SOME ASPECTS OF THE EFFECTS OF UREASE AND NITRIFICATION INHIBITORS ON UREA, AMMONIUM AND NITRATE CONTENTS IN THE SOIL

Major thesis presented in partial fulfilment of the requirements for the award of the degree of Master of Science.

by

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Dedication

Affectionately dedicated to the one I love, CECILIA AISHETU AMELOKO.

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1. INTRODUCTION.

Urea has a higher nitrogen content than most other nitrogen fertilizers. In comparison the theoretical nitrogen content of urea is about 46 % as opposed to 35 % in ammonium nitrate, 21 % in ammonium sulphate, 16 % in calcium nitrate and 15 % in Chilean nitrate. The high percentage of nitrogen in urea makes it a relatively cheaper source of nitrogen. This is especially the case in countries where high cost of transportation leads to high prices of fertilizers. Urea can be made into prills (an advantage over some other nitrogen fertilizers) and can be granulated. Urea can be handled with relative ease and does not require safety precautions as ammonium nitrate and calcium nitrate and for these reasons urea is more preferred in world commerce (Tomlinson, 1970).

The technical advantages in conjunction with the high nitrogen content of urea have focused attention of many research workers on how the high percentage of nitrogen can be used to advantage. In doing so attempts are made in various degrees to find ways and means of improving the efficiency of urea. These include methods of inhibiting urea decomposition in soils caused by rapid hydrolysis of urea to ammonium carbonate through the activity of urease (Bremner and Douglas, 1971). The formation of ammonium carbonate causes rise of soil pH and subsequent liberation of gaseous ammonia particularly when the pH is 7 and above thus, leading to nitrogen losses.

Under favourable conditions the ammonium ions formed during hydrolysis undergo oxidation reactions (nitrification) to form nitrate. The formation of nitrate lends nitrogen to losses by leaching and denitrification under anaerobic conditions. However, by prolonging the life time of ammonium, it is possible to eliminate the problem of leaching and denitrification thus, increasing the efficiency of urea in crop production. In order to bring about this, the use of nitrification inhibitors low in side effects (particularly on hydrolysis) must be sought.

In the work hereafter, the fate of urea when incubated as such, in presence of some urease inhibitors, a nitrification inhibitor and in presence of a urease and a nitrification inhibitor together was studied. The effects of these treatments on the germination of maize was also examined. In this work too, the nutritive value of urea in comparison with some other nitrogen fertilizers was examined.

This study therefore, has the following objectives:

1) To study the inhibitory effects of some urease and nitrification inhibitors on urea incubated in soils and the effects of these treatments on maize germination.

2) To compare the nutritive value of urea with that of some commonly used nitrogen fertilizers including a comparison of nitrogen as urea, NH_4^+ and NO_3^- in a water culture.

2. LITERATURE REVIEW.

As pointed out in the introduction, in this chapter more emphasis will be placed on the inhibitory effects of urease inhibitors and nitrification inhibitors than on the fertilizer value of urea.

Urea, when added to the soil first undergoes hydrolysis. This is a reaction in which urea is transformed into ammonium carbonate. This reaction is of chemical nature and or acts in presence of the enzyme urease.

$$(NH_2)_2CO + 2H_2O \longrightarrow (NH_4)_2CO_3$$

This catalytic reaction is most favourable under normal conditions of moisture and temperature.

The rapidity and extent of hydrolysis of urea in the soil depends on the microbiological activity of the soil which in turn is related to soil temperature and pH (Bhavanandan, 1970). Related to this fact, Anderson, 1952; Gibson, 1930; Soulide and Clark, 1958 found that urea hydrolyses slowly in alkaline, calcareous and sandy soils containing little organic matter while, hydrolysis occurs most rapidly in grassland and forest soils.

Urea is hydrolysed under soil moisture contents varying from nearly air-dry to water-logged conditions. Delaune and Patrick (1970) in their study of urea conversion to ammonia under two moisture conditions of one third bar and water-logged, found no apparent difference in urea hydrolysis under both conditions. The authors further examined the effect of pH on urea hydrolysis under both water-logged and one third bar moisture conditions. In their study the pH of the soils was adjusted over a range of pH units by thoroughly mixing various amounts of soils with either potassium hydroxide or 2N hydrochloric acid. Urea was added to the soils and the amount hydrolysed determined. Their finding pointed out that hydrolysis of urea occurs over a wide

range of pH with no hydrolysis below pH 4 and above pH 10. Hydrolysis under the two moisture conditions were found to be similar, with both giving maximum conversion of urea at pH 8. Lack of urea hydrolysis above the pH 10 was attributed to the rapid destruction of urease above this pH, while that below pH 4 was explained on the basis of denaturation of urease (Thomson et al, 1956).

Investigators have shown that urea hydrolysis is greatest in surface soils and decreases with depth in samples incubated at field moisture content (Gasser, 1964).

Urease activity is destroyed by heating the soil and decreased by adding de-activators or inhibitors. Sources of the enzymes in the soil are: micro-organisms in which the enzymes may exist intracellular (the enzymes are present inside the cells of the ureolytic micro-organisms) and crop residues as well as plant parts in which the enzymes exist extracellular (released from disrupted plant and microbial cells). Lloyd and Sheaffe (1973) in their study of the activity of urease in the soil noted that a good number of bacteria can hydrolyse urea in the soil. The authors found that about 17 - 30 % of the total bacterial population including aerobes, micro-aerophiles and anaerobes could hydrolyse urea. In their experiment, one of the soils used received urea for at least 10 years and yet the proportion of ureolytic bacteria (24 %) was similar to that found in soils that did not receive any urea. Lloyd and Sheaffe points out that addition of urea to soils low in available carbon did not increase the total urease activity and size of the bacterial population or the ratio of ureolytic to non-ureolytic bacteria. They, however, found that on addition of carbon (as glucose) with urea to the soil, urease activity and size of the bacterial population both increased, but the ratio of ureolytic to non-ureolytic bacteria in the entire population remained unchanged.

The direct effect of urea hydrolysis is the temporary increase in soil pH. An increase in pH above 7 may lead to release of ammonia

from ammonium carbonate with the formation of the more stable ammonium bicarbonate (Bhavanandan and Fernando, 1970).

$$(NH_4)_2CO_3 \longrightarrow NH_4CO_3 + NH_3$$

The free ammonia released can damage germination and growth of crops (Cooke et al, 1962). The free ammonia may also volatalize resulting in the loss of nitrogen. Gaseous loss of urea-N as ammonia, rise in pH and damage to germinating seedlings and young plants constitute some of the problems in using urea as a fertilizer.

Ammonia volatalization can be reduced or minimized by mixing urea with the soil. Meyer et al found that urea must be incorporated with the soil for maximum efficiency unless rain falls or irrigation is given immediately after surface application. When urea is below the surface of the soil, the ammonia released on hydrolysis could be absorbed by the soil. The ability of the soil to absorb ammonium ions depends on the cation exchange capacity (CEC) of the soil. Thus, losses of nitrogen will be greater from soils with low and less from soils with high CEC. Gasser (1964) in his study of factors affecting losses of ammonia from urea and ammonium sulphate applied to soils, found losses of ammonia from surface-applied urea to very from 2 to 30 % of nitrogen applied and accordingly found that losses were most from sandy soils and less from clay soils. The amount of ammonia lost also depends on other factors: Anderson, Ernst and Massey in 1960 found that increasing pH of the soil, increases losses because more ammonia is lost from limed than from unlimed soils. More ammonia is lost from dried soils than from wet ones. Gasser compared ammonia losses at $25^{o}C$ and at 5 - $10^{o}C$ and found that at $25^{o}C$ there was increase in losses of ammonia from urea applied to some soils and decrease in losses from other soils. Also the period of losses was decreased for soils at 5 - $10^{o}C$ from 30 - 70 days to 10 - 14 days for the soils at $25^{o}C$.

Both toxicity and loss of ammonia to the air can be lessened by mixing urea with acid salts which neutralize the ammonia (Low and Piper, 1961).

These authors showed that germination is improved by mixing urea with mono ammonium phosphate and superphosphate. Cooke in 1962 reported in his work that the best way of improving urea as a fertilizer would be to inhibit the enzymatic transformation of urea to ammonium carbonate long enough to allow the fertilizer to be washed into the soil.

The idea of Cooke about urease inhibitors was quickly picked up by research workers who found a good number of compounds to retard more or less the conversion of urea to ammonium by soil urease. Notably amongst these compounds are certain quinones which include hydroquinone, p-benzoquinone, 2,3-dimethyl-p-benzoquinone, 2,5-dimethyl-p-benzoquinone, 2,6-dimethyl-p-benzoquinone, 2,5-dichloro-p-benzoquinone, 2,6-dichloro-p-benzoquinone, polyhydric phenols such as catechol, some esters like sodium-p-chloromercuribenzoate and phenyl mercuric acetate (Bremner and Douglas, 1973). Bremner and Douglas compared the effectiveness of these inhibitors in addition to acetohydroxamic acid, and found that 2,5-dimethyl-p-benzoquinone had the greatest ability to retard urea hydrolysis in soils and reduced gaseous loss of urea-N as ammonia. In one of their experiments with sandy soil incubated at 20^{o}C for 14 days, they found that gaseous loss of urea-N was reduced from 61.1 % to 0.3 % by addition of 2,5-dimethyl-p-benzoquinone (2.3 parts/100 parts of urea). The use of heavy metals as urease inhibitors has been reported. Bhavanandan and Fernando (1970) in their work on the influence of copper on urease activity in soils, listed silver, mercury, cadmium and lead as known inhibitors for urease. In their work inhibition of urease activity by copper was studied using copper sulphate or Perenox (cuprous oxide - a fungicide containing 50 % copper) and they found out that both forms of copper inhibited urease activity in the soil at concentrations above 100 ppm with the Perenox causing greater inhibition than copper sulphate at equivalent amounts. Toluene, an organic compound is also found to inhibit urease activity. Lloyd and Sheaffe found that toluene although an inhibitor of microbial activity is known to partly inhibit urease and induce lysis.

Certain compounds either individually or in combination function as antimetabolites towards soil bacteria, fungi and actinomycetes which produce urease (Peterson and Walter, 1970). In this way the rate of hydrolysis of urea in the soil is regulated neither by limiting the solubility of urea nor by inhibiting the activity of the urease, but by controlling the microbial production of the enzyme. Pyridine-3-sulphonic acid, oxythiamine chloride, desthiobiotin, gama-benzene hexachloride and o-chloro-p-aminobenzoic acid are known antimetabolites and of these pyridine-3-sulphonic acid is most preferred.

On the whole, effects of inhibitors differ markedly for different soils. Study of effects of various urease inhibitors showed that 2,5-dimethyl-p-benzoquinone (DBQ) proved most effective, while aceto-hydroxamic acid was the least effective.

The ammonium ions formed during hydrolysis may in addition to volatilization be fixed in the lattice of clay minerals by the same mechanism as operative in potassium fixation, thus leading to low efficiency of urea.

