UREASE ACTIVITY, AMMONIA VOLATILISATION AND RELATED MICROBIOLOGICAL ASPECTS IN SOME SOUTH AFRICAN SOILS

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Summary

Urease activity of various soils was indexed employing toluene sterile and non-sterile soils. The two methods indicated that part played by micro-organisms. Apparatus used, soil depth, increment, depth of placement and pattern distributions are discussed in relation to volatile loss as are influences of soil moisture, reaction, temperature and drying-out. Urea on trash benefits fungi but invariably results in high losses. KCl and $MgSO_4$ retard otherwise rapid pH changes and appear to check losses.

Great variability in loss-potential between soils parallels differences in base exchange capacity, the low value of sandy soils giving rise to higher losses than for other soils. Sharp rise in loss from sands and the more moderate loss from clay soils, reach peak losses around the seventh to eighth days.

The distribution and type of ureolytic bacteria isolated and differential loss depending on pretreatment is discussed. Respiratory quotient and loss, or urease index, were correlated indicating a possible short-cut in determining urease activity and loss-potentials in soils.

Introduction

The high nitrogen content, ease of application and relative cheapness of Urea have established its popularity in South Africa, as evidenced by the recent commissioning of a large commercial plant for its mass production. In some quarters, however, there exists a tendency to accept, somewhat uncritically, rather substantial volatile-loss claims, and furthermore, that toxicity[17] is a possible outcome of Urea application.

Information on the factors and conditions resulting in volatilisation in South Africa is noticeably lacking and the investigations reported are intended to clarify circumstances under which losses may be expected as well as to gain insight into microbiological aspects of Urea application and hydrolysis.

Materials and Methods

For volatilisation studies, a closed-circuit acid-trap method, similar to that of Ernst and Massey was employed except that negative pressure, Nesslerisation and twenty-four ounce glass-jars were substituted. The apparatus (Figure 1) was arranged in battery, served by a water-trap pump, the rate flow through

