexation and electrostatic adsorption¹⁶. The combination of these materials was hypothesized to create a dual-mechanism ammonium buffering system, integrating ion exchange stabilization with carbon-mediated adsorption and pH modulation.

The proposed bentonite-leonardite composite may be particularly relevant for low-technology or semi-intensive freshwater aquaculture systems where advanced biofiltration infrastructure is limited. Such systems are commonly found in parts of Southern Europe, Southeast Asia, and Sub-Saharan Africa¹. Potential applications include tilapia (*Oreochromis niloticus*), carp (*Cyprinus carpio*), and catfish (*Clarias* spp.) culture under static or low water-

exchange conditions, where ammonia accumulation represents a major production constraint⁹.

To date, no study has evaluated the combined application of bentonite and leonardite for ammonium mitigation under aquaculture-relevant nitrogen loading conditions. Although both materials have individually been reported as effective natural adsorbents, their combined application remains largely unexplored in aquaculture systems. The rationale for selecting a bentonite–leonardite mixture is based on their complementary physicochemical properties. Bentonite provides a high cation exchange capacity through its montmorillonite structure, while leonardite contributes humic substances with functional groups capable of surface complexation and pH modulation. This dual functionality was hypothesized to enhance ammonium buffering performance under dynamic nitrogen release conditions typical of aquaculture systems.

The experimental design was therefore structured to evaluate the intrinsic ammonium adsorption performance of bentonite and leonardite mixtures under controlled conditions. While real aquaculture systems involve complex biological processes, isolating physicochemical adsorption mechanisms provides fundamental baseline data prior to application in dynamic systems such as recirculating aquaculture systems or pond environments.

Materials and methods

Experimental design

The experimental design consisted of 8 groups with 3 replicates per group. Commercial fish feed containing 47.5% crude protein was added at a dose of 0.5 g per experimental unit in order to simulate organic nitrogen loading typical of intensive aquaculture feeding conditions. In aquaculture systems, ammonia is primarily generated through protein catabolism and excretion by fish, while the decomposition of uneaten feed and organic matter represents an additional nitrogen source. In the present study, feed-derived nitrogen was used to simulate realistic ammonification dynamics rather than equilibrium chemical dosing commonly applied in laboratory adsorption studies using ammonium salts such as NH_4Cl .

Each experimental unit consisted of 500 mL of dechlorinated tap water placed in 500 mL glass containers. The experiment was conducted under controlled laboratory conditions at room temperature (22–24 °C). No aeration, mechanical mixing, or agitation was applied during the experimental period in order to simulate static water conditions comparable to low water-exchange aquaculture systems. A single addition of 0.5 g commercial fish feed was applied at the beginning of the experiment (Day 0), and no additional feed was supplied during the 12-day experimental period. The feed dose was selected to ensure measurable

ammonium production during the experimental period and to simulate organic nitrogen loading conditions typical of intensive aquaculture systems. The contact time between the adsorbents and ammonia was monitored over a 12-day period, during which $\text{NH}_4^+\text{-N}$, pH, temperature, and dissolved oxygen were periodically measured ^{18, 19}.

To ensure experimental robustness, all treatments were conducted in triplicate under identical environmental conditions, and a control group without adsorbent was included for direct comparison. The use of feed-derived nitrogen rather than synthetic ammonium salts was specifically selected to simulate progressive ammonification processes commonly observed in aquaculture systems. Although simplified, this approach enables evaluation of adsorption performance under dynamic nitrogen release conditions, thereby increasing the ecological relevance and experimental validity of the study.

Adsorbent treatments

A total of 3 g of adsorbent material was added once at the beginning of the experiment (Day 0) to each experimental unit, corresponding to a final concentration of 6 g L⁻¹. The experimental treatments were arranged as follows:

- (1) B: 3 g bentonite (6 g/L)
- (2) BL1: 2.5 g bentonite + 0.5 g leonardite
- (3) BL2: 2 g bentonite + 1 g leonardite
- (4) BL3: 1.5 g bentonite + 1.5 g leonardite
- (5) BL4: 1 g bentonite + 2 g leonardite
- (6) BL5: 0.5 g bentonite + 2.5 g leonardite
- (7) L: 3 g leonardite (6 g/L)
- (8) C: Control (no adsorbent)

Each treatment was conducted in triplicate (n = 3), resulting in a total of 24 experimental units