Under aerobic conditions, however, ammonium is readily oxidized into nitrate. This is a biological process brought by chemo-litho autotrophic micro-organisms (mimeograph by A.van Diest, 1975). During the processes of nitrification ammonium is converted to nitrite by the representatives of the genus Nitrosomonas while the genus Nitrobacter is responsible for the oxidation of nitrite to nitrate. Both bacteria function most effectively under neutral to slightly alkaline conditions (pH 6.8 – 8.0) and the limiting acidity for the activities of these bacteria in the soil is about pH 4.0 (Waksman, 1927).

Reactions:

$$NH_4^+ + 2H_2O \longrightarrow NO_2^- + 8H^+ + 6e^- + \text{energy}$$

$$3/2O_2 + 6H^+ + 6e^- \longrightarrow 3H_2O$$

$$NH_4^+ + 3/2\ O_2 \longrightarrow NO_2^- + 2H^+ + H_2O + \text{energy (Nitrosomonas)}$$

$$NO_2^- + H_2O \longrightarrow NO_3^- + 2H^+ + 2e^- + \text{energy}$$

$$\tfrac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$$

$$NO_2^- + \tfrac{1}{2}O_2 \longrightarrow NO_3^- + \text{energy (Nitrobacter)}$$

It has been reported that the oxidation of NO_2^- to NO_3^- proceeds at a faster rate than the oxidation of NH_4^+ to NO_2^-. Therefore, nitrite accumulation is not a common phenomenon in soils except under alkaline conditions where the activity of Nitrobacter is retarded by the poisonous effect of NH_3 (Campbell and Lees, 1967). Since nitrite is phytotoxic to plants(Court et al, 1969), accumulation of nitrite in soils therefore leads to less effective use of urea. Under anaerobic conditions, both NO_2^- and NO_3^- can be reduced or denitrified by certain chemical reactions (Soulides and Clark, 1958) and this leads to losses of gaseous nitrogen.

From the reaction equations above, oxidation of NH_4^+ into NO_3^- is accompanied by the production of hydronium ions, a phenomenon which increases the soil acidity. Therefore, nitrification of NH_4^+ halts the local and temporary rise of pH caused by the transformation of urea into ammonium carbonate. As the process of nitrification continues, the pH gradually decreases.

In general therefore, oxidation of ammonium increases the susceptibility of mineral nitrogen to losses by leaching and denitrification. It increases the soil acidity and produces nitrite toxicity under certain conditions. By prolonging the persistence of fertilizer in ammonium form, chemical inhibition of ammonium oxidation could increase the efficiency of a given quantity of nitrogen in crop production. In such chemicals used as inhibitors, side effects are undesirable. The use of chemicals to inhibit ammonium oxidation has long been known. Known nitrifying inhibitors include the following: 2-chloro-6(trichloromethyl) pyridine which is popularly known as N-serve, cyanoquamidine, thiourea, 2-amino-4-chloro-6-methyl-pyridine (A.M.),

sulphur thiozole (S.T.), 4-amino-1,2,4-triazole (A.T.C.) and 2,4-diamino-6-trichloro methyl-S-triazine (CL-1580). Of these inhibitors, 2-chloro-6(trichloromethyl) pyridine has been most extensively studied. It has been reported that this compound, N-serve appears to be more or less specific for autotrophic ammonium oxidizing organisms. Shattuck and Alexander in 1963 found that N-serve affects both Nitrosomonas and Nitrobacter, but the activity of the former organism was inhibited much more than that of the latter. The authors further found that the nitrate production by several strains of the nitrifying fungus Aspergillus flavus was unaffected by the concentration of the inhibitor which effectively controlled ammonium oxidation by Nitrosomonas and Nitrobacter. The specificity of N-serve in reducing ammonium oxidation by Nitrosomonas and Nitrobacter as well as the marked reduction of oxidation that occurs in presence of this inhibitor is one of the reasons for supposing that these bacteria are predominantly responsible for ammonium oxidation in soils. In addition to the above named nitrifying inhibitors other workers have shown that many organic sulphur compounds like methionine, cystine and others inhibit nitrification of ammonium in soils and several of these sulphur compounds retard the growth of Nitrosomonas europaea (Bremner and Bundy, 1974). Jensen and others found that the inhibiting effects of these sulphur compounds are due to the formation of volatile sulphur compounds by microbial decomposition of the compounds. Bremner and Bundy (1974) studied the effects of some volatile sulphur compounds like methyl mercaptan, dimethyl sulphide, carbon sulphide, dimethyl disulphide and hydrogen disulphide on nitrification in the soil and they found that the five compounds tested retarded nitrification when added to the soil treated with 2 mg of NH_4^+ -N as ammonium sulphate. Them inhibitory effects decrease in the following order: carbon disulphide $\gg$ dimethyl disulphide $>$ methyl mercaptan $>$ dimethyl sulphide $>$ hydrogen sulphide. The authors also found that the effectiveness of these compounds to inhibit nitrification decrease with time (days) and explained the finding on the basis that soils have the ability to sorb volatile sulphur compounds, thereby decreasing the inhibitory effects of these compounds on nitrification. Work on

chromatography by Banwart and Bremner in 1974 showed that moist soils have the capacity of sorbing volatile compounds used and the ability of moist soils to do so decreases in the following order: $H_2S > CH_3SH > CH_3SCH_3 > CH_3SCH_3 > CS_2$. Bremner and Bundy compared the effectiveness of these sulphur compounds as nitrifying inhibitors with the patented nitrifying inhibitors (N-serve, AM & ST) and found that CS_2 was more effective than N-serve, AM and ST in inhibiting nitrification. The effect was even very marked when applied as little as 2 parts per 10^6 parts of soil.

Effects of nitrifying inhibitors on urea nitrogen transformation in soils have been studied. Bundy and Bremner (1974) in their studies with N-serve, A.T.C. and CL-1580 found that these compounds have little if any affect on urea hydrolysis but they retarded nitrification of ammonium and increased gaseous loss of urea-N as ammonia. They found that N-serve had no effect on urea hydrolysis when applied at the rate of 10 μg/g of soil but that A.T.C. and CL-1580 of the same amount slightly reduced the amount of urea hydrolysed. In 1973, the same authors investigated the effects of some urease inhibitors on nitrification and compared with those of N-serve, AM and ST which are known inhibitors for nitrification. Most of the urease inhibitors studied had little effects on nitrification in ammonium treated soils when applied at the rate of 10 μg/g of soil, but had a marked inhibitory effect when applied at the rate of 50 μg/g of soil. However, none of the urease inhibitors tested inhibited nitrification as effectively as N-serve. Phenyl mercuric acetate has more effective inhibitory action on nitrification than AM and ST and greatly inhibited nitrification when applied at the rate of 50 μg/g of soil.

Various authors have discussed the suitability of urea as a fertilizer. Prominent amongst these authors is J.K.R.Gasser (1964). Also the fertilizer value of urea particularly in tropical regions has been published in the Annotated Bibliographies nos. 1589 and 1570 of the Commonwealth Bureau of Soils. Therefore, for general information about urea as a fertilizer see Gasser (Urea as a fertilizer:

Soils and Fertilizers, vol XXVII 175 - 180 (1964)) and Annotated Bibliography of the Commonwealth Bureau of Soils nos. 1589 and 1570.

3. EXPERIMENTS.

A total of four experiments were conducted for this report. Two of the chapters 3.1 - 3.4 were directed to the study of effects of urease and nitrification inhibitors and the subsequent effects of these treatments on germination of maize, while the last two were to assess the value of urea as a plant nutrient (chapter 3.5) or fertilizer (chapter 3.6) and compare urea with some other nitrogen sources or fertilizers.

3.1 Effects of N-serve, DBQ and $CuSO_4$ on Urea Incubated at Room Temperature ($20^{o}C$). "Low Level of Urea".

N-serve is believed to inhibit the activities of nitrifying bacteria, thus preserving the nitrogen in ammonium form. DBQ and $CuSO_4$ retard the activities of urease, thus controlling the rate at which urea is hydrolysed to ammonium carbonate which may lead to ammonia volatalization. This experiment is designed to see the effects of these inhibitors on urea hydrolysis and nitrification following this hydrolysis of low levels of urea-N.

3.1.1 Materials and methods.

Two batches of Eng-soil were used. One batch of pH KCl 3.66 and the other of pH 4.64. Eng-soil is a Dutch sandy soil (12 % $<16\mu$ and some pebbles) with 2.7 % organic matter. The soil has a high content of available phosphate due to manuring in earlier times with sheep manure. The nitrogen, potassium, magnesium and micro-nutrient status of the soil is low. Before using, the soil samples were air-dried. For the incubation trials a low level of 100 mg N/kg of soil (3.1) was used. The samples were incubated at room temperature ($20^{o}C$). For analysis of urea, nitrate, ammonium and pH wet soil samples were taken (see 3.1.2)

3.1.2 Sample preperations and treatments.

The following treatments were used for the trial with low level of urea.

(a) BLANK: Blanks were prepared by mixing 2.6 kg of each soil sample (low and high pH) with 260 ml of distilled water to bring the moisture content to 60 % of the water holding capacity. From each mixture, 120 g portions were weighed out into small experimental pots and incubated at room temperature ($\pm$ 20°C). Moisture conditions were maintained by weighing and adding water from time to time. Wet samples from one pot of each pH level were taken immediately (zero day of treatment) and analysed for NO_3^-, $(NH_2)_2CO$, NH_4^+ and pH according to methods given below. The rest of the pots bearing soil samples, excepting 10 of each pH level in which maize seeds were sown (see treatment with maize in 3.4), were incubated at the room temperature as above. After 3, 7, 11, 17, 23, 31 and 37 days samples (two pots at a time) were analysed for NO_3^-, $(NH_2)_2CO$, NH_4^+ and pH.

(b) SOIL + UREA: In addition to 2.6 kg of the dry soil, 560 mg of urea dissolved in 360 ml of water was added and thoroughly mixed. The weighing of 120 g portions, incubation and analysis of the samples were exactly the same as described under (a).

(c) SOIL + UREA + N-SERVE: Preperation as in (b) plus 5.0 ml of N-serve per 2.6 kg of dry soil. (N-SERVE 24 E, diluted 1:100).

(d) SOIL + UREA + DBQ: As in (b) plus 30 mg of 2,5-dimethyl-p-benzoquinone (DBQ) per 2.6 kg of dry soil.

(e) SOIL + UREA + $CuSO_4$: As in (b) plus 50 ml of a $CuSO_4$ solution (prepared by dissolving 3.250 g $CuSO_4$ in 250 ml water) per 2.6 kg of dry soil.

(f) SOIL + UREA + $CuSO_4$ + N-SERVE: As in (b) plus additional 5.0 ml N-serve and 50 ml of $CuSO_4$ solution per 2.6 kg dry soil.

3.2 Effects of N-serve (Single and Double Dose) and DBQ on Urea Incubated at Room Temperature (20°C). "High Level of Urea".

Only the inhibitory actions of N-serve and DBQ were studied at this level of urea. The functions of both inhibitors are as described in section 3.1 above. This experiment was carried out first (Nov. 1976). The level of 1000 ppm N is about ten times the normal application for arable crops.