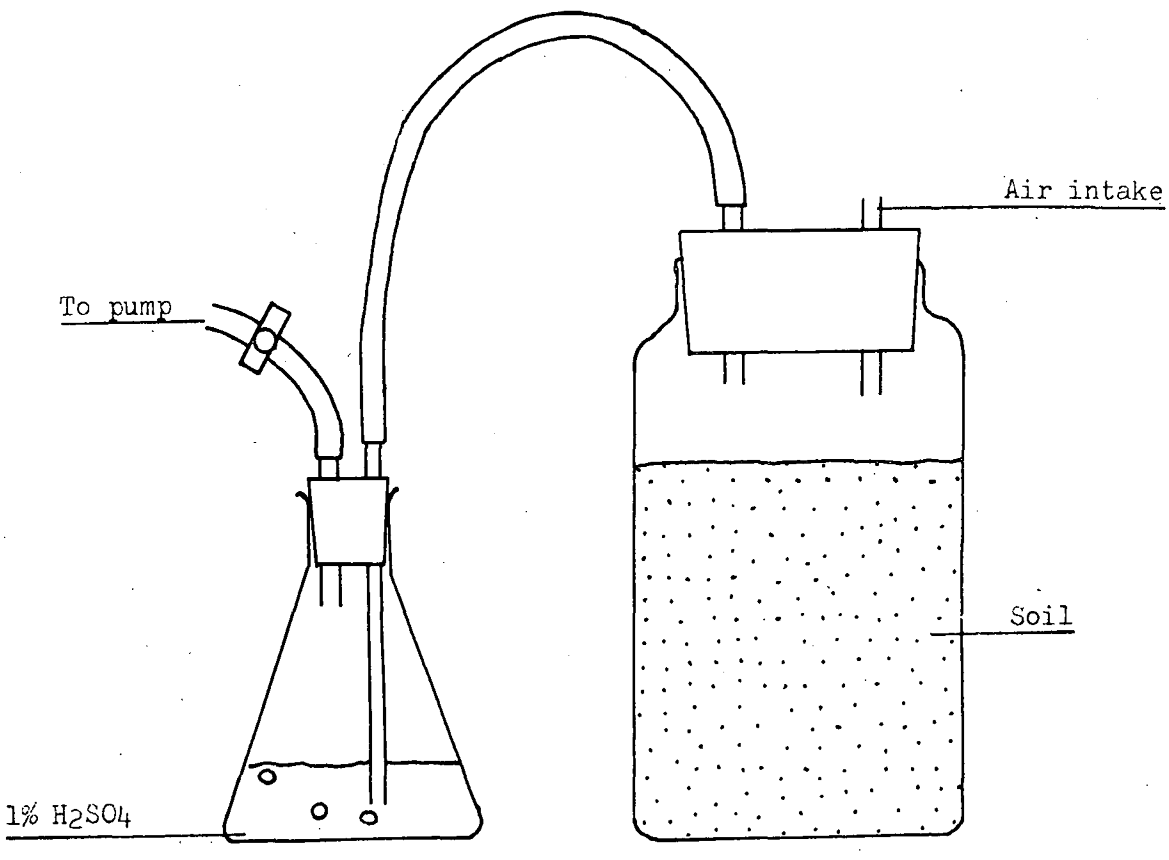

Figure 1—Ammonia volatilisation apparatus

each flask being adjusted to a slow even flow of air-bubbles, not exceeding one litre per hour. Purified air and humidity control were unnecessary as the blanks revealed no ammoniacal contamination, and relative humidity never at the extreme values found[14] to depress ammonia losses. Temperatures, unless stated, were in the range 20°C.±3°C. (ambrient).

Urease activity was determined by two methods, namely that of Strojanovic[19] (except that Nesslerisation was substituted), and a modification dispensing with toluene sterilisation. Isolations were made on enrichment broth[20] and ureas soil-extract agar. Respiration studies were conducted in a macro-respirometer.[4] Soils investigated were a Table Mountain sandstone (T.M.S. ordinary), Recent (grey) and Recent (red) sands, the latter being found in two pH ranges, 5.3 and 8.5 and several clay and clay-loam soils, relevent data on which is given where applicable. Generally the soils were air-dried, screened in a 2 mm. sieve and moisture content adjusted prior to placing in the glass jars whereafter the soil was firmed down to a level surface. One third of the soil's moisture-holding capacity was inavariably employed to minimise evaporation.

Urease Activity

Toluene and toluene-free methods were applied to a series of soils for purposes of comparing methods as well as individual soil hydrolytic ability. Results from both methods are given in Table 1 below:

Table 1

Urease index of sterile versus non-sterile soils. (96 hours at 37°C.)

Soil	Hydrolytic ability as % ammonia formed	
	No toluene added	Toluene added
Black Dolerite	99.00%	45.40%
Lower Ecca Shale	94.00%	83.20%
Dwyka	89.70%	72.30%
Alluvium	60.50%	46.20%
T.M.S. (Mist Belt)	59.20%	42.90%
Recent (red) Sand (pH 8.5)	44.30%	28.40%
Red Dolerite	40.30%	33.60%
Tugela Schist	28.20%	20.10%
Recent (red) Sand (pH 5.3)	17.30%	10.80%
T.M.S. (ordinary)	17.25%	Nil
Middle Ecca Shale ..	15.50%	6.70%
Recent (grey) Sand	2.86%	2.50%

Very considerable differences in hydrolytic ability of soils and in method-results are apparent from the above table. Toluene sterilisation permits evaluation of "residual" enzyme in the absence of active bacteria capable of replacing or increasing the amount of urease present (no toluene added). The sterile soil samples show lower urease activity in most cases with T.M.S. (ordinary) exhibiting no detectable activity. The difference between the two columns is assumed to indicate the additional enzymic potential supplied by the ureolytic soil population and varies considerably from soil to soil, the exception being the Recent (grey) sand for which no satisfactory explanation is presentable. The small conversion factor of this latter soil in no way reflects a low volatilisation loss-potential since the saturated soil conditions existing in the above tests cannot be compared with the normal conditions applicable to volatilisation experiments in which adequate facilities for removal or dispersal of the end product of hydrolysis exist. In addition, the amount of urea hydrolysed in a given time has been shown[8] to vary with amount of urea added, and furthermore, where small amounts of urease exist, high concentrations of urea, such as those used in indexing experiments, inhibit the enzymatic reaction velocity.[10] Under normal soil conditions, the apparently small urease content of the Recent (grey) sands is considered as adequate to account for rapid hydrolysis of the added urea. Subsequent rapid losses, through lack of retaining influences on the ammonia, would encourage a more rapid 'left to right' enzymatic reaction.

The toluene method of urease determination is useful inasmuch as it permits the recording of periodic fluctuations in soil urease content, a condition which is known to parallel the fluctuations of microbiological population, the one being influenced virtually to the same extent by factors influencing the other.[10]

As an evaluation method for absolute hydrolytic ability however, it is felt that due consideration for the viable ureolytic bacterial population is lacking, thus in toluene sterilised soils, Lower Ecca Shale emerges as the most rapid and efficient converter of Urea to ammonia with black Dolerite only a little over half as efficient. In non-sterile soil, however, the latter soil emerges as the most efficient with Lower Ecca Shale, second. The implications of this are that the latter soil has a greater retaining capacity for "residual" urease, and whereas adsorption and release of urease by and from clay minerals has been postulated,[18] consideration of the clay mineral contents[15] of the above soil types (Table 1) did not reveal a common factor to which adsorption and release of urease could, in all cases, be satisfactorily correlated.