Experimental system characteristics

Dechlorinated tap water was used as the experimental medium in order to establish standardized conditions and minimize environmental variability. The water did not originate from an active aquaculture pond, tank-based production unit, or recirculating aquaculture system (RAS). Instead, the system represented a simplified experimental model designed to evaluate adsorption performance under controlled conditions. This experimental design aimed to minimize biological variability and primarily evaluate the physicochemical adsorption performance of bentonite and leonardite. The simulated system represents conditions comparable to static aquaculture environments where ammonia accumulation may occur due to uneaten feed and limited water exchange. No aeration, mechanical mixing,

or agitation was applied during the experimental period in order to simulate low water-exchange static conditions and to observe natural diffusion-based adsorption. Natural leonardite and Ca-bentonite were taken from Kütahya Kimya (Kütahya, Türkiye) and Samsa Industrial Materials (Tokat, Türkiye), respectively.

Water quality assessment

Water parameters (dissolved oxygen, $\text{NH}_4^+\text{-N}$, pH, temperature) were determined by using a multiparameter instrument (YSI Professional Plus Series).

Nitrogen generation in the experimental system

Ammonia generation in the experimental system resulted from the microbial degradation of the single feed addition applied at the beginning of the experiment. Although the initial $\text{NH}_4^+\text{-N}$ concentration was low, progressive ammonification occurred during the experimental period through mineralization of organic nitrogen compounds derived from feed residues. Therefore, the experimental design represents a single organic nitrogen pulse under batch conditions rather than a fixed initial ammonium concentration.

Experimental nitrogen loading description

In this study, ammonia was generated from a single addition of 0.5 g commercial fish feed at the beginning of the experiment (Day 0). No additional feed was supplied during the 12-day experimental period. Therefore, all treatments were exposed to an identical organic nitrogen load. Although the measured initial $\text{NH}_4^+\text{-N}$ concentration was low, progressive ammonification occurred through microbial degradation of feed residues, leading to increasing ammonium concentrations over time. Thus, the experimental model represents a single organic nitrogen pulse under batch conditions rather than a fixed initial ammonium solution.

Data analysis

NH_3 and TAN values in the study were calculated from $\text{NH}_4^+\text{-N}$, water temperature, and pH values ^{8, 20, 21} The ammonia and TAN values were computed using the following formula ²².

$$\text{pK}(\text{NH}_3) = \frac{2726.3}{273 + ^\circ\text{C}} + 0.0963 \quad 1$$

$$\text{NH}_3\text{-N} = 10^{(\text{pH} - \text{pK}(\text{NH}_3))} \times \text{NH}_4^+\text{-N} \quad 2$$

$$\text{TAN} = \text{NH}_3\text{-N} + \text{NH}_4^+\text{-N} \quad 3$$

BET (Brunauer-Emmett-Teller), XRF (X-ray fluorescence), SEM (scanning electron microscopy) with EDS (energy-dispersive X-ray spectroscopy), and FT-IR (Fourier transform infrared spectroscopy) analyses of the leonardite and Ca-bentonite employed in this study

were conducted at the Kastamonu University using a Quantachrome Nova Touch LX4, Spectro Xepos II, FEI Quanta FEG 250, and Bruker Alpha instruments, respectively.

Removal efficiency (%) and adsorption capacity were calculated on day 12 using commonly applied adsorption equations ²³.

$$\text{Removal efficiency (\%)} = \frac{C_0 - C_e}{C_0} \times 100$$

$$\text{Adsorption Capacity (mg g}^{-1}\text{)} = \frac{(C_0 - C_e) \times V}{m}$$

Where:

C_0 = maximum ammonium concentration measured in the control group (mg L⁻¹)

C_e = equilibrium ammonium concentration (mg L⁻¹)

V = solution volume (L)

m = mass of adsorbent used in the experiment (g)

Statistical analysis

Statistical analyses were performed using Minitab version 17. All data are expressed as mean $\pm$ standard error (mean $\pm$ SE). Differences among experimental groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's HSD post-hoc test for multiple comparisons. A significance level of $p < 0.05$ was applied for all statistical analyses.

Results

Initial Water Quality Parameters

At the beginning of the experiment (Day 0), the baseline water quality parameters were measured prior to adsorbent addition. Initial values were as follows:

Temperature: 18.4 ± 0.02 °C, Dissolved oxygen: 1.42 ± 0.01 mg L⁻¹, pH: 8.49 ± 0.01 , NH₄⁺-N: 0.01 ± 0.01 mg L⁻¹. Using the Emerson equilibrium equation ⁸, the calculated initial NH₃ concentration was below the toxic threshold (<0.05 mg L⁻¹). These baseline values were statistically similar across all experimental groups ($p > 0.05$).

