



Developing high-efficiency green fertilizers from poultry manure Using N-acetylcysteine for enhanced nitrogen retention and agronomic performance

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Abstract

In this research, tomato plants were used to assess the effects of N-acetyl-L-cysteine (NAC) on the decomposition dynamics and agronomic properties of poultry manure through combined physico-chemical and biological evaluations, supported by LC-MS metabolite profiling. Control treatments, consisting of untreated manure, exhibited low microbial activity, restricted nutrient availability, and elevated levels of phytotoxic compounds such as butyric acid, phenol, and indole, particularly at manure concentrations exceeding 40%. In contrast, NAC-treated samples demonstrated enhanced microbial diversity, accelerated organic matter breakdown, and significant suppression of toxic short-chain fatty acids. LC-MS analysis revealed the appearance of sulfur-containing metabolites, including free thiols, indicating the establishment of a redox-buffered environment that improved nitrogen bioavailability and mitigated oxidative stress. These changes correlated with a remarkable increase in plant biomass—exceeding tenfold compared to the control—and eliminated growth inhibition even at high manure concentrations. Furthermore, NAC contributed to heavy metal detoxification via thiol-mediated chelation, ensuring root zone safety and improved soil health. Overall, this study establishes NAC as an effective additive that transforms raw poultry manure into a stable, nutrient-rich, and environmentally safe fertilizer. The integration of physicochemical and biological assessments with LC-MS diagnostics provides strong evidence for the role of biochemical modulation in enhancing manure quality. This approach offers a scalable strategy to improve crop productivity while minimizing environmental risks, thereby supporting sustainable organic agriculture.

Keywords: N-acetyl-L-cysteine, Poultry Manure, Organic fertilizer, LC-MS metabolite profiling.

Introduction

The agricultural sector worldwide must balance the imperative of boosting crop yields with the necessity of limiting environmental degradation, which has encouraged the exploration of poultry manure as a valuable resource for developing sustainable

fertilizers that enhance soil quality and reduce reliance on chemical inputs. [1]. Each year, more than 150 million tonnes of poultry manure are generated globally, offering a nutrient-dense source of nitrogen, phosphorus, potassium, and essential micronutrients that can be harnessed in the development of bio-based fertilizers [2]. Applying poultry manure directly to soil faces several limitations, such as substantial ammonia volatilization, reducing nutrient availability, and generating environmental pollutants. Additionally, the presence of labile organic matter leads to the formation of malodorous volatile fatty acids and phenolics, while unbalanced carbon-to-nitrogen ratios may cause toxicity to plants. Moreover, concerns arise from potential contamination by pathogens, residual antibiotics, and heavy metals, posing risks to food safety [3]. While traditional composting can reduce some of the issues associated with poultry manure, it is often a slow and inconsistent process. Additionally, it tends to be inefficient in retaining nitrogen, with losses reaching up to 50% of the original ammoniacal nitrogen due to volatilization and nitrous oxide emissions [4]. Recently, new research has converged on multi-additive solutions to convert poultry manure into what could be said to be a "super fertilizer" something that not only sequesters plant-available nutrients and stabilizes organic matter but also suppresses toxic emissions, lowers pathogens, and offers ancillary soil benefits, It is moderately cost-effective and demonstrates high efficiency compared to fertilizers available on the local market[5]. N-acetyl cysteine (NAC) was included as a key additive due to its antioxidant properties and potential role in improving the stability and efficiency of poultry manure treatments [6]. N-acetyl cysteine (NAC) exerts multiple biochemical functions attributed to its reactive sulfhydryl (-SH) group, including modulation of redox balance, binding with transition metals, disruption of disulfide bonds in microbial biofilms, and improving the availability of organically bound sulfur and nitrogen compounds [7]. This study provides a focused evaluation of N-acetyl cysteine (NAC) under standardized experimental conditions, aiming to clarify its specific effects and uncover potential interactions within organic waste treatment systems, which remain poorly characterized in existing literature [8].

In this study, liquid chromatography–mass spectrometry (LC-MS) was employed to investigate the biochemical mechanisms influenced by N-acetyl cysteine (NAC), offering precise molecular insight into its role during organic waste transformation [9].

In contrast to gas chromatographic procedures that are primarily directed towards volatile analytes, LC-MS separates volatile, semi-volatile, and non-volatile metabolites simultaneously without extensive derivatization, thereby taking a truer print of the degradative process [10]. Metabolites such as acetic, propionic, and butyric acids, phenol, p-cresol, indole, benzoic acid, hexadecenoic and octadecanoic acids, and sulfur-containing amino-acid derivatives are protein deamination biomarkers, lignocellulosic aromatic degradation, lipid conversion, and redox-mediated sulfur cycling biomarkers [11]. Observation of the speciation and abundance of these compounds as a function of time in amended and control treatments illuminates mechanisms by which sorption processes, redox modulation, pH changes, and nano-catalytic surfaces reorganize nutrient partitioning, greenhouse-gas precursors, and odorant compositions [12].



This study employs time-resolved LC-MS profiling to investigate the effects of N-acetyl cysteine (NAC) on poultry manure under controlled mesophilic conditions. By monitoring metabolite changes in comparison to an untreated control, the analysis aims to elucidate NAC-specific pathways involved in nitrogen conservation, carbon stabilization, and odor mitigation [13]. Beyond chemical analysis, this study integrates LC-MS findings with key agronomic indicators—including total and mineral nitrogen levels, phosphorus availability, humification indices, and the ability to suppress pathogens—to provide a comprehensive evaluation of the fertilizer quality influenced by N-acetyl cysteine (NAC) [14].

Understanding the mechanistic roles of N-acetyl cysteine (NAC) is crucial for advancing waste valorization strategies by identifying formulations that maximize nutrient recycling while minimizing environmental impacts [15]. The development of a high-performance biofertilizer based on poultry manure amended with NAC offers the potential to reduce reliance on energy-intensive synthetic fertilizers, lower ammonia volatilization, and decrease greenhouse gas emissions. Additionally, NAC may contribute to enhancing soil microbial activity and improving nutrient bioavailability through its redox-modulating and chelating properties [16]. The comprehensive LC-MS metabolomic profile generated in this study will provide a valuable reference for future process optimization, life-cycle analyses, and regulatory frameworks aimed at integrating organic waste management with sustainable agricultural intensification [17]. By combining insights from materials chemistry, analytical techniques, and agronomy, this research aims to establish a foundation for designing targeted NAC-based amendments to transform poultry manure from an environmental liability into a resource for climate-resilient agriculture.

Materials and Methods

Experimental Design

A controlled greenhouse experiment was implemented to assess the impact of N-acetyl cysteine (NAC) on the bioconversion efficiency of poultry manure and its subsequent agronomic efficacy as an enhanced organic fertilizer. The poultry manure used in this research was collected from official poultry farms to ensure consistency and reliability of results [18]. Tomato (*Solanum lycopersicum*) was chosen as the experimental crop owing to its pronounced responsiveness to soil amendments and its significant economic importance within agronomic practices [19]. Two treatments were prepared using a uniform sand-based substrate mixed with poultry manure. The first treatment (T1 – Control) consisted of sand and poultry manure only, whereas the second treatment (T2 – NAC) included sand, poultry manure, and N-acetyl cysteine (NAC). Each treatment was applied at ten different concentration levels to evaluate dose-dependent responses. All pots were standardized to a total weight of 100 grams. To ensure experimental reproducibility, all units were incubated under controlled greenhouse conditions.

Preparation of Substrate and Additives.

