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Research paper

Evaluation of Some Adsorbents and Modified Bentonite for Mitigation of Mycotoxin Deoxynivalenol (DON) Produced by Fusarium Graminearum

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Abstract

Deoxynivalenol (DON) is a prevalent mycotoxin mainly produced by Fusarium graminearum, which frequently contaminates cereals like maize, wheat, and barley both in the field and during storage. Human exposure to this toxin occurs directly through plant-based foods (such as grains) or indirectly through animal-derived products (including kidney, liver, milk, and eggs). Clinically, DON induces a range of adverse effects in animals, such as gastrointestinal distress, vomiting, anorexia, bloody diarrhea, reproductive issues, abortion, and even death. Consequently, its presence in animal feed poses a serious threat to livestock health and industry. Using mycotoxin-binding agents is a key approach to mitigate this risk by adsorbing mycotoxins in the aqueous environment, preventing their absorption, and facilitating the excretion of the toxin-adsorbent complex via feces. In this study, two organic adsorbents (activated carbon with different mesh sizes and leonardite) and seven mineral adsorbents (bentonite, two dolomite types, diatomite, perlite, vermiculite, and zeolite) were screened for DON reduction potential. Organic adsorbents, achieving 80-100% DON removal. However, they were excluded due to their non-selective adsorption and nutrient depletion. Among the mineral adsorbents, bentonite showed the highest DON adsorption (52%). Acid modification of bentonite further improved its performance to 60%, an enhancement confirmed by BET and FTIR analyses. Adsorption isotherm studies revealed that the process was best described by the Redlich–Peterson model (R^2 = 0.92), indicating incorporating both Langmuir and Freundlich behaviors. The Freundlich isotherm suggested favorable adsorption ($1/n < 1$).

1. Introduction

Mycotoxins are toxic secondary metabolites produced by specific fungal species such as Aspergillus, Fusarium, Penicillium, Claviceps, and Alternaria. These secondary metabolites are capable of causing pathological alterations in both humans and animals [1]. When nutrients and moisture are available—typically at a water activity (aw) level greater than 0.6—mold growth and subsequent mycotoxin production can occur under a wide range of climatic conditions and on diverse solid or liquid substrates. As a result, mycotoxins are considered natural contaminants in a variety of food and feed products, particularly cereals, as well as fruits, nuts (e.g., hazelnuts and almonds), oilseeds, forages, and derived products intended for human or animal consumption [4]. Trichothecenes are a group of chemically related mycotoxins mainly produced by fungus Fusarium [5]. These compounds are sesquiterpenoid metabolites characterized by a common 12,13-epoxytrichothec-9-ene core structure. Trichothecenes are generally classified into two major types, A and B, based on the functional groups attached at the C-8 position. Type A trichothecenes typically contain a hydrogen atom or an ester moiety at C-8 and include highly toxic members such as T-2 toxin, its acetylated metabolite HT-2, and diacetoxyscirpenol (also known as anguidine). Among them, T-2 toxin is particularly potent and is estimated to be approximately ten times more toxic than deoxynivalenol (DON) in mammals. In contrast, Type B trichothecenes are distinguished by the presence of a ketone group at C-8 and include DON and nivalenol (NIV) as their primary representatives [6, 7]. DON, a naturally occurring mycotoxin, is primarily produced by Fusarium graminearum and is one of the most prevalent contaminants in cereal grains worldwide [8].

Trichothecenes exert various toxic effects on eukaryotic cells, including inhibition of protein synthesis, cytotoxicity, and induction of apoptosis [9, 10].

The impact of DON on human health may occur following the consumption of contaminated food products such as oats, barley, wheat, maize, or other cereal grains. It has also been detected in buckwheat, sorghum, popcorn, and various processed foods commonly consumed by humans, including flour, bread, noodles, beer, and malt [11]. Although DON is generally not considered a significant threat to public health, some cases of acute exposure have been associated with short-term nausea and vomiting [12]. Additional symptoms reported in human include diarrhea, abdominal pain, headache, dizziness, and fever [13]. According to Commission Regulation (EU) 2023/915, the maximum permitted level of DON is 1250 µg/kg in unprocessed common cereals and 750 µg/kg in cereal flour, while in feed, it ranges from 900 to 5000 µg/kg depending on the animal species and age [14].