Morphology and crystalline structure of utilized minerals

The BET (Brunauer-Emmett-Teller) surface area analysis and XRF (X-ray fluorescence) results of the leonardite and bentonite samples used in the study are presented in Table 1.

Table 1 XRF values of powder leonardite and bentonite at the end of the experiment.

	Leonardite	Bentonite
SiO ₂ (%)	8.98	66.11
Al ₂ O ₃ (%)	4.47	20.35
MgO (%)	0.19	3.13
K ₂ O (%)	0.34	0.87
CaO (%)	3.05	2.92
Na ₂ O (%)	0.01	5.39
Fe ₂ O ₃ (%)	0.88	3.48
P ₂ O ₅ (%)	0.31	0.13
SiO ₂ /Al ₂ O ₃	2.00	3.25
Specific Surface Area (BET) (m ² /g)	7.872	22.0163

Scanning electron microscopy (SEM) was used to examine the pore structure and surface morphology of the mineral powders (Fig. 1). Bentonite exhibited a layered plate-like morphology typical of clay minerals, indicating the presence of interlayer spaces that may facilitate ion exchange processes. Leonardite showed irregular porous carbonaceous structures with heterogeneous surface features.

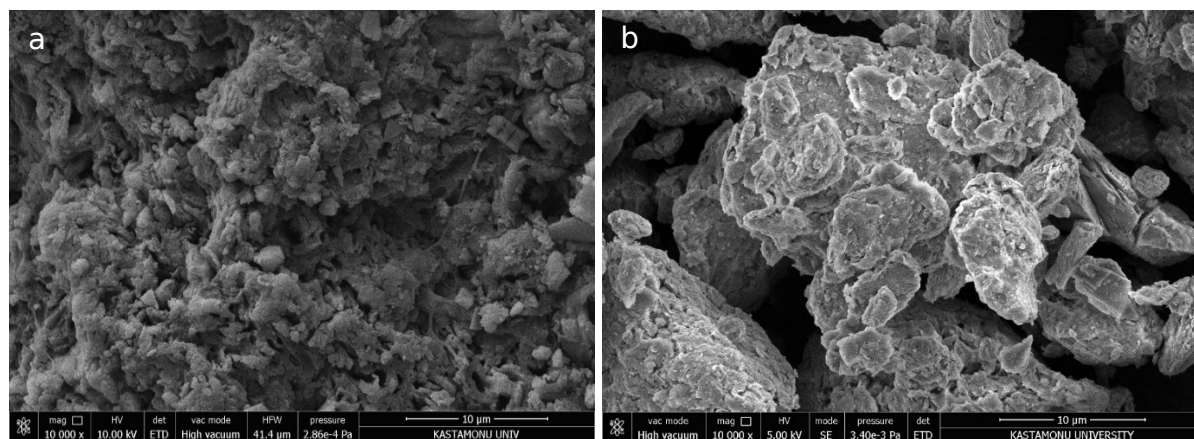


Fig. 1. SEM images of leonardite (a) and bentonite (b)

Energy dispersive X-ray spectroscopy (EDS) analysis confirmed the elemental composition of both minerals (Fig. 2). The spectra revealed the presence of Na, Ca, Mg, Al, Fe and Si in the leonardite particles. Both minerals showed considerable silicon content. The elemental composition trends identified by EDS were consistent with the bulk elemental composition obtained by XRF analysis ²⁴.

