A STUDY OF THE EFFECTS OF ADDITION OF COPPER COMPOUNDS TO SOILS ON THE METABOLISM OF NITROGEN

By

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ABSTRACT

Incubation studies were made on the effects of adding copper compounds to soils on transformations involving nitrogen, in particular ammonification, nitrification and immobilisation. The copper compounds were added in different forms (copper sulphate, copper phosphate and copper oxide) and their effects on nitrogen changes were studied immediately after treatment and also after the copper compounds were allowed to react with moist soil for some time.

The effects of copper compounds on nitrogen changes were studied in relation to soil type and pH, moisture content, and type of added nitrogen and organic matter.

The fractionation of copper in soils treated with copper compounds were studied by extraction with a number of reagents. The contribution of organic and inorganic soil fractions to the fixation of copper and other trace elements was also studied.

The relationship between levels of extractable copper and changes in the extent of transformations involving nitrogen during incubation were assessed.

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DEDICATED TO MY BELOVED MOTHER

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INTRODUCTION

The biological transformations involving the conversion of one form of nitrogen to another in the soils are dependent on many factors (e.g. pH, temperature, moisture content etc.) and these have been widely studied.

Soils may become contaminated with heavy metals through application of mining, industrial, and sewage wastes and also with residues of pesticides, all of which may contain heavy metals. In addition, there are some soils which are naturally high in heavy metals as a result of these being contained in the parent rock materials.

The work reported in this thesis is concerned with the study of the effects of one of these heavy metals, namely copper, on microbiological conversions involving nitrogenous compounds, in particular, the processes of ammonification, nitrification and nitrogen immobilization. Incubation studies were used so that the effects of adding copper to soils on these processes could be accurately studied in relation to variations in soil pH, moisture content and aeration. In addition, the effects of different copper compounds immediately after addition to soil as well as after allowing the copper compounds to equilibrate with the soil were studied.

The contributions of the organic and inorganic soil fractions on the extent and mode of fixation of copper in soils were also studied.

REVIEW OF LITERATURE

A. General outlines and some definitions in the nitrogen cycle.

Nitrogen is an indispensable component of all life on earth. It is the key building block of the protein molecule upon which all life is based. Its study therefore is of much importance. The availability of nitrogen to plants is of prime importance, since the plants depend on the adequate supply of this element in a form usable to them for the synthesis of their nitrogenous constituents. Animals also, in turn, depend on plants for their energy and synthetic processes. Thus a mutual give and take of nitrogen amongst the plant and animal kingdom of nature is prevailing in a cyclic order.

Plant and animal residues are being constantly added to soils by natural phenomenon. In soils these residues undergo various biological transformations to form a cycle in which nitrogen changes its form due to the activities of the soil microorganisms. A small part of the atmospheric nitrogen is converted to organic nitrogenous compounds by microorganisms,

either free-living or in symbiosis with plants. Lightning also converts a little of the atmospheric nitrogen into ammonia and nitrate. In soils nitrogen occurs principally in organic complexes of microbial origin. The plant and animal residues are the materials from which the reserve soil nitrogen supply is derived.

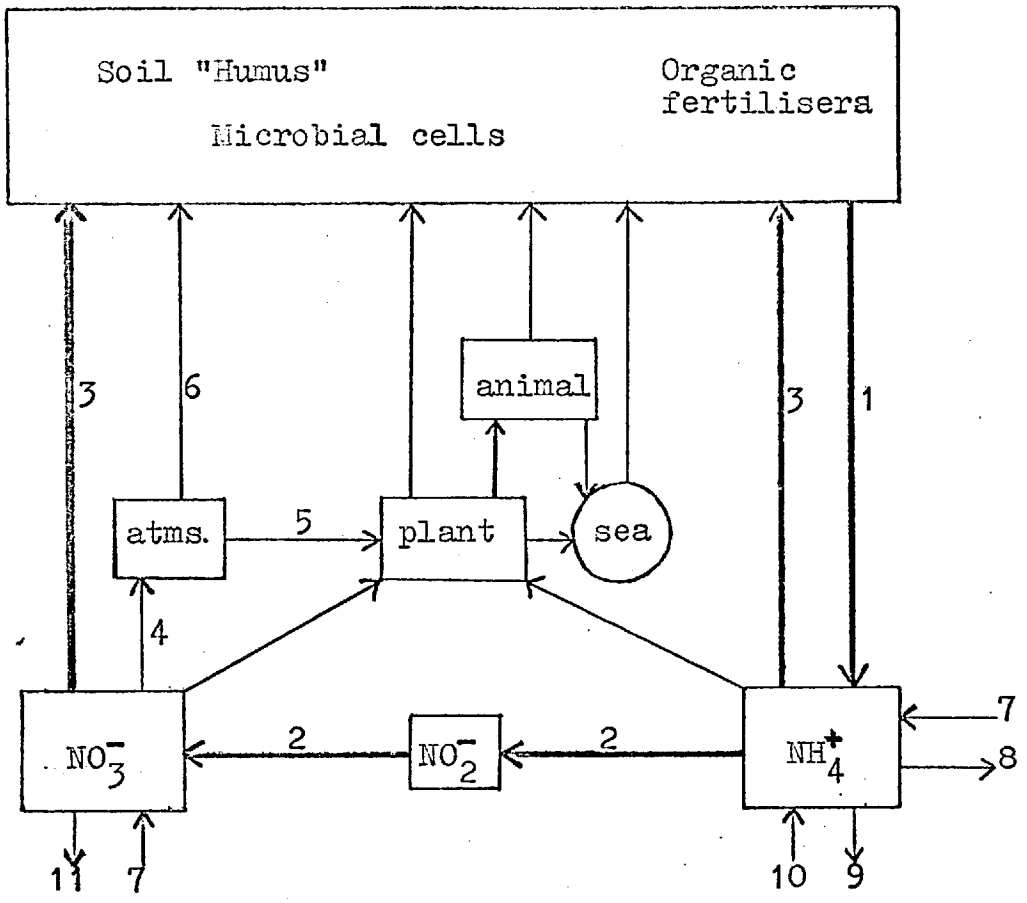
Nitrogen uptake by plants is only possible through the ammonium and nitrate forms. The organic nitrogenous compounds in soils must therefore be transformed into ammonium compound by certain microorganisms. This ammonium is either used by the plants or microorganisms or further oxidised to nitrate, via nitrite, by certain other groups of microorganisms and is then utilised by the plants or leached or denitrified.

This series of biological transformations of nitrogen which result in the formation of nitrogenous compounds readily available to plants and microorganisms is called the Nitrogen Cycle and is illustrated in Fig. 1.

The various terms used are defined as follows:-

Mineralisation:- Mineralisation of nitrogen is defined as the release of the organically bound nitrogen in soil humus and organic materials added to soils and the subsequent conversion of this nitrogen into inorganic forms. This process is analogous to the liberation of carbon dioxide from the carbonaceous materials in that both the transformations result

Fig. 1 - The Nitrogen Cycle



(The deep lines -1,2,3 - indicate the processes studied)

Key to the figure

1. Ammonification (Mineralisation)
2. Nitrification
3. Immobilisation
4. Denitrification
5. Symbiotic fixation
6. Non-Symbiotic fixation
7. Gain by fertiliser, rain etc.
8. Loss of ammonia through volatilisation
9. Loss of ammonia through "fixation"
10. Gain by release of "fixed" soil ammonium
11. Loss of nitrate by leaching

in the release of the elements in inorganic form.

Ammonification:- The first step of nitrogen mineralisation in which ammonium is formed from the organic nitrogen is called ammonification.

Nitrification:- The formation of nitrate from the ammonium compound is called nitrification. It is purely a biological process carried out by two groups of microorganisms. First, nitrite is formed and then this nitrite is rapidly oxidised to nitrate by the organisms. The whole process is called nitrification.

Immobilisation:- The microbial assimilation of inorganic nitrogen in forming their cells, a process opposite to mineralisation, is called nitrogen immobilisation. It results in the decrease of inorganic nitrogen or conversion of "available" form of nitrogen to "unavailable" form in soils. It is dominant when energy-rich materials are added to soils.

Denitrification:- The sequence of steps that result in gaseous loss of nitrogen is known as denitrification. It is microbial reduction of nitrate and nitrite with the formation of molecular nitrogen and, in some instances, nitrous oxide, which occurs under anaerobic or waterlogged conditions in the presence of energy-rich materials.

Nitrogen Fixation:- The biological transformations of atmospheric nitrogen into organic nitrogenous compounds in the

soil is called nitrogen fixation. It involves two processes:-

(a) Non-Symbiotic Fixation. There are certain free-living microorganisms which are able to use for their growth the elemental nitrogen of the soil air. The nitrogen is incorporated into the bodies of the microorganisms and left in the form of protein and related compounds in the soil. Since these organisms use the soil organic matter as a source of energy and are not directly associated with higher plants, the transformation is called non-symbiotic fixation.

(b) Symbiotic Fixation. There are certain microorganisms which fix atmospheric nitrogen in close association with the legumes and other nitrogen-gathering plants. The classical example of symbiotic fixation is the association between bacteria of the genus Rhizobium and plants of the Leguminosae. The bacteria live in symbiosis with their hosts and bring atmospheric nitrogen into organic combinations, which is available for the nutrition of the host plants and from which, in turn, bacteria draw their nitrogenous constituents.

B. Mineralisation of organic nitrogen (ammonification).

Mineralisation of nitrogen as already defined is the conversion of organic nitrogen into inorganic form which is usable by the plants. The first product of this conversion is ammonia. The organisms which form ammonia from organic compounds are unable to cause further transformations of ammonia

to more completely oxidised inorganic compounds, such as nitrite or nitrate. Consequently ammonia may be considered as the inorganic end product resulting from the activities of the microbes upon the organic substances. Therefore, the process of mineralisation can best be designated as ammonification.

Earlier workers (1,2,3,4) studied the factors such as aeration, organic matter, temperature, pH etc. involved in the mineralisation of organic compounds in soils and divided the whole process into two steps; (a) ammonification which is the conversion of organic nitrogen into ammonia and (b) nitrification, the formation of nitrate, via nitrite, from ammonia.

The fact that the process of mineralisation is mainly carried out by soil microorganisms was shown by Muntz and Coudon (5). They said that the process is purely a biological one as they could not detect ammonia from sterilised soil whereas much ammonia was found in the unsterilised soil. This was also confirmed by Etinger-Tulczynska in a recent study (6). However, some evidence has been brought forward which shows that ammonification and nitrification in soils are also made possible by sunlight, which is termed as photo-ammonification (7,8) ; but the validity of this idea is in doubt.

In ammonification, a very large diversity of bacteria, fungi and actinomycetes e.g. Achromobacter, Bacillus, Mucor, Penicillum, Aspergillus etc. can liberate ammonia from organic

compounds (9,10,11,12). Marchal (13) found that Bacillus mycoides was one of the most common soil organisms and one that attacks soil protein most energetically. McLean and Wilson (14) found that fungi rather than bacteria are responsible for the large accumulation of ammonia in soils rich in organic nitrogenous substances.

In soils, about 98% of the nitrogen occurs in organic form (15,16). The nitrogen-containing fraction of the soil, mainly composed of proteins, nucleic acids and their derivatives, are metabolised by the microorganisms. The metabolism is catalysed by the enzymes such as proteinases, peptidases, amidases etc. The products of these enzymatic processes are more simple nitrogenous compounds or ammonia (9,17). The whole process can be summarised as follows (18,19,20):-

A. Nucleic acids (purine and pyrimidine bases) ⟶ mononucleotides ⟶ ammonia.

B. Proteins ⟶ metaproteins ⟶ proteoses ⟶ peptones ⟶ polypeptides ⟶ amino acids ⟶ ammonia.

The process of nitrogen mineralisation in soils is dependent on a number of environmental factors. Physical and chemical conditions of the soil habitat, such as temperature, moisture, pH, aeration, the total nitrogen status of the soil and the inorganic nutrient supply, will govern the activities

of the soil flora and the rate of mineralisation (9,21). The rate of mineralisation of organic nitrogen, usually measured as mineral nitrogen (ammonium + nitrate) is frequently correlated with the total nitrogen content of the soil (22).

Temperature affects the mineralisation sequence as each biochemical step is catalysed by a temperature sensitive enzyme produced by the microorganisms whose growth is, in turn, conditioned by temperature. The optimum temperature for ammonification is usually between 40°C and 65°C depending on the soil (9,23,24,25). Within the range for the optimum temperature for ammonification, the higher the temperature, the faster is the process of ammonification (24,26,27,28).

Ammonification in soils can take place over a wide range of moisture contents, since the ammonifying population of the soil includes aerobes and anaerobes. It can occur even at the wilting point % (pF 4.2) although the rate is slow (29,30). Ammonification increases with increasing moisture content up to 40 to 75% of the maximum water holding capacity, depending on the soils and then declines with further increasing moisture content but still continues even under waterlogging (31,32,33,34,35,36).

The process of mineralisation is influenced by the pH of the soil. If all other factors are favourable, the production of inorganic nitrogen (ammonium + nitrate) is greater in

neutral than in acid soils (9). But as both acid-sensitive and acid-resistant soil microorganisms bring about ammonification, the process continues over a wide range of pH of the habitat (37,38). In acid soils, the process is depressed, but is not eliminated. Organic nitrogenous compounds tend to accumulate in acid soils because of the slow mineralisation. When acid soils are limed the process is stimulated as the pH of the soil is brought closer to the optimum for the active microflora (9,22,39,40,41,42). However, it has been found that there is little increase in mineralisation or nitrification when the pH of the soil is increased above about 6.5 (43,44).

C. Nitrification.

The conversion of ammonium nitrogen to nitrite or nitrate is called nitrification. During the process of ammonification, the nitrogen atom remains in a reduced state. But the plants, in general, prefer the element in oxidised form, that is, in nitrate-nitrogen form (9). Organic nitrogenous compounds can not be directly converted to nitrate, and ammonium generally must first be liberated as a consequence of the mineralisation (ammonification) process.

Nitrification is a process of enzymic oxidation brought about by certain microorganisms. Initial investigations on the process were carried out by many workers (45,46,47,48).

They observed that the process is a biological one. However, some evidence of nitrification due to sunlight is available, but the validity of this is in doubt (7,8). Schloesing and Muntz (45) were the first who experimentally gave the evidence that nitrification is a biological process. Winogradsky (48) was the first to isolate the organisms (Nitrosomonas and Nitrobacter) involved in the nitrification process. Later on Lees and Quastel (49) studying the biochemical transformations in nitrification by soil perfusion technique confirmed that nitrification in soil is entirely a biological process.

The organisms responsible for nitrification are all autotrophic and are divided into two groups (50,51,52,53):-

1. Ammonia oxidisers:- This group comprises the genus Nitrosomonas, Nitrosococcus, Nitrosocystis and Nitrosoglea.
2. Nitrite oxidisers:- This group comprises the genus Nitrobacter and Nitrocystis

Some evidence has also been found that heterotrophic organisms might be implicated in nitrification. Bacteria, actinomycetes and fungi are capable of producing nitrite or nitrate from ammonia and organic nitrogenous compounds in pure culture (54,55). Barrit (56) had advanced the idea that heterotrophic organisms can be involved in nitrification. It was also found by some other workers (57,58,59) that heterotrophic organisms such as Achromobacter and Corynebacter take

part in nitrification. Aspergillus flavus can convert ammonia to nitrate (60,61).

The reaction mechanism of the enzymic oxidation of ammonium compounds carried out by two highly specialised groups of aerobic autotrophic bacteria is given below (62):-

1. Ammonia oxidisers:-

$$\underline{\text{Nitrosomonas}}: \quad 2NH_4^+ + 3O_2 \xrightarrow[\text{oxidation}]{\text{enzymic}} 2NO_2^- + 2H_2O + 4H^+ + \text{energy}$$

2. Nitrite oxidisers:-

$$\underline{\text{Nitrobacter}}: \quad 2NO_2^- + O_2 \xrightarrow[\text{oxidation}]{\text{enzymic}} 2NO_3^- + \text{energy}$$

Kluyver and Donker (63) studied the possibility of an intermediate product during the oxidation of ammonium to nitrite. They indicated that the oxidation goes through the production of hydroxylamine and hyponitrous acid and then to nitrite. This pathway was also confirmed by Hofman and Lees (64). In recent years, Anderson (65,66,67) proposed the following pathway for the oxidation:-

$$NH_4^+ + O \longrightarrow NH_2OH \longrightarrow NO \longrightarrow NO_2^-$$

Nitrification takes place on the surface of soil and humus particles where ammonium is absorbed (68,69), but the presence of a solid phase is not necessary for the growth of the nitrifying bacteria (70,71,72).

The process of nitrification is markedly affected by

temperature. The optimum temperature for nitrification falls between 25°C and 37°C (73,74,75). Frederick (75) observed a low rate of nitrification at low temperature and explained that it is due to the initial low numbers of the nitrifying microorganisms. When the temperature was gradually raised, the nitrification rate increased up to an optimum. Above 37°C the process falls very rapidly (76,77). Some workers (77,78) reported that the optimum temperature falls between 24°C and 30°C. Many workers studying the temperature effects on the process of nitrification reported that below 5°C and above 40°C, the rate of nitrification is very slow (79,80,81,82,83).

Oxygen is necessary for all autotrophic species concerned, making adequate aeration essential. Because moisture affects the aeration regime of the soil, the water status of the microbial habitat has a marked influence upon the nitrate production. The optimum moisture level for nitrification varies considerably with different soils, ranging from 20% to 80% of the maximum water holding capacity of the soils (84,85, 86,87,88). At very low moisture content the process is either very slow or negligible. Below 5% mwhc moisture content, no nitrification occurs because the bacterial proliferation at this moisture content is retarded (29,32,89). In field conditions, Calder (84) and Simpson (90) reported that in soils of Uganda nitrate accumulation ceased when the moisture level dropped below 10% mwhc of the soils, whilst 22-23% moisture

content seemed to be most favourable for nitrification. Low moisture content inhibited nitrite oxidisers more than ammonium oxidisers (76). In waterlogged conditions, the supply of oxygen is inadequate for the microorganisms and hence the oxidation of ammonia stops (91). The texture of the soils affects nitrification through its influence on aeration. It was reported that under controlled experimental conditions in the laboratory, the artificial aeration stimulated nitrification (9,92).

Amongst the environmental factors that influence nitrification markedly, the pH of the habitat is the main one. In acid medium, nitrification cannot proceed. The optimum pH as observed by many workers for nitrification falls between 6.5 and 9.0 (93,94,95,96,97). Anderson (94) observed that 8.6 is the optimum pH for the oxidation of ammonia whereas the pH values of the soil for the formation of hydroxylamine and nitrite were 8.6 and 7.2 respectively. It was reported that the oxidation of nitrite is restricted to a narrow range of pH, limited in acid medium by the dissolution of an active site of the enzyme system (98). Harmsen and Schreven (38) and Allison (99) observed that at high pH, accumulation of nitrite takes place because nitrite is stable under alkaline conditions. On the other hand, Reuss and Smith (100) reported some accumulation of nitrite even in acid soils.

Although nitrification markedly falls off below pH 6.0, and is negligible below pH 5.0, yet occassionally it continues even in acid soils of pH 4.0 or below (9,101). The reason for this as given by Bollen and Wright (102) is due to the fact that microorganisms in such acid medium obtain their essential supply of bases from the decomposition of the organic litter in the soil. Premi (103) has also observed that nitrate accumulated even in acid soils (pH 5.1). Addition of lime to acid soils stimulates nitrification, the rate of production of nitrate being proportional to the amount of lime added up to the optimum pH (99,104,105,106).

Nitrification of ammonium sulphate added to soils tends to reduce the soil pH due to the conversion of ammonium to nitric acid and also to the accumulation of sulphate residues. The extent of reduction of pH increases with decreasing buffer capacity of the soil (107,108). Many workers observed that nitrification of added ammonium sulphate takes place rapidly in naturally calcareous soils and in acid soils the process is stimulated when calcium carbonate is added (109,110,111,112). When high concentrations of ammonium sulphate are added to soils of high pH, a temporary toxic effect on nitrification occurs because of the accumulation of free ammonia and nitrite. This effect quickly disappears as the free ammonia and nitrite are lost through volatilisation, leaching etc. and eventually the pH of the soil falls and hence nitrification becomes

normal (113,114,115,116,117,118)

D. Immobilisation of inorganic nitrogen.

The microbial assimilation of inorganic nitrogen produced during mineralisation is called immobilisation. It leads to the biosynthesis of the complex molecules of microbial protoplasm from ammonium and nitrate. Thus mineralisation and immobilisation take place simultaneously in soils. The organisms responsible for mineralisation are also responsible for immobilisation under different environmental conditions.

Both mineralisation and immobilisation of nitrogen in soils are markedly affected by the carbon:nitrogen (C/N) ratio of the added organic matter (21,119). The active decomposition of the organic matter implicates an actively multiplying microflora with a simultaneous assimilation of nitrogen for its growth processes. In the decomposition, the organic matter must have a nitrogen content in excess of the microbial requirements to favour mineralisation. The critical C/N ratio falls between 20 and 25:1. Above this critical value immobilisation is predominant and below this value mineralisation is predominant (9,99,120).

Nitrogen is a key nutrient substance for microbial growth and hence for organic matter breakdown in the soil. If the nitrogen content of the substrate is high i.e. the C/N ratio is lower than the critical value, the microflora satisfies

its needs from this source and additional nitrogen is not necessary. If, however, the substrate is poor in nitrogen i.e. C/N ratio is higher than the critical value, the decomposition of the organic matter is slow. An additional source of nitrogen is required for the maximum rate of decomposition and biological immobilisation of nitrogen (121,122). Nitrogen-free materials such as carbohydrates greatly favour immobilisation. The rate of immobilisation is proportional to the amount of organic matter present in or added to the soil and also related to the degree of resistance to microbial attack of the organic matter (123,124).

Decomposition of cellulose takes place rapidly when ammonium sulphate is added as a source of additional nitrogen (125,126). Blasco and Cornfield (127) observed that the addition of cellulose to soils depressed nitrogen mineralisation. If no nitrogen is added, cellulose can remain in soils without complete degradation for even more than two months (128). Lueken and others (129) observed that in cellulose-treated soils a substantial nitrogen deficiency occurs and when extra nitrogen is added, the rate of decomposition is greatly increased.

E. Decomposition of organic matter and the evolution of carbon dioxide.

The organic matter of the soil is comprised of a variety

of substances of plant, animal and microbial origin. These substances consist of proteins and other nitrogenous compounds, of carbohydrates (cellulose, hemicelluloses, starches, pectins, saccharides, glucosides etc.) and their derivatives, of fats, waxes, lignins, tannin, resins, alkaloids and mineral matter. Thus the soil organic matter is a complex of substances, the composition of which depends on the transformations of these individual substances by the activities of the microorganisms (93,130). The biological and chemical transformations which involve the breakdown of the organic matter in soil or added to the soil serve two functions for the heterotrophic groups of microflora, providing energy for their growth and supplying carbon for the formation of their cells (9,131).

The organisms responsible for the degradation of the organic materials vary with the type of the material. Cellulose is attacked by Clostridium, Achromobacter, Aspergillus, Verticillum, Fusarium, Penicillum, Hormodendrum etc. (132,133 134,135). Hemicellulose by Bacillus, Actinomycetes, Aspergillus, Streptomyces etc. (9,136). Lignin by Agrobacterium, Flaviobacterium, Pseudomonas, Hyphomycetes, Basidiomycetes, Marasmius, Clavaria etc. (137,138,139,140). Hydrocarbons by Methanomonas, Pseudomonas, Corynebacterium, Mycobacterium, Practinomyces, Nocardia etc. (141,142,143).

Decomposition of organic matter results in the production

of carbon dioxide. The biological transformations of organic carbon into the inorganic state in the form of carbon dioxide is called carbon mineralisation which is analogous to nitrogen mineralisation. The evolved CO_2 is partly used by the micro-organism themselves and partly lost to the atmosphere (9,144). This is an enzymic oxidation process which can be represented as follows (62):-

$$-(C,4H) + O_2 \xrightarrow[\text{oxidation}]{\text{enzymic}} CO_2 + 2H_2O + \text{energy}.$$

Carbon & Hydrogen compound

The rate of release of carbon dioxide indicates the rate at which soil organic matter is being attacked and is dependent on the organic matter content of the soil (9,130). When fresh organic matter is added to soil, the mineralisation of the native organic matter is also stimulated (145,146). In the mineralisation of organic carbon, high microbial activity is characterised by high oxygen uptake and increased carbon dioxide production associated with rapid decomposition of the organic materials (147,148). In the early stages of decomposition, the CO_2 production is rapid but slows down at later stages because of the accumulation of more resistant substances (149).

Environmental factors, such as temperature, pH, moisture, aeration and available mineral elements affect the production

of CO_2 and the decomposition of organic matter (9). Increasing the temperature from low level to high level, increases the rate of decomposition of organic matter causing the increase in the production of CO_2 (150,151,152).

Moisture content of the soil influences the microbial activities and hence the production of carbon dioxide. The production of carbon dioxide is low at low moisture contents, but increases with moisture up to 50 to 100% mwhc, depending on the soil (153,154,155,156,157).

Decomposition of organic matter and the production of CO_2 are also markedly affected by pH of the environment. Each bacterium, fungus and actinomycete that are responsible for the process has an optimum pH for growth and a range outside of which no cell proliferation takes place. The decomposition of organic matter and CO_2 production is usually most rapid in neutral soils and is stimulated when acid soils are limed (9).

F. Effect of copper on nitrogen transformations in soils.

Swaine (158), in a review on the trace element contents of soils, concluded that the "usual" content of copper in soils from many parts of the world is in the approximate range of 2-100 ppm. However, instances of soils having much higher levels of copper occurred frequently e.g. some Scotish soils having 250-5000 ppm copper, Hungarian, Finish and Spanish soils having up to 3000 ppm copper. Some of these high values

may have resulted from contamination of soils by mining and industrial wastes of high copper content. The problem of contamination of soils in this way is likely to increase with increasing industrialisation. Other methods by which the content of copper (and other metallic elements) may be increased in soils are through the use of copper-containing pesticides, contamination from city and industrial smoke, and the use of sewage materials as manures (particularly sewage from industrial areas where factories discharge wastes containing metallic elements into the sewage system).

Some work has been reported on the effects of increasing soil copper content on the microbiological processes involving nitrogen transformations in soils. The results appear to be rather contradictory.

Lipman and associates (159) observed that the addition of copper to soils stimulated ammonification, while others (160) observed that copper had no effect on ammonification of tankage and dried blood. Other workers (161,162,163) found that copper had either no effect or inhibited ammonification.

Lees (164) found that the addition of copper compounds to soil increased its nitrifying power. Anderson (94) observed that in the oxidation of ammonia to hydroxylamine and nitrite, the presence of copper was required. Fred (165) observed no effect of copper added compounds on nitrification. Lipman

and others (160,166) found that copper added at low levels (less than 125 ppm) was toxic to nitrification, but at higher levels it had a stimulating effect on nitrification. Other workers (167,168,169) observed no effect or inhibitory effect on nitrification by the addition of copper.

Premi (103) and Premi and Cornfield (170) found that 100 ppm copper increased ammonification slightly in aerobic soil, 1000 ppm copper had no effect, whilst 10,000 ppm copper decreased it considerably. In anaerobic incubation, 100 to 10,000 ppm copper had no effect on ammonification. In neutral aerobic soil, they found increases in nitrification with 100 ppm copper as sulphate and 1000 and 10,000 ppm copper as carbonate. 100 and 1000 ppm of copper stimulated the accumulation of nitrate with prolonged incubation when nitrate had initially been immobilised by sucrose in neutral soil in aerobic conditions.

THE OBJECT OF THE PRESENT WORK

The work reported in this thesis was concerned with the incubation studies of the effects of adding copper compounds to soils on some of the transformations involving nitrogen, specifically, ammonification, nitrification and immobilisation. The copper compounds were added in different forms (cupric sulphate -$CuSO_4.5H_2O$, cupric orthophosphate -$CuHPO_4$ and copper oxide -CuO) and their effects on the nitrogen changes

in soils were studied immediately after addition and also after the copper compounds were allowed to react with the moist soil for some time.

The effects of copper compounds on nitrogen changes during incubation was studied in relation to soil type, moisture content, source of added nitrogen, type of organic matter added and soil pH (using either soils of naturally different pH and also some in which pH was altered by prior treatment with calcium carbonate or ferrous sulphate).

The fractionation of copper in soils treated with copper compounds was studied by extraction with a number of solvents. The contribution of organic and inorganic soil fractions to the fixation of copper and certain trace elements was also studied.

As a matter of routine copper and other trace elements were extracted after incubation using suitable solvents based on the fractionation study.

CHAPTER II

MATERIALS AND METHODS

CHAPTER II

MATERIALS AND METHODS

A. MATERIALS

Soils.

Most of the studies reported in this thesis were done on three types of soil, the characteristics of which are given below. The soils were all from cultivated areas and were sampled to 6-inch depth, spread on brown paper to air dry and ground to pass through 2mm sieve.

Silwood soil:- A sandy soil (Bagshot sand) according to the United States Soil Survey Classification. It contains sand 81%, silt 11%, clay 5.5%, organic carbon 2.1% and has cation exchange capacity 7.6 m.equiv. per 100g, total nitrogen 0.18%, pH 5.4, maximum water holding capacity 42% and total copper (extracted by boiling 6N HCl) 12 ppm. (Methods used to determine these values are described later).

Harlington soil:- A sandy loam soil. It contains sand 55%, silt 25%, clay 16%, organic carbon 2% and has cation exchange capacity 14.0 m.equiv. per 100g, total nitrogen 0.16%, pH 6.6, maximum water holding capacity 50% and total copper 40 ppm.

London clay soil:- A clay soil. It contains sand 40%, silt 16%, clay 42%, organic carbon 3.4% and has cation exchange capacity 20.0 m.equiv. per 100g, total nitrogen 0.32%, pH 7.1,

maximum water holding capacity 60% and total copper 8 ppm.

Copper compounds.

The copper compounds used in this study were in the form of finely ground Analar cupric sulphate ($CuSO_4.5H_2O$), cupric orthophosphate ($CuHPO_4$), ~~and~~ copper acetate ($Cu(CH_3COO)_2.H_2O$), and cupric oxide (CuO).

Organic materials.

The organic materials used were dried, finely ground samples (Christie and Norris Mill) of cellulose (44% C), sucrose (42% C), dried blood (12.5% N, 37% C), grass (0.9% N, 39% C) and wheat straw (0.3% N, 40% C).

B. METHODS

Method of altering soil pH.

In those studies where pH was a variable it was necessary to treat samples of soil with varying levels of chalk ($CaCO_3$) to increase and with varying levels of ferrous sulphate ($FeSO_4.7H_2O$) to decrease the pH. 500g samples of air dried 2mm sieved soil were mixed with levels of finely ground chalk ranging from 0.1 to 0.5% and with levels of finely ground ferrous sulphate from 0.2 to 3.2%. The treated samples were placed in plastic or glass flower pots with the drainage holes covered by glass cloth. Water was added to each pot in an amount equivalent to 50% of the maximum water holding capacity of each soil. The pots were left at laboratory temperature,

with water being added periodically to keep them moist. Every two weeks sufficient water was added to obtain about 2-inch of leachate. This was continued for 2-3 months and at the end of the treatment period about 4-inch of water was added. Leaching was done in order to remove soluble salts and nitrate. The soil was allowed to drain and then spread on brown paper to air dry. The samples were then ground and passed through 2mm sieve and their pH values were determined. In this way for each soil type a wide range of pH levels were obtained, and suitable samples were selected for study.

Method of addition of organic materials.

The finely ground organic materials were mixed thoroughly with air dried soil at particular levels of application. Sucrose, being soluble in water, was sometime added in solution in the water used to bring the soil to the selected moisture content.

Method of addition of copper compounds to soil.

(a) Where incubation was done soon after addition of copper compounds:- Where $CuSO_4.5H_2O$ was added this was done by adding the salt dissolved in sufficient water to raise the soil moisture content to 50% mwhc. The soil sample (usually 10g) was spread on a petri dish, the copper solution was added, and the contents allowed to air dry. The contents were then

rewetted and again allowed to air dry. This was repeated three more times, the whole process taking 10-15 days. This allowed the copper compounds to react evenly with the soil.

$CuHPO_4$ and CuO at appropriate levels of copper were added in finely ground form to the air dried soil in bulk and thoroughly mixed in. Suitable amount (usually 10g) was then weighed out for incubation study.