The widespread occurrence of DON in major agricultural commodities causes acute and chronic toxicity in humans and animals and leads to significant economic losses, thus necessitating effective contamination control strategies. Various biological, chemical, and physical approaches have been employed for the detoxification of mycotoxins in grains and processed food products [15-17]. Among these, the use of mycotoxin adsorbents as feed additives is considered one of the most promising and widely adopted strategies for reducing the risk of mycotoxicosis in farm animals and minimizing the carryover of mycotoxins into animal-derived products [18, 19]. These adsorbents act by binding mycotoxin molecules within the gastrointestinal tract, thereby reducing their intestinal ab-sorption and subsequently systemic toxicity [19, 20].

To date, three main categories of detoxification strategies for DON have been reported in the literature: biological, chemical, and physical methods [21-25].

Physical detoxification methods, rely on the use of various adsorbent mate-rials added to contaminated diets. They act as “chemical sponges” that bind mycotoxins in the gastrointestinal tract, preventing their absorption and distribution to target organs in animals [18]. The effectiveness of adsorption depends on the chemical structure of both the binding agent and the mycotoxin [26]. Key factors include the physical structure of the adsorbent—such as

pore size, surface accessibility, overall charge, and charge distribution—as well as the physicochemical properties of the mycotoxin, including polarity, solubility, molecular shape, and charge distribution [27].

Various natural materials have demonstrated potential for binding myco-toxins in animal feed [26], including Bentonite [28], diatomite [29], zeolite [30], leonardite [31], and activated carbon [32]. Dolomite, vermiculite, and perlite, three naturally occurring miner-als, have shown promising results in the adsorption of various contaminants such as heavy metals, organic pollutants, and certain mycotoxins [33-35]. Given their favorable physicochemical characteristics, we hypothesize that these minerals could also exhibit a high affinity for DON. To the best of our knowledge, this study is the first to systematically evaluate the DON adsorption capacity of do-lomite, vermiculite, and perlite under controlled laboratory conditions, thereby providing new insight into their potential use as alternative, low-cost adsorbents in feed detoxification strategies.

2. Materials and Methods

2.1. Chemicals and Reagents

Deoxynivalenol (DON) standard (≥98% purity) was purchased from Sig-ma-Aldrich (St. Louis, MO, USA). All solvents used in this study, including methanol, acetonitrile, and nitric acid, were of HPLC-grade and obtained from Carlo Erba Reagents (Milan, Italy). Surfactant Hexadecyltrimethylammonium bromide (HDTMA) was purchased from Merck (Darmstadt, Germany). Dextrose and agar were purchased from Merck (Darmstadt, Germany). Purified water obtained from a Milli-Q system was used in all extraction and analytical procedures. A toxigenic Fusarium graminearum isolate F48 was obtained from the Mycotoxin Laboratory of the Iranian Research Institute of Plant Protection, Tehran, Iran.

2.2. Production of DON by Fusarium graminearum

Potato Dextrose Agar (PDA) medium (39 g/l) was prepared, autoclaved at 121 °C for 21 minutes using an autoclave (Systec DX-150, Germany), and poured into sterile Petri dishes.

Five small pieces (1×1 cm) of a 7-day-old culture of toxigenic, F. gramine-arum F48 were placed into 250 ml Erlenmeyer flasks containing 20 g autoclaved rice (moisture content 28%). The flasks were transferred to an incubator for 21 days to allow fungal growth and DON production.

2.3. Extraction of DON

After 21 days, DON was extracted from the fungal biomass using water: methanol mixtures in a ratio of 25:75 (v/v), with slight modifications to previous methods [36].

A portion of the fungal culture was weighed using an analytical balance (Sarto-rius ED124S, Germany), and 10 times its weight of the extraction solvent was added. The mixture was shaken for 2 hours using a laboratory shaker (IKA HS 501 digital, Germany) at 130 rpm. It was then centrifuged at 12,000 rpm for 15 minutes using a refrigerated centrifuge (MSE Micro Centaur, UK). The super-natant containing DON was collected and analyzed.

2.4. DON- adsorption assay

The adsorbents screened for DON removal included bentonite, diatomite, magnetic dolomite, granulated dolomite, leonardite, vermiculite, perlite, zeolite, and activated carbon with various mesh sizes, as well as leonardite. All adsorbents were purchased from Medicinal Plants and Drugs Research Institute, Shahid Beheshti University.

Ten mg of each adsorbent was weighed using a microbalance and placed into 20 mL amber glass vials. Extracted DON was added to the 100mM phosphate buffer (pH = 7.0) at a final concentration of 25 µg/mL, and the total volume was adjusted to 2 mL at a ratio of 200:1 (adsorbent: toxin) The toxin-buffer mixture (2 mL) was added to each adsorbent and shaken in the dark at 145 rpm for 2 hours at 39 °C. Afterward, the suspensions were centrifuged at 12,000 rpm for 15 minutes. The supernatants were transferred into an HPLC vial for analysis—control containing only toxin-buffer mixture (without adsorbent).