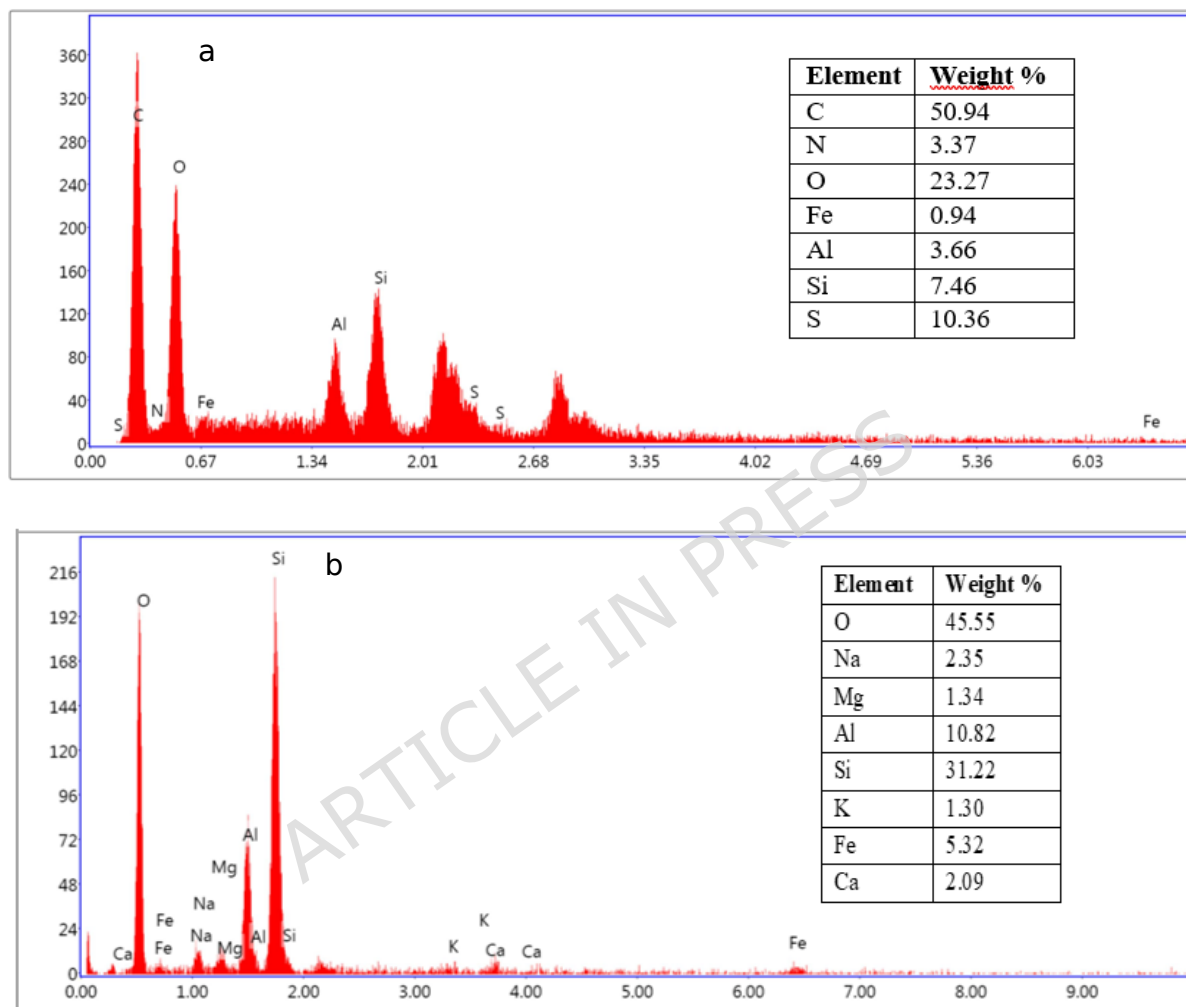


Fig. 2. EDS analyses of leonardite (a) and bentonite (b)

The FT-IR spectra of leonardite and bentonite are presented in Fig. 3a-b. For leonardite, absorption bands observed at 1019.06, 795.15 and 772.27 cm^{-1} correspond to the stretching vibration of Si-O groups, while bands at 690.47, 525.69 and 464.14 cm^{-1} correspond to

bending vibrations associated with quartz structures 25, 26.



Fig. 3. FT-IR analyses of leonardite (a) and bentonite (b)

For bentonite, the FT-IR spectrum obtained within the wavenumber range of 400–4000 cm^{-1} showed characteristic peaks associated with clay minerals. The absorption peak at 3628 cm^{-1} and the broad peak at 3438 cm^{-1} correspond to O–H stretching vibrations of Al–OH groups and bound water, respectively ^{27, 28}. The band at 1635 cm^{-1} corresponds to the bending vibration of O–H bonds in water molecules associated with the silicate structure. Additional peaks at 1114.57, 999.25, 916.56, 794.45, 619.11, 517.52 and 455.92 cm^{-1} correspond to stretching vibrations of functional groups such as Si–O–Al, Si–O, Al–Al–OH, Al–O and Mg–OH within the tetrahedral and octahedral sheets of the clay structure ^{27, 29}.

Water parameters

At the onset of the experiments, the water temperature, dissolved oxygen, pH, and ammonium concentrations were measured as 18.4 ± 0.02 °C, 1.42 ± 0.01 mg L⁻¹, 8.49 ± 0.01 , and 0.2 ± 0.01 mg L⁻¹, respectively. No significant differences were observed between the groups ($p > 0.05$).