(b) Where incubation was done after allowing the copper compounds to react with the soil for sometime:- 500g portions of air dry soil were mixed with CuO or $CuHPO_4$ at levels of 100, 1000 and 10,000 ppm Cu. The mixtures were placed in pots, wetted to 50% mwhc and allowed to stand for three months at laboratory temperature with addition of water when necessary to maintain the soil in a moist condition. In one series using $CuHPO_4$ suitable amounts of $CaHPO_4$ were also added in order to equalise the PO_4 added due to addition of varying levels of $CuHPO_4$. After the soils were held for three months at room temperature they were air dried and ground to pass through 2mm sieve.

C. ANALYTICAL METHODS USED

Determination of maximum water holding capacity of soil.

10g of air dried 2mm sieved soil was weighed into a weighed porcelain crucible with porous bottom. The crucible was then placed on a shallow dish and water was added so that

the level of water in the dish was at the height of the bottom of the crucible. After being kept overnight, the crucible was removed, the outside was dried, and the crucible with contents was then weighed. A blank was done using an empty crucible to allow for water absorbed by the porous bottom. The value obtained (ml of water per 10g soil) represents 100% maximum water holding capacity (mwhc). This value is equal to a pF of approximately zero (32).

Determination of pH of soils and organic materials.

The pH of soils and organic materials was determined by shaking one part by weight of solid material with one volume of water (usually 10g of material + 10 ml of water). A Pye model 79 pH meter was used.

Determination of total nitrogen in soils and organic materials.

The Kjeldahl digestion method using conc. H_2SO_4 and K_2SO_4 with addition of copper sulphate as catalyst was used. The reaction mixture was transfered to a distillation apparatus and the ammonia was distilled, after the addition of excess of alkali, into 2% (w/v) boric acid which was then titrated against standard H_2SO_4 (171).

Determination of cation exchange capacity of soil.

This was done by an ammonium acetate method (172).

Determination of organic carbon in soils and organic materials.

This was done by a wet combustion method (173).

Mechanical analysis of soil.

This was done by a pipette method (171).

Method of incubation of soil.

10g portions of air dried 2mm sieved soil (mixed beforehand with appropriate levels of organic materials and/or copper compounds) were weighed into 4"x1" diam. flat-bottomed tubes (incubation tubes). Depending on what moisture content was required during incubation sufficient water was added from a burette. Where sucrose was added a solution of appropriate concentration was made and this was used to bring the soil to the required moisture content.

For supplying oxygen and absorbing CO_2 during incubation a modified BaO_2 method (174) was used. This involved placing 0.2g of BaO_2 in a glass vial (1.5"x0.5" diam.) and adding 0.1 ml of saturated $Ba(OH)_2$ solution. This mixture was spread over the inside walls by rotating the vial. Sufficient of these vials were prepared for each experiment, depending on the number of incubation tubes used, and until ready for use the vials were closed with polythene stoppers. Before putting for incubation a BaO_2 vial was placed on the surface of the soil in each incubation tube, and the tube was closed with a

rubber bung. The vials were changed periodically depending on the soil treatment ; where organic materials were added the vials were changed after incubation periods ranging from 2-7 days, but in untreated soils the vials were changed after periods ranging from 2-4 weeks.

In those experiments where release of CO_2 was measured, the CO_2 content of the vial was determined by reaction with 2N HCl in a calcimeter (175). The calcimeter was calibrated using pure $BaCO_3$.

All treatments, including the controls, in incubation experiments were done in duplicate.

Methods of treating soils for determination of pH and extraction of mineral nitrogen and metallic elements.

A technique was developed based on the use of successive extraction with reagents so that pH, mineral nitrogen (ammonia and nitrate) and metallic elements could be determined. In general, this involved firstly the addition of 10 ml of water followed by, shaking and pH measurement. This was followed by addition of 10 ml of 1N NaOAc (pH 7.0), followed by shaking for 2 minutes. The tube was then centrifuged for about 5 minutes and the supernatent liquid was then poured into a filter paper or a plug of cotton wool held in a filter funnel. This treatment extracted nitrate, water soluble and exchangeable ammonium, and exchangeable cations (including water

soluble and exchangeable metallic elements). The soil residue left in the tube was then treated with 20 ml of Morgan's reagent (0.5N acetic acid - 0.75N sodium acetate), the tube shaken for 2 minutes, centrifuged and the filtrate was obtained as already described. The Morgan's reagent was used since this is a well known extract for determining "active" or "available" nutrients in soils (176,177). The results obtained for nutrients extracted by sodium acetate reagent were added to those obtained using Morgan's reagent to give the true Morgan-extractable nutrients. The process was repeated after addition of 20 ml of 0.1N EDTA-Na (pH 4.0). The EDTA reagent extracted the chelated form of elements from the soil. Pizer et al (178) used EDTA-NH_4, but Premi (103) showed that sodium form of the reagent gave results very similar to the ammonium form.

In some experiment only Morgan's reagent followed by EDTA was used and in others only Morgan's reagent or EDTA was used after determination of pH as described above. It was shown that both Morgan's reagent and EDTA were as effective as sodium acetate in extracting nitrate and exchangeable ammonium from soils.

Determination of ammonium and nitrate nitrogen in soil extracts.

Suitable aliquots of the sodium acetate, Morgan or EDTA

extracts (depending on which was the first extract used, since it was shown that all the ammonium and nitrate was removed in the first extract) were analysed for ammonia and nitrate using the microdiffusion method of Bremner and Shaw (179). The determination of ammonium + nitrate was modified by Premi and Cornfield (180) using 0.5M $FeSO_4.7H_2O$ in 1N H_2SO_4 instead of titanous sulphate reagent recommended by Bremner and Shaw.

Determination of metallic elements in soil extracts.

Copper was determined in all extracts in all experiments. Zn, Fe and Mn were determined in all extracts in some of the experiments. All these elements were determined by atomic absorption spectroscopy using the Unicam SP 90 Atomic Absorption. This involved direct aspiration of the extracts into the instrument preceded, where necessary, by dilution by a known amount of solvent or water.

Determination of total metallic elements in soils and organic materials.

The total metallic elements in soils and organic materials were determined by extraction with boiling 6N HCl followed by atomic absorption spectroscopic determination of the elements in the filtrates. Premi and Cornfield (181) studied this proceedure and showed that it gave satisfactory results for total metallic element contents in plant and

organic materials, including organic manures.

For the determination of total metallic elements in soils the boiling 6N HCl proceedure was also used. Although it is known that this method may not extract metallic elements completely from certain resistant soil minerals, it was considered to be satisfactory for characterising the metallic element contents. The A.E.A proceedure (182) which has long been used for determining potentially available major elements in soils, is in fact very similar to the boiling 6N HCl proceedure used here.

CHAPTER III

INCUBATION STUDY OF THE EFFECTS OF ADDITION OF COPPER AS SULPHATE ON NITROGEN MINERALISATION, NITRIFICATION AND EXTRACTABLE TRACE ELEMENTS IN RELATION TO SOIL pH.

Experiment 1.

Effects of addition of varying levels of copper (as sulphate) on nitrogen mineralisation, nitrification and extractable trace elements in Silwood soils adjusted to different pH and without and with addition of ammonium sulphate (100 ppm N).

Experiment 2.

Effects of addition of varying levels of copper (as sulphate) on nitrogen mineralisation, nitrification and extractable trace elements in Harlington soils adjusted to different pH and without and with addition of ammonium sulphate (100 ppm N).

Experiment 3.

Effects of addition of varying levels of copper (as sulphate) on nitrogen mineralisation, nitrification and extractable trace elements of six soils of naturally different pH.

CHAPTER III

INCUBATION STUDY OF THE EFFECTS OF ADDITION OF COPPER AS SULPHATE ON NITROGEN MINERALISATION, NITRIFICATION AND EXTRACTABLE TRACE ELEMENTS IN RELATION TO SOIL pH.

INTRODUCTION

This chapter will report studies on the effects of adding different levels of Cu on the nitrogen transformations in several types of soil, particularly in relation to soil pH. In the first two experiments Silwood soil (sand) and Harlington soil (sandy loam) which had been adjusted to different pH levels, as described in Chapter II, were set up for incubation with varying levels of added Cu as $CuSO_4.5H_2O$ and without and with addition of 100 ppm N as $(NH_4)_2SO_4$ before incubation. In the third experiment six different soils varying naturally in pH over a wide range were studied without addition of N.

Ammonia and nitrate values were determined initially (zero incubation) and after 3 weeks of incubation at 33% mwhc and 30°C. Trace elements were determined after 3 weeks of incubation by successive extraction with 0.5N NaOAc (pH 7.0), Morgan's reagent (pH 4.8) and 0.1N EDTA-Na (pH 4.0).

Experiment 1

Effects of addition of varying levels of copper (as sulphate) on nitrogen mineralisation, nitrification and extractable trace elements in Silwood soils adjusted to different pH and without and with addition of ammonium sulphate (100 ppm N).

METHODS

Silwood soils which had been adjusted to pH levels of 4.8, 6.0 and 7.4 were used. Copper was added as $CuSO_4.5H_2O$ at 0 (control), 100 and 1000 ppm on the dry soil basis to 10g portions of soil. The method used was to apply $CuSO_4.5H_2O$ in solution to the soil samples spread on the petri dishes followed by alternate wetting and drying four times over 10-15 days as described in Chapter II. The dry samples were then transfered to incubation tubes. Water, containing sufficient dissolved $(NH_4)_2SO_4$ to supply 100 ppm N on the soil basis, was added to bring soil moisture to 33% mwhc. Half tubes were thus treated (N-treated) and half treated with water only.

The tubes were incubated for 3 weeks at 30^oC using the BaO_2 method for absorbing CO_2 and supplying oxygen. After incubation soil pH was determined followed by successive extraction with 0.5N NaOAc, Morgan's reagent (pH 4.8) and 0.1N EDTA-Na (pH 4.0) as described in Chapter II. Ammonia and

nitrate nitrogen were determined in the NaOAc extract and Cu, Zn, Fe and Mn were determined in this extract and also in the Morgan and EDTA extracts, using the methods already described.

Abbreviations used in the text.

In order to save space the following abbreviations will be used in the text:-

NaOAc-Cu means the amount of Cu extracted by 0.5N NaOAc (pH 7.0) and expressed on the dry soil basis. Similarly Morgan-Cu means the amount of Cu extracted by Morgan's reagent (pH 4.8), and EDTA-Cu that extracted by 0.1N EDTA-Na (pH 4.0). This also applies to other trace elements.

RESULTS

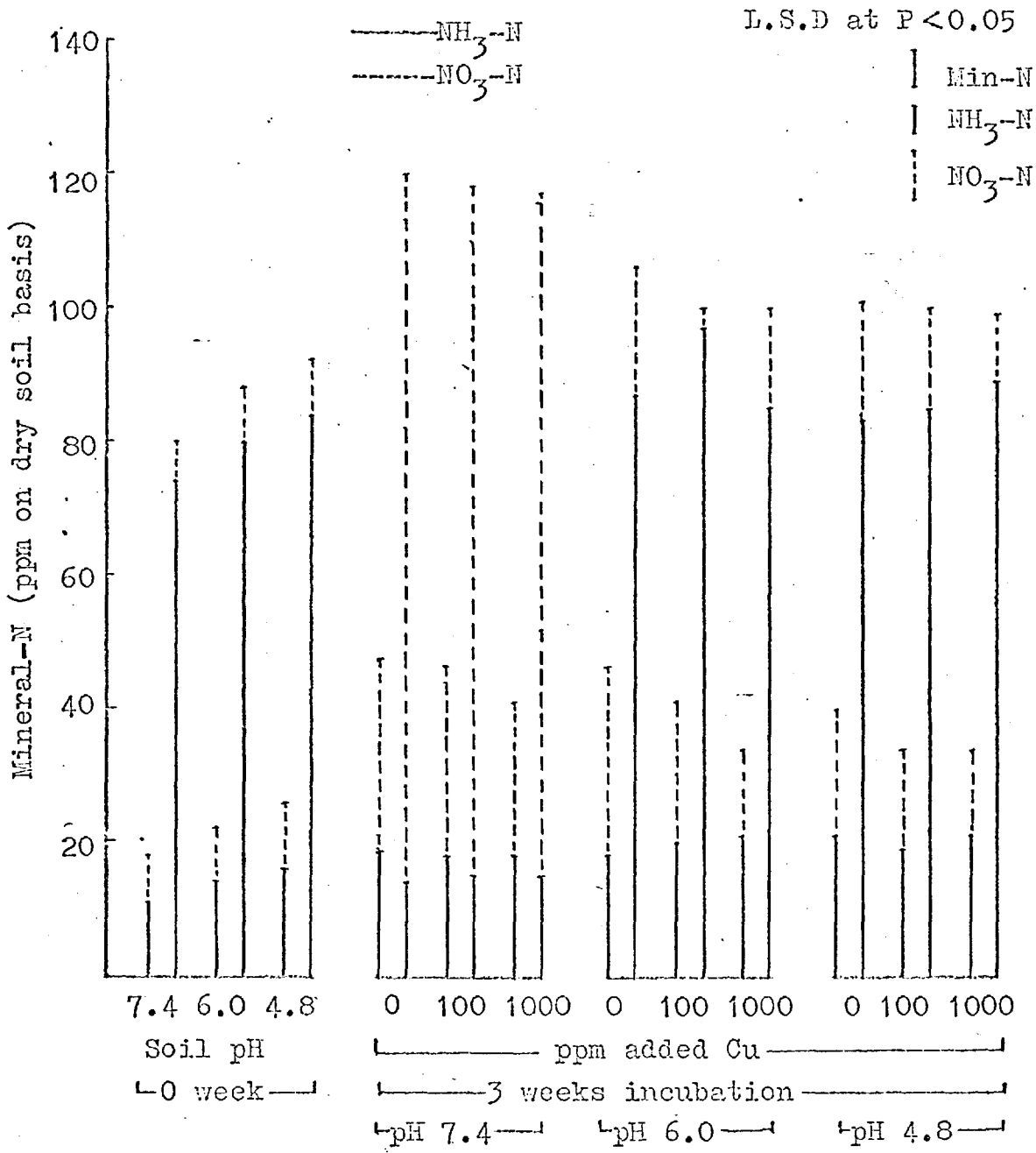
Mineral nitrogen levels.

The results for mineral-N levels initially (zero incubation) and after 3 weeks of incubation are shown in Fig. 2 and Table 1. The total line indicates mineral-N level, the continuous line ammonia-N, and the dashed line nitrate-N. The differences required for significance (L.S.D) at $P < 0.05$ are also shown on the figure and in the table for each form of mineral-N.

In the soil of pH 7.4 virtually all the added ammonia-N was converted to nitrate-N after 3 weeks incubation. There

Fig:- 2

Levels of ammonia- and nitrate-N initially (zero incubation) and after 3 weeks of incubation of Silwood soils (pH 7.4, 6.0 and 4.8) treated with 0, 100 and 1000 ppm Cu (as sulphate) and without and with added ammonium sulphate (100 ppm N).



For each pair the left-hand value represents no added N and the right-hand value addition of 100 ppm N.

Table 1

Levels of ammonia- and nitrate-N initially (zero incubation) and after 3 weeks incubation of Silwood soils (pH 7.4, 6.0 and 4.8) treated with 0, 100 and 1000 ppm Cu (as sulphate) and without and with added ammonium sulphate (100 ppm N).

(Results are given in ppm on dry soil basis)

Initial values

Soil of pH	NO_3-N		NH_3-N		Min-N	
	Nil N	Added N	Nil N	Added N	Nil N	Added N
7.4	7	6	11	74	18	80
6.0	8	8	14	80	22	88
4.8	10	8	16	84	26	92

After 3 weeks incubation

Soil of pH	Added Cu	NO_3-N		NH_3-N		Min-N	
		Nil N	Added N	Nil N	Added N	Nil N	Added N
7.4							
	0	28	106	19	14	47	120
	100	28	103	18	15	46	118
	1000	23	102	18	15	41	117
6.0							
	0	28	19	18	87	46	106
	100	21	3	20	97	41	100
	1000	13	15	21	85	34	100
4.8							
	0	19	17	21	84	40	101
	100	15	15	19	85	34	100
	1000	13	10	21	89	34	99

L.S.D at $P < 0.05$ for

Min-N = 5.2, NH_3-N = 3.8 and NO_3-N = 6.9

were no significant differences in mineral-N levels after incubation due to addition of 100 or 1000 ppm Cu, although there was trend for these values to decrease with increasing levels of Cu. Where no N was added nitrate-N accumulation was not significantly affected by 100 or 1000 ppm Cu.

In the soil of pH 6.0 mineral-N accumulation was less than that in the soil of pH 7.4. Where no N was added both levels of Cu significantly decreased mineral-N accumulation and nitrate-N accumulation decreased significantly with increasing levels of Cu. Where N was added Nitrate-N accumulation was decreased significantly by 100 ppm Cu, but not by 1000 ppm Cu.

In the soil of pH 4.8 there was even less accumulation of mineral-N than in the soils of higher pH. Where no N was added mineral-N accumulation was decreased significantly to the same extent by both levels of Cu, but where N was added Cu levels had no effect on mineral-N accumulation. Where no N was added there was a small but significant accumulation of nitrate-N in the absence of Cu, but no accumulation with either level of added Cu. Where N was added significant amounts of nitrate-N accumulated where 0 or 100 ppm Cu, but not where 1000 ppm Cu, was added.

Trace element levels.

Extractable trace elements after 3 weeks incubation are

shown in Tables 2a, 2b and 2c for soils of initial pH 7.4, 6.0 and 4.8 respectively. Soil pH and L.S.D values after incubation are also shown in the tables. Because of the widely different levels of extractable Cu which sometimes occurred due to Cu addition, L.S.D values were calculated separately for 0, 100 and 1000 ppm Cu-treated soils. For Zn, Fe and Mn, L.S.D values were calculated by combining the data from all soils. The Morgan-extractable values shown in the tables represent the levels obtained in the Morgan extract plus those obtained in the NaOAc extract, in other words these would be the levels obtained if the Morgan reagent had been used without the previous extraction with NaOAc. Similarly EDTA levels of trace elements indicated are those obtained by adding NaOAc, Morgan and EDTA extractable values.

Very small amounts of Cu were extracted by NaOAc from soil of pH 7.4 irrespective of the level of Cu addition. Fair amounts were extracted from soils of pH 6.0 and 4.8, but then only where 1000 ppm Cu had been added. Morgan extractable Cu (Morgan-Cu) was increased slightly by 100 ppm Cu, and to considerable extent by 1000 ppm Cu at all pH levels ; the extent of the increase due to the Cu treatment increased with increasing soil pH. EDTA-Cu increased to an even greater extent with levels of applied Cu, and there were no great differences due to soil pH.

Table 2a

Extractable trace elements and soil pH after 3 weeks of incubation of Silwood soil (pH 7.4) treated with 0, 100 and 1000 ppm Cu (as sulphate) and without and with added ammonium sulphate (100 ppm N).

(Results are given in ppm on dry soil basis)

Treatments Cu	Treatments N	Extract used	Cu	Zn	Fe	Mn	Soil pH
0	0	0.5N-NaOAc	0	0.4	2.1	0.2	7.5
100	0		0	0.4	2.0	0.2	7.5
1000	0		1.3	0.5	1.4	0.8	7.2
0	100		0	0.4	1.9	0.2	7.3
100	100		0	0.5	2.3	0.4	7.4
1000	100		3.2	1.9	2.4	4.0	6.9
0	0	Morgan reagent	0	3.7	6.0	1.9	
100	0		3.0	5.1	6.2	2.5	
1000	0		161	6.7	5.7	5.1	
0	100		0	3.8	6.2	2.3	
100	100		3.0	5.9	6.7	3.4	
1000	100		161	23	7.2	14	
0	0	0.1N-EDTA-Na	3.1	29	116	32	
100	0		49	23	110	29	
1000	0		624	25	119	31	
0	100		3.0	22	120	32	
100	100		50	28	119	33	
1000	100		638	50	122	37	

L.S.D at $P < 0.05$ for Cu at 0 = 0.14, at 100 = 1.05 at 1000 = 7.6 ; Zn = 3.3 ; Fe = 4.5 ; Mn = 2.2

Table 2b

Extractable trace elements and soil pH after 3 weeks of incubation of Silwood soil (pH 6.0) treated with 0, 100 and 1000 ppm Cu (as sulphate) and without and with added ammonium sulphate (100 ppm N).

(Results are given in ppm on dry soil basis)

Treatments Cu	Treatments N	Extract used	Cu	Zn	Fe	Mn	Soil pH
0	0	0.5N-NaOAc	0	1.9	1.7	0.4	5.9
100	0		0.7	2.3	1.7	0.7	5.8
1000	0		88	5.7	1.6	32	5.1
0	100		0	2.1	2.2	0.5	5.8
100	100		0.7	2.7	2.3	1.0	5.8
1000	100		90	6.7	1.9	32	5.1
0	0	Morgan reagent	0	5.8	3.7	1.1	
100	0		4.1	8.0	3.7	1.7	
1000	0		243	12	4.2	49	
0	100		0	5.6	5.0	1.4	
100	100		3.6	7.7	4.7	2.4	
1000	100		241	14	4.3	49	
0	0	0.1N-EDTA-Na	3.1	20	105	5.3	
100	0		54	24	105	5.8	
1000	0		708	23	113	51	
0	100		3.2	25	115	5.9	
100	100		52	26	110	6.8	
1000	100		701	25	106	51	

L.S.D at $P < 0.05$ for Cu at 0 = 0.14, at 100 = 1.05 at 1000 = 7.6 ; Zn = 3.3 ; Fe = 4.5 ; Mn = 2.2

Table 2c

Extractable trace elements and soil pH after 3 weeks of incubation of Silwood soil (pH 4.8) treated with 0, 100 and 1000 ppm Cu (as sulphate) and without and with added ammonium sulphate (100 ppm N).

(Results are given in ppm on dry soil basis)

Treatments Cu	Treatments N	Extract used	Cu	Zn	Fe	Mn	Soil pH
0	0	0.5N-NaOAc	0	1.0	2.2	8.5	4.9
100	0		0	1.4	2.1	13	4.7
1000	0		93	5.5	1.2	26	4.4
0	100		0	1.3	2.2	11	4.8
100	100		0	2.1	2.2	13	4.7
1000	100		93	5.5	1.2	26	4.1
0	0	Morgan reagent	0	5.0	7.0	17	
100	0		6.5	6.5	6.9	22	
1000	0		309	13	8.8	33	
0	100		0	6.0	6.8	18	
100	100		6.0	7.4	7.0	21	
1000	100		311	14	8.6	36	
0	0	0.1N-EDTA-Na	2.9	12	107	77	
100	0		55	13	107	29	
1000	0		699	18	123	37	
0	100		2.9	13	101	27	
100	100		54	15	101	28	
1000	100		701	21	123	40	

L.S.D at $P < 0.05$ for Cu at 0 = 0.14, at 100 = 1.05 at 1000 = 7.6 ; Zn = 3.3 ; Fe = 4.5 ; Mn = 2.2

NaOAc extractable Zn was not affected by level of added Cu either in the presence and/or absence of applied N in the soil of pH 7.4 and in soils of pH 6.0 and 4.8 it was increased significantly only where 1000 ppm Cu was added. The presence of added N has little effect. At pH 7.4 Morgan-Zn was increased only by 1000 ppm Cu but only where N was also applied, whilst at pH 6.0 and 4.8 it was increased whether or not N was applied. EDTA-Zn was little affected by Cu or N treatments except for increases where 1000 ppm Cu was added with N in soils of pH 7.4 and 4.8.

The levels of Fe extracted by NaOAc, Morgan reagent or EDTA were little affected by either Cu or N treatments in all soils. However, EDTA-Fe was considerably higher than Fe extracted by the other two reagents.

In the soil of pH 7.4 NaOAc extractable Mn was increased by 1000 ppm Cu, but only where N was also added, but the increase, although significant, was of low magnitude. In the two soils of lower pH NaOAc-Mn was increased to a fair extent by 1000 ppm Cu both with and without addition of N. Morgan-Mn responsed to treatments as did NaOAc-Mn, except that the extent of increases was somewhat greater with the former extract.

The soil pH after incubation was little affected due to addition of 100 ppm Cu, but was decreased by 0.3-0.8 units

by addition of 1000 ppm Cu. However, comparison of pH values with and without N addition shows that part of this decrease was due to the application of $(NH_4)_2SO_4$.

Experiment 2

Effects of addition of varying levels of copper (as sulphate) on nitrogen mineralisation, nitrification and extractable trace elements in Harlington soils adjusted to different pH and without and with addition of ammonium sulphate (100 ppm N).

METHODS

Harlington soils which had been adjusted to pH levels of 5.1, 5.9 and 7.3 were used. The experimental proceedures were exactly the same as those described in experiment 1 for Silwood soils.

RESULTS

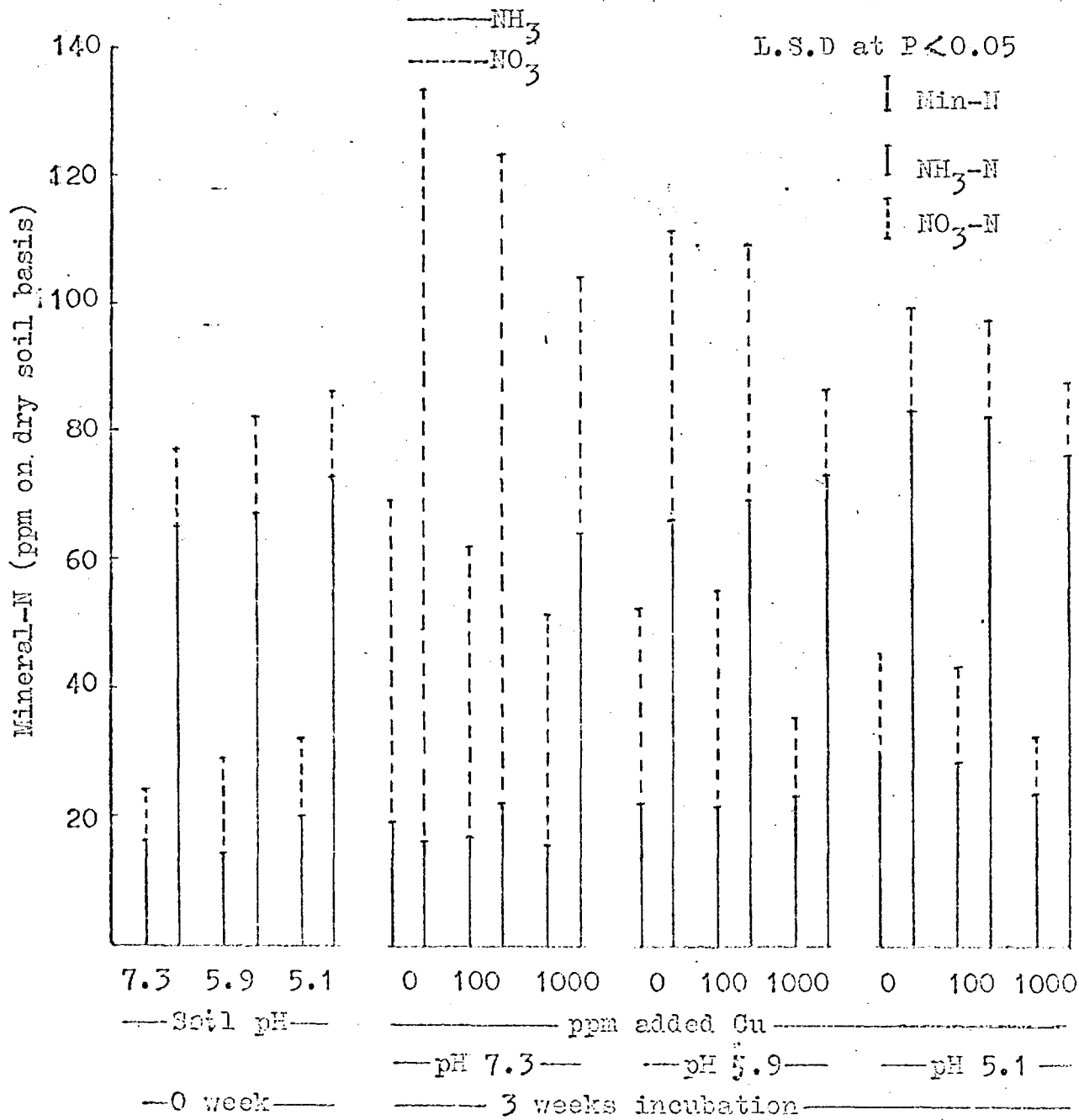
Mineral nitrogen levels.

Figure 3 and Table 3 present the results for mineral-N levels initially (zero incubation) and after 3 weeks of incubation, and also show the L.S.D values at $P < 0.05$ for each form of mineral-N.

In the soil of pH 7.3 mineral-N accumulation decreased

Fig:- 3

Levels of ammonia- and nitrate-N initially (zero incubation) and after 3 weeks of incubation of Harlington soils (pH 7.3, 5.9 and 5.1) treated with 0, 100 and 1000 ppm Cu (as sulphate) and without and with added ammonium sulphate (100 ppm N).



For each pair the left-hand value represents no added N and the right-hand value addition of 100 ppm N.

Table 3

Levels of ammonia- and nitrate-N initially (zero incubation) and after 3 weeks incubation of Harlington soils (pH 7.3, 5.9 and 5.1) treated with 0, 100 and 1000 ppm Cu (as sulphate) and without and with added ammonium sulphate (100 ppm N).

(Results are given in ppm on dry soil basis)

Initial values

Soil of pH	Added Cu	NO_3-N		NH_3-N		Min-N	
		Nil N	Added N	Nil N	Added N	Nil N	Added N
7.3		8	12	16	65	24	77
5.9		15	15	14	67	29	82
5.1		12	13	20	73	32	86

After 3 weeks incubation

7.3							
	0	50	117	19	16	69	133
	100	45	101	17	22	62	123
	1000	36	40	16	64	52	104
5.9							
	0	30	45	22	66	52	111
	100	34	40	21	69	55	109
	1000	12	13	23	73	35	86
5.1							
	0	15	16	31	83	46	99
	100	15	15	28	82	43	97
	1000	9	11	23	76	32	87

L.S.D at $P < 0.05$ for

Min-N = 5.0, NH_3-N = 4.3 and NO_3-N = 6.0

significantly with increasing levels of Cu addition both in the absence and presence of added N. The 1000 ppm Cu level decreased nitrogen mineralisation by 42% where no N was added and by 52% where N was added. Where no N was added nitrate-N accumulation was not affected by 100 ppm Cu but significantly decreased by 1000 ppm Cu.

In the soil of pH 5.9 where no N was added mineral-N accumulation was not affected by 100 ppm Cu, but was completely inhibited by 1000 ppm Cu. Where N was added mineral-N accumulation was significantly decreased by 100 ppm Cu, and completely inhibited by 1000 ppm Cu. Where no N was added nitrate-N accumulation was unaffected by 100 ppm Cu but was completely inhibited by 1000 ppm Cu, and where N was added nitrate-N accumulation was decreased by 100 ppm Cu and completely inhibited by 1000 ppm Cu.

In the soil of pH 5.1 mineral-N accumulation was not affected by 100 ppm Cu but was completely inhibited by 1000 ppm Cu whether or not N was added. There were no significant changes in nitrate-N accumulation due to incubation irrespective of Cu and/or N additions.

Trace element levels.

Extractable trace elements after 3 weeks of incubation are shown in Tables 4a, 4b and 4c for soils of initial pH values 7.3, 5.9 and 5.1 respectively. The L.S.D values for

Table 4a

Extractable trace elements and soil pH after 3 weeks of incubation of Harlington soil (pH 7.3) treated with 0, 100 and 1000 ppm Cu (as sulphate) and without and with added ammonium sulphate (100 ppm N).

(Results are given in ppm on dry soil basis)

Treatments Cu	Treatments N	Extract used	Cu	Zn	Fe	Mn	Soil pH
0	0	0.5N-NaOAc	0	1.2	1.0	0.2	7.3
100	0		0	0.7	1.0	0.2	7.2
1000	0		3.6	2.3	1.0	0.2	6.6
0	100		0	1.4	1.0	0.2	6.8
100	100		0	1.3	1.0	0.2	6.7
1000	100		4.3	2.2	2.0	0.2	6.5
0	0	Morgan reagent	0	16	3.4	1.7	
100	0		4.8	15	3.2	1.9	
1000	0		209	25	3.3	2.4	
0	100		0	15	3.2	2.1	
100	100		4.3	20	3.1	2.7	
1000	100		204	26	4.4	2.5	
0	0	0.1N-EDTA-Na	28	72	273	112	
100	0		140	65	253	102	
1000	0		749	74	263	97	
0	100		28	66	263	102	
100	100		144	76	263	103	
1000	100		729	83	264	93	

L.S.D at $P<0.05$ for Cu at 0 = 1.5, at 100 = 4.1 at 1000 = 16.5 ; Zn = 6.8 ; Fe = 10.6 ; Mn = 3.6

Table 4b

Extractable trace elements and soil pH after 3 weeks of incubation of Harlington soil (pH 5.9) treated with 0, 100 and 1000 ppm Cu (as sulphate) and without and with added ammonium sulphate (100 ppm N).

