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Effects of ZnO-Based Smart Urea Coating on Ammonium Release and Characterization of Soil Ureolytic Bacteria in Loamy Soil

Fathia Meidy Nurindriana[1,✉], M. Wasilul Lutfi[2], Atiqah Aulia Hanuf[2,3], Soemarno[2], Sugeng Prijono[2]

[1] Study Program of Soil and Water Management, Department of Soil Science, Agriculture Faculty, Brawijaya University, INDONESIA.
[2] Department of Soil Science, Agriculture Faculty, Brawijaya University, INDONESIA.
[3] Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, TAIWAN.

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Corresponding Author:
✉ fathia.meidyn@gmail.com
(Fathia Meidy Nurindriana)

ABSTRACT

The application of ZnO-coated urea is a promising strategy to reduce nitrogen losses and improve zinc availability in soils. This study aimed to evaluate the effects of ZnO-coated urea on soil ammonium concentrations and ureolytic bacterial populations. A completely randomized design was used with five treatments:control (CU), uncoated urea (CU) and ZnO-coated urea at three different coating (bulk ZnO (NCU-Z), ZnO NPs (NCU-ZNP), and ZnO NPs combined with bentonite NPs (NCU-ZBNP)), each with four replications. Soil samples were incubated on sterile jars under controlled conditions for 8 weeks and used loamy soil as a medium. Ammonium concentrations were measured using the spectrohotometry, while ureolytic bacteria were enumerated using the total plate count (TPC). NCU characterization results revealed inconsistencies in coating thickness, however, it has shown significant results on soil ammonium content and total ureolytic bacteria population. Results on the number of ureolytic bacterial populations showed that NCU-ZNP treatment could reduce the number of bacteria up to 77.8%. The research results also showed that the NCU-ZBNP treatment significantly maintained soil ammonium levels that were 1.76 times higher compared to CU.These findings suggest that ZnO-coated urea can slow nitrogen transformation and potentially improve nitrogen use efficiency while influencing soil microbial communities.

1. INTRODUCTION

Agriculture relies heavily on fertilizers to optimize productivity, with global consumption reaching 134.2 kg/ha in 2022 and projected to rise by 2.2% by 2025; nitrogen fertilizer demand alone reached 144 Mt in 2023 (IFA, 2025; World Bank, 2025). In Indonesia, subsidized nitrogen fertilizer use increased by 50.7% in 2024, and as a tropical region it faces higher nitrogen losses due to rainfall, solar radiation, and humidity, which accelerate fertilizer dissolution, nitrogen mineralization, and leaching (Huddell *et al*., 2020; Yu *et al*., 2022). Urea, the most widely used nitrogen fertilizer (46% N), is highly susceptible to volatilization and leaching, with losses of 16–21% under conventional use (Ransom *et al*., 2020; Hayashi *et al*., 2022; Tapia-Hernández *et al*., 2022). To address these losses, Controlled-Release Fertilizer (CRF) technology has been developed, employing hydrophobic coatings to regulate nutrient release and improve urea efficiency by 23–48%, thereby delaying dissolution and reducing nitrogen losses (Jingjing et al., 2015; Zheng et al., 2021).

Conventional CRF coatings lose functionality once urea is fully released, prompting the development of alternative materials that not only regulate nutrient release but also enhance soil quality. Among these, zinc oxide (ZnO) provides dual benefits: as a source of the essential micronutrient zinc (Zn^{2+}) for phytoenzyme production and as an inhibitor of ureolytic bacteria and urease activity (Cheng *et al*., 2020; Beig *et al*., 2022; Abdelmeged *et al*., 2023; Lahive *et al*., 2023). Bentonite, a smectite clay dominated by montmorillonite, offers strong binding capacity that slows urea release (Baker *et al*., 2022; Yadav *et al*., 2023). More recently, CRF coatings have incorporated nanotechnology to improve performance by reducing particle size, increasing surface area, and strengthening material bonds (Vejan *et al*., 2021 Helal *et al*., 2023). Both natural ZnO and ZnO nanoparticle (NP)-coated urea have significantly influenced microbial biomass and nitrogen mineralization compared to conventional urea (Sadiq *et al*., 2021; Abdullah *et al*., 2022), although data on bacterial population dynamics and ammonium availability remain limited.

Transforming ZnO into nanoparticle form enhances its contact with bacteria and capacity to suppress urease, while bentonite NPs improve soil adhesion, create micropores, and immobilize Zn ions, modulating bioavailability. Together, ZnO and bentonite NPs form Nano-coated Urea (NCU), a novel formulation designed to regulate microbial activity and reduce nitrogen loss by targeting ureolytic bacteria, which hydrolyze urea into ammonia that may volatilize or convert to ammonium, thereby lowering fertilizer efficiency (Fowler *et al*., 2015; Nurhidayati, 2017; Grzyb *et al*., 2021; Mekonnen *et al*., 2021).

$$CO(NH_2)_2 + 2\,H_2O \xrightarrow{\text{Urease}} 2\,NH^{4+} + CO_3^{2-} \quad (1)$$

The integration of ZnO nanoparticles (NPs), bentonite NPs, and polymer materials in Nano-coated Urea (NCU) represents a novel coating strategy that can modulate soil microbial communities responsible for key nitrogen transformations, including mineralization, fixation, and various denitrification pathways. Urease-producing bacteria are particularly affected, as enhanced urea availability can stimulate their abundance and diversity; however, the incorporation of nanomaterials enables controlled urea dissolution within safe thresholds. By restricting ureolytic activity and slowing the conversion of urea to ammonium, NCU offers both agronomic and environmental advantages. Yet, previous studies have not quantified bacterial populations via the Total Plate Count (TPC) method or assessed ammonium levels as indicators of urea decomposition under high-Zn conditions. This study addresses these gaps, underscoring the potential of NCU to optimize ammonium availability while regulating number of ureolityc bacteria, thereby supporting sustainable agriculture and maintaining soil health.