2.5. Modification of Raw Bentonite

Most natural bentonites are characterized by low toxicity, low cost, wide-spread availability, and selective adsorption capacity [37, 38]. These properties, combined with their high efficiency in adsorbing mycotoxins, make them economically viable for detoxification purposes. In the present study, two types of modifications were applied to enhance the adsorption capacity of raw bentonite: acid modification and surfactant modification.

2.5.1. Acid Modification

A 0.1 N nitric acid and 0.1 N HCl (1:20 w/v, 65 °C) significantly enhanced the Aflatoxin B1 adsorption capacity of bentonite were selected (data not published). This treatment achieved a solution that was used for activation. The bentonite-to-acid ratio was kept constant at 1:20 (w/v) throughout the experiment. The mixtures were shaken at 150 rpm for 2 hours at 65 °C using a Heidolph Unimax 1010 shaker with incubator 1000 (Germany). The acid-treated bentonite was then filtered through a Buchner funnel using filter paper, and the collected solids were dried overnight in an oven (Memmert, Germany) at 100 °C.

2.5.2. Surfactant Modification

The second modification method involved the use of a quaternary alkylammonium surfactant, hexadecyltrimethylammonium bromide (HDTMA). Based on the bentonite analysis (cation exchange capacity, CEC, of 100–110 mEq/100 g), two modification levels were tested: 0.5 × CEC and 3 × CEC. Accordingly, 166.6 mg or 1 g of HDTMA was used to activate 1 g of bentonite for the 0.5 × CEC and 3 × CEC treatments, respectively. The procedure was as follows: 1 g of raw bentonite was suspended in 25 mL of distilled water and shaken at 150 rpm at room temperature for 30 minutes. The calculated amount of HDTMA was then added, and the mixture was shaken overnight at 150 rpm at room temperature. Finally, the suspension was filtered through a Buchner funnel using filter paper, and the modified bentonite was dried overnight in an oven at 60 °C.

2.6. Adsorption Isotherm Studies

To investigate the adsorption characteristics of DON onto the most effective adsorbent (acid-modified bentonite with 0.1 N HNO_3), adsorption isotherm studies were conducted using a range of DON concentrations. The tested concentrations were 10, 15, 25, 30, 40, 50, 70, 85, and 100 µg/mL. The adsorbent dose was maintained at 4 mg, and the solution volume was kept constant at 2 mL throughout the experiments. Adsorption isotherm studies were conducted to characterize the interaction between DON and the most effective adsorbent, acid-modified bentonite (0.1 N HNO_3). After two hours of shaking under dark conditions at 145 rpm, the suspensions were centrifuged (12,000 rpm, 15 min). The DON concentration in the supernatant was then quantified by HPLC using a calibrated standard curve. The experimental data were fitted to four adsorption isotherm models—Langmuir, Freundlich, Redlich–Peterson, and Temkin—to evaluate the equilibrium adsorption behavior. The adsorption capacity (q_e) and percentage of DON adsorption (%A) were calculated using the following equations:

$$q_e = (C_0 - C_e) \times V / m$$
$$\%A = ((C_0 - C_e) / C_0) \times 100$$

Where C_0 and C_e (mg/L) represent the initial and equilibrium concentrations of DON, respectively; V is the solution volume (L), and m is the weight of the adsorbent (g).

Linearized forms of the Langmuir, Freundlich, Redlich–Peterson, and Tem-kin isotherm models were used to fit the experimental data. The corresponding regression equations and model parameters are presented in Table 2. Details of the model assumptions and interpretations can be found in the referenced literature [39-44].

2.7. Analytical Characterization and Quantification of Adsorbents

To assess both the adsorption performance and physicochemical properties of the adsorbents, a combination of analytical techniques was employed, including high-performance liquid chromatography (HPLC), Brunauer–Emmett–

Teller (BET) surface area analysis, and Fourier-transform infrared (FTIR) spectroscopy.

The quantification of DON was carried out using a high-performance liquid chromatography system (Waters 2695, USA), equipped with a dual-wavelength absorbance detector (DAD 2487) set at 218 nm and a Knauer C18 analytical column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase consisted of ultrapure water (solvent A) and acetonitrile (solvent B), and gradient elution was performed as described in Table 1. The injection volume was 20 µL, and the flow rate was maintained at 1 mL/min.