(Results are given in ppm on dry soil basis)

Treatments Cu	Treatments N	Extract used	Cu	Zn	Fe	Mn	Soil pH
0	0	0.5N-NaOAc	0	3.0	0.8	0.3	5.9
100	0		0	5.5	0.8	0.3	5.8
1000	0		2.5	11	0.8	51	5.6
0	100		0	3.1	0.8	0.3	5.9
100	100		0	4.6	0.8	0.3	5.9
1000	100		2.5	10	0.8	52	5.7
0	0	Morgan reagent	0	16	2.5	0.9	
100	0		3.3	20	2.6	0.8	
1000	0		208	35	2.5	92	
0	100		0	18	2.4	0.9	
100	100		3.3	18	2.6	0.9	
1000	100		208	35	2.6	93	
0	0	0.1N-EDTA-Na	26	70	214	136	
100	0		72	82	202	121	
1000	0		718	92	213	156	
0	100		28	73	213	131	
100	100		72	70	207	121	
1000	100		712	89	219	157	

L.S.D at $P < 0.05$ for Cu at 0 = 1.5, at 100 = 4.1 at 1000 = 16.5 ; Zn = 6.8 ; Fe = 10.6 ; Mn = 3.6

Table 4c

Extractable trace elements and soil pH after 3 weeks of incubation of Harlington soil (pH 5.1) treated with 0, 100 and 1000 ppm Cu (as sulphate) and without and with added ammonium sulphate (100 ppm N).

(Results are given in ppm on dry soil basis)

Treatments Cu	Treatments N	Extract used	Cu	Zn	Fe	Mn	Soil pH
0	0	0.5N-NaOAc	0	6.8	3.2	33	5.4
100	0		0.8	13	4.5	39	5.3
1000	0		60	21	2.6	61	4.9
0	100		0	6.6	3.1	31	5.3
100	100		0.8	7.0	3.7	37	5.3
1000	100		58	20	3.2	60	4.9
0	0	Morgan reagent	0.9	25	11	54	
100	0		9.5	33	14	67	
1000	0		297	48	16	98	
0	100		1.0	30	11	53	
100	100		9.4	29	15	63	
1000	100		290	47	17	98	
0	0	0.1N-EDTA-Na	34	65	551	106	
100	0		140	79	614	121	
1000	0		907	82	488	131	
0	100		36	79	590	112	
100	100		154	71	545	112	
1000	100		900	79	562	130	

L.S.D at $P < 0.05$ for Cu at 0 = 1.5, at 100 = 4.1 at 1000 = 16.5 ; Zn = 6.8 ; Fe = 10.6 ; Mn = 3.6

trace elements and soil pH after incubation are also shown in these tables.

No or non-significant amounts of Cu were extracted by NaOAc from control or 100 ppm Cu-treated soils. Where 1000 ppm Cu was added negligible amounts of Cu were extracted from soils of pH 7.3 and 5.9, but fair amounts from soil of pH 5.1. Morgan-Cu was little affected by 100 ppm Cu addition but was increased considerably by 1000 ppm Cu in soils of all pH levels. The extent of increases were similar for all soils. Where 100 ppm Cu was added EDTA-Cu was greater in soils of pH 7.3 and 5.1 than in the soil of pH 5.9. In general, EDTA-Cu increased considerably with levels of addition of Cu at all pH levels. With 100 ppm added Cu EDTA extracted virtually all the native and added Cu ; with 1000 ppm added Cu 69-72% of the total Cu was extracted from soils of pH 7.3 and 5.9, and 89-90% of the total Cu from the soil of pH 5.1. The addition of N before incubation had little effect on the levels of Cu extracted by any of the reagents.

NaOAc-Zn was not affected by any Cu level in the soil of pH 7.3 and was significantly increased, but only by 1000 ppm Cu in the soils of lower pH. Morgan-Zn increased significantly with decreasing soil pH irrespective of added Cu level, and only 1000 ppm Cu increased Morgan-Zn significantly. EDTA-Zn was high in all soils irrespective of pH or N treatments. Cu

addition sometime increased and sometime decreased EDTA-Zn, and although these effects were sometime significant, they were not consistently related to levels of Cu addition. The addition of N before incubation had little effect on levels of Zn extracted by any of the reagents.

For each of the three extracts in the soil of each pH level Cu and/or N additions had little effect on EDTA-Fe. However, soil pH influenced EDTA-Fe level, in that values were higher in soil of pH 5.1 for each type of extract than in the soils of pH 5.9 or 7.3.

NaOAc-Mn was very low with all treatments in soil of pH 7.3 ; in soil of pH 5.9, 100 ppm Cu had no effect on but 1000 ppm Cu increased NaOAc-Mn to considerable extent. In the soil of pH 5.1 NaOAc-Mn was high even in the control soil, but was significantly increased by 1000 ppm Cu. The effects of Cu and N treatments on Morgan-Mn levels were similar to those on NaOAc-Mn levels for soils of each pH level, except that the magnitude of the effects was somewhat greater with Morgan reagent. EDTA-Mn levels were not much affected by Cu and/or N treatments and the magnitude of the effects, though significant, were of low order. There was a general trend for EDTA-Mn to decrease with increasing Cu addition in soil of pH 7.3, but to have the reverse effects in the two soils of lower pH.

Soil pH decreased with increasing level of Cu. The maximum decrease in pH was 0.7 units and the minimum 0.2 units. The N treatment decreased soil pH by amounts ranging from 0-0.5 units.

Experiment 3

Effects of addition of varying levels of copper (as sulphate) on nitrogen mineralisation, nitrification and extractable trace elements of six soils of naturally different pH.

In this experiment 6 soils of loam texture covering a wide range of pH were used. These soils had been selected from a large number of loamy soils obtained from various parts of southern England. In this experiment the effects of Cu was studied only on mineralisation and nitrification of native nitrogen.

METHODS

The methods used were as described in the previous experiments except that no N treatment was given in this experiment.

RESULTS

Mineral nitrogen levels.

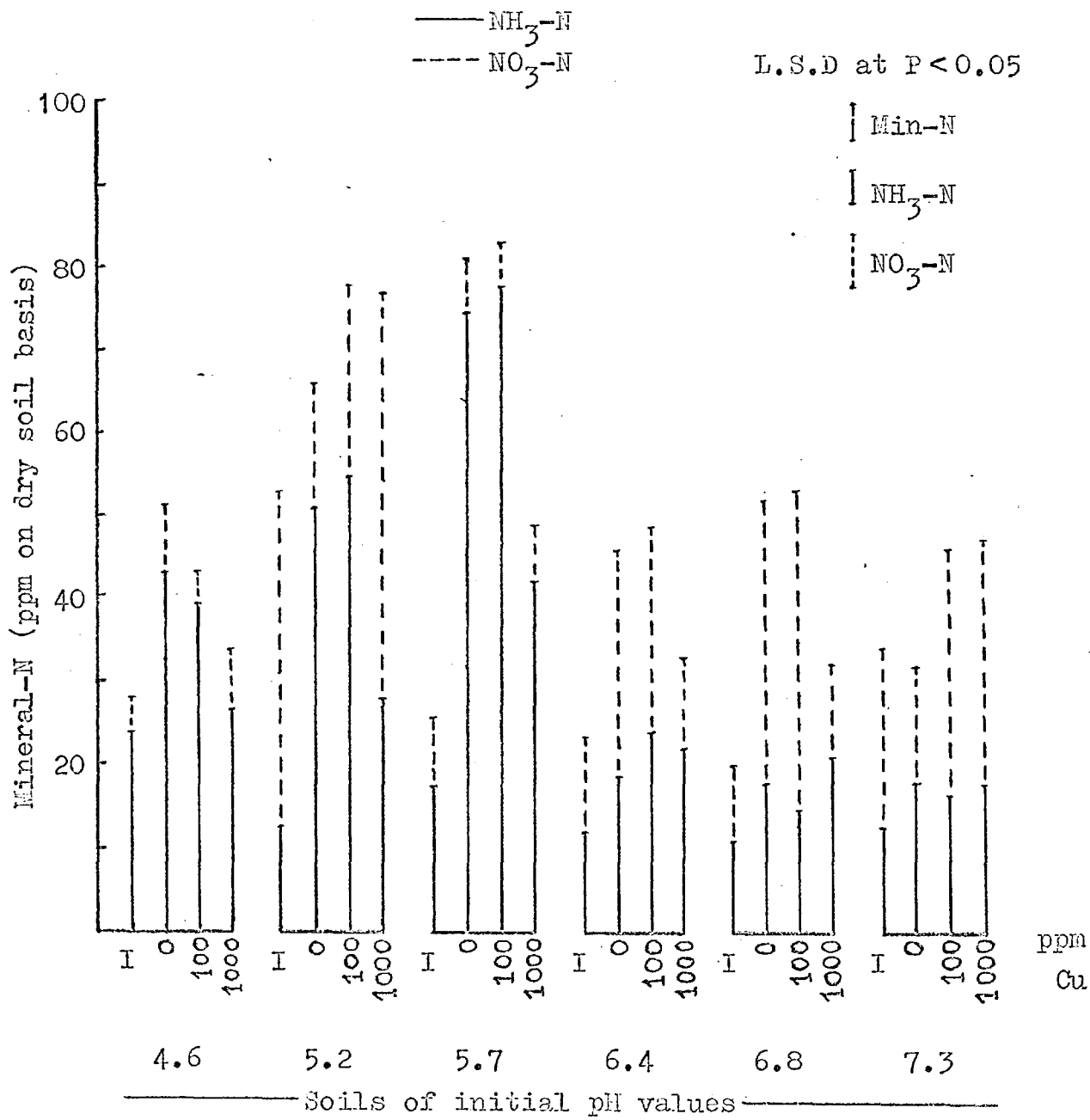
Figure 4 and Table 5 present results for mineral-N levels initially and after 3 weeks incubation. The L.S.D values at $P < 0.05$ are also presented.

The pattern of mineral-N and nitrate-N accumulation due to incubation varied considerably depending on the soil pH. The soils of pH 5.2 and 7.3 were the only two soils to show the significant increases in mineral-N accumulation due to addition of both levels of Cu, with no differences between Cu levels. The soil of pH 4.6 showed a significant decrease in mineral-N accumulation with increasing level of Cu. In the soils of pH 5.7, 6.4 and 6.8 100 ppm Cu had no effect on mineral-N accumulation, whilst 1000 ppm Cu depressed it.

The initial nitrate-N values in soils of pH 4.6 and 5.7 were low and nitrate-N accumulation was not significantly affected by either level of Cu in these soils. In the soil of pH 5.2 nitrate-N decreased with incubation in the control soil, but the extent of this decrease was less where 100 ppm Cu was added ; where 1000 ppm Cu was added there was a significant small increase in nitrate-N accumulation. The soils of pH 6.4 and 6.8 behaved similarly in that 100 ppm Cu had no effect on nitrate-N accumulation during incubation, whilst 1000 ppm Cu decreased nitrate-N accumulation. The soil of

Fig:- 4

Levels of ammonia- and nitrate-N initially (zero week incubation "I") and after 3 weeks of incubation of 6 different soils (pH 4.6, 5.2, 5.7, 6.4, 6.8 and 7.3) treated with 0, 100 and 1000 ppm Cu as sulphate.



For each soil "I" represents initial (zero week) and the rest values after 3 weeks of incubation.

Table 5

Levels of ammonia- and nitrate-N initially (zero incubation) and after 3 weeks incubation of 6 different soils (pH 4.6, 5.2, 5.7, 6.4, 6.8 and 7.3) treated with 0, 100 and 1000 ppm Cu (as sulphate).

(Results are given in ppm on dry soil basis)

Initial values

Soil pH	Added Cu	NO_3-N	NH_3-N	Min-N	Soil pH	Added Cu	NO_3-N	NH_3-N	Min-N
4.6		4	24	28	5.2		40	13	53
5.7		8	18	26	6.4		11	12	23
6.8		9	11	20	7.3		21	13	34

After 3 weeks incubation

4.6					5.2				
	0	8	43	51		0	15	51	66
	100	4	39	43		100	23	55	78
	1000	7	27	34		1000	49	28	77
5.7					6.4				
	0	6	75	81		0	27	19	46
	100	5	78	83		100	25	24	49
	1000	7	42	49		1000	11	22	33
6.8					7.3				
	0	34	18	52		0	14	18	32
	100	38	15	53		100	29	17	46
	1000	11	21	32		1000	29	18	47

L.S.D at $P < 0.05$ for

Min-N = 3.7, NH_3-N = 4.2 and NO_3-N = 6.2

pH 7.3 was the only one to show increased accumulation of nitrate with addition of both levels of Cu, with no difference between the Cu levels.

Trace element levels.

Extractable trace elements after 3 weeks of incubation are shown in Tables 6a, 6b and 6c for soils of initial pH 4.6 and 5.2, 5.7 and 6.4, and 6.8 and 7.3 respectively. Soil pH levels after incubation and L.S.D at $P < 0.05$ for trace elements are also shown in these tables.

The NaOAc reagent did not extract any Cu from the control soils of any pH. The lower limit of determination of Cu was 0.2 ppm on the soil basis. Non-significant amounts of Cu were extracted even where 100 ppm Cu was added in all the soils. With 1000 ppm Cu fair amounts of Cu were extracted from the soils of low pH levels. NaOAc- Zn tended to increase with increasing level of Cu and to decrease with increasing soil pH. NaOAc-Fe was low in all soils and was not related to soil pH or level of Cu addition. Irrespective of Cu treatment NaOAc-Mn was high in soils of pH 4.6 and 5.2 but decreased considerably with increasing soil pH ; in general, 100 ppm Cu had no effect on NaOAc-Mn, whilst 1000 ppm Cu increased it in all soils.

In all soils Morgan-Cu was increased to a small extent by 100 ppm Cu but to a considerable extent by 1000 ppm Cu ;

Table 6a

Extractable trace elements and soil pH after 3 weeks of incubation of different soils (pH 4.6 and 5.2) treated with 0, 100 and 1000 ppm Cu (as sulphate).

(Results are given in ppm on dry soil basis)

0.5N NaOAc

Treat-ments	Soil of initial pH 4.6					Soil of initial pH 5.2				
	Cu	Zn	Fe	Mn	pH	Cu	Zn	Fe	Mn	pH
0	0	2.5	2.4	87	5.0	0	1.3	3.6	57	5.7
100	1.0	2.9	2.9	88	4.9	0.9	1.8	2.7	62	5.4
1000	54	8.6	2.6	122	4.5	36	4.8	1.5	100	4.7
	Morgan's reagent									
0	0	9.3	6.3	153		0	7.4	7.5	122	
100	5.3	11	6.6	153		3.9	8.4	6.5	128	
1000	254	31	6.0	190		223	15	6.0	174	
	0.1N EDTA-Na									
0	6.6	32	108	204		4.1	33	177	190	
100	50	41	107	201		54	34	165	195	
1000	644	51	101	231		693	38	172	225	

L.S.D at $P < 0.05$ for Cu at 0 = 0.4, at 100 = 1.1 at 1000 = 7.5 ; Zn = 3.1 ; Fe = 4.2 ; Mn = 2.3

Table 6b

Extractable trace elements and soil pH after 3 weeks of incubation of different soils (pH 5.7 and 6.4) treated with 0, 100 and 1000 ppm Cu (as sulphate).

(Results are given in ppm on dry soil basis)

Soil of pH	Treatments	Extract used	Cu	Zn	Fe	Mn	Soil pH
5.7		0.5N-					
	0	NaOAc	0	0.7	2.0	6.2	6.1
	100		1.0	0.9	2.9	8.9	5.9
	1000		20	2.0	1.2	11	5.1
	0	Morgan	0	3.2	4.2	13	
	100	reagent	3.4	4.3	8.0	19	
	1000		180	7.4	3.6	22	
	0	0.1N-	2.4	11	80	24	
	100	EDTA-NA	39	13	129	30	
	1000		625	15	76	30	
6.4		0.5N-					
	0	NaOAc	0	0.6	1.3	0.6	6.3
	100		0	0.6	1.1	0.6	6.0
	1000		13	2.3	1.0	15	5.4
	0	Morgan	0.	4.2	3.3	1.6	
	100	reagent	3.2	5.6	2.9	2.1	
	1000		141	10	2.9	28	
	0	0.1N-	12	30	112	27	
	100	EDTA-Na	70	29	114	26	
	1000		701	33	100	46	

L.S.D at $P < 0.05$ for Cu at 0 = 0.4, at 100 = 1.1 at 1000 = 7.5 ; Zn = 3.1 ; Fe = 4.2 ; Mn = 2.3

Table 6c

Extractable trace elements and soil pH after 3 weeks of incubation of different soils (pH 6.8 and 7.3) treated with 0, 100 and 1000 ppm Cu (as sulphate).

(Results are given in ppm on dry soil basis)

Soil of pH	Treat-ments	Extract used	Cu	Zn	Fe	Mn	Soil pH
6.8	0	0.5N-NaOAc	0	0.9	2.2	0.6	6.6
	100		0	1.3	2.2	1.3	6.3
	1000		11	5.6	1.3	14	5.7
	0	Morgan reagent	0	18	4.5	1.4	
	100		1.9	27	4.9	3.8	
	1000		115	39	3.7	29	
	0	0.1N-EDTA-Na	2.0	70	104	23	
	100		36	77	94	24	
	1000		487	92	95	45	
7.3	0	0.5N-NaOAc	0	0.3	1.4	2.5	7.7
	100		0	0.4	1.4	1.7	7.4
	1000		0.7	0.4	1.4	4.3	7.2
	0	Morgan reagent	0	4.8	4.2	29	
	100		1.1	5.0	4.1	20	
	1000		40	7.3	4.3	33	
	0	0.1N-EDTA-Na	4.6	41	45	79	
	100		36	40	41	58	
	1000		394	43	41	80	

L.S.D at $P<0.05$ for Cu at 0 = 0.4, at 100 = 1.1 at 1000 = 7.5 ; Zn = 3.1 ; Fe = 4.2 ; Mn = 2.3

the extent of increase in the extractable Cu due to 1000 ppm Cu addition decreased with increasing soil pH. Negligible amounts of Cu were extracted by the Morgan's reagent from all control soils. There was a general trend for Morgan-Zn to decrease with increasing soil pH, although the soil of pH 6.8 had an unusually high value. This soil also had an EDTA-Zn value approximately twice as high as did the other soils. Morgan-Zn was usually little affected by 100 ppm Cu in any of the soils, but was increased by 1000 ppm Cu, particularly in soils of low pH. Morgan-Fe tended to decrease with increasing soil pH but was not affected by Cu at either level. Morgan-Mn was little affected by 100 ppm Cu at any pH, but was usually increased by 1000 ppm Cu in most of the soils.

EDTA-Cu decreased with increasing soil pH and was non-significant in the soil of pH 7.3. EDTA-Cu increased considerably with increasing level of added Cu in all soils. Approximately 39-70% of the 1000 ppm Cu applied was extracted by EDTA, with the two higher pH soils giving the lowest values. EDTA-Zn showed a trend for increasing with level of Cu applied, the trend tending to be more significant with the lower pH soils. There was tendency for EDTA-Fe to decrease slightly in all soils with increasing level of Cu addition. EDTA-Mn was unaffected by 100 ppm Cu but was increased somewhat by 1000 ppm Cu, except in the soil of pH 7.3 where no level of added Cu increased Mn.

Soil pH decreased with increasing addition of Cu, and the extent of decrease due to 1000 ppm Cu ranged from 0.5-1.0 units.

GENERAL DISCUSSION FOR CHAPTER III

Effects of Cu treatments on nitrogen mineralisation and nitrification.

Since nitrogen mineralisation and, in particular, nitrification in soils are affected by pH the effects of Cu on these processes must be considered in relation to the decrease in pH resulting from additions of both $CuSO_4.5H_2O$ and $(NH_4)_2SO_4$. The acidifying action of copper sulphate arises from the sulphate residue. The acidifying action of ammonium sulphate arises from the sulphate residue and also the conversion of ammonium to nitrate. Nitrification is decreased to a greater extent with decreasing pH than is ammonification (37,38,93,94,95). The former process is due to autotrophic bacteria whose activity is decreased with decreasing pH. Ammonification is brought about by a wide range of microorganisms (bacteria, fungi, actinomycetes) some of which may be susceptible to low pH but others, particularly fungi, can function even at low pH levels.

Copper treatments usually had little effect on nitrogen mineralisation in Silwood soil, but had a somewhat greater

effect in Harlington soil. In Silwood soils where mineralisation was significantly altered the effect was a decrease in mineralisation which was somewhat greater where no N than where N was added. In this soil pH was decreased by a maximum of 0.8 units by the highest level of copper sulphate and by a maximum of 0.3 units by ammonium sulphate. It appeared therefore that Cu treatments had little effect on nitrogen mineralisation in this soil even allowing for changes in pH. Nitrification of added ammonium sulphate in the soil of initial pH 7.4 was virtually complete irrespective of level of added Cu, indicating that even the 1000 ppm Cu level was not toxic to the nitrifying organisms. In the soils of initial pH 6.0 and 4.8 nitrification of added ammonium sulphate was low even in the absence of added Cu presumably because of the relatively low pH of these soils. The slight decreases in nitrification due to Cu additions indicated a toxic effect to nitrification. The Silwood soils of all pH levels therefore appeared to have a considerable capacity for converting added Cu^{2+} to forms in which the Cu had no effect on microbiological processes involving ammonification and nitrification.

In contrast to relatively small effects of Cu on nitrogen changes in Silwood soil, Cu additions generally decreased both mineralisation and nitrification to a fair extent in Harlington soil. The effects were of the same order irrespective of the absence or presence of added ammonium sulphate.

In general, the effect of adding 1000 ppm Cu was only about twice as great as adding 100 ppm Cu. This indicated that the Harlington soil had a lower capacity than the Silwood soil for overcoming the toxic effects of Cu. In Harlington soil pH was decreased by a maximum of 0.7 units by the 1000 ppm Cu level. This decrease in pH in itself seemed insufficient to account for the reduction in mineralisation and nitrification, and the reductions must be ascribed to the direct toxic effects of Cu. The extent of reduction in nitrification due to Cu was greater in the soil of initial pH 7.3 than in the soil of pH 5.9. In the soil of pH 5.9 nitrification was decreased by 100 ppm Cu and prevented by 1000 ppm Cu. In the soils of pH 5.1 there was no nitrification even in the control soil, and Cu additions had no effect on nitrification.

It is perhaps surprising that the Silwood soil had a greater effect than did the Harlington soil in decreasing the toxic effect of Cu on nitrogen mineralisation and nitrification. Both soils have virtually the same organic carbon content, but the Harlington soil is considerably higher in clay and silt than the Silwood soil, and because of these the former soil might be expected to have a greater effect in detoxifying Cu through adsorption and cation exchange. Thus soil texture in itself is of no value in indicating the extent to which added Cu may be detoxified. Possibly the types of clay mineral present may be more important in this respect.

In the experiment where 6 different loam soils of pH ranging from 4.6 to 7.3 Cu additions had no consistent effects on nitrogen mineralisation and nitrification in relation to soil pH. There was a general trend for the proportion of nitrate-N to ammonia-N to increase with pH in control soils. This would be expected in view of the increasing activity of nitrifying organisms with increasing pH. However, where Cu was added two of the soils (pH 5.2 and 7.3) showed increases in mineralisation and nitrification, whilst the other soils were either unaffected or showed decreases in both processes. Where increases in mineralisation and nitrification occurred these may have been due to the fact that in the soils concerned Cu may have been limiting both processes. Anderson (94) and Lees (164) showed that Cu was an essential trace element for nitrification and it can be presumed that this element is also essential for the activity of at least some of the ammonifying organisms. The main effect of pH was noted in the fact that 100 ppm Cu decreased mineralisation only in the soil of pH 4.6, indicating that even this low level of Cu was toxic to mineralising organisms. The low pH of this soil has probably partially prevented the added Cu^{2+} from being converted to non-toxic forms. In soils of higher pH (except for those two soils of pH 5.2 and 7.3 which showed increases in mineralisation and nitrification) 100 ppm Cu did not affect mineralisation presumably because the added Cu

was converted to forms non-toxic to this process. In the soils of pH 6.4 and 6.8 100 ppm Cu had no effect on nitrification, , whilst 1000 ppm Cu prevented it. In general, the toxic effects of Cu were more apparent at low than at high soil pH.

Extractable trace element levels and their relationship to nitrogen changes.

Sodium acetate (NaOAc), by definition, extracts exchangeable forms of cations. The Morgan's reagent also extracts exchangeable cations, but in addition, extracts a portion of of non-exchangeable forms. The latter fraction is not well defined, so that no specific form of the cation can be named. Presumably it extracts part of the chelated form of trace elements in soils. However, the Morgan's reagent has long been used for assessing the status of cations in soils with respect to plant growth, or in other words it has been shown to be a fairly reliable indicator of availability of nutrients to plants. EDTA extract removes exchangeable cations and, in addition, removes much of the chelated forms, including that which is removed by Morgan's reagent.

NaOAc-Cu was very low (less than 1 ppm) in the control and where 100 ppm Cu was added in soils of all pH levels. This indicated that added Cu was converted almost entirely to non-exchangeable forms. Even where 1000 ppm Cu was added most of the added Cu was converted to non-exchangeable forms,

particularly in soils of pH greater than 6.0, but even in soils of low pH more than 90% of the added Cu was found to be non-exchangeable. This indicates that all soils at all pH levels have a considerable capacity for immobilising added soluble Cu^{2+} in non-exchangeable form. However, much of this fixed Cu was removable by EDTA extraction at all pH levels, indicating that the added Cu was largely fixed in chelated form. However, there were differences due to pH and between soils in the extent of fixation of added Cu in chelated form. Except for the Silwood soil with both levels of added Cu there was trend for the extent of fixation in chelated forms to decrease with increasing pH, but even in high pH soils more than 33% of the Cu added at either level was extractable with EDTA.

The question arises as to which of the three extractants tested is the most useful for indicating deficiency or toxicity of Cu to nitrogen mineralisation and nitrification. It is interesting to note that relatively strong reagents have been recommended for determining available Cu in soils by other workers. Thus HCl (pH 2.0) (183) and 0.5N HNO_3 (184) have been recommended as methods for assessing soil Cu status. These reagents would almost certainly extract at least part of the chelated and difficultly soluble forms of Cu from soils. These workers as well as Pizer et al (178) (who used 0.1N EDTA, pH 4.0) showed that their reagents were suitable for

indicating Cu status of the soil with respect to plant growth. It is clear, therefore, that the plants are able to use forms of Cu other than exchangeable form. If it is assumed that microorganisms can utilise the same forms of Cu in soils as do plants, then the results obtained in this study confirm those obtained by these other workers. This is shown mainly in the fact that exchangeable soil Cu is of no value in indicating the "availability" of Cu to nitrogen mineralising and nitrifying organisms. Thus the very low exchangeable Cu in all soils where 100 ppm Cu was added was not related to changes in either process which sometimes occurred. The same reasoning can be applied in general to the Morgan reagent, which, although it extracted more Cu from soils than did NaOAc, was not related to toxic or stimulating effects of added Cu. The EDTA extract was possibly more satisfactory in this respect, but even this extractant did not always indicate whether soil Cu levels would affect the nitrogen transformation processes. This is evident when comparing the results for the six loam soils differing in pH treated with 1000 ppm Cu. The EDTA-Cu levels in those two soils which showed increased nitrification were of the same order as in the two other soils where nitrification was completely inhibited. On the other hand in the Harlington soil the extent of decrease in nitrification was roughly correlated with the extent of increase in EDTA-Cu. In this soil nitrification

was decreased when EDTA-Cu was 72 ppm or higher. However, in one of the loam soils (pH 6.4) nitrification was not affected even when EDTA-Cu was 70 ppm. This indicates that the critical EDTA-Cu level for affecting nitrification varies with factors other than texture, e.g. type of clay, and quantity of organic matter present.

Exchangeable Zn (NaOAc-Zn) was not generally affected by 100 ppm Cu in any of the soils but was usually increased somewhat by 1000 ppm Cu, but then only in the more acid soils. This could have been due to the direct effect of added Cu^{2+} in replacing Zn^{2+} in the exchange complex, or to the ability of Cu to form more stable chelates in the soil thus displacing Zn from chelated form. The pattern of Morgan-Zn values in relation to Cu treatment was generally similar to that of NaOAc-Zn, although the magnitude of the values were somewhat higher. Other workers have recommended extractants of moderately low pH i.e. N-KCl + acetic acid, pH 3.2 (185) and N-ammonium acetate + acetic acid, pH 4.6 (186). These reagents are similar to Morgan's reagent used in this study and indicates that this reagent is a better indicator of "availability" of Zn than is NaOAc.

Copper additions at either level both in the absence and presence of added N generally had little effect on the amounts of Fe removed by any of the extracts, even though extractable

Fe levels varied with soil pH and soil type. This indicates that Cu additions probably would have no effect on availability of Fe in these soils.

There was definite trend for both NaOAc-Mn and Morgan-Mn to be increased by 1000 ppm Cu, but not usually by 100 ppm Cu, whilst EDTA-Mn was generally little affected. Mn can exist in soils in the manganous form (Mn (II)) and in higher oxidation states (Mn (IV) and possibly Mn (III)) (187,188). As soil pH increases so the proportion of Mn (IV) increases and that of Mn (II) decreases, and the reverse effect occurs as soil pH decreases. These transformations arise from both microbial and chemical mechanisms. Over the pH range of 5.5 to 8.0 microbial effects predominate, whilst below pH 5.5 both effects are operative. Although 100 ppm Cu had little effect on exchangeable Mn, 1000 ppm Cu generally increased exchangeable Mn, sometimes to a considerable extent. The higher level of Cu clearly accelerated the conversion of Mn (IV) to Mn (II) even in some of the soils of high pH and low pH, although the effects were generally greatest in soils of intermediate pH. Although 1000 ppm Cu reduced pH slightly, the increased Mn (II) cannot be ascribed entirely to this. It appears, therefore, that Cu has direct effect in increasing Mn (IV) to Mn (II). This is shown very clearly in the Harlington soil of pH 5.9 where the Cu treatment decreased pH by only 0.2 units, whilst exchangeable Mn was increased from

0.3 to 52 ppm. It is possible that the Cu has directly stimulated the activity of microorganisms concerned with conversion of Mn (IV) to Mn (II). In addition, the high levels of exchangeable Mn in the Cu-treated soils may have had some influence in decreasing nitrification. The effects of Cu in increasing exchangeable Mn (which is usually considered to be available to plants) could be beneficial in soils of high pH (in which Mn may be deficient to plants). On the other hand in soils of lower pH where Mn (II) is usually high the increase in Mn (II) due to Cu addition could raise the available soil Mn to toxic levels.

SUMMARY OF CHAPTER III

An incubation study (3 weeks at 30°C and 33% mwhc) was carried out on the effects of adding 100 and 1000 ppm Cu as sulphate with and without addition of 100 ppm N as ammonium sulphate on nitrogen mineralisation and nitrification in (a) a sand (Silwood soil), (b) a sandy loam (Harlington soil) both of which had been adjusted to different pH levels before incubation and (c) six soils of loam texture differing naturally in pH. In addition, soil pH and the levels of Cu, Zn, Fe and Mn extracted by 0.5N NaOAc (pH 7.0), Morgan's reagent (pH 4.8) and 0.1N EDTA-Na (pH 4.0) were determined after incubation.

The Cu treatments had little effects on N mineralisation in the Silwood soil even allowing for changes of pH, but decreased mineralisation in Harlington soil to a fair extent with increasing level of addition of Cu. Nitrification was decreased only slightly in Silwood soil at any pH but was decreased to somewhat greater extent in Harlington soil. The extent of reduction in nitrification due to Cu was greater in the soil of pH 7.3 than in the soil of pH 5.9. In the latter soil nitrification was completely inhibited by 1000 ppm Cu. In general, the effects of Cu treatment in the absence or presence of added ammonium sulphate were the same.

In the six loam soils, with pH ranging from 4.6 to 7.3, Cu additions had no consistent effects on mineralisation or nitrification in relation to soil pH. Two of the soils (pH 5.2 and 7.3) showed increases in mineralisation and nitrification, whilst the other soils were unaffected or showed decreases in both processes. In general, the toxic effects of Cu were more apparent at low than at high soil pH.

Most of the added Cu^{2+} was converted to non-exchangeable forms by the end of incubation, and most of this was found in chelated form. There was a trend for the extent of fixation of added Cu in chelated form to decrease with increasing pH. Exchangeable Zn and Morgan-Zn were decreased by 1000 ppm Cu but then only in acid soils, but chelated Zn was little

affected by level of Cu. Extractable Fe levels in all soils at any pH were little affected by addition of either level of Cu. 1000 ppm Cu stimulated the conversion of the higher oxidation states of Mn to Mn^{2+} particularly in soils of intermediate pH.