2. MATERIALS AND METHODS

2.1. Experimental Design

This research was conducted from August 2023 to September 2024 in Pujon Kidul Village, Malang Regency, Indonesia. Soil samples for the incubation medium were collected from the 0–20 cm top layer of loamy soil, air-dried, and sieved to 2 mm. Laboratory analyses were carried out at the Applied Physics Laboratory, Faculty of Mathematics and Natural Sciences, and the Biology Laboratory, Department of Soil, Faculty of Agriculture, Brawijaya University.

The experiment followed a Randomized Complete Block Design (RCBD) with 120 incubation jars, consisting of five treatments, four replications, and six destructive sampling periods. Treatments included soil without fertilizer (control, C), conventional urea (CU), and coated urea produced with bulk ZnO, ZnO nanoparticles (NPs), or a combination of ZnO NPs and bentonite NPs. Coating was performed using a pan rotary granulator, and treatment details are presented in Table 1. Each jar contained 100 g of soil adjusted to field capacity by adding 8.5 mL of distilled

Table 1. Reseach treatment

Code	Treatment	Fertilizer materials		
		Urea	Main coating	Other polymers
C	Without fertilizer treathment	-	-	-
CU	Conventional urea	200 g of urea	-	-
NCU-Z	NCU with bulk ZnO coating		15 g of ZnO bulk	5 mL of molasses and 20 mL of distilled water
NCU-ZNP	NCU with ZnO NP coating		15 g of ZnO NPs	
NCU-ZBNP	NCU with combination of ZnO NP and Bentonite NP coating		7.5 g of ZnO NPs combined with 7.5 g of bentonite NPs	

Note: NP was nano particles material

water and maintained by watering at two-week intervals. Fertilizer was applied according to treatments: 1.78 g for urea, 1.68 g for coated formulations, and none for the control. Incubation was conducted at temperature of 27 °C (average) in a closed room, and samples were collected periodically at designated Weeks After Incubation (WAI).

Soil ammonium (NH_4^+) concentrations were determined on days 0, 1, 2, 4, 6, and 8 WAI using spectrophotometry. Ureolytic bacteria were enumerated by the pour plate method on Urea Agar Based (UAB) medium, incubated at 30 °C for 42 hours, and expressed as CFU/g fresh soil. Data were analyzed by Analysis of Variance (ANOVA), and treatment means were compared using Duncan's New Multiple Range Test (DMRT) at the 5% significance level. All statistical analyses were conducted using R-Studio version 2025.05.0+496.

Table 2. Research parameter

Parameter	Object	Method	Unit
Fertilizer Characterization	Code Material and NCU Fertilizer	PSA (Particle Size Analyzer)	-
		SEM (Scanning Electron Microscopy)	-
		EDS (Energy-Dispersive X-ray Spectroscopy)	-
		FTIR (Fourier-Transform Infrared Spectroscopy)	-
Fertizer Dissolution		Visual	-
Number of Ureolytic Bacteria	Incubation Soil	Total Plate Count (TPC)	CFU/g fresh soil
Soil ammonium		Spectrophotometry (UV-VIS)	Persent (%)

Note: Sampling on material and NCU was just carried out before the treatment was applied; Sampling on TPC and ammonium was carried on 0, 1, 2, and 4 Weeks After Incubation (WAI)

Table 3. Initial soil analysis

Parameter	Value	Categorize
Water Content (%)	15.9	-
Bulk Density (g cm^{-3})	1.5	High
Particle Dencity (g cm^{-3})	1.95	-
Soil texture	Loam (35% sand; 45% silt; 20% clay)	Loamy
pH	6.8	Nuetral
EC (µS)	782	-
Total Nitrogen (%)	0.18	Low
Organic Carbon (%)	1.04	-
Total-Zn (ppm)	65.2	Low (digest HNO_3/$HClO_4$ method)
Available-Zn (ppm)	7.6	-
TPC (x 10^6 CFU. g^{-1})	1.69	-

Note: The categories were determined based on the Technical Guidelines from BSIP (Eviati *et al*., 2023; Kurnia *et al*., 2023).

The soil early analysis includes nutrient content, soil texture conditions, and the number of microorganisms before treatment, as shown in Table 3. This analysis can also be used as comparative data after the study with the application of fertilizer. Soil data's bellow carried out before the research shows the soil has a loamy texture. This data (Table 3) can also be used as supporting data for the main data. The framework can be seen in the image below (Figure 1).

2.2. Nano Coating Urea Production Fertilizer Coating Material Production

Fertilizer coating material production starting from bulk ZnO and bentonite were first calcined at 400 °C for 2 hours to remove residual organic matter, followed by sieving through a 45 µm mesh. Materials requiring nano-sizing were further processed using dry planetary ball milling with ceramic balls (3 mm) at 400 rpm for 3 hours, maintaining a 1:10 material-to-ball mass ratio. The milled products were subsequently sieved (45 µm) to ensure uniform particle size before use as urea coating materials.

Nano-fertilizer coatings were prepared using a pan rotary granulator by combining 200 g of urea with 15 g of coating material (ZnO, nano-ZnO, or a mixture of nano-bentonite and nano-ZnO), 5 mL of molasses, and 20 mL of distilled water. The granules were rotated at 60 rpm under heating (50–90 °C) for ~20 minutes until uniformly coated and dried, yielding fertilizer ready for subsequent trials.