At the end of the experiment, the addition of bentonite and leonardite did not significantly alter the temperature and dissolved oxygen values, but there were significant differences in pH, NH_4^+ -N, and NH_3 . All treatments recorded mean temperatures between max. 19.58 ± 0.12 °C and min 19.24 ± 0.10 °C, and mean dissolved oxygen between max. 0.28 ± 0.07 mg L⁻¹ and min. 0.23 ± 0.05 mg L⁻¹. The mean lowest values of NH_4^+ -N were recorded in treatment BL5 (2.11 ± 0.34 mg L⁻¹), while the mean highest value was in treatment Control (21.98 ± 2.76 mg L⁻¹).

The mean lowest values of pH were recorded in treatment L (7.34 ± 0.05), while the highest was in treatment B (7.87 ± 0.05) (Table 2).

Table 2 Mean temperature, dissolved oxygen, pH and NH_4^+ -N values in leonardite and bentonite added groups at the end of the experiments at 8 different treatment (mean $\pm$ SE)

Experimental groups*	Temperature (°C)	Dissolved Oxygen (mg L ⁻¹)	pH	NH_4^+ -N (mg L ⁻¹)
Bentonite	19.40 ± 0.10	0.28 ± 0.07	7.87	8.84 ± 1.45^b
BL1	19.46 ± 0.10	0.28 ± 0.07	7.76	5.30 ± 0.93^{bc}
BL2	19.25 ± 0.10	0.28 ± 0.07	7.66	3.76 ± 0.66^{bc}
BL3	19.28 ± 0.11	0.27 ± 0.06	7.60	2.96 ± 0.52^c

BL4	19.24±0.10	0.26±0.06	7.49	2.43±0.38 ^c
BL5	19.31±0.10	0.25±0.05	7.41	2.11±0.34 ^c
Leonardite	19.58±0.10	0.23±0.05	7.34	4.78±0.70 ^{bc}
Control	19.58±0.12	0.23±0.05	7.78	21.98±2.76 ^a

Different superscript letters within the same column indicate statistically significant differences at $p < 0.05$ according to Tukey's HSD test.

NH₃ concentrations, which can have toxic effects on aquatic organisms and are undesirable to reach toxic levels in aquaculture units, are presented in Fig. 4.