CHAPTER IV

EFFECTS OF ADDITION OF COPPER AS OXIDE AND PHOSPHATE ON NITROGEN MINERALISATION AND NITRIFICATION IN SOILS RECEIVING DRIED BLOOD DURING INCUBATION (A) IMMEDIATELY FOLLOWING ADDITION AND (B) SOME TIME AFTER ADDITION OF COPPER COMPOUNDS.

Experiment 1. Effects of Cu levels on N-mineralisation in soils where CuO was applied 3 months before incubation.

Experiment 2. Comparison of effects of Cu as $CuHPO_4$ and CuO on N-mineralisation immediately following addition of Cu compounds.

Experiment 3. Comparative effects of added Cu levels on N-mineralisation in 3 soils where $CuHPO_4$ was applied 3 months before incubation.

Experiment 4. Comparative effects of added Cu levels on N-mineralisation in calcareous soils of varying carbonate content immediately following $CuHPO_4$ addition.

CHAPTER IV

EFFECTS OF ADDITION OF COPPER AS OXIDE AND PHOSPHATE ON NITROGEN MINERALISATION AND NITRIFICATION IN SOILS RECEIVING DRIED BLOOD DURING INCUBATION (A) IMMEDIATELY FOLLOWING ADDITION AND (B) SOME TIME AFTER ADDITION OF COPPER COMPOUNDS.

INTRODUCTION

In the previous chapter experiments were described which dealt with the effects of adding Cu in soluble form on N transformations during incubation, preceded by 10-15 days of wetting and drying in order to allow the added Cu to react with the soil. This treatment and also the addition of ammonium sulphate resulted in decreases in soil pH, so that it was sometime difficult to separate the effects of the Cu treatments from changes in pH on nitrogen transformations.

In the experiments in this chapter Cu was added in two forms (CuO and $CuHPO_4$) which were found to have no effects on soil pH. Both of these forms of Cu are virtually insoluble in water. In addition, these forms are possibly more similar to those forms of Cu which could find their way into soils through application of potentially polluting materials (e.g. industrial and mining wastes).

Where soils have been polluted with Cu compounds there

would be occassions where some time might elapse between pollution and use of the soil for growing crops. This effect was simulated in some of the experiments in this chapter by keeping the soil moist for some time after addition of CuO or $CuHPO_4$ and before using these soils for incubation experiments. In addition, much higher levels of added Cu (up to 10,000 ppm) were used in some experiments. Although such high levels of Cu pollution would probably rarely occur in soils, it was considered that their effects should be studied in order to cover the widest possible range of likely pollution. In all experiments dried blood was added to Cu-treated as well as control soils in order to simulate the effects of the presence of an easily decomposable source of organic nitrogen and to accentuate differences due to Cu treatment on nitrogen transformations.

Experiment 1

Effects of 100, 1000 and 10,000 ppm Cu (as oxide) on nitrogen mineralisation and nitrification of Silwood and Harlington soils after 6 weeks incubation. (Soils kept moist for 3 months after addition of CuO and 200 ppm N as dried blood added just before incubation).

METHODS

500g portions of air dried 2mm sieved Silwood soil (pH 6.1) and Harlington soil (pH 6.5) were separately mixed with 100, 1000 and 10,000 ppm Cu (soil basis) as CuO. The various mixtures were placed in glass flower pots having a layer of glass cloth over the drainage holes. Controls for both soils (no Cu added) were also placed in pots. Water was added to all pots to bring moisture contents to 50% mwhc. The pots were held at laboratory temperature (18-22°C) for 3 months and water was added periodically to maintain them moist. After 3 months 2-inch of water was added to all pots to leach out some of the soluble salts. The soils were allowed to drain and then to air dry. 10g samples were then set up for incubation after mixing in 200 ppm N as finely ground dried blood and water was added to bring moisture content to 50% mwhc. The BaO_2 method was used for aeration and absorption of CO_2. After 6 weeks of incubation the samples were extracted

with Morgan's reagent. Suitable aliquots of the extracts were analysed for ammonia and nitrate.

RESULTS

Results for ammonia- and nitrate-N levels on the air dry soil basis initially (zero week incubation) and after 6 weeks incubation are shown in Fig. 5 and Table 7. The L.S.D values at $P < 0.05$ are also indicated on the figure and in the table.

The initial values (zero week incubation) shown represent the effects of the Cu treatments on mineral-N levels during the 3 months pre-incubation period when the soils were held moist at room temperature. During this pre-incubation period mineral-N accumulated entirely as nitrate in all soils including the controls. However, there were differences in the extent of nitrate accumulation due to level of added Cu. In both soils 100 ppm Cu had no effect on, whilst 1000 ppm Cu decreased nitrate accumulation. In the Silwood soil 10,000 ppm Cu decreased nitrate accumulation, whilst in the Harlington soil it had no effect.

During the 6 weeks of incubation of Silwood soil at 30°C the extent of mineralisation of the organic N of dried blood was low, being less than 19% of the N added. 100 ppm Cu had no effect on this mineralisation, but mineralisation decreased slightly, although significantly, with increasing level

Fig. 5

Levels of ammonia- and nitrate-N initially (zero week) and after 6 weeks of incubation of Silwood and Harlington soils treated with 0, 100, 1000 and 10,000 ppm Cu as oxide. (200 ppm N as dried blood added to all before incubation).

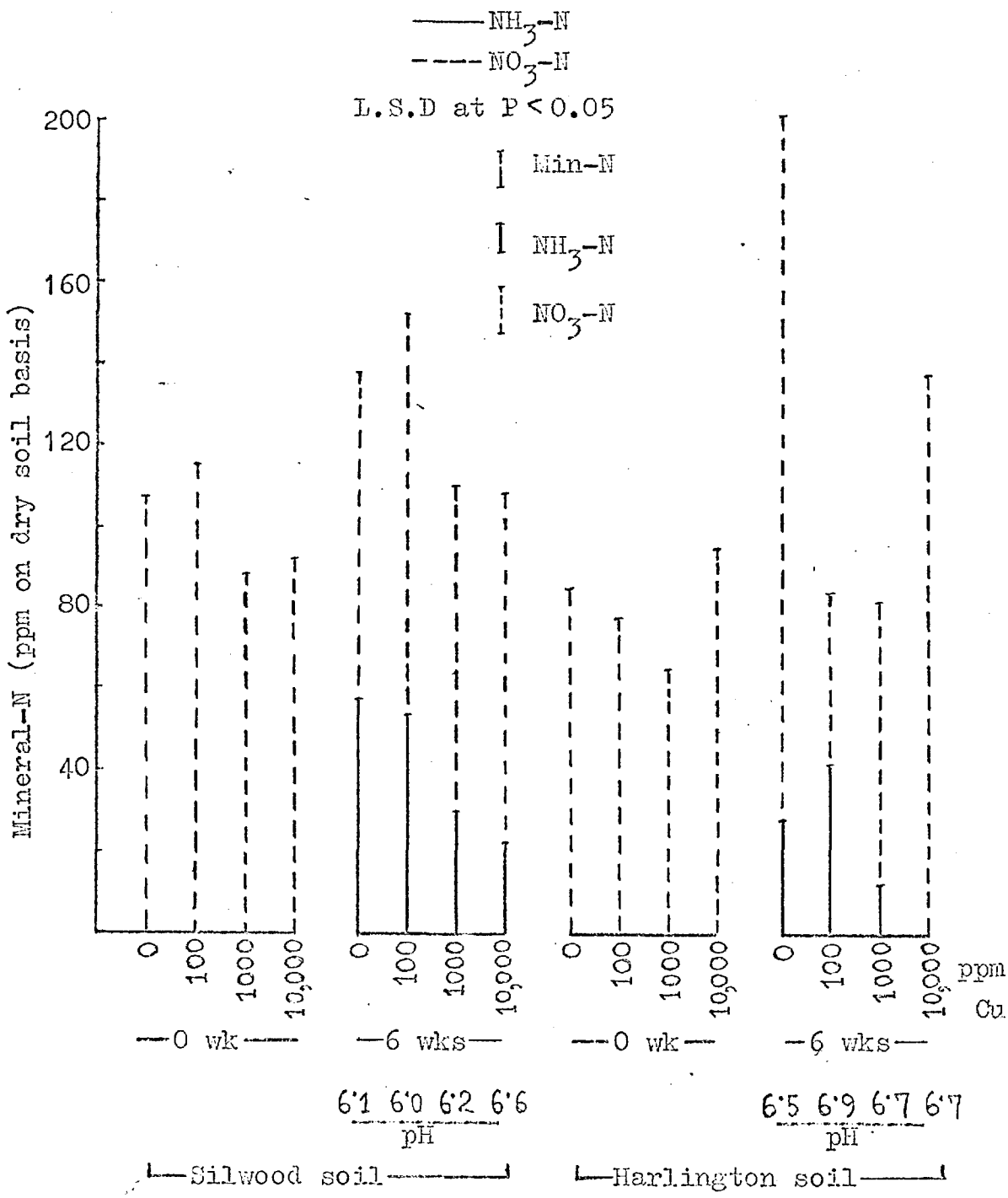


Table 7

Levels of ammonia- and nitrate-N initially (zero incubation) and after 6 weeks of incubation of Silwood and Harlington soils treated with 0, 100, 1000 and 10,000 ppm Cu (as oxide) (200 ppm N as dried blood added to all just before incubation).

(Results are given in ppm on dry soil basis)

Soil type	Added Cu	Initial values			After incubation		
		NO_3-N	NH_3-N	Min-N	NO_3-N	NH_3-N	Min-N
Silwood	0	108	0	108	80	58	138
	100	115	0	115	98	54	152
	1000	88	0	88	80	30	110
	10,000	92	0	92	85	23	108
Harlington	0	84	0	84	172	28	200
	100	77	0	77	42	41	83
	1000	64	0	64	69	12	81
	10,000	94	0	94	137	0	137

L.S.D at $P < 0.05$ for

Min-N = 7.6, NH_3-N = 5.5 and NO_3-N = 11.5

of added Cu. The nitrogen mineralised from dried blood accumulated as both ammonia and nitrate in all treatments. The extent of ammonia-N accumulation decreased with increasing level of Cu. The extent of nitrate-N accumulation was increased by 100 ppm Cu, but was unaffected by 1000 and 10,000 ppm Cu.

In the Harlington soil during the 6 week incubation period 58% of the added organic N was mineralised where no Cu was added. Where 100 and 1000 ppm Cu were added mineralisation was considerably reduced, to 8% or less of the organic N added, whilst with 10,000 ppm Cu it was reduced to 22%. The mineralised N accumulated as both ammonia and nitrate in all Harlington soils except where 10,000 ppm Cu was added, where it accumulated entirely as nitrate. Nitrification was decreased to the greatest extent by 100 ppm Cu, and to a lesser extent with further increasing level of Cu.

DISCUSSION

During the 3 months pre-incubation period where the soils were kept moist at room temperature, the presence of mineral-N entirely as nitrate (zero week incubation values in Fig. 5) indicated that even the 10,000 ppm Cu level did not inhibit nitrification during this period. However, the accumulation of ammonia during incubation with addition of dried blood

indicated that, except for the Harlington soil receiving 10,000 ppm Cu, ammonification rate exceeded nitrification rate. There was a definite trend for increasing Cu levels to decrease the proportion of mineral-N accumulating as ammonia. Although the extent of mineralisation of organic N of dried blood was relatively low, it is clear that the presence of Cu was effective in stimulaing nitrification over ammonification.

The general effects of added Cu in decreasing mineralisation of N was greater for the Harlington than for the Silwood soil. This is probably a direct effect of the Cu treatments and not due to any effect of pH since in any case the CuO treatment resulted in slight increases in pH. Cu additions had greater effect in decreasing nitrification in the Silwood than in the Harlington soil. The accumulation of mineral-N entirely as nitrate in the Harlington soil where 10,000 ppm Cu was added indicated that even this high level of Cu did not suppress nitrification. In fact nitrate accumulation was being limited by mineral-N production in this soil.

Experiment 2

Effects of 100, 1000 and 10,000 ppm Cu (as oxide and phosphate) on nitrogen mineralisation and nitrification of Harlington soil after 3 weeks incubation. (Soils incubated immediately after treatment with Cu compounds and 200 ppm N as dried blood added).

METHODS

The main purpose of this experiment was to compare the effects of Cu added as CuO and $CuHPO_4$ on N mineralisation and nitrification in Harlington soil treated with dried blood. The Harlington soil had previously been treated with 0.5% $CaCO_3$ to raise its pH to 7.4. This was done to ensure that pH was not limiting nitrification. The samples were set up for incubation as described in the previous experiment, using finely ground CuO in one series and finely ground $CuHPO_4$ in another series. The levels of added Cu were 100, 1000 and 10,000 ppm Cu on dry soil basis and a control soil without added Cu was also included. Dried blood was added to all samples, including the control. Moisture was added to 33% mwhc, and the tubes were incubated at 30°C for 3 weeks. After incubation the samples were extracted with 0.1N EDTA-Na (pH 7.0) and the extracts were analysed for ammonia and nitrate and Cu. EDTA-Na (pH 7.0) was used rather than EDTA-Na (pH 4.0)

since the soils studied in this and in the rest of the experiments in this chapter contained free chalk. The use of EDTA-Na of different pH levels for extracting Cu from soils is reported in the appendix.

RESULTS

Results for ammonia-N and nitrate-N initially and after 3 weeks of incubation are shown in Fig. 6 and Table 8. Table 8 also shows extractable Cu values.

Although the accumulation of mineral-N and nitrate-N differed considerably depending on the level of added Cu, there were no significant differences in the accumulation of either form of N due to the source of added Cu. In the control soil (no Cu added) only 12% of the added organic N was mineralised and there was no accumulation of nitrate during incubation. Mineralisation of nitrogen and nitrification increased with level of added Cu up to 1000 ppm. 28% of the added organic N was mineralised where 100 ppm Cu and 42% where 1000 ppm Cu were added. Where 10,000 ppm Cu was added only 23% of the added organic N was mineralised, but mineralisation even with this high level of Cu was significantly greater than where no Cu was added. Mineral-N accumulated entirely as ammonium where no Cu was added and largely as nitrate with all levels of added Cu. Although ammonium-N accumulated

Fig. 6

Levels of ammonia- and nitrate-N initially (zero week) and after 3 weeks of incubation of Harlington soil (pH 7.4) treated with 0, 100, 1000 and 10,000 ppm Cu as (a) CuO and (b) $CuHPO_4$. (200 ppm N as dried blood added to all before incubation).

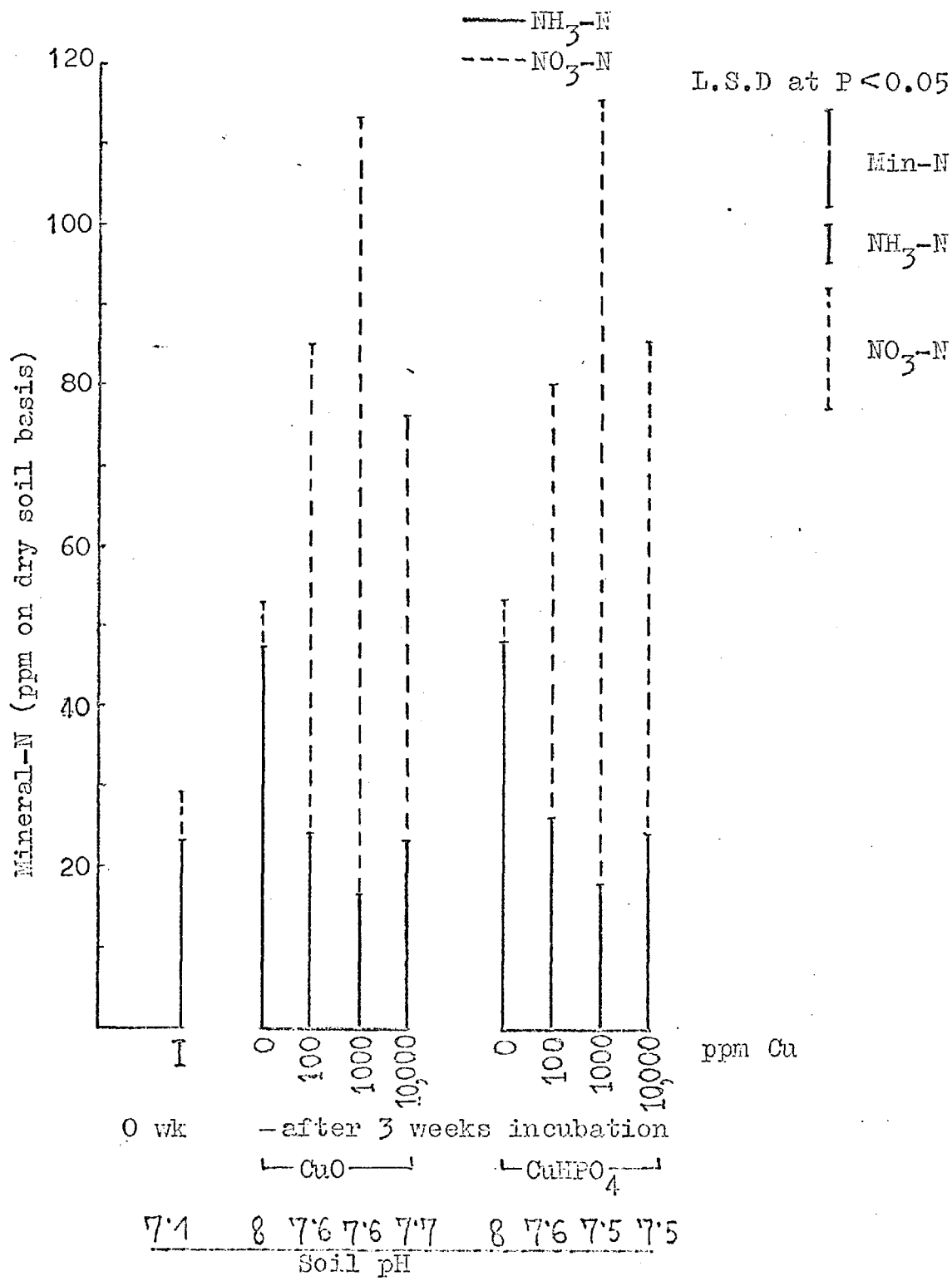


Table 8

Levels of ammonia- and nitrate-N initially (zero incubation) and after 3 weeks of incubation and extractable Cu after incubation of Harlington soil (pH 7.4) treated with 0, 100, 1000 and 10,000 ppm Cu (as CuO and $CuHPO_4$). (200 ppm N as dried blood added to all just before incubation).

(Results are given in ppm on dry soil basis)

Initial values

Min-N = 29

NH_3-N = 23

NO_3-N = 6

Mineral-N after incubation

Added Cu	CuO			$CuHPO_4$		
	NO_3-N	NH_3-N	Min-N	NO_3-N	NH_3-N	Min-N
0	5	48	53	5	48	53
100	61	24	85	54	26	80
1000	96	17	113	97	18	115
10,000	53	23	76	61	24	85

L.S.D at $P<0.05$ for

Min-N = 12.3, NH_3-N = 4.5 and NO_3-N = 14.8

Cu values after incubation

Added Cu	CuO	$CuHPO_4$
0	16	16
100	20	40
1000	110	200
10,000	313	1050

during incubation of the control soil there was no significant accumulation of ammonia where the Cu compounds were added. After incubation soil pH was decreased by 0.3 to 0.5 units, compared with the control, by addition of the Cu compounds, but the Cu treatments had little effect on pH before incubation.

EDTA-Na (pH 7.0) extracted 4%, 9% and 3% of the applied Cu where CuO was added, and 24%, 18% and 10% where $CuHPO_4$ was added at 100, 1000 and 10,000 ppm Cu respectively.

DISCUSSION

The Harlington soil used in this experiment had previously been adjusted to pH 7.4 by addition of $CaCO_3$ in order to ensure that conditions were optimum for nitrification. Under these conditions it was clear that nitrification and mineralisation of the organic N of dried blood were limited by lack of Cu. The increase in both processes due to addition of even 100 ppm Cu confirmed this. 1000 ppm Cu increased both processes further. Even 10,000 ppm Cu was not toxic to either process, in that both processes were still higher than in the control. The accumulation of some ammonia with all treatments indicated that mineralisation was not limiting nitrification. The higher accumulation of ammonia in the control than in the Cu-treated soils is reflected in the higher pH of the control

soil compared with the latter soils.

Of particular interest is the fact that both forms of Cu (CuO and $CuHPO_4$) had virtually identical effects on both mineralisation and nitrification. This is in spite of the fact that the levels of "active" Cu, as measured by extraction with EDTA, were considerably higher where $CuHPO_4$ than where CuO had been added (Table 8). However, even the maximum "active" Cu level (1050 ppm where 10,000 ppm Cu as phosphate had been added) represents only 10% of the added Cu. This soil appears to have considerable capacity for converting Cu to non-active forms, possibly carbonates or basic carbonates. The lack of any difference in effects between CuO and $CuHPO_4$ also indicated that the phosphate anion itself did not influence mineralisation of nitrogen or nitrification, and also did not influence the effects of Cu on these processes.

It is not possible to determine from the data obtained in this experiment what the critical level of EDTA-Cu would be for suppressing nitrogen mineralisation or nitrification. The critical level would be something greater than 1050 ppm Cu (the value obtained where 10,000 ppm Cu as phosphate had been added).

Experiment 3

Effects of 100, 1000 and 10,000 ppm Cu (as phosphate) on nitrogen mineralisation and nitrification of Silwood, Harlington and Clay soils after 6 weeks incubation. (Soils kept moist for 3 months after addition of $CuHPO_4$ and sufficient $CaHPO_4$ to equalise PO_4 addition. 200 ppm N as dried blood added just before incubation.

METHODS

In this experiment Cu was added as $CuHPO_4$ at varying levels, and extra PO_4 was also added as $CaHPO_4$ at rates calculated to bring the added PO_4 in all soils, including the controls, to the same levels. The purpose of this was to eliminate the effects of added PO_4 as a variable. Although added Ca varied, this was considered unimportant since the 3 soils used had previously been treated with 1-2% $CaCO_3$ in order to make them slightly calcareous. After the treatments the soils were kept moist in pots for 3 months in order to allow the added materials to react with the soils before they were air dried and set up for incubation. During this pre-incubation period all the soils were leached with 2-inch of water at monthly intervals in order to remove most of the nitrate and other soluble salts. After air drying the soils were set up for incubation as previously described with

addition of 200 ppm N as dried blood. The samples were wetted to 40% mwhc and the incubation was done for 6 weeks at 30°C. The soils were extracted after incubation with EDTA-Na (pH 7.0) for the determination of ammonia- and nitrate-N and extractable Cu.

RESULTS

The results for ammonia- and nitrate-N levels initially and after 6 weeks of incubation are shown in Fig. 7 and Table 9. Table 9 also shows extractable Cu values and soil pH levels after incubation.

The initial values for ammonia and nitrate (zero week incubation in Fig. 7) indicated the amounts of these constituents which accumulated during the 3 months pre-incubation period less those amounts extracted by the 3 leachings which were given during the pre-incubation period. In the Silwood and Harlington soils there were no differences due to any level of Cu treatment in ammonia and nitrate levels. In the Clay soil there were no differences due to Cu additions in ammonia levels, but the nitrate level where 10,000 ppm Cu had been added was significantly higher than where the lower levels of Cu or no Cu had been added.

In the Silwood soil after incubation 100 ppm Cu had no effect on, but 1000 and 10,000 ppm Cu significantly increased,

Fig. 7

Levels of ammonia- and nitrate-N initially (zero week) and after 6 weeks of incubation of Silwood, Harlington and Clay soils (all of pH 7.2) treated with 0, 100, 1000 and 10,000 ppm Cu as phosphate. (200 ppm N as dried blood also added).

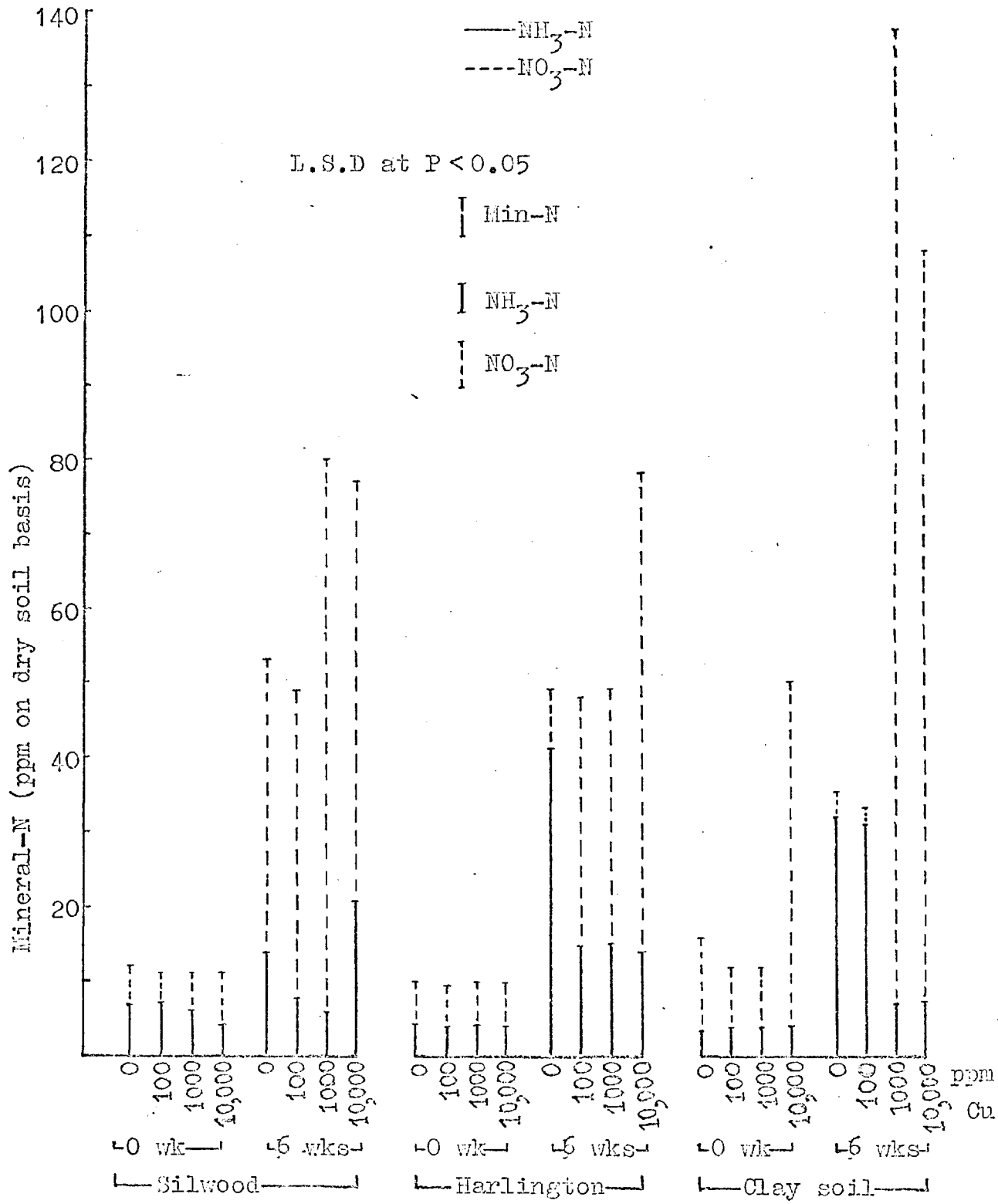


Table 9

Levels of ammonia- and nitrate-N initially and after 6 weeks incubation of Silwood, Harlington and Clay soils (all of pH 7.2) treated with 0, 100, 1000 and 10,000 ppm Cu (as $CuHPO_4$). Soil pH levels and extractable Cu values after incubation are also given.

(Results are given in ppm on dry soil basis)

Added Cu	Initial values NO_3-N	NH_3-N	Min-N	After incubation NO_3-N	NH_3-N	Min-N	pH	Cu
Silwood soil								
0	5	7	12	39	14	53	7.5	3.0
100	4	7	11	41	8	49	7.5	34
1000	5	6	11	74	6	80	7.3	265
10,000	7	4	11	55	22	77	7.2	1450
Harlington soil								
0	6	4	10	7	42	49	7.5	14
100	6	4	10	33	15	48	7.7	42
1000	6	4	10	34	15	49	7.4	255
10,000	6	4	10	64	14	78	7.4	1150
Clay soil								
0	13	3	16	3	32	35	7.5	4.7
100	8	4	12	2	31	33	7.8	30
1000	8	4	12	130	7	137	7.6	240
10,000	46	4	50	101	7	108	7.5	925

L.S.D at $P < 0.05$ for

Min-N = 4.6, NH_3-N = 3.6 and NO_3-N = 5.8

the extent of mineral-N accumulation. Mineralisation of the organic N of the added dried blood was 20%, 19%, 35% and 33% where 0, 100, 1000 and 10,000 ppm Cu respectively had been added. Nitrate accumulation during incubation was unaffected by 100 ppm Cu but was increased by 1000 and 10,000 ppm Cu, the effect being significantly greater with 1000 ppm Cu. Ammonia accumulation was significantly decreased by 100 and 1000 ppm Cu and significantly increased by 10,000 ppm Cu.

In the Harlington soil after incubation 100 and 1000 ppm Cu had no significant effects on, whilst 10,000 ppm Cu increased, mineral-N accumulation. Mineralisation of the organic N of added dried blood was 20%, 19%, 20% and 34% where 0, 100, 1000 and 10,000 ppm Cu respectively had been added. Nitrate accumulation during incubation was 4 times greater with 100 and 1000 ppm Cu and 8 times greater with 10,000 ppm Cu than in the control soil. Ammonia accumulation was decreased significantly to the same extent by all levels of added Cu.

In the Clay soil after incubation mineral-N accumulation was not affected by 100 ppm Cu but was greatly increased by 1000 ppm Cu, and increased to a moderate extent by 10,000 ppm Cu. The extent of mineralisation of the added organic N was 10%, 10%, 63% and 29% where 0, 100, 1000 and 10,000 ppm Cu respectively had been added. In the control soil there was a slight but significant decrease in nitrate due to incubation.

Nitrate accumulation was unaffected where 100 ppm Cu was added, but was increased considerably by 1000 ppm Cu, in particular, and to a lesser extent by 10,000 ppm Cu. In the control and 100 ppm Cu-treated soils mineral-N accumulated mainly as ammonia, whilst in the 1000 and 10,000 ppm Cu-treated soils it accumulated almost entirely as nitrate.

The Cu treatments had little effects on soil pH (Table 9) after incubation. Maximum differences in pH values from the control soils were 0.3 units for the Silwood, 0.2 units for the Harlington and 0.3 units for the Clay soil.

The pattern of EDTA extractable Cu (Table 9) in relation to level of added Cu was generally similar for the 3 types of soil. Where 100 ppm Cu was added EDTA extracted 25-31% of the applied Cu. Where 1000 ppm Cu was added 24-26% of the added Cu was extracted, and where 10,000 ppm Cu was added 9-14% of the added Cu was extracted. There was a general trend for extractable Cu to decrease in the order Silwood, Harlington and Clay soil.

DISCUSSION

The soils used in this experiment had been treated some time previously with sufficient $CaCO_3$ to make them slightly calcareous, and PO_4 additions had been equalised by adding $CaHPO_4$ in inverse amounts in relation to $CuHPO_4$ additions.

In this way the effects of varying pH and varying Ca levels were eliminated.

The significant effect of 10,000 ppm Cu in increasing nitrification in the Clay soil even during the 3 months pre-incubation period indicated that this soil was deficient in Cu with respect to nitrification. This effect was shown even more clearly during the subsequent 6 week incubation period, where both mineralisation of nitrogen and nitrification were greatly increased. However, under the very suitable conditions for nitrification obtained during incubation, the 10,000 ppm Cu level appeared to be slightly toxic to both N mineralisation and nitrification compared with the 1000 ppm Cu treatment. By contrast the Silwood and Harlington soils showed maximum stimulation of nitrification with the 10,000 ppm Cu, although the extent of increase in nitrification in these two soils due to this level of Cu addition was less than in the Clay soil. These differences were almost certainly due to differences in the clay contents of these three soils. The high clay content of the Clay soil was possibly able to detoxify the effects of Cu to a greater extent than the Harlington soil (sandy loam) and Silwood soil (sandy soil). These differences due to texture were also reflected in the greater effects in increasing nitrification due to 100 ppm Cu in the Silwood and Harlington than in the Clay soil.