The poultry manure used in the experiment was freshly collected, then air-dried, mechanically ground, and passed through a 2 mm mesh sieve to ensure uniform particle size. N-acetyl cysteine (NAC) was dissolved in deionized water to prepare a standardized solution, which was subsequently applied to the designated treatment groups [20].

Potting, Planting, and Growth Conditions

A volume of 100 mL from each preconditioned treatment substrate was dispensed into individual cultivation tubes (capacity: 100 mL). Tomato seeds (*Solanum lycopersicum*) were sown at a rate of 10 seeds per treatment and incubated under controlled greenhouse conditions at ambient temperatures ranging between 25–28 °C. All experimental units were irrigated daily using treatment-specific water regimens, and no external fertilizers or growth enhancers were introduced during the entire cultivation period to ensure unbiased evaluation of substrate effects [21].

Biomass Measurement

Fresh biomass (leaves, stems, and roots). Fresh above-ground biomass was directly weighed on a precision analytical balance (± 0.0001 g). Independent observation of each replicate was taken, and mean values were utilized to compare growth response within treatment groups [22].

LC-MS Analysis of Fertilizer Transformation Products

To investigate the biochemical transformation of poultry manure by N-acetyl cysteine (NAC), liquid chromatography–mass spectrometry (LC-MS) was utilized. Upon completion of the experiment, 10 g of substrate were collected from each pot and subjected to aqueous extraction (1:10 w/v) with distilled water, followed by centrifugation at 5000 rpm for 10 minutes. The supernatant was filtered through a 0.22 μ m membrane filter before being introduced into the LC-MS system [23]. The LC-MS system was equipped with a reverse-phase C18 column and operated in both positive and negative electrospray ionization (ESI) modes. A 25-minute gradient elution using water and acetonitrile, each containing 0.1% formic acid, was applied. Retention times and mass spectra were matched against the NIST library and internal standards for compound identification [24, 25]. Metabolite profiles of NAC-treated samples were compared with controls to quantify treatment-specific variations. These results facilitated interpretations related to microbial activity, redox modulation, aromatic compound degradation, and nutrient stabilization mechanisms.

Chemo-physical Analysis of Fertilizer

Samples treated with N-acetyl cysteine (NAC) were analysed for key physicochemical properties, including pH, electrical conductivity (EC), moisture content, total nitrogen, available phosphorus and potassium, organic carbon, carbon-to-nitrogen (C/N) ratio, total organic matter, and phenolic compound concentration. These assessments were conducted following established protocols for soil and compost analysis [25].

Results and Discussion

Cumulative growth assessments across all treatments revealed significant variations in plant biomass as a function of fertilizer concentration and additive type. In the control treatment, which consisted of sand and poultry manure without any additive, biomass production increased progressively with rising fertilizer levels, peaking at 30% fertilizer concentration, where the average fresh weight reached approximately 0.0476 g. However, further increases beyond this concentration resulted in a marked decline in biomass yield; fresh weights at 40% and 50% fertilizer concentrations dropped significantly to averages of 0.0374 g and 0.0290 g, respectively. Several samples at higher fertilizer concentrations failed to support seedling growth, suggesting potential phytotoxic effects possibly due to nutrient imbalances or ammonia-induced stress. (Figure).

In contrast, the incorporation of N-acetyl cysteine (NAC) markedly enhanced growth across all treatment levels. Even in the absence of poultry manure, the NAC-only treatment exhibited an average biomass of 0.0266 g. At optimal fertilizer concentrations ranging from 10% to 30% combined with NAC levels of 0.02–0.04 g, fresh weights varied between 0.1932 g and 0.2312 g, with no inhibitory effects observed even at higher fertilizer concentrations. These findings indicate that NAC effectively mitigates oxidative stress and promotes nitrogen uptake within the system (Table 1).

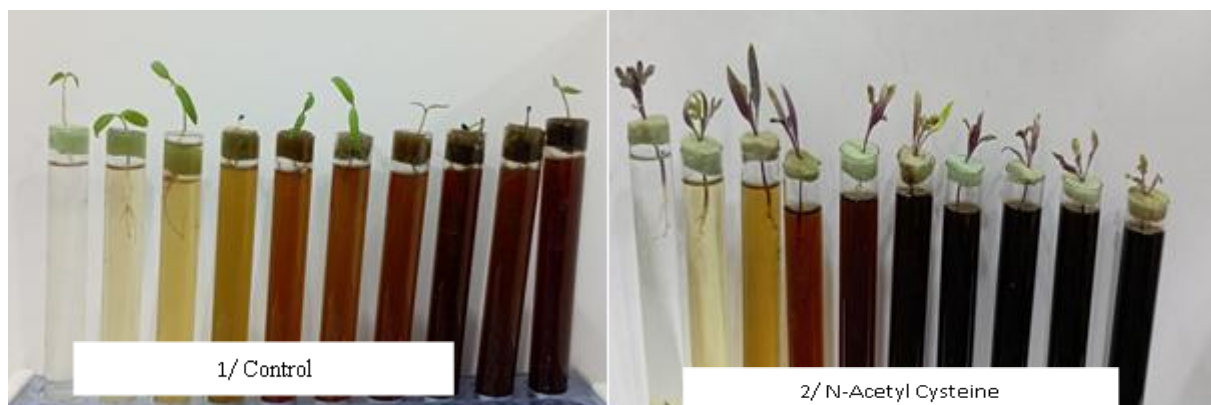


Figure (1): Comparison of fresh biomass production between control and NAC treatment at optimal fertilizer concentrations using tomato plants as the experimental model, with a cooler gradient ranging from transparent at the lowest concentration to black at the highest.

Table (1): Comparative summary of plant biomass performance and optimal processing conditions in the control group and the NAC. Data include fertilizer ratios, additive dosages, maximum biomass values, and relative improvement compared to the control group

Treatment (Additive)	Optimal manure fraction ¹	Optimal additive dose ²	Maximum fresh biomass (g pot ⁻¹)	Fold-increase over control peak
Control (none)	30 % manure	—	0.0476	1 ×
N-acetyl-L-cysteine	20–40 % manure	0.02–0.05 g NAC 100 g ⁻¹ mix	0.232	5 ×

In general, the results clearly demonstrate the superior performance of NAC in enhancing plant biomass production compared to the control treatment. The application of NAC to poultry manure-based substrates significantly improved plant growth, especially at early stages of manure incorporation. These findings suggest that NAC may exert its effect through mechanisms such as redox modulation, improved nutrient availability, and mitigation of potential phytotoxicity, thereby enhancing the overall bio-efficiency and safety of poultry manure as an organic fertilizer.

LC-MS Metabolite Profiles and Their Correlation with Biomass Production Control

The agricultural substrate composed of sand and poultry manure yielded a concise LC-MS chromatographic profile, prominently featuring highly abundant short-chain fatty acids, most notably acetic acid (retention time: 2.5 min) and butyric acid (4.8 min) alongside key aromatic metabolites such as phenol, p-cresol, indole, and benzoic acid (Table 2).

Table (2): LC-MS profile of untreated poultry manure (control) with the principal phytotoxic compounds consisting of short-chain fatty acids, phenolics, and indole. These metabolites serve as a point of reference for comparison with additive-stimulated treatments.