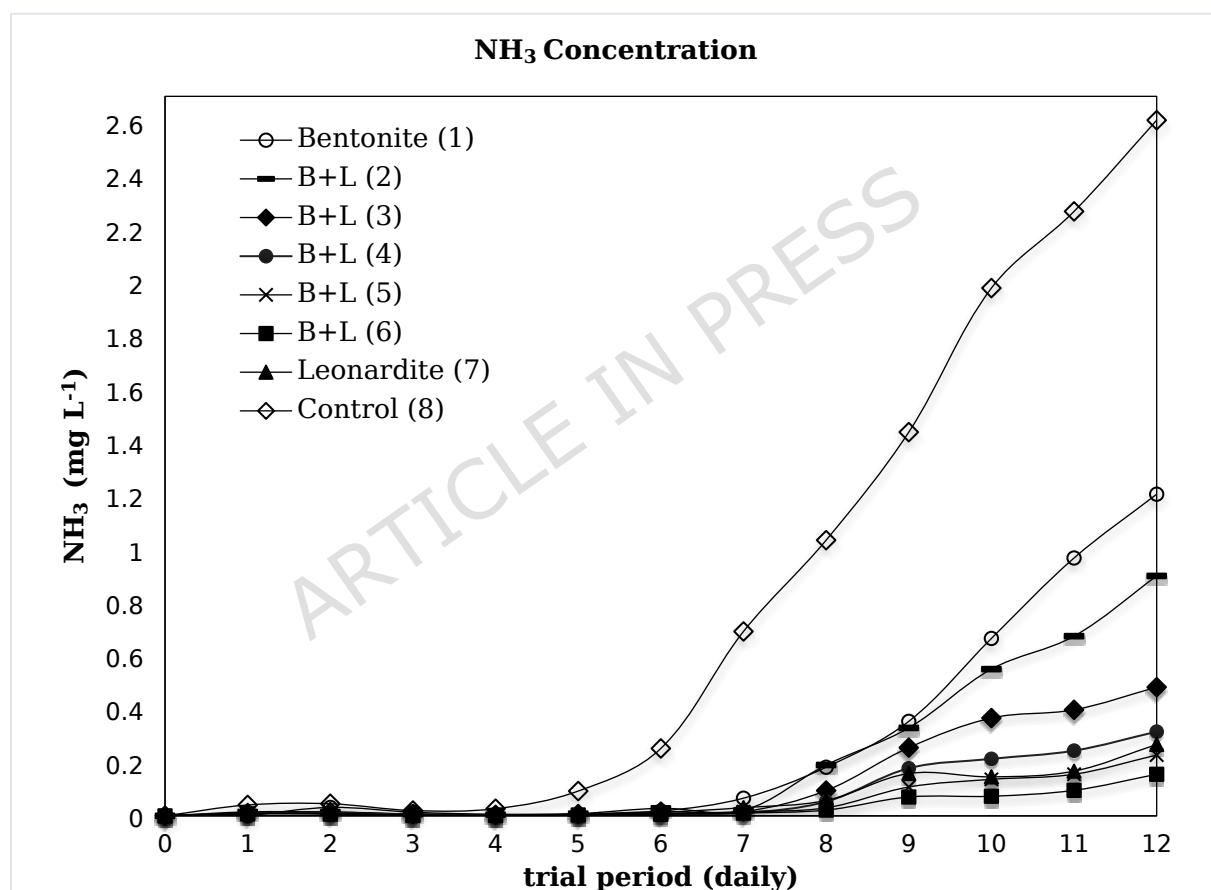


Fig. 4. Ammonia concentration changes measured in the experimental groups during the experiment

Analysis of NH₃ concentrations revealed that levels in the control group were consistently higher than those in the experimental groups throughout the study period ($p < 0.05$). Toxic thresholds for aquatic organisms (>0.05 mg L⁻¹) were exceeded on day 2 in the control group, on day 6 in the bentonite-only group, and on day 8 in the BL1, BL2, BL3, and leonardite (L)

groups, and on day 9 in the BL4 ($0.11 \pm 0.03 \text{ mg L}^{-1}$) and BL5 ($0.07 \pm 0.02 \text{ mg L}^{-1}$) groups ($p < 0.05$). Acute toxicity levels of NH_3 vary among species; however, concentrations approaching 2.0 mg L^{-1} are reported to be lethal for many freshwater species under typical aquaculture conditions. While the lethal level of 2 mg L^{-1} for aquatic organisms was reached in the control group on the day 10 experimental measurement, this limit was not reached in the other groups.

The maximum TAN concentration in the control group reached 48.01 mg L^{-1} . In contrast, all treatment groups exhibited adsorption of ammonium from the water column. Removal efficiency increased progressively with increasing leonardite proportion in the mixture. The bentonite-only treatment (B) achieved 48.4% removal, whereas the BL5 formulation (0.5 g bentonite + 2.5 g leonardite) demonstrated the highest performance with 87.7% removal. Similarly, apparent adsorption capacity increased from 3.66 mg g^{-1} in the bentonite-only group to 6.64 mg g^{-1} in BL5. These findings indicate a clear dose-dependent enhancement of ammonium mitigation with increasing leonardite content ($p < 0.05$) (Table 3).

Table 3 Ammonium removal efficiency (%) and adsorption capacity of experimental groups at the end of the experiment

Experimental Groups	Max NH_3 (mg/500 ml)	Max NH_4^+ (mg/500 ml)	Removal Efficiency (%)	Adsorption Capacity (mg g^{-1})
B	1.60	23.43	48.4%	3.66
BL1	0.9	14.77	67.5%	5.11
BL2	0.49	10.07	77.8%	5.89
BL3	0.32	8.60	81.1%	6.13
BL4	0.23	6.70	85.2%	6.45
BL5	0.16	5.57	87.7%	6.64
L	0.27	11.23	75.3%	5.70
C	2.61	45.4	-	-