The accumulation of ammonia, even though generally in small amounts, indicated that nitrification was not being limited by mineralisation of N. The differences in texture were also reflected in the effects of the treatments on ammonia accumulation during incubation. In the relatively coarse-textured Silwood and Harlington soils ammonia accumulation was decreased even by 100 ppm Cu, but was unaffected by this level of Cu in the Clay soil. This indicated the higher fixing capacity for Cu of the Clay compared with the two other soils.

The general trend for EDTA extractable Cu to decrease with increasing fineness of texture also indicated the ability of increasing clay content in fixing Cu. However, differences in "active" Cu due to soil type were fairly small. It appeared that slightly calcareous soils of this type can tolerate more than 10,000 ppm total Cu and more than about 1000 ppm "active" Cu (EDTA extraction) without any deleterious effects on N mineralisation or nitrification.

Experiment 4

Effects of 1000 and 10,000 ppm Cu (as phosphate) on nitrogen mineralisation and nitrification of five naturally calcareous soils ($CaCO_3$ contents ranging from 0.5% to 22%) after 6 weeks incubation. (Soils incubated immediately after treatment with Cu compound and 200 ppm N as dried blood added just before incubation).

METHODS

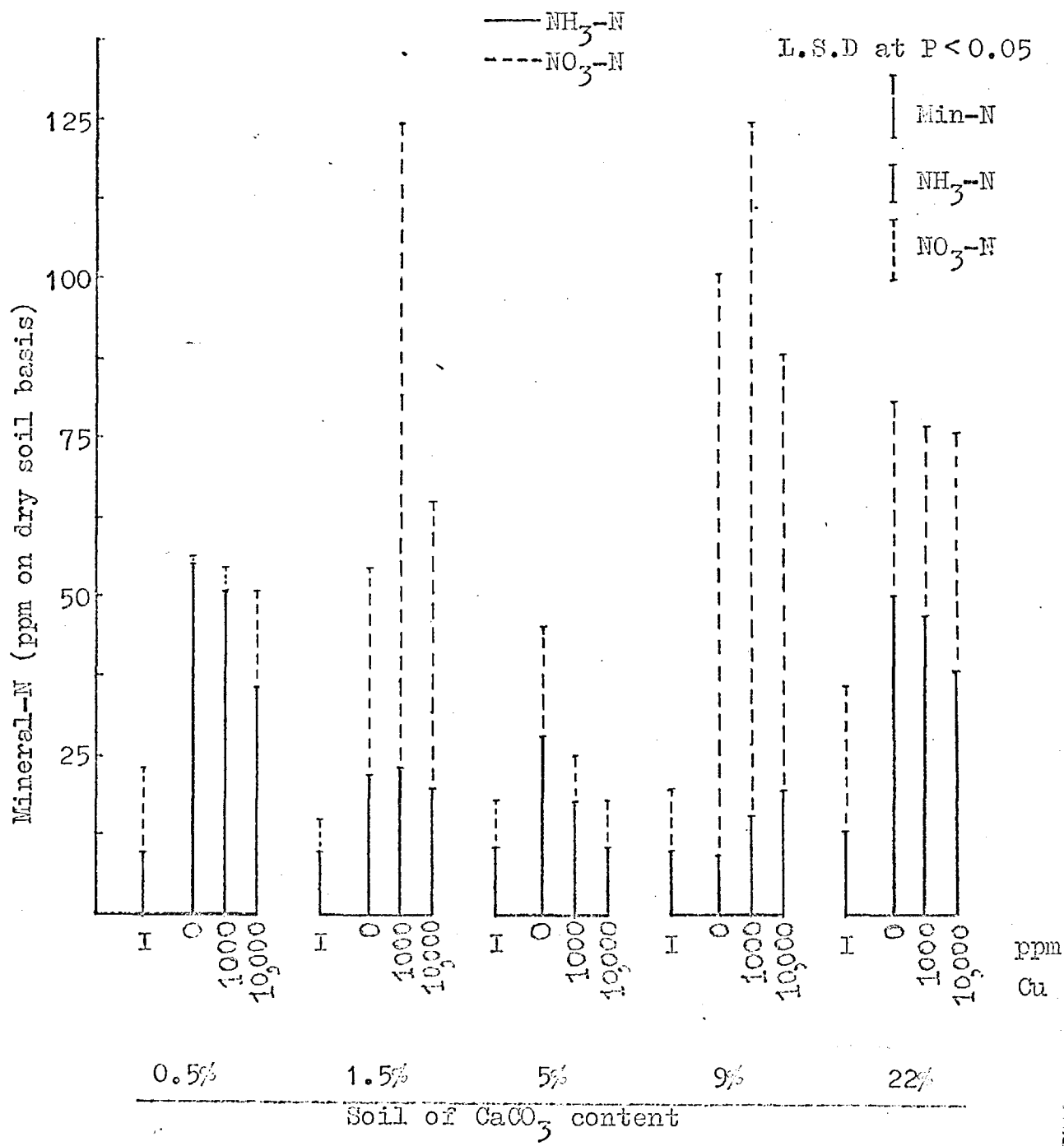
In this experiment five naturally calcareous sandy loam soils were selected which contained a wide range of carbonate contents, ranging from 0.5% to 22% $CaCO_3$. Cu was added as $CuHPO_4$ at 1000 and 10,000 ppm levels and 200 ppm N as dried blood was added before incubation. A control (no added Cu) was also set up for each soil. Incubation (30°C and 40% mwhc) was done for 6 weeks. The samples were then extracted with EDTA-Na (pH 7.0) and the extracts were analysed for ammonia- and nitrate-N and Cu.

RESULTS

Results for ammonia- and nitrate-N initially (before incubation) and after 6 weeks of incubation are shown in Fig. 8 and Table 10. Results for EDTA extractable Cu after incubation are also shown in Table 10. Results are presented in

Fig. 8

Levels of ammonia- and nitrate-N initially (zero week) and after 6 weeks incubation of 5 naturally calcareous soils (0.5, 1.5, 5, 9 and 22% $CaCO_3$) treated with 0, 1000 and 10,000 ppm Cu as phosphate. (200 ppm N as dried blood added).



For each soil "I" represents initial (zero week) and the rest values after 6 weeks incubation.

Table 10

Levels of ammonia- and nitrate-N initially and after 6 weeks incubation of 5 naturally calcareous soils (0.5-22% $CaCO_3$) treated with 0, 1000 and 10,000 ppm Cu (as phosphate) and with addition of 200 ppm N as dried blood. Extractable Cu levels after incubation are also indicated.

(Results are given in ppm on dry soil basis)

Soil of $CaCO_3$ content	Added Cu	Initial values			After incubation			
		NO_3-N	NH_3-N	Min-N	NO_3-N	NH_3-N	Min-N	Cu
0.5%	0	14	9	23	1	55	56	6.4
	1000	14	9	23	3	51	54	560
	10000	14	9	23	15	36	51	1650
1.5%	0	5	10	15	32	22	54	3.7
	1000	5	10	15	101	23	124	230
	10000	5	10	15	45	20	65	1100
5%	0	7	11	18	18	28	46	2.0
	1000	7	11	18	7	18	25	198
	10000	7	11	18	7	11	18	1178
9%	0	10	10	20	92	9	101	2.0
	1000	10	10	20	108	16	124	215
	10000	10	10	20	68	20	88	1025
22%	0	23	13	36	31	50	81	1.6
	1000	23	13	36	30	47	77	175
	10000	23	13	36	38	38	76	613

L.S.D at $P < 0.05$ for

Min-N = 9.0, NH_3-N = 5.8 and NO_3-N = 9.4

increasing order of calcium carbonate contents of the soils.

Although there were considerable differences in the extent of mineralisation and nitrification in relation to Cu treatments among the 5 soils, the responses were not related to the carbonate contents of the soils. Two of the soils (0.5% and 22% $CaCO_3$) showed no significant changes in mineral-N accumulation due to either level of added Cu. In one soil (5% $CaCO_3$) both levels of added Cu prevented mineralisation of nitrogen. In two other soils (1.5% and 9% $CaCO_3$) 1000 ppm Cu increased mineral-N accumulation, whilst 10,000 ppm Cu either had no effect on (soil containing 1.5% $CaCO_3$) or slightly decreased mineral-N accumulation (9% $CaCO_3$ soil).

The soil with 0.5% $CaCO_3$ was the only one in which initial nitrate disappeared during incubation. 1000 ppm Cu did not prevent, whilst 10,000 ppm Cu prevented, this disappearance. However, the treatments did not result in any accumulation of nitrate during incubation. In the soil containing 5% $CaCO_3$ both levels of added Cu prevented the accumulation of nitrate during incubation. In the soil containing 22% $CaCO_3$, 1000 ppm Cu had no effect, whilst 10,000 ppm Cu increased nitrate accumulation. In the soil containing 9% $CaCO_3$ nitrate accumulation was slightly increased by 1000 ppm Cu and slightly decreased by 10,000 ppm Cu. In the soil containing 1.5% $CaCO_3$ nitrate accumulation was considerably increased by 1000 ppm Cu and slightly increased by 10,000 ppm Cu.

In all soils except that containing 9% $CaCO_3$ ammonia accumulation increased during incubation in the controls (no added Cu). In soils containing 0.5%, 5% and 22% $CaCO_3$ ammonia accumulation decreased with increasing levels of Cu but was unaffected by either Cu level in the other two soils (1.5% and 9% $CaCO_3$).

There was a general trend for EDTA-Cu after incubation (Table 10) to decrease with increasing carbonate contents in the control soils and also where 1000 and 10,000 ppm Cu were added. 17-56% of the added Cu was extracted where 1000 ppm Cu was added and 6-16% where 10,000 ppm Cu was added.

DISCUSSION

Although there were considerable differences in the effects of Cu on N mineralisation and nitrification among the five calcareous soils studied, there were no consistent trends in relation to the $CaCO_3$ contents of these soils. In addition, both processes were affected differently in relation to level of applied Cu, with no consistent relationship to $CaCO_3$ content. These soils were all sandy loams, hence differences could not be accounted for in terms of clay content. Presumably other factors, which were not measured were affecting the differences in response to Cu treatments.

Only one soil (5% $CaCO_3$) showed significant decreases in

nitrogen mineralisation and nitrification with increasing Cu level. Only one soil (0.5% $CaCO_3$) showed disappearance of nitrate during incubation when no Cu or 1000 ppm Cu was added, but there was no disappearance of nitrate when 10,000 ppm Cu was added. The disappearance of nitrate was probably due to immobilisation by decomposing crop residues, and this immobilisation was prevented by the presence of 10,000 ppm Cu. Premi (103) noted a similar effect when a soil containing nitrate was incubated with addition of sucrose and the high level of Cu.

The general decrease in "active" Cu (EDTA extraction) with increasing level of soil carbonate at both levels of applied Cu indicated that increasing carbonate content was able to fix Cu in inactive form. However, even in the soil containing 22% $CaCO_3$, the "active" Cu value was 613 ppm.

GENERAL DISCUSSION FOR CHAPTER IV

The importance of soil pH in relation to the effects of Cu on nitrogen mineralisation and nitrification can be seen by comparing the results for the Harlington soil in experiment 1 with the same soil in experiment 2. In the former case the samples had an average pH of 6.7 and in the latter case of 7.7. In the former case N mineralisation and nitrification was inhibited, whilst in the latter case both processes were

increased, by all levels of Cu. It appears, therefore, that there is a critical pH somewhere between 6.7 and 7.7 below which Cu can be toxic and above which it can be stimulating to both the N mineralising and nitrifying organisms.

The comparative effects of incubation immediately after adding Cu compounds and incubation some time after Cu treatments can be seen from experiments 3 and 4, where all the soils contained free cacium carbonate. In experiment 3 where incubation was delayed for 3 months after addition of $CuHPO_4$, 10,000 ppm Cu stimulated N mineralisation and nitrification in all the 3 soils studied. On the other hand in experiment 4 where incubation was done immediately following Cu treatments, 10,000 ppm Cu either had no effect on or decreased nitrogen mineralisation, and either increased nitrification slightly or decreased it depending on soil type. It appears, therefore, that during the pre-incubation period both N mineralising and nitrifying organisms were able to adapt themselves to the high concentrations of Cu, so that during subsequent incubation the Cu was not toxic to either process. In addition, both groups of organisms appear to have been stimulated by Cu. An alternative explanation may be that during the pre-incubation period "active" Cu was decreased to low levels by the high pH and carbonate contents of the soils.

It appears that calcareous soils have a considerable capacity for preventing the toxic effects of Cu on nitrogen

transformations providing sufficient time is allowed for reactions to take place. In Chapter III where the effects of Cu were studied on more acid soils than those used in the experiments described in this chapter, even a 1000 ppm Cu often had toxic effects on N mineralisation and nitrification. The results obtained with calcareous soils in this chapter indicated that if acid soils become contaminated with high levels of Cu compounds their toxic effects on subsequent nitrogen transformations could be overcome by treating them with sufficient calcium carbonate to make them slightly calcareous. Not only will the toxicity of Cu be overcome in this way but there is a likelihood that the N mineralisation and nitrification processes would be stimulated.

SUMMARY OF CHAPTER IV

Nitrogen mineralisation and nitrification were studied during incubation of soils treated with varying levels of Cu (100-10,000 ppm, soil basis) as CuO or $CuHPO_4$. Incubation was done either immediately following additions of Cu compounds and, in some cases, calcium carbonate as well, and also after the treated soils had been kept moist for 3 months. The effects of these treatments were also studied with 5 naturally calcareous soils. In all experiments dried blood to supply 200 ppm N on dry soil basis was added just before incubation. Aerobic

moisture contents (33-50% mwhc) were used during incubation (30°C) for periods ranging from 3 to 6 weeks. Morgan's reagent and 0.1N EDTA-Na (pH 7.0) were used to extract ammonium and nitrate-N and Cu.

Addition of Cu as oxide or hydrogen phosphate had the same effects on N transformations. Nitrogen mineralisation and nitrification were stimulated by 100 ppm Cu in Silwood soil (pH 6.0) ; 1000 and 10,000 ppm Cu decreased nitrogen mineralisation but increased nitrification. In Harlington soil (pH 6.5) 100-10,000 ppm Cu decreased N mineralisation and nitrification to a fair extent. In Harlington soil of pH 7.6 both N mineralisation and nitrification increased with level of Cu up to 1000 ppm ; both processes increased even with 10,000 ppm Cu, but to a lesser extent than with 1000 ppm Cu.

In calcareous soils where incubation was done some time after Cu treatments 100 ppm Cu had no effect on N transformations, whilst 1000 and 10,000 ppm Cu usually increased both processes to a fair extent. On the other hand, treatment of calcareous soils with 1000 ppm Cu sometimes increased and sometimes decreased N mineralisation and nitrification ; 10,000 ppm Cu either had no effect on or decreased both processes. In calcareous soils there was no relationship between the effects of Cu on N transformations and calcium carbonate contents (0.5-22%) of the soils.

In the soils of high pH and those containing free calcium carbonate most of the added Cu was fixed against extraction by EDTA-Na. Although it was not possible to determine from the data in these experiments what the critical level of EDTA-Cu would be for suppressing N mineralisation and nitrification, this level would appear to be something greater than 1000 ppm Cu, providing there was a period of 3 months between addition of Cu compounds and incubation.

CHAPTER V

EFFECTS OF ADDITION OF COPPER COMPOUNDS TO SOILS TREATED WITH ORGANIC MATERIALS ON NITROGEN TRANSFORMATIONS DURING INCUBATION.

Experiment 1. Effects of addition of various amounts of sucrose on mineral nitrogen levels of Silwood soil (treated with 100 ppm N as ammonium nitrate) during 12 weeks of incubation.

Experiment 2. Effects of addition of 100, 1000 and 10,000 ppm Cu (as oxide) on mineral nitrogen levels of Silwood and Harlington soils (receiving 100 ppm N as potassium nitrate and 0.2% sucrose) during 12 weeks of incubation.

Experiment 3. Effects of 1000 ppm Cu (as oxide) on mineral nitrogen levels during 18 weeks of incubation of Harlington soil treated with 1% grass or straw (100 ppm N added as potassium nitrate).

CHAPTER V

EFFECTS OF ADDITION OF COPPER COMPOUNDS TO SOILS TREATED WITH ORGANIC MATERIALS ON NITROGEN TRANSFORMATIONS DURING INCUBATION.

INTRODUCTION

The previous two chapters have described experiments on the effects of varying types and levels of copper compounds on nitrogen mineralisation of native organic N and added N sources. The transformations involved were mineralisation of organic N and nitrification. Another important part of the nitrogen cycle (Chapter I) is that involving immobilisation of inorganic forms of nitrogen during decomposition of materials of wide C/N ratio in soils. The materials responsible for immobilisation are such things as root residues and top residues which are ploughed back into the soil. These residues consist mainly of mixtures of cellulose, hemicellulose and proteinaceous materials, and simple carbohydrates. In materials where nitrogen is absent or low the usual course of their decomposition in soils involves a temporary immobilisation of mineral nitrogen present due to the nitrogen requirements of the organisms decomposing the materials. In time, however, the nitrogen immobilised as organic nitrogen in the microbial protoplasm is again re-mobilised as mineral nitrogen.

This chapter will describe incubation experiments on the effects of added Cu compounds on the whole process of immobilisation followed by re-mobilisation. In order to ensure that immobilisation was not to be limited by lack of mineral N in soils, either ammonium and/or nitrate forms were added with the organic materials before incubation.

The first experiment where no Cu compound was added was in the nature of a preliminary experiment to determine a suitable level of sucrose to be added to a soil, so that only a single level could be used in the subsequent experiments where the effects of addition of a Cu compound were to be studied.

Experiment 1

Effects of addition of various amounts of sucrose on mineral nitrogen levels of Silwood soil (treated with 100 ppm N as ammonium nitrate) during 12 weeks of incubation.

METHODS

10g portions of Silwood soil (pH 6.0) were weighed into sufficient number of incubation tubes to allow for duplicate analysis initially and after 5 periods of incubation. Ammonium nitrate, to supply 100 ppm N on the soil basis, was added to

all tubes, dissolved in the water used to bring soil moisture content to 33% mwhc. The various sucrose levels (0, 0.05, 0.1 and 0.2%, soil basis) were also dissolved in the water used to bring the soils to 33% mwhc. Thus ammonium nitrate was not a "treatment", but was added to ensure that decomposition of sucrose was not limited by lack of mineral nitrogen. The tubes were incubated at 30°C. Duplicate tubes of each sucrose level, including the control, were removed after 48 hours and after 1, 3, 6, and 12 weeks of incubation, extracted with 0.5N NaOAc (pH 7.0), and the extracts were analysed for ammonia- and nitrate-nitrogen.

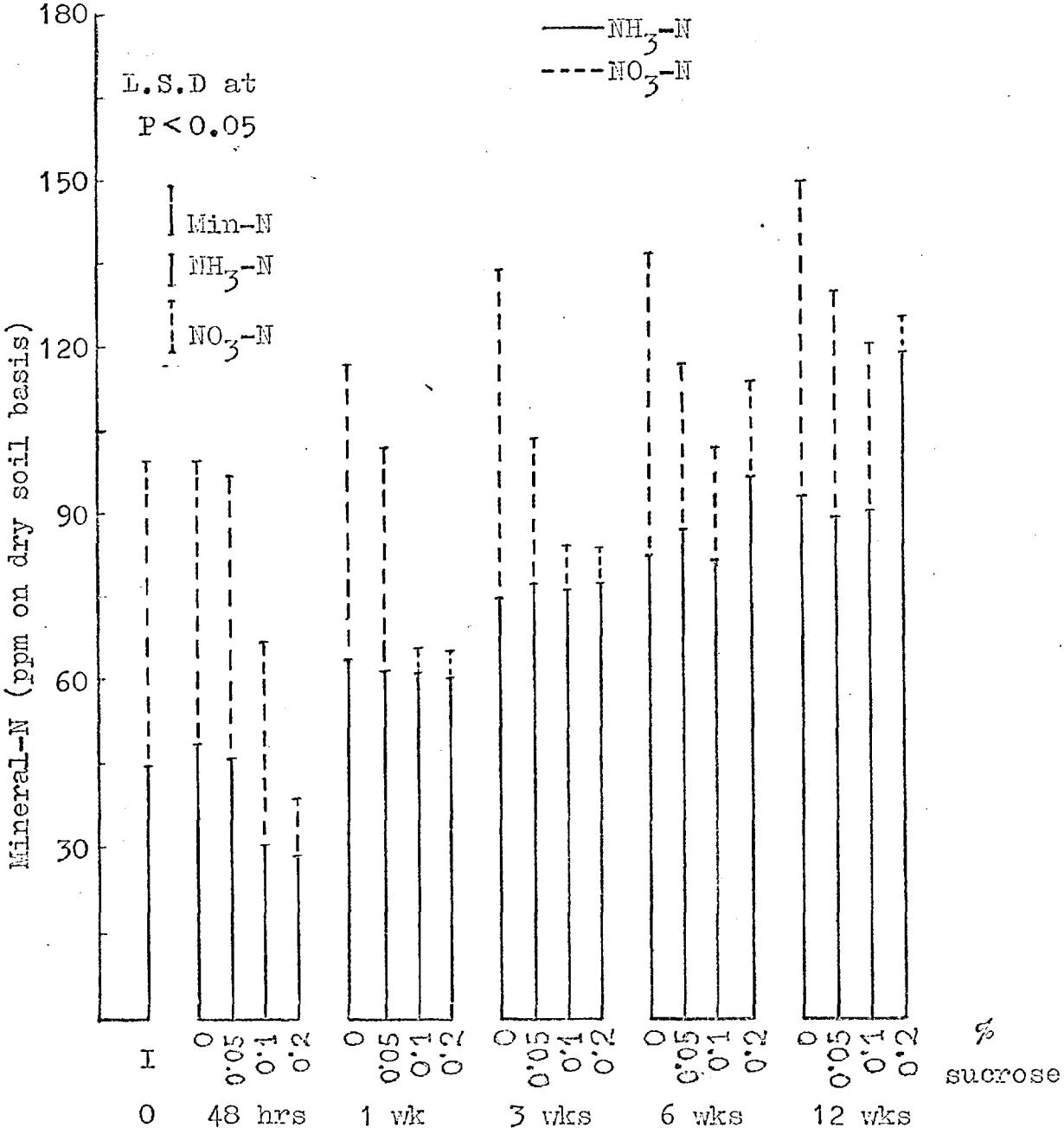
RESULTS

Results for mineral-N (ammonia- and nitrate-N) initially (zero week incubation) and after various incubation periods are shown in Fig. 9 and Table 11. L.S.D values at $P<0.05$ are also presented on the figure and in the table.

0.05% sucrose had no effect on mineral-N levels after 48 hours, but slightly decreased these levels during all other periods of incubation. 0.1% sucrose and, in particular, 0.2% sucrose decreased mineral-N levels to the greatest extent during 48 hours of incubation, but increased with further incubation up to 12 weeks. However, even after 12 weeks of incubation mineral-N levels were somewhat lower than in the

Fig:- 9

Levels of ammonia- and nitrate-N initially (zero week) and after 48 hrs. and after 1, 3, 6 and 12 weeks of incubation of Silwood soil treated with 0, 0.05, 0.1 and 0.2% sucrose and 100 ppm N as ammonium nitrate before incubation.



"I" represents initial value (zero week incubation).

Table 11

Levels of ammonia- and nitrate-N initially (zero incubation) and after 48 hrs. and 1, 3, 6, and 12 weeks of incubation of Silwood soil treated with 0, 0.05, 0.1 and 0.2% sucrose. (100 ppm N as ammonium nitrate added before incubation).

(Results are given in ppm on dry soil basis)

Initial values

Min-N = 100, NH_3-N = 45 and NO_3-N = 55

After incubation

Sucrose added	NO_3-N			NH_3-N			Min-N		
	48 hrs	1 wk	3 wks	48 hrs	1 wk	3 wks	48 hrs	1 wk	3 wks
0	51	53	59	49	64	75	100	117	134
0.05%	50	40	26	47	62	78	97	102	104
0.1%	36	4	7	31	62	77	67	66	84
0.2%	10	4	6	29	61	78	39	65	84
	6 wks	12 wks		6 wks	12 wks		6 wks	12 wks	
0	54	57		83	93		137	150	
0.05%	30	41		87	89		117	130	
0.1%	20	30		82	91		102	121	
0.2%	17	6		97	119		114	125	

L.S.D at $P < 0.05$ for

Min-N = 8.7, NH_3-N = 5.4 and NO_3-N = 8.8

control (no sucrose). All levels of sucrose resulted in a higher mineral-N level after 12 weeks of incubation than that present initially, but the highest increase in mineral-N during incubation occurred in the control soil.

In those treatments where mineral-N was decreased, nitrate was usually decreased to a greater extent than was ammonium. With incubation beyond 48 hours where mineral-N increased with time this increase was accounted for to a greater extent by ammonium than by nitrate. There was a distinct trend for the proportion of nitrate to ammonium to decrease with increasing level of sucrose.

Initial pH of all soils was 5.7 (zero incubation), and pH increased with time ; after 12 weeks it ranged from 6.1 where no sucrose was added to 6.6 where 0.2% sucrose was added.

DISCUSSION

Significant immobilisation of mineral-N occurred even after 48 hours of incubation where 0.1-0.2% sucrose was added. during 48 hours to 1 week period re-mobilisation of initially immobilised N occurred where 0.2%, but not where 0.1% sucrose was added. It is clear that the level of sucrose has important effect on the rate of immobilisation, with 0.2% level having the maximum effect.

The microorganisms involved in immobilisation had a

distinct preference for nitrate over ammonia, this effect becoming more apparent with increasing level of sucrose. With 0.2% sucrose 82% of the initial nitrate was immobilised, whilst only 36% of the initial ammonium N was immobilised. Although most of the immobilisation of nitrate occurred during 48 hours, there was still some immobilisation up to 1 week of incubation, as shown by decreasing nitrate level after this period. However, during the 48 hours to 1 week period the increasing ammonium levels indicated that there was also some re-mobilisation of mineral-N. With longer incubation the accumulation of re-mobilised N mainly as ammonium is rather surprising in view of the fact that the soil pH tended to increase slightly (0.2-0.4 units) with level of sucrose up to 12 weeks of incubation. However, the pH values of the soils (6.1-6.6) were probably on the border line where nitrification was tending to be inhibited by low pH. After 12 weeks of incubation mineral-N levels with all treatments were higher than initial levels and mineral-N accumulation was accounted for entirely by increased ammonium. The extra mineral-N had presumably arisen from mineralisation of native organic N. However, all the sucrose treatments significantly decreased the extent of re-mobilisation of the native organic N. It is possible that the microbial residues, left after immobilisation was complete, had an inhibitory effect on mineralisation of native organic N. In addition, these residues also

decreased nitrification during the re-mobilisation phase, as shown by the decreasing nitrate levels with increasing initial sucrose level after 12 weeks of incubation.

The main purpose of this experiment was to determine what would be a suitable level of sucrose to be used in the subsequent experiment where the effects of Cu were to be studied. It appeared that the 0.2% sucrose level would be the most suitable, since this gave high immobilisation of mineral-N during the initial phase and also had the greatest effect in decreasing nitrification during the latter phases. It was also decided to use an 1 week incubation period to measure the immobilisation phase, since, even though some re-mobilisation was occuring in this period, the lowest nitrate values were obtained after 1 week of incubation.

Experiment 2

Effects of addition of 100, 1000 and 10,000 ppm Cu (as oxide) on mineral nitrogen levels of Silwood and Harlington soils (receiving 100 ppm N as potassium nitrate and 0.2% sucrose) during 12 weeks of incubation.

METHODS

The Silwood and Harlington soils used had been treated with 0, 100, 1000 and 10,000 ppm Cu as CuO on dry soil basis and kept moist for 3 months at room temperature (as described in Chapter II). The soils were then air dried and ground to pass a 2mm sieve. The soils were set up for incubation in the usual way with sufficient replicate to allow for duplicate analysis initially (zero week incubation) and after 1 and 12 weeks of incubation. 0.2% sucrose and 100 ppm N as KNO_3, both on dry soil basis, were added in the water used to bring the soils to 50% mwhc. Initially and after 1 and 12 weeks of incubation ($30^{\circ}C$) duplicate samples were extracted with Morgan reagent and the extracts were analysed for ammonia and nitrate.

RESULTS

Results for ammonia and nitrate and mineral-N for both soils are shown in Fig. 10 and Table 12. Table 12 also shows

Fig:- 10

Levels of ammonia- and nitrate-N initially (zero week) and after 1 and 12 weeks incubation of Silwood and Harlington soils treated with 0, 100, 1000 and 10,000 ppm Cu as oxide and 0.2% sucrose and with addition of 100 ppm N as KNO_3.

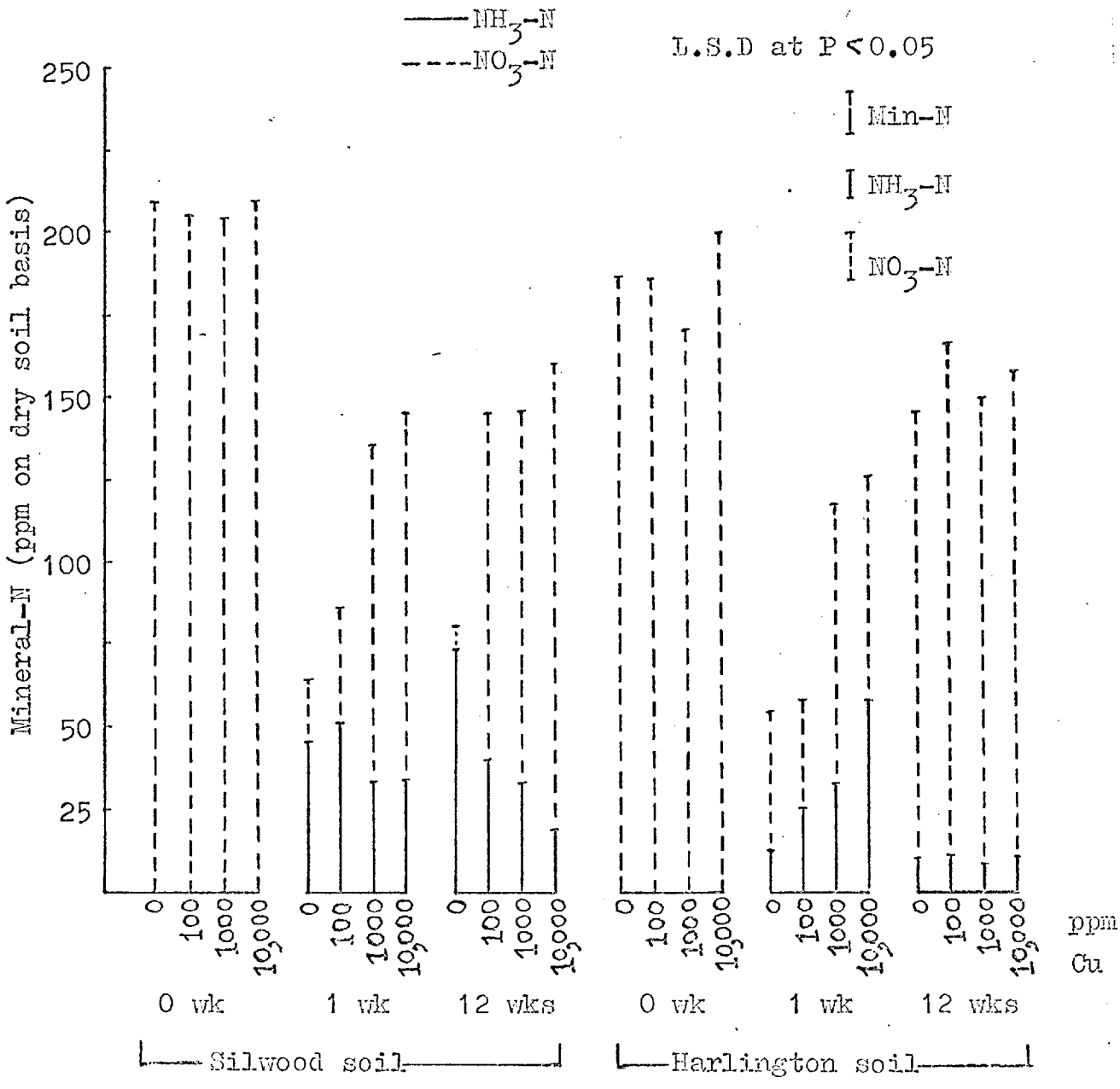


Table 12

Levels of ammonia- and nitrate-N initially (zero incubation) and after 1 and 12 weeks of incubation of Silwood and Harlington soils treated with 0, 100, 1000 and 10,000 ppm Cu (as CuO). (100 ppm N as potassium nitrate and 0.2% sucrose added before incubation).