3.2.1 Materials and methods.

Again two batches of Eng-soil at two pH KCl levels 4.1 and 6.4 respectively were used. The Eng-soil is as described in 3.1.1 and also the reagents, methods of incubation, sampling and analysis are as described in 3.1.1. The urea level in this experiment is 1000 ppm N.

3.2.2 Treatments.

(a) SOIL + UREA: 2.8 kg of each soil was weighed out and 352.8 ml of water with 5.6 g of urea was added and thoroughly mixed. The moisture content of the soil came in this way to about 60 % of its water holding capacity. 200 g portions were weighed into experimental pots and incubated at room temperature. The moisture conditions of the samples were maintained by weighing and adding water from time to time. On the very day of these treatments (day 0), samples from two pots were analysed for urea, nitrate, ammonium and pH. Subsequently analysis of the above species was done in duplicate after 2, 7, 10, 14 and 21 days of incubation for both pH levels (4.1 & 6.4).

(b) SOIL + UREA + N-SERVE: As in (a), but + 2.5 ml of N-serve added with the urea solution after thoroughly mixing.

(c) SOIL + UREA + DBQ: As in (a), but plus 0.14 g DBQ (see 3.1.2 treatment) thoroughly mixed with urea.

(d) SOIL + UREA + DOUBLE DOSE OF N-SERVE: As in (b) plus extra 2.5 ml of N-serve.

3.3 Analysis of Soil Samples.

Analysis carried out included urea-N, NO_3^-, NH_4^+ and pH.

3.3.1 Urea-N.

The principle of urea analysis in pure solutions and in soil/plant extracts is based on the reaction of urea with diacetyl monoxime and thiosemicarbazide under acidic conditions and specified temperature to produce a red-coloured compound, the intensity of which can be determined colorimetrically. The method used in this work is that derived from the study of Arni Kyllingsbak and the modified procedure.*

Diacetyl monoxime-potassium nitrate solution (DAM-KNO_3) was prepared by dissolving 2.5 g diacetyl monoxime and 2.0 g potassium nitrate in 100 ml water. Thiosemicarbazide (TSC) was prepared by dissolving 0.25 g TSC in 100 ml of water. The acid mixture of 37 % hydrochloric acid and 85 % phosphoric acid was prepared by mixing 175 ml of hydrochloric acid with 100 ml of phosphoric acid and making up the mixture up to 500 ml. The colouring reagent was prepared immediately before use and it was obtained by adding 30 ml of diacetyl monoxime-potassium nitrate solution and 12 ml of thiosemi-carbazide solution to 450 ml of the acid mixture.

The standard solution was prepared by dissolving 0.2144 g urea in a 500 ml volumetric flask using 1 M KCl-PMA solution (prepared by dissolving 75 g KCl in one liter volumetric flask followed by addition of 100 ml of a 50 ppm solution of phenyl mercuric acetate (PMA) and making up to the mark with water). The solution contained 200 ppm urea-N. Standards were prepared by diluting 5 ml of the standard solution to 100 ml with 1 M KCl-PMA solution. From the diluted solution (concentration 10 ppm urea-N) 0, 5, 10, 20 and 30 ml were pipetted into 100 ml volumetric flasks and made up to mark with 1 M KCl-PMA solution. These solutions contained 0, 0.5, 1, 2, and 3 ppm urea-N respectively.

* The extraction procedure and the colorimetric determination was modified by Ameloko(1977) as part of the fulfilment of the minor thesis Soil and Plant Analysis of the M.Sc.Course.

10 ml of the extract obtained by shaking 10 g of the soil sample with 100 ml of 1 M KCl-PMA solution and 10 ml of each of the standards were pipetted into 50 ml volumetric flasks followed by 30 ml of the colouring reagent and the flasks were heated in a water bath at 80°C for 35 minutes. The flasks were cooled and made upto mark with water and the extinction of the solutions was measured at 508 nm using a 1 cm cuvette. The standards after diluting 10 ml of each to 50 ml contained 0, 0.1, 0.2, 0.4 and 0.6 ppm urea-N respectively. The urea-N contents of the soil samples were obtained by referring to the curve plotted using results of the standard series (see fig A). In tables 1a, 2a, 3a and 4a the results of the determinations are given and in figures 1a, 2a, 3d and 4d the data are graphically represented.

3.3.2 Nitrate.

The nitrate contents of the soil extracts were measured with the nitrate specific ion electrode. The nitrate ion electrode of model no. 476134 (Corning) was used together with a mV meter having an accuracy of ± 0.5 mV. The soil was extracted with 0.01 M solution of $CuSO_4$. A copper sulphate medium was used to prevent loss of nitrate by biological activity. Copper sulphate solution of 0.01 M was prepared by dissolving 2.5 g $CuSO_4.5H_2O$/liter. A standard solution of 1000 ppm NO_3^- was prepared by dissolving 1.6308 g KNO_3/liter.

For the extraction, 20 g of soil was weighed into a polythene bottle and 50 ml of copper sulphate solution was added. The mixture was shaken mechanically for 15 minutes before filtering over fluted filter paper. About 20 ml of the extract was transferred into a 100 ml beaker for the measurement.

Standard series were prepared by pipetting from the standard solution 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0 and 25.0 ml into 250 ml volumetric flasks and made upto mark with copper sulphate solution. 20 ml of each of these solutions was pipetted into 100 ml beakers.

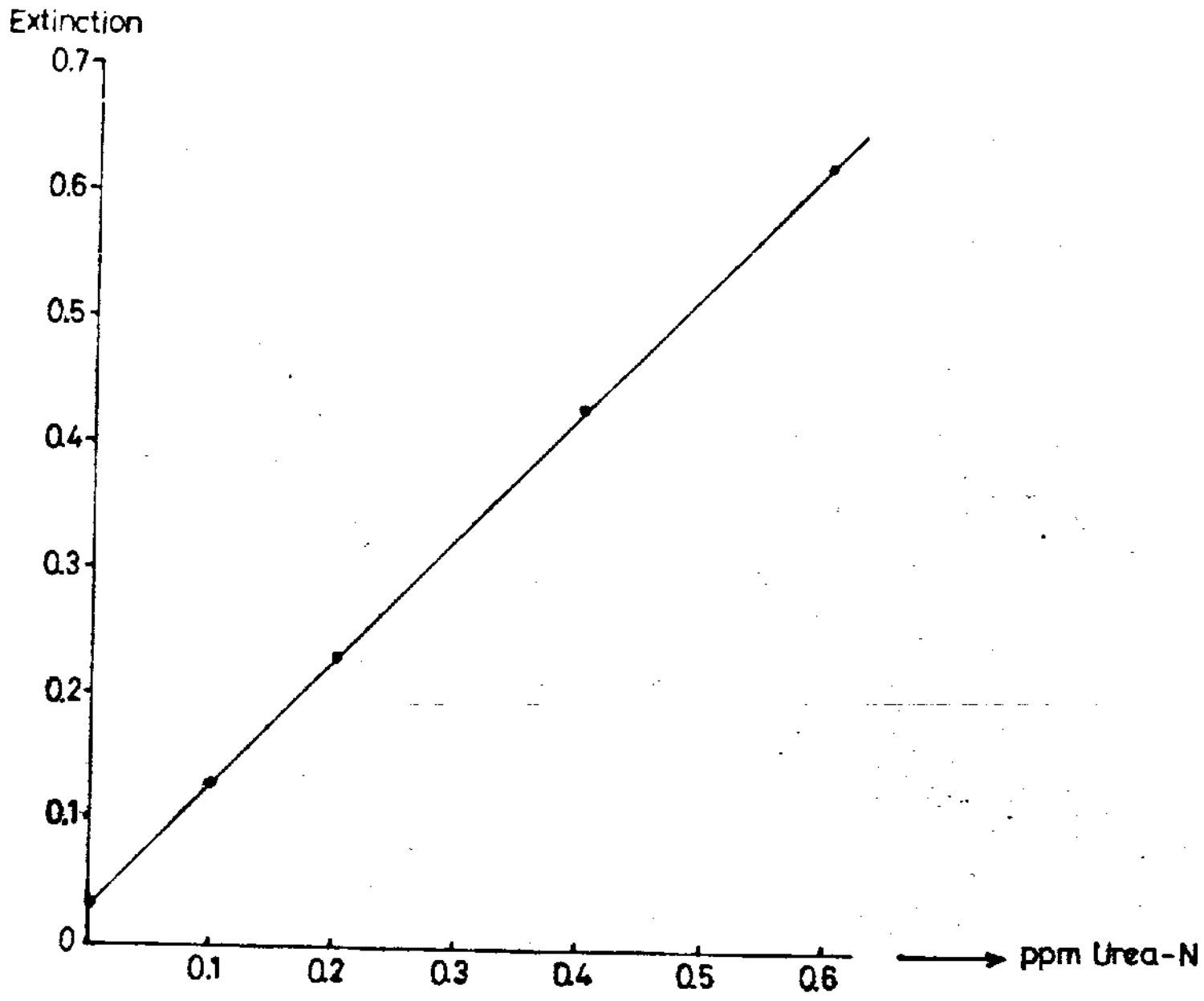


Fig. A Standard line for urea determination.

The standards have the concentrations of 2, 4, 10, 20, 30, 40, 60, 80 and 100 ppm NO_3^- respectively.

The potentials of the standards and the samples were measured by bringing the electrodes in the solution and stirring at constant speed while taking readings within 15 to 30 seconds after the drift has ceased.

The nitrate contents were computed by referring to the graph of standards (see fig. 8). Results of the determinations are given in tables 1b, 2b, 3b, 4b and illustrations in figures 1c, 2c, 3b and 4b.

3.3.3 Ammonium.

Ammonium concentrations were determined according to the method of Novozamsky and Houba (1977). Using this method, 20 g of each of the samples was treated with 50 ml of 1 M KCl and shaken for 2 hours at room temperature (see also 3.3.4). After shaking and filtering a 5 ml aliquot was pipetted and the pH raised by adding 0.1 ml of 10 M NaOH.

Standard solutions with concentrations of 10^{-5} to 10^{-2} M ammonia were prepared using ammonium sulphate $(NH_4)_2SO_4$ p.a. Also the pH values of the standard series were raised using NaOH.

The ammonium contents of the standards and samples were measured using a gas diffusion electrode as ammonia sensor. Measurements were carried out on a Beckman pH ASAR-I digital type potentiometer using the mV scale. The contents of ammonium were finelly computed by referring to the standard curve obtained from the standard series (a typical standard curve is shown in figure C). Increase of pH by addition of NaOH sets free ammonia gas ($NH_4^+ \rightleftharpoons NH_3 + H^+$) to which the electrode is sensitive. Tables 1c, 2c, 3c and 4c show the results of the analysis while figures 1b, 2b, 3c and 4c give graphical representations.