Discussion

The objective of this study was to evaluate the capacity of bentonite-leonardite mixtures to buffer ammonium accumulation under conditions simulating nitrogen dynamics in aquaculture systems. In intensive aquaculture environments, ammonia is primarily generated through protein catabolism and metabolic excretion by fish, while additional nitrogen may originate from feed decomposition. Therefore, the present study examined the effects of bentonite and leonardite used individually and in combination on the removal of ammonium nitrogen originating from fish feed in a simulated aquaculture environment. Previous studies have shown that combining natural adsorbents can enhance ammonia removal efficiency in aquatic systems. For example, ³⁰ investigated the removal of ammonia using a zeolite-bentonite mixture at different water temperatures and reported that the highest removal efficiency occurred at 18 °C. Similarly, ³¹ examined ammonia removal using a leonardite and clinoptilolite-type zeolite mixture and demonstrated that the clinoptilolite: leonardite combination improved ammonia removal while maintaining optimal pH conditions in freshwater aquaria. In addition, ³² reported that biochar-bentonite hydrochar composites possess micro- and mesoporous structures, enabling effective ammonia adsorption in aquaculture systems and achieving complete ammonia removal from a Koi fish tank within 60 minutes. The enhanced performance of such combinations is generally attributed to the complementary adsorption properties of different materials, particularly when carbon-based adsorbents with activated carbon-like characteristics, such as leonardite, are combined with clay minerals like bentonite. Similar improvements in adsorption efficiency have been reported for several adsorbent mixtures ^{18, 26, 31, 33, 34}. However, to the best of our knowledge, the adsorption performance of a bentonite-leonardite mixture has not previously been investigated, and the present study therefore provides the first evaluation of this combination for ammonium removal in aquaculture systems.

Montmorillonite (MMt), the primary component of bentonite, consists of extremely fine particles and exhibits a high swelling capacity. These properties enable MMt to form nearly impermeable membranes, restricting water movement. As a result, MMt is not ideal for use as an adsorbent in fixed-bed adsorption systems for water or wastewater treatment. To utilize this clay mineral effectively in such systems, surface modification is necessary. One potential solution to this challenge is the incorporation of another porous material to support and enhance the distribution of MMt ¹⁸.

pH is a critical parameter influencing both fish health and the ammonia-ammonium equilibrium in aquatic systems. In the present study, the reduction in pH observed in treatments containing higher proportions of leonardite may have contributed to improved ammonium removal efficiency. Ammonia nitrogen exists in water in two forms: ionized ammonia (NH_4^+) and un-ionized ammonia (NH_3) ³⁵. The equilibrium between these species

is strongly pH-dependent, with decreasing pH shifting the balance toward NH_4^+ ^{7, 21}. Because natural adsorbents preferentially retain the cationic NH_4^+ form, this shift can enhance adsorption efficiency. In contrast, NH_3 is uncharged and therefore interacts weakly with cation exchange sites. Natural clay minerals possess negatively charged structural sites resulting from isomorphic substitution within aluminosilicate layers, which enables electrostatic attraction and exchange of cationic species such as NH_4^+ . In aquaculture systems, ammonia is considered one of the most critical water quality parameters after dissolved oxygen, and elevated NH_3 concentrations are known to be harmful to fish. Concentrations exceeding approximately 0.05 mg L^{-1} NH_3 are generally associated with adverse effects in many freshwater species ^{18, 30, 36, 37, 38}. Previous studies have shown that natural mineral materials such as zeolite ³⁹, leonardite ³¹, diatomite ⁴⁰ and bentonite ^{30, 41} can influence water quality parameters and contribute to ammonia control in aquaculture systems, with their effectiveness depending on their structural properties and environmental conditions.

In this study, pH values decreased as the amount of leonardite increased. Considering the NH_3 limit values for fish farming, the limit values (0.05 mg L^{-1}) were reached in the control group on the second day of the study, while this value was found to be the same in groups BL1, BL2, BL3, and L on the day 8 of the experimental measurement, and in groups BL4 and BL5 on the day 9 of the experimental measurement. Anytime the un-ionized ammonia (NH_3) is higher than 0.05 mg L^{-1} , the fish are being damaged. As the concentration rises above 0.05 mg L^{-1} , it causes more and more damage ^{7, 42}. Experimental conditions were optimized to ensure a decline in ammonium nitrogen levels from 2-3 times the “maximum allowable concentration” ($5\text{--}7.5 \text{ mg L}^{-1}$) to $0.2\text{--}0.8 \text{ mg L}^{-1}$. The analysis revealed that sorption capacity is maximized when the bentonite loading is reduced to 0.01 g L^{-1} , provided that the ammonium ion concentration in the solution exceeds the “maximum allowable concentration” by 2-3 times. Considering operational factors such as treatment duration, purification efficiency, sorbent dosage, material and labor costs, a bentonite concentration of 0.01 g L^{-1} was deemed the most efficient for decreasing ammonium ion levels from 3 times “the maximum allowable concentration” to a safe threshold.

