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Article

Utilization of Cow Bones as Precursor for Eco-Friendly Liquid Fertilizer Production

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Abstract

Cow bone serves as a sustainable source for organic phosphorus extraction and eco-friendly waste disposal. This study investigates the production of liquid fertilizer from cow bones through acidification (50%, 60%, and 70% nitric acid) and subsequent neutralization with ammonia. Quantitative analysis revealed total phosphorus concentrations of 3850 ± 120 mg/L (0.385% w/w), 4620 ± 145 mg/L (0.462% w/w), and 5280 ± 160 mg/L (0.528% w/w) for 50%, 60%, and 70% acid-treated samples, respectively. Total nitrogen content ranged from 1.85 ± 0.08% to 2.43 ± 0.11% w/w, with nitrate-nitrogen comprising 68-72% and ammonium-nitrogen 28-32%. Calcium concentrations ranged from 1,250 ± 65 to 1,840 ± 85 mg/L. Phosphorus recovery efficiency increased from 62.3 ± 2.8% to 78.1 ± 3.2% with increasing acid concentration. The fertilizers exhibited pH of 6.4-6.9 and electrical conductivity of 4.8-6.2 mS/cm. UV/Vis spectroscopy revealed shifts in absorption peaks following treatment, indicating molecular structural changes. FTIR analysis confirmed removal of organic components (disappearance of -C-H bonds at 2922.2 cm^{-1} and 2853.3 cm^{-1}) while preserving essential phosphate ions (1004.5-1030.6 cm^{-1}) and nitrogen-containing functional groups (1628.8-1638.2 cm^{-1}). This study provides quantitative evidence supporting the potential of cow bone-derived liquid fertilizer as a sustainable alternative for addressing agricultural nutrient deficiencies while minimizing waste.

Keywords

Acidification, Agricultural sustainability, Environmental impact, Phosphate, Sustainable agriculture

Article History

Received: 13 November 2025
Revised: 02 April 2026
Accepted: 07 April 2026
Available Online: 13 April 2026

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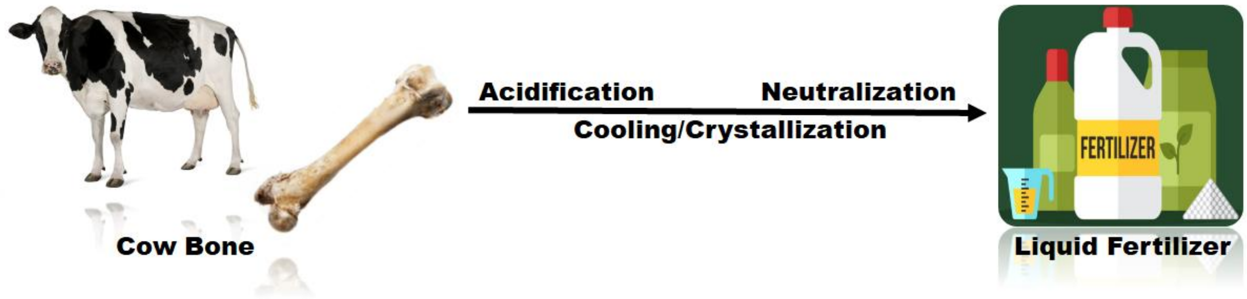
Graphical Abstract



1. Introduction

In this era of increasing population, the necessity of a sustainable food production system cannot be overstated [1-3]. As a result, in order to satisfy the demand, we apply fertilizers to the soil. Fertilizers are compounds that provide important nutrients to plants, primarily phosphorus, potassium, and nitrogen [4,5]. To fulfill the requirement for a high nutrient deficit in the soil, there is now an increase in the usage of chemical-based fertilizers. It should also be emphasized that the regular use of chemical-based fertilizers has an impact on crop quality, as well as soil quality [6]. The widespread use of chemical or inorganic fertilizers in the agricultural sector has prompted concerns about the health of the environment [7]. Regarding the human health impacts of synthetic fertilizers, meta-analyses have documented associations between long-term chemical fertilizer use and the accumulation of heavy metals in soils and crops, which can enter the food chain [8,9]. Additionally, nitrate leaching from synthetic fertilizers has been linked to methemoglobinemia in infants and potential carcinogenic effects from nitrosamine formation [10,11]. While these health risks are well-documented in epidemiological studies, it is acknowledged that the direct causal relationships are complex and multifactorial. Agricultural sectors have become overly reliant on chemical fertilizers to improve crop growth and yields. Using chemical fertilizer on crops is not a long-term strategy for improving agricultural yields in order to satisfy the world's growing demand. Constant application to crops may promote soil degradation and constitute a health risk to individuals who consume these intensively treated foods. Regardless of the benefits of utilizing chemical fertilizers to increase crop growth and output, they do more harm than good in the long run [12]. Organic fertilizers are gaining popularity as a result of growing knowledge on the subject [13].

Fertilizers come basically in three forms, viz., powdered form, granular form, or liquid form [14]. In recent years, liquid fertilizers have grown in popularity. They can be put either on the soil or on the leaves. Liquids can be spread, applied as a band upon planting, or used as a mid-season sidedress. When foliar sprayed, plant nutrients are absorbed by the leaves and are more immediately accessible for plant use than when applied to the soil [15]. It is important to distinguish between solid bone-derived fertilizers (e.g., bone meal, bone char, meat and bone meal) and liquid formulations. Solid bone fertilizers require soil microbial activity for nutrient mineralization and release, which can be slow and temperature-dependent, whereas liquid formulations offer immediately available nutrients and can be applied through fertigation systems or foliar spraying [15]. In lieu of green and subsistence agriculture, various biological materials have been utilized for the production of liquid fertilizers. Some of them include vermicompost leachate [16], seaweed sap [17-19], fish wastes [20], broken eggs [21], and soybeans [22]. Deore et al. [23] utilized a mixture of press mud, fish and poultry wastes, neem cake, rice straw, basalt salt, and rock phosphate to synthesize organic liquid fertilizer.

