

Article

Improving Nitrate Fertilization by Encapsulating Zn-Al Layered Double Hydroxides in Alginate Beads

Vander L. N. Nunes ^{1,*}, Richard L. Mulvaney ¹, Reinaldo B. Cantarutti ², Frederico G. Pinto ³ and Jairo Tronto ³

¹ Department of Natural Resources and Environmental Sciences, University of Illinois, Turner Hall, 1102 S. Goodwin Ave., Urbana, IL 61801, USA; mulvaney@illinois.edu

² Departamento de Solos e Nutrição de Plantas, Universidade Federal de Viçosa, Av. P. H. Rolfs-s/n, 36.570-900 Viçosa, Brazil; cantarutti@ufv.br

³ Universidade Federal de Viçosa, Instituto de Ciências Exatas e Tecnológicas, Campus Rio Paranaíba, Rodovia BR 354, km 310, CEP: 38810-000 Rio Paranaíba, MG, Brazil; fredericogarcia.ufv@gmail.com (F.G.P.); jairotonto@gmail.com (J.T.)

* Correspondence: vlenn@illinois.edu; Tel.: +1-217-419-2744

Received: 5 September 2020; Accepted: 12 October 2020; Published: 13 October 2020



Abstract: Layered double hydroxides (LDH) are anionic clays that have potential as slow-release fertilizers; however, their formulation as powders makes them difficult to apply, and their slow-release properties are impaired due to instability under acidic conditions. In the work reported, Zn-Al LDH containing interlayered $^{15}\text{NO}_3^-$ was synthesized for use as powder (LDH-N) or for encapsulation in alginate beads (LDH-AN), and then authenticated by X-ray diffraction, attenuated total reflectance-Fourier transform infrared spectroscopy, and elemental analyses. The two LDHs were compared to K^{15}NO_3 for evaluating their slow-release properties through (i) a kinetic study of NO_3^- release in water under dynamic conditions, and (ii) a growth chamber experiment designed to estimate fertilizer N uptake efficiency (FNUE) by growing pearl millet (*Pennisetum glaucum* L.) on an acidic Oxisol in the absence of N losses. Both LDH materials exhibited slow-release properties in the kinetic studies, and NO_3^- release was reduced for LDH-AN as compared to LDH-N. Because of these properties, FNUE measurements in the growth chamber experiment should have been lower with the LDHs than with K^{15}NO_3 , but this was not the case for LDH-N, which was attributed to the structural instability of powdered LDH in the presence of soil acidity and to the exchange of NO_3^- by more competitive anions such as CO_3^{2-} . A significant decrease in FNUE was observed for LDH-AN, demonstrating retention of slow-release behavior that most likely resulted from the presence of a physicochemical barrier having high cation-exchange and buffering capacities while limiting exposure to soil acidity and anion exchange. Alginate encapsulation expands the practical potential of LDH for slow-release NO_3^- fertilization.

Keywords: layered double hydroxides; alginate; ^{15}N ; fertilizer nitrogen uptake efficiency; nitrate; slow-release fertilizer

1. Introduction

The growing demand for food and feed by a burgeoning world population leaves no alternative to modern cereal production systems with intensive use of synthetic N fertilizers. Most of these fertilizers supply NH_4^+ , reflecting universal dependence on the Haber-Bosch process for NH_3 synthesis [1]. Unfortunately, there are negative consequences of applying NH_4^+ , such as soil acidity generated by nitrification and the greater concern of gaseous N loss through NH_3 volatilization following application of the three major ammoniacal fertilizers: anhydrous NH_3 , urea, and urea- NH_4NO_3 (UAN)

solutions [2–4]. A further limitation arises from the microbial preference for immobilization of NH_4^+ over NO_3^- [5–8], which promotes heterotrophic oxidation of soil organic C with concurrent depletion of soil N [9,10] and has been implicated in lower fertilizer N uptake efficiency (FNUE) for NH_4^+ than NO_3^- [11,12] when plant N uptake was not limited by leaching or denitrification. This difference would be favored by NO_3^- storage in vacuoles [13,14], whereas NH_4^+ accumulation is toxic to most plant species [15].

Except for the use of NH_4NO_3 in UAN solutions and of $\text{CaNH}_4(\text{NO}_3)_3$ (CAN) as a major N source in some parts of the world, NO_3^- fertilizers currently have a very limited presence in the commercial marketplace, due in part to their low N content that makes them more expensive and less practical than ammoniacal fertilizers. Owing to the inherent risk of N loss by leaching or denitrification, NO_3^- applications must be synchronized with crop N demand, creating a logistical complication that becomes especially problematic for large farming operations.

A slow-release matrix could enhance the convenience of NO_3^- fertilization, and has become feasible by utilizing Layered Double Hydroxides (LDHs) that have been employed in a wide variety of applications, including the removal of anionic pollutants from natural water and wastewater [16], and for the production of fertilizer materials supplying NO_3^- , phosphate, Zn, or borate [17–21].

Besides occurring naturally, LDHs can be synthesized by various techniques, the most common involving coprecipitation of di- and trivalent cations with continuous pH adjustment of an alkaline medium containing the desired anion in the absence of CO_3^{2-} [22]. The product obtained has the empirical formula $[\text{M}^{2+}_{(1-x)}\text{M}^{3+}_{(x)}(\text{OH})_2] \text{A}^{m-}_{(x/m)} \cdot n\text{H}_2\text{O}$, where M represents the two metallic cations differing in valence, A the interlayer anion of valence m , and x the mole fraction of the trivalent cation [i.e., $\text{M}^{3+}/(\text{M}^{2+} + \text{M}^{3+})$]. This fraction determines the permanent, positive charge created by isomorphic substitution of M^{3+} for M^{2+} , generating anion-exchange capacity (AEC) that typically ranges from 200 to 300 $\text{cmol}_c \text{kg}^{-1}$ [16]. Anion mobility is inversely related to charge density, and is reduced as a consequence of the physical protection afforded by the layered structures of LDHs [23].

When these materials are used as fertilizers, the rate of nutrient release is enhanced by soil acidity, which reduces their structural stability [24]. The liberated hydroxyl groups become a source of alkaline buffering that retards further loss of layer integrity, thereby retaining some of the original slow-release behavior that only disappears when the buffering capacity has been completely overcome by acidity [20]. The detrimental impact of soil acidity on the fertilizer value of LDHs is exacerbated by the prevalent practice of applying these materials as powders [18,20,24], which greatly increases the surface area for exposure to acidity and precludes accurate applications under field conditions.