A calibration curve was plotted using standard DON concentrations of 1, 2, 3.5, 5, 7.5, and 10 µg/mL. According to Commission Regulation (EU) 2023/915, the maximum permitted level of DON is 1250 µg/kg in unprocessed common cereals and 750 µg/kg in cereal flour, while in feed, it ranges from 900 to 5000 µg/kg depending on the animal species and age [14].

The Brunauer–Emmett–Teller (BET) theory explains the physical adsorption of gas molecules on solid surfaces and provides the foundation for determining the specific surface area of materials [45]. To evaluate the specific surface area and porosity characteristics, BET analysis was carried out on the best-performing adsorbent, namely acid-modified bentonite (treated with 0.1 N HNO_3), both before and after modification. The measurements were performed using nitrogen adsorption at 77 K with a BELSORP MINI surface area analyzer (BEL Japan). Prior to the analysis, the sample was degassed under vacuum for 2 hours. The analysis was conducted ac-cording to ISO standard 15901-2.

FTIR analysis was performed to identify the functional groups and characterize the structure of the most effective adsorbent, i.e., bentonite modified with 0.1 N HNO_3. The spectra were recorded in the wave-number range of 400–4000 cm^{-1} using the KBr pellet method at a resolution of 4 cm^{-1}, with a TENSOR 27 FTIR spectrometer (Bruker, Germany), both before and after acid modification.

Table 1. Gradient elution program used for the analysis of deoxynivalenol by HPLC

Time (min)	Flow Rate (mL/min)	Water (%)	Acetonitrile (%)	Injection Volume (µL)
1	1.0	90	10	20
15	1.0	90	10	20
17	1.0	90	10	20
20	1.0	10	90	20
22	1.0	10	90	20
32	1.0	90	10	20

2.8. Statistical analysis

Experiments were arranged in a completely randomized design. Data were analyzed using SAS software (v. 9.1.3). Analysis of variance (ANOVA) was applied, and Duncan's multiple range test was used for mean separation.

3. Results and discussion

3.1. Adsorption of DON by Mineral Adsorbents

The DON standard appeared at a retention time of 7.0 minutes. The concentration of DON produced by F. graminearum F48 isolate was determined to be 866.5 µg/mL based on the calibration equation.

Evaluation of raw bentonite at an adsorbent-to-toxin ratio of 200:1 (w/w) demonstrated its superior adsorption capacity among the mineral adsorbents tested. The initial DON concentration of 25.03 µg/mL was reduced to 12.15 µg/mL post-treatment, corresponding to a removal efficiency of 52.0% (Table 2).

Considerable differences were observed between the two dolomite types (Table 2). Magnetic dolomite demonstrated substantially enhanced adsorption capacity, reducing DON concentration from 25.03 µg/mL to 12.26 µg/mL (51.0% efficiency). In contrast, granulated dolomite showed limited efficacy, achieving only 24.0% removal (19.02 µg/mL residual concentration). These results suggest that modifying dolomite into its magnetic form considerably enhances its binding capacity for DON. Vermiculite exhibited moderate adsorption capacity, reducing DON concentration to 17.77 µg/mL (29.0% efficiency). Perlite showed negligible adsorption activity,

with only a minimal reduction in DON concentration from 25.03 µg/mL to 25.02 µg/mL (Table 2). Zeolite demonstrated poor adsorption characteristics, achieving only 7.5% removal efficiency (23.15 µg/mL residual concentration; Table 2). Diatomite exhibited limited adsorption capacity, reducing DON concentration to 21.27 µg/mL (15.0% efficiency; Table 2).

Table 2. Efficiency of Mineral Adsorbents for Deoxynivalenol (DON) Removal from Phosphate Buffer (pH 7.0) at a 200:1 Adsorbent: Toxin Ratio after 2 h incubation at 39 °C with shaking at 145 rpm in the dark.

Adsorbent	Adsorption (%)
Bentonite	52 ± 1.43*
Vermiculite	29 ± 1.02
Granulated Dolomite	24 ± 1.00
Magnetic Dolomite	51 ± 1.41
Perlite	2 ± 0.17
Zeolite	7.5 ± 0.56
Diatomite	15 ± 0.79

* Mean ± standard deviation (n = 3).