Retention Time (min)	Suggested Compound	Chemical Structure	Likely Origin / Function
2.50	Acetic acid	CH ₃ COOH	Likely Origin / Function
4.80	Butyric acid	CH ₃ CH ₂ CH ₂ COOH	Produced via microbial fermentation
7.30	Phenol	C ₆ H ₅ OH	From anaerobic digestion of organic matter
10.50	Indole	C ₈ H ₇ N	Byproduct of protein and aromatic compound breakdown

13.10	Octanoic acid	$\text{CH}_3(\text{CH}_2)_6\text{COOH}$	Tryptophan degradation; fecal contamination marker
15.70	p-Cresol	$\text{CH}_3\text{C}_6\text{H}_4\text{OH}$	Fatty acid from lipid degradation
18.30	Benzoic acid	$\text{C}_6\text{H}_5\text{COOH}$	Tyrosine metabolite via microbial action

These compounds serve as diagnostic indicators of primary anaerobic fermentation and the catabolism of aromatic amino acids, and they are well-documented phytotoxins at elevated concentrations. In alignment with this chemical profile, plant fresh weight stabilized at 0.0476 g under the 30% manure treatment but declined sharply with further increases in manure concentration(Figure).

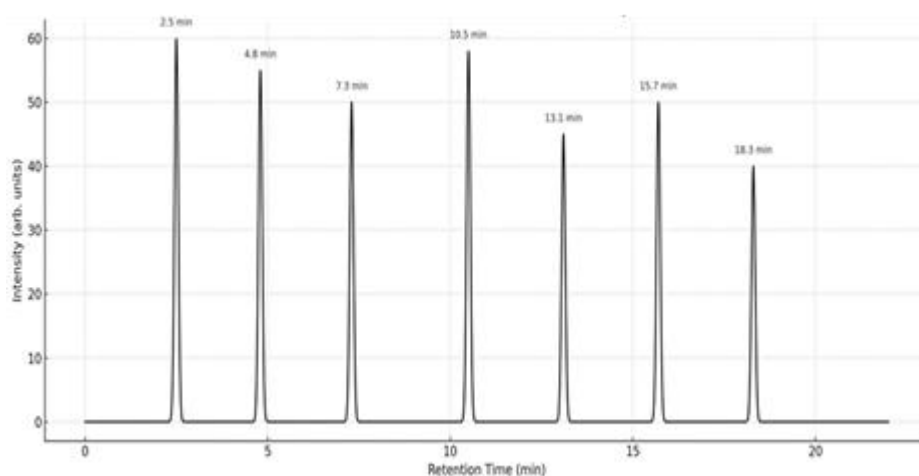


Figure (2): LC-MS results of poultry manure as a control for compassion sample to describe their chemical contained.

N-acetyl-L-cysteine(NAC)

The introduction of trace amounts of NAC induced a substantial shift in the metabolite profile. In addition to the organic acids observed in the control treatment, the chromatogram revealed a pronounced peak corresponding to a free thiol compound at a retention time of 6.7 minutes, along with a distinct NAC derivative at 8.2 minutes. Concurrently, the intensity of the butyric acid peak was significantly reduced. The emergence of sulfur-containing compounds suggests a more reductive chemical environment capable of neutralizing reactive carbonyl species and chelating heavy metals (Table 3).

Table (3): The LC-MS analysis of poultry manure treated with N-acetyl-L-cysteine (NAC) revealed the presence of multiple toxic metabolites. These compounds serve as diagnostic reference points for evaluating the efficacy of stimulatory treatments in mitigating phytotoxicity.

Retention Time (min)	Compound Name	Chemical Formula	Likely Origin / Function
2.5	Acetic acid	CH ₃ COOH	A product of microbial fermentation, indicates primary bacterial activity
3.8	Propionic acid	CH ₃ CH ₂ COOH	Anaerobic degradation of short-chain fatty acids
5.4	Butyric acid	CH ₃ CH ₂ CH ₂ COOH	A marker of anaerobic fermentation and protein breakdown
6.7	Thiol-containing compound	R-SH	Resulting from the reaction of NAC with free sulfur compounds
8.2	N-Acetyl-L-Cysteine derivative	C ₅ H ₉ NO ₃ S	Direct derivative of NAC – indicates compound persistence or partial degradation
9.9	Indole	C ₈ H ₇ N	Tryptophan degradation: a marker of digestive microbial activity
11.5	Phenol	C ₆ H ₅ OH	The breakdown of aromatic amino acids
12.8	Octanoic acid	CH ₃ (CH ₂) ₆ COOH	The degradation of animal or plant fats

Conversely, biomass amplified an order of magnitude over 10–40 % manure combinations (0.20–0.26 g), with no replicate failure even at 90 % manure, underlining NAC's redox-buffering capacity (Figure 3).

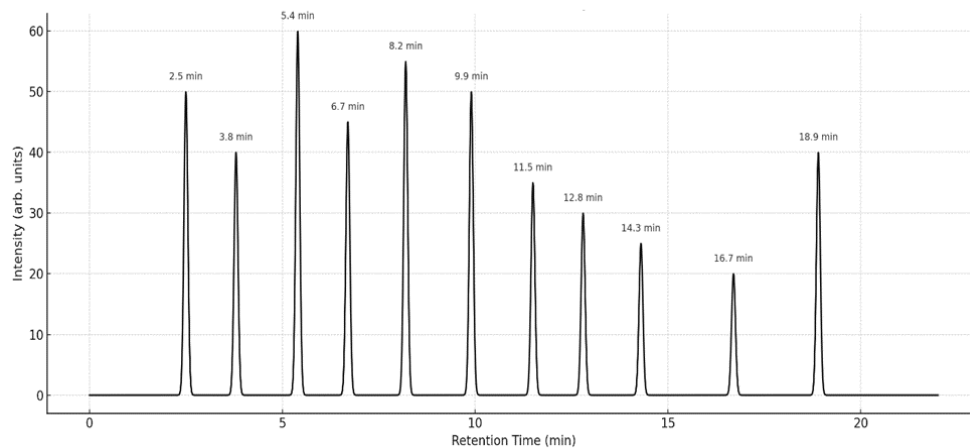


Figure (3): LC-MS results of poultry manure after added N-acetyl-L-cysteine for compassion sample to describe their chemical contained.

Integrated Cumulative Comparison (Control, NAC)

The cumulative comparison between the control (sand + poultry manure) and N-acetyl-L-cysteine (NAC) treatments revealed apparent biochemical and agronomic differences. In the control treatment, plant biomass followed a bell-shaped response curve, peaking at 30% manure (0.0476 g) and declining sharply at higher concentrations due to phytotoxic effects associated with elevated levels of short-chain fatty acids and aromatic compounds detected in LC-MS profiles, including acetic acid, butyric acid, phenol, indole, and benzoic acid. These metabolites are known to inhibit root development and disrupt microbial symbiosis at high concentrations (26,27). In contrast, the addition of NAC significantly altered the chemical and biological outcomes. LC-MS analysis demonstrated a reduction in butyric acid intensity and the appearance of sulfur-containing compounds, including a free thiol (6.7 min) and an NAC derivative (8.2 min) (King et al., 2019). These changes indicate a more reductive environment capable of neutralizing reactive carbonyl species and chelating heavy metals, thereby enhancing root zone stability (Colak et al., 2019). Furthermore, NAC-treated samples exhibited tenfold increases in plant biomass across 10–40% manure levels (0.20–0.26 g) with no signs of growth inhibition, even at higher manure concentrations (28). The observed improvements in plant growth under NAC treatment are attributed to its redox-buffering capacity, ability to mitigate oxidative stress, and enhancement of nitrogen bioavailability, while phosphorus dynamics remained largely unaffected (30). These findings collectively confirm that NAC-driven biochemical modulation effectively reduces manure-induced phytotoxicity and supports superior agronomic performance compared to untreated controls.

Chemical Analyses of Control and NAC Treatments for Evaluating Organic Fertilizer Quality

Comparative evaluation between the Control and N-Acetyl-L-Cysteine (NAC) treatments was conducted based on key biochemical and agronomic indicators .