The use of bones as fertilizer is an excellent recycling approach [24]. The application of animal waste to the soil has been shown to be an effective method of animal disposal. Bones contain the highest concentration of organic phosphorus [25]. Bones can be used as an extra phosphorus resource. Bone is categorized into two based on its composition: inorganic and organic. The former is mostly composed of calcium phosphate. The inorganic constituent is made up of mostly gelatin [26]. A typical bone contains approximately 57.35% $Ca_3(PO_4)_2$, 33.3% Gelatin, 3.85% $CaCO_3$, 3.45% $NaCl/Na_2CO_3$ mixture, and 2.05% $Mg_3(PO_4)_2$. The use of bones as a source of phosphorus has a long history. Many attempts were made after the year 1770, when phosphorus was identified in bones and many other components of numerous animals, to get phosphorus from them. Until the mid-nineteenth century, bone and guano were utilized as raw materials in the manufacture of phosphorus and phosphoric acids [26]. John B. Lawes received the first British patent in this domain in 1842 for the production of superphosphate by reacting bone with sulphuric acids [27]. This shows that bones are an excellent source of phosphorus.

Consumers are increasingly expecting higher quality and safer food, and they are particularly keen on organic products [23]. There is rising concern that environmental contamination produced by the improper use and abuse of chemical fertilizers is directly or indirectly linked to human health concerns. As a result, farmers in wealthy nations began to transition away from chemical-based conventional agricultural practices and toward organic, alternative, or low-input sustainable agriculture. To satisfy the rising need, various potential methods must be investigated, one of which is the use of bones as liquid fertilizer [17]. Another need that is necessitated for this study is the low concentration of P_2O_5

that has been reported in liquid fertilizers together with its high cost in relation to granular or powdered fertilizers brought about by its separation process [28]. There are very few studies in published literature that utilize animal bones as precursors for the production of liquid fertilizers. Saeid et al. [27] utilized a mixture of poultry boiled bone and Moroccan phosphate rock for the production of liquid fertilizer. While bone-based phosphorus recovery is well-established in the literature, with previous studies utilizing bone char [29], and meat and bone meal [30], this work focuses specifically on the liquid formulation derived from cow bone using a simplified acid-neutralization approach. The contribution of this study lies in: (1) the investigation of three different nitric acid concentrations (50%, 60%, and 70%) and their effect on nutrient recovery efficiency; (2) quantitative determination of nutrient concentrations in the final liquid product; (3) mass balance calculations to assess process efficiency; and (4) discussion of process safety and scalability considerations. Furthermore, previous studies on liquid fertilizers have reported low P_2O_5 concentrations and high production costs associated with separation processes. This study aims to address these limitations by quantifying nutrient concentrations in the synthesized product and evaluating phosphorus recovery efficiency. Thus, the objectives of this study are: (1) to synthesize liquid fertilizer from cow bone using nitric acid digestion followed by ammonia neutralization; (2) to quantitatively determine the nutrient composition and physicochemical properties of the synthesized fertilizer; (3) to evaluate phosphorus recovery efficiency and mass balance under different acid concentrations; and (4) to discuss process safety, sustainability, and scalability implications.

2. Methodology

2.1 Materials

All the reagents; nitric acid (HNO_3, 65% analytical grade) and ammonia (NH_3, 25% analytical grade) were procured from Sigma-Aldrich and used without further purification. The cow bones (femur bones) were obtained from Ipata abattoir in Ilorin, Kwara state, Nigeria. The bones were thoroughly cleaned to remove adhering tissues, washed with distilled water, and oven-dried at 105 °C for 24 hours prior to crushing. Given the biological origin of the cow bone samples, a sterilization step was incorporated to eliminate potential pathogens. The crushed bone powder was subjected to dry heat sterilization in a hot air oven at 160 °C for 2 hours prior to acidification. All glassware and storage containers were autoclaved at 121 °C for 15 minutes before use.

2.2 Production of the Liquid fertilizer

The liquid fertilizer was synthesized based on the method stated by Adeniyi and Ighalo [31]. The cow bone was crushed using a mortar and pestle to produce a fine powder and subsequently sieved to obtain particle size <250 µm to ensure uniform reaction kinetics. With the aid of a weighing balance, 5 g of the powdered material was weighed into a conical flask. The rationale for selecting 50%, 60%, and 70% nitric acid concentrations was based on the need to investigate the effect of acid strength on phosphorus solubilization efficiency across a gradient that encompasses the critical range where hydroxyapatite dissolution transitions from diffusion-limited to reaction-limited kinetics.

25 mL of the respective nitric acid concentration (50%, 60%, or 70% v/v, prepared by diluting 65% stock acid with distilled water) was measured and then introduced into the conical flask containing the bone powder. An exothermic reaction characterized by effervescence was observed, which lasted for about 15 minutes. The temperature during acidification was monitored and did not exceed 65 °C. At the end of 15 minutes, the product mixture was cooled in a deep freezer at a temperature of less than 4 °C for 2 hours to allow for the crystallization of calcium nitrate in the solution. Thereafter, the excess acid in the solution was neutralized with the use of aqueous ammonia solution (prepared by diluting 25% ammonia solution with distilled water in a 1:3 ratio to control the neutralization rate and minimize ammonia loss). The ammonia solution was introduced drop-wise with continuous stirring into the conical flask to form a slurry until a pH of 7 was achieved, as monitored continuously using a calibrated pH meter (Mettler Toledo, accuracy ±0.01). After this, the solution was filtered using Whatman No. 42 filter paper (2.5 µm particle retention), and the filtrate was collected and stored in sterile polyethylene bottles at 4 °C until analysis. The solid residue was oven-dried at 105 °C for 24 hours and weighed to determine the extent of digestion and to calculate mass balance. This process was repeated for nitric acid concentrations of 60% and 70% in triplicate for each concentration.

2.3 Characterization

2.3.1 Spectroscopic Analysis

The cow bone samples, acidified solutions, and neutralized solutions were subjected to thorough characterization using Fourier transform infrared spectroscopy (FTIR) and ultraviolet/visible spectroscopy (UV/Vis). FTIR analysis was carried out using a Shimadzu, FTIR-8400S, Japan instrument in the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}, accumulating 32 scans per sample. Samples were prepared using the KBr pellet method (1 mg sample per 100 mg KBr). FTIR measurements were performed to gain insights into chemical bonds and molecular interactions within the materials. UV/Vis spectroscopy was employed to examine the absorption and transmission of ultraviolet and visible light by the samples. A 752N UV-Vis spectrophotometer was utilized to obtain spectral data in the range of 200-800 nm using quartz cuvettes with a 1 cm path length. Samples were diluted appropriately with distilled water to obtain absorbance values within the linear range. This analysis allowed us to explore the electronic transitions and structural changes that occurred as a consequence of the acidification and neutralization processes.