Differences in urease activity between Recent (red) sand at pH 8.5 and pH 5.3 is possibly accountable by the differences in predominant ureolytic bacteria found. The higher urease index of the alkaline sand is of little significance in volatilisation, where, without the influence of a buffer solution as used in indexing, the acid sand rapidly increases in alkalinity to a value not far removed from that found naturally in the alkaline sand. This rise to alkaline values permits *both* forms of ureolytic bacteria to develop, thereby enhancing the urease content of the initially acid sand. Indirect evidence of this lies in the fact that little difference in volatile loss in encountered in volatilisation from acid and alkaline sands.

Ammonia Volatilisation

(a) Depth, Increment, Placement and Pattern

Preliminary experiments showed that depth of soil was a critical factor in true evaluation of ammonia losses. The unrealistic shallow soil depths imposed by petri-dishes[23] and other such containers,[14] did not permit of adequate downward dispersion of urea, and/or moisture, the result being increased volatile losses.

Increasing increments of urea resulted in increasing losses, but even here, the limitations of depth imposed by the glass jars had to be considered since a minimum depth of six cms. was established for a 200 lbs. rate-equivalent of urea. All subsequent estimates were conducted on ten cms. of soil depth with a maximum equivalent of 200 lbs./acre.

Placement of urea at depth in soils of high loss-potential, restricted volatilisation considerably, becoming non-detectable at eight cms. in T.M.S. (ordinary). This fact suggests a practical means of volatilisation control in the field.

Aggregations of pellets were confirmed to result in greater volatile losses[13] than scattered patterns of pellet distribution, although the results obtained were not consistantly significant between replications.

(b) Soil Moisture

The T.M.S. (ordinary) and Recent sands showed increasing ammonia losses for moisture values up to one half of their maximum water holding capacities, whereafter excess mositure resulted in a retaining effect of ammonia i.e. restrictive product removal. Reports purportrating the highest losses to come from near-saturation or saturation point are based on the use of shallow containers to which large increments of urea were added. Results concerning loss of ammonia from soils at various soil moisture values are in agreement with some[14,21] but at variance with other[12,13] reports. There is general agreement, however, that little or no loss occurs from air-dry soil.

(c) Soil Reaction

Low base exchange and adsorption capacity of T.M.S. (ordinary) and Recent sands permit of rapid changes in pH following additions of urea, as shown in Table 2 below:

Table 2

Influence of urea on pH changes in a Recent (red) sand of pH 5.8 and 7% moisture

Time (hours)	Change in pH due to urea additions			
	=50 lbs./acre	=100 lbs./acre	=150 lbs./acre	=200 lbs./acre
2	6.0	6.1	6.2	6.2
24	7.0	7.5	8.0	8.4
48	8.1	8.5	8.8	8.7
72	8.5	8.9	8.9	9.0
96	8.8	8.9	8.6	8.9
144	7.5	8.2	8.4	8.8

The initially acid reaction of this sand has been found to be of little consequence to volatilisation data when compared to its alkaline (pH 8.5) counterpart since little difference in total ammonia lost, over a seven day period, were recorded the actual values being 21.5 per cent lost for the initially acid sand and 26.8 per cent for the alkaline sand, both at 5 per cent moisture. At one-half moisture holding capacity, the respective losses were 30.0 per cent and 34.0 per cent. Alkaline reaction has been found to stimulate ammoniacal loss irrespective of ammoniacal nitrogen-form applied (Table 3)

Table 3

Volatilisation of Ammonia from Recent (red) sands (7% moisture) receiving three forms of nitrogen

Nitrogen Form	Sand pH 5.3		Sand pH 8.5	
	after 7 days	after 14 days	after 7 days	after 14 days
Urea	21.9%	24.5%	27.3%	27.4%
Am. sulphate	0.8%	0.8%	19.0%	19.0%
Am. nitrate	Nil	Nil	4.0%	4.3%

Clay and clay-loam soils, possessing higher base exchange values, generally showed low loss-potentials even at initial neutral to alkaline pH vlaues but such were greater losses than for the same soils in the acid range.

Table 4

Effect of pH on clay soils on volatilisation losses, after seven days at ambient

Soil	Treatment (Urea =200 lbs.)	pH		% lost as ammonia
		Before	After	
Black Dolerite ..		5.4	5.5	0.00%
Black Dolerite ..	+lime (=3 tons)	6.6	6.8	1.56%
Lower Ecca Shale		5.4	6.0	2 60%
Alluvium		7.8	7.8	6.50%
T.M.S. (ordinary)		5.3	7.2	21.00%

The interesting feature is that the recorded[2] base exchange values of the above soils (in order of increasing loss-potential) are as follows:

Black Dolerite 34.5, Lower Ecca Shale 18.5, Alluvium 15.6 and T.M.S. (ordinary) 5.6 mgm.—equivalents/100 grams soil.

