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**RESEARCH ARTICLE**

# Urease Enzyme Activity in Surface Soils under Various Land use Types in Minna, Southern Guinea Savanna

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## ABSTRACT

The study sites were some vegetation types in Minna, Southern Guinea Savanna. The objectives of this study were to determine the activity of urease at surface soil depth under three vegetation types and to estimate the effect of vegetation types on physical, chemical, and biological properties of soil. Soil samples were collected diagonally on July 11 and 12, 2014, with the aid of sterilized auger, bulked, air dried, and screened through 2 mm and 0.5 mm sieves for physicochemical and biological properties determination according to standard methods. The treatments were three vegetation types (fallow, teak, and gmelina vegetation), at three soil depths (0–5 cm, 5–10 cm, and 10–15 cm), fitted into a completely randomized design having three replicates. The results obtained demonstrated that urease activity is an indicator of nitrogen status of soil. High urease activity may signify nitrogen stress. The higher the $NH_4$-N volatilized, the higher the urease activity. Teak vegetation had the highest urease activity of 14.47 µg $NH_4$-N/g of soil at depth 0–5 cm while fallow had the lowest of 0.82 µg $NH_4$-N/g of soil at depth 10–15 cm. Low values (<2 µg $NH_4$-N/g of soil) were obtained under gmelina at 0–5 cm and 10–15 cm soil depth, respectively. Fallow and gmelina vegetation which produced the lowest urease activity averagely should, therefore, be recommended as suitable land use types in Minna, Southern Guinea Savanna.

**Key words:** Activity, biological, chemical, depth, enzyme, land use types, physical, soil, urease, vegetation

## INTRODUCTION

Soils receive inputs through various means including naturally, human activities, and plant death. These inputs can affect chemical balance and acidity like the application of fertilizers such as urea for the supply of nitrogen. Nitrogen is a crucial element for plant growth, but the most plant can only use it in the form of ammonia or nitrate. Many animals excrete urea in their urine; soil microorganisms remediate animal urine, producing urease to transform the urea to ammonia, which is then readily accessible to plants. Urea is a product of decarboxylation of certain amino acids; it can be hydrolyzed to ammonia and carbon dioxide by bacteria containing the enzyme urease. Urease is a nickel protein of microorganisms and plants that are used in clinical assays of plasma urea concentrations.

When urea fertilizers are applied to the soil, an enzyme called urease begins converting them to ammonia gas. If this conversion takes place below the soil surface, the ammonia is almost instantaneously converted to $NH_4$-N, which is bound to soil particles. If conversion takes place on the soil surface or on surface residue, there is a potential for ammonia gas to escape back into the atmosphere through a process called ammonia volatilization.

Volatilization losses depend on environmental conditions at the time of application. This loss can be substantially reduced if a urease inhibitor is used with fertilizer. Schwab and Murdock

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(2010) stated that urease inhibitors improve nitrogen use efficiency. It works by slowing one of the processes within the nitrogen cycle, thereby reducing nitrogen loss.

Urea added to soils as fertilizer or as animal urine is hydrolyzed enzymatically by soil urease and the resulting release of ammonia and the rise in pH can lead to several problems including damage to germinating seedlings and young plants, nitrite toxicity, and volatilization of urea N as ammonia, which may cause air and water pollution problems (http://en.m.wikipedia.org/wiki/Ammonia_volatilization_from_urea). Urease activity in soil may originate from plant residues which are rich sources of urease (soybean seeds, watermelon seeds, pumpkin seeds, jack bean seeds, and winged bean seeds), animal waste, or soil microbes containing urease. However, there is no direct evidence for the production of urease by plant roots. It is also reported to be present in animal intestine and excreta. Therefore, the addition of plant materials and animal wastes may supply urease to the soil. Joachim *et al*. (2008) reported that soil urease could be microbial origin. Huan Guo *et al*. (2012) also identified some species of bacteria, yeast, and fungi which contained urease.