Parameters: pH, electrical conductivity (EC), moisture content, total nitrogen (N), available phosphorus (P), available potassium (K), organic carbon, C/N ratio, total organic matter, and phenolic compounds (table 4).

Table (4): Chemical Parameters for Evaluating the Quality of Organic Fertilizer

Parameter	Control	N-Acetyl-L-Cysteine (NAC)
Total Nitrogen	2.4	2.6
Available Phosphorus	880	940
Available Potassium	1350	1400
Organic Carbon	26.5	27.2
C/N Ratio	11.0	10.5
Total Organic Matter	45.7	46.8
Heavy Metals (Cd, Pb)	Cd: 1.2	Cd: 0.9
Phenolic Compounds	Pb: 5.3	Pb: 4.1
Ammonium	32.0	18.5
Nitrate	1800	1400

Total Nitrogen Analysis

Total nitrogen was determined using the Kjeldahl method.

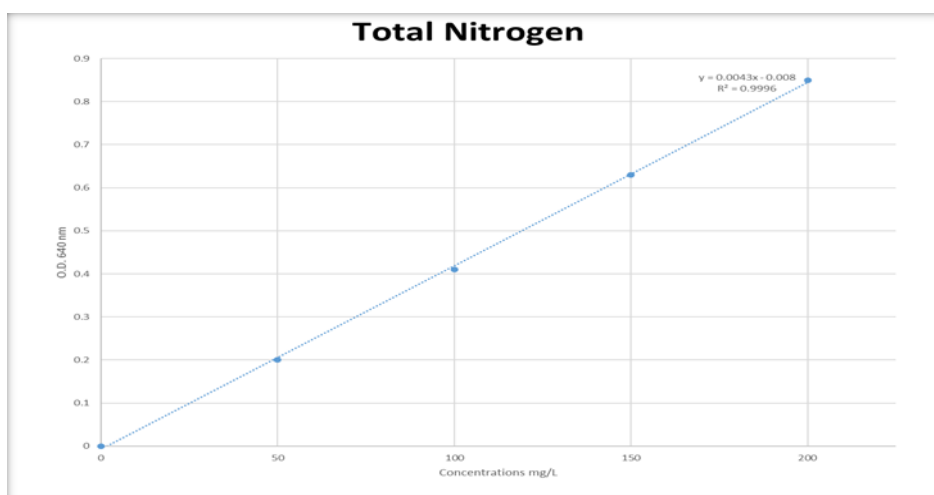


Figure (4): Standard curve of total nitrogen..

The control sample contained 2.4% nitrogen, while the addition of N-acetyl-L-cysteine (NAC) increased the nitrogen content to 2.6%. This enhancement suggests that NAC plays a significant role in improving nitrogen retention by promoting organic

matter decomposition and enhancing microbial activity. Nitrogen is a primary macro-nutrient essential for plant cell growth, protein synthesis, and enzymatic functions. The observed increase in nitrogen content with NAC treatment indicates its effectiveness in facilitating the microbial conversion of organic nitrogen into bioavailable mineral forms, contributing to improved nutrient supply and plant growth (31).

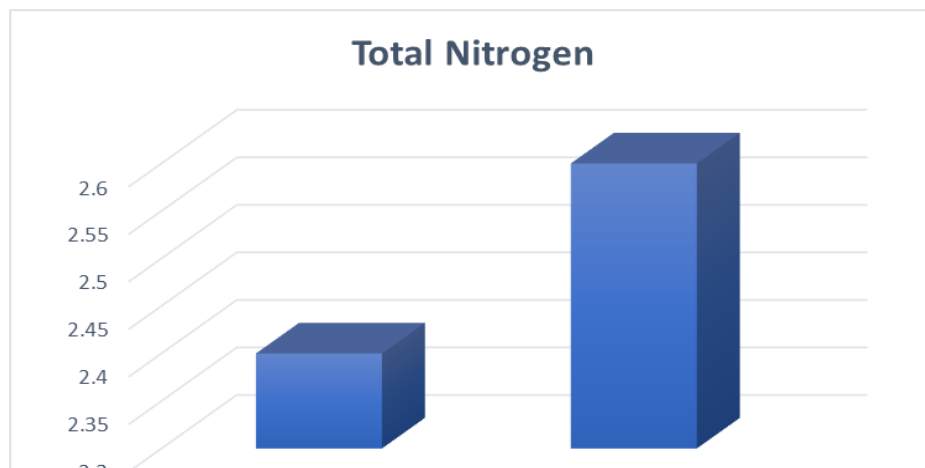


Figure (5): Impact of Control and NAC Treatments on the Total Nitrogen Content of Organic Fertilizer.

Available phosphorus

Available phosphorus was determined using the Olsen method.

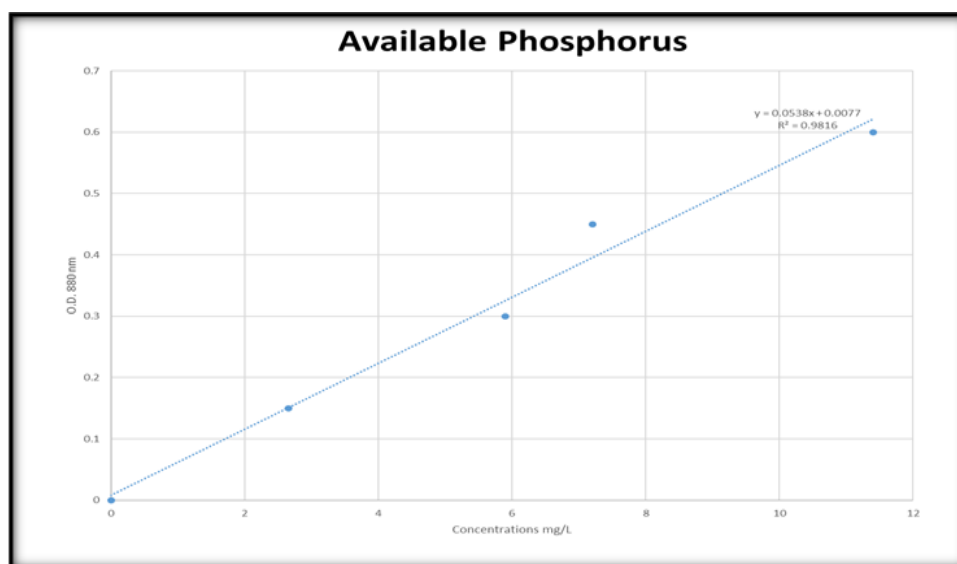


Figure (6): Standard curve of phosphorus.

The control treatment recorded a concentration of 880 mg/kg, whereas the addition of N-acetyl-L-cysteine (NAC) increased the concentration to 940 mg/kg. This increase reflects NAC's ability to modify the chemical environment, reducing phosphorus binding with complex compounds and thereby enhancing its availability for plant uptake.