3.2. Adsorption of DON by Organic Adsorbents

The adsorption performance of activated carbon was evaluated across three mesh sizes, with corresponding HPLC chromatograms presented in Table 3. Activated carbon with a mesh size of 30–100 achieved 80% adsorption efficiency, reducing DON concentration from 25.07 µg/mL to 5.01 µg/mL, while the 100-300 mesh size variant showed marginally improved efficiency of 83%, reducing concentration to 4.26 µg/mL. Notably, activated carbon with a mesh size > 300 exhibited the highest performance among all tested adsorbents, achieving complete DON removal by reducing the concentration to below the detection limit, corresponding to 100% adsorption. Leonardite demonstrated a substantial adsorption capacity, reducing the deoxynivalenol concentration to 4.01 µg/mL, which corresponds to an adsorption efficiency of approximately 84% (Table 3).

Table 3. Efficiency of organic Adsorbents for Deoxynivalenol (DON) Removal from Phosphate Buffer (pH 7.0) at a 200:1 Adsorbent: Toxin Ratio after 2 h incubation at 39 °C with shaking at 145 rpm in the dark.

Adsorbent	Adsorption (%)
Activated Carbon (mesh 30–100)	80 ± 1.16*
Activated Carbon (mesh 100-300)	83 ± 1.12
Activated Carbon (mesh>300)	100 ± 0.00
Leonardite	84 ±1.85

* Mean ± standard deviation (n = 3).

3.3. Adsorption of DON by Modified Bentonite

3.3.1 Acid-Modified Bentonite

Chemical modification with 0.1 N HNO_3 (1:20 w/v, 65 °C) significantly enhanced the adsorption capacity of bentonite. This treatment achieved the highest efficiency among modified clays, reducing the DON concentration from 25.04 µg/mL to 10.02 µg/mL, which corresponds to a 60.0% removal rate (Table 4; Figure 1).

Table 4. Efficiency of acid-modified and surfactant-modified bentonite for Deoxynivalenol (DON) Removal from Phosphate Buffer (pH 7.0) at a 200:1 Adsorbent: Toxin Ratio after 2 h incubation at 39 °C with shaking at 145 rpm in the dark HDTMA.

Adsorbent	Adsorption (%)
raw bentonite	51 ± 1.20**
HCl 0.1N-modified bentonite	50 ± 1.05
HNO_3 0.1N-modified bentonite	60 ± 1.18
HDTMA-modified bentonite (0.5× CEC) *	55 ± 1.38
HDTMA-modified bentonite (3× CEC)	55 ± 1.25

*Hexadecyl trimethyl ammonium bromide (HDTMA) at cation exchange capacity (CEC) levels: 0.5× CEC

** Mean ± standard deviation (n = 3).

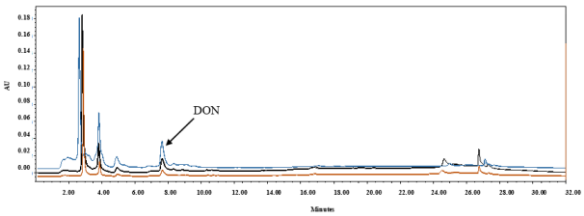


Figure 1. HPLC chromatograms of DON before and after treatment with raw and acid-modified bentonite. Blue: control (untreated DON); Black: DON after treatment with raw bentonite; Gold: DON after treatment with bentonite modified using 0.1 N nitric acid (1:20 w/v, 65 °C).

3.3.2 Cationic Surfactant-Modified Bentonite

Modification with hexadecyltrimethylammonium bromide (HDTMA) at both 0.5× and 3× the cation exchange capacity resulted in identical performance, reducing the DON concentration from 25.04 µg/mL to 11.26 µg/mL (55.0% removal efficiency; Table 4; Figure 2).

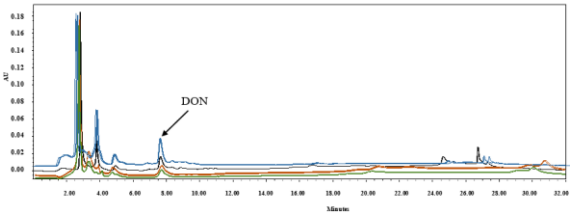


Figure 2. High-performance liquid chromatography (HPLC) analysis of deoxynivalenol (DON) before and after adsorption treatment using raw and surfactant-modified bentonite. Chromatograms are shown for untreated DON control (blue), raw bentonite (black), and bentonite modified with hexadecyltrimethylammonium bromide (HDTMA) at two cation exchange capacity (CEC) levels: 0.5× CEC (orange) and 3× CEC (green).