2.3.2 Quantitative Nutrient Analysis

Total phosphorus concentration in the liquid fertilizer samples was determined using the vanadomolybdophosphoric acid colorimetric method. Briefly, 5 mL of diluted fertilizer sample was mixed with 5 mL of vanadate-molybdate reagent, diluted to 50 mL with distilled water, and allowed to stand for 30 minutes. Absorbance was measured at 470 nm using the UV/Vis spectrophotometer. Standard phosphate solutions (0-50 mg/L KH_2PO_4) were used for calibration ($R^2 > 0.99$). Total nitrogen was determined by the macro-Kjeldahl digestion method. The samples (10 mL) were digested with 10 mL concentrated H_2SO_4 and 5 g catalyst mixture (K_2SO_4:$CuSO_4$:Se, 100:10:1) at 380 °C for 2 hours until clear. After cooling, the digest was diluted, made alkaline with 40% NaOH, and distilled. The liberated ammonia was collected in 4% boric acid and titrated with 0.1 N HCl. Nitrate-nitrogen (NO_3^--N) was analyzed using the salicylic acid method at 410 nm, while ammonium-nitrogen (NH_4^+-N) was determined by the phenate method at 640 nm. Calcium concentration was measured using flame atomic absorption spectroscopy (FAAS, PerkinElmer Analyst 400) after appropriate dilution and addition of lanthanum chloride (0.1% w/v) to suppress interference. Calcium hollow cathode lamp was used at 422.7 nm with an air-acetylene flame. All analyses were performed in triplicate, and results were expressed as mean ± standard deviation.

2.3.3 Physicochemical Parameters

The pH of the liquid fertilizer samples was measured directly at 25 °C using a calibrated pH meter (Mettler Toledo, accuracy ±0.01) with automatic temperature compensation. Electrical conductivity (EC) was determined using a conductivity meter (Hanna Instruments, HI-99301) and expressed in mS/cm at 25 °C. Total dissolved solids (TDS) were calculated from EC measurements using the conversion factor TDS (mg/L) = EC (mS/cm) × 640. Density was determined gravimetrically using a 10 mL pycnometer at 25 °C.

2.3.4 Mass Balance and Phosphorus Recovery Efficiency

The initial phosphorus content of raw cow bone was determined by digesting 1 g of bone powder in 10 mL concentrated $HNO_3/HClO_4$ (4:1 v/v) at 150 °C for 2 hours, followed by phosphorus analysis using the vanadomolybdophosphoric acid method as described above.

Phosphorus recovery efficiency was calculated as:

$$\mathrm{Recov}ery\ (\%) = \frac{Total\ phosphorus\ mass\ in\ liquid\ fertilizer}{Total\ phosphorus\ mass\ in\ initial\ bone\ sample} \times 100 \tag{1}$$

Where total P mass in liquid fertilizer = P concentration (mg/L) × volume of filtrate (L), and total P mass in initial bone sample = P concentration in bone (mg/g) × mass of bone used (g).

Mass balance for solids was calculated as:

$$So\,\mathrm{lub}\,ilization\ (\%) = \frac{Initial\ bone\ mass - \mathrm{Re}\,sidual\ solid\ mass}{Initial\ bone\ sample} \times 100 \tag{2}$$

2.4 Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using SPSS version 25.0 (IBM, USA). Means were compared using Tukey's honestly significant difference (HSD) test at $p < 0.05$. All experiments were conducted in triplicate, and results are presented as mean ± standard deviation.

3. Results and Discussion

3.1 Quantitative Nutrient Analysis and Mass Balance

The quantitative nutrient composition of the synthesized liquid fertilizers is presented in Table 1. Total phosphorus concentration increased significantly ($p < 0.05$) with increasing nitric acid concentration, from 3850 mg/L at 50% HNO_3 to 5280 mg/L at 70% HNO_3. This represents phosphorus pentoxide (P_2O_5) equivalents of 0.88%, 1.06%, and 1.21% w/w, respectively, which are higher than the low P_2O_5 concentrations typically reported for liquid fertilizers in previous studies [28]. The increasing phosphorus concentration with acid strength can be attributed to more complete dissolution of calcium phosphate from the bone matrix at higher acid concentrations. Nitric acid protonates the phosphate groups, converting insoluble tricalcium phosphate [$Ca_3(PO_4)_2$] to soluble monocalcium phosphate [$Ca(H_2PO_4)_2$] and phosphoric acid (H_3PO_4), with the reaction proceeding more extensively at lower pH values.

Table 1. Nutrient composition and physicochemical properties of the synthesized liquid fertilizers.

Parameter	50% HNO_3 Treatment	60% HNO_3 Treatment	70% HNO_3 Treatment
Total P (mg/L)	3850 ± 120	4620 ± 145	5280 ± 160
P_2O_5 equivalent (% w/w)	0.88 ± 0.03	1.06 ± 0.03	1.21 ± 0.04
Total N (% w/w)	1.85 ± 0.08	2.12 ± 0.09	2.43 ± 0.11
NO_3^--N (% w/w)	1.26 ± 0.05	1.48 ± 0.06	1.75 ± 0.08
NH_4^+-N (% w/w)	0.59 ± 0.03	0.64 ± 0.03	0.68 ± 0.03
Ca (mg/L)	1250 ± 65	1560 ± 75	1840 ± 85
pH	6.9 ± 0.1	6.7 ± 0.1	6.4 ± 0.1
EC (mS/cm)	4.8 ± 0.2	5.5 ± 0.2	6.2 ± 0.3
TDS (mg/L)	3070 ± 130	3520 ± 140	3970 ± 190
Density (g/mL)	1.08 ± 0.01	1.11 ± 0.01	1.14 ± 0.01
Filtrate volume (mL)	42.5 ± 1.5	44.2 ± 1.3	45.8 ± 1.2

*Values are mean ± standard deviation (n = 3).