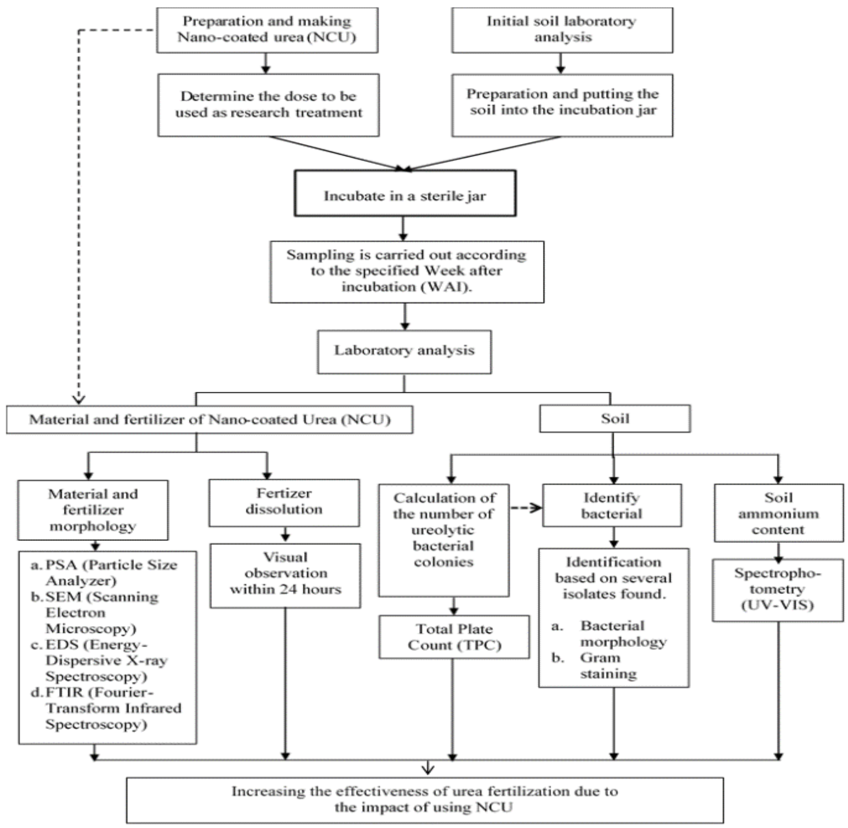

Figure 1. Research framework

2.3. NCU Characterization

Both the coating materials and the final Nano-coated Urea (NCU) were characterized using SEM (Scanning Electron Microscopy), EDS (Energy Dispersive Spectroscopy), and XRD (X-ray diffraction) analyses. The PSA (Particle Size Analyzer) as well applied to raw materials. Solubility was tested by soaking 2 g of fertilizer in 100 mL of distilled water for 24 h, followed by visual observation of dissolution, disintegration, or residue.

2.4. Preparation of Ureolytic Bacteria Isolation

Bacterial isolation was performed from refrigerated soil samples using the pour plate method on Urea Agar Based (UAB) medium. A 0.85% NaCl physiological saline solution was prepared as the extraction medium, while the UAB medium contained NaCl, peptone, glucose, KH_2PO_4, phenol red, and agar in distilled water. Urea was excluded during preparation and added aseptically after sterilization. All media and equipment were sterilized by autoclaving at 121 °C and 1 MPa for 45 minutes before.

Soil suspensions (5 g soil in 45 mL saline) were homogenized and serially diluted to 10^{-5}–10^{-6}. A 40% urea solution (dissolving 40 g of sterilized urea in 100 mL of distilled water) was prepared under laminar airflow and added to the medium at 40–50 °C using requires sterile filter paper. Serial dilution (0.1 mL) from the 10^{-4} to 10^{-6} dilutions were inoculated onto sterile plates using the pour plate method. Cultures were incubated for 48 hours (2 × 24 h) to allow the growth of ureolytic bacterial colonies for further analysis.

2.5. Counting Ureolytic Bacteria

The population of ureolityc bacteria was quantified using the Total Plate Count (TPC) method, which assumes that each viable microbial cell in suspension can develop into a colony under suitable growth conditions. The abundance of ureolytic bacteria was expressed as colony-forming units per gram (CFU/g), while their activity was further represented by fitting population. The total population of isolated bacteria was then calculated using Equation 2, where *fp* is serial dilution.

$$\text{CFU} = \frac{((\Sigma \text{ colony} \times fp))}{(\text{soil sample})} \quad (2)$$

2.6. Soil Ammonium Analysis

Prior to sample analysis, a standard series was prepared to establish the regression value of the calibration curve. Ammonium (NH_4^+) content was determined by weighing 1 g of soil into a film vial, followed by the addition of 10 mL of 2 M KCl solution and homogenization on a shaker for 60 minutes. The suspension was filtered, and 2 mL of the filtrate was transferred into a reaction flask, to which 4 mL each of sodium phenate and sodium tartrate solutions were added. After vortex mixing and a 10-minute reaction, 4 mL of 5% NaOCl was introduced, followed by vortexing and an additional 10-minute incubation. This procedure was repeated for all samples, which were subsequently analyzed using a UV–VIS spectrophotometer at a wavelength of 205 nm. The readings are used to calculate the soil ammonium content (Eq. 3), expressed as a percentage (%) or in ppm.