(Results are given in ppm on dry soil basis)

Soil type	Added Cu	Initial values			After incubation					
		NO_3-N	NH_3-N	Min-N	NO_3-N		NH_3-N		Min-N	
					1 wk	12 wks	1 wk	12 wks	1 wk	12 wks
Silwood native NO_3-N = 108 ppm	0	208	0	208	17	6	46	74	63	80
	100	205	0	205	35	105	51	40	86	145
	1000	203	0	203	102	113	33	33	135	146
	10,000	209	0	209	111	141	34	19	145	160
Harlington native NO_3-N = 86 ppm	0	186	0	186	42	136	12	9	54	145
	100	184	0	184	32	155	25	10	57	165
	1000	170	0	170	84	141	32	8	116	149
	10,000	199	0	199	93	147	32	10	125	157

L.S.D at $P < 0.05$ for

Min-N = 11.6, NH_3-N = 6.4 and NO_3-N = 13.1

pH after incubation

Added Cu	Silwood soil			Harlington soil		
	0 wk	1 wk	12 wks	0 wk	1 wk	12 wks
0	4.9	6.6	6.6	5.8	6.7	6.2
100	5.0	6.5	6.0	6.0	6.8	6.2
1000	5.1	6.3	5.8	6.1	6.9	6.3
10,000	5.1	6.4	5.9	6.2	7.0	6.7

soil pH values initially and after each period of incubation of both the soils.

Silwood soil:- After 1 week of incubation mineral-N levels were decreased in all soils, including the control (nil Cu), compared with the initial values. The greatest decrease occurred in the control soil and the decrease was less with increasing levels of Cu ; the differences were significant between 0 and 100, and between 100 and 1000 ppm Cu, but not between 1000 and 10,000 ppm Cu. The differences in nitrate levels due to Cu treatments were similar to the differences in mineral-N levels. During this period significantly more ammonia accumulated in the control and 100 ppm Cu-treated soils than in the soils treated with 1000 and 10,000 ppm Cu.

After 12 weeks of incubation of the control soil there was a slight but significant increase in mineral-N, accounted for entirely by ammonium, compared with 1 week of incubation. In the 100 ppm Cu-treated soil there was a fair increase in mineral-N, accounted for entirely by nitrate. With 1000 and 10,000 ppm Cu there were only slight increases in mineral-N between 1 and 12 weeks of incubation. There was a trend for ammonium levels at 12 weeks to decrease with increasing levels of Cu.

Harlington soil:- After 1 week of incubation mineral-N levels were significantly lower than initial levels with all

treatments, including the control. Mineral-N was decreased to a great extent with 0 and 100 ppm Cu than with 1000 and 10,000 ppm Cu treatments. Nitrate levels between 0 and 1 week were decreased to a greater extent by 0 and 100 ppm Cu than by 1000 and 10,000 ppm Cu ; 100 ppm Cu had no significant effects on nitrate change, whilst 10,000 ppm Cu decreased nitrate significantly more than did 1000 ppm Cu. There was a significant trend for ammonia accumulation to increase with increasing levels of Cu.

After 12 weeks of incubation, compared with 1 week, mineral-N increased to great extent in the control and 100 ppm Cu-treated soils than in the 1000 and 10,000 ppm Cu-treated soils. After this period mineral-N levels were of the same order in all soils. The ammonia which accumulated in the Cu-treated soils during the first week of incubation decreased to low levels by 12 weeks of incubation, so that mineral-N levels were accounted for almost entirely as nitrate in all treatments.

DISCUSSION

In this experiment the mineral-N added initially was in nitrate form, since the previous experiment had shown that the immobilising organisms had a distinct preference for nitrate over ammonia. It was considered, therefore, that

addition of KNO_3 rather than $(NH_4)_2SO_4$ would result in bigger changes during the immobilisation phase.

There were differences due to Cu treatments in both the immobilisation phase (0-1 week) and re-mobilisation phase (1-12 weeks) between the two types of soil used. During the immobilisation phase 100 ppm Cu had no effect in the Harlington soil, but decreased immobilisation in the Silwood soil. The Harlington soil (sandy loam) was higher in clay than the Silwood soil (sand), so that the former soil probably had a greater effect in converting the low level of added Cu to "inactive" forms. With 1000 and 10,000 ppm Cu, where the extent of immobilisation of mineral-N was decreased to about the same extent in both soils, this indicated that these high levels of Cu were sufficient to satisfy the Cu fixing capacity of both soils. The other main point of difference between two soils occurred during re-mobilisation phase, where the effects of all levels of Cu had disappeared in the Harlington soil by 12 weeks of incubation, in that there was little difference in mineral-N levels due to Cu treatments. In the Silwood soil, on the other hand, all Cu levels increased the re-mobilisation of N. This again indicated the higher Cu fixing capacity of the Harlington soil.

The ammonia which accumulated in both soils during both periods of incubation must have come from mineralisation of

native organic N, since neither soil contained ammonia initially. It is interesting to note that ammonification was occuring during the first week of incubation concurrently with nitrate immobilisation. This showed clearly that mineralisation and immobilisation processes with respect to N can occur simultaneously. Another difference due to soil type can be seen in the effects of Cu on ammonia accumulation during both phases. In the Harlington soil during the immobilisation phase, where ammonia accumulation increased with level of Cu, this indicated an effect of Cu in increasing ammonification over immobilisation (it is possible that ammonia was also being immobilised during this period, but this type of experiment can only indicate the excess of mineralisation over immobilisation of N or vice versa). During the re-mobilisation phase in the Silwood soil, although little extra mineral-N was produced due to Cu treatments, the main effect of Cu treatments was to increase nitrate levels at the expense of ammonia levels. This indicated that all levels of added Cu were still active with respect to nitrification.

The Silwood soil is of particular interest in the relationship between Cu treatments and soil pH. It is seen that nitrate accumulation after 12 weeks was considerably higher with all Cu levels than in the control, and in particular, nitrate accumulation increased somewhat with Cu levels. This is in spite of a fair decrease in pH (0.7-0.8 units with the

two higher levels of Cu) compared with the control soil. This indicated an ability of Cu in stimulating nitrification even at low pH.

Experiment 3

Effects of 1000 ppm Cu (as oxide) on mineral nitrogen levels during 18 weeks of incubation of Harlington soil treated with 1% grass or straw (100 ppm N added as potassium nitrate).

This experiment involved a study of the effects of adding 1000 ppm Cu on ammonia and nitrate levels during incubation of Harlington soil treated with 1% grass or 1% straw. These materials are commonly incorporated into soils, the former by ploughing in a grass sod and the latter by ploughing in cereal straw residues.

METHODS

Harlington soil (pH 6.3) which had been treated with 1000 ppm Cu as CuO was used in this experiment. Sufficient control soils (no organic materials added) as well as soils receiving 1% finely ground grass or wheat straw on the soil basis were prepared. These treatments were done on one batch of the soil containing no added Cu and another batch containing 1000 ppm Cu. Usual amounts (10g portions) were weighed

out into each incubation tube and 100 ppm N as KNO_3 on the soil basis dissolved in the water used to bring the soil moisture content to 50% mwhc was added to each tube. Sufficient replicates were set up for incubation to allow for analysis in duplicate initially and after 3, 6, 12 and 18 weeks of incubation (30°C). After the various incubation periods duplicate tubes of each treatment were extracted successively with Morgan reagent and 0.1N EDTA-Na (pH 7.0). Ammonia and nitrate and Cu were determined in the Morgan extract, and Cu was determined in the EDTA extract. The first analysis was done after 3 weeks of incubation, since Cornfield (189) showed that with the Harlington soil maximum immobilisation of mineral-N by the decomposition of 1% grass or straw levels occurred after 3 weeks of incubation.

RESULTS

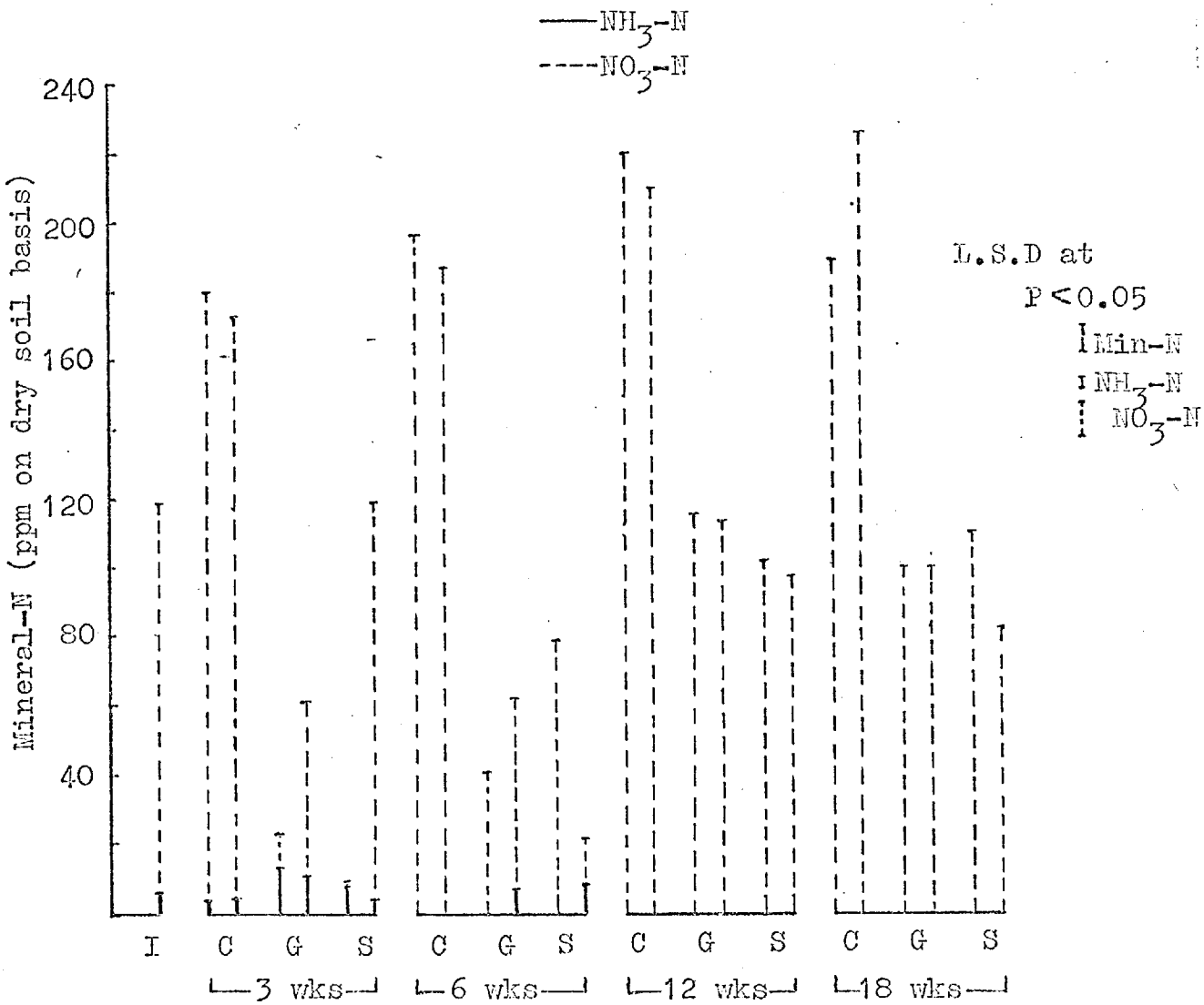
Results for ammonia- and nitrate-N levels initially and after each incubation period are shown in Fig. 11 and Table 13.

Copper levels in both extracts are not given since it was found that the grass and straw treatments had no significant effects on extractable Cu levels after any period of incubation either in untreated or in Cu-treated soils.

Initial levels of ammonia- and nitrate-N were the same in all soils irrespective of treatments. This initial value is

Fig:- 11

Levels of ammonia- and nitrate-N initially (zero week) and after 3, 6, 12 and 18 weeks of incubation of Harlington soil treated with 1% grass or straw and with and without addition of 1000 ppm Cu as oxide and with addition of 100 ppm N as potassium nitrate.



I = Initial, C = Control, G = Grass, and S = Straw

For each pair the left-hand value represents no added Cu and the right-hand value addition of 1000 ppm Cu.

Table 13

Levels of ammonia- and nitrate-N initially (zero incubation) and after 3, 6, 12 and 18 weeks incubation of Harlington soil treated with 1% grass and straw, and with and without 1000 ppm Cu (as CuO). (100 ppm N as KNO_3 added to all).

(Results are given in ppm on dry soil basis)

Initial values

Min-N = 119, NH_3-N = 6 and NO_3-N = 113.

After incubation

Treatments org. matt.	Cu	NO_3-N Weeks 3	6	NH_3-N Weeks 3	6	Min-N Weeks 3	6	pH after 6 weeks
Cont	0	176	197	4	0	180	197	6.3
Grass	0	10	41	13	0	23	41	6.6
Straw	0	1	78	8	0	9	78	6.7
Cont	1000	169	187	4	0	173	187	6.5
Grass	1000	50	55	11	7	61	62	6.8
Straw	1000	115	13	4	9	119	22	7.0
		Weeks 12	18	Weeks 12	18	Weeks 12	18	pH after 18 weeks
Cont	0	220	189	0	0	220	189	6.5
Grass	0	116	120	0	0	116	120	6.7
Straw	0	102	130	0	0	102	130	6.7
Cont	1000	210	226	0	0	210	226	6.6
Grass	1000	114	120	0	0	114	120	7.0
Straw	1000	98	82	0	0	98	82	7.2

L.S.D at $P < 0.05$ for

Min-N = 7.8, NH_3-N = 2.8 and NO_3-N = 9.0

shown only once on the far left-hand side of the Fig. 11. In the control soil (no organic material added) mineral-N increased with time of incubation up to 12 weeks and then decreased somewhat. 1000 ppm Cu decreased mineral-N accumulation slightly in the control soil after all incubation periods except after 18 weeks where mineral-N accumulation was higher than in the control.

After 3 weeks of incubation mineral-N fell to very low levels where grass or straw was present without Cu treatment. With Cu treatment mineral-N decreased to a lesser extent where grass was present, and there was no decrease where straw was present. With further incubation of up to 6 weeks mineral-N increased where grass was added without Cu, but was unaffected when added with Cu ; where straw was added without Cu mineral-N increased, but when added with Cu mineral-N decreased to a considerable extent. With further incubation mineral-N levels increased irrespective of treatments with organic materials or Cu, except for a slight decrease after 18 weeks incubation where straw was added with Cu. The greatest increase occurred during the 6-12 weeks period, and the increases were usually relatively small during the 12-18 weeks incubation period. By 18 weeks, although mineral-N with grass and the straw treatments had reached approximately the same levels as that present initially (zero week incubation), these levels were only half to two-thirds of those present in the control soil

(no organic matter treatment) after 18 weeks of incubation.

There was no significant accumulation of ammonia after any period of incubation except after 3 weeks with grass treatment without added Cu. Thus mineral-N values were accounted for entirely or almost entirely as nitrate.

DISCUSSION

The main difference in the effects of Cu in affecting nitrogen transformations occurred early in incubation. Cu completely prevented immobilisation of N during the first 3 weeks of incubation where straw was added, but only decreased the extent of immobilisation of N by about 60% where grass was added. This indicated a strong inhibitory effect of Cu on the activity of those organisms responsible for the decomposition of straw, whilst those organisms which were decomposing grass had their activity decreased to only a limited extent. During decomposition of organic materials in soils different types of heterotrophic organisms, such as bacteria, fungi and actinomycetes are involved. Different types of organisms have different mineral nitrogen requirements. Alexander (9) stated that the N requirements for proliferation increased in the order bacteria, fungi and actinomycetes. It is quite likely that the organisms responsible for decomposing straw are somewhat different from those decomposing grass ; the straw

would be higher in lignin and carbohydrates than the grass and this could result in the development of different types of microorganisms involved in their decomposition. It is well known that Cu compounds are more toxic to fungi than to other forms of microorganisms (slightly soluble Cu compounds have long been used for controlling fungal diseases on plants). Although it is not known specifically what types of micro-organisms are affected by adding Cu compounds to soils, it is likely that the activity of the common soil fungi could be decreased by Cu treatments. Thus the differential effects of Cu on the extent of immobilisation of mineral nitrogen during the early stages of incubation depending on whether straw or grass was present could be accounted for if the fungal population was higher where straw was added.

The effects of Cu in preventing immobilisation of N during the first 3 weeks of incubation where straw was added disappeared during the next 3 weeks of incubation. Thus Cu had not strictly prevented immobilisation but had only delayed it where straw was added compared with where grass was added. This could have been due to (a) fungi had in time become tolerant to the added Cu and proliferated normally during the 3-6 weeks incubation period, (b) bacteria and actinomycetes proliferated to a much greater extent during the second than during the first 3 weeks period and were entirely responsible for nitrogen immobilisation, or (c) copper which was present

in "active" form during the first 3 weeks incubation was deactivated during the second and third 3 weeks through formation of organic compounds, produced by decomposition of straw, resulting in the conversion of active Cu to inactive chelated forms, thus eliminating the toxic effect of Cu on fungal activity.

During incubation beyond 6 weeks the differential effects of Cu depending on whether straw or grass had been added had disappeared, in that N levels after 12 weeks of incubation were of the same order whether or not Cu had been added. However, the differential effects of Cu again appeared during 12-18 weeks period of incubation, where re-mobilisation continued in the absence of Cu, but further, slight but significant, immobilisation occurred where Cu was added. The reason for this is not clear, but may have been due to the fact that Cu had again become partially active through decomposition of chelated Cu compounds formed earlier in incubation ; the active Cu released then decreased mineralisation of organic N. In addition, the organisms decomposing the organic compounds will require further mineral nitrogen. Thus it appears where relatively stable forms of organic materials such as straw are added to soils, the effects of Cu on transformations involving N can persist for relatively long period during the decomposition of such materials. On the other hand transformations of N during decomposition of materials of relatively

narrow C/N ratio such as grass would be affected for only a limited period by the presence of Cu compounds.

The fact that with all treatments only about a half to two-thirds of the mineral-N immobilised initially was recovered during subsequent mineralisation up to 18 weeks indicates that a fair proportion of the immobilised N was held in very stable combination, probably in the form of lignin-protein-like compounds. It seems unlikely that mineral N would have increased further with incubation beyond 18 weeks since Cornfield (189) who did a similar experiment with the same soil (but without Cu additions) found that there was little further N mineralisation during 18-24 weeks incubation period.

GENERAL DISCUSSION FOR CHAPTER V

The experiments in this chapter have shown that the addition of Cu compounds to soils containing decomposable organic materials had significant effects on that portion of the nitrogen cycle involving immobilisation of mineral N in organic forms and subsequent re-mobilisation of the initially immobilised N.

Differences in the extent of these transformations were affected by the level of Cu addition, and the effects of Cu were generally greater during the initial (immobilisation) phase than during the subsequent re-mobilisation phase. Even

a relatively low addition of Cu (100 ppm in experiment 2 where sucrose was added) significantly decreased the extent of immobilisation of N early in incubation. Although the higher levels of Cu had a greater effect in this respect the general lack of difference between 1000 and 10,000 ppm Cu indicates that soils have the capacity for eliminating effects of even very high levels of Cu. The general lack of effect of any Cu level during re-mobilisation is possibly more important from the practical point of view, and indicates that in soils which have inadvertantly become contaminated with Cu compounds, these materials have only a temporary effect, at least on those microorganisms concerned with nitrogen transformations.

In experiment 3 where straw and grass were compared the main effect to be noted was the ability of Cu to delay the decomposition of straw as compared with that of grass. This could be of significance in a field soil contaminated with Cu in that decomposable residues such as straw could persist for longer periods than normal. This could be a disadvantage in that if straw is ploughed in the late autumn (as is commonly done) it may not have decomposed completely by the time the crop is sown in the following spring ; if the straw should then start decomposing during early growth of the plants the immobilisation of mineral N resulting from this could deprive the plants of nitrogen at this stage of growth.

Once a soil has become contaminated with Cu compounds it would be extremely difficult to remove Cu. Although leaching, through application of large amount of irrigation water, would seem to be the only possible way of doing this, it is most unlikely that this would be effective. Experiments in Chapter III have shown that even when large amounts of Cu have been added to soils virtually all this Cu was held in forms which were not extractable with NaOAc, indicating that negligible exchangeable Cu was present. This indicates that leaching would be ineffective in displacing Cu to the lower soil depths out of the rooting zone.

SUMMARY OF CHAPTER V

The effects of adding CuO on mineral N levels during incubation ($30^{o}C$) of soils receiving decomposable organic materials (sucrose, grass and straw) at aerobic moisture levels (33-50% mwhc) were studied. Potassium nitrate was added initially at 100 ppm N (soil basis) to ensure that nitrogen was not limiting the decomposition of organic materials.

A preliminary experiment to determine the optimum levels of sucrose to be added (together with 100 ppm N as NH_4NO_3) without any Cu addition was carried out. This showed that the maximum immobilisation of mineral N occurred after 48 hours of incubation where 0.2% sucrose was added, and after 48 hrs.

or 1 week of incubation where 0.1% sucrose was added. Maximum immobilisation of nitrate occurred after 1 week of incubation. Nitrate was immobilised to a greater extent than was ammonium. With incubation beyond 3 weeks nitrogen was remobilised mainly in the form of ammoinium, and the proportion of re-mobilised nitrate to ammonium decreased with increasing initial level of sucrose.

In the second experiment the effects of 100, 1000 and 10,000 ppm Cu (as CuO) on mineral-N levels during incubation of two soils receiving 0.2% sucrose and 100 ppm N as KNO_3 were studied. In the sandy (Silwood) soil the extent of immobilisation of mineral-N after 1 week of incubation decreased with increasing level of added Cu. In the sandy loam (Harlington) soil 100 ppm Cu had no effect on, whilst 1000 and 10,000 ppm Cu decreased immobilisation of mineral-N. After another 11 weeks of incubation of the sandy soil mineral-N levels resulting from re-mobilisation of nitrogen were approximately the same at all Cu levels, and were higher than in the control. In the sandy loam soil mineral-N level at the end of incubation was approximately the same with all treatments, including nil Cu. The proportion of ammonium to nitrate N at the end of incubation decreased with increasing level of Cu in the sandy soil, but was not affected by level of Cu in the sandy loam soil.

In the third experiment the effects of 1000 ppm Cu (as

CuO) on mineral-N levels during incubation for 3 to 18 weeks of a sandy loam (Harlington) soil treated with 1% grass and 1% straw (with 100 ppm N as KNO_3) were studied. The Cu treatment prevented any immobilisation of mineral-N during the first 3 weeks of incubation where straw was added, but decreased somewhat the extent of immobilisation where grass was added. With incubation up to 6 weeks maximum immobilisation of mineral-N occurred where Cu was added with straw, whilst re-mobilisation occurred where no Cu was added. With further incubation up to 18 weeks the effects of Cu largely disappeared with both the grass and straw treatments, except for a tendency for further immobilisation to occur during 12-18 weeks period where Cu was added with straw.

CHAPTER VI

EFFECTS OF VARYING MOISTURE CONTENTS ON MINERAL NITROGEN LEVELS DURING INCUBATION OF SOIL TREATED WITH COPPER COMPOUNDS AND WITH AND WITHOUT ADDITION OF NITROGEN.

Experiment 1. Effects of varying soil moisture contents and addition of 1000 ppm Cu (as sulphate) on mineral nitrogen levels during 3 weeks incubation of Silwood soil.

Experiment 2. Effects of varying soil moisture contents and addition of 1000 ppm Cu (as oxide) on mineral nitrogen levels during 6 weeks incubation of Silwood soil treated with 200 ppm N as dried blood.

CHAPTER VI

EFFECTS OF VARYING MOISTURE CONTENTS ON MINERAL NITROGEN LEVELS DURING INCUBATION OF SOIL TREATED WITH COPPER COMPOUNDS AND WITH AND WITHOUT ADDITION OF NITROGEN.

INTRODUCTION

In the work described up to now aerobic moisture levels were used, since virtually all crops are grown under these conditions. However, there is one species, namely rice, which is commonly grown under waterlogged, and hence apparently, anaerobic conditions. In addition, excessive rainfall combined with poor drainage may result in temporary anaerobic conditions even in soils where aerobic crops are grown.

The first experiment in this chapter reports on the effects of addition of 1000 ppm Cu as sulphate on ammonia and nitrate values during incubation ($30^{\circ}C$) at varying moisture levels. In the second experiment CuO was added at 1000 ppm Cu and dried blood at 200 ppm N was also added using the same varying moisture levels. The moisture levels used were 20, 40, 75, and 100% mwhc which were equivalent to pF values of 4.2, 2.5, 1.5 and 0 respectively.

Experiment 1

Effects of varying soil moisture contents and addition of 1000 ppm Cu (as sulphate) on mineral nitrogen levels during 3 weeks incubation of Silwood soil.

METHODS

Silwood soil of pH 6.0 was used. Sufficient samples were set up in duplicate to allow for analysis after 3 weeks of incubation ($30^{\circ}C$) with moisture variables of 20, 40, 75 and 100% mwhc, and with and without addition of 1000 ppm Cu as sulphate on the soil basis. The $CuSO_4.5H_2O$ was finely ground and mixed with the air dry soil before addition of water. After incubation the samples were extracted with 0.1N EDTA-Na (pH 7.0) and the extracts were analysed for ammonia- and nitrate-N and Cu.

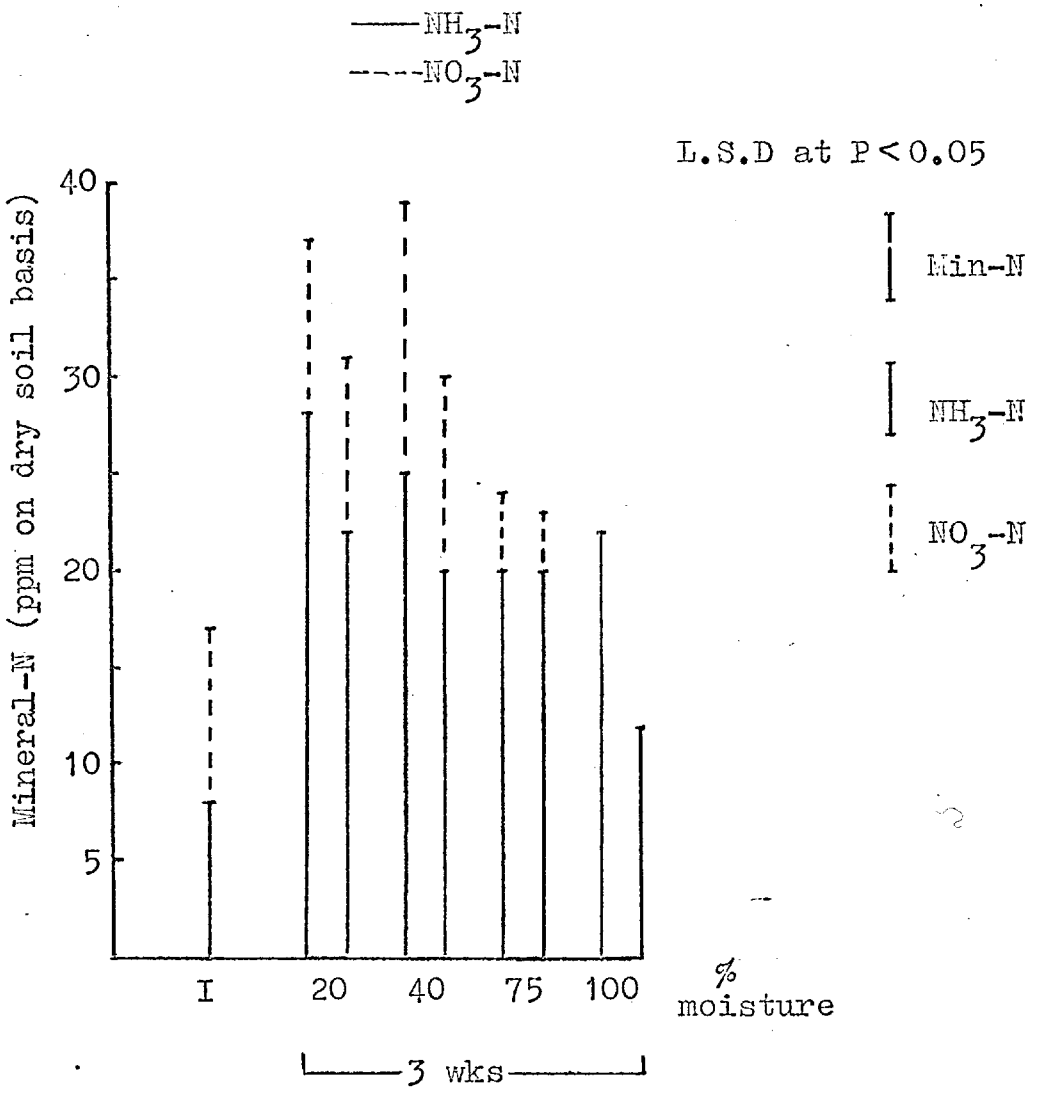
RESULTS

Results for ammonia- and nitrate-N initially (zero week incubation) and after 3 weeks of incubation are shown in Fig. 12 and Table 14. Table 14 also shows results for EDTA extractable Cu and pH initially and after incubation.

During incubation mineral-N increased to the greatest extent at 20 and 40% mwhc with or without added Cu. The Cu

Fig:- 12

Effects of varying levels of moisture with and without 1000 ppm Cu (as sulphate) on ammonia- and nitrate-N levels of Silwood soil after 3 weeks of incubation.



"I" represents initial value (0 wk incubation).

For each pair of each moisture content the left-hand value represents no added Cu and the right-hand value addition of 1000 ppm Cu.

Table 14

Levels of ammonia- and nitrate-N, pH values and Cu levels initially (zero week incubation) and after 3 weeks incubation at varying moisture levels (20, 40, 75 and 100% mwhc) of Silwood soil with and without addition of 1000 ppm Cu (as sulphate).

(Results are given in ppm on dry soil basis)

Treatments		Initial values					After incubation				
% moisture	Cu	NO_3 N	NH_3 N	Min N	Cu	pH	NO_3 N	NH_3 N	Min N	Cu	pH
20	0	9	8	17	6	6.0	9	28	37	2.8	6.3
40	0	9	8	17	6	6.0	14	25	39	2.5	6.4
75	0	9	8	17	6	6.0	4	20	24	2.1	6.6
100	0	9	8	17	6	6.0	0	22	22	1.9	7.2
20	1000	9	8	17	800	5.0	9	22	31	673	5.4
40	1000	9	8	17	800	5.0	10	20	30	660	5.4
75	1000	9	8	17	800	5.0	3	20	23	548	5.5
100	1000	9	8	17	800	5.0	0	12	12	505	5.6

L.S.D at $P<0.05$ for

Min-N = 4.2, NH_3-N = 3.4 and NO_3-N = 4.2

treatment decreased the extent of mineral-N accumulation to about the same extent at both of these moisture contents. At 75% mwhc mineral-N accumulation was less than at lower moisture contents and Cu had no effect on mineral-N accumulation. At 100% mwhc there was a significant, though small, increase in mineral-N without Cu, whilst with Cu there was a significant decrease compared with the initial value.

Nitrate levels were not affected by incubation at 20% mwhc either in absence or presence of Cu. At 40% mwhc nitrate increased without Cu, but was unaffected where Cu was added. At 75% mwhc nitrate decreased to the same extent with or without added Cu, whilst at 100% mwhc initial nitrate had completely disappeared with and without added Cu.

Ammonia accumulation due to incubation was decreased by Cu at all moisture contents except at 75% mwhc where it was unaffected.

EDTA extractable Cu after 3 weeks of incubation of the Cu-treated soils decreased with increasing soil moisture contents (Table 14). Without added Cu pH after 3 weeks of incubation increased with increasing moisture level, whilst with added Cu there was little difference in pH due to moisture contents.

Experiment 2

Effects of varying soil moisture contents and addition of 1000 ppm Cu (as oxide) on mineral nitrogen levels during 6 weeks incubation of Silwood soil treated with 200 ppm N as dried blood.