(d) Temperature

The temperature range for urease has been placed at 30°C. to 40°C., the same range found to result in greatest volatile-loss from sandy soils (Table 5).

Table 5

Effect of temperature on volatilisation from a Recent (red) sand, pH 5.3 at 7% moisture, after 48 hours. Urea=200 lbs./acre

Temperature	% lost as ammonia
Ambient (20°C.)	1.5%
30°C.	2.0%
37°C.	20.3%
40°C.	19.8%
47°C.	11.6%
50°C.	12.0%
57°C.	1.5%
60°C.	0.85%
67°C.	0.30%

The above results have been interpreted as indicating hydrolytic velocity, rather than purely physical release of ammonia from ammonium carbonate, in view of the marked decrease in loss above 40°C. Greatest loss, and probably hydrolysis, occurred between 37°C. and 40°C. temperatures which are not uncommon to Natal soils.

Optimum growth temperatures for ureolytic organisms on culture are in the range 28°C. to 30°C. but these are not necessarily optimum for urease formation and are below the range recorded for greatest volatilisation/hydrolysis. With allowance for discrepancies between *culture* and *natural soil*—optimum growth temperatures, it is highly probable that optimum growth, optimum urease formation and optimum hydrolysis temperatures will be found to occur frequently under field conditions.

Maximum temperature for ureolytic bacterial growth has been placed[3] at 45°C. but this should preclude the continuation of bacterial-free enzymatic hydrolysis.

(e) Drying-out, Plant Litter and Other Salts

Drying-out influences on soil are difficult to reproduce in the laboratory. Warmed air imposes temperature changes to the soil and whilst being the case in the field, a separation of the two is desirable for better individual assessment. Sulphuric acid and calcium chloride were used as *cold* drying influences which served to illustrate an acceleration of volatile loss from soils upon drying out.

In all cases where urea was applied over plant litter (trash), extremely variable results were obtained but generally a high loss-potential was indicated. Greater losses occurred from old trash (24 months) than from trash a few months old due to the former having a higher bacterial count. Degree of wetness was of major importance, little to no loss occurring from air-dry trash, greatest loss from damp trash and a depressed loss from saturated trash due probably to re-adsorption of ammonia in the excess moisture.

Magnesium sulphate, potassium chloride, or both, applied together with urea resulted in reduced losses. Urea alone resulted in an 11.3 per cent loss from a Recent (grey) sand of pH 5.6 at 6.4 per cent moisture content. Urea with potassium chloride resulted in a 6.17 per cent loss, Urea with magnesium sulphate, 4.05 per cent loss and urea with both salts, 3.5 per cent loss. Chloride ion antagonism of urease[18] possibly accounts for a recovery of only 71.5 per cent ammonia (including that volatilised) from the chloride treatments. A marked tendency to delay the otherwise precipitous rise to alkaline pH values was a further outcome of these salt additions, a possible explanation for which exists in a double decomposition theory between the salts.

(f) Loss-Potential between various Soil Types

An experiment embracing all the foregoing variables was not practical on a laboratory scale and consequently the loss-potential was determined under uniform conditions for a number of important soil groups in the South African Sugar Industry. Table 7.

Very great differences in loss-potential were established, the loss ranging from nil (black Dolerite) to 56.2 per cent in the Recent (grey) sand. High losses seemed to closely parallel low base exchange values. That is, base exchange values of 6 mgm.-equivalents or less may be predicted to result in high losses as well as a significant shift in soil reaction, whereas values of 15 mgm.-equivalents or more would result in low loss and only a slight alteration of pH.

Sandy and sandy-loam soils of a loose, open, texture (Recent sand and T.M.S. ordinary), and in particular, possessing low base exchange qualities, rapidly give up the enzymatic reaction's end-product thus facilitating the reactions left to right tendency, even though these soils may possess seemingly small amounts of urease (Table 1). The more gradual losses of the clay and clay-loam soils indicates strong ammonia retentive forces within such soils which prevent rapid dispersal of the end-product but, as a result of their higher urease content, the lack of product dispersal or removal does not apparently result in a slower rate of conversion of urea to ammonia through restrained product removal. Whereas the total loss from these latter soils is small, the period over which the loss occurs is longer—a highly satisfactory state of affairs since sooner or later moisture (as rainfall) will create conditions conducive to minimising even these small losses.

Considerable variation in recorded loss data within a soil group is neither uncommon nor unexpected since variations in urease content and/or microbiological activity are known to occur,[19] the former being used to some extent as a measure of monthly or periodic microbial fluctuation. Such fluctuations are known to occur not only with time of year but also with conditions prevailing at the time of sampling. Generally, however, high loss-potential from sands and low loss-potential from clay and clay-loams were recorded irrespective of sampling conditions or time of year.