$$\text{Ammonium } (\%) = \text{C} \times \left(\frac{\text{v}}{(1{,}000 \text{ mL})}\right) \times \left(\frac{100}{\text{m}}\right) \times \text{fk} \quad (3)$$

where *C* is ppm curve, ν is filtrate volume (mL), and *m* is sample mass (mg)

3. RESULTS AND DISCUSSION

3.1. Characteristic of Nano Coating Urea

3.1.1. Particle Size Analysis (PSA)

Particle size distribution analysis was conducted on ZnO nanoparticles (NPs) and bentonite NPs to evaluate whether the ball-milled products fulfilled the nano-size criterion (<1 µm). As shown in Figure 2a, ZnO NPs predominantly achieved the nano-scale, with 66% of particles measuring 26.68 nm and all particles classified within the nanoparticle category suitable for fertilizer coating. In contrast, bentonite NPs (Figure 2b) did not fully meet this classification, as particles >1 µm remained dominant, although a fraction of nano-sized particles was present. Despite this limitation, bentonite was retained as a coating material due to its partial nano-scale fraction with potential functional benefits.

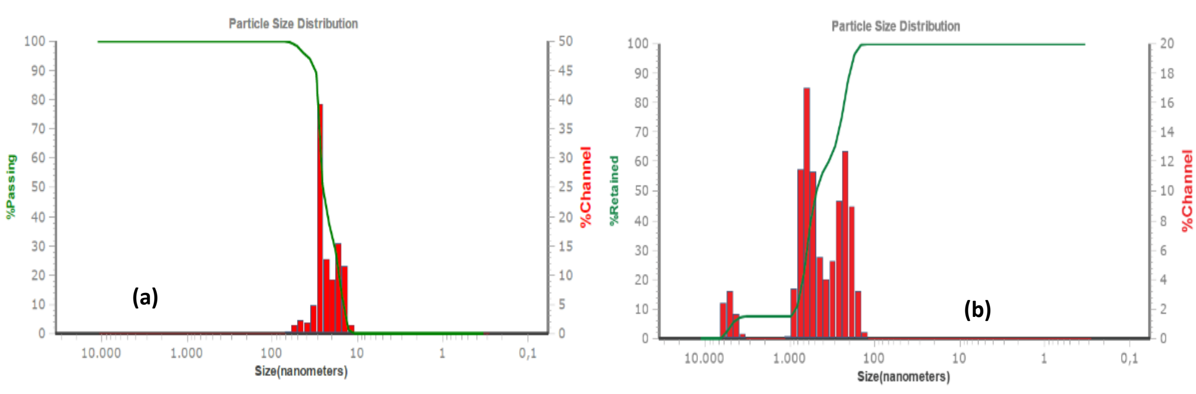

Figure 2. Particle size distribution resulted from PSA: (a) ZnO NP PSA; (b) Bentonite NP PSA

The PSA results also indicated heterogeneous particle size distribution, primarily attributed to agglomeration during milling. Previous studies highlight milling parameters such as temperature, speed, duration, and ball-to-material ratio—govern the extent of agglomeration (Pohshna & Mailapalli, 2023; Qi & Wang, 2024). While the applied milling conditions were effective for ZnO, they were less suitable for bentonite, as particles tended to adhere to tube walls and densify under combined friction and heat. Suboptimal milling settings may also induce re-aggregation of initially nano-sized particles, resulting in stronger cohesive bonds (Maleki & Karimi-Jashni, 2017;

Zhang *et al.*, 2020). These findings underscore the need to optimize milling parameters according to the physicochemical characteristics of each material, as intrinsic differences between ZnO and bentonite strongly affect their capacity to achieve stable nano-scale structures (Pohshna & Mailapalli, 2023).

Bentonite, a smectite clay mineral (montmorillonite), is characterized by a high density of negative surface charges (Baker *et al.*, 2022). During milling, its large specific surface area and pressure-induced effects promote agglomeration. The bentonite used in this study contained 63.62% silica (Si), whereas a previous study with 54.96% Si content reported that prolonged milling and heavier ceramic balls dispersed more particles, expanded surface area, and further increased agglomeration (Maleki & Karimi-Jashni, 2017). In contrast, ZnO particles, stabilized by their hexagonal wurtzite crystal structure, are less prone to aggregation under high pressure and temperature (Molefe *et al.*, 2015), which explains their more consistent nano-scale distribution.

3.1.2. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) at 200X magnification (100 µm scale) revealed that the coating layers in NCU-Z, NCU-ZNP, and NCU-ZBNP treatments were consistently thin (< 50 µm) and uneven (Figure 3). Cross-sectional images obtained from fractured granules confirmed the presence of irregular white outer layers, indicating that the coating process did not produce a uniform protective barrier.

The heterogeneity of coating thickness is likely attributable to non-uniform deposition during the pan rotary granulation process and an insufficient ratio of coating material to urea (Dubey & Mailapalli, 2019). Manual application of the coating solution and the hygroscopic nature of urea further exacerbated uneven layer formation, thereby limiting the ability of Nano-coated Urea (NCU) to effectively reduce solubility and prolong nutrient release. Similar limitations have been reported in zeolite-based Controlled-Release Fertilizers (CRF), where irregular coatings persisted despite higher thicknesses of 135–150 µm due to poorly controlled application intervals and volumes (Dubey (Dubey & Mailapalli, 2019). Achieving finer particle size distribution (<50 µm) and optimized formulation is therefore critical to enhance coating uniformity and efficiency. Supporting evidence from nano-silica coatings (<100 µm) demonstrated that increasing coating thickness by 35.2% improved resistance to dissolution by up to 300% (Shen *et al.*, 2019). These results underscore the importance of tailoring both formulation and application methods to maximize the performance of NCU.