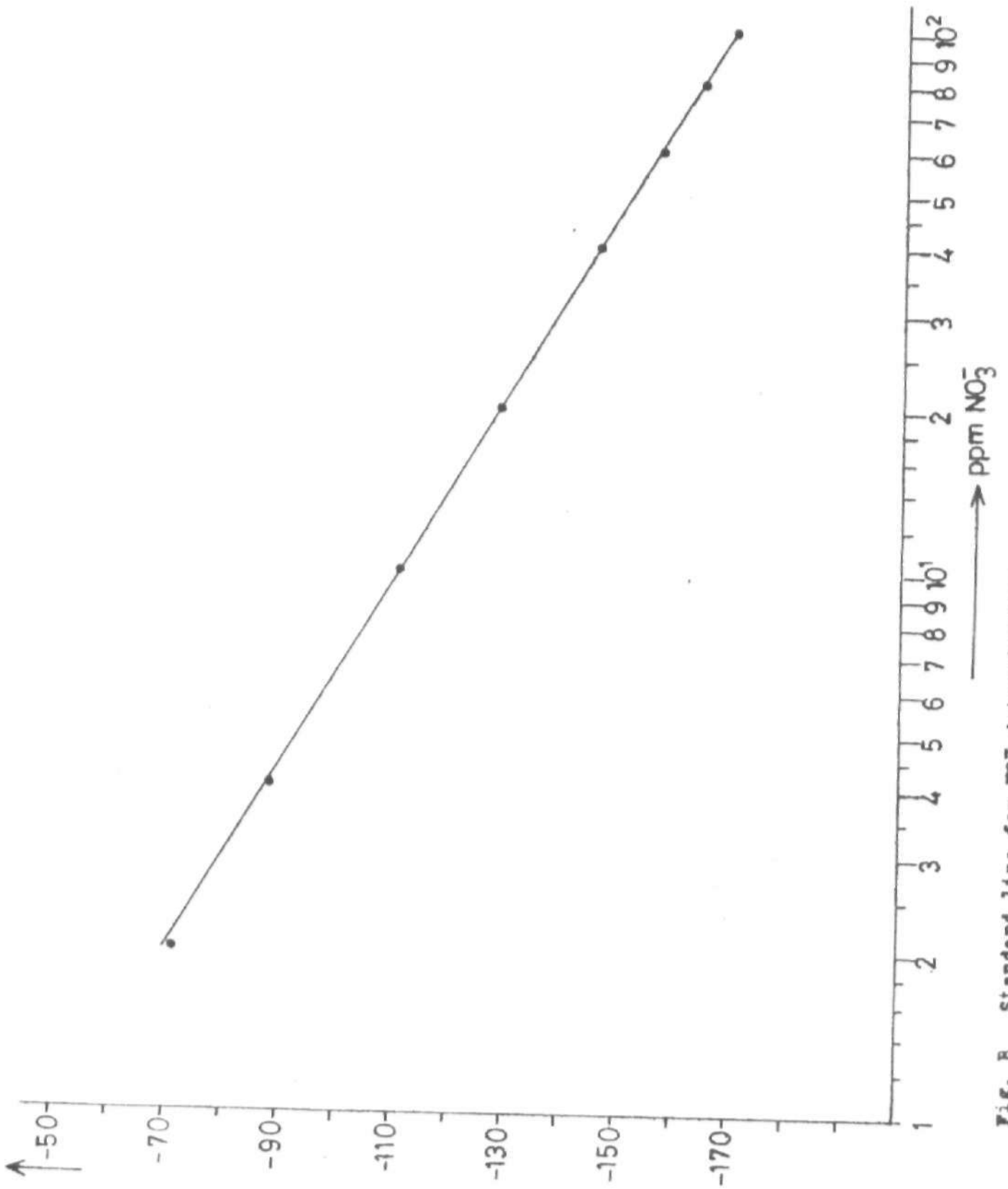


Fig. B Standard line for NO_3^- determination.

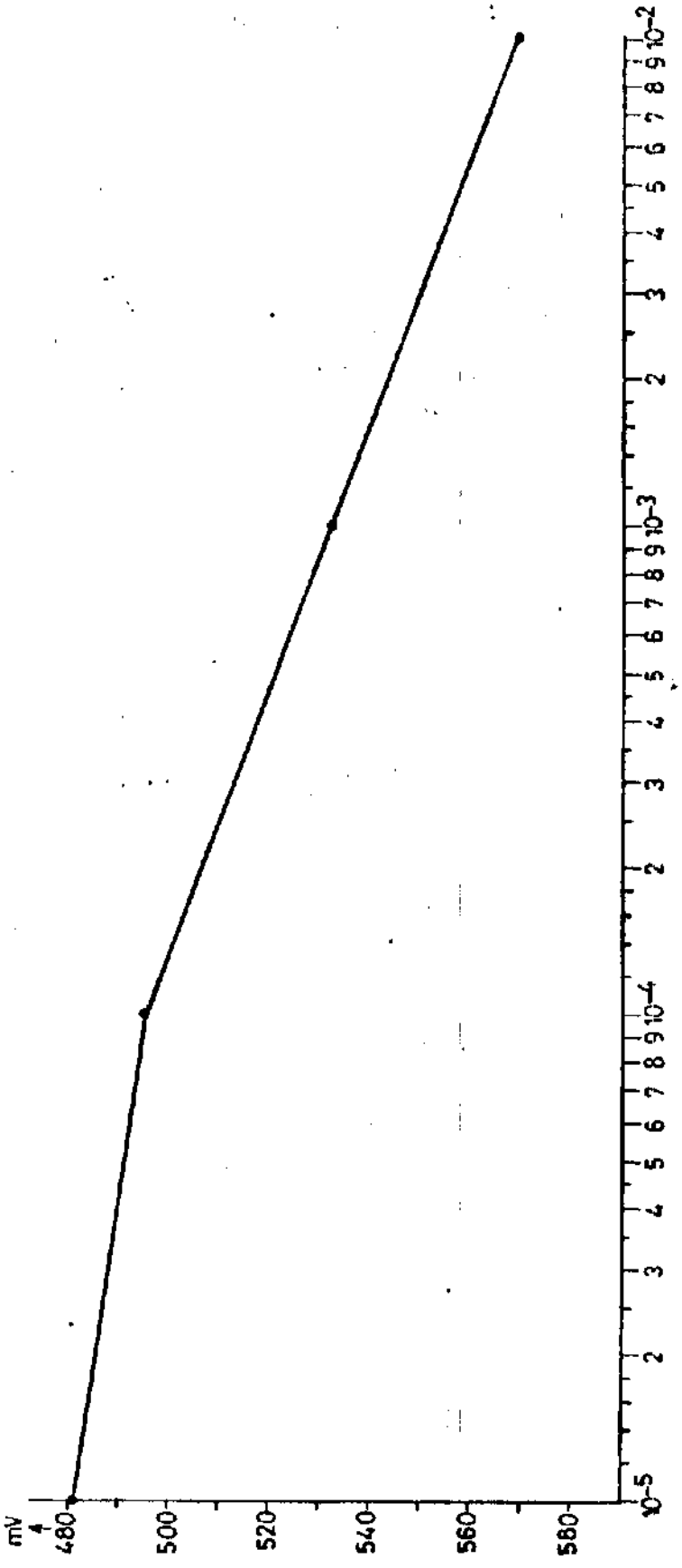


Fig. 6 Standard curve for the determination of ammonium (NH_4^+).

Part of the analysis of NH_4^+ was done with the modified indophenol-blue method (Novozamsky et al, 1974). This procedure was used because measurement of low NH_4^+ concentrations with the electrode was taking too much time before reaching equilibrium.

3.3.4 pH KCl.

For the pH measurement a method used by the Department of Soils and Fertilizers of the Agricultural State University, Wageningen, was applied in this study. According to this method 20.0 g of each sample was shaken for 2 hours with 50 ml of 1 M KCl (prepared by dissolving 74.5 g KCl and making the volume upto mark in a 1000 ml volumetric flask). After shaking, the suspension was left to settle making the supernatant solution to reach equilibrium with the soil solution before measuring the pH potentiometrically. Tables 1d, 2d, 3e and 4e show the results of the determinations. Variation of pH under various treatments are illustrated in figures 1d, 2d, 3e and 4e. Since the extractant and the procedure for the extraction for pH and NH_4^+ determinations are the same, the extracts for pH after filtration were used for the ammonium determination (3.3.3).

3.3.5 Results.

Table 1 Contents(based on ppm oven dry (105°C) soil) of Urea-N (table 1a), NO_3-N (1b), NH_4-N (1c), Urea+NO_3+NH_4 (1d) and pH (1e) in soil samples incubated with urea at a low level (100 mg N per kg soil) in Eng-soil with a low pH (means of duplicates).

Table 1a. Urea-N

Treatment	0	3	7	11	17	23	36	39	days after incubation
a. Blank (without Urea)	8.5	6.0	1.0	0.6	-*	-	-	-	
b. + Urea	100.5	68.2	10.5	2.2	-	-	-	-	
c. + Urea + N-serve	100.5	72.5	27.5	4.3	-	-	-	-	
d. + Urea + DBQ	100.5	89.6	36.6	22.8	3.0	-	-	-	
e. + Urea + $CuSO_4$	100.5	75.3	31.4	17.9	2.6	-	-	-	
f. +Urea+$CuSO_4$ +N serve	100.5	84.3	44.4	9.6	3.4	-	-	-	

* not measured supposed to be zero.

Table 1b. NO_3 - N

a.	4.0	4.9	7.8	6.3	7.5	9.4	9.3	9.7
b. see	4.2	6.4	8.0	7.5	10.3	10.6	15.5	18.8
c. table 1a	4.2	5.7	7.9	5.9	10.5	8.5	10.3	10.1
d.	4.2	6.2	10.3	6.6	7.5	8.4	9.4	12.9
e.	4.2	6.4	6.6	5.0	6.4	6.6	8.4	6.3
f.	4.2	5.4	3.0	4.7	8.5	5.4	7.2	4.4

Table 1c. NH_4-N

Treatment	0	3	7	11	17	23	31	39	days after incubation
a.	1.6	8.6	.*	6.6	8.3	6.8	8.2	6.1	
b. see	5.2	47.1	.	91.7	85.5	97.8	86.4	74.7	
c. table 1a	5.2	40.3	.	122.8	93.6	113.6	113.9	98.3	
d.	5.2	17.9	.	91.4	105.2	111.0	111.1	95.8	
e.	5.2	19.8	.	92.5	96.3	116.1	109.1	91.3	
f.	5.2	19.7	.	88.0	107.5	121.1	122.5	90.6	

* . results not reliable.

Table 1d. Urea-N + NO_3 -N + NH_4-N

a.	14.1	19.5	.*	13.4	15.7	16.2	17.5	15.8
b. see	109.9	121.6	.	101.4	95.8	108.4	101.9	93.5
c. table 1a	109.9	118.5	.	134.0	104.1	122.1	124.2	108.4
d.	109.9	113.8	.	120.8	115.7	119.5	120.6	108.7
e.	109.9	101.4	.	116.4	105.3	122.7	118.0	97.5
f.	109.9	109.5	.	102.3	119.4	128.3	129.7	95.1

Table 1e. pH KCl.

a.	3.7	4.0	3.9	3.8	3.4	.*	3.5	3.7
b. as table	3.7	4.0	4.2	4.1	3.8	.	3.8	3.5
c. 1a	3.7	4.0	4.3	4.3	3.8	.	4.0	3.7
d.	3.7	4.0	4.3.	4.3	4.2	.	4.1	3.8
e.	3.7	3.9	4.1	4.1	4.3	.	4.0	3.7
f.	3.7	3.9	4.1	4.1	4.2	.	4.0	3.7

* . results not reliable.