Nitrogen is an essential nutrient for plant growth; it can only be taken up by plant in the form of nitrate and some in ammonia nitrogen. $NH_4$-N losses occur when urea (added to soils as fertilizer or as animal urine) is hydrolyzed mainly due to the activity of the enzyme, urease. In most arable soils, urea is rapidly converted to ammonia and $CO_2$ by soil urease and this leads to several problems including nitrite and ammonia toxicities to young seedling plants, and loss of ammonia through volatilization, which may cause air and water pollution problems.

Urease is important because it breaks down urea to ammonia which is eventually oxidized to nitrate, thereby supplying nitrogen in the form

**Table 1:** The effect of soil depth and vegetation type on urease activity and soil texture

| Soil | Urease activity (µg $NH_4$-N/g soil) | Sand (g/kg) | Silt (g/kg) | Clay (g/kg) |
|---|---|---|---|---|
| Depth (D) (cm) | | | | |
| 0–5 | 6.222 | 732 | 108 | 160 |
| 5–10 | 2.878 | 692 | 110 | 198 |
| 10–15 | 1.906 | 707 | 95 | 198 |
| LSD (0.05) | 0.920 | NS | NS | 1.181 |
| Vegetation (v) | | | | |
| Fallow | 2.061 | 738 | 74 | 188 |
| Teak | 6.883 | 681 | 125 | 194 |
| Gmelina | 2.061 | 712 | 114 | 174 |
| LSD (0.05) | 0.920 | NS | NS | 1.181 |
| Interaction | | | | |
| D X V | ** | NS | NS | NS |

Significant at $P < 0.05$, ** – significant at $P < 0.01$, LSD: Least significant difference

**Table 2:** The effect of soil depth and vegetation type on urease activity and chemical properties

| Treatments | OC g/kg | TN g/kg | AVP mg/kg | Ph $H_2O$ | Ca cmol/kg | Mg cmol/kg | Na cmol/kg | K cmol/kg |
|---|---|---|---|---|---|---|---|---|
| Depth (D) | | | | | | | | |
| 0–5 | 24.844 | 0.0941 | 2.301 | 6.807 | 0.129 | 0.069 | 1.295 | 1.003 |
| 5–10 | 17.218 | 0.110 | 1.862 | 6.784 | 0.124 | 0.095 | 0.694 | 0.484 |
| 10–15 | 14.271 | 0.108 | 2.189 | 6.762 | 0.115 | 0.084 | 0.588 | 0.521 |
| LSD (0.05) | 2.2579 | NS | NS | NS | NS | NS | NS | 0.239 |
| Vegetation (v) | | | | | | | | |
| Fallow | 14.329 | 0.099 | 2.681 | 6.758 | 0.118 | 0.080 | 0.696 | 0.719 |
| Teak | 18.373 | 0.103 | 1.721 | 6.859 | 0.128 | o. 079 | 0.854 | 0.734 |
| Gmelina | 23.631 | 0.110 | 1.949 | 6.737 | 0.121 | 0.089 | 1.028 | 0.554 |
| LSD (0.05) | 2.2579 | NS | NS | NS | NS | NS | NS | NS |
| Interaction | | | | | | | | |
| D X V | * | NS | NS | ** | NS | NS | NS | NS |

NS: Not significant, *Significant at $P < 0.05$, **Significant at $P < 0.01$, OC: Organic carbon, Av. P: Available Phosphorus, TN: Total nitrogen, Ca: Calcium, Mg: Magnesium, Na: Sodium, K: Potassium. LSD: Least significant difference

that is useable by plant for growth. However, an increase in urease activity could mean nitrogen use inefficiency or increasing nitrogen loss. Hence, the need to estimate the level of urease activity that can encourage retention of $NH_4$-N is against volatilization loss.