Both difficulties can be addressed by incorporating LDHs within microspherical capsules that limit soil contact and can easily be applied. An ideal encapsulating agent is alginate, a natural polysaccharide obtained from brown marine algae that is economical, nonhazardous, and biodegradable. Hydrogel beads are readily formed by the dropwise introduction of an alginate sol to an aqueous solution containing Ca^{2+} or another suitable polyvalent cation, and can be conveniently loaded with the material of interest such as clays, polymers, or nonionic fertilizers [25–27].

The present project was undertaken with the goal of developing a slow-release NO_3^- fertilizer that would be suitable for a single pre-plant application. The work involved the synthesis and characterization of powdered Zn-Al LDH containing $^{15}\text{NO}_3^-$ with or without alginate encapsulation, and was followed by studies comparing the two LDH materials and K^{15}NO_3 with respect to (i) the rate of NO_3^- release evaluated dynamically, and (ii) FNUE determined in a growth chamber experiment designed to rigorously test the slow-release behavior of these materials by application to an acidic Oxisol.

2. Materials and Methods

2.1. Synthesis of Zn-Al LDHs Interlayered with $^{15}\text{NO}_3^-$

The preparation of a Zn-Al LDH interlayered with NO_3^- (LDH-N) was carried out by the constant pH coprecipitation method detailed by De Roy et al. [28]. In this method, 120 mL of an aqueous

solution 0.26 mol L^{-1} in $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 0.13 mol L^{-1} in $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ was dispensed using a peristaltic pump for dropwise addition ($\approx 0.3 \text{ mL min}^{-1}$) into a sealed reaction chamber containing 800 mL of a 0.13 mol L^{-1} solution of K^{15}NO_3 ($\approx 1.0 \text{ atom } \% \text{ }^{15}\text{N}$) that was stirred vigorously throughout the 6-h period of cation addition. To minimize the presence of CO_3^{2-} that would compete with NO_3^- , both solutions were prepared using ultrapure water obtained from a Milli-Q[®] system (MilliporeSigma, Burlington, MA, USA) that had been previously boiled under an atmosphere of N_2 for removal of CO_2 , and a similar atmosphere was maintained throughout LDH-N synthesis. Concurrent with cation addition, the solution pH was controlled between 8.75 and 9.00 by dispensing 1.0 mol L^{-1} KOH from a potentiometric titrator (Quimis Q799; Diadema, Brazil). Following synthesis, the contents of the reaction chamber were filtered under vacuum through JP41 filter paper (Quanty, Londrina, Brazil) in a 15-cm (dia.) Büchner funnel. The solid material was scraped from the filter paper into a Pyrex[®] petri dish (15-cm dia.), transferred to a desiccator for drying over silica gel, and crushed to pass through a 0.15 mm screen.

2.2. Synthesis of Zn-Al LDHs with $^{15}\text{NO}_3^-$ in Alginate Beads

Ten g of LDH-N and 1 g of sodium alginate (SA) obtained from Sigma-Aldrich (St. Louis, MO, USA) were weighed into a 100-mL beaker, 50 mL of deionized water was added, and a sol was prepared by stirring magnetically until complete homogeneity was achieved in approximately 1 h. After transfer to a 50-mL burette, the gelatinized product was dispensed by dropwise addition into a 100-mL beaker containing 50 mL of 0.2 mol L^{-1} $\text{Ca}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, forming alginate beads with LDH-N (LDH-AN). A 2 mm screen was used to separate the $\text{Ca}(\text{NO}_3)_2$ solution from the beads, which were then transferred to a 15-cm (dia.) Pyrex[®] petri dish for drying over silica gel in a desiccator to constant mass (Figure S1).

2.3. Characterization of Materials Studied

For X-ray powder diffraction (XRD) analysis to determine crystal structure, a slightly excessive amount of SA, KNO_3 , LDH-N, or LDH-AN was transferred to the cavity in a sample holder, and a microscope slide was subsequently used to compress and level the sample, such that powder filled the entire cavity and was flush with the top surface of the sample holder. The XRD analysis was accomplished by using a Shimadzu XRD-6000 instrument (Shimadzu Scientific Instruments, Inc., Kyoto, Japan) equipped with a Cu cathode and a graphite monochromator producing X-ray radiation at 1.54 \AA , 30 kV, and 30 mA, which was operated to provide a scanning range of $4\text{--}70^\circ$ in 0.01° increments with 10 s per increment.

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was used to compare NO_3^- orientation in the four materials studied. Spectra were recorded with a Jasco FTIR 4100 spectrophotometer (Jasco Deutschland GmbH, Pfungstadt, Germany), configured to obtain 256 scans over a wavelength range of 4000 to 500 cm^{-1} .

Elemental analyses of LDH-N and LDH-AN for C, H, and N were performed with a PerkinElmer 2400 Series II CHNS/O Analyzer (PerkinElmer, Waltham, MA, USA). These materials were also analyzed for Zn and Al with the use of an Agilent 240FS atomic absorption spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA), following complete dissolution by digestion with 6 mol L^{-1} HCl at 100°C . Direct combustion mass spectrometry was employed for ^{15}N analysis of the two LDH materials, and also K^{15}NO_3 , using an ANCA-GSL instrument (Sercon, Crewe, UK). Table 1 shows elemental analyses for the four materials studied, along with ^{15}N analyses for KNO_3 , LDH-N, and LDH-AN.

2.4. Rate of NO_3^- Release from Zn-Al LDHs and KNO_3

To compare the kinetics of NO_3^- release from LDH-N, LDH-AN, and KNO_3 , a stirred-flow study was conducted by utilizing a peristaltic pump (Dionex UltiMate 3000; Thermo Fisher Scientific, Waltham, MA, USA) to supply deionized water for continuous flow (1 mL min^{-1}) through a 15 mL reactor that was stirred magnetically (500 rev min^{-1}) and connected for discharge to microcentrifuge tubes in a fraction collector. In the procedure used, water flow was initiated after introducing the

fertilizer material into the reactor and positioning a cellulose filter (0.45 μm) to retain particulates, and the outlet flow was delivered for automated collection of 40 samples in 80 min, prior to colorimetric NO_3^- analyses by the method of Cataldo et al. [30].

Table 1. Chemical properties of sodium alginate (SA) Zn-Al layered double hydroxides with interlayered NO_3^- (LDH-N) in the form of powder, or Zn-Al layered double hydroxides with interlayered NO_3^- and encapsulated in alginate beads (LDH-AN). Data reported as a mean of triplicate determinations. O: determined by difference; KNO_3 and SA: as reported by Fisher Scientific [29]; na: natural abundance (0.366 atom% ^{15}N).