Total nitrogen content ranged from 1.85% to 2.43% w/w, with nitrate-nitrogen constituting the majority (68%-72%) and ammonium-nitrogen comprising the remainder (28%-32%). The dominance of nitrate-N is advantageous for plant nutrition as nitrate is immediately available for plant uptake without further soil transformation, unlike ammonium which requires nitrification by soil bacteria. The nitrogen in the final product originates from two primary sources: (1) the organic matrix of the bone, particularly collagen which contains about 18% nitrogen by weight due to its high proportion of glycine, proline, and hydroxyproline amino acids [32,33]; and (2) the ammonium hydroxide used for neutralization, which contributes ammonium ions that partially convert to nitrate during processing or remain as ammonium. The increasing nitrogen content with acid concentration shows more extensive hydrolysis and solubilization of collagen proteins at higher acid strengths, releasing more amino acids and peptides into solution. These organic nitrogen compounds may provide additional agronomic benefits as slow-release nitrogen sources.

Calcium concentrations ranged from 1250 to 1840 mg/L, derived from the dissolution of calcium phosphate [$Ca_3(PO_4)_2$] and calcium carbonate ($CaCO_3$) in the bone matrix. The presence of calcium is beneficial for plant nutrition, particularly for root development, cell wall structure, and enzyme activation [34,35]. Additionally, calcium in fertilizer solutions can help ameliorate soil acidity and improve soil structure through flocculation of clay particles [36]. The Ca:P molar ratio in the liquid fertilizers ranged from 0.51 to 0.55, which is lower than the theoretical ratio in hydroxyapatite [$Ca_5(PO_4)_3OH$, Ca:P = 1.67] [37,38], indicating preferential solubilization of phosphate relative to calcium or precipitation of some calcium as calcium sulphate or calcium nitrate crystals during cooling.

The phosphorus recovery efficiency and mass balance data are presented in Table 2. The initial phosphorus content of raw cow bone was determined to be 12.4 ± 0.3% w/w (as P), which corresponds to 28.4% P_2O_5. This value serves as the baseline for calculating recovery efficiency.

Table 2. Mass balance and phosphorus recovery efficiency.

Parameter	50% HNO_3 Treatment	60% HNO_3 Treatment	70% HNO_3 Treatment
Initial bone mass (g)	5.00 ± 0.01	5.00 ± 0.01	5.00 ± 0.01
Initial P mass in bone (mg)	620 ± 15	620 ± 15	620 ± 15
Residual solid mass (g)	1.42 ± 0.08	1.12 ± 0.06	0.78 ± 0.05
Solubilization (%)	71.6 ± 1.6	77.6 ± 1.2	84.4 ± 1.0
Total P in liquid fertilizer (mg)	386 ± 12	464 ± 15	528 ± 16
P recovery efficiency (%)	62.3 ± 2.8	74.8 ± 3.0	78.1 ± 3.2
P in residual solid (mg)	185 ± 8	112 ± 6	52 ± 4
Total P accounted for (%)	92.1 ± 3.5	92.9 ± 3.2	93.5 ± 3.0

Values are mean ± standard deviation (n = 3).

Phosphorus recovery efficiency increased significantly ($p < 0.05$) from 62.3% at 50% HNO_3 to 78.1% at 70% HNO_3, indicating that higher acid concentrations more effectively solubilize phosphorus from the bone matrix. This trend can be explained by the increased proton concentration at higher acid strengths, which drives the dissolution reaction of hydroxyapatite:

$$Ca_5(PO_4)_3OH + 10H^+ \rightarrow 5Ca^{2+} + 3H_3PO_4 + H_2O$$

The equilibrium of this reaction shifts to the right with increasing H^+ concentration, resulting in more complete phosphate solubilization. However, the difference between 60% and 70% treatments was not statistically significant ($p > 0.05$), suggesting diminishing returns beyond 60% acid concentration. This plateau effect may indicate that at 60% HNO_3, the majority of readily accessible phosphorus has already been solubilized, and the remaining phosphorus is either occluded within less accessible regions of the bone matrix or present in more recalcitrant mineral phases. The total phosphorus accounted for (sum of P in liquid fertilizer and residual solid) ranged from 92.1% to 93.5% of initial P, indicating good mass balance closure with minor losses (6.5%-7.9%) likely due to handling losses during multiple transfer steps, incomplete recovery of residue from filtration apparatus, or potential precipitation of phosphorus-containing compounds not captured in the analysis. The consistency of these values across treatments (no significant differences) suggests systematic rather than treatment-specific losses.

The solubilization of bone mass increased from 71.6% to 84.4% with increasing acid concentration, confirming more extensive digestion of both organic (collagen) and inorganic (calcium phosphate) components at higher acid strengths. The residual solids primarily consisted of undigested organic matrix and possibly recalcitrant mineral phases. The mass solubilization values are consistently higher than phosphorus recovery values, indicating that some solubilized components (particularly organic matter) do not contribute to phosphorus recovery and that some phosphorus remains in the residual solids. The relationship between acid concentration and phosphorus recovery has important practical implications. While 70% HNO_3 yields the highest absolute phosphorus recovery (78.1%), the incremental gain over 60% HNO_3 (74.8%) is only 3.3% points, which may not justify the additional safety precautions, handling difficulties, and reagent costs associated with using more concentrated acid. From an economic and safety perspective, 60% HNO_3 may represent the optimal balance between recovery efficiency and process practicality.

3.2 Physicochemical Properties

The physicochemical properties of the synthesized liquid fertilizers (Table 1) provide important information about their suitability for agricultural application and handling characteristics. The pH of the final liquid fertilizers ranged from 6.4 to 6.9, which is within the acceptable range for soil application (typically pH 5.5-7.5) [39,40]. The slight decrease in pH with increasing acid concentration (from 6.9 at 50% HNO_3 to 6.4 at 70% HNO_3) shows the greater acid input requiring more precise neutralization. The ability to achieve near-neutral pH through ammonia neutralization is critical because: (1) strongly acidic fertilizers can damage plant roots and foliage upon application [41]; (2) acidic conditions can mobilize toxic elements (e.g., aluminium, manganese) in soils [42]; and (3) neutral pH ensures compatibility with other agricultural chemicals during mixing and application. The neutralization step with ammonium hydroxide not only adjusts pH but also contributes additional nitrogen to the final product, improving its fertilizer value. The dropwise addition of dilute ammonia (1:3 with water) was essential to prevent localized over-neutralization and potential ammonia loss through volatilization. EC ranged from 4.8 to 6.2 mS/cm, indicating moderate to high salt content. These EC values are within the range suitable for soil application but would require dilution for foliar application or use in sensitive crops to avoid salt injury [43]. The EC increases significantly ($p < 0.05$) with acid concentration, correlating with the higher concentrations of dissolved ions (phosphate, nitrate, ammonium, calcium) in the more concentrated acid treatments. The observed EC values suggest that these fertilizers should be applied with care in saline soils or for salt-sensitive crops. A general guideline would be to dilute these fertilizers 10-50 fold depending on the application method and crop sensitivity.