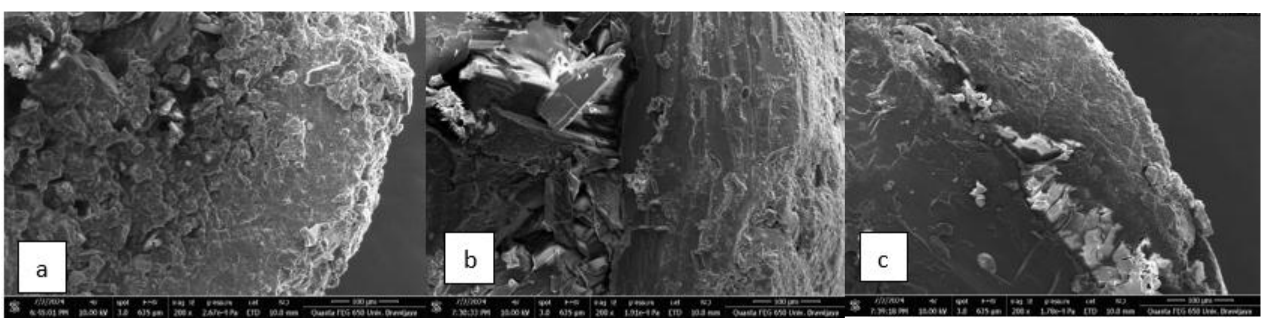

Figure 3. SEM Result. Note: a) NCU-Z treatment; b) NCU-ZNP treatment; c) NCU-ZBNP treatment

Table 4. Material and NCU's EDS result

Object	Weight (%)	
	Silica (Si)	Zinc (Zinc)
ZnO natural / bulk powder	17.73	46.34
ZnO nanoparticles powder	34.65	32.14
Bentonite nanoparticles powder	63.62	0
NCU-Z treatment	0.10	4.97
NCU-ZNP treatment	0	2.24
NCU-ZBNP treatment	0.47	1.33

SEM analysis revealed that the coating layer was uneven and thin (< 50 µm), indicating suboptimal performance. While the applied NCU can be used in tropical soils, improvements in coating quality are needed to enhance resistance to hydrolysis and reduce nitrogen immobilization. In soils with high bulk density (1.5 g cm^{-3}) and silt content (~45%), released ammonium may become adsorbed onto soil colloids, increasing susceptibility to denitrification and ammonia volatilization, which can reach up to 40% under tropical conditions (Cantarella *et al*., 2018; Wallace *et al*., 2020).

3.1.3. Energy Dispersive X-ray Spectroscopy (EDS)

The EDS results show that the raw powders are enriched in their characteristic elements, with ZnO bulk powder containing the highest zinc level (46.34 wt%) and bentonite nanoparticles dominated by silica (63.62 wt%). In contrast, all NCU treatments exhibited much lower elemental contents, consistent with the presence of only thin coating layers on the urea surface (Figure 4). Specifically, NCU-Z retained moderate zinc (4.97 wt%), NCU-ZNP contained reduced zinc without silica, and NCU-ZBNP incorporated both elements at low levels. These findings indicate that the nano-coating process successfully deposited functional layers while minimizing the overall elemental proportion.

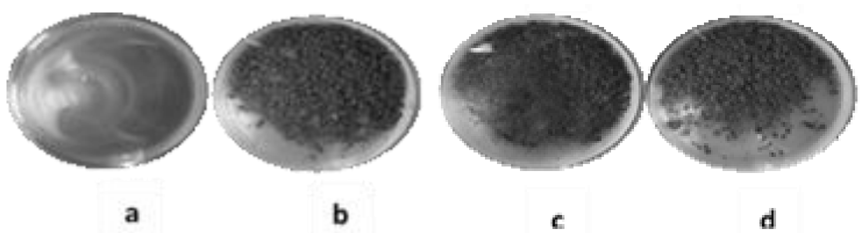

Figure 3. Dissolution of NCU Result. Note: Treatment C; a) ZnO nanoparticles; b) NCU-Z; c) NCU-ZNP; d) NCU-ZBNP

The overall reduction in Zn content following fertilizer incorporation is likely linked to suboptimal coating performance, including inadequate thickness, poor material ratios, and insufficient homogenization, which often result in thin and irregular layers (Abdullah *et al*., 2022; Degryse *et al*., 2024). The EDS spectra of NCU-ZNP and NCU-ZBNP further suggest that nanoparticles are more prone to agglomeration than bulk materials, thereby reducing coating uniformity and Zn retention (Dimkpa *et al*., 2020). Material composition also contributes to coating efficiency, as adhesion depends on the physicochemical interaction between coating agents and the urea surface. Previous studies have demonstrated that the addition of binders, such as molasses or paraffin, enhances coating stability and improves adhesion of Zn-based materials to fertilizer granules (Sadiq *et al*., 2021). These findings highlight that optimization of formulation and the integration of binding agents are essential strategies to increase Zn retention and improve the performance of Nano-coated Urea (NCU).

3.1.4. Fertilizer Dissolution

The solubility test demonstrated clear differences in dissolution behavior among treatments. After 24 hours of immersion in distilled water, the control (C) and NCU-ZNP were fully dissolved, whereas NCU-Z and NCU-ZBNP remained partially intact (Figures 4b and 4d). In both NCU-Z and NCU-ZBNP, the urea core dissolved completely within 24 hours, but the coating capsules persisted, indicating that the applied layers provided enhanced water resistance and stronger bonding compared with the other treatments.