Material	Elemental Analysis (g kg^{-1})						Atom% ^{15}N
	C	H	O	N	Zn	Al	
KNO_3	0	0	475	139	0	0	1.844
SA	333	42	518	0	0	0	na
LDH-N	4	30	499	45	282	138	1.064
LDH-AN	51	34	458	48	277	132	1.022

2.5. Growth Chamber Experiment

2.5.1. Soil Studied

A Brazilian Oxisol (Typic Hapludox [31]), collected at a depth of 20 cm from a pasture site in the vicinity of Viçosa, Minas Gerais, was air-dried and then pulverized to <2 mm using a mortar and pestle. Table 2 shows physicochemical properties determined as detailed by Nunes et al. [32].

Table 2. Physicochemical properties of the Brazilian Oxisol used in the growth chamber experiment.

Property	Value
pH (soil:water, 1:1)	5.6
Organic C (g kg^{-1})	18.4
Total N (g kg^{-1})	1.26
Available P (mg kg^{-1})	3.8
Exchangeable K (mg kg^{-1})	39
Exchangeable Ca ($\text{cmol}_c \text{ kg}^{-1}$)	1.46
Exchangeable Mg ($\text{cmol}_c \text{ kg}^{-1}$)	0.57
Potential acidity ($\text{cmol}_c \text{ kg}^{-1}$)	7.5
Sand (%)	34
Silt (%)	12
Clay (%)	54
Water-holding capacity (%)	32

2.5.2. Procedure

Seeds of pearl millet (*Pennisetum glaucum* L., cultivar BR1501) were placed on sterilized paper towels moistened with deionized water, beginning a 2-day period for germination at 30 °C. After 1 day, a suitable potting medium was prepared by homogenizing 150 g of soil with 100 g of nutrient-free sand (>0.5 mm) in a polyethylene bag, and the mixture was then transferred to each of the 40 350-mL (12-oz) polypropylene storage containers that had been painted to exclude light (Figure S2). The three ^{15}N sources (LDH-N, LDH-AN, and KNO_3) were applied at rates of 50, 150, and 300 mg N kg^{-1} , leaving four pots to serve as unlabeled controls in a completely randomized design with four replicates. Using a spatula, the fertilizers were spread uniformly across the pots and incorporated to a depth of 0.5–1 cm, and sufficient deionized water was then added to each pot so the medium would be at 70% water-holding capacity and the pots were weighed to allow subsequent watering. The following day, eight healthy seedlings of uniform size were transplanted to be evenly spaced in each pot, and the pots were transferred to a growth chamber providing 12 h of light and dark periods at a constant

temperature of 25 °C. Thinning was done 5 days after transplanting, so as to obtain a final stand of six plants per pot, and 10 mL of a nutrient solution, mostly following Novais et al. [33], was applied to supply N (10 mg kg⁻¹), P (215 mg kg⁻¹), K (200 mg kg⁻¹), Ca (120 mg kg⁻¹), Mg (24 mg kg⁻¹), S (40 mg kg⁻¹), Zn (4 mg kg⁻¹), Cu (1.3 mg kg⁻¹), Fe (1.5 mg kg⁻¹), B (0.8 mg kg⁻¹), Mn (3.6 mg kg⁻¹), and Mo (0.1 mg kg⁻¹).

The plants were harvested 25 days after transplanting (Figure S3), by cutting the stem to within 0.5 cm of the soil surface using scissors that were cleaned with ethanol between samples to prevent isotopic cross-contamination. After inverting the pot to collect the medium by hand, the roots were separated by gentle washing with deionized water. Shoot and root samples were collected in paper bags, followed by drying for at least 72 h in a forced-air oven at 65 °C. The samples were subsequently weighed for dry matter determination, and then segmented to <3 mm using scissors that were cleaned between samples as previously described. After samples were thoroughly mixed, a subsample of each was transferred to fill approximately half the volume of a 2-mL microcentrifuge tube, a 3-mm stainless steel ball was dropped into the tube, and the tube was sealed for grinding to <150 µm using a Retsch MM 400 mixer mill (Retsch GmbH, Haan, Germany). Direct combustion mass spectrometry was employed for total N and ¹⁵N analyses.

2.6. Data Analyses

Data generated by XRD and ATR-FTIR were plotted using Origin version 7.0 software (OriginLab Corp., Northampton, MA, USA). Identification of diffraction peaks was carried out by a comparison with the hydrotalcite diffractogram from the diffraction equipment database [34] and with a series of Zn-Al LDHs described in the literature [35,36]. Interlayer distances were calculated from 2θ values by using Bragg's equation, $n\lambda = 2d_{hkl} \sin\theta$, where n is the diffraction order, λ is the wavelength of the X-ray, d_{hkl} is the interlayer distance for the hkl peak, and θ is Bragg's angle as determined by the diffraction peak. ATR-FTIR spectra were analyzed by comparison with LDH-N described in the literature [37,38].

From dry matter, total N, and isotopic data collected in the growth chamber experiment, the percentage of FNUE was calculated for shoots and roots as $N_t/N_f \times [(SR - B)/(F - B)] \times 100$, where N_t (mg pot⁻¹) is the total N determined for the plant sample analyzed, N_f (mg pot⁻¹) is the amount of fertilizer ¹⁵N applied, and the remaining variables represent isotopic enrichments (atom % ¹⁵N) measured for the shoot or root sample analyzed (SR), the fertilizer N applied (F), and the corresponding plant sample (shoot or root) collected from the unlabeled control pots (B).

Microsoft® Excel was used to generate descriptive statistics from replicate data collected in the growth chamber study. Past version 3.22 [39] was utilized for analysis of variance (ANOVA) and for mean comparisons involving Tukey's procedure ($p < 0.05$ to 0.001).