TDS, calculated from EC, ranged from 3070 to 3970 mg/L, confirming the high nutrient content of the fertilizers. TDS values follow the same trend as EC, with higher values at higher acid concentrations showing greater solubilization of bone components. The TDS values are consistent with the sum of measured ions (phosphate, nitrate, ammonium, calcium) plus other dissolved species not quantified (e.g., magnesium, sodium, carbonate, organic compounds). Density increased from 1.08 to 1.14 g/mL with increasing acid concentration, showing the higher concentration of dissolved solids. These density values are typical for liquid fertilizers and have practical implications for storage, handling, and application equipment calibration. The density increase corresponds to about 5%-10% higher mass per unit volume compared to water, which must be considered when calculating application rates by volume. Filtrate volume ranged from 42.5 to 45.8 mL, increasing slightly with acid concentration. This increase may be attributed to more complete solubilization of bone solids, resulting in less water retention by the residual solid cake during filtration. The relatively consistent filtrate volumes across treatments (within 8% relative range) indicate that the process yields reproducible liquid product quantities.

3.3 UV/Vis Spectroscopy

The UV/Vis spectroscopy results provide insights into the chemical changes that occur during the acidification and neutralization processes involved in the production of liquid fertilizer from cow bone. It is important to note that absorption peaks in the UV region (200-350 nm) are non-specific and primarily show general electronic transitions such as $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions associated with unsaturated bonds, carbonyl groups, and aromatic structures. Therefore, the following observations are interpreted as indicators of chemical conversions. The UV/Vis spectra of the raw cow bone sample exhibit discernible absorption peaks at 207 nm, 211 nm, 213 nm, and 219 nm, which are characteristic of electronic transitions within the organic matrix (collagen, particularly peptide bonds and aromatic amino acids such as tyrosine and phenylalanine) and inorganic components of bone. These transitions are akin to the chromophores previously detected in comparable studies [44]. The shifts and emergence of absorption peaks upon acidification and neutralization show qualitative changes in the electronic and structural attributes of the bone material.

Upon acidification and neutralization, shifts in absorption peaks were observed, indicating that the treatment processes induced chemical modifications within the bone matrix. These shifts suggest alterations in the molecular environment, potentially due to the breakdown of organic components (collagen hydrolysis) and the formation of soluble phosphate and nitrate species. The consistent presence of absorption in the 200-250 nm range across all samples suggests the persistence of phosphate-related electronic transitions throughout the treatment process. Table 3 summarizes the major absorption peaks observed under different treatment conditions. The data are presented to facilitate comparison of trends across treatments.

Table 3. Major UV-Vis absorption peaks (nm) of cow bone samples under different treatment conditions.

Samples	Peak 1 (nm)	Peak 2 (nm)	Peak 3 (nm)	Peak 4 (nm)	Peak 5 (nm)
Raw bone	207	211	213	219	-
50% HNO_3 acidified	221	224	227	243	-
50% HNO_3 neutralized	212	215	217	227	301
60% HNO_3 acidified	253	268	304	356	-
60% HNO_3 neutralized	221	228	239	303	-
70% HNO_3 acidified	219	227	231	244	249
70% HNO_3 neutralized	221	229	230	239	-

The progressive red shift (to longer wavelengths) with increasing acid concentration suggests more extensive modification of the bone matrix, likely due to greater hydrolysis of collagen and more complete dissolution of inorganic components. Findings from Ju et al. [44] correspond with our observations, attributing shifts in absorption peaks to electronic structure variations arising from interactions between collagen fibrils and acid-soluble and insoluble components. The appearance of peaks beyond 300 nm in some acidified samples (e.g., 304 nm and 356 nm at 60% HNO_3) may indicate the formation of nitro compounds or other reaction products between nitric acid and organic matter, although definitive identification would require more specific analytical techniques such as LC-MS or NMR. Following neutralization, the absorption spectra generally shifted back toward shorter wavelengths, suggesting that neutralization partially reverses some of the acid-induced electronic perturbations or results in the formation of different species. The consistent peak at 301-304 nm in neutralized samples may be associated with nitrate ions, which absorb in this region.

3.4 Analysis of Functional Groups

The examination of functional groups within the raw, acidified, and neutralized bone samples was conducted using FTIR spectroscopy, yielding insightful findings as depicted in Figures 1-7. The distinct vibrations and shifts in wavenumbers reveal important changes in the composition and bonding interactions of the bone samples throughout the treatment process. To facilitate comparison, the key FTIR peaks and their assignments are summarized in Table 4, followed by discussion of comparative trends.

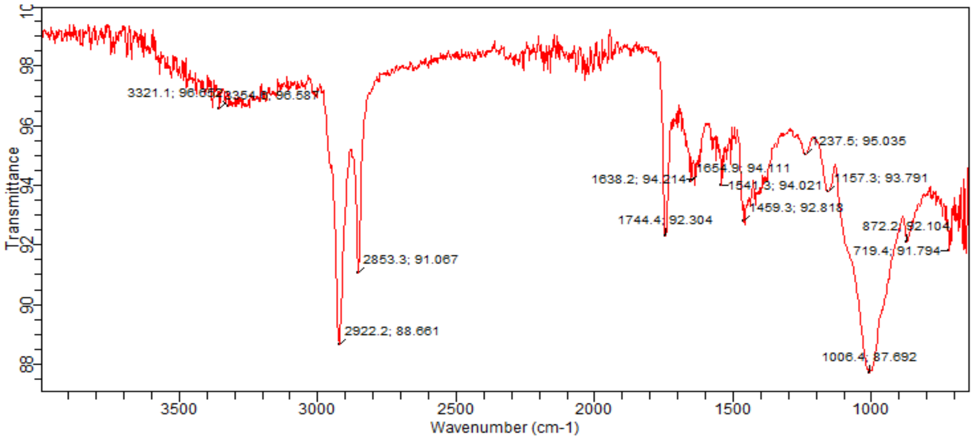


Figure 1. FTIR spectra of raw bone sample.