Table 2 . As table 1 but for Eng soil with high pH.

Table 2a. Urea.

Treatment	0	3	7	11	17	23	31	38 days after incubation
a. Blank(without Urea)	5.6	0.0	-*	-	-	-	-	-
b. + Urea	101.2	37.5	1.1	-	-	-	-	-
c.+ Urea +N - Serve	101.2	43.3	1.4	-	-	-	-	-
d.+ Urea + DBQ	101.2	82.5	29.1	21.2	0.7	-	-	-
e. + Urea + $CuSO_4$	101.2	59.8	11.5	0.5	-	-	-	-
f.+Urea+$CuSO_4$+N-serve	101.2	48.1	14.9	0.5	-	-	-	-

* . not measured supposed to be zero

Table 2b. NO_3^- - N

a.	2.5	4.8	8.0	11.8	12.3	12.7	16.8	17.7
b. see table 2a	2.4	5.8	13.6	18.0	38.4	56.6	71.6	100.0
c.	2.4	3.5	3.9	3.4	5.6	6.7	7.3	6.8
d.	2.4	2.8	4.0	3.6	5.5	10.9	14.3	14.0
e.	2.4	4.1	10.0	12.2	15.4	34.4	40.7	38.9
f.	2.4	3.2	4.5	3.1	4.4	5.9	6.1	5.1

Table 2c. NH_4^+ - N

a.	1.7	4.4	.*	13.3	1.9	0.5	2.0	1.2
b. see table 2a.	5.3	69.6	.	107.1	72.0	61.5	44.6	26.0
c.	5.3	87.3	.	101.4	119.2	124.9	116.3	107.4
d.	5.3	34.6	.	116.8	105.7	103.9	104.7	103.6
e.	5.3	37.5	.	100.0	75.6	93.3	90.1	53.0
f.	5.3	54.7	.	125.3	88.4	112.0	105.9	96.0

* . results not reliable

Table 2d. Urea-N + NO_3-N + NH_4^+ - N

Treatment	0	3	7	11	17	23	31	38
a.	9.8	9.7	.*	25.8	14.2	13.2	18.8	18.4
b. see table 2a	108.7	112.6	.	125.0	110.4	118.1	116.1	127.0
c.	108.7	134.1	.	104.8	124.8	131.6	122.7	114.2
d.	108.7	119.9	.	141.6	111.8	114.9	119.1	117.6
e.	108.7	101.4	.	112.8	91.0	127.6	130.9	91.9
f.	108.7	106.0	.	128.9	92.8	118.0	112.0	101.4

Table 2e pH KCl

a.	4.6	4.9	4.7	4.7	4.5	.*	4.5	4.2
b. see table 2a	4.6	5.4	5.6	5.2	5.0	.	4.1	3.8
c.	4.6	5.4	5.6	5.4	5.9	.	4.2	4.9
d.	4.6	5.3	5.7	5.6	6.1	.	5.2	4.9
e.	4.6	5.0	5.3	5.2	5.9	.	4.8	4.3
f.	4.6	5.0	5.3	5.2	5.6	.	5.0	4.7

* . results not reliable.

Table 3. Contents (ppm based on ovendry (105°C) soil) of Urea-N (table 3a) NO_3-N(3b), NH_4-N(3c), (Urea + NO_3 + NH_4)-N(3d) and pH (3e) in soil samples incubated with Urea at a high level (993 mg N per kg soil) in Eng soil with low pH.

Table 3a. Urea - N

Treatment	0	2	7	10	14	21	28	days after incubation
a. Soil + Urea	1070	792	871	684	594	327	199	
b. +Urea+N-serve single dose		878	832	684	590	382	114	
c. + Urea + DBQ		892	882	823	812	853	708	
d. +Urea+N-serve double dose		861	776	693	570	321	112	

Table 3b. NO_3 - N

a.		10	11	11	11	12	15
b. as table 3a		10	10	10	11	12	15
c.		9	10	10	10	10	13
d.		9	10	8	11	11	14

Table 3c. NH_4 - N

a.		114	258	284	523	650	896
b. as table 3a		78	240	272	415	642	783
c.		34	89	111	153	217	355
d.		57	211	297	345	693	890

Table 3d. Urea-N + NO_3-N + NH_4-N

a.		916	1140	979	1140	989	1030
b. as table 3a.		966	1082	966	1016	1036	912
c.		935	981	944	975	1080	1076
d.		927	997	999	926	1025	1016

Table 3e pH-KCl

a.	4.2	4.6	5.1	5.8	6.6	7.0	7.6
b. as table 3a		4.6	5.0	5.8	6.5	7.2	7.5
c.		4.4	4.7	4.7	5.0	5.2	6.0
d.		4.6	5.0	5.8	6.4	7.1	7.6

Table 4. As table 3 but for Eng soil with high pH

Table 4a. Urea-N

Treatment	0	2	7	10	14	21	28 days after incubation
a. Soil + Urea	852	897	577	231	99	65	64
b. +Urea+N--serve single dose		707	555	317	119	67	51
c. + Urea + DBQ		844	794	712	690	544	418
d. +Urea+N--serve double dose		763	642	320	164	70	65

Table 4b. NO_3^- - N

a.	11	13	12	13	13	16
b. as table 4a	11	11	12	11	12	13
c.	10	11	10	10	12	14
d.	11	11	12	12	10	14

Table 4c. NH_4^+ -N

a.	235	347	578	701	565	559
b. as table 4a	208	326	344	637	550	502
c.	109	179	208	293	307	355
d.	207	316	357	609	551	540

Table 4d. Urea-N + NO_3-N + NH_4^+ -N

a.	1143	957	821	813	643	639
b. as table 4a	926	892	673	767	629	566
c.	963	985	930	993	863	787
d.	981	969	689	785	631	619

Table 4e. pH KCl.

a.	6.6	6.3	7.8	8.2	8.3	8.1	7.9
b. as table 4a		7.3	7.7	8.2	8.4	8.0	7.8
c.		6.9	7.0	7.3	7.5	7.8	8.0
d.		7.3	7.7	8.2	8.4	8.2	7.9

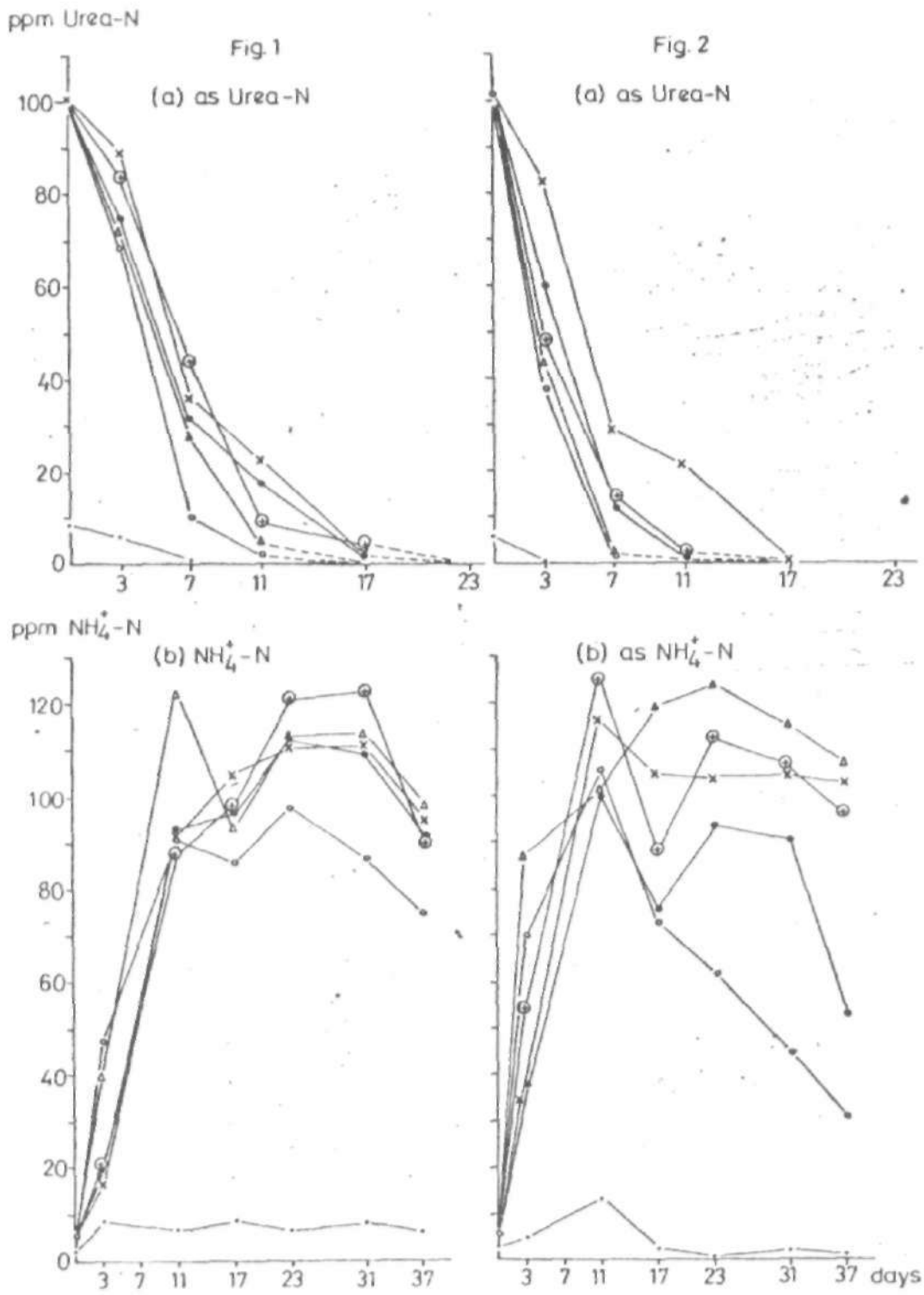


Fig. 1 and 2 Changes in urea and NH_4 contents of Eng-soil with (left) low and (right) high pH and incubated with (o) 10 mg urea / 100 g of soil; (Δ) as in the foregoing plus N-serve; (x) as in the foregoing plus urea inhibitor (DBQ see text); (•) as in the foregoing plus $CuSO_4$; ⊕ as in the foregoing plus $CuSO_4$ and N-serve.