3. Results and Discussion

3.1. Characterization

X-ray diffractograms are presented in Figure 1 for KNO₃, alginate, LDH-N, and LDH-AN. The XRD pattern in Figure 1a exhibits well-defined peaks typical of a pure salt with high crystallinity. In contrast, Figure 1b shows a single weak broad diffraction peak at 13.32° but nothing else, indicating the same amorphous character documented by diffractograms previously obtained for alginate [40,41]. The X-ray diffraction pattern of the pristine LDH (Figure 1c) exhibits the presence of basal peaks (00 ℓ) [(003), (006)] related to the stacked layers characteristic of the LDH structure. The basal spacing distance (d) calculated from the position of the (003) and (006) peaks was 7.54 Å, coinciding with the value observed for NO₃⁻ intercalation in Zn-Al LDHs [35,36]. The residual presence of KNO₃ is indicated by the four peaks labeled as (*), which coincide with the corresponding peaks obtained for KNO₃ (Figure 1a) but are absent from the diffractogram obtained for LDH-AN (Figure 1d). In all other respects Figure 1c,d show the same pattern, verifying that pristine LDH-N was successfully incorporated into alginate beads.

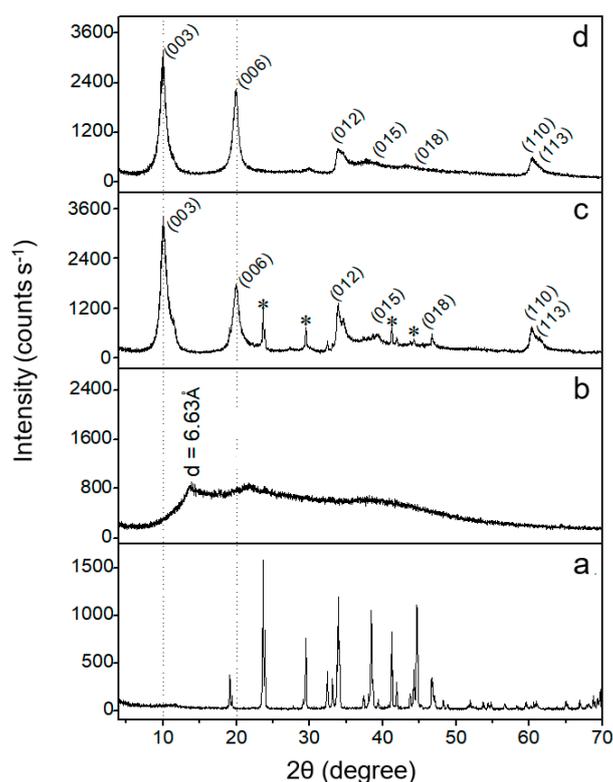


Figure 1. Diffractograms of (a) KNO_3 , (b) alginate, (c) Zn-Al layered double hydroxides with interlayered NO_3^- (LDH-N) in the form of powder, and (d) Zn-Al layered double hydroxides with interlayered NO_3^- and encapsulated in alginate beads (LDH-AN). (*) KNO_3 phase.

Figure 2 shows the functional group composition of the four materials studied when characterized by ATR-FTIR spectroscopy. The two bands noted in the spectrum obtained for KNO_3 (Figure 2a) are attributable to the vibrational modes of NO_3^- at 1350 (ν_3) and 820 cm^{-1} (ν_2) [42,43]. In the alginate spectrum (Figure 2b), the presence of the broad band in the region between 3000 and 3700 cm^{-1} is associated with OH stretching vibration; the bands at 1600 and 1420 cm^{-1} are due to symmetric and asymmetric stretching vibrations of carboxylate (COO^-) groups; and the band at 1030 cm^{-1} corresponds to the stretching vibration of C-O-C groups [44]. For LDH-N (Figure 2c), the spectrum shows a very broad band in the region between 3600 and 3000 cm^{-1} assigned to OH stretching of the H_2O molecules and hydroxyl groups present in the inorganic layers. The band related to the bending mode of water molecules is observed at 1640 cm^{-1} , while the bands at 1350 and 820 cm^{-1} confirm the presence of interlayered NO_3^- and the band at 560 cm^{-1} is assigned to the Al/Zn-OH translation mode of LDH layers [45,46]. The spectrum of LDH-AN presented in Figure 2d shows bands related to the presence of both alginate and LDH-N as evidenced by bands at 1600 , 1420 , and 1030 cm^{-1} due to vibration stretching of alginate functional groups, and by those at 1350 and 820 cm^{-1} related to modes of vibration of interlayered NO_3^- present in LDH.

3.2. Kinetic Study

Based on previous work demonstrating slow-release properties for LDHs [17,19,21,47], the same behavior was expected in the present study. Confirmation is provided by Figure 3, which compares KNO_3 , LDH-N, and LDH-AN in terms of NO_3^- release in a continuous stirred flow system using deionized water as the liquid phase. The results show a very different pattern for the two LDH materials than for KNO_3 , in that NO_3^- release occurred much more gradually and was far from complete. In both respects, the decrease was larger for LDH-AN than for LDH-N, as would be expected from the much lower surface area of alginate spheres as compared to powdered LDH.

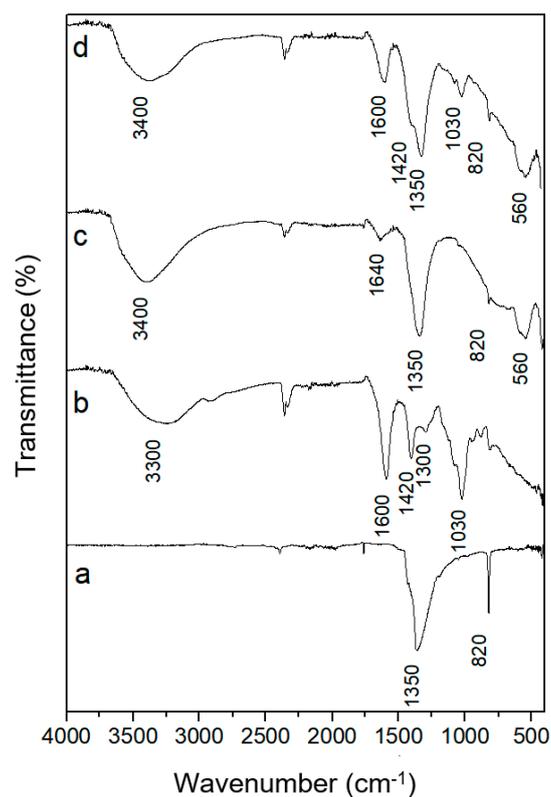


Figure 2. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) spectra of (a) KNO_3 , (b) alginate, (c) Zn-Al layered double hydroxides with interlayered NO_3^- (LDH-N) in the form of powder, and (d) Zn-Al layered double hydroxides with interlayered NO_3^- and encapsulated in alginate beads (LDH-AN).