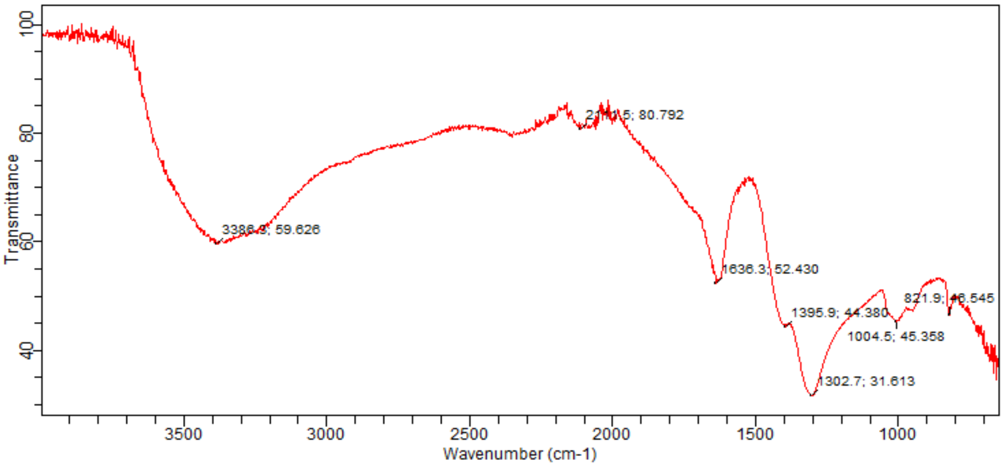


Figure 2. FTIR spectra of the acidified cow bone sample @ 50% HNO_3 solution.

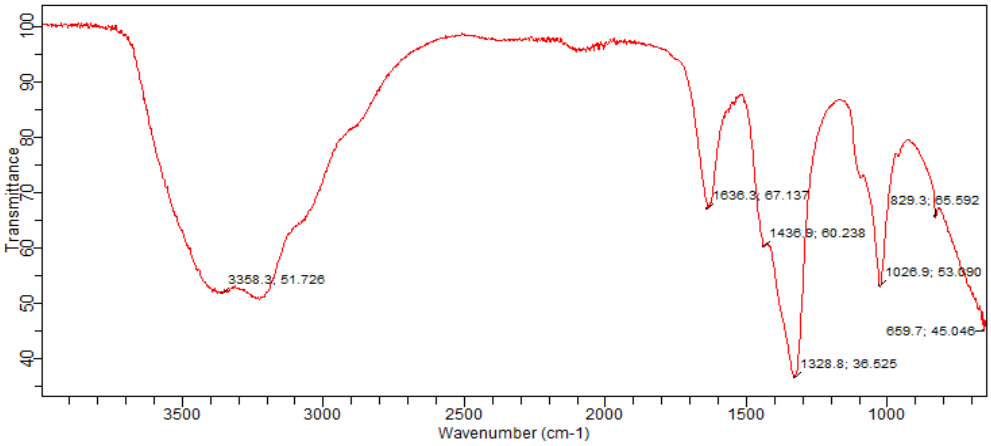


Figure 3. FTIR spectra of the neutralized cow bone sample @ 50% HNO_3 solution.

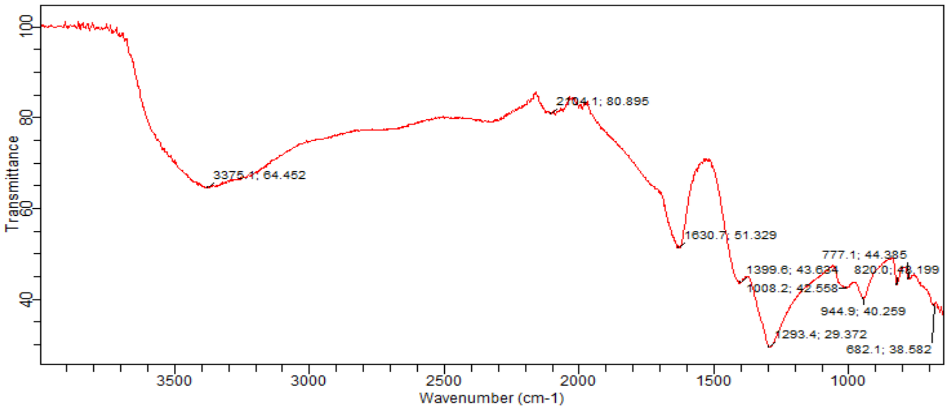


Figure 4. FTIR spectra of the acidified cow bone sample @ 60% HNO_3 solution.

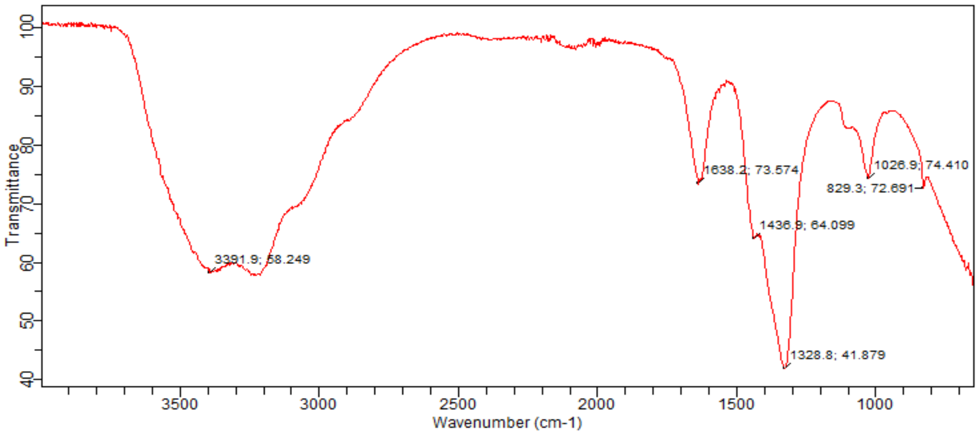


Figure 5. FTIR spectra of the neutralized cow bone sample @ 60% HNO_3 solution.