Microbiological Aspects

The majority of organisms isolated were from the sandy and sandy-loam soils as well as a black Dolerite and were found to be gram-positive, sporeforming, urease positive bacilli of which *Bacillus pasturi, B.lentus* and *B.sphaericus*—like forms were provisionally identified. Other isolates resembled *Sarcina ureae* and, from trash, *Proteus spp.* Several other bacilli and cocci exhibited urease activity to a lesser extent but were not identified further. Distribution of these organisms in the soils was found to be as follows:

Recent (red) sand (acid): *B. lentus; B. pasteuri; Sarcina spp.*

Recent (red) sand (alkaline): *B. pasteuri; B. sphaericus.*

T.M.S. (ordinary) (acid): *B. sphaericus.*

Black Dolerite (acid): *B. lentus; Bacillus spp.; Cocci; B. sphaericus.*

The steno-responsive (little growth above or below pH 8.5 to 8.8) and eury-responsive (wide response to pH) ureolytic classification, although based primarily on pure culture observation is still tenable in considering these organisms under natural conditions, but whereas *B. pasteuri*-type organisms were found[5] to be largely steno-responsive this organism's ability to survive as spores, or exist as adapted strains, in soils of neutral to acid reaction, should not be precluded. In any event, both steno- and eury-responsive ureolytic organisms are ideally suited to the alkaline Recent sand, a condition in keeping with the latter's relatively high urease index. The acid sands, whilst theoretically too acid for optimum steno-responsive development, may readily support both groups once initial hydrolytic action of eury-responsive bacteria and/or "residual" (bacterial-free) urease hydrolytic action has proceeded sufficiently to raise the pH to the alkaline range (Table 2). Such pre- or conditioning-hydrolysis would further serve to supply the nutritional ammoniacal requirements[6] of the steno-responsive group.

Since re-wetted air-dried sand invariably gave a greater volatile loss and urease index than fresh field soil, the latter placed directly into glass jars was compared with re-wetted air-dried soil and soil which had been steamed for four hours. Moisture contents were adjusted, and ammonia lost from applied urea was determined after seven days. Fresh soil lost 27.6 per cent re-wetted air-dried 31.0 per cent and steamed soil, after an initial loss-free period, showed a 46.0 per cent loss. In the latter case, steaming would induce spore formation and probably destroyed the residual urease content of the soil. Addition of urea then resulted in virtual selective-enrichment-like culture conditions favouring development of ureolytic organisms, the initial nutritional nitrogen demand being made available by the steaming process. Similarly, air-drying will induce spore formation in the ureolytic (and non-ureolytic) organisms but upon re-wetting and adding of urea the (probably) unharmed residual urease and/or inherent nitrogen content of the soil provides the nutritional nitrogen demand of the ureolytic organisms which, in the presence of urea, develop in virtually enrichment-culture-like conditions. The complex population present in the fresh soil will similarly become enriched in ureolytic forms once the urea is introduced, but the multi-various population initially present incurrs a greater biological nitrogen demand and this may account in part for the observed lower volatile loss in this sample. Thus, differences in volatile loss are thought to be due to a difference in response to added urea, the fresh soil being enriched in ureolytic forms but the air-dried and steamed soils being selectively enriched by virtue of spore inducing pre-treatments.

When urea was applied over trash samples, a more extensive fungal mycelial network was observed than when ammonium sulphate or nitrate was applied. In addition the trash receiving urea was darker, after prolonged incubation, possibly indicating a more rapid breakdown. Three forms of Basidiomycetes were observed and in one instance fruiting bodies successfully obtained which was not the case with other treatments. Additions of urea, at a later stage, to one of the control samples, brought about a similar result.

Ureolytic organisms were isolated at eight to ten cm. depths in soil indicating adequate urease sources well within the upper rooting zones provided conditions are condusive to growth of organisms and urease production. Micro-organisms, however, are not the sole source of urease in soil.[24]

Inter-relationship between volatilisation, urease activity and respiration quotient was sought by means of a macro-respirometer,[4] the only modification being the introduction of a second (acid-trap) vial in addition to that present for CO_2 absorption. Interest in soil colour was aroused from a report[12] that hydrolytic activity was predominant in dark coloured soils as compared to lighter coloured soils. Association of colour with urease activity is certainly in evidence in the following table, but the value and precise implications of such are not as yet fully appreciated.