The superior performance of NCU-ZBNP is attributed to the synergistic interaction between ZnO and bentonite nanoparticles, which form stronger composite bonds and improve resistance to water (Maleki & Karimi-Jashni, 2017). By contrast, NCU-ZNP showed no improvement over NCU-Z, likely due to inconsistencies in coating formulation that limited encapsulation efficiency and resulted in similar dissolution patterns (Dimkpa *et al*., 2020). Although bentonite enhanced interparticle bonding, SEM analysis revealed that the coatings in this study were thin (<50 µm) and uneven, making them prone to dissolution and accelerating nitrogen release. Previous studies have shown that thicker coatings, approaching 1 mm as in nano-silica applications, can extend nitrogen release for up to 65 days (Shen *et al*., 2019). These findings underscore the importance of optimizing both coating composition and thickness to improve the controlled-release performance of NCU in tropical agricultural use.

3.2. Effect Of Nano-coating Urea to Ureolityc Bacteria Population

The effects of Nano-Coated Urea (NCU) application on soil ureolytic bacterial populations are summarized in Table 5. Bacterial counts increased at one week after incubation (WAI) across all treatments but declined thereafter. The peak occurred at 1 WAI under conventional urea (CU), whereas the control (C) and NCU treatments peaked at 2 WAI. The CU treatment showed the fastest decline, with reductions of 20.5% by 2 WAI and 34.6–85.7% over the incubation period. Significant differences in bacterial populations were observed in NCU-Z, NCU-ZNP, and NCU-ZBNP compared with both C and CU at 4, 6, and 8 WAI, except for NCU-ZNP at 6 WAI. The delayed peak in C suggests reliance on native soil organic matter (Setiawati *et al*., 2019), while the rapid increase under CU reflected high ammonium concentrations that supported microbial proliferation (Grzyb *et al*., 2021; Liu *et al*., 2025).

Over the 8-WAI period, bacterial populations gradually declined, likely due to prolonged exposure to zinc released from NCU coatings. Statistical analysis confirmed that Zn-based coatings modulated microbial dynamics, presumably through Zn^{2+} release from soil colloids or coating polymers. Previous studies have shown that Zn^{2+} disrupts ureolytic metabolism and damages bacterial cells (Rajput *et al*., 2018; Abdullah *et al*., 2022; Lahive *et al*., 2023). This inhibitory effect is expected to reduce ammonia formation in soil and thereby improve nitrogen-use efficiency.

Table 5. The result of numbered ureolityc bacteria

Treatment	0 WAI	1 WAI	2 WAI	4 WAI	6 WAI	8 WAI
C	1.3×10^6	2.4×10^6 c	2.6×10^6	2.1×10^6 a	1.7×10^6 a	1.7×10^6 a
CU	1.4×10^6	3.9×10^6 a	3.1×10^6	2.1×10^6 a	1.8×10^6 a	1.8×10^6 a
NCU-Z	1.3×10^6	2.7×10^6 b	2.8×10^6	1.3×10^6 b	1.3×10^6 b	1.0×10^6 b
NCU-ZNP	1.4×10^6	2.4×10^6 c	$2.8.\ 10^6$	1.7×10^6 ab	0.8×10^6 c	0.4×10^6 d
NCU-ZBNP	1.3×10^6	$2.0.\ 10^6$ d	2.5×10^6	1.5×10^6 b	1.1×10^6 b	0.7×10^6 c
*	ns	s	ns	s	s	s

Note: TPC's unit was (Colony Forming Unit) CFU g^{-1}. * label "ns" was not significant result and "s" was significant result based on ANOVA. Alphabetic code was result from DMRT 5%.

The application of NCU in tropical agricultural soils, particularly those with properties similar to the sampling site, requires careful consideration of soil constraints (Table 3). NCU formulations containing Zn must also be managed to avoid excessive contamination, since Zn serves as both an essential micronutrient for plants and a potential stress factor for soil microbes. Under incubation conditions, Zn-coated urea has been shown to reduce ammonium availability by 41.1–76.4%, which may also influence microbial community dynamics when applied directly in the field. The release of Zn^{2+} can affect not only ureolytic bacteria but also total bacterial opulations, as previous studies have reported that a concentration of 1000 µg/L reduced general bacterial counts by 5% within 24 hours, leading to increased ammonium accumulation (Prywer *et al*., 2024). Moreover, bacterial species such as Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Acinetobacter baumannii exhibit varying resistance to Zn^{2+}, with tolerance levels ranging from 0.25 to 5000 µg/mL (Aliyu *et al*., 2023; Jasim *et al*., 2023; Mekonnen *et al*., 2021).

A total of 12 bacterial isolates were obtained and characterized based on morphology, Gram staining, and urease activity (Table 6). Of these, 58% were Gram-negative, and 57% of Gram-negative isolates exhibited the highest urease activity. The predominance of Gram-negative bacteria, with thinner cell walls, may explain their greater susceptibility to Zn^{2+} ions from NCU coatings (Grzyb *et al*., 2021). Although certain isolates demonstrated high urease activity, the controlled Zn release and regulated urea dissolution from NCU coatings can limit overall ureolytic activity, contributing to more sustainable nitrogen release in soil systems.

3.3. Effect of Nano Coating Urea on Soil Ammonium

The influence of coating materials on soil ammonium availability is presented in Table 7. Across treatments, ammonium concentrations increased up to 6 WAI. The NCU-Z treatment (urea coated with natural ZnO) continued to increase even at 8 WAI, while conventional urea (CU) peaked at 1 WAI, reflecting faster release compared to coated formulations. Statistical analysis confirmed significant differences at all observation points, with the control (C) consistently showing the lowest ammonium concentrations. Notably, CU surpassed the coated treatments after 6 WAI, indicating that coatings were most effective in delaying ammonium release during the early stages.