### Objectives

The objectives of the study are as follows:
(i) To determine the activity of urease at surface soil depth under three different vegetations.
(ii) To estimate the effect of vegetation types on soil physical, chemical, and biological properties on urease activity.

## METHODOLOGY

### Study area

The study site was the forest soils of the Federal University of Technology, Gidan Kwano, Minna, which is within the Southern Guinea Savannah vegetation zone with a subhumid tropical climate. The forest has a global positioning system location of latitude 09°31'214"N and longitude 06°27'604"E with an elevation of 233 m. Minna has an average annual relative humidity of 48.9% and average monthly relative humidity ranges 21% in February–73% in August, while the average annual temperature of Minna is hot at 27°C.

### Soil description and biodiversity

The geology of the area is made up of basement complex rocks. The forest was developed 10 years ago and has different tree vegetation such as gmelina (*Gmelina arborea*), teak (*Tectona grandis*), and cashew (*Anacardium occidentale*), with each vegetation covering 4.5 ha. Both the intrarow and the interrow spacing of the trees are 3 m. The predominant trees, shrubs, and weeds of the forest reserve are *Piliostigma reticulatum*, *Nauclea latifolia*, *Eucalyptus camaldulensis*, *Butyrospermum parkii*, *Prosopis africana*, and *Tridax procumbens*. The forest contains biota such as butterflies, mushrooms, grasshopper, ants, birds, termites, centipedes, beetles, millipedes, houseflies, earthworms, spider, dragonflies, and other features such as anthills, termite hills, rock outcrops, and gully channels.

### Treatment and experimental design

The experiment was factorial (two factors which are vegetation types and soil depth). The treatments were three vegetation types (teak, gmelina, and fallow), at three depths (0–5 cm, 5–10 cm, and 10–15 cm), fitted into completely randomized design, having three replicates for each treatment.

### Soil sampling and analysis

#### *Physicochemical properties determination*

Soil samples were collected from three different vegetation of the forest; teak, gmelina, and fallow vegetation. The samples were collected randomly from 10 points on each vegetation type at depth of 0–5 cm, 5–10 cm, and 10–15 cm. Thereafter, samples were bulked according to their depth, air-dried, and crushed to pass through a 2 mm sieve and 0.5 mm sieve for physicochemical analysis according to standard methods outlined by the International Soil Reference and Information Center/Food and Agricultural Organization [Tables 1 and 2].[1-5]

### Urease activity assay

#### *Preparation of reagents*

To prepare potassium chloride (2.5 M)-silver sulfate (100 parts/million) solution, 100 mg of reagent grade $Ag_2SO_4$ was dissolved in 700 ml of water, then 188 g of reagent grade KCl (Guangdong Guanghua Sci-Tech Co., Ltd.) was dissolved in the solution and the solution was diluted to 1 L, while THAM buffer (pH 9.0) 0.05 M was prepared by dissolving 6.1 g of tris(hydroxymethyl)aminomethane in 700 ml of water, then the pH of the solution was brought to 9.0 by addition of 0.2 M sulfuric acid and was diluted with water to 1 L. To prepare 0.2 M urea solution, 1.2 g of urea (Guangdong Guanghua Sci-Tech Co., Ltd.) was dissolved in 80 ml of THAM buffer; thereafter, the solution was diluted to 100 ml with THAM buffer before storage in a refrigerator. The reagents for the determination of ammonium (magnesium oxide, boric acid-indicator solution, and 0.005 N sulfuric acid) were prepared as described by Ekologija, Gupta and Bhardway, Guo *et al*., Food and Agricultural Organization.[6-10]