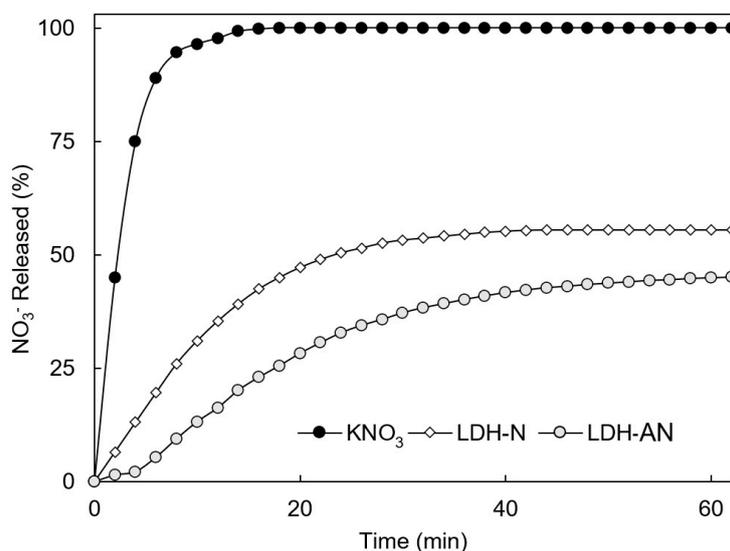


Figure 3. Dissolution of KNO_3 , Zn-Al layered double hydroxides with interlayered NO_3^- (LDH-N) in the form of powder, and Zn-Al layered double hydroxides with interlayered NO_3^- and encapsulated in alginate beads (LDH-AN), by deionized water in a continuous stirred flow system. Percentage dissolution shown for 60 min, assuming KNO_3 was completely dissolved. Data reported as a mean of three replicate samples.

3.3. Growth Chamber Experiment

To more realistically evaluate the fertilizer potential of the two Zn-Al LDH materials under acidic conditions that would differentiate their slow-release properties, a growth chamber experiment was conducted that compared FNUE when $^{15}\text{NO}_3^-$ was supplied to a tropical Oxisol by applying 50–300 mg N kg^{-1} as KNO_3 , LDH-N, or LDH-AN (Figure S2). The efficiency of ^{15}N uptake was enhanced by (i) densely planting a forage crop with high N demand (pearl millet) in a soil-sand mixture with very limited N supply, (ii) using pots without drainage holes so that leaching losses were prevented, and (iii) taking care to ensure that soil moisture content never exceeded 70% WHC, thereby controlling denitrification. In keeping with soils typical of the tropics, the Oxisol studied was used without previous pH adjustment, and soil acidity would have been exacerbated by the high planting density (6 plants per 100 cm^2).

Figure 4 compares dry matter production with and without the three N sources studied. Regardless of the N rate applied, dry matter was significantly lower with LDH-AN than with LDH-N or KNO_3 (Figure S4), which is consistent with the slow-release pattern documented by Figure 3 that was more pronounced for LDH-AN than for LDH-N.

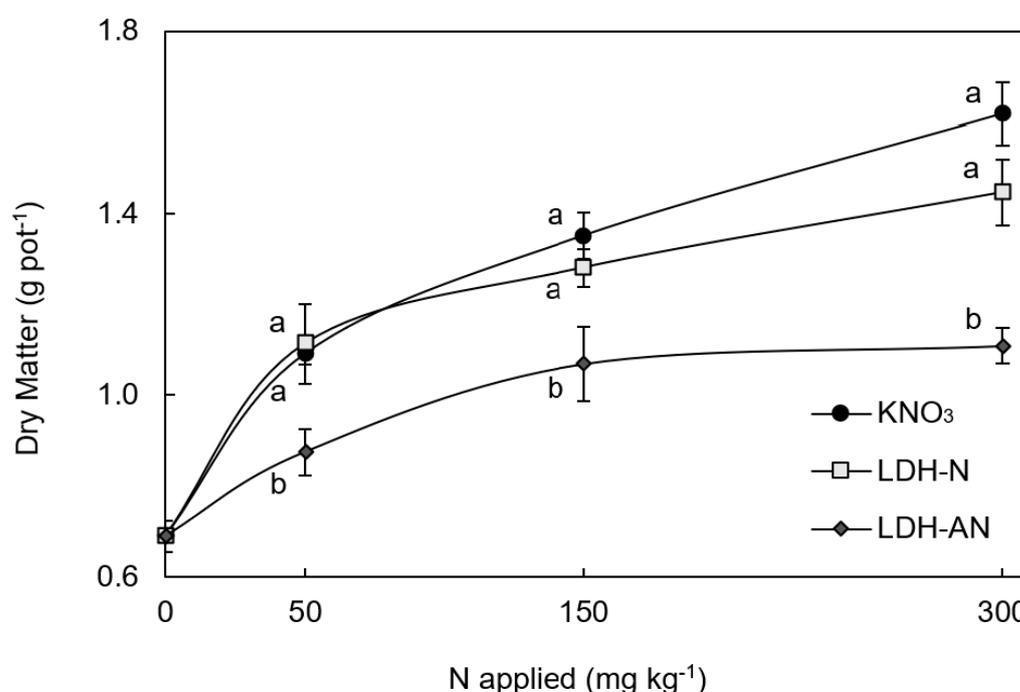


Figure 4. Dry matter determined for pearl millet (*Pennisetum glaucum* L.) grown 25 days after transplanting in a growth chamber study with an Oxisol involving application of 50, 150, or 300 mg ^{15}N kg^{-1} as KNO_3 , Zn-Al layered double hydroxides with intercalated NO_3^- (LDH-N) in the form of powder, or Zn-Al layered double hydroxides with interlayered NO_3^- and encapsulated in alginate beads (LDH-AN). Data shown for shoots plus roots as a mean from four replicate pots, with standard error bars. At each N rate, treatments having the same lowercase letter do not differ significantly ($p < 0.05$) by Tukey's test.

The FNUE calculated from N and ^{15}N data obtained for shoots plus roots are reported by Figure 5. Regardless of the N rate applied, FNUE was significantly ($p < 0.001$) lower for LDH-AN than for KNO_3 or LDH-N. The data in Figure 5 show that, when applied to an acidic soil, LDH-AN was highly effective for slowing the release of NO_3^- , whereas LDH-N was not effective.