Table 6. Isolate of ureolityc bacteria

Code	Shape	Margin	Elevation	Texture	Appearance	Optical	Pigment	Gram*	EC (µS)
A3	Filamentous	Filamentous	Convex	Rough	Dull	Opaque	Cream	+	2.15
A6	Irregular	Undulate	Flat	Rough	Dull	Pendaflor	Cream	+	2.59
A7	Irregular	Undulate	Umbonate	Rough	Dull	Pendaflor	Cream	-	11.63
A8	Irregular	Undulate	Convex	Smooth	Glistening	Opaque	Cream	-	41.92
A12	Circular	Entire	Convex	Smooth	Glistening	Opaque	Cream	-	35.55
A15	Irregular	Undulate	Convex	Smooth	Glistening	Opaque	Cream	+	31.03
A22	Circular	Entire	Flat	Smooth	Glistening	Opaque	Cream	+	8.89
A24	Beralun	Entire	Convex	Smooth	Glistening	Opaque	White	+	16.07
A27	Irregular	Undulate	Umbonate	Smooth	Glistening	Opaque	White	-	12.22
A31	Circular	Entire	Umbonate	Smooth	Glistening	Transparent	White	-	30.22
A36	Circular	Entire	Convex	Smooth	Glistening	Opaque	Cream	-	11.18
A37	Irregular	Undulate	Convex	Rough	Dull	Transparent	White	-	41.55

Note: * label "+" was positive gram's and "-" was was negative.

Table 7. The result of soil ammonium

Treatment	0 WAI	1 WAI	2 WAI	4 WAI	6 WAI	8 WAI
C	0.002	0.005 c	0.008 e	0.022 e	0.085 d	0.025 e
CU	0.003	0.048 a	0.098 a	0.135 a	0.223 b	0.160 d
NCU-Z	0.004	0.021 b	0.045 b	0.093 c	0.221 b	0.442 a
NCU-ZNP	0.004	0.022 b	0.029 c	0.121 b	0.262 a	0.240 b
NCU-ZBNP	0.002	0.019 b	0.025 d	0.080 d	0.166 c	0.204 c

Note: Soil ammonium's unit was present (%). Same letters following mean values in the same column was not significant based on DMRT 5%.

The rapid ammonium release from coated urea is attributed to immediate hydrolysis upon contact with soil moisture, consistent with previous studies showing that uncoated urea hydrolyzes within 15 minutes to 4 hours after application (Dubey & Mailapalli, 2019; Ray *et al*., 2018; Sadiq *et al*., 2021). By contrast, coated urea exhibited delayed yet sustained ammonium availability, exceeding CU after 6 WAI. This trend aligns with reports that coatings can extend nutrient release by up to 10 days, depending on the material used (Hayashi *et al*., 2022; Tapia-Hernández *et al*., 2022), thereby enhancing nutrient-use efficiency.

The choice of coating materials plays a critical role in regulating nutrient dynamics. Bentonite contributes to slower leaching due to its clay mineral properties and strong bonding capacity (Neto *et al*., 2023), while zinc not only provides an essential nutrient but also supports microbial control (Abdullah *et al*., 2022; Beig *et al*., 2022). Nanoparticle-based coatings further improve controlled-release performance through increased surface area and reduced micropore size, allowing finer regulation of ammonium availability (Vejan *et al*., 2021; Hussain *et al*., 2022; Helal *et al*., 2023). Collectively, these findings highlight the potential of mineral- and nano-based coatings to sustain soil ammonium availability and improve nitrogen management in agricultural systems.

3.4. Relation of Ureolityc Bacteria Population and Soil Ammonium Content

The regression analysis between ureolytic bacterial populations and soil ammonium content demonstrated highly variable relationships across the incubation period (Figure 5). At the early stages (0–2 WAI), the correlations were consistently positive, with particularly strong associations observed at 1 and 2 WAI (Table.8; Figure 5a; 5b), influence factor of the number of ureolytic bacteria which had the greatest influence on the ammonium content was shown at 2 WAI at 66.9%. These results indicate that bacterial activity played a critical role in accelerating urea hydrolysis and enhancing ammonium release during the initial phase of fertilizer application. In contrast, from 4 to 8 WAI (Figure 5c; 5d), the correlation shifted to negative values, suggesting that increasing bacterial abundance was instead associated with reduced ammonium concentrations. During this later stage, ureolytic bacteria contributed only 22.6-25.1% to ammonium availability.