### Method for assay of urease activity

About 5 g of soil (0.5 mm) was placed in a 50 ml

volumetric flask, 0.2 ml of toluene and 9 ml of THAM buffer were added; thereafter, the flask was swirled for a few seconds to mix the contents. About 1 ml of 0.2 M urea solution was then added; the flask was swirled again for a few seconds and was stoppered and placed in an incubator at 37°C. After 2 h, the stopper was removed and 35 ml of KCl-$Ag_2S0_4$ was added to the solution, the flask was swirled for a few seconds and was allowed to stand until the contents have cooled to room temperature (5 min). Then, the contents were made to volume (50 ml) by addition of KCl-$Ag_2SO_4$ solution, after which, the flask was stoppered and inverted several times to mix the contents. Ammonium N in the resulting soil suspension was then determined by pipetting 20 ml aliquot of the suspension into a 100 ml distillation flask, designed for use with the steam distillation apparatus described by Bremner and Edwards (1965). The ammonium N released by steam distillation of this aliquot was determined with 0.2 g of MgO for 5 min as described by Bremner and Keeney (1966).

Control was performed to allow for ammonium N not derived from urea through urease activity, by following the procedure described for the assay of urease activity, but the addition of 1 ml of 0.2 M urea solution was done after the addition of 35 ml of KCl-$Ag_2S0_4$ solution. The KCl-$Ag_2SO_4$ solution was prepared by addition of KC1 to $Ag_2SO_4$ solution as specified ($Ag_2S0_4$ will not dissolve in KC1 solution). The soil suspension analyzed for ammonium was mixed thoroughly immediately before using it for ammonium analysis.

Urease activity of the soil was computed as follows:

$$\text{Urea N } (\mu g/g \text{ soil}) = \frac{(a\text{-}b) \times 70 \times v}{w \times al}$$

Where,

a= titer value of the sample

b= titer value of blank

v= volume of extract

w= weight of soil

al= volume of aliquot used for distillation

## Data analysis

Analysis of variance was used to assess treatment difference. Least significant difference was used to separate means where significant differences were observed at 5% probability level while correlation matrix was used to correlate urease activity with soil physicochemical properties.

Globally, urea has been the most used form of nitrogen fertilizer accounting for 46% of all usage because it is the cheapest form of solid N fertilizer to produce and has the highest N content. It is, however, one of the most inefficient forms of N fertilizer due to losses which have been reported to occur by $NH_3$ volatilization. When urea is added to the soil, it is first hydrolyzed to $NH_4$+N by urease enzymes. In this study, the interaction between vegetation and soil depth significantly affected urease activity at $P < 0.01$ implying that the effect of vegetation on urease activity could depend on the depth of the soil underneath the vegetation. This is at variance with the report of Sima *et al*. (2013) who observed that the effect of forest communities and various depths on soil urease activity was not significant.

Table 3 revealed that teak vegetation recorded the highest urease activity of 14.47 µg $NH_4$-N/g of soil at 0–5 cm implying that 14.47 µg $NH_4$-N was volatilized under teak vegetation compared with lower values of 1.87 µg and 2.33 µg $NH_4$-N volatilized under gmelina and fallow vegetations, respectively. This is in agreement with the work of Reddy *et al.* (1987) who reported that soils under vegetation have higher urease activity compared to vegetation-free soils. The lowest urease activity recorded by fallow is consistent with the report of Speir *et al*. (1980) who maintained that urease activity under fallow soils consistently decreased.

**Table 3:** Effect of interaction between soil depth and vegetation type on urease activity

| Treatments | Vegetation | | |
|---|---|---|---|
| Depth (cm) | Fallow | Teak | Gmelina |
| 0–5 | $2.33^{bcd}$ | $14.47^{a}$ | $1.87^{cde}$ |
| 5–10 | $3.03^{bc}$ | $2.80^{bc}$ | $2.80^{bc}$ |
| 10–15 | $0.82^{e}$ | $3.38^{b}$ | $1.52^{de}$ |
| SE ± | | 0.54 | |

Means in the same column followed by the same letter are not significantly different at $P > 0.05$.