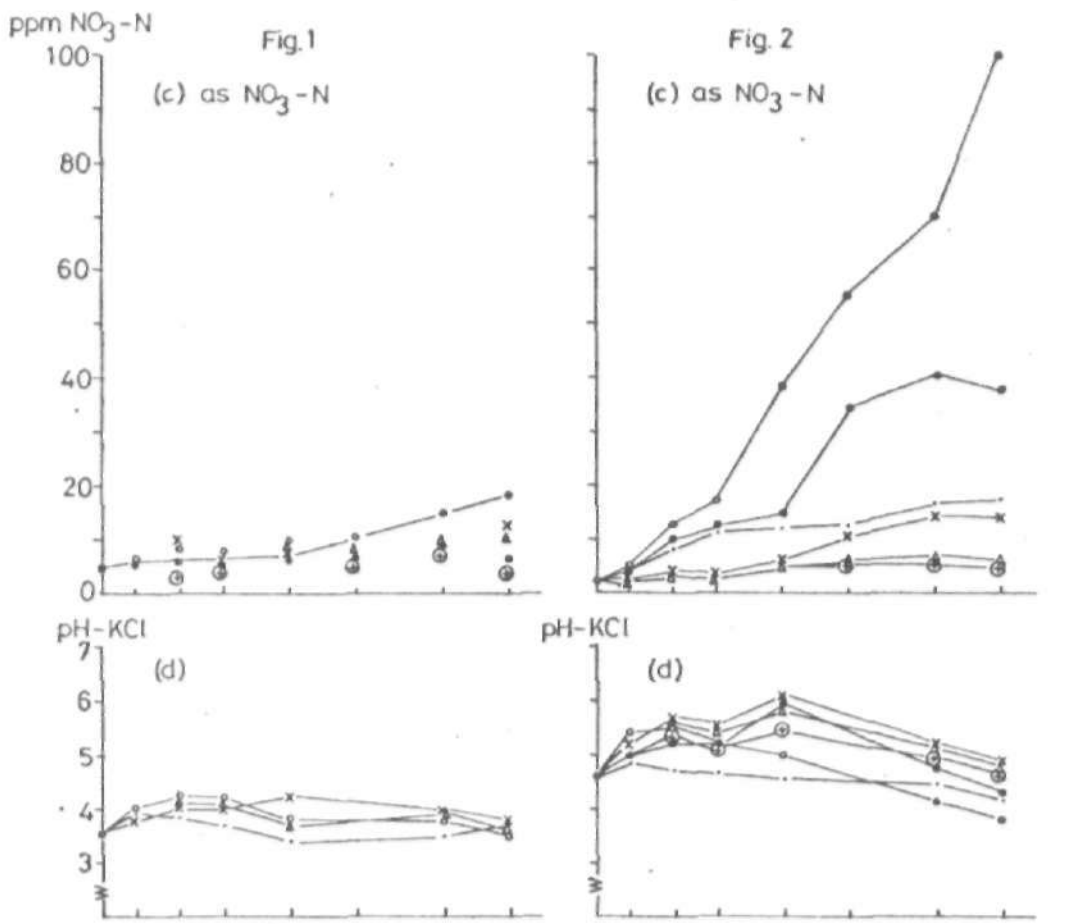


Fig. 1 and 2 Changes in NO_3^-, pH and Σ(urea + NO_3 + NH_4) contents of Eng-soil with (left) low and (right) high pH and incubated with (o) 10 mg / 100 g of soil; Δ as in the foregoing + N-serve; x as in the foregoing plus DBQ; • as in the foregoing plus $CuSO_4$; ⊕ as in the foregoing plus $CuSO_4$ and N-serve.

Fig.3

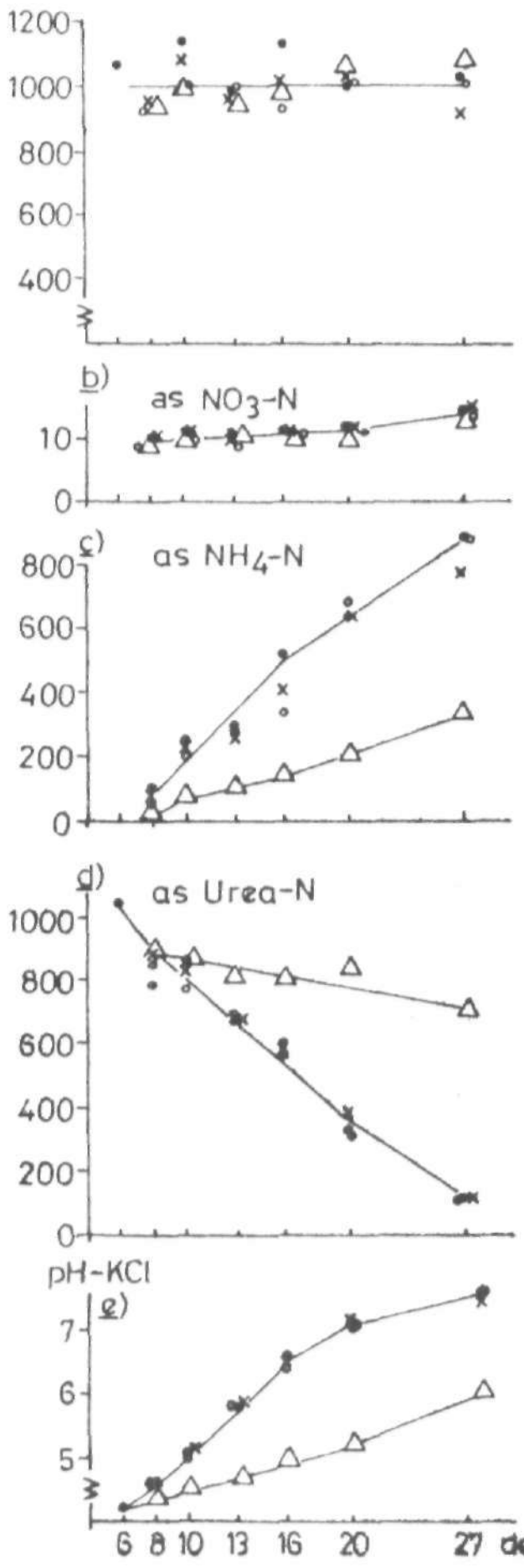


Fig.4

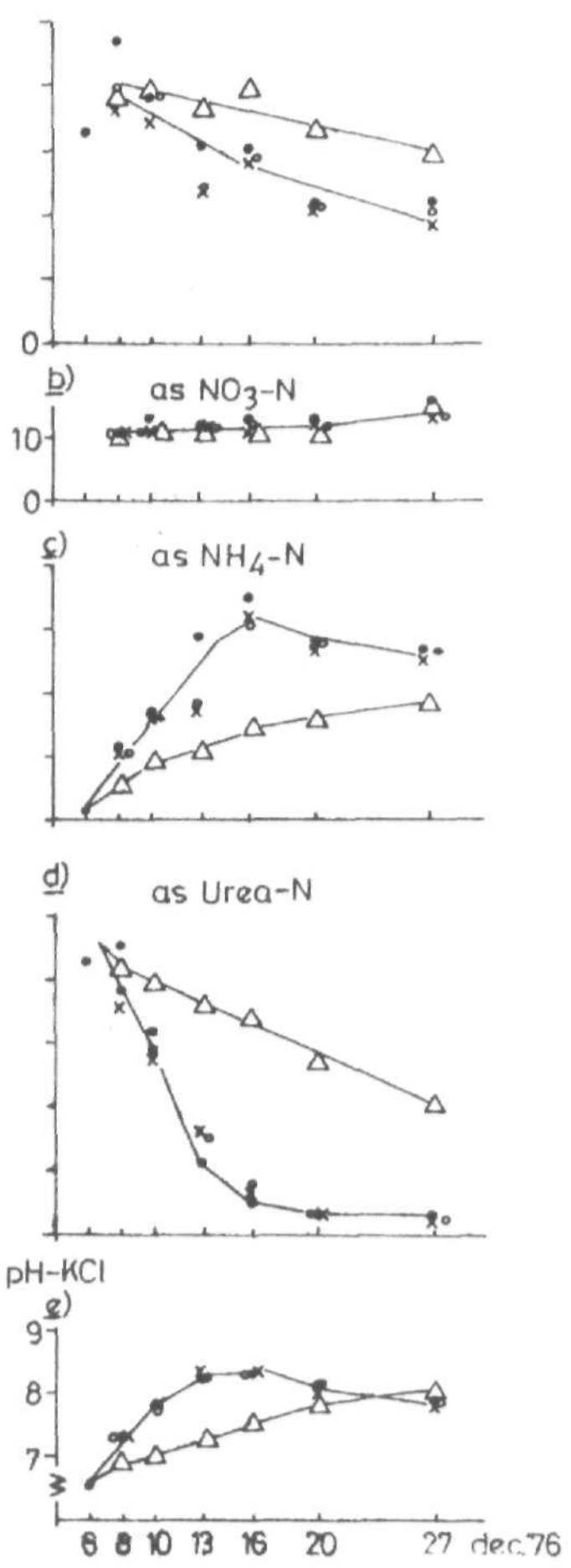


Fig. 3 and 4 Changes in pH, Urea, NH_4 and NO_3 contents of 'Eng' soil with (Fig.3) low and (Fig.4) high pH and incubated with (•) 200 mg Urea per 100 g of soil; (x) as foregoing plus N serve (single dose); (△) as (•) + Urea inhibitor (DBφ, see text) and (o) as (•) plus N serve, double dose.

3.3.6 Results and discussion.

Results obtained in the experiment with low level of urea and low pH (pH KCl 3.66) (fig. 1a and table 1a) showed that between 17 and 23 days after incubation, all the urea added to the soil was completely hydrolysed no matter which urease inhibitor was present. It is also evident from table 1a and figure 1a that all the urea added to the soil without urease inhibitor was completely hydrolysed about the 11th day (treatments: urea only and urea + N-serve). At this pH level, there seems to be no difference in the inhibitory effects of 2,5-dimethyl-p-benzoquinone and copper sulphate. Since there is clear evidence of hydrolysis at this low pH, these results contradict the findings of Delaune and Patrick (1970) who concluded that at pH values below 4 no urea hydrolysis occurs because of the denaturation of urease.

The results with the low level of urea and at a somewhat higher pH (pH KCl 4.64) presented in table 2a and illustrated by figure 2a show evidence of a higher rate of hydrolysis. In this case urea without inhibitors was hydrolysed completely about the 7th day and urea with inhibitors was completely hydrolysed about the 17th day. The steeper slopes of the curves in figure 2a compared with those in figure 1a are evidences of the higher rate of hydrolysis. At pH 4.64 there is also an indication that DBQ is a more effective inhibitor than copper sulphate.

The presence of N-serve presented no obstacle to urea hydrolysis at both pH levels as can be seen in the figures 1a and 2a by comparing treatments urea and urea + N-serve.

A close survey of the experiment with the higher level of urea at pH 4.19 and pH 6.58 reveals also a higher rate of hydrolysis of urea at higher pH than at lower pH. Inhibitory action of DBQ is well illustrated (figs. 3d and 4d). Also the non-interference of

N-serve on the rate of hydrolysis of urea is clearly shown for the higher N-doses (tables 3a,4a and figures 3d, 4d) for the low as well as for the high pH.

The rate of hydrolysis can be assesed either from the rate of decomposition of urea as above or from the rate of formation of ammonium. Tables 1c and 2c together with figures 1b and 2b give impressions of the rate of ammonium formation resulting from urea hydrolysis. From these data, it is also clear that the rate of ammonium formation is greater at higher than at lower pH. The maximum is found at about 11 and 23 days respectively. As can be expected ammonification is slower in the presence of urease inhibitor DBQ. Tables 3c and 4c and figure 3c and 4c also support the faster ammonification at higher pH as well as retardation of this process in the presence of urease inhibitor.