Table 6

Relationship between soil colour, urease index, soil respiration and volatilisation (28°C./7 days)

Soil	Colour	CO_2/O_2	% Ammonia lost	Urease index (No toluene)
Black Dolerite	Black	0.071	0.00%	99.0%
Lower Ecca Shale	Black	0.064	0.96%	94.0%
T.M.S. (Mist Belt)	Dark Brown	0.055	0.40%	59.2%
Dwyka	Dark Grey	0.053	1.28%	89.7%
Tugela Schist	Medium Dark-Brown ..	0.038	1.58%	28.2%
T.M.S. (ordinary)	Light Grey	0.023	7.76%	17.2%
Recent (red) Sand	Light Red	0.023	10.00%	17.3%
Middle Ecca Shale..	Medium Grey-Brown ..	0.016	4.56%	15.5%
Alluvium	Dark Brown-Grey	0.012	7.76%	60.5%

The following three total correlation coefficients (r) were calculated from the above results, and from these, a fourth correlation, namely the partial correlation between urease index (u) and percentage ammonia lost (A) for fixed values of respiratory quotient (Q), calculated.

$$r_{QA} = -0.8465 \quad \ldots \quad (1)$$

$$r_{Qu} = +0.8011 \quad \ldots \quad (2)$$

$$r_{uA} = -0.6678 \quad \ldots \quad (3)$$

$$r_{uA \cdot Q} = 0.0324 \text{ (NS)} \quad \ldots \quad (4)$$

The latter partial correlation (4) is the correlation between (A) and (u) eliminating the effect of(Q). Thus the spurious correlation ruA is the result of ammonia lost and urease index being highly correlated with respiratory quotient. Urease index, therefore, does not indicate the loss-potential of a particular soil. Respiratory quotient, on the other hand, does indicate loss-potential and approximate urease index value.

The results are not unexpected since production of urease enzyme by bacteria should be reflected in the bacterial cells respiration, whilst the spurious correlation of urease index to loss-potential has been discussed under "Urease Activity" and is well illustrated by the low urease index value of Recent (grey) sand and the latter's high loss-potential.

The correlation of respiratory quotient to loss-potential confirms the findings, earlier, that fresh soil (which is known to possess a higher respiration than air-dried or steamed soil) has a lower loss-potential than either air-dried or steamed soils, by virtue of possessing a greater and more varied (less homogeneous) microflora.

General Conclusions

Whilst the evidence of enzymatic hydrolysis in the absence of micro-organisms[9] has been confirmed, it is felt that consideration of direct bacterial influence is a more desirable means of evaluating hydrolytic potential of a soil. The wide differences between soils in this respect are not unexpected and the apparently low urease indices of some soils are nevertheless quite adequate in practice for efficient urea hydrolysis.

In volatilisation studies, the technique by which evaluation of losses is made should entail careful consideration of apparatus, sample depth and increment or rate-equivalent applied per unit depth of soil in the sample container. Closed-circuit systems, unlike the open systems[22,23] permit daily loss evaluations to be made and on this basis are more desirable.

Placement of urea at depth suggests a practical means for minimising volatile losses, the depth at which no loss occurred being eight cms. (three inches) or more. The suggested depth of Parish, Ross and Figon[16] of six inches would be quite satisfactory for South African conditions.

The practice of applying urea pellets in, or as close as possible to, the row would seem to be less desirable than application to the inter-row *and* the row since aggregations of pellets were confirmed as resulting in greater losses than well spaced or scattered patterns of distribution.

Dry soil is not likely to result in volatile losses but losses increase with increasing soil moisture up to approximately one-half the total moisture holding capacity value in sandy soils, whereafter readsorption of the ammonia in excess moisture will probably occur. However, rapid soil drying in conjunction with high temperatures, may well lead to increased volatile loss. Prolonged rainfall followed by cool cloudy conditions would be ideal for penetration of urea to depths from which losses would be minimised.

In sandy soils of low base exchange capacity, acid soil reactions are of little consequence in reducing volatile losses. Such soils do, however, show remarkably little loss of ammonia when in receipt of ammonium sulphate or nitrate. The alkaline sandy soils do show losses in respect of the latter fertiliser forms. Whereas acid clay soils show little loss in comparison to sandy soils, an increased loss is noticeable with neutral or alkaline reaction.

The temperatures at which volatilisation (and probably hydrolysis) is greatest are not uncommon, but fortunately not persistent, in Natal sugarcane soils.[15] The more usual temperatures of 20°C. to 30°C. are admirably suited to both optimum growth and enzyme formation[24] of ureolytic bacteria.

Applications of urea over trash are not considered to be advisable in spite of the beneficial effects on micro-organisms and trash decomposition, unless sufficiently soaking rains are guaranteed in which case the trash blanket may act as an efficient preventor of subsequent rapid drying-out of the top-soil. Since drying-out conditions are difficult to apply under laboratory conditions, the effects of this factor may only be inferred or presumed, but nevertheless must not be underestimated.

The effects of potassium chloride and/or magnesium sulphate are possibly worthy of greater research, their apparent effect being one of delayed pH alteration and ammonia loss in sandy soils. The latter may result from a urease inhibition by chloride ions.