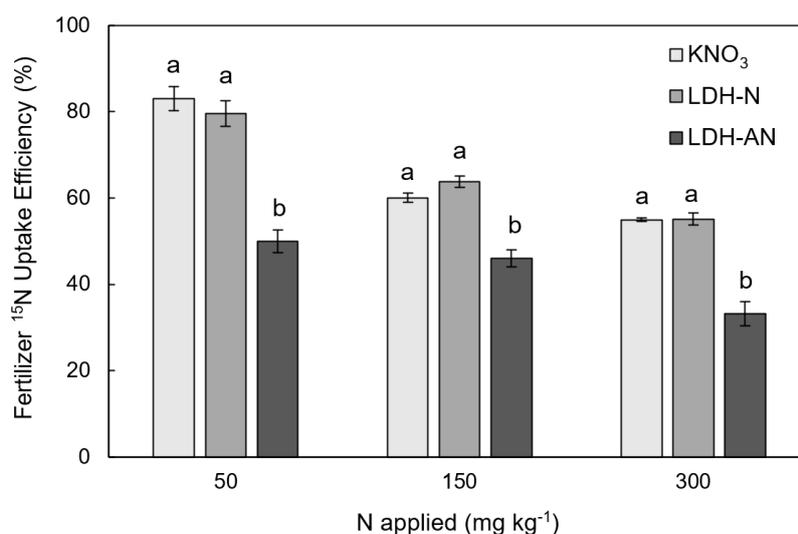


Figure 5. Fertilizer N uptake efficiency (FNUE) determined for pearl millet (*Pennisetum glaucum* L.) grown 25 days after transplanting in a growth chamber study with an Oxisol involving application of 50, 150, or 300 mg ¹⁵N kg⁻¹ as KNO₃, Zn-Al layered double hydroxides with interlayered NO₃⁻ (LDH-N) in the form of powder, or Zn-Al layered double hydroxides with interlayered NO₃⁻ and encapsulated in alginate beads (LDH-AN). Data shown for shoots plus roots as a mean from four replicate pots, with standard error bars. Treatments having the same lowercase letter do not differ significantly ($p < 0.001$) by Tukey's test.

The complete absence of slow-release behavior with LDH-N can be attributed to (i) soil acidity that would have degraded the Zn-Al LDH structure, (ii) the surface area associated with a broadcast application of powder, and (iii) weak NO₃⁻ retention by anion exchange. Owing to the presence of structural hydroxyl groups, LDHs are inherently reactive toward H₃O⁺ ions and are solubilized in the presence of acidity that exceeds their alkalizing capacity [16,20,24,48]. In the present study, excess acidity would have existed when LDH-N was applied at a rate of 50 or 150 mg N kg⁻¹, but probably not with 300 mg N kg⁻¹ unless there was substantial acidification during the uptake of basic cations by a dense stand of pearl millet. The effect of soil acidity on degrading the powdered LDH-N would have been intensified by the broadcast application that was made with subsequent incorporation to maximize soil exposure of this material. This exposure would have promoted the exchange of NO₃⁻ by other anions that are much more competitive for adsorption due to higher valence and/or charge density [16,49]. The most effective competitor would have been CO₃²⁻, which is retained more tightly than other monovalent or divalent anions [16,23] and would have been formed in abundance in the proximity of an alkalized LDH, owing to root and microbial respiration promoted by the densely planted stand of millet.

The use of alginate in conjunction with LDH was initially undertaken as a means to avoid the difficulties inherent to fertilization with powders, but the slow-release properties documented by Figure 5 for LDH-AN leave no doubt regarding the value of alginate for protecting LDHs from acidic attack. These properties cannot be due merely to a reduction in surface area by reformulating the powder as alginate beads, since recent work by Everaert et al. [19] showed that P uptake efficiency for an acidic Oxisol did not differ significantly in comparing powdered and granulated Mg-Al LDH. More likely, the high cation-exchange and buffering capacities of alginate [32,50] provided physicochemical protection, arising not only from a limitation in LDH contact with soil, but also because the cationic buffering moderated acidity in the microenvironment surrounding the alginate beads and created an electrostatic barrier that reduced NO₃⁻ exchange by competitive anions.

The fertilizer materials were each applied at three different N rates to ascertain whether a higher rate would enhance the slow-release properties of LDH materials by prolonging their presence,

which would decrease FNUE. Such a decrease was indeed observed with either LDH-N or LDH-AN (Figure 5), but also occurred with KNO_3 and thus is not indicative of slower N release. The most plausible explanation for this decline is the inverse relationship that normally exists between fertilizer N rate and FNUE.

4. Summary and Conclusions

For potential use as a slow-release NO_3^- fertilizer, an anionic clay was prepared by synthesizing Zn-Al LDH containing interlayered $^{15}\text{NO}_3^-$ and formulated as either powder (LDH-N) or alginate beads (LDH-AN). The latter formulation was superior to the former when their slow-release properties were evaluated relative to K^{15}NO_3 in a kinetic experiment using water as the liquid phase. Moreover, LDH-AN was effective for minimizing the loss of these properties in a growth chamber study with an acidic Oxisol, as documented by significantly lower FNUE than for LDH-N or K^{15}NO_3 . Alginate encapsulation expands the practical potential of LDH for slow-release NO_3^- fertilization.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2504-3129/1/2/11/s1>, Figure S1: Zn-Al- $^{15}\text{NO}_3$ layered double hydroxides encapsulated in alginate beads (LDH-AN), Figures S2–S4: growth chamber experiment with pearl millet (*Pennisetum glaucum* L.).

Author Contributions: J.T., F.G.P. and V.L.N.N. synthesized and characterized the LDHs utilized; R.B.C. and V.L.N.N. performed the stirred-flow and greenhouse experiments; V.L.N.N. and J.T. analyzed the data; R.L.M. and V.L.N.N. wrote the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded in part by FAPEMIG (The Foundation for Research of the State of Minas Gerais), process: APQ-02360-18.