3.3.3 Adsorption Isotherm Analysis

Given that bentonite modified with 0.1 N nitric acid demonstrated the highest adsorption efficiency for DON among all mineral adsorbents tested, adsorption isotherm models were employed to further elucidate the underlying adsorption mechanism (Figure 3; Table 5). The optimal model was selected based on the coefficient of determination (R^2) and the congruence between experimental data and model-predicted values. As summarized in Table 5, the isotherm models ranked in order of agreement with experimental data were Redlich–Peterson> Freundlich > Temkin> Langmuir. The isotherm plots are presented in Appendix A

Table 5. Parameters and correlation coefficients (R^2) of fitted isotherm models for DON adsorption onto HNO_3 0.1N-modified bentonite.

Isotherm Model	Equation	Parameter	Value
Redlich–Peterson	y = 1.369x + 0.0219	A	0.9508
		B	1.369
		R^2	0.9197
Freundlich	y = –0.369x – 0.0505	KF	0.9507
		1/n	–0.369
		R^2	0.4542
Temkin	y = –0.1388x + 0.8026	BT	–0.1388
		KT	0.1000
		R^2	0.2909
Langmuir	y = –6.1451x + 4.144	KL	–0.7643
		qm	0.2413
		R^2	0.2399

The Redlich–Peterson isotherm is a hybrid model that incorporates features of both the Freundlich and Langmuir models, introducing three parameters into its empirical equation. Due to the higher R^2 value of the Freundlich isotherm (0.4542) compared to that of the Langmuir isotherm (0.2399), it can be inferred that the Freundlich model better represents the behavior described by the Red-lich–Peterson equation.
Moreover, the fact that the 1/n value in the Freundlich model is less than one indicates a favorable adsorption process. The corresponding isotherm plots are provided in Figure 3.

3.3.4 BET Analysis

Acid activation significantly enhanced the specific surface area of bentonite, with measured values increasing from 17.368 m^2/g for raw bentonite to 29.965 m^2/g following modification with 0.1 N nitric acid. Total pore volume similarly increased after acid treatment, from 3.9905 cm^3/g to 6.8847 cm^3/g (Figure 4, Table 6). This improvement is attributed to the removal

of impurities—including the exchange of K^+, Na^+, and Ca^{2+} cations with H^+ ions—as well as the leaching of Al^{3+}, Fe^{3+}, and Mg^{2+} from the octahedral and tetrahedral sheets of the bentonite structure. Detailed BET results are summarized in Table 6, and the corresponding nitrogen adsorption isotherms for both raw and acid-modified bentonite are pro-vided in Appendix B1.

Table 6. BET surface area and pore volume of raw and acid-modified bentonite.

Adsorbent	Surface Area (m^2/g)	Pore Volume (cm^3/g)
Raw bentonite	17.368	3.9905
HNO_3 0.1N-modified bentonite	29.965	6.8847

3.3.5. FTIR Spectral Analysis

FTIR spectra of raw and nitric acid-modified bentonite (0.1 N) are presented in appendix B.2, Figure 5. Key absorption bands include Si–O–Si bending vibrations at 468 cm^{-1}, O–Si–O vibrations of cristobalite at 521 cm^{-1}, O–Si–O bending at 623 cm^{-1}, and Si–O–Al vibrations at 794 cm^{-1}. A noticeable decrease in the intensity of these bands was observed after acid treatment. Additional bands were identified at 1040 cm^{-1} and 1085 cm^{-1} (Si–O stretching), 1639 cm^{-1} (H–O–H bending of water), 3444 cm^{-1} (O–H stretching), and 3624 cm^{-1} (Si–O–H stretching). These spectral changes support the structural modifications indicated by BET analysis.

4. Conclusion

This study evaluated the efficiency of various natural and modified mineral adsorbents for the removal of deoxynivalenol (DON), a commonly occurring mycotoxin in contaminated grains. Among all the tested mineral adsorbents, ac-id-modified bentonite demonstrated the highest binding capacity, corresponding to an adsorption efficiency of 60%. The BET analysis also confirmed a significant increase in surface area and pore volume following acid treatment, which can explain the improved adsorption behavior. To better understand the adsorption mechanism, four isotherm models were fitted to the experimental data: Redlich–Peterson, Freundlich, Langmuir, and Temkin models. The Redlich–Peterson model yielded the best fit (R^2 = 0.9197), suggesting that the adsorption process did not follow purely monolayer or multilayer behavior, but rather a hybrid mechanism. However, the relatively low R^2 values for other models indicate that none of the tested isotherms captured the experimental behavior with high accuracy, possibly due to surface heterogeneity and the complexity of DON interactions with mineral surfaces. Furthermore, vermiculite, granulated dolomite, and perlite were tested for DON adsorption in this study for the first time. Among them, magnetic dolomite showed moderate performance (51% removal), while vermiculite and granulated dolomite achieved 29% and 24% removal, respectively. In contrast, perlite had almost no effect on DON levels (approximately 2%). These findings suggest that although some of these materials are low-cost and naturally abundant, further modification would be necessary to enhance their adsorption efficiency.