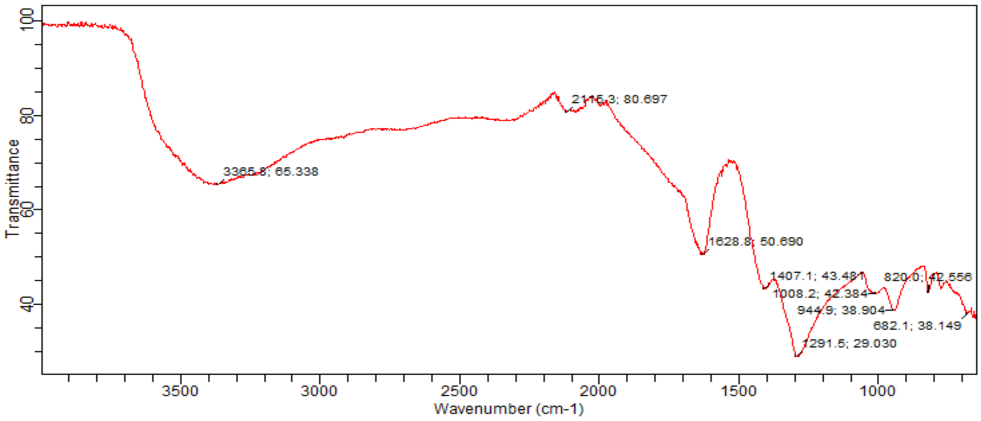


Figure 6. FTIR spectra of the acidified cow bone sample @ 70% HNO_3 solution.

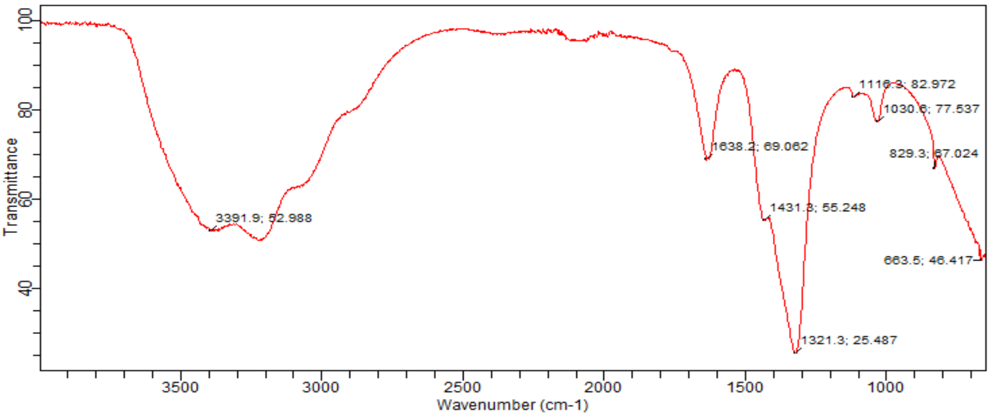


Figure 7. FTIR spectra of the neutralized cow bone sample @ 70% HNO_3 solution.

Table 4. Summary of key FTIR peaks and assignments for raw, acidified, and neutralized bone samples.

Wavenumbers (cm^{-1})	**Assignment**	**Raw Bone**	**50% HNO_3**	**60% HNO_3**	**70% HNO_3**	**Neutralized**	**Interpretation**
3700-3000	O-H stretching (bonded hydroxyl)	3321, 3355	3386	3375	3366	3358-3392	Surface water present in all samples; reduction in peak multiplicity after treatment indicates partial dehydration
2922, 2853	C-H stretching (aliphatic)	Present	Absent	Absent	Absent	Absent	Complete removal of organic components (collagen) after acidification
1630-1640	N-H bending (primary amine)	1638	1636	1631	1629	1636-1638	Nitrogen retention throughout treatment; slight shift indicates modification of amine environment
1450-1410	CO_3^{2-} asymmetric stretching	1459	1396	1400	1407	1431-1437	Carbonate preservation; shift indicates altered chemical environment in acidified vs neutralized states
1000-1030	PO_4^{3-} stretching	1006	1005	1008	1008	1027-1031	Phosphate preservation; slight shift to higher wavenumbers after neutralization

As can be seen from the spectra of raw bone sample (Figure 1), at wavenumbers 3321.1 cm^{-1} and 3354.8 cm^{-1} stretching vibrations of bonded hydroxyl groups were observed. This showed the presence of adsorbed surface water on the raw bone samples. The adsorbed surface water was retained on the sample after acidification with varying concentrations and even after subsequent neutralization as observed in 3386.3 cm^{-1} and 3358.5 cm^{-1} for the sample with 50% HNO_3 and its subsequent neutralization respectively; 3375.1 cm^{-1} and 3391.9 cm^{-1} for the sample with 60% HNO_3 and its subsequent neutralization respectively; 3365.8 cm^{-1} and 3391.9 cm^{-1} for the sample with 70% HNO_3 and its subsequent neutralization respectively. However, an evident observation is that some peaks representing surface water present in the raw bone samples was consistently missing after acidification at all acid concentrations. This change can be attributed to the hygroscopic and dehydrating action of concentrated nitric acid, which removes loosely bound water molecules from the bone surface [45]. The remaining single peak represents more strongly bound structural water or hydroxyl groups within the mineral phase.

The presence of organic materials was observed in the raw bone samples at wavenumbers 2922.2 cm^{-1} and 2853.3 cm^{-1} which signifies the presence of stretching vibrations from -C-H bonds characteristic of aliphatic hydrocarbons in collagen and other organic matrix components. The spectra for the acidified and subsequent neutralized samples lacked the presence of the -C-H bonds across all treatment conditions, which signifies the removal of organic materials by the concentrated acids from the sample. This confirms that the acidification process effectively hydrolyzes and solubilizes the collagen matrix, leaving behind the inorganic mineral phase (primarily calcium phosphate) which then reacts with the acid to form soluble phosphate species. The complete disappearance of these peaks, regardless of acid concentration, indicates that even the lowest acid concentration (50%) is sufficient to fully degrade the organic component under the reaction conditions employed. A similar result was obtained by Akindoyo et al. [45] after exposing cow bone to ultrasound followed by calcination technique.