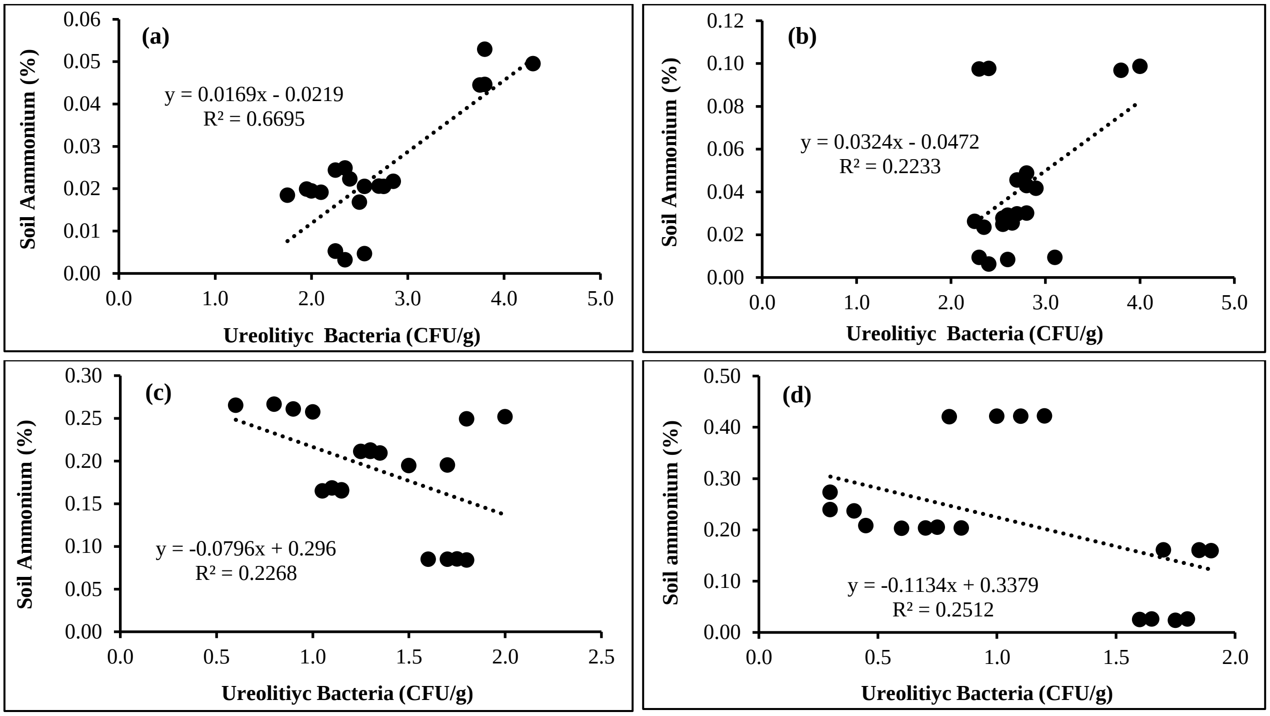

Figure 4. Relation of number of ureolityc bacteria and soil ammonium content at: (a) 1 WAI; b. 2 WAI; c. 4 WAI; d. 8 WAI.

Table 8. Correlation of soil ammonium content (%) and ureolityc bacteria population

	TPC M0	TPC M1	TPC M2	TPC M4	TPC M6	TPC M8
AMO M0	0.059					
AMO M1		**0.818**				
AMO M2			**0.472**			
AMO M4				-0.049		
AMO M6					**-0.476**	
AMO M8						**-0.501**

The positive correlations at the beginning of incubation can be explained by the controlled-release properties of nano-coated urea (NCU). Although ZnO- or bentonite-based coatings delay urea dissolution, diffusion still occurs through micropores and microcracks. Previous studies reported that ZnO-coated urea begins releasing within 15 minutes and can dissolve completely within 140 minutes under waterlogged conditions (Sadiq *et al.*, 2021). This gradual dissolution maintains a constant nutrient supply, allowing ureolytic bacteria to hydrolyze urea into ammonium, which in turn supports bacterial proliferation (Wang *et al.*, 2015).

By 4–8 WAI, however, coating degradation due to osmotic pressure and hydrolytic processes likely accelerated urea release. The resulting ammonium was not only assimilated by ureolytic bacteria but also rapidly oxidized by nitrifying bacteria into nitrite (NO_2^-) and nitrate (NO_3^-) (Farzadfar *et al.*, 2021; Jin *et al.*, 2017; Noll *et al.*, 2019). Consequently, higher bacterial populations promoted faster urea hydrolysis, but concurrent microbial demand and nitrification reduced ammonium accumulation. These results highlight the time-dependent interactions between coating stability, microbial hydrolysis, and nitrogen transformation pathways that collectively regulate soil nitrogen dynamics during incubation.

4. CONCLUSIONS

This study aimed to evaluate the effects of ZnO-coated urea on soil ammonium availability and ureolytic bacterial populations. The results demonstrate that Nano-Coated Urea (NCU), formulated with ZnO nanoparticles, bentonite

NPs, and polymer materials, can optimize ammonium availability while regulating bacterial populations. The eight-week incubation showed that ZnO-coated urea slowed urea dissolution, leading to more sustained ammonium release than conventional urea, while Zn^{2+} availability contributed to bacterial suppression. These findings highlight the role of NCU in modulating microbial dynamics and improving nitrogen-use efficiency.

Although promising, the characterization tests revealed that the coatings remained thin and uneven, indicating a need for improved formulation and consistency in production. As this experiment was conducted under controlled laboratory conditions, further field-based studies are necessary to confirm the results. Integrating ZnO-coated urea into fertilizer management could reduce nitrogen losses, address zinc deficiency, and support more sustainable agricultural practices.

AUTHOR CONTRIBUTION STATEMENT

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
FMN	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓
MWL	✓	✓		✓	✓	✓	✓					✓	✓	✓
AAH	✓	✓		✓	✓	✓				✓		✓	✓	✓
Soe	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
SP	✓	✓		✓	✓	✓			✓	✓	✓	✓	✓	✓

C: Conceptualization	Fo: Formal Analysis	O: Writing - Original Draft	Fu: Funding Acquisition
M: Methodology	I: Investigation	E: Writing - Review & Editing	P: Project Administration
So: Software	D: Data Curation	Vi: Visualization	
Va: Validation	R: Resources	Su: Supervision	

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