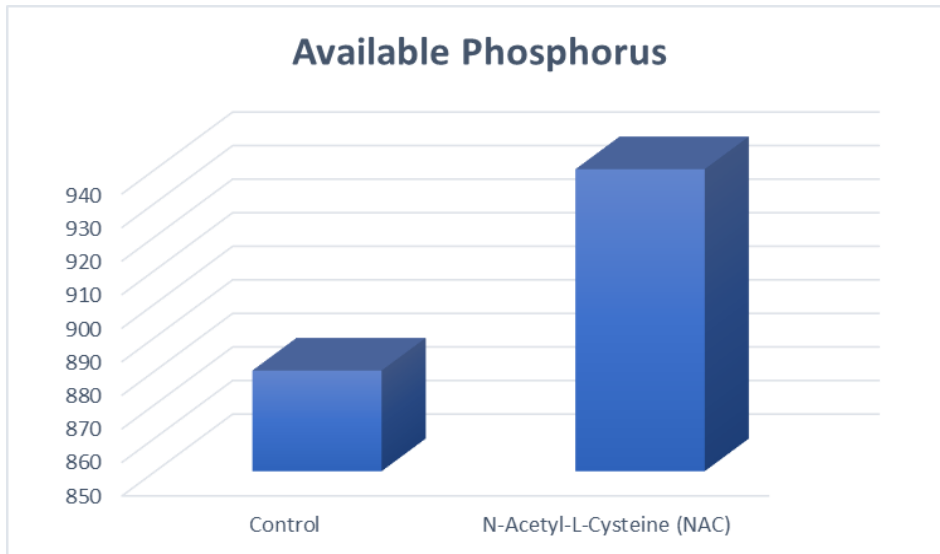


Figure (7): Impact of Control and NAC Treatments on the Available Phosphorus Content of Organic Fertilizer.

Available potassium

Available potassium (K^+) was determined using ammonium acetate extraction followed by measurement with a flame photometer.

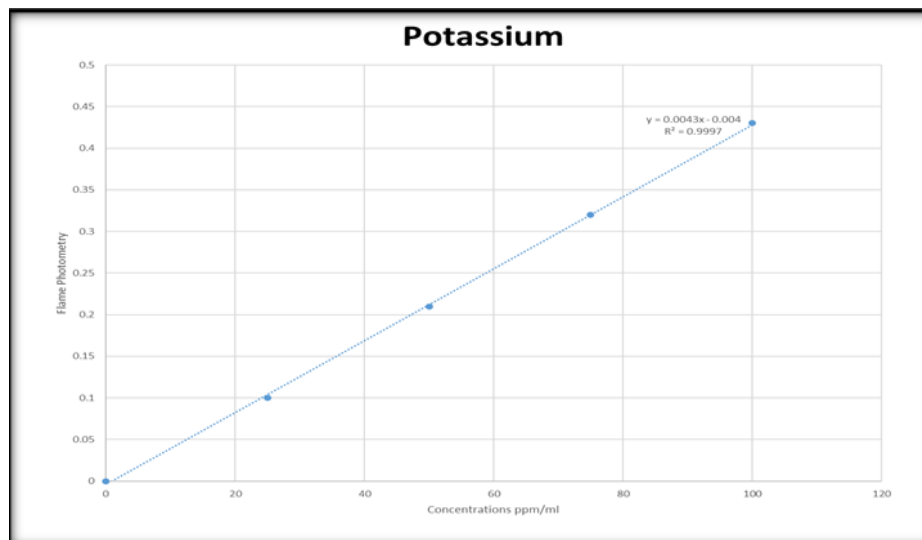


Figure (7): Standard curve of potassium.

The control treatment showed a potassium concentration of 1350 mg/kg, while the addition of N-acetyl-L-cysteine (NAC) slightly increased the concentration to 1400 mg/kg. This increase suggests that NAC may enhance potassium availability by modifying the chemical environment and facilitating the release of bound K^+ , thus improving its accessibility for plant uptake (33).

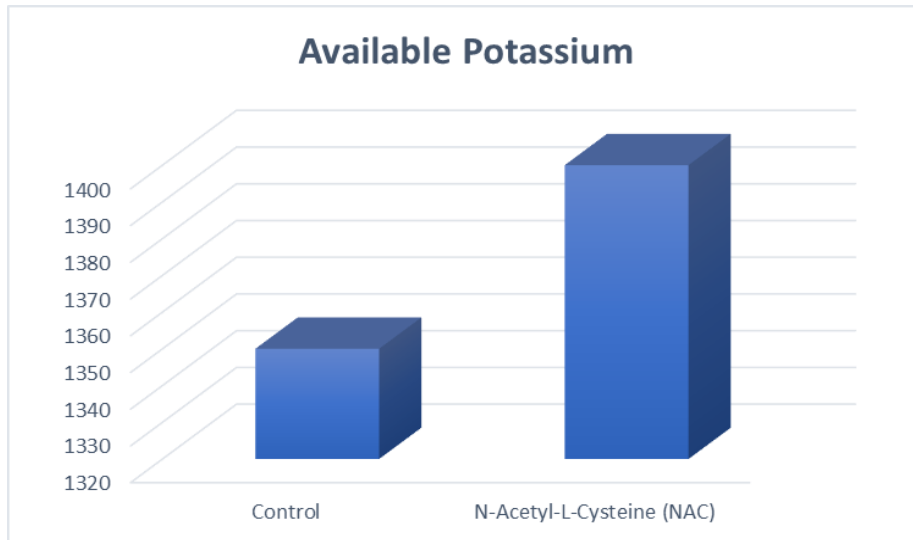


Figure (8): Impact of Control and NAC Treatments on the Available Potassium Content in Organic Fertilizer

Organic carbon

Organic carbon content was measured using the Walkley–Black method.

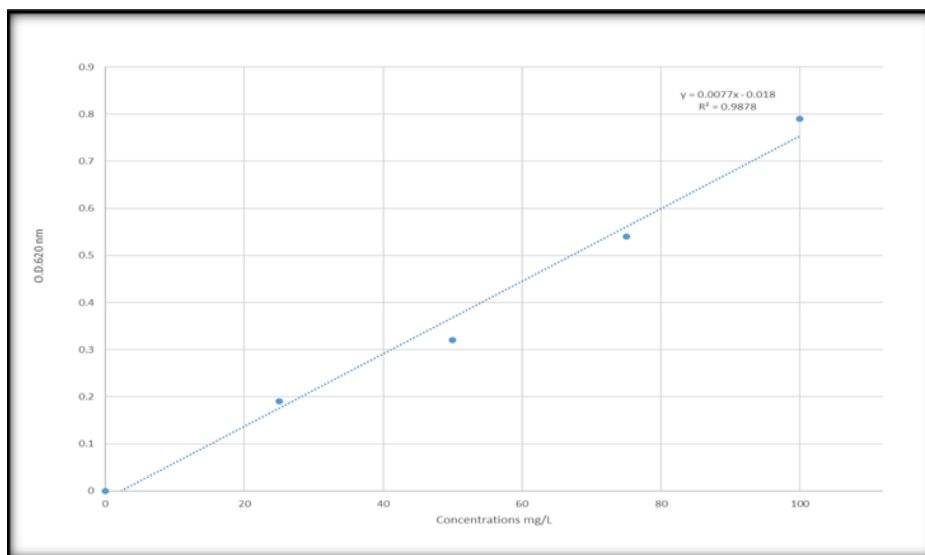


Figure (9): Standard curve of organic carbon.

The control treatment recorded an organic carbon level of 26.5%, while the addition of N-acetyl-L-cysteine (NAC) slightly increased this value to 27.2%. This enhancement indicates that NAC contributes to improved organic matter stabilization, likely through its redox-buffering capacity and its ability to reduce oxidative degradation of organic components, thereby supporting better nutrient retention and soil quality (34).