Since ammonium ions formed will under favourable conditions be converted to nitrate, after some time there will be a reduction in the amount of NH_4^+ and gradual accumulation of nitrate. Evidences of ammonium conversion and the building up of nitrate are clearly given by table 2b (treatments with urea and urea + $CuSO_4$) and illustrated by figure 2c (low level of urea and high pH). At low pH level 3.66, nitrification is only indicated for the treatment with urea and even then at a very slow rate (fig. 1c). The slow rate of nitrification here is a result of the low pH which is unfavourable for the micro-organisms responsible for nitrification. Figure 2c illustrates so well the inhibitory action of N-serve as nitrification inhibitor and from the same figure it looks as if DBQ and $CuSO_4$ have some inhibitory action on nitrification. At higher level of urea and at pH 4.19 and 6.58, nitrification is very slow as can be seen from illustration of figures 3b and 4b. In all cases, nitrification hardly occurred in presence of N-serve. Both single and double doses of N-serve seem to produce the same effect and at the same rate.

As a result of the formation of ammonium carbonate, the pH increases. The increase is less pronounced in soils which were originally at lower pH. In soil samples that received a low amount of urea, the pH reached a maximum of 4.1 and 6.1 starting from 3.7 and 4.6 respectively. The decrease in pH which followed is due to acidifying effect of the nitrification process. For samples that received higher levels of urea, the pH increased from 4.2 till 7.6 and from 6.6 till above 8. In the last case a pH decrease followed after the maximum was reached. The presence of DBQ did not stop the increase in pH as can be seen from figures 3c and 4c, but the rate of ammonification which leads to increase in pH is less fast than with other treatments.

Under ideal situations the sum of urea + NO_3^- + NH_4^+ expressed as N should equal at any time to the amount of urea N added to the soil at the start of the experiment (assuming no mineralization of organic material of the soil and or no denitrification of NO_3^- takes place). As can be seen from the summation in the tables, average values of the original amounts + the values of the blanks are given. In figures illustrating these data, there is quite a scattering of points due to uncontrolled errors in sampling and determinations. The results of the blanks (for urea, NH_4^+ and NO_3^-) show the picture of a soil without substantial mineralization and nitrification. Nevertheless it is possible that some NH_4^+ is incorporated in the organic matter (the micro-organisms in the soil might have used some NH_4^+ for their growth). It is obvious that the increase in pH above 7 as recorded in samples with a high level of urea and high pH may cause volatilization of nitrogen as ammonia and this seems a more likely explanation for the results found.

3.4 Germination of Maize in Soils Incubated with Urea with and without Urease and Nitrification Inhibitors.

The main objective of this experiment is to investigate if the treatments as described in 3.1 have any effect on the germination of maize.

If it is true that high concentrations of ammonia are injurious and high levels of urea do not injure germination of seeds, keeping urea as such should be favourable under normal circumstances.

3.4.1 Materials and methods.

Two batches of Eng-soil, low and high pH with low level of urea as described in the previous chapter were used. At the start of the experiment a batch of dry maize seeds and another of wetted seeds (3 hours in demineralized water) were used. The purpose of wetting was to accelerate germination. A nutrient solution containing P,K, Mg, Fe and the other trace elements was added to each pot. For both soil samples (low and high pH), 4 maize seeds were sown in two pots of each treatment (a - f). This was done on day zero with wet and dry seeds and in the same way on day 7 and 17 with dry seeds only. After sowing, the pots were incubated at room temperature ($20^{o}C$). The moisture contents of the samples were maintained by weighing and spraying with water from time to time until measurements of the lengths of the seedlings were taken and these were done after 11, 15 and 21 days after sowing. The results as the mean lengths per seedling in cm are given in table 5 (at the time of measurement there was only one leaf).

3.4.2 Results and discussion.

In this experiment (table 5) there is no regular behaviour of the plants as a result of differences in treatments. As can be noticed, there seems to be no evidence of retardation in growth due to high urea or ammonium concentrations because plants on the blanks are as good as those on urea, urea + N-serve and urea + DBQ treated soils. May be this can be expected as the pH increase is very modest and stays well below pH 7. A close examination of the data reveals retardation in growth in treatments with copper sulphate and copper sulphate + N-serve. Under this condition, the retardation must be linked with copper and not with N-serve.

Table 5. Length (cm) of maize seedlings grown in soil samples with low and high pH and incubated with Urea with and without Urease and nitrification inhibitors.

Treatment	Sowing:	0	wet 0	7	17	days after incubation
Soil low pH	measuring:	11	11	15	21	days after sowing
a. Blank		6.0	5.0	6.0	9.5	
b. + Urea		6.0	4.5	6.0	8.0	
c. + Urea + N-serve		9.0	5.5	5.5	6.0	
d. + Urea + DBQ		5.0	7.0	3.0	3.0	
e. + Urea + $CuSO_4$		3.5	3.0	5.0	6.0	
f. + Urea + $CuSO_4$ + N-serve		3.5	3.0	8.0	1.5	
Soil high pH						
a.		8.0	7.0	5.5	6.0	
b. as for low pH		7.0	6.0	7.0	7.0	
c.		7.0	6.0	7.5	4.5	
d.(see above)		6.0	6.0	8.0	3.0	
e.		4.5	3.5	8.0	7.0	
f.		6.5	4.5	5.5	3.0	

Looking at the results in columns 1 and 2, no difference in growth as a result of wetting the seeds can be seen. A delay in sowing (on day 7 or 17 after incubation) shows no clear differences between the treatments. In fact, there is no difference between the blank and urea only and the negative effect of copper is more clearly seen.

3.5 Urea Compared with Nitrogen as Nitrate and as Ammonium; Water Culture.

The general belief is that under field culture conditions nitrogenous fertilizers like urea and ammonium sulphate undergo biological and chemical changes so that most of the nitrogen is taken up as NO_3^-. In the case of urea, it is first hydrolysed in presence of urease into ammonium carbonate and the ammonium ion is then oxidized into nitrate ion before being absorbed by plants. Likewise, in ammonium sulphate, the ammonium ion may be oxidized or taken up as such.

This water culture experiment was done to investigate urea as a nitrogen source and compared with nitrogen as nitrate and ammonium with maize as a test crop.

3.5.1 Materials and methods.

The experiment was carried out in three containers, one for each nutrient solution with Radiometer equipment (automatic burette) to keep the pH of the culture solutions at a constant level of 5.0. The nutrient solutions were aerated (see Slangen, 1971). Maize seeds were germinated on gravel put in sieves and these were put on top of the culture solutions so that the roots could reach the solutions but the gravel was prevented from being wetted. The composition of the nutrient solutions are given in table 6. This experiment will be evaluated in detail by L.J.M.van Loon and M.L.van Beusichem*.For this

* The author refers with thanks and pleasure to both workers for supplying part of the data.

Table 6. Composition of nutrient solutions (meq/L) used for maize in water culture.

	Urea	NH_4-N	NO_3-N
Urea	5 (mm ol/l)	-	-
$(NH_4)_2SO_4$	-	7	-
$NH_4\ H_2PO_4$	-	3	-
$NaNO_3$	-	-	2
KNO_3	-	-	8
K_2SO_4	8	8	-
NaN_2PO_4	-	-	3
Na_2SO_4	6	2	7
$MgSO_4$	2	2	2
$CaCl_2$	1	1	1
FeEDTA	1 ml of stock solution	1 ml	1 ml
Trace elements	1 ml of stock solution*	1 ml*	1 ml*
N-serve	2 ml of stock solution	2 ml	2 ml

* The stock solution for the trace elements contained the following (per 1l of stock solution).

2.86 g H_3BO_3
1.55 g $Mn\ SO_4-10H_2O$
0.08 g $CuSO_4-5H_2O$
0.22 g $ZnSO_4-7H_2O$
0.0196 g $(NH_4)_6MO_7O_{24}.4H_2O$

thesis the dry matter yields of maize for the various treatments are presented. The plants were harvested (aerial parts and roots separately) weighed, dried and reweighed to determine the dry matter yields.

3.5.2 Harvest and measurements.

The first harvest was after 12 days, then followed by two other harvests after 20 and 28 days of treatments. The shoots were separated from the roots. The roots were washed and rinsed with distilled water. The plants were dried in an oven at 70^{o}C for 48 hours after which the oven dry weights were taken. Table 7 give the results of the weights.

3.5.3 Results and discussion.

Table 7. Dry weight (g/plant) of maize on water culture.

A = after 12 days.
B = after 20 days.
C = after 28 days.

		Nitrogen source		
		Urea	NH_4-N	NO_3-N
A	shoots	0.32	0.35	0.29
	roots	0.09	0.10	0.09
B	shoots	1.31	1.15	1.18
	roots	0.24	0.21	0.22
C	shoots	2.87	3.34	2.88
	roots	0.43	0.52	0.43

From these results, it may be said that upto the period of 28 days after sowing there was no significant difference in dry matter yields between the three treatments. However, NH_4-N seemed to produce more yield than others on the 28th day, but urea and NO_3-N showed no difference at all.

From the results available uptill now from this experiment, it cannot be said that urea was absorbed by maize plants as such. It is possible that urea was hydrolysed (the presence of urease in plant tissue has been reported in the literature) and that NH_4^+ formed was used by the plants. Since the system is not sterile, complete absence of urease activity cannot be neglected. Besides this hydrolysis affected by urease, the equilibrium $(NH_2)_2CO + 2H_2O \longrightarrow (NH_4)_2CO_3 \rightleftharpoons 2NH_4^+ + CO_3^{--}$ gives a more or less constant but low concentration of NH_4^+ in the nutrient solution. Nevertheless the yield of maize with urea as a N-source is not lower than that with NO_3-N uptil one month after germination and under the conditions as given before.

3.6 Comparison of Urea with other Nitrogen Fertilizers under Field Conditions.

In previous experiments, the trials were done under more or less controlled conditions. To be able to assess the value of urea as a fertilizer it is necessary to carry out trials under natural field conditions, where plants are faced with many hazards.

This experiment was therefore designed to study and compare the practical significance of 6 nitrogen fertilizers including urea on crop growth (yield and chemical composition) and the effects on the soil as regards the pH. The treatment combinations were not completely randomized per block, but for statistical analysis, the trials were considered as factorial designs in randomized blocks. The experiment belongs to the permanent field trials of the Department of Agricultural Chemistry of the Agricultural State University, Wageningen the Netherlands.

3.6.1 Experimental design; materials and methods.

The trials were carried out on the Dutch sandy soil (Eng-soil), the characteristics which have been described earlier on in the first chapter. As regards treatments, the following fertilizers were used.

a) NPK - a compound fertilizer (12 - 10 - 18)

b) Ammonium nitrate + limestone (26 % N)

c) Ammonium sulphate (21 % N)

d) Chilean nitrate (15.5 % N)

e) Urea (46 % N)

f) Sulphur coated urea (35 % N)

The fertilizers were applied at three levels viz. 84, 120 and 120^{+} kg nitrogen/ha.(Explanation for 120^{+} further in this text). The layout of the experimental field is shown below.