The loss-potential from various soil groups varies with percentage clay and/or base exchange capacity of the soil. The sandy soils generally have a high loss-potential for increments of 200 lbs. urea/acre and more. Rates of 217 lbs. urea/acre have been reported[14] as resulting in no appreciable loss, but for the sandy soils reported here, it was found that whereas little loss occurred for rate-equivalents up to 100 lbs. urea/acre, increasingly greater losses were recorded for increments above this. The curve of loss against time reveals the maximum losses to occur within the first seven to eight days whereafter a gradual tailing-off is noticeable in sandy soils. Where losses are obtained from clay soils, the rate of loss is more gradual extending over a similar period but noticeably more linear.

Table 7

DAILY LOSS OF AMMONIA FROM EIGHT SOIL TYPES RECEIVING 200 LBS. UREA. TEMPERATURE AMBIENT.

Soil	Texture	Moisture per cent*	pH		Base exchange capacity	Daily loss (percentage as ammonia)†										
			Before	After		1	2	3	4	5	6	7	8	9	10	11
T.M.S. (ordinary)	Coarse sandy loam ..	10.0%	5.3	7.2	6.0	0	3.5	8.2	9.1	11.4	17.6	21.0	22.0	23.9	23.9	23.9
Recent (red) Sand	Sand	9.5%	5.3	7.3	3.5	1.0	5.5	9.5	17.0	22.1	25.8	31.3	37.2	39.6	41.7	42.3
Tugela Schist	Clay-loam	13.0%	5.3	7.2	15.0	0	0.09	1.4	1.9	4.3	5.3	5.8	7.0	8.6	9.0	9.4
Black Dolerite	Clay	17.0%	5.4	5.5	34.5	0	0	0	0	0	0	0	0	0	0	0
Lower Ecca Shale	Clay-loam	12.0%	5.4	6.0	18.5	0	0.08	1.3	1.6	2.5	2.6	2.6	2.6	4.3	4.3	4.5
Recent (grey) Sand ..	Sand	8.0%	6.8	6.3	2.0	0.1	7.6	15.1	23.6	30.0	35.7	40.8	47.2	50.8	55.1	56.2
Middle Ecca Shale	Clay-loam	10.0%	7.7	7.7	15.6	0	1.2	1.5	2.4	4.3	5.2	6.1	8.0	8.4	8.7	9.0
Alluvium	Silty-clay-loam	15.0%	7.8	7.8	15.6	0	1.5	3.1	3.7	5.3	5.8	6.5	7.9	8.8	9.0	9.2

*Given as one-third of the holding capacity.
†Accumulated daily total.

The microbiological flora of these soils possess a number of organisms showing strong ureolytic activity. The distribution of the various types is roughly in accordance with the ureolytic bacterial classification which factor is of little consequence in sandy soils where rapid changes of pH (to the alkaline side) are undoubtedly the outcome of urea applications. There is no reason to doubt the presence of adequate urease activity in South African sugar cane soils.

Statistical evaluation of the respirometer studies reveals a possible short-cut for estimations of a soil's loss-potential and urease index, the separate experiments formerly necessary now being combined in a single operation with minimum apparatus requirements.

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Mr. J. Wilson, in the chair, said that what happened to the nitrogen placed in the soil was of great interest. There had been rumours of the losses to be expected when urea was used. It was of importance to the grower and particularly to the Experiment Station to assess how far these rumours were based on fact. The work described by the author represented the initial effort made by the Experiment Station to elucidate the position.

The paper indicated many problems in the practical application of the findings noted in the laboratory. The results, when one got down to the possible factors likely to affect loss of nitrogen, in any form were rather horrifying.

Mr. du Toit, the President, said that in his Presidential Address he had stated that he thought the study of microbiology might help considerably in elucidating nitrogen problems. With the introduction of urea a year or two ago and the subsequent claims of very high losses the author set out to examine what was taking place in the soil itself. He had now given some basic information on the subject.

It was interesting to note that in all soils there was evidence of high hydrolytic activity. In Recent Sands, within a week, all the urease added was hydrolised to the ammoniacal form. It was to be expected that in the sandy soil the small amount of ammonium carbonate formed could raise the pH to the alkaline condition and a certain amount of ammonia be lost. The loss figures shown however were obviously in excess of what would happen in the field, because the work done was carried out under somewhat artificial conditions, but the danger was that such losses could take place if the urea was added in fairly moist sandy soil and a period of drying out followed. The losses shown however did not appear to be very excessive.

Confirmatory field experimental work would have to be done before one could assess the value of urea as compared with other forms of nitrogen. Field experiments which will elucidate this point are now in progress.

Mr. Fisher said he started using urea three years ago and right from the start found a fall-off in yield. The following year he carried out a test of urea against ammonium sulphate and again urea showed up badly. Now he would not use urea any more in his Table Mountain sandstone Mist-Belt soil.

The Chairman asked if Mr. Fisher had actually weighed the cane at harvest and pointed out there could be many other complicating factors so that urea itself might not be to blame for poor results.