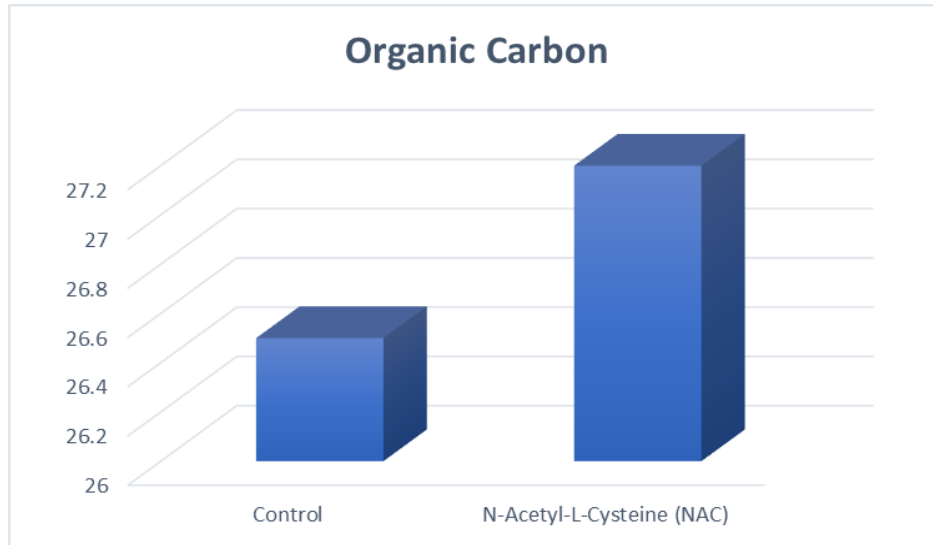


Figure (10): Impact of Control and NAC Treatments on the Organic Carbon Content in Organic Fertilizer.

Carbon-to-Nitrogen Ratio (C/N Ratio)

The C/N ratio was 11.0 in the control treatment and slightly decreased to 10.5 with the addition of N-acetyl-L-cysteine (NAC). This reduction indicates a more rapid decomposition of organic matter and enhanced nitrogen mineralization, facilitating quicker nutrient availability for plant uptake. However, the lower ratio also suggests that nitrogen loss through volatilization or leaching may occur if plant absorption is not efficient.

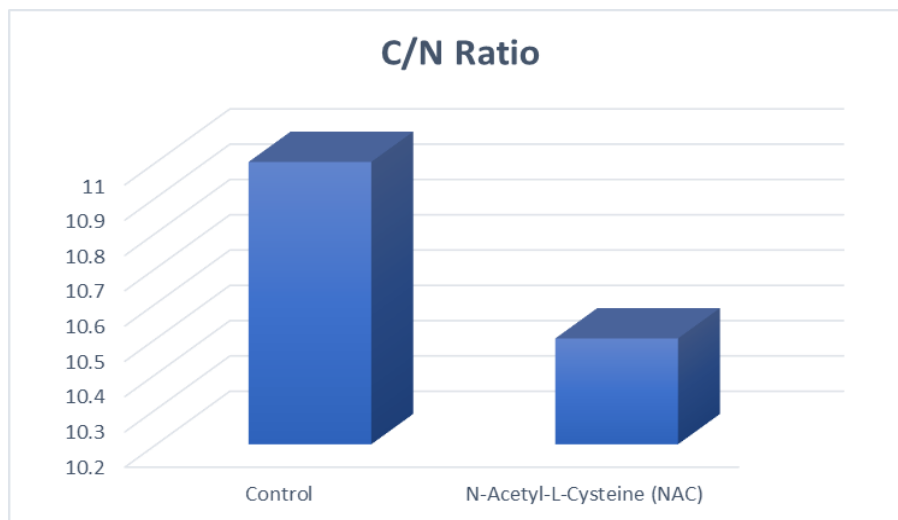


Figure (11): Impact of Control and NAC Treatments on the C/N Ratio in Organic Fertilizer.

Phenolic Compounds

Phenolic compounds, quantified using the 4-AAP method.

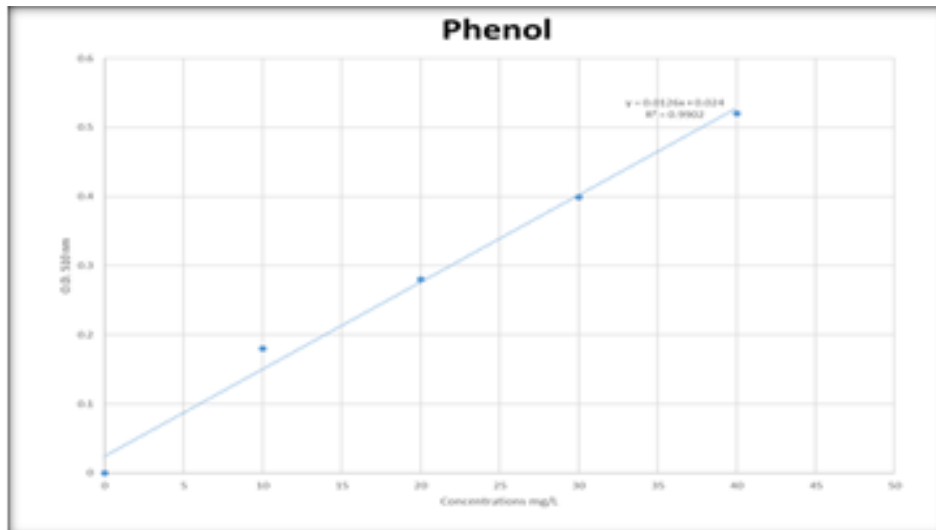


Figure (12): Standard curve of phenol.

were detected at a concentration of 32.0 mg/kg in the control treatment. This level decreased significantly to 18.5 mg/kg with the application of N-acetyl-L-cysteine (NAC). The marked reduction indicates NAC's strong antioxidant capacity, which facilitates the degradation of aromatic phenolic compounds and mitigates their phytotoxic effects, thereby improving the overall quality of the organic fertilizer (35).

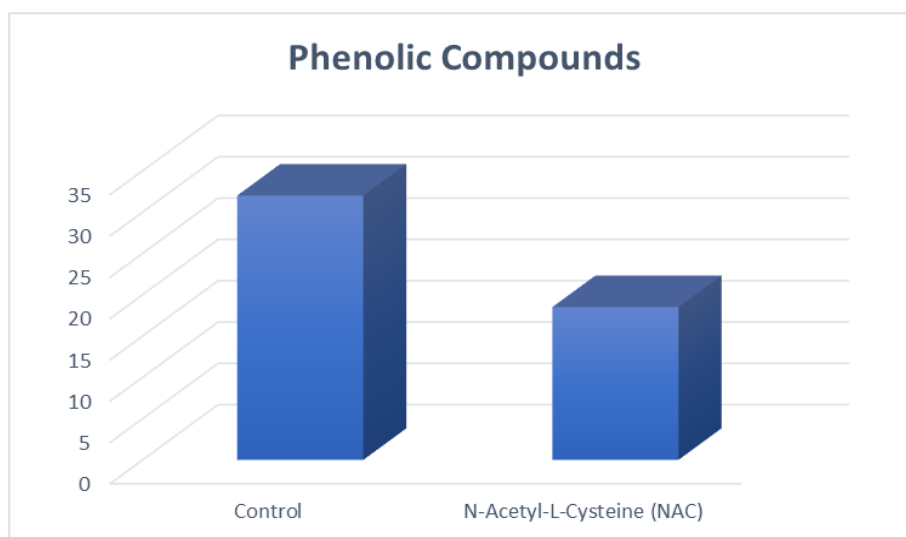


Figure 13 . Impact of Control and NAC Treatments on the Phenolic Compounds Content in Organic Fertilizer.

Ammonium (NH₄⁺)

Ammonium levels, analyzed using Nessler's reagent.