Acknowledgments: We thank the CNPq (Brazilian National Council for Scientific and Technological Development) for supporting a PhD fellowship for the senior author. We also thank the Department of Soils and Plant Nutrition, Federal University of Viçosa, and the Layered Compounds Laboratory at Federal University of Viçosa, campus Rio Paranaíba, for providing research facilities and supplies used in conducting the research described.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Smil, V. *Enriching the Earth. Fritz Haber, Carl Bosch, and the Transformation of World Food Production*; MIT Press: Cambridge, MA, USA, 2001.
- Nelson, D.W. Gaseous losses of nitrogen other than through denitrification. In *Nitrogen in Agricultural Soils*; Agronomy 22; SSSA: Madison, WI, USA, 1982; Volume 22, pp. 327–363. [[CrossRef](#)]
- Bremner, J.M. Recent research on problems in the use of urea as a nitrogen fertilizer. In *Nitrogen Economy in Tropical Soils*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1995; pp. 321–329.
- Bouwman, A.F.; Van Vuuren, D.P.; Derwent, R.G.; Posch, M. A global analysis of acidification and eutrophication of terrestrial ecosystems. *Water Air Soil Pollut.* **2002**, *141*, 349–382. [[CrossRef](#)]
- Jansson, S.L. Tracer studies on nitrogen transformations in soil. *Ann. Roy. Agric. Coll. Sweden.* **1958**, *24*, 101–361.
- Rice, C.W.; Tiedje, J.M. Regulation of nitrate assimilation by ammonium in soils and in isolated soil microorganisms. *Soil Biol. Biochem.* **1989**, *21*, 597–602. [[CrossRef](#)]
- Recous, S.; Mary, B.; Faurie, G. Microbial immobilization of ammonium and nitrate in cultivated soils. *Soil Biol. Biochem.* **1990**, *22*, 913–922. [[CrossRef](#)]
- Jensen, E.S. Nitrogen immobilization and mineralization during initial decomposition of ^{15}N -labelled pea and barley residues. *Biol. Fert. Soils* **1997**, *24*, 39–44. [[CrossRef](#)]
- Khan, S.A.; Mulvaney, R.L.; Ellsworth, T.R.; Boast, C.W. The myth of nitrogen fertilization for soil carbon sequestration. *J. Environ. Qual.* **2007**, *36*, 1821–1832. [[CrossRef](#)]
- Mulvaney, R.L.; Khan, S.A.; Ellsworth, T.R. Synthetic nitrogen fertilizers deplete soil nitrogen: A global dilemma for sustainable cereal production. *J. Environ. Qual.* **2009**, *38*, 2295–2314. [[CrossRef](#)]
- Powlson, D.S.; Johnston, A.E.; Jenkinson, D.S. The nitrogen cycle in the Broadbalk wheat experiment: Recovery and losses of ^{15}N -labelled fertilizer applied in spring and inputs of nitrogen from the atmosphere. *J. Agric. Sci.* **1986**, *107*, 591–609. [[CrossRef](#)]

12. Recous, S.; Machet, J.M.; Mary, B. The fate of labelled ^{15}N urea and ammonium nitrate applied to a winter wheat crop. *Plant. Soil* **1988**, *112*, 215–224. [[CrossRef](#)]
13. Martinoia, E.; Heck, U.; Wiemken, A. Vacuoles as storage compartments for nitrate in barley leaves. *Nature* **1981**, *289*, 292–294. [[CrossRef](#)]
14. Granstedt, R.C.; Huffaker, R.C. Identification of the leaf vacuole as a major nitrate storage pool. *Plant. Physiol.* **1982**, *70*, 410–413. [[CrossRef](#)] [[PubMed](#)]
15. Gerendás, J.; Zhu, Z.; Bendixen, R.; Ratcliffe, R.G.; Sattelmacher, B. Physiological and biochemical processes related to ammonium toxicity in higher plants. *Z. Pflanzenernähr. Bodenk.* **1997**, *160*, 239–251. [[CrossRef](#)]
16. Goh, K.H.; Lim, T.T.; Dong, Z. Application of layered double hydroxides for removal of oxyanions: A review. *Water Res.* **2008**, *42*, 1343–1368. [[CrossRef](#)]
17. Silva, V.D.; Kamogawa, M.Y.; Marangoni, R.; Mangrich, A.S.; Wypych, F. Layered double hydroxides as matrices for nitrate slow release fertilizers. *Rev. Bras. Ciên. Solo* **2014**, *38*, 272–277. [[CrossRef](#)]
18. Benício, L.P.F.; Constantino, V.R.L.; Pinto, F.G.; Vergütz, L.; Tronto, J.; da Costa, L.M. Layered double hydroxides: New technology in phosphate fertilizers based on nanostructured materials. *ACS Sustain. Chem. Eng.* **2016**, *5*, 399–409. [[CrossRef](#)]
19. Everaert, M.; Degryse, F.; McLaughlin, M.J.; De Vos, D.; Smolders, E. Agronomic effectiveness of granulated and powdered P-exchanged Mg–Al LDH relative to struvite and MAP. *J. Agric. Food Chem.* **2017**, *65*, 6736–6744. [[CrossRef](#)]
20. Castro, G.F.; Ferreira, J.A.; Eulálio, D.; de Souza, S.J.; Novais, N.R.F.; Tronto, J. Layered double hydroxides: Matrices for storage and source of boron for plant growth. *Clay Miner.* **2018**, *53*, 79–89. [[CrossRef](#)]
21. Songkhum, P.; Wuttikhun, T.; Chanlek, N.; Khemthong, P.; Laohhasurayotin, K. Controlled release studies of boron and zinc from layered double hydroxides as the micronutrient hosts for agricultural application. *Appl. Clay Sci.* **2018**, *152*, 311–322. [[CrossRef](#)]
22. Leroux, F.; Besse, J.P. Polymer interleaved layered double hydroxide: A new emerging class of nanocomposites. *Chem. Mater.* **2001**, *13*, 3507–3515. [[CrossRef](#)]
23. Miyata, S. Anion-exchange properties of hydrotalcite-like compounds. *Clay Miner.* **1983**, *31*, 305–311. [[CrossRef](#)]
24. Halajnia, A.; Oustan, S.; Najafi, N.; Khataee, A.R.; Lakzian, A. Effects of Mg–Al layered double hydroxide on nitrate leaching and nitrogen uptake by maize in a calcareous soil. *Commun. Soil Sci. Plant Anal.* **2016**, *47*, 1162–1175. [[CrossRef](#)]
25. Alcantara, A.C.S.; Aranda, P.; Darder, M.; Ruiz-Hitzky, E. Bionanocomposites based on alginate–zein/layered double hydroxide materials as drug delivery systems. *J. Mater. Chem.* **2010**, *20*, 9495–9504. [[CrossRef](#)]
26. Wang, Y.; Liu, M.; Ni, B.; Xie, L. κ -Carrageenan–sodium alginate beads and superabsorbent coated nitrogen fertilizer with slow-release, water-retention, and anticompaction properties. *Ind. Eng. Chem. Res.* **2012**, *51*, 1413–1422. [[CrossRef](#)]
27. Shan, L.; Gao, Y.; Zhang, Y.; Yu, W.; Yang, Y.; Shen, S.; Yun, J. Fabrication and use of alginate-based cryogel delivery beads loaded with urea and phosphates as potential carriers for bioremediation. *Ind. Eng. Chem. Res.* **2016**, *55*, 7655–7660. [[CrossRef](#)]
28. De Roy, A.; Forano, C.; El Malki, K.; Besse, J.P. Anionic clays: Trends in pillaring chemistry. In *Expanded Clays and Other Microporous Solids*; Springer: New York, NY, USA, 1992; pp. 108–169.
29. Fisher Scientific. 2020. Available online: <https://www.fishersci.com> (accessed on 3 September 2020).
30. Cataldo, D.A.; Maroon, M.; Schrader, L.E.; Youngs, V.L. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun. Soil Sci. Plant Anal.* **1975**, *6*, 71–80. [[CrossRef](#)]
31. United States Department of Agriculture, Natural Resources Conservation Service (USDA, NRCS) Soil Taxonomy. 1999. Available online: https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_051232.pdf (accessed on 21 September 2020).
32. Nunes, V.L.N.; Mulvaney, R.L.; Tronto, J.; Cantarutti, R.B. Potential of alginate and mesoporous carbon to improve the efficiency of fertilizer urea. *Commun. Soil Sci. Plant Anal.* **2020**. [[CrossRef](#)]
33. Novais, R.F.; Neves, J.C.L.; Barros, N.F. Ensaio em ambiente controlado. In *Métodos de Pesquisa em Fertilidade do Solo*; EMBRAPA-SEA: Brasília, Brazil, 1991; pp. 189–255.
34. International Centre for Diffraction Data. 2020. Available online: <http://www.icdd.com/> (accessed on 3 September 2020).