METHODS

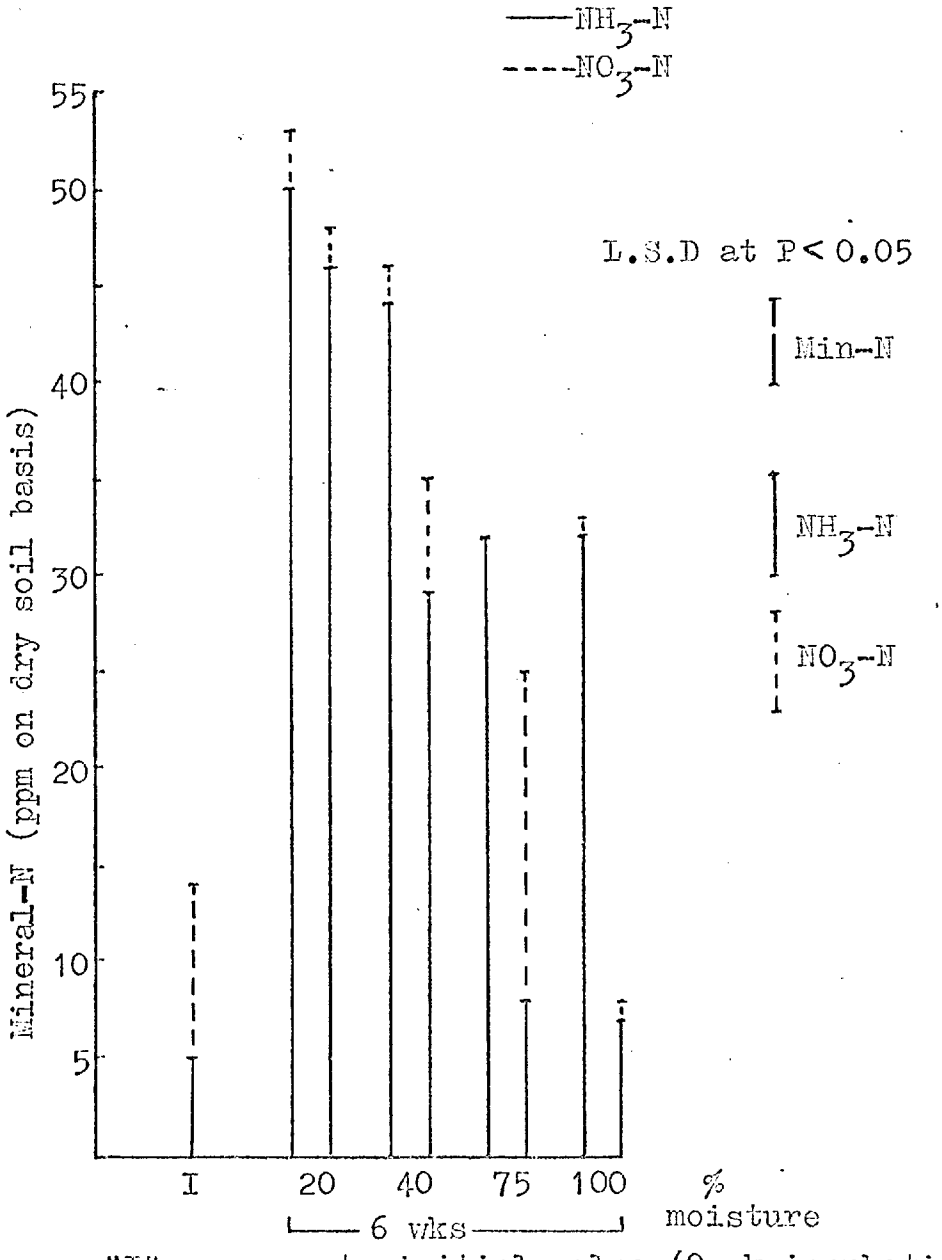
Silwood soil (2mm sieved) was treated with 0.2% calcium carbonate, placed in a pot and kept moist at about 50% mwhc for 3 months at room temperature. The soil was then air dried and ground to pass through a 2mm sieve. Treatments were as in the previous experiment except that 1000 ppm Cu was added as CuO and 200 ppm N as dried blood was added to all, including the control, soils. The moisture levels used during incubation ($30^{\circ}C$) were also the same as in the previous experiment, but incubation was for 6 weeks. Extraction after incubation was also the same as for the previous experiment.

RESULTS

Results for ammonia- and nitrate-N initially and after 6 weeks of incubation are shown in Fig. 13 and Table 15. The table also shows results for EDTA extractable Cu and pH initially and after incubation.

Fig:- 13

Effects of varying levels of moisture with and without 1000 ppm Cu (as oxide) on ammonia- and nitrate-N levels of Silwood soil made slightly calcareous and with addition of 200 ppm N (as dried blood) after 6 weeks of incubation.



"I" represents initial value (0 wk incubation)

For each pair of each moisture content the left-hand value represents no added Cu and the right-hand value addition of 1000 ppm Cu.

Table 15

Levels of ammonia- and nitrate-N, pH values and Cu levels initially (zero week incubation) and after 6 weeks incubation at varying moisture levels (20, 40, 75 and 100% mwhc) of Silwood soil with and without addition of 1000 ppm Cu (as oxide). (200 ppm N as dried blood added to all).

(Results are given in ppm on dry soil basis)

Treatments		Initial values					After incubation				
% moisture	Cu	NO_3 N	NH_3 N	Min N	Cu	pH	NO_3 N	NH_3 N	Min N	Cu	pH
20	0	9	5	14	3.2	6.8	3	50	53	2.0	7.2
40	0	9	5	14	3.2	6.8	2	44	46	2.0	7.4
75	0	9	5	14	3.2	6.8	0	32	32	1.2	7.7
100	0	9	5	14	3.2	6.8	1	32	33	0.9	7.4
20	1000	9	5	14	88	6.8	2	46	48	164	7.5
40	1000	9	5	14	88	6.8	6	29	35	266	7.3
75	1000	9	5	14	88	6.8	17	8	25	290	7.3
100	1000	9	5	14	88	6.8	1	7	8	288	7.2

L.S.D at $P < 0.05$ for

Min-N = 4.2, NH_3-N = 5.4 and NO_3-N = 4.6

Mineral-N accumulation due to incubation in the absence of Cu decreased with increasing moisture contents up to 75% mwhc, but was not further affected at 100% mwhc. The Cu treatment decreased mineral-N accumulation to a small extent at 20, 40, and 75% mwhc, but to considerable extent at 100% mwhc. In fact mineral-N content after incubation at 100% mwhc was less than that present initially.

In the control soils (nil Cu) nitrate was significantly less after incubation at all moisture levels than that present initially. In the Cu-treated soils nitrate levels were also less after incubation than intially at moisture content of 20 and 100% mwhc ; at 40% mwhc Cu treatment had no significant effect on nitrate level, whilst at 75% mwhc Cu treatment significantly increased the nitrate level compared with the initial value.

Copper decreased ammonia accumulation, compared with the corresponding controls, to a small extent during incubation at 20% mwhc and to a fair extent at 40% mwhc, whilst at 75 and 100% mwhc Cu virtually prevented any ammonia accumulation.

EDTA extractable Cu after incubation were significantly lower with the 20% mwhc moisture level than with the other moisture levels, among which there were no significant differences due to moisture level. There were only small differences in pH (0.1-0.4 units) after incubation due to the Cu

treatment at all moisture levels.

GENERAL DISCUSSION FOR CHAPTER VI

In experiment 1 the generally low accumulation of nitrate even at the aerobic moisture levels of 20 and 40% mwhc was due to the low pH of the soils. Without added Cu soil pH was 6.3-6.4 and this was on the border line where nitrification was being limited by pH. In the Cu-treated soils where pH was 5.4, it was not surprising that no nitrate accumulated during incubation. In the second experiment, where a soil of high pH with addition of dried blood was used there was no nitrification even at 20 and 40% mwhc either in the absence or presence of Cu. This is probably due to the high accumulation of ammonia, arising from the mineralisation of dried blood, inhibiting the activity of nitrifying organisms. However, at 75% mwhc there was a significant accumulation of nitrate only where Cu was added. It appeared, therefore, that Cu was able to overcome the effects of high ammonia levels in inhibiting nitrification, but only at this particular moisture level.

In both experiments mineral-N accumulation during incubation was greater at 20-40% mwhc than at 75-100% mwhc even in the absence of Cu. At 75-100% mwhc anaerobic effects were apparent as shown by partial or complete disappearance of nitrate originally present. Under anaerobic conditions nitrate

can disappear by (a) denitrification (190,191) and/or (b) microbial assimilation of nitrate originally present (192). Under anaerobic conditions there is incomplete mineralisation of organic materials and the relatively simple intermediate products formed would then decompose further, and the micro-organisms decomposing them would preferentially utilise nitrate over ammonia. The ability of 1000 ppm Cu to overcome the anaerobic effects at 75% mwhc in experiment 2, resulting in the accumulation of significant amount of nitrate must be due to a direct effect of Cu in stimulating nitrification even under this partially anaerobic condition. Under truly anaerobic condition of 100% mwhc Cu had no such effect, in that nitrate initially present was virtually completely immobilised in both experiments irrespective of addition of Cu.

In both experiments Cu had considerably greater effects in decreasing mineralisation of nitrogen at the higher than at the lower moisture content. Thus Cu appeared to be more toxic to mineralising organisms under partially or completely anaerobic conditions than under aerobic conditions. Mineralisation of N under aerobic conditions is due to both bacteria and fungi, whilst under anaerobic conditions it is due mainly to fungi. This could explain the greater toxic effects of Cu on N mineralisation at 100% mwhc than at lower moisture contents, in view of the toxicity of Cu compounds on fungal activity.

The generally higher levels of chelated Cu (EDTA extractable Cu) in experiment 1 than in experiment 2 is probably due to the lower pH in the former than in the latter experiment and possibly also to the fact that Cu was added as sulphate in the former and as oxide in the latter experiment. In addition, the ability of dried blood, added in experiment 2, in fixing some of the Cu in a form non-exchangeable by EDTA may also have accounted for the difference. This is confirmed by the fact that EDTA-Cu was much lower initially than after incubation at all moisture contents ; presumably Cu strongly fixed by dried blood initially was released when dried blood decomposed during incubation.

SUMMARY OF CHAPTER VI

The effects of varying soil moisture content (20, 40, 75 and 100% mwhc) during incubation of Silwood soil treated with 1000 ppm Cu (as sulphate or oxide) on ammonia and nitrate levels were studied without and with addition of dried blood.

In the first experiment where 1000 ppm Cu was added as sulphate, but no N was added, mineralisation of N and ammonia accumulation were decreased by Cu treatment at all moisture levels except at 75% mwhc where it were unaffected. The Cu treatment had no effect on nitrate accumulation at any of the moisture levels.

In the second experiment where 1000 ppm Cu was added as oxide and 200 ppm N was added as dried blood mineral nitrogen accumulation was decreased by Cu at all moisture levels, but to a greater extent at 100% mwhc than at the lower moisture levels. Nitrate accumulation was increased by Cu at 75% mwhc, but was not affected at other moisture levels. Ammonia accumulation was decreased by Cu at all moisture levels, but to considerably greater extent at 75% and 100% mwhc than at 20 or 40% mwhc.

Where no N was added the levels of EDTA extractable Cu after incubation decreased with increasing moisture content. Where N (dried blood) was added EDTA extractable Cu after incubation was higher at 40-100% mwhc than at 20% mwhc.

CHAPTER VII

CONTRIBUTION OF INORGANIC AND ORGANIC SOIL FRACTIONS TO FIXATION OF COPPER IN SOILS.

Experiment 1. Fractionation of Cu, Zn, Fe and Mn in normal and humus-free Silwood and Harlington soils treated with 100 ppm Cu as sulphate.

Experiment 2. Contribution of inorganic and organic soil fractions to fixation of Cu^{2+} against water extraction.

Experiment 3. Contribution of inorganic and organic soil fractions to fixation of Cu^{2+} against 0.5N NaOAc extraction.

CHAPTER VII

CONTRIBUTION OF INORGANIC AND ORGANIC SOIL FRACTIONS TO FIXATION OF COPPER IN SOILS.

INTRODUCTION

This chapter reports on experiments on the fixation of Cu by normal Silwood and Harlington soils compared with the same soils in which organic matter (humus) had been destroyed by treatment with hydrogen peroxide. In this way the results obtained with the humus-free soils would indicate the contribution of the inorganic soil fraction to fixation, whilst the differences in results between the normal and humus-free soils would indicate the contribution of the organic soil fraction.

In the first experiment the normal and humus-free soils were treated with $CuSO_4.5H_2O$ and, after allowing reaction for some time, the Cu was fractionated by successive extraction with 0.5N NaOAc (pH 7.0), Morgan's reagent and 0.1N EDTA-Na (pH 4.0). In this experiment Zn, Fe and Mn were also included in the fractionation although these elements were not added. In the other two experiments the fixation of Cu by normal and humus-free Silwood and Harlington soils was determined by shaking the soils with varying concentrations of Cu^{2+} dissolved in water and in 0.5N NaOAc.

Preparation of humus-free soils:- 100g portions of Silwood and Harlington soils (air dried and 2mm sieved) were placed in 500 ml beaker and treated with 20 ml of water and 15 ml of 100vol (30%) hydrogen peroxide. The beakers were heated gently with constant stirring until boiling. A few drops of caprylic alcohol were added when frothing became excessive. After cooling another 15 ml portion of H_2O_2 was added and the mixture was again brought to boiling. The H_2O_2 treatment was given once more and the contents boiled. After cooling the soils were separated from the solution and washed with water several times by centrifuging and filtering and then allowed to air dry and ground to pass an 0.5mm sieve.

Experiment 1

Fractionation of Cu, Zn, Fe and Mn in normal and humus-free Silwood and Harlington soils treated with 100 ppm Cu as sulphate.

METHODS

10g portions of normal and humus-free Silwood and Harlington soils (0.5mm sieved) were placed in petri dishes and treated with aqueous solution of $CuSO_4.5H_2O$ so as to supply 100 ppm Cu on the soil basis in an amount of water equal to

the maximum water holding capacities of the soils. The soils were then allowed to air dry at room temperature. The soils were then rewetted with water and again allowed to air dry and this process was repeated once more. The three cycles of wetting and air drying took about 2 weeks. After final air drying the soils were ground to pass an 0.5mm sieve. 2g portions of each type of soil were then successively extracted with 20 ml portions of 0.5N NaOAc (pH 7.0), Morgan's reagent and 0.1N EDTA-Na (pH 4.0), centrifuging and filtering each extract. The extracts were then analysed for Cu, Zn, Fe and Mn.

RESULTS

Results for normal and humus-free Silwood and Harlington soils are shown in Table 16. It will again be mentioned that Morgan extractable values include NaOAc extractable values and EDTA extractable values include Morgan and NaOAc values.

Copper:- The normal Silwood soil retained significantly less Cu against extraction with EDTA than did the humus-free soil, but the reverse was true for the Harlington soil. No Cu was extracted from the normal Silwood and Harlington soils by NaOAc, whilst significant though small amounts were extracted from both the humus-free soils. The biggest difference due to humus removal occurred in Morgan extract, which removed

Table 16

Contribution of Cu, Zn, Fe and Mn (successive extraction with 0.5N NaOAc, Morgan reagent and 0.1N EDTA-Na, pH 4.0) in normal and humus-free soils treated with 100 ppm Cu (as sulphate).

(Results are given in ppm on dry soil basis)

Soil type	Extracts used	Soil fraction	Cu	Zn	Fe	Mn
Silwood	0.5N NaOAc	Normal	0	2.9	5	17
		H-Free	6.0	2.5	0	12
	Morgan reagent	Normal	17	14	10	31
		H-Free	50	16	375	25
	0.1N EDTA-Na	Normal	66	25	100	45
		H-Free	75	26	462	34
Harlington	0.5N NaOAc	Normal	0	3.5	5	5
		H-Free	13	7.0	0	58
	Morgan reagent	Normal	15	30	10	15
		H-Free	49	29	350	86
	0.1N EDTA-Na	Normal	92	75	328	149
		H-Free	80	53	505	103
L.S.D at $P < 0.05$			1.1	2.7	4.5	2.2

about half the added Cu from humus-free soils, but only about one-sixth from the normal soils.

Zinc:- The amount of Zn extracted by all the reagents from both types of soil were generally of the same order irrespective of removal of humus, except for higher values of EDTA-Zn in the normal than in the humus-free Harlington soil.

Iron:- The removal of humus had a significant, though relatively small, effect on NaOAc-Fe from both soils compared with the effect of removal of humus on Morgan-Fe, which was increased by about 35 times by removal of humus. The removal of humus also increased EDTA-Fe to a fair extent, more so with the Silwood than with the Harlington soil.

Manganese:- The removal of humus decreased somewhat the EDTA-Mn in both soils. Humus removal slightly decreased NaOAc-Mn and Morgan-Mn in the Silwood soil, but increased both forms to a fair extent in the Harlington soil.

DISCUSSION

The Silwood soil contains 5.5% and Harlington soil 16% clay. However, both soils contain very similar amounts of organic carbon, 2.1% in the Silwood and 2.0% in the Harlington soil. If the conventional factor of 1.724 (171) is used to convert organic carbon to humus this gives humus values of 3.6% for Silwood and 3.4% for Harlington. Thus any

differences in the effects of removal of humus on fixation of mineral elements between the two soil types can be ascribed to the differences in their clay content. This difference is shown particularly in the effects of humus removal on retention of added Cu in exchangeable form (0.5N NaOAc). Thus although all the added Cu was fixed in non-exchangeable form in both soils, the removal of humus resulted in less fixation of added Cu in exchangeable form in the Silwood than in the Harlington soil. This indicates that humus has the ability to convert exchangeable Cu to non-exchangeable form. This ability is more pronounced in Harlington than in Silwood soil.

If the truly chelated forms of Cu are considered (the difference between EDTA and NaOAc extractable values) it is seen that removal of humus had little effect on the extent of conversion of added Cu to chelated forms in Silwood soil, but decreased by about 27% the extent of chelation of the added Cu in the Harlington soil. This difference must be due to the differences in clay contents between these two soils.

The Morgan extract is a measure of potential availability of Cu to plants and probably also to the microorganisms. The much higher Morgan-Cu values in humus-free than in the normal Silwood and Harlington soils indicate that the presence of humus would decrease the effects of toxic levels of Cu on plants where soil Cu levels are high. On the other hand where soil

Cu levels are low the presence of humus could decrease Cu to such low levels that plants may suffer from lack of Cu. It is well known that plants grown on reclaimed organic soils frequently tend to suffer from Cu deficiency and this is presumably due to the ability of such soils to absorb Cu very strongly in chelated form (193).

Extractable Zn, Fe and Mn from normal and humus-free soils generally showed no consistent differences except for Fe, which was increased to fair extent in EDTA extract and to a considerable extent in the Morgan extract after removal of humus from both soils. This indicates an important effect of humus in maintaining extractable Fe at relatively low levels, presumably by chelation. However, this conclusion cannot be considered very definite since the hydrogen peroxide treatments to destroy humus may have brought some of these elements into solution and part of these elements may have been lost during separation of the humus-free soils from the solutions.

Experiment 2

Contribution of inorganic and organic soil fractions to fixation of Cu^{2+} against water extraction.

METHODS

1g samples of normal and humus-free Silwood and Harlington soils were shaken for 30 minutes with 10 ml of aqueous solution of copper acetate containing 100, 200, 500 and 1000 ppm Cu. The solution was separated from the soil by centrifuging and filtering. Cu was determined in the solution (filtrate).

RESULTS

Results are shown in Fig. 14 and Table 17a. In the table the results are presented in terms of total fixed Cu (obtained by subtracting Cu present in solution after shaking from that present initially for each concentration of Cu used) and also Cu fixed by the organic soil fraction (obtained by subtracting results for humus-free soils from those of normal soils). The table also shows the percentage contributions in fixation of Cu by the inorganic and organic fractions. Table 17b shows total, inorganically and organically fixed Cu after shaking the soils with 10 ml of 500 ppm Cu in water, and also the

Fig:- 14

Levels of Cu fixed by total, inorganic, and organic fractions of Silwood and Harlington soils against water extraction.

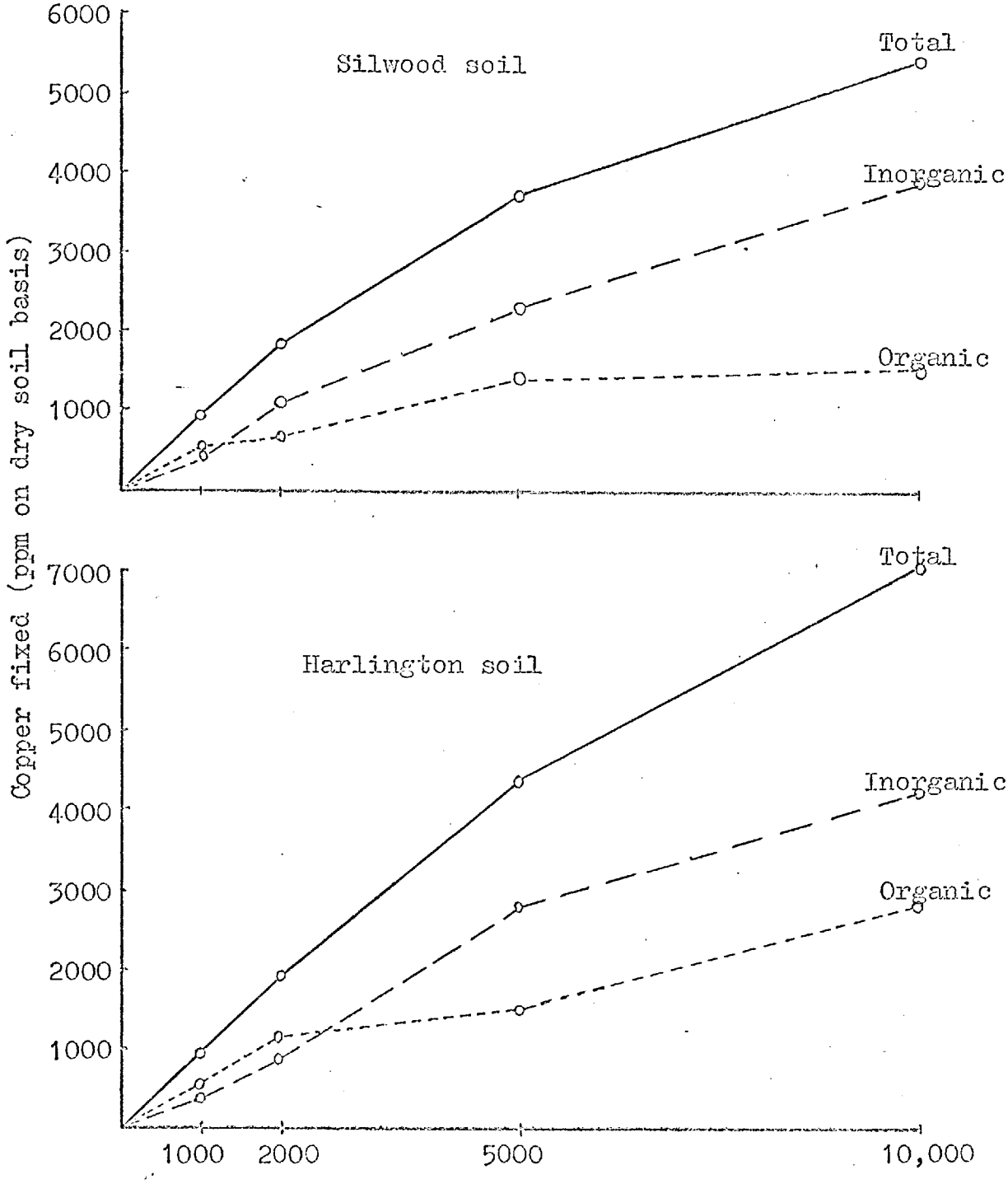


Table 17a

Levels of Cu fixed by total, inorganic and organic soil fractions against water extraction.

(Results are given in ppm on dry soil basis)

Soil type	Added Cu	Total fixed	Inorganically		Organically	
			Value obtained	% of total	Value obtained	% of total
Silwood	1000	965	450	47	515	53
	2000	1820	1100	60	720	40
	5000	3725	2300	62	1425	38
	10,000	5400	3900	72	1500	28
Harlington	1000	987	400	40	587	60
	2000	1953	850	43	1103	57
	5000	4300	2800	65	1500	35
	10,000	7000	4200	60	2800	40

Table 17b

Levels of total, inorganically, and organically fixed Cu (in m.equiv. per 100g soil) after shaking with aq. soln. supplying 5000 ppm Cu^{2+} (soil basis) and Cu fixed by humus (in m.equiv. per 100g humus).

Cu fixed by	Silwood	Harlington
Total	11.6	13.4
Organic	4.4	4.6
Inorganic	7.2	8.8
Fixed by humus	124	138

extent of Cu fixation by humus (assuming % humus = % organic C x 1.724) all expressed in m.equiv. per 100 grams.

In both soils Cu was fixed to a greater extent by the organic than by the inorganic soil fraction at the lowest level of added Cu. However, with increasing level of added Cu the proportion of Cu fixed by the inorganic fraction increased, whilst that fixed by the organic fraction decreased. With the highest level of Cu 72% of the Cu was fixed in inorganic form in the Silwood soil and 60% in the Harlington soil. The extent of fixation of Cu increased linearly in both soils with the amount of Cu added up to 2000 ppm on the soil basis, but the extent of fixation decreased with further increasing level of added Cu. With the lowest level of Cu both Silwood and Harlington soils fixed approximately the same amount of Cu in organic and inorganic forms. In the Harlington soil the amount fixed by the inorganic fraction increased to a greater extent with increasing Cu than in the Silwood soil.

At the highest level of added Cu (10,000 ppm soil basis) the Silwood soil fixed 16.9 m.equiv. and the Harlington soil 21.9 m.equivalent Cu per 100g soil against extraction with water (Table 19 in the following experiment 3). At the 5000 ppm Cu (soil basis) the figures for fixation by Silwood soil were 11.6, 4.4 and 7.2, and by Harlington soil 13.4, 4.6 and 8.8 for total, organically and inorganically fixed Cu respectively (Table 17b). The amounts of Cu fixed by Silwood- and

Harlington-Humus at the 5000 ppm Cu level (using the conversion factor 1.724 x Organic C) were 124 and 138 m.equiv. per 100g humus respectively.

Experiment 3

Contribution of inorganic and organic soil fractions to fixation of Cu^{2+} against 0.5N NaOAc extraction.

METHODS

The methods used were the same as described in the previous experiment except that 0.5N NaOAc was used instead of water and the concentrations of Cu in NaOAc were 100, 200 and 500 ppm (to supply 1000, 2000 and 5000 ppm Cu on soil basis).

RESULTS

Results are shown in Fig. 15 and Table 18a. The manner in which the results are set out are as described in the previous experiment, except that a 10,000 ppm Cu level (soil basis) was not included in this experiment.

In both soils Cu was fixed to a greater extent by the organic than by the inorganic fraction at all levels of added Cu, but there were relatively small differences in the percentage fixed in either form due to Cu levels. The highest

Fig:- 15

Levels of Cu fixed by total, inorganic, and organic fractions of Silwood and Harlington soils against 0.5N NaOAc extraction.

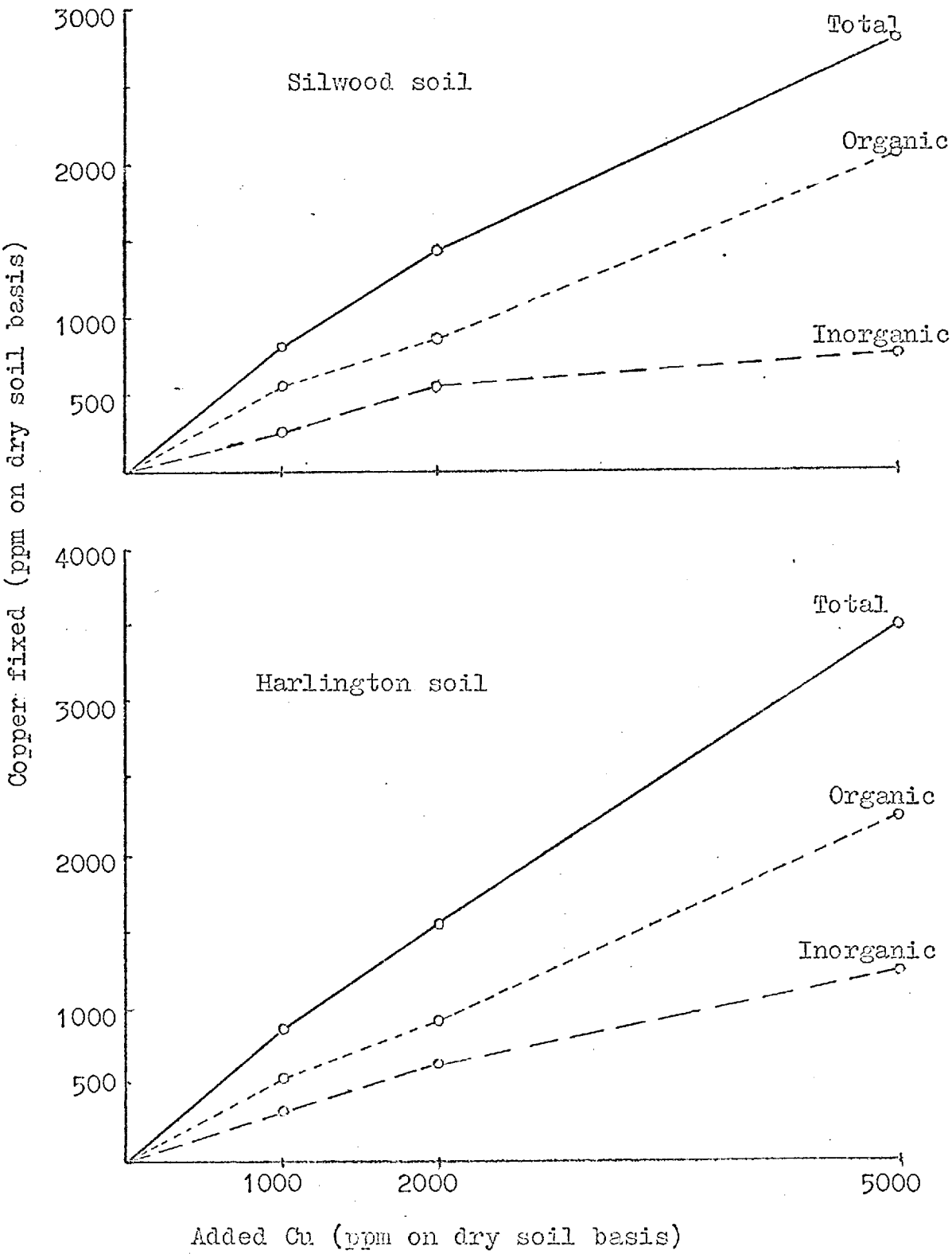


Table 18a

Levels of Cu fixed by total, inorganic and organic soil fractions against 0.5N NaOAc extraction.

(Results are given in ppm on dry soil basis)

Soil type	Added Cu	Total fixed	Inorganically		Organically	
			Value obtained	% of total	Value obtained	% of total
Silwood	1000	812	250	31	562	69
	2000	1425	550	38	875	62
	5000	2800	750	27	2050	73
Harlington	1000	875	337	39	538	61
	2000	1570	650	41	920	59
	5000	3500	1250	36	2250	64

Table 18b

Levels of total, inorganically, and organically fixed Cu (in m.equiv. per 100g soil) after shaking with 0.5N NaOAc soln. supplying 5000 ppm Cu^{2+} (soil basis) and Cu fixed by humus (in m.equiv. per 100g humus).

Cu fixed by	Silwood	Harlington
Total	8.8	10.9
Organic	6.4	7.0
Inorganic	2.4	3.9
Fixed by humus	178	207

Table 19

Levels of total Cu fixed by normal soils from water and NaOAc solution supplying 1000-10,000 ppm Cu (soil basis).

(Results are given in m.equiv. per 100g soil)

Soil type	Added Cu	Water	NaOAc	Exchangeable
Silwood	1000	3.0	2.5	0.5
	2000	5.7	4.5	1.2
	5000	11.6	8.8	2.8
	10,000	16.9	-	-
Harlington	1000	3.1	2.7	0.4
	2000	6.1	4.9	1.2
	5000	13.4	10.9	2.5
	10,000	21.9	-	-

Cation exchange capacity for Silwood soil = 7.6 m.equiv. per 100g

" " " " Harlington soil = 14.0 m.equiv. per 100g

proportion of inorganic fixation occurred with 2000 ppm added Cu, whilst the highest proportion of organic fixation occurred with 5000 ppm added Cu in both soils. In both soils the extent of organic fixation of Cu increased almost linearly with increasing Cu level up to 5000 ppm. On the other hand the extent of inorganic fixation increased linearly with amount of Cu added up to 2000 ppm, but fell off with the 5000 ppm Cu, more so in the Silwood than in the Harlington soil.

At the 5000 ppm Cu level the Silwood soil fixed 8.8, 6.4 and 2.4 m.equiv. and the Harlington soil fixed 10.9, 7.0 and 3.9 m.equiv. Cu per 100g soil in total, organically and inorganically bound forms respectively. The amount of Cu fixed by humus at 5000 ppm applied Cu level was 178 m.equiv. per 100g humus for Silwood-humus and 207 m.equiv. per 100g humus for Harlington-humus (Table 18b).