Mr. Fisher said that he had not weighed the cane at harvest but he had no doubt that the poor result from urea existed.

Dr. Shuker related that under irrigation in Rhodesia urea was being used with success.

Mr. W. J. G. Barnes said the Doornkop growers had very much the same type of soil as Mr. Fisher and were very disappointed with the results from urea.

The Chairman said that steps were now being taken to lay down trials in these areas. In maize and other crops, sulphate of ammonia often gave plants a healthy green appearance but this apparent beneficial effect was rarely reflected in the final yields which were the main criterion.

Mr. A. C. Barnes related that work done in Jamaica on this subject showed that urea was definitely inferior to sulphate of ammonia as a fertilizer for sugarcane in certain soils. The trouble was traced to biuret.

Mr. Anderson said that the amount of biuret in South African-produced urea was very low and as sugarcane generally was a very tolerant crop he doubted that the biuret present, in the concentration it existed in locally produced urea, would have any adverse effect.

Mr. du Toit thought that in the British West Indies they experienced a slightly lower response from urea than that obtained from sulphate of ammonia, but only on the average. From Mauritius came similar claims. In Natal only two experiments had been harvested. In both cases there was a slightly lower response to urea, but there was no significant difference between equal quantities of nitrogen applied as ammonium sulphate or as urea. Complaints from the Doornkop area were that urea gave no response, but these complaints could not be substantiated on close examination.

Mr. A. C. Barnes said the author had touched briefly on the placement of urea at depth in soils of high loss potential, and this aspect he thought should be studied further.

Mr. Anderson believed there was some zinc deficiency in the Doornkop area which might be localised in certain areas where urea was applied and this might have a bearing on Mr. Fisher's observations.

Mr. Fisher suggested reports from fertilizer distributors might show a switch from urea in certain areas. At Entumeni, some years ago, urea was used by many growers but he knew there was a decided switch from it now.

Mr. Grice said that three years ago Natal Estates, Ltd. decided to use urea because it was a cheaper form of nitrogen to apply. The saving of R3.50 per acre thus obtained was important.

He considered fertilizer should be applied on the row of cane and not between rows, because the root system in the ratoon crop was developing in the row and not between the rows. Application to the rows led to an appreciable reduction in the cost of applying fertilizer and yield results amply confirmed this policy.

pH values had a large effect on response to urea in certain areas and he asked the author if growers should condition soil by the use of lime.

Mr. Anderson replied that in a sandy soil loss of ammonia could be minimised by distributing the pellets of urea between rows and thus reducing its concentration. Alkaline conditions led to a great loss of ammonia so he was against the use of lime as a soil conditioner.

The Chairman said it was peculiar that one grower whose conditions would suggest that urea could be used to advantage did not consider it of value, while another, where the conditions could be considered the worst possible, found it extremely effective and advocated its use. This pointed to the fact that one grower had been judging by appearance while the other had weighed his crop.

Mr. Anderson agreed that losses did occur in sandy soils and Mr. Grice had conditions which were detrimental to retention of ammonia from urea. However the soil around cane roots under a canopy of leaves was probably more moist than that between the rows and was therefore more retentive. Mr. Grice was furthermore able to apply the urea at a time when conditions were suitable for the downward movement of this substance and its subsequent hydrolysis.

Mr. Wyatt said urea was not used in the U.S.A. cane fields. At Doornkop it was used for economic reasons as mentioned by Mr. Grice, but previously Doornkop did not use a great deal of nitrogenous fertilizer, so the response to heavier applications of nitrogen in the form of urea was at first welcomed, but now the tendency was towards top-dressing with other forms of nitrogen while still applying some urea in the furrow.

Mr. de Lange said that urea had been used on the entire area of Doornkop Estate but it had been found that the responses on the lower areas which were predominantly of a sandy nature were not as good as on our Mist-belt soils where the organic matter is of a much higher level.

It was, however, also observed that even on Mist-belt soils, that an apparently better response was achieved by deep placement of urea using a combination sub-soiling fertilizing tool.

Mr. Halse asked if it would be beneficial to apply urea in irrigation water rather than in the dry state, especially in the case of a trash layer.

Mr. Anderson replied that he considered application in irrigation water would lead to better penetration of the soil.

Mr. du Toit said he considered factors other than the mere application of urea should be considered. Other causes for lack of response, such as had been mentioned by Mr. Fisher and the Doornkop growers, should be examined fuither. Such things as change in pH, first towards alkalinity and then back to acidity, might lead to the unavailability of a trace element where it occurred in low concentration.

The Chairman thought that it was significant that the areas from which complaints about urea emanated were the areas in which incipient zinc deficiency was now being found. It was just possible that a slight realignment of pH could be the responsible factor, and the zinc position was being actively investigated at the present time.