35. Inayat, A.; Klumpp, M.; Schwieger, W. The urea method for the direct synthesis of ZnAl layered double hydroxides with nitrate as the interlayer anion. *Appl. Clay Sci.* **2011**, *51*, 452–459. [[CrossRef](#)]
36. Yasaei, M.; Khakbiz, M.; Ghasemi, E.; Zamanian, A. Synthesis and characterization of ZnAl-NO₃(-CO₃) layered double hydroxide: A novel structure for intercalation and release of simvastatin. *Appl. Surf. Sci.* **2019**, *467*, 782–791. [[CrossRef](#)]
37. Arizaga, G.G.C.; Mangrich, A.S.; da Costa Gardolinski, J.E.F.; Wypych, F. Chemical modification of zinc hydroxide nitrate and Zn–Al-layered double hydroxide with dicarboxylic acids. *J. Colloid Interface Sci.* **2008**, *320*, 168–176. [[CrossRef](#)]
38. Li, W.; Liu, A.; Tian, H.; Wang, D. Controlled release of nitrate and molybdate intercalated in Zn-Al-layered double hydroxide nanocontainers towards marine anticorrosion applications. *Colloid Interface Sci. Commun.* **2018**, *24*, 18–23. [[CrossRef](#)]
39. Paleontological Statistics Software Package for Education and Data Analysis (PAST). 2020. Available online: https://palaeo-electronica.org/2001_1/past/issue1_01.htm (accessed on 3 September 2020).
40. Kim, S.G.; Lim, G.T.; Jegal, J.; Lee, K.H. Pervaporation separation of MTBE (methyl tert-butyl ether) and methanol mixtures through polyion complex composite membranes consisting of sodium alginate/chitosan. *J. Membr. Sci.* **2000**, *174*, 1–15. [[CrossRef](#)]
41. Ionita, M.; Pandele, M.A.; Iovu, H. Sodium alginate/graphene oxide composite films with enhanced thermal and mechanical properties. *Carbohydr. Polym.* **2013**, *94*, 339–344. [[CrossRef](#)] [[PubMed](#)]
42. Miller, F.A.; Wilkins, C.H. Infrared spectra and characteristic frequencies of inorganic ions. *Anal. Chem.* **1952**, *24*, 1253–1294. [[CrossRef](#)]
43. Klopogge, J.T. Infrared and Raman spectroscopy of naturally occurring hydrotalcites and their synthetic equivalents. In *The Application of Vibrational Spectroscopy to Clay Minerals and Layered Double Hydroxides*; The Clay Minerals Society: Aurora, CO, USA, 2005; pp. 203–238.
44. Larosa, C.; Salerno, M.; de Lima, J.S.; Meri, R.M.; da Silva, M.F.; de Carvalho, L.B.; Converti, A. Characterisation of bare and tannase-loaded calcium alginate beads by microscopic, thermogravimetric, FTIR and XRD analyses. *Int. J. Biol. Macromol.* **2018**, *115*, 900–906. [[CrossRef](#)]
45. Legrouiri, A.; Badreddine, M.; Barroug, A.; De Roy, A.; Besse, J.P. Influence of pH on the synthesis of the Zn–Al–nitrate layered double hydroxide and the exchange of nitrate by phosphate ions. *J. Mater. Sci. Lett.* **1999**, *18*, 1077–1079. [[CrossRef](#)]
46. Ahmed, A.A.A.; Talib, Z.A.; bin Hussein, M.Z. Thermal, optical, and dielectric properties of Zn–Al layered double hydroxide. *Appl. Clay Sci.* **2012**, *56*, 68–76. [[CrossRef](#)]
47. Komarneni, S.; Newalkar, B.L.; Li, D.; Gheyi, T.; Lopano, C.L.; Heaney, P.J.; Post, J.E. Anionic clays as potential slow-release fertilizers: Nitrate ion exchange. *J. Porous Mater.* **2003**, *10*, 243–248. [[CrossRef](#)]
48. Mishra, G.; Dash, B.; Pandey, S. Layered double hydroxides: A brief review from fundamentals to application as evolving biomaterials. *Appl. Clay Sci.* **2018**, *153*, 172–186. [[CrossRef](#)]
49. Wang, S.L.; Wang, P.C. In situ XRD and ATR-FTIR study on the molecular orientation of interlayer nitrate in Mg/Al-layered double hydroxides in water. *Colloids Surf. A Physicochem. Eng. Asp.* **2007**, *292*, 131–138. [[CrossRef](#)]
50. Papageorgiou, S.K.; Katsaros, F.K.; Kouvelos, E.P.; Nolan, J.W.; Le Deit, H.; Kanellopoulos, N.K. Heavy metal sorption by calcium alginate beads from *Laminaria digitata*. *J. Hazard. Mater.* **2006**, *137*, 1765–1772. [[CrossRef](#)] [[PubMed](#)]