Unlike conventional adsorption studies that use predefined ammonium solutions, the present experimental design simulated aquaculture conditions in which ammonia is generated progressively through the mineralization of protein-rich feed and associated nitrogen transformation processes occurring in aquaculture systems. In actual aquaculture environments, ammonia originates primarily from metabolic nitrogen excretion by fish as well as from feed decomposition. The experimental approach used in this study therefore aimed to reproduce the progressive nitrogen release associated with feed-derived organic

matter rather than relying on instantaneous ammonium addition. Consequently, the adsorption performance observed in this study reflects the buffering capacity of the adsorbents under dynamic nitrogen release conditions rather than equilibrium adsorption alone. This approach improves the ecological relevance of the results and allows a more realistic evaluation of adsorption performance under aquaculture-like conditions ^{43, 44}.

Although leonardite alone exhibited substantial ammonium removal (75.3%), its combination with bentonite significantly enhanced performance. The progressive increase in removal efficiency from BL1 to BL5 suggests a synergistic interaction between the clay mineral structure of bentonite and the carbon-rich composition of leonardite. The highest apparent adsorption capacity (6.64 mg g^{-1}) was achieved in BL5, representing an approximately 81% increase compared to bentonite alone and a 16.5% improvement over leonardite alone. These findings confirm that the combined system performs better than individual components, indicating a complementary adsorption mechanism.

The superior performance of leonardite-rich mixtures can be explained by multiple interacting mechanisms. Bentonite provides a negatively charged layered structure with high cation exchange capacity (CEC), enabling NH_4^+ substitution for exchangeable interlayer cations. However, the relatively moderate performance of bentonite alone (48.4% removal) suggests that ion exchange alone is insufficient under progressive ammonification conditions. Leonardite, in contrast, contains humic substances rich in carboxylic and phenolic functional groups, which promote electrostatic attraction and surface complexation with ammonium ions. The increased removal efficiency observed in BL4 and BL5 treatments indicates that carbon-mediated adsorption plays a dominant role. Additionally, the gradual pH reduction observed with increasing leonardite proportion likely shifted the $\text{NH}_3/\text{NH}_4^+$ equilibrium toward the ionized form (NH_4^+), which is preferentially retained by adsorbent surfaces. This dual effect chemical equilibrium modulation and adsorption provides a plausible explanation for the 87.7% removal achieved in BL5.

The findings of this study will contribute to the gap in the literature by presenting the first scientifically original data, showing that the use of leonardite, an economical and natural carbon source, together with the clay mineral bentonite, has a positive effect on ammonium ion removal.

While the present study was conducted under controlled laboratory conditions without biological components such as fish or active biofiltration, the results provide a mechanistic understanding of ammonium adsorption under simplified conditions. In real aquaculture systems, additional factors such as continuous feeding, microbial activity, aeration, and water exchange may influence nitrogen dynamics and adsorption efficiency. Therefore, the results obtained in this study should be interpreted as baseline adsorption performance rather than direct operational outcomes. Further research is required to validate the

performance of bentonite-leonardite mixtures under dynamic and biologically active aquaculture conditions.

Conclusion

In conclusion, bentonite-leonardite mixtures demonstrated substantial ammonium buffering capacity under experimental conditions simulating nitrogen release from fish feed in static freshwater aquaculture systems, indicating their potential applicability as natural adsorbents for ammonia control in aquaculture environments. The highest performance was achieved in the BL5 formulation (0.5 g bentonite + 2.5 g leonardite), which resulted in 87.7% removal of peak NH_4^+ concentration and an apparent adsorption capacity of 6.64 mg g^{-1} .

The enhanced mitigation observed in composite treatments may be explained by the combined effects of montmorillonite-mediated cation exchange and humic substance-based surface complexation. In addition, the pH reduction observed in leonardite-containing treatments may have shifted the $\text{NH}_3/\text{NH}_4^+$ equilibrium toward the ionized NH_4^+ form, which is more readily retained by adsorbent surfaces.

Although ammonia toxicity thresholds vary among species and environmental conditions, the delayed increase in NH_3 levels observed in leonardite-rich treatments indicates improved buffering stability under static nitrogen loading.

Nevertheless, given that the present study was conducted under simplified and controlled laboratory conditions, the results should be interpreted as preliminary evidence of adsorption potential rather than direct representation of performance in operational aquaculture systems. While the findings indicate that bentonite-leonardite mixtures may offer a promising low-cost approach for ammonium buffering, further validation under dynamic, aerated, and biologically active conditions is required to fully assess their practical applicability and long-term effectiveness.

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