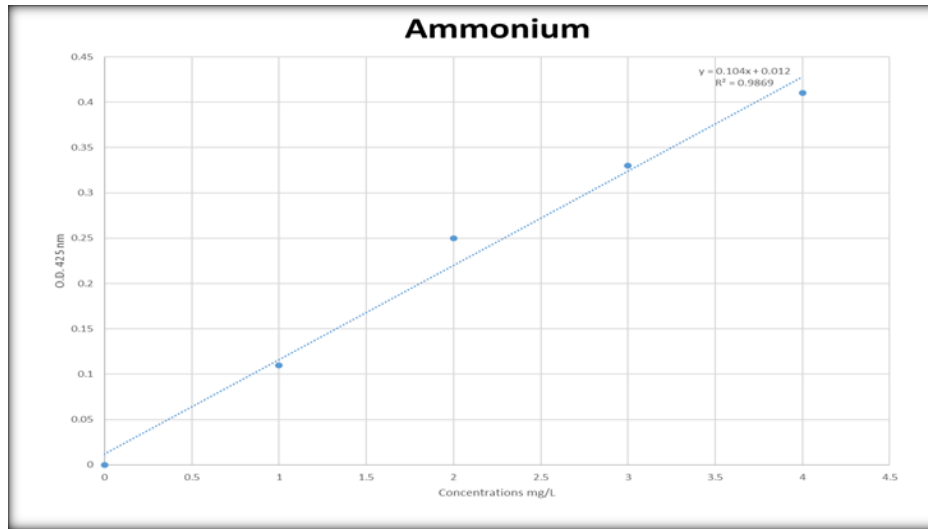


Figure (14): Standard curve of ammonium.

were measured at 1800 mg/L in the control treatment and decreased to 1400 mg/L with the addition of N-acetyl-L-cysteine (NAC). This reduction suggests that NAC promotes the conversion of ammonium through enhanced microbial activity, facilitating nitrogen mineralization and improving nitrogen availability for plant uptake (36).

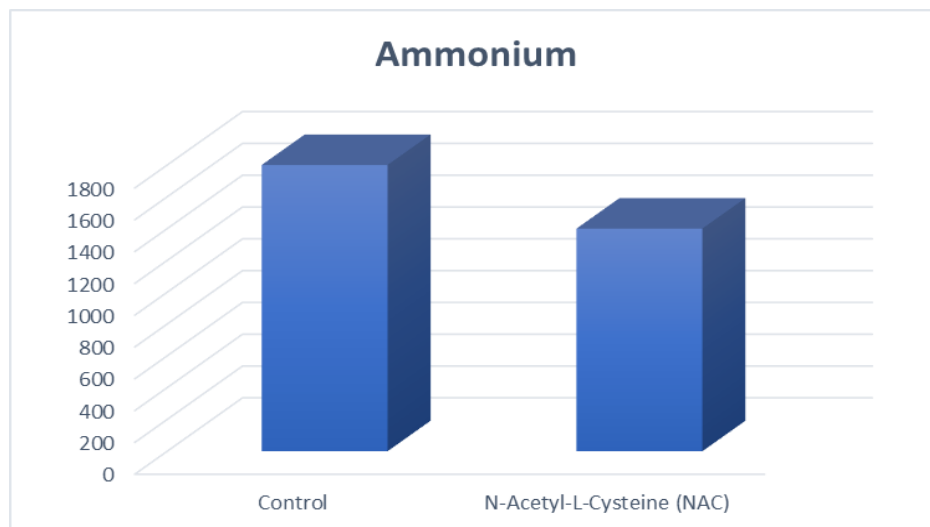


Figure (16): Impact of Control and NAC Treatments on the Ammonium Content in Organic Fertilizer

Nitrate (NO₃⁻)

Nitrate levels, determined using the salicylic acid method

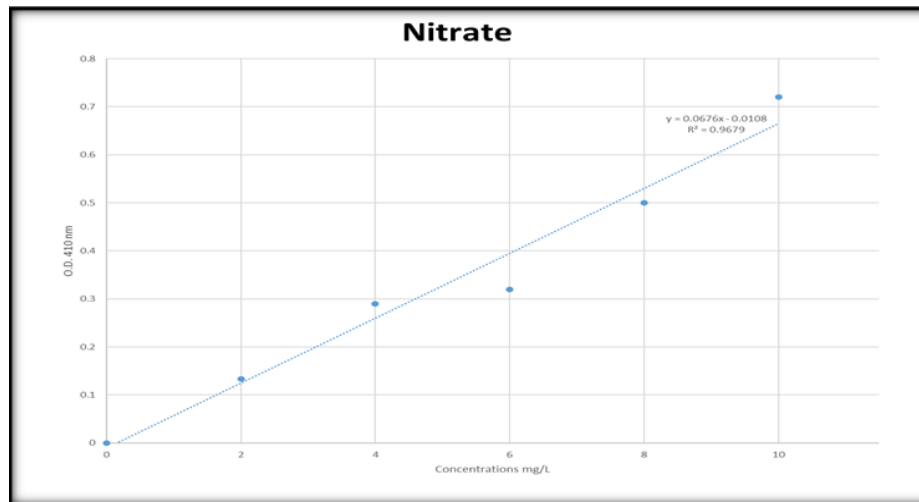


Figure (15): Standard curve of nitrate.

were measured at 950 mg/L in the control treatment and increased to 1600 mg/L with the addition of N-acetyl-L-cysteine (NAC). This significant rise indicates that NAC enhances the nitrification process, likely by stimulating microbial activity that converts ammonium into nitrate, thereby improving nitrogen cycling and increasing the availability of this essential nutrient for plant growth (36).

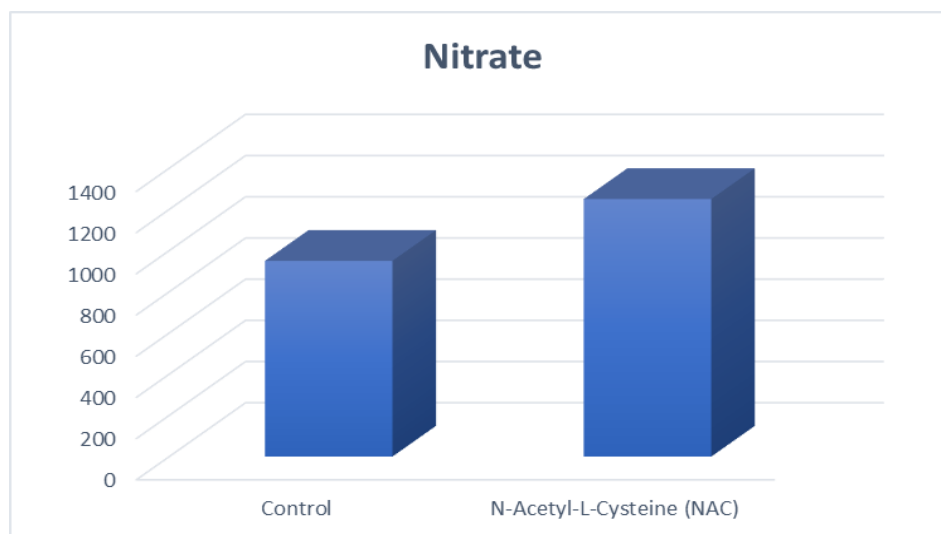


Figure (16): Impact of Control and NAC Treatments on the Nitrate Content in Organic Fertilizer.

Heavy Metals (Cd, Pb)

In the control treatment, cadmium (Cd) and lead (Pb) concentrations were recorded at 1.2 mg/kg and 5.3 mg/kg, respectively. Following the addition of N-acetyl-L-cysteine (NAC), these levels decreased to 0.9 mg/kg for Cd and 4.1 mg/kg for Pb. This reduction indicates that NAC contributes to mitigating heavy metal toxicity, likely

through its thiol (-SH) groups, which have the ability to chelate and immobilize toxic metals, thereby reducing their bioavailability and enhancing root zone safety (37).