The vibration at 1459.3 cm^{-1} is characteristics of asymmetric carbonate ion (CO_3^{2-}) stretching obtained for the raw sample. This carbonate, originating from the small amount of calcium carbonate in bone (about 3.85% of bone composition), was preserved after the various treatments of the sample, though with shifts in peak position. This vibration was observed at 1395.9 cm^{-1}, 1399.6 cm^{-1}, and 1407.1 cm^{-1} for the acidified samples at 50%, 60%, and 70% HNO_3 concentrations, respectively, and 1436.9 cm^{-1}, 1436.9 cm^{-1}, and 1431.3 cm^{-1} for the neutralized samples of 50%, 60%, and 70% HNO_3 concentrations, respectively. The shift to lower wavenumbers in acidified samples compared to the raw bone indicates protonation of carbonate ions or alteration of the carbonate coordination environment under acidic conditions. The partial reversal toward the original wavenumber upon neutralization suggests that some of these changes are reversible through pH adjustment, though complete restoration is not achieved, indicating some permanent modification of the carbonate environment.

The peak at 1006.4 cm^{-1} for the raw sample is attributed to phosphate ion (PO_4^{3-}) stretching vibrations. This is the most critical functional group from a fertilizer perspective, as it represents the phosphorus nutrient source. The various treatments did not tamper with the phosphate content of the sample as was observed in the FTIR spectra. PO_4^{3-} vibrations were observed at 1004.5 cm^{-1}, 1008.2 cm^{-1}, and 1008.2 cm^{-1} peaks for the acidified samples at 50%, 60%, and 70% HNO_3 concentrations, respectively, and 1026.9 cm^{-1}, 1026.9 cm^{-1}, and 1030.6 cm^{-1} for the neutralized samples of 50%, 60%, and 70% HNO_3 concentrations, respectively. The slight shift to higher wavenumbers in neutralized samples compared to acidified samples shows changes in the phosphate speciation and coordination environment. In acidified conditions, phosphate exists primarily as $H_2PO_4^-$ and H_3PO_4, while upon neutralization to pH 7, the dominant species shifts toward HPO_4^{2-} and PO_4^{3-}, which have different vibrational frequencies. The persistence and intensity of these peaks across all treatment conditions quantitatively confirms that phosphorus, the primary nutrient of interest, is retained throughout the production process.

The peak at 1638.2 cm^{-1} is attributed to the presence of nitrogen-hydrogen bond of a primary amine originating from the collagen protein. This vibration was observed at 1636.3 cm^{-1}, 1630.7 cm^{-1}, and 1628.8 cm^{-1} for the acidified samples at 50%, 60%, and 70% HNO_3 concentrations, respectively, and 1636.3 cm^{-1}, 1636.2 cm^{-1}, and 1638.2 cm^{-1} for the neutralized samples of 50%, 60%, and 70% HNO_3 concentrations, respectively. The shift to lower wavenumbers in acidified samples indicates protonation of amine groups under acidic conditions (forming $-NH_3^+$), which alters the N-H bond strength and vibrational frequency. Upon neutralization, the peaks shift back toward the original position, indicating deprotonation and restoration of the amine environment. The persistence of these peaks, though with modified positions, confirms that nitrogen from the organic matrix is retained in the final product, consistent with the quantitative nitrogen analysis showing 1.85%-2.43% total nitrogen.

4. Conclusion

This study has successfully demonstrated the synthesis of liquid fertilizer from cow bone through nitric acid digestion followed by ammonia neutralization. Quantitative nutrient analysis confirmed that the synthesized liquid fertilizers contain significant concentrations of plant-essential nutrients: phosphorus (3850-5280 mg/L as P, equivalent to 0.88-1.21% P_2O_5), nitrogen (1.85%-2.43% w/w), and calcium (1250-1840 mg/L). Phosphorus recovery efficiency increased with acid concentration, ranging from 62.3% at 50% HNO_3 to 78.1% at 70% HNO_3. However, the difference between 60% and 70% treatments was not statistically significant, suggesting that 60% HNO_3 may represent the optimal balance between recovery efficiency and process practicality. Mass balance analysis showed good closure (92%-94% of initial phosphorus accounted for), with solubilization of bone mass ranging from 71.6% to 84.4% depending on acid concentration. The final products exhibited favourable physicochemical properties for agricultural application, with pH of 6.4-6.9 and EC of 4.8-6.2 mS/cm, indicating suitability for soil application with appropriate dilution. UV/Vis spectroscopy revealed shifts in absorption peaks following acidification and neutralization, indicating chemical transformations within the bone matrix. These observations are general indicators of molecular modification. FTIR analysis confirmed the removal of organic components (disappearance of -C-H bonds) while preserving essential phosphate ions and nitrogen-containing functional groups, providing molecular-level evidence of nutrient retention throughout the production process. This study provides a quantitative foundation for the potential utilization of cow bone-derived liquid fertilizer as a sustainable approach to nutrient recycling and waste valorization in agriculture.

However, this study has limitations that should be acknowledged. The fertilizer efficacy has not been validated through greenhouse or field trials, and nutrient availability to plants remains to be demonstrated. Additionally, the long-term stability of the liquid fertilizer during storage and potential changes in nutrient speciation over time were not investigated. Future work should focus on: (1) greenhouse and field trials to validate fertilizer efficacy on crop growth and yield; (2) investigation of nutrient release kinetics and plant uptake efficiency; (3) optimization of production parameters to maximize nutrient recovery while minimizing acid consumption; (4) life cycle assessment to evaluate environmental impacts compared to conventional fertilizers; (5) exploration of alternative acids and neutralization agents; and (6) scale-up studies to assess process economics and practicality at commercial scale.

Funding

This study was not supported by any specific funding from public, commercial, or non-profit organizations.

Conflict of Interest

The author confirms that there are no known financial or personal conflicts of interest that could have influenced the findings or interpretations presented in this study.

Data Availability Statement

The data supporting the findings of this study are available upon reasonable request from the corresponding author.

Generative AI Statement

The authors declare that no Generative AI was used in the creation of this manuscript.

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