**Table 4:** Effect of interaction between soil depth and vegetation type on organic carbon content

| Treatments | Vegetation | | |
|---|---|---|---|
| Depth (cm) | Fallow | Teak | Gmelina |
| 0–5 | $21.67^{b}$ | $26.17^{a}$ | $26.69^{a}$ |
| 5–10 | $12.65^{c}$ | $14.21^{c}$ | $24.79^{a}$ |
| 10–15 | $8.67^{d}$ | $14.73^{c}$ | $19.41^{b}$ |
| SE ± | | 1.32 | |

Means in the same column followed by the same letter are not significantly different at $P > 0.05$.

Averagely, regardless of vegetation, urease activity decreased with depth. This is consistent with the report of Reddy *et al*. (1987) who observed that $NH_4$-N volatilization is higher at the surface of the soil probably as a result of higher microbial proliferation and activity at the rhizosphere. It is also in line with the report of Roberge *et al*. (1968) who maintained that urease distribution decreased in quantity in deeper genetic horizons of soil profiles.

The consequence of such reaction is that the $NH_4$-N emitted into the atmosphere can accentuate climate change and aggravate global warming over a long period of time. Averagely, gmelina vegetation and fallow had similar urease activity, suggesting that variation in plant species marginally affected their urease activity. Although Gupta *et al*. (1990) reported that urease activity was greater in the grassland soils followed by forest soils and uncultivated soils in that order, this work has demonstrated that teak vegetation had greater urease activity than gmelina/fallow in that arrangement.[11-21]

The soil reaction in $CaCl_2$ was significantly affected by the interaction between vegetation and soil depth. Soils under teak were averagely less acidic than those under fallow and gmelina. The highest urease activity of 14.47 µg $NH_4$-N recorded under teak was obtained at soil reaction 6.2. This is below the pH optimum of jack bean urease activity of 6.5–7 reported by Lai and Tabatabai (1992) and above pH optimum of <5.87 reported by Silva and Perera (1971). The result also revealed that urease activity did not consistently increase with pH. The highest urease activity recorded in this work was, however, observed at the highest pH of 6.2. Nevertheless, Brzezinska *et al.* (2001) observed in their work that increase of urease activity, in general, occurs with a relative increase in pH while Nannipieri *et al*. (1978) reported that generally, soil urease is most active at slightly alkaline pH optimum (8.3).

Urease activity correlated positively with sand, silt, Ca, Na, and K contents of soil, respectively, but correlated positively and significantly with organic carbon content, pH in $CaCl_2$, and water. This may be because Ca, Na, and K most likely increased the pH of soil, hence increased urease activity and $NH_4$-N volatilization. Singh *et al.* (1991) reported that urease activity correlated positively with silt

**Table 5:** Effect of interaction between soil depth and vegetation type on pH in $H_2O$

| Treatments | Vegetation | | |
|---|---|---|---|
| Depth (cm) | Fallow | Teak | Gmelina |
| 0–5 | 6.88$^{a}$ | 6.90$^{a}$ | 6.64$^{d}$ |
| 5–10 | 6.70$^{cd}$ | 6.84$^{ab}$ | 6.81$^{ab}$ |
| 10–15 | 6.69$^{cd}$ | 6.83$^{ab}$ | 6.76$^{bc}$ |
| SE ± | | 0.04 | |

Means in the same column followed by the same letter are not significantly different at $P > 0.05$.

**Table 6:** Effect of interaction between soil depth and vegetation type on pH in $CaCl_2$

| Treatments | Vegetation | | |
|---|---|---|---|
| Depth (cm) | Fallow | Teak | Gmelina |
| 0–5 | 6.18$^{a}$ | 6.20$^{a}$ | 5.97$^{d}$ |
| 5–10 | 5.96$^{cd}$ | 6.14$^{ab}$ | 6.10$^{ab}$ |
| 10–15 | 6.01$^{cd}$ | 6.08$^{ab}$ | 6.06$^{bc}$ |
| SE ± | | 0.03 | |

Means in the same column followed by the same letter are not significantly different at $P > 0.05$.