Activated carbon, particularly with smaller mesh sizes, showed high removal capacities—up to 100% in the finest mesh tested. However, its application is not recommended for use in animal feed, as its non-selective adsorption may result in the unintended removal of essential organic and inorganic nutrients from the gastrointestinal tract of livestock. This raises concerns about its potential negative impacts on animal health and nutrient availability.

In summary, acid-modified bentonite emerges as a promising candidate for DON mitigation in contaminated feed. Future research should focus on surface engineering approaches to enhance the selectivity and efficiency of low-cost minerals, along with comprehensive evaluation under simulated gastrointestinal conditions, assessment of multi-mycotoxin adsorption capabilities, and in vivo validation to ensure practical applicability and animal safety.

Appendix A

Appendix A.1

The following figures illustrate the isotherm model fits for the adsorption of DON onto acid-

modified bentonite. Each model was fitted based on the experimental data, and the best fit was evaluated using R^2 values (as summarized in Table 2).

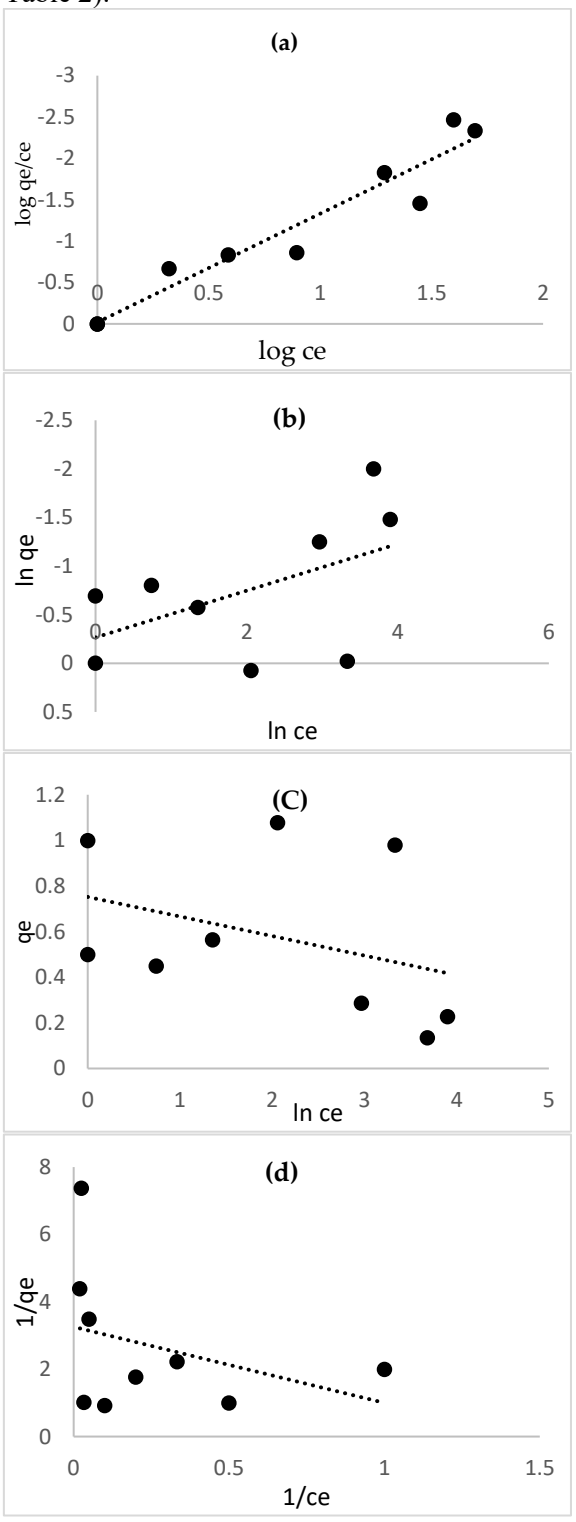


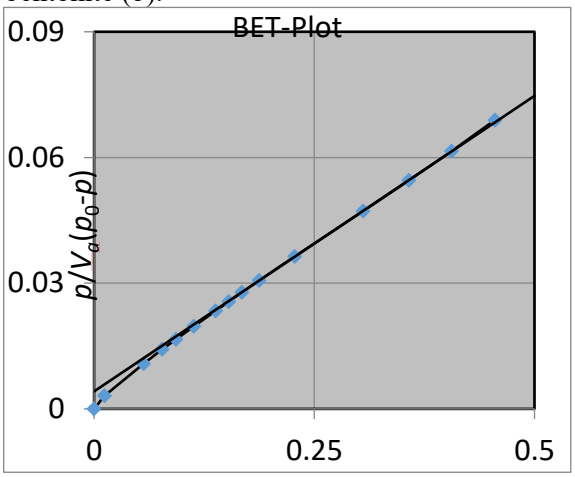
Figure 3. Linearized isotherm models for the adsorption of deoxynivalenol (DON) onto acid-modified bentonite, (a) Redlich–Peterson model, (b) Freundlich model, (c) Temkin model, (d) Langmuir model. The fitting parameters and R^2 values are presented in Table 3.

Appendix B

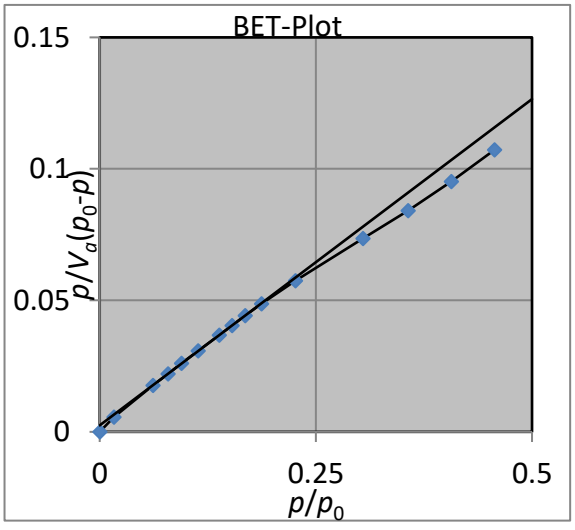
Appendix B.1