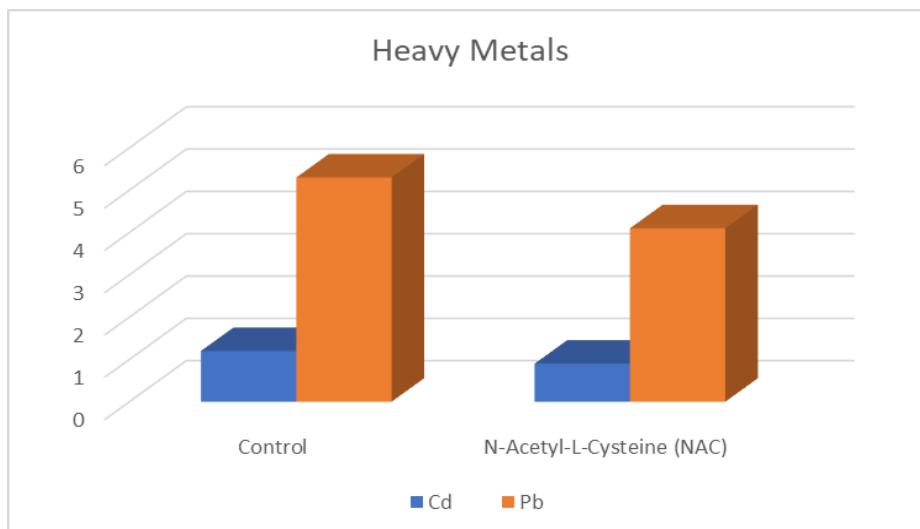


Figure (19): Impact of Control and NAC Treatments on the Heavy Metals Content in Organic Fertilizer.

Physical Analysis of Control and NAC Treatments to Assess the Quality of Organic Fertilizer

The physical parameters of the produced fertilizer included pH, electrical conductivity (EC), and moisture content (Table 5).

Table (5): Physical Parameters for Evaluating the Quality of Organic Fertilizer

Parameter	Control	N-Acetyl-L-Cysteine
Ph	6.5	6.8
Electrical (dS/m)	3.2	2.9
Moisture Content (%)	25	24

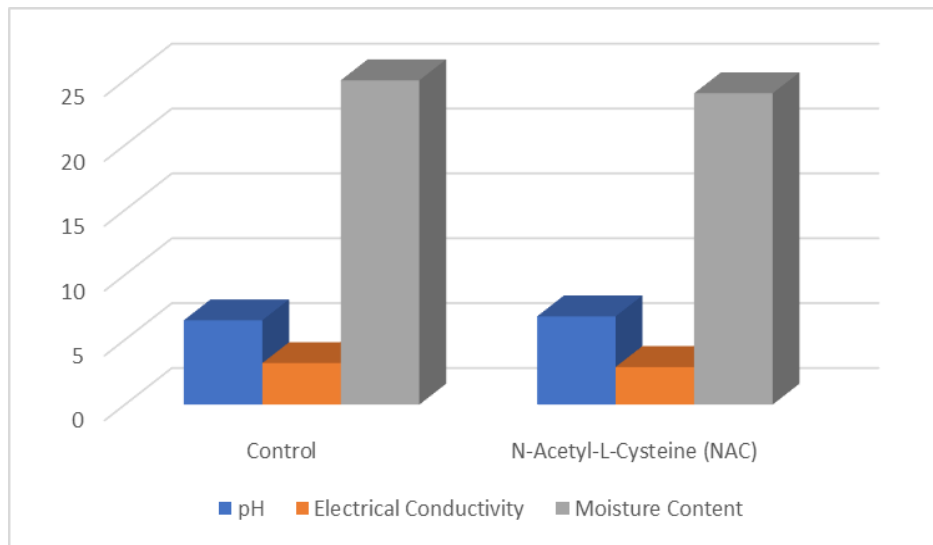


Figure (17): Comparison of Physical Property Parameters in Control and NAC Treatments

Biological Analysis of Control and NAC Treatments to Assess the Quality of Organic Fertilizer

In the control treatment, microbial counts were maintained at baseline levels, reflecting the limited biological activity typically observed in raw poultry manure. The addition of N-acetyl-L-cysteine (NAC) resulted in a moderate increase in microbial populations, indicating improved environmental conditions within the substrate. This enhancement is likely attributed to NAC's role in reducing oxidative stress and stabilizing the microbial habitat, thereby supporting the decomposition process and nutrient availability. These findings emphasize the significance of promoting microbial activity as a critical factor in determining fertilizer quality, aligning with studies that link improved biological conditions to enhanced agricultural performance (38).

This research demonstrates the significant potential for targeted biochemical modification to improve the agronomic value of poultry manure, supported by plant growth trials and LC-MS metabolite profiling. The integration of growth data with metabolomic analysis reveals how N-acetyl-L-cysteine (NAC) modulates poultry manure decomposition and enhances plant biomass accumulation. The control treatment, consisting of sand mixed with poultry manure without additives, exhibited a typical bell-shaped biomass response curve, peaking at 30% manure concentration (0.0476 g) before declining sharply at higher concentrations ($\geq 40\%$) due to phytotoxicity and seedling mortality. LC-MS profiling identified the presence of phytotoxic short-chain fatty acids such as acetic acid and butyric acid, alongside aromatic compounds including phenol and indole, all known to inhibit root development and microbial symbiosis [40-41]. These results highlight the inherent limitations of applying raw poultry manure at elevated levels.

In contrast, NAC addition markedly improved biomass production at all manure concentrations, with fresh weight increasing more than tenfold compared to the control

at optimal NAC doses (0.02–0.04 g NAC with 10–30% manure), without growth inhibition even at 90% manure. LC-MS analysis of NAC-treated samples showed a significant reduction in butyric acid and the appearance of sulfur-containing metabolites, including free thiols and NAC derivatives, indicating a shift toward a reductive, redox-buffered environment that mitigates oxidative stress and enhances nitrogen bioavailability [42-43]. Phosphorus speciation remained largely unaffected by NAC treatment. Furthermore, NAC's thiol groups likely contributed to heavy metal detoxification via chelation, improving root zone safety and overall plant health.

The chemical and biological enhancements observed with NAC addition, including increased total nitrogen content, elevated nitrate levels, decreased phenolic compounds, and moderated ammonium concentrations, further support its role in improving nutrient cycling and reducing manure-induced phytotoxicity. These effects combined to significantly broaden the usable concentration range of poultry manure for safe and effective plant growth, demonstrating NAC's capacity to transform raw organic waste into a more stable and agronomically beneficial fertilizer.

This study highlights the capacity of N-acetyl-L-cysteine (NAC) to remediate poultry manure by reducing phytotoxicity and enhancing nutrient bioavailability, thereby substantially improving plant biomass production compared to untreated controls. LC-MS metabolite profiling confirmed that NAC alters manure decomposition by decreasing toxic short-chain fatty acids and generating sulfur-containing metabolites that support a reductive, stabilized environment conducive to plant growth. NAC supplementation increased nitrogen availability and reduced heavy metal toxicity through chelation, contributing to safer and more efficient organic fertilizer use.

Significantly, NAC extended the effective range of poultry manure application, enabling productive growth at manure concentrations that inhibited plants in the control treatment. These findings establish NAC as a promising additive for optimizing the agronomic and environmental performance of poultry manure-based fertilizers, supporting sustainable organic agriculture through biochemical modulation of organic waste.

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