**Table 7:** Correlation matrix between pairs of soil properties

| Soil | Urease | Sand | Silt | Clay | OC | TN | Av. P | pH ($H_2O$) | pH ($CaCl_2$) | Ca | Mg | Na | K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Urease | | | | | | | | | | | | | |
| Sand | 0.05 | | | | | | | | | | | | |
| Silt | 0.04 | −0.92** | | | | | | | | | | | |
| Clay | −0.21 | −0.38 | −0.02 | | | | | | | | | | |
| OC | 0.41* | 0.03 | 0.26 | −0.67* | | | | | | | | | |
| TN | −0.19 | 0.02 | −0.02 | −0.00 | −0.02 | | | | | | | | |
| Av. P | −0.11 | 0.28 | −0.24 | −0.16 | −0.11 | −0.01 | | | | | | | |
| pH ($H_2O$) | 0.46* | −0.08 | 0.17 | −0.18 | 0.17* | −0.19 | −0.17 | | | | | | |
| pH ($CaCl_2$) | 0.49* | −0.03 | 0.37 | −0.26 | 0.09 | −0.23 | −0.30 | 0.97 | | | | | |
| Ca | 0.19 | −0.39* | 0.51* | −0.19 | 0.24 | −0.12 | −0.52* | 0.36 | 0.11 | | | | |
| Mg | −0.26 | 0.06 | −0.15 | 0.21 | −0.06 | 0.24 | 0.16 | −0.32 | −0.23 | −0.49* | | | |
| Na | 0.06 | 0.15 | −0.06 | −0.24 | 0.25 | 0.14 | −0.05 | −0.42* | −0.44 | 0.13 | 0.41* | | |
| K | 0.29 | 0.28 | −0.11 | −0.45* | 0.21 | −0.20 | 0.10 | 0.15 | 0.09 | 0.21 | 0.29 | 0.28 | |

NS: Not significant, *Significant at $P < 0.05$, **Significant at $P < 0.01$, OC: Organic carbon, Av. P: Available phosphorus, TN: Total nitrogen, Ca: Calcium, Mg: Magnesium, Na: Sodium, K: Potassium

while Six *et al.* (2000) attributed it to high surface activity of silt that improved soil organic matter aggregation and protection. Sand and silt possibly increased urease activity due to the presence of macrospores that allow gaseous exchange of $NH_3$ between the rhizosphere and atmosphere. The positive and significant correlation between urease activity and organic carbon is consistent with the report of Reynolds *et al*. (1985) who worked on cultivated soils. This may be as a result of greater hydrolysis of urea and ammonia volatilization, particularly if urea fails to move into the soil.

Conversely, urease activity correlated negatively with clay, Total N, Available P, and magnesium contents of soil, respectively. This is in agreement with the report of Makboul and Ottow (1979) who noted that the addition of clay to the soil decreased urease activity. The report of Ekologija (2008) that increase in available P lowered urease activity is consistent with this result and suggests that high phosphorus level decreased microbial activity (Wynn, 1982) and ammonium volatilization. The negative correlation between magnesium and urease activity observed in this study is at variance with the report of Wyszkowski *et al.* (2003). The report of Speir *et al.* (1980) that urease activity is highly correlated with total N did not support our finding probably because the total N obtained in this study contained higher level of $NH_4$-N than any other form of nitrogen. USDA (2010) corroborated that urease activity may be suppressed by $NH_3$-based nitrogen fertilizer because ammonia is a product of urease activity.[20-29]

In conclusion, the result has demonstrated that changes in urease activity can be used as an indirect indicator of the variation in the pool of potentially available N in a soil. Soils under teak vegetation that produced the highest urease activity implied higher $NH_4$-N volatilization which has a consequence on global warming. Fallow land and gmelina vegetation with the lowest urease activities, respectively, should be considered as suitable land use in the tropics since $NH_4$-N volatilization was minimized [Tables 4-7].

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