The following figure illustrates the BET adsorption isotherms of nitrogen for raw and acid-modified bentonite samples. These isotherms were used to calculate surface area and pore volume using the Brunauer–Emmett–Teller (BET) method. The corresponding values are summarized in Table 6 in the main text.

Figure 4. BET nitrogen adsorption isotherms of nitric acid-modified bentonite (a) and raw bentonite (b).



(a)



(b)

Appendix B.2

The figure below presents the Fourier Transform Infrared (FTIR) spectra of raw bentonite and bentonite modified with 0.1 N nitric acid. The spectra were recorded in the wavenumber range of 400–4000 cm^{-1}. Characteristic peaks corresponding to Si–O, Al–O, and OH functional groups are observed. Modifications in the intensity and position of absorption bands confirm structural and surface changes upon acid treatment.

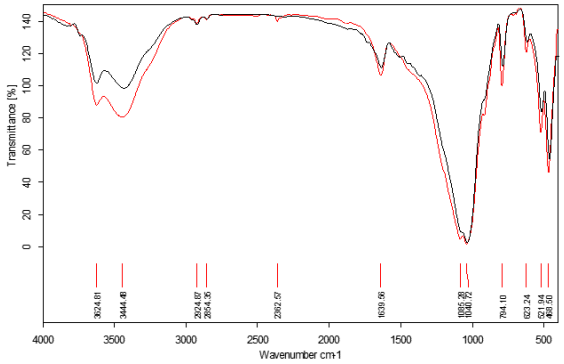


Figure 5. FTIR spectra of raw bentonite (red) and nitric acid (0.1 N)-modified bentonite (black). Key peaks include Si–O–Si bending at 468 cm^{-1}, O–Si–O and Si–O–Al vibrations at 521, 623, and 794 cm^{-1}, and Si–O stretching at 1040 and 1085 cm^{-1}. The broad band at 3444 cm^{-1} is attributed to O–H stretching, and the peak at 1631 cm^{-1} corresponds to water bending vibrations.

Abbreviations

DON	Deoxynivalenol
FTIR	Fourier Transform Infrared
BET	Brunauer–Emmett–Teller
HPLC	High Performance Liquid Chromatography
CEC	Cation Exchange Capacity
aw	Water activity
PDA	Potato Dextrose Agar
rpm	Revolutions Per Minute
EU	European Union
HDTMA	Hexadecyltrimethylammonium bromide
KBr	Potassium Bromide

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