Table 8. Layout of field trial with 6 nitrogen fertilizers:

Block	Fertilizer	plot No.	Kg N/ha	plot No	Kg N/ha	plot No.	Kg N/ha
	NPK	12	120^{+}	24	120	36	84
	Chilean nitrate	11	120^{+}	23	120	35	84
I	Ammonium sulphate	10	120^{+}	22	120	34	84
	Sulphur coated urea	9	120^{+}	21	120	33	84
	Ammonium nitrate+lime	8	120^{+}	20	120	32	84
	Urea	7	120^{+}	19	120	31	84
	Ammonium nitrate+lime	6	84	18	120	30	120^{+}
	Urea	5	84	17	120	29	120^{+}
II	NPK	4	84	16	120	28	120^{+}
	Ammonium sulphate	3	84	15	120	27	120^{+}
	Chilean nitrate	2	84	14	120	26	120^{+}
	Sulphur coated urea	1	84	13	120	25	120^{+}

The pH of the soil is likely to decrease because of some of the treatments with nitrogen as NH_4^+ and in fairly high amounts. In order to neutralize the effect of these pH changes, calculated quantities of lime ($CaCO_3$) or sulphur were applied with 120 kg nitrogen/ha (treatment 120^+). Calculated as CaO, the amounts of lime added to each fertilizer were 61, 46, 40, 15 and 0 kg per 100 kg fertilizer for ammonium sulphate, urea, sulphur coated urea, NPK and ammonium nitrate + limestone respectively. 17 kg S per 100 kg fertilizer was added to Chilean nitrate. These amounts are established from field experiments on arable land with N-fertilizers in Holland.

The trial was started in 1972 with an initial pH of ± 4.4 and in 1973, 1974 and 1975, the pH of the soil of each treatment was measured. In 1975 summer wheat was grown as the test crop and in 1976 two varities of Kale (Brassica oleracea var Sabauda(L) Schulz which in Dutch are called "spitskool" and "savooiekool") were used as test crops. At the end of each growing season, the yields and in most cases the chemical composition of the harvested crops were determined and recorded. In 3.6.2 part of the data (as far as significant) are given and attention is given to the position of urea compared with other N-carriers.

3.6.2 Results and discussion.

Only the summarized results are presented in the following tables. The basic data and examples of the statistical analysis are available in the Laboratory of Soils and Fertilizers de Dreyen 3, Wageningen. The data for 1972, 1973 and 1974 were worked out by Mrs.H.O.Anjorin, 1975.

The data support the well known fact that Chilean nitrate has a pH raising effect which in this case is an average of 0.2 pH unit (the data for 1974 are not very reliable). The compound fertilizer (NPK), urea and ammonium nitrate + lime seem not to have any significant influence on pH under the conditions in question. Ammonium

Table 9a. Changes in pH KCl from 1973 -1975 in Eng-soil dressed with different nitrogen fertilizers (pH KCl in 1972 4.4)

Fertilizer	1973	1974	1975
NPK	4.6	4.3	4.5
Ammonium nitrate + lime	4.3	4.2	4.5
Ammonium sulphate	4.2	4.0	4.2
Chilean nitrate	4.7	4.0	4.8
Urea	4.4	4.1	4.4
Sulphur coated urea	4.3	4.2	4.3

Table 9b. Yields of grain and straw (kg/plot) of summer wheat in 1975 with different nitrogen fertilizers.

Fertilizer	grain	straw
NPK	4.79	7.52
Ammonium nitrate + lime	4.80	7.97
Ammonium sulphate	4.74	7.22
Chilean nitrate	4.07	7.10
Urea	5.24	7.63
Sulphur coated urea	4.32	7.07

Table 9c. Chemical composition of grains of summer wheat in meq/kg at 3 levels of fertilizer.

Fertilizer levels kg/ha N	Na	K	Ca	Mg	Cation(C)	H_2PO_4	Cl	SO_4	Anions(A)	C-A	K-org
84	-	-	20	-	275	-	see(1)	5	-	101	-
120	-	-	22	-	292	-	below	8	-	114	-
120+	-	-	26	-	291	-		7	-	116	-
mean $\bar{x}$	3 ± 0.4	150 ± 18.0		111 ± 1.41		143 ± 1.43			174 ± 1.79		1779 ± 862

(1) The effect of N-source is significant at 5% level i.e. NPK 27, Ammonium nitrate 26, Ammonium sulphate 24 chilean nitrate 27, urea 26 and sulphur coated urea 25 meq of Cl/kg.

sulphate as can be seen has a pH decreasing effect. This holds also for sulphur coated urea (with the extra effect of the sulphur). In general it is known that the final pH decrease for urea is less than for ammonium sulphate - the widely used N-fertilizer before urea was introduced.

Results in table 9b show no significant difference in yields of straw. There is, however, a significant difference in yields of grain. The differences of the means were not tested, but it is quite clear that urea belongs to the group of fertilizers that gave the higher yield of grain as well as straw.

From the summarized data of table 9c the chemical composition of the grains (the data for straw are not yet available), it can be seen that the effects are very small and are related to the amount of fertilizers. Only for chloride it is shown that there is a slight influence of the type of fertilizer.

Table 9d. Yields of two varities of Kale, Brassica oleracea (called "spitskool" and "savooiekool") under six N-fertilizers.

Fertilizer	Fresh weight (kg/100 pl)	
	"Spitskool"	"Savooiekool"
NPK	43.5	37.7
Ammonium nitrate + lime	46.4	38.5
Ammonium sulphate	41.7	31.3
Chilean nitrate	45.7	37.3
Urea	49.4	37.4
Sulphur coated urea	43.0	34.2

It can be seen from this table that the yields of both types of kale are better or among the best when urea is used as a fertilizer. (It is only for spitskool that the fertilizer effect is significant at 5 % level).

Table 9e (i) Chemical composition (meq/kg) of Brassica's in N-types trials 1976: mean values of observations with no effects of treatments.

	Mg	H_2PO_4	NO_3	SO_4	Sum anions	N total	N-organic
$\bar{x}$	307	172	409	344	1311	3271	2864
$\sigma_{\bar{x}}$	6.7	1.1	27.3	9.3	31.8	55.6	37.1

Table 9e (ii) As in 9e(i), but Ca and C-A contents(meq/kg)Significance of the amounts of nitrogen.

	84	120	120^+(kg N/ha)
Ca	914	853	980
C-A	900	1018	1091

Table 9e(iii) As in (i), but sum of cations(C) and C-A contents(meq/kg). Significance of types of fertilizers.

	NPK	AN-L	Amm-S	Chilean-N	Urea	S-Urea
Sum of cations	2297	2310	2275	2540	2182	2286
C-A	1071	1013	829	1244	843	1014

Table 9e (iv) As in (i) for Na & Cl contents (meq/kg).Significance of types and amounts of fertilizers and of the interaction.

Fertilizer	Level of fertilizers				Levels of fertilizers				
	Na	84	120	120^+	$\bar{x}$	Cl	84	120	120^+	$\bar{x}$
NPK		46	58	55	53		319	187	174	226
AN-L		44	50	47	47		397	428	359	394
Amm-A		36	35	44	38		475	576	524	525
Ch-N		385	536	621	514		297	269	265	277
Urea		38	44	40	41		482	541	340	455
S-U		52	50	37	46		472	481	352	435
$\bar{x}$		100	129	141	123		407	413	335	385

The results of the chemical analysis of "savooiekool" (table 9e) also give no idea about an extraordinary behaviour of urea as a fertilizer. The somewhat lower C-A value for urea (the same for ammonium sulphate) compared with that for Chilean nitrate and other fertilizers, supports the idea that most of the nitrogen is taken up as NH_4^+. This is also in good agreement with the results of the incubation trials (chapter 3.1) where no nitrification was found at pH values around 4.

The high contents of Na with Chilean nitrate are quite understandable because the amount of this compound was not compensated for in the other treatments. The same holds for chloride in NPK compound in which potassium is given as potassium sulphate and there is no correction for Cl in which form potassium is given in other treatments.

4. SUMMARY AND CONCLUSIONS.

Hydrolysis of urea is affected by the pH of the soil and can be limited to a slight extent with inhibitors such as 2,5-dimethyl-p-benzoquinone and copper sulphate with the former being more effective than the latter.

The formation of NH_4^+ and increase of pH which accompanied hydrolysis did not show injurious effects on germination of maize. However, it has to be emphasized that the amount of nitrogen, 100 mg N (as urea) per kg of soil is comparable with the normal dressing (1 ha = $2,5 * 10^6$ kg $\longrightarrow$ 250 kg N/ha), but in most experiments described in literature, higher levels of urea-N were used. With this moderate level of urea, the pH increase and the NH_4^+ accumulation will likewise be moderate (as shown in chapter 3) so that losses of nitrogen are no problem. That losses can occur, is shown in the incubation trial with 1000 mg N (as urea) per kg of soil (chapter 4). Even at pH levels around 7, the values are below those at which according to equilibrium, free ammonia can be expected in substantial amounts. However, losses are substantial in our experiment uptil 40 % with no doubt that they were caused by volatilization.

As said before, urease inhibitors have some effects, but in this experiment, within a period of 17 days all urea was hydrolysed so that only seeds that germinate very fast can profit from the low ammonium concentrations. Besides functioning as urease inhibitors, 2,5-dimethyl-p-benzoquinone (DBQ) and copper sulphate acted as nitrification inhibitors (comparable to N-serve). On the other hand N-serve a well known nitrification inhibitor is more specific and does not limit hydrolysis of urea. Since urease inhibitors described in the literature are related to either DBQ (quinones like hydroquinone; p-benzoquinone; 2,3-dimethyl-p-benzoquinone; 2,6-dimethyl-p-benzoquinone and 2,5-dichloro-p-benzoquinone) or to copper (as a heavy metal), it seems reasonable to conclude that these inhibitors when used in the soil are not specific enough and are less effective

than expected. Nevertheless there is no clear evidence about the chemical and or the enzymatic part of urea hydrolysis. Of course the accumulation of the products of hydrolysis of urea could be limited if nitrification takes place as quickly as the hydrolysis. This is not, however, the case and in this respect a very conclusion of the experiments is that at low pH values (4.0 - 4.5) even after 30 days of incubation no nitrification in Eng-soil took place (fig. 1c). Even under more favourable conditions of higher pH the process is relatively slow and cannot be accelerated substantially by adding a carbon source like glucose to stimulate the growth of micro-organisms as was seen in a small scale experiment not described here. In that experiment addition of glucose stimulated the hydrolysis of urea, but not the nitrification.

Connected with the behaviour of urea in soils which is the main subject of study here, it is clear from the water culture experiment (chapter 5) that urea can be used as a source of nitrogen at least for a period of 28 days as was tested for maize.

When urea was tested under field conditions, evidences from yields of crops proved that urea could be as good as other commonly used nitrogen fertilizers and could even be slightly better in some cases. This conclusion, however cannot be a general one because it is a well known fact that effectiveness of a fertilizer differs according to a number of factors not discussed here and this also holds for urea.

Aspect of uptake of urea or NH_4^+ by plants and the transportation together with the incorporation in plants are left out of discussion in this work.

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