DISCUSSION FOR EXPERIMENTS 2 AND 3

Copper fixed against extraction with water includes exchangeable Cu, chelated Cu and also Cu held very strongly in forms which are not extracted by EDTA. The results obtained in experiment 1 of this chapter (Table 16) confirmed the last point. The Cu fixed against extraction with NaOAc would exclude exchangeable Cu by definition, but would include chelated and very strongly held forms of Cu. For the purpose of

this discussion the very strongly held forms of Cu will also be considered as chelated forms (it is possible that chelating agents stronger than EDTA could extract more Cu than EDTA).

When a soil is shaken with a solution containing Cu^{2+} there will be competition between exchange position and chelating position in the soil for the Cu ions. At low concentrations of added Cu (1000 ppm in Silwood and 1000-2000 ppm in Harlington soil) the organic fraction fixed more Cu than did the inorganic fraction against water extraction. With increasing Cu level, however, the inorganic fraction was more effective than the organic fraction in fixing Cu against water extraction. This indicates a stronger bonding capacity by the organic than by inorganic materials in the soil. It seems likely that the organic fraction fixes Cu mainly by covalent and chelate bonds and the inorganic fraction mainly by ionic bonds, so it is not surprising that the organic fraction should compete successfully with the inorganic fraction when the supply of Cu^{2+} is limited. However, when adequate Cu^{2+} is present the organic fraction is first satisfied, and the inorganic fraction can then fix more Cu. This is shown clearly with the Silwood soil receiving the higher levels of Cu, where there was no difference in organically fixed Cu between the 5000 and 10,000 ppm Cu levels, whilst the inorganic fraction fixed more Cu from the 10,000 ppm Cu level. In the Harlington soil on the other hand organic fixation of Cu was greater with

10,000 than with 5000 ppm Cu. It appeared, therefore, that the organic fraction of the Harlington soil has a greater capacity for fixing Cu than has the Silwood soil. This fact is shown up in the greater extent of fixation of Cu by Harlington-humus than by Silwood-humus against water (Table 17b) and against NaOAc extraction (Table 18b).

The most important difference between water extraction and NaOAc extraction with respect to the proportions of added Cu fixed by organic and inorganic soil materials is seen in the fact that organic material fixed a higher amount and proportion of the applied Cu than did the inorganic material at all levels of applied Cu in both soils, whereas against water extraction inorganic material fixed the same or greater amounts of Cu than did the organic material. The inorganic fraction of the Silwood soil appeared to have been almost satisfied by 2000 ppm applied Cu where NaOAc was used, as shown by only a small increase in fixed Cu between 2000 and 5000 ppm applied Cu. Cu fixed against extraction by NaOAc would, by definition, exclude exchangeable Cu. Any Cu fixed would, therefore, be fixed by covalent or chelate bonds. In the Silwood soil the relatively low level of inorganically fixed Cu even with 5000 ppm added Cu indicates a low capacity of the inorganic material to fix Cu by covalent or chelate bonds. In the Harlington soil on the other hand the rather greater extent of inorganic Cu fixation with 5000 than with 2000 ppm applied Cu indicates

that the inorganic fraction has not yet been satisfied. This is not surprising in view of the higher clay content of the Harlington (16% clay) than that of the Silwood (5.5% clay) soil.

It can be seen from the Table 19 that where 10,000 ppm Cu was applied the extent of total fixation of Cu against extraction with water exceeded the cation exchange capacities (as determined by the ammonium acetate method) of both soils. With the Silwood soil this occurred even where 5000 ppm Cu was applied. This apparently shows that providing sufficient Cu is present for fixation the exchange positions can be satisfied and further Cu can be fixed by chelation. The difference in total fixation between water and NaOAc extractions for 1000-5000 ppm Cu levels represents the amount of Cu fixed in exchangeable form (Table 19). It is seen that even with the 5000 ppm applied Cu level exchangeable Cu in both soils was considerably less than the cation exchange capacities of the soils. This indicates that Cu is preferentially fixed by chelation, presumably by the organic fraction, rather than by exchange. This indicates that a fair proportion of the exchange positions would still be available for adsorption of the other cations, e.g. Ca^{++} and K^{+}, which cannot be held by chelation.

SUMMARY OF CHAPTER VII

A study was made of the contribution of inorganic and organic soil fractions to fixation of Cu in Silwood and Harlington soils by reacting normal and humus-free (hydrogen peroxide treated) soils with Cu^{2+} and determining the extent and mode of fixation of Cu.

In the first experiment 100 ppm Cu (soil basis) as $CuSO_4.5H_2O$ was allowed to react with normal and humus-free soils which were then extracted successively with 0.5N NaOAc, Morgan reagent and 0.1N EDTA-Na (pH 4.0). All the added Cu was fixed in non-exchangeable form in the normal soils, but there was some fixation of Cu in exchangeable form in humus-free soils. The Morgan reagent extracted much more Cu from the humus-free than from the normal soils. The normal Silwood soil retained significantly less Cu against extraction with EDTA than did the humus-free soils, but the reverese was true for the Harlington soils. Extractable values for Zn, Fe and Mn are also presented.

In experiment 2 the extent of fixation of Cu against water extraction by normal and humus-free soils was determined by shaking 1g sample with 10 ml of aqueous solution of copper acetate so as to supply 1000, 2000, 5000 and 10,000 ppm Cu on the soil basis. Experiment 3 was similar to experiment 2 except that fixation of Cu against 0.5N NaOAc extraction was

determined and the Cu levels were used only up to 5000 ppm (soil basis). With the lower concentrations of added Cu the organic soil fraction fixed more Cu than did the inorganic fraction against water extraction, but the reverse was true with the higher levels of added Cu. On the other hand the organic soil fraction fixed more Cu than did the inorganic fraction against NaOAc extraction in both soils with all levels of added Cu. The Harlington-humus fixed more Cu than did the Silwood-humus. The higher inorganic fixation in chelated form in the Harlington than in the Silwood soil is due to the higher clay content of the former soil. At the higher levels of added Cu the amount of total Cu fixed by the normal soils exceeded the cation exchange capacities (as determined by the ammonium acetate method) of the soils. The amounts of exchangeable Cu present in these soils were considerably less than the cation exchange capacities of the soils, indicating that Cu can be fixed by chelation even before all the exchange positions have been filled.

CHAPTER VIII

EFFECTS OF ADDITION OF GRASS, STRAW AND SUCROSE TO SOILS TREATED WITH 10,000 ppm COPPER AS OXIDE ON DOWNWARD MOVEMENT OF COPPER, ZINC, IRON AND MANGANESE DUE TO LEACHING WITH WATER.

CHAPTER VIII

EFFECTS OF ADDITION OF GRASS, STRAW AND SUCROSE TO SOILS TREATED WITH 10,000 ppm COPPER AS OXIDE ON DOWNWARD MOVEMENT OF COPPER, ZINC, IRON AND MANGANESE DUE TO LEACHING WITH WATER.

This experiment reports on the mobility of Cu under the influence of leaching water applied to a 10-inch column of soil the upper 1 inch of which was mixed with 10,000 ppm Cu as CuO. The effects of addition of 3 organic materials (grass, straw and sucrose) mixed with the upper inch of soils were studied. The mobilities of native Zn, Fe and Mn were also determined.

METHODS

The experiment was carried out with 0.75-inch bore glass tubing of 14-inch length and the bottom of each tube was closed with rubber bung having a hole to take a 3-inch length of 3mm bore glass tubing. A disc of glass cloth was placed on the top of the rubber bung. 90g of Silwood soil (pH 5.2, air dried and 2mm sieved) were placed in each of eight such tubes and after tamping down another disc of glass cloth was placed on top of the soil. A separate 100g sample of Silwood soil was mixed with 10,000 ppm Cu (as CuO) on the soil basis. 10g duplicate portions of this Cu-treated soil were then mixed

with (1) 2% by weight of ground dried grass, (2) 2% by weight of ground wheat straw plus ground ammonium nitrate at a rate of N equal to one-hundreth of the weight of straw and (3) 2% by weight of ground sucrose plus N as ammonium nitrate at one-fiftieth the weight of sucrose. In addition, two control 10g quantities of Cu-treated Silwood soil were weighed out. The 10g quantities of control and treated soils were placed on top of the soils already in the column. Thus there were two control columns and two columns for each of the organic matter treatments. Finally, after another tamping, a disc of glass cloth was placed on top of the soil in each column.

All the columns were wetted by sub-irrigation by lowering them gradually into water contained in large measuring cylinders until the top of the soils appeared moist. The columns were then clamped upright in stands and left for 1 week at room temperature (18-22^{o}C). 2 inches of water was then applied to each column and the effluents were analysed for Cu. After standing for another 1 week 2 inches of water was again added and the process was repeated until eight such treatments in all had been given, so that in all 16 inches of water was applied.

After final leaching the columns were kept for 3 weeks so as to partially air dry. The top 1 inch of each column was removed and then successively the 1-2", 2-4", 4-7" and 7-10"

depths were removed and allowed to air dry separately. 5g portons of the air dried samples were then extracted with 10 ml of 0.1N EDTA-Na (pH 4.0) and the extracts were analysed for Cu, Zn, Fe and Mn.

RESULTS

Results are shown in Fig. 16 and Table 20, where the concentrations of EDTA-extractable Cu, Zn, Fe and Mn (in ppm on soil basis) are shown for each soil layer of each treatment, including the control. Because of the widely different concentrations of Cu in the various soil layers it must be noted that the results for Cu in Fig. 16 are presented in 3 different scales. It must also be noted that there are different scales for each of the 4 elements.

No Cu was found in the effluent from any of the leachates of any of the columns.

The results obtained for the 4 elements in different layers of soil were as follows:-

Copper:- The sucrose treatment approximately trebled the amount of Cu found in the 0-1" and 1-2" soil layers compared with the respective control for each layer, whilst grass and straw had no significant effects. However, the levels of Cu found in the 0-1" layer were approximately 20 times greater than those found in the 1-2" layer. The effect of sucrose

Fig:- 16

Levels of Cu, Zn, Fe and Mn in different layers of soil column the top 1-inch of which treated with 10,000 ppm Cu (as CuO) and grass, straw and sucrose.

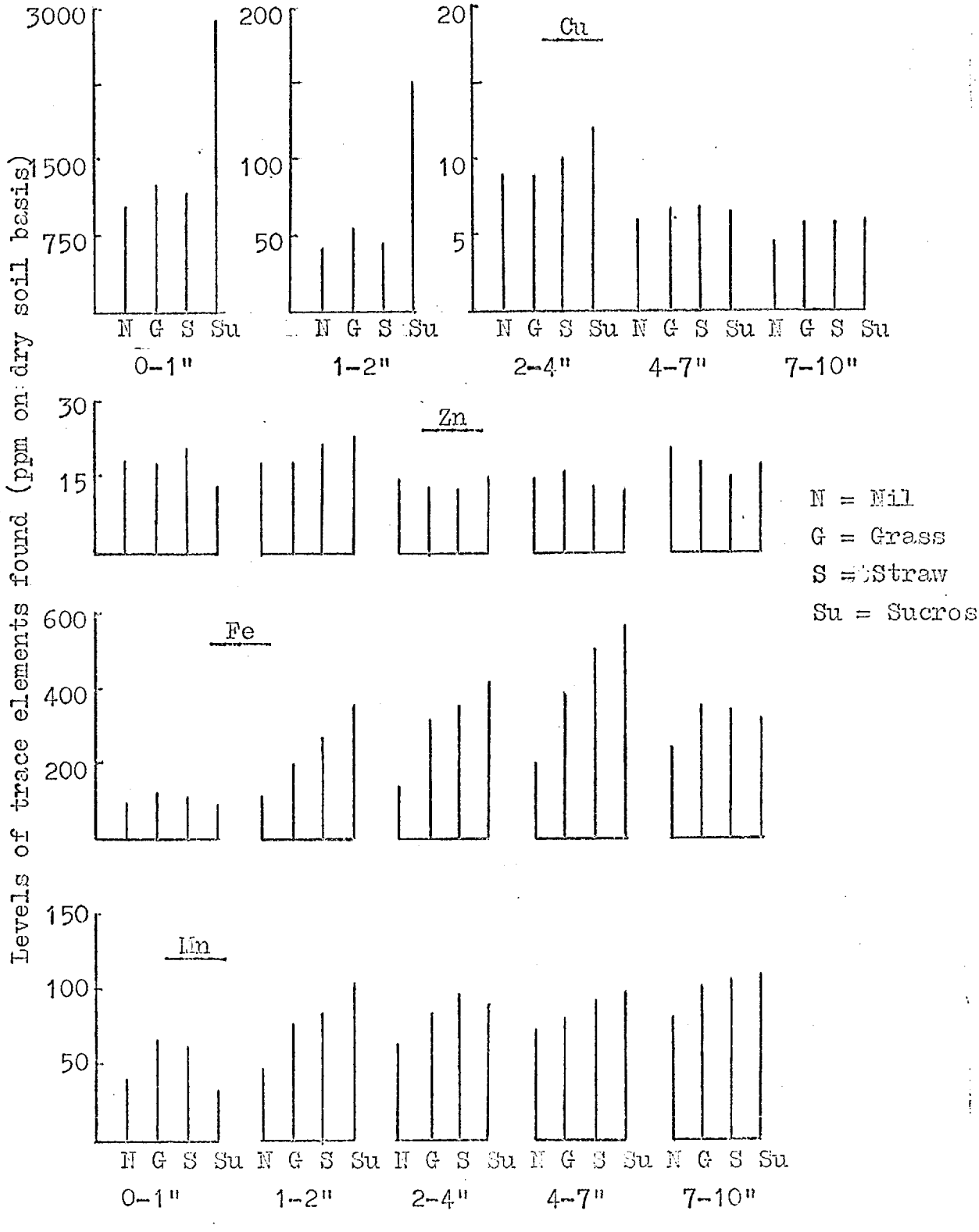


Table 20

Levels of Cu, Zn, Fe and Mn in different layers of soil treated with grass, straw and sucrose.

Treatments	0-1"				1-2"			
	Cu	Zn	Fe	Mn	Cu	Zn	Fe	Mn
Cont	1050	18	101	39	41	18	110	47
Grass	1250	18	128	67	55	18	195	77
Straw	1186	21	110	62	45	22	273	87
Sucrose	2870	13	85	34	149	23	360	103
	2-4"				4-7"			
Cont	9.0	14	140	65	6.0	14	202	75
Grass	9.0	13	318	86	6.7	16	385	83
Straw	10	13	355	98	6.8	13	505	93
Sucrose	12	14	420	90	6.5	12	565	96
	7-10"							
Cont	4.3	20	250	83				
Grass	5.8	18	555	103				
Straw	5.7	14	545	106				
Sucrose	5.8	17	525	110				

L.S.D at $P < 0.05$ for Cu at 0-1" = 350, 1-2" = 15 and for (2-4" to 7-10") = 1.5 ; Zn = 4.2, Fe = 65 and Mn = 16.

treatment in increasing Cu persisted into the 2-4" layer, but not into the lower layers. Grass and straw had no effect on Cu levels in the lower layers. The levels of Cu found in the 2-4" soil layer were only 3-6 ppm greater than that present in the soil before treatment with CuO. In the 4-7" and 7-10" layers the Cu concentrations were not significantly different from that present in the original soil.

Zinc:- The EDTA-Zn levels due to organic matter treatments although sometimes significant, showed only a little downward movement. The only effect of any magnitude was a tendency for sucrose treatment to decrease Zn in the 0-1" and increase it in the 1-2" layer.

Iron:- EDTA-Fe increased somewhat with depth of the soil layers in the control. None of the organic matter treatment significantly affected Fe compared with the control in the 0-1" layer, whilst all treatments increased Fe in all the lower layers compared with the respective controls. Sucrose had a greater effect than straw which, in turn, had a greater effect than did grass in this respect. However, in the lowest layer (7-10") there were no differences in Fe among the organic matter treatments. There was also a trend for Fe to increase with increasing depths of layers with all organic matter treatments down to 4-7" layer although the effect due to grass persisted to the 7-10" layer.

Manganese:- The pattern of movement of Mn was similar in some respect to that of Fe, except that the magnitude of differences due to the organic matter treatments were not as great as with Fe. Grass and straw, but not sucrose, increased EDTA-Mn in the 0-1" layer, whilst all organic materials increased it in the 1-2" layer, with sucrose having the greatest effect. In the lower layers all organic materials increased EDTA-Mn to about the same extent.

DISCUSSION FOR CHAPTER VIII

The considerable effects of sucrose in increasing active Cu (EDTA-Cu) in the zone of application (0-1" layer) as well as in the 1-2" layer is due to the more rapid decomposition of sucrose than of the other materials. Since the soils were not analysed until 2 months after leaching started the effect of sucrose in increasing active Cu persisted in spite of the fact that sucrose must have decomposed completely during earlier stages. Thus there was no tendency for active Cu to revert to non-active form (non-extractable with EDTA) even after the stimulating effect of sucrose had disappeared. In spite of the considerable increase in active Cu in the 0-1" layer due to sucrose only about 5% of this active Cu was leached into the 1-2" layer, and only a very small amount into the 2-4" layer. It is clear, therefore, that there is only

very little downward movement of Cu due to leaching even in the presence of easily decomposable organic material such as sucrose.

The grass and straw applied to the upper inch of soil had no significant effect on active Cu in this zone or on any of the lower layers. Although these materials must have decomposed more slowly than the sucrose it is clear that their decomposition products had no influence in increasing active Cu. The main decomposition product of all these materials would be CO_2. The carbonic acid formed in the soil solution appears, therefore, to have the ability of converting inactive Cu to active form. Only a very small proportion of the active form would be exchangeable and water soluble as indicated by the relatively small downward movement of Cu. Other decomposition products of the organic materials would be organic acids and although these would themselves decompose rapidly they could also exert some effect in bringing Cu into a mobile form. It is clear, however, that even if this did occur where grass and straw were added some other reaction product was formed which opposed any tendency for Cu to be mobilised in leachable form. This other product was probably the residues (humin and humic acid) formed from the decomposing grass and straw and these materials were probably effective in converting any mobile Cu which tended to be formed into immobile form.

The organic matter treatments generally had negligible

effects on the mobility of Zn in the columns. The only effect of any magnitude was a decrease in the active Zn in the zone of application due to sucrose treatment, but this was accompanied by a similar increase in active Zn in the 1-2" layer. The effect of sucrose on Zn mobility was not apparent in any of the lower layers. The mechanism involved in the mobilisation of Zn in the 0-1" layer and in the immobilisation of Zn in the 1-2" layer are probably similar to those occurring with Cu. Results for Cu and Zn cannot strictly be compared since the mobility of Cu is due to added Cu, whilst that of Zn was due to native Zn. However, Zn appeared to have moved more easily from the zone of application of sucrose than had Cu, but both elements were almost entirely retained in the 1-2" layer and the organic matter treatments did not result in further downward movement of either element.

Unlike Cu and Zn which moved downward to only a limited extent and then only with the sucrose treatment, downward movement of Fe was increased to a fair extent by all the organic materials added to the upper 1 inch soil layer. In the zone of application of organic materials the treatments had little effects on the levels of active Fe (EDTA extractable), indicating that mobile Fe resulting from the treatments was leached to lower layers. All organic materials had similar effects in this respect in the zone of application, but there were significant differences due to organic material

treatments in the lower layers. Their effectiveness increased in the order grass, straw, sucrose and this order of effectiveness is positively correlated with rates of decomposition of these materials in soil. The mechanism of the mobility of Fe is probably due to the formation of mobile chelates in the treated layer which are soluble in water and move to lower layers where they be rendered immobile. The mechanism is probably similar to that occurring during podzolisation. Differences due to type of organic materials persisted in all layers, except the lowest, where there were no significant difference in active Fe among the three organic materials used.

The effects of organic materials on the mobility of Mn were in general, similar to their effects on Fe mobility, except that the extent of movement of Mn was not as great as that of Fe and there were not such consistent effects due to type of organic matter applied in the upper zone. Apart from the superiority of sucrose in increasing active Mn in 1-2" layer as compared with grass and straw, there were no significant difference in the lower layers. In addition to the ability of decomposing organic materials to mobilise Mn in chelated forms the higher oxides of Mn present in soil can also be reduced by microbial activity to Mn^{2+} (194,195). The much lower active Mn due to sucrose treatment compared with grass and straw treatment in the treated zone (0-1") is probably due to the greater activity of sucrose in mobilising Mn by the

reducing effect resulting in greater amount of leaching of Mn out of the treated zone.

SUMMARY OF CHAPTER VIII

10-inch columns of Silwood soil (pH 5.2) were placed in glass tubes and held in a vertical position for leaching. The upper inch of soil in each tube was mixed with 10,000 ppm Cu as CuO and duplicate tubes were treated in the top 0-1" layer with 2% by weight of (1) ground dried grass, (2) ground wheat straw plus ammonium nitrate-N at 1% of the weight of the straw, and (3) ground sucrose plus ammonium nitrate-N at 2% of the weight of sucrose. In addition, duplicate control tubes, without added organic materials, but with CuO mixed in the upper layer were also set up. After initial wetting by sub-irrigation water was added in 2"-amount to each tube at weekly intervals over 8 weeks.

No Cu could be detected in any of the leachates from any of the treatments. After partial drying the soils in each tube were separated into 0-1", 1-2", 2-4", 4-7" and 7-10" layers and, after air drying were analysed for Cu, Zn, Fe and Mn by extracting with 0.1N EDTA-Na (pH 4.0).

The levels of EDTA-Cu were not significantly affected, compared with the control, by grass and straw in any of the soil layers. The sucrose treatment increased EDTA-Cu to a

considerable extent in 0–1" and 1–2" layers, but had negligible or non-significant effects in the lower layers.

The organic matter treatments had small though sometime significant effect on EDTA-Zn in 0–1" layer accompanied by a corresponding increase in the 1–2" layer with the sucrose treatment.

None of the treatments affected EDTA-Fe in the 0–1" layer, whilst all treatments increased EDTA-Fe in the lower layers. There was a significant trend for EDTA-Fe to increase with depth of soil and the effects of the treatments increased in the order grass, straw, sucrose.

The grass and straw, but not the sucrose, treatments increased EDTA-Mn in the 0–1" layer, whilst all treatments increased it in the 1–2" layer, the sucrose having the greatest effect. In the lower layers all organic materials increased EDTA-Mn to about the same extent.

CHAPTER IX

GENERAL DISCUSSION AND CONCLUSION

CHAPTER IX

GENERAL DISCUSSION AND CONCLUSION

In the soils where no N or ammonium sulphate was added Cu addition decreased mineralisation of N more in the sandy loam Harlington than in the sandy Silwood soil. Where dried blood was added the same effects were noted with respect to N mineralisation, but nitrification was decreased more in the sandy than in the sandy loam soil. In fact in the latter soil nitrate accumulation was being limited by mineral-N production.

Decrease in pH due to addition of $CuSO_4.5H_2O$ was not sufficient to account for reduction in mineralisation of N and nitrification and the reduction must be ascribed to a direct toxic effect of Cu. Where 6 different loam soils of widely ranging pH were used Cu additions had no consistent effects on N mineralisation or nitrification in relation to soil pH. Where increases in N mineralisation and nitrification occurred this may have been due to the fact that in these soils Cu may have been limiting both processes. In general, the toxic effect of a given level of added Cu were more apparent at low than at high soil pH.

If a soil should become contaminated with high levels of Cu to such an extent that microbial processes and plant growth were being adversely affected then the obvious method of

overcoming this would be to increase soil pH, if this was intially low, to higher levels by liming. This is commonly done in order to eliminate toxic effects of Fe, Mn and Al which frequently occur in soils of low pH. In addition, fixation of phosphate in non-available form in acid soil is commonly prevented by increasing soil pH before application of phosphate fertilisers. Because it was considered that deficiency of Cu with respect to N mineralisation, and in particular, to nitrification would be most apparent in soils of high pH, the studies involving the addition of dried blood (a relatively easily mineralisable source of organic N) were done with soils of high pH and calcareous soils. Since Cu contamination would arise most likely in soils through the addition of insoluble Cu compounds (industrial, mining, and sewage wastes, and pesticides) the effects of addition of CuO and $CuHPO_4$, both water insoluble, were studied. Addition of Cu in these forms also had the advantage in that there was very little change in soil pH in comparison with addition of Cu as sulphate. Thus alteration in pH due to addition of Cu compound was eliminated.

Addition of Cu as CuO or $CuHPO_4$ had virtually identical effects on both mineralisation of N and nitrification, even though the levels of EDTA-extractable Cu by the end of incubation were considerably higher where $CuHPO_4$ than where CuO had been added.

In high pH, but non-calcareous, soils both N mineralisation and nitrification increased with level of added Cu up to 1000 ppm. With 10,000 ppm Cu, although mineralisation was less than with the lower levels of Cu, it was still higher than in the controls. This indicates that soil has a considerable capacity for converting added Cu to non-active forms, presumably carbonates or basic carbonates. The lack of any difference in effects between CuO and $CuHPO_4$ indicated that the PO_4 anion in itself did not influence N mineralisation or nitrification and also did not influence the effects of Cu on these processes.

In three soils which had been pre-treated with sufficient $CaCO_3$ to render them slightly calcareous 100 ppm Cu had no effect on N mineralisation but nitrification was increased in one soil. With 1000 ppm added Cu N mineralisation was usually increased, whilst nitrification was increased in all soils. With 10,000 ppm added Cu, N mineralisation was always increased, although usually to a lesser extent than with 1000 ppm Cu. This indicates that the slightly calcareous soils could tolerate the presence of considerable amounts of Cu without any deleterious effects on either N mineralisation or nitrification. The differences in responses of soils of varying texture indicate that the clay soil was able to detoxify the effects of Cu to a greater extent than were the soils of coarser texture. On the other hand where only 100 ppm Cu was added, the clay

soil immobilised Cu completely so that it had no effect on nitrification, whereas the soils of coarser texture showed increased nitrification.

In 5 natural soils containing amounts of $CaCO_3$ ranging from 0.5% to 22% there were no consistent effects on nitrogen mineralisation and nitrification due to Cu addition in relation to $CaCO_3$ contents. These soils were all of sandy loam texture, hence differences could not be accounted for in terms of clay contents. Presumably other factors, which were not measured, were affecting the differences in response to Cu treatments.

In those experiments on the effects of addition of Cu compounds on N changes in soils containing decomposable organic materials the results differed depending on the nature of the organic materials and the levels of added Cu. The effects of Cu were generally greater during the initial immobilisation phase than during the subsequent re-mobilisation phase. Thus all levels of Cu (100-10,000 ppm) decreased the extent of immobilisation of N, but had little effect on re-mobilisation during the later stages of incubation. Where straw and grass were compared the main effect noted was the ability of Cu to delay the decomposition of straw, as indicated by delayed immobilisation of N, compared with that of grass.

In the study on the effects of Cu on N mineralisation in relation to moisture contents during incubation Cu was more

toxic to mineralising organisms under partially or completely anaerobic than under aerobic conditions. Since N mineralisation under aerobic conditions is due to both bacteria and fungi, whilst under anaerobic conditions it is due mainly to fungi, the greater toxicity of Cu under anaerobic conditions is probably due to the well-known toxicity of Cu on fungal activity. The ability of added Cu to stimulate nitrification under partially anaerobic conditions (75% mwhc) can be ascribed to a direct effect of Cu in stimulating nitrification or to its ability in decreasing denitrification and/or microbial assimilation (immobilisation) of nitrate.

The fractionation studies of Cu fixed by normal and humus-free (hydrogen peroxide treated) soils indicated that a higher amount and proportion of the applied soluble Cu was fixed by the organic than by the inorganic fraction. Where a high level of soluble Cu was allowed to react with the soils the extent of total fixation of Cu against extraction with water exceeded the cation exchange capacities (as determined by N ammonium acetate method) of the soils. This indicates the presence of chelating position, probably organic, which are able to fix amounts of Cu in excess of the cation exchange capacities of the soils. On the other hand where lower levels of Cu were reacted with the soils the Cu was preferentially fixed by chelation rather than by exchange. This indicates that providing sufficient organic matter is present in a soil

added Cu may be held by relatively strong (chelated) fixation and exchange positions would still be available for adsorption of other cations. The well known fact that plants grown on soils high in organic matter frequently tend to suffer from Cu deficiency without any deficiencies of other cations can be explained on this basis.

When Cu was added as Cu^{2+} it was all or virtually all converted to non-exchangeable forms (non-extractable with NaOAc). ~~EDTA~~). This applied particularly to soils of pH greater than 6.0, but even in soils of lower pH more than 90% of the added Cu was found to be non-exchangeable. However, much of the fixed Cu was present in chelated form as indicated by the high levels of EDTA-extractable Cu. There was a trend for the extent of fixation of Cu in chelated forms to decrease with increasing soil pH.

Exchangeable Cu in soils was of no value in indicating the "availability" of Cu to N mineralising and nitrifying organisms. Cu extracted by the Morgan reagent (0.5N acetic acid - 0.75N sodium acetate) was also of little value in this respect, since even though it extracted more Cu from soils than did NaOAc, the results were not related to toxic or stimulating effects of Cu. Even the EDTA extractable Cu (which includes exchangeable and chelated forms) did not always indicate the critical levels of Cu with respect to effects on

N mineralisation and nitrification. Only in the Harlington soil was the extent of decrease in nitrification correlated with the extent of increase in EDTA-Cu. However, the critical level of Cu in this soil differed from that in another soil of similar texture. It is clear that each type of soil must be studied separately to determine the critical levels of Cu, as determined by extraction methods, which would affect nitrification. Presumably the type of clay and the quantity and quality of organic matter are important.

There is a possibility that some of the apparently direct effects of added Cu in affecting N mineralisation and nitrification may be due to the effects of Cu in altering the availability of other trace elements to the microorganisms concerned. Exchangeable Zn was not much affected by addition of low Cu levels to soils, but was increased somewhat by 1000 ppm Cu, but then only in acid soils. This was probably due to the ability of Cu to form more stable chelates in soils, thus displacing Zn from chelated into exchangeable form.

A high level of Cu (1000 ppm) sometimes increased exchangeable Mn to considerable extents and this increase could not be accounted for by the slight change in pH due to Cu treatments. Presumably Cu has a direct effect in stimulating the activity of microorganisms concerned with the conversion of Mn(IV) to Mn(II) compounds. This indicates that Cu addition

to soils may increase the availability of Mn to plants. This might be an advantage in soils of high pH, where Mn is sometimes deficient to plants. On the other hand in soils of low pH, where Mn(II) is usually high, the increase in Mn(II) due to Cu addition could raise available soil Mn to toxic levels.

Even though the levels of Fe removed by various extractants differed considerably with soil pH and soil type, the addition of Cu had little effects on extractable levels of Fe, indicating that Cu is unlikely to affect the availability of Fe to plants.

Some discussion of methods of eliminating Cu toxicity in soils which have been inadvertently treated with high levels of Cu compounds may be made. This would be applicable not only to the effects of Cu on N changes in the soils but also to its effects on toxicity to plants. All the experiments have shown that even where Cu was added in water soluble form most of this was fixed very rapidly in non-exchangeable form. This indicates that any attempt to remove Cu by downward leaching through the application of large amounts of irrigation water would not be successful. This was confirmed in experiment where Cu was applied in the top 1-inch layer of a column of soil and where there was negligible downward movement of Cu due to application of 16-inch of water over 2 months period. Even the application of organic materials in the same layer

as added Cu, in the hope that mobility of Cu would be increased, had negligible effects on downward movement. In fact the only positive effect noted was the ability of Cu applied in the upper zone to increase the downward movement of Fe and Mn.

Another possible method of eliminating toxic level of Cu could be the application of some materials to react with active Cu to convert it to inactive form. The addition of phosphate in water soluble form in the hope that inactive Cu phosphate might be precipitated was not borne out by the experiment comparing the effects of adding Cu as CuO and $CuHPO_4$. The virtually identical effects which both these sources of Cu had on N mineralisation and nitrification indicate that application of phosphate would be of little value in eliminating the toxic effects of Cu.

Since it was found that the toxic effects of Cu on N mineralisation and nitrification were greater at low than at high pH, raising soil pH by liming would be a feasable method of reducing Cu toxicity. This is commonly done to decrease toxicity of Fe, Mn and Al in acid soils.

The addition of rotted or stable organic materials such as farmyard manure, compost, or peat or decomposable materials such as plant residues could also be helpful in decreasing the toxic effects of Cu, because of the ability of organic materials to hold Cu in chelated form, or even in stronger forms, which would not be available to plants.

APPENDIX

1. Effects of pH of 0.1N EDTA-Na on extractable levels of Cu, Zn, Fe and Mn from soils of different pH.

2. Effects of various levels of Cu compounds on Morgan-extractable phosphate during incubation of soils.

3. Effects of addition of sewage sludge on mineral nitrogen levels during incubation of soil treated with 0.2% sucrose and 100 ppm N as KNO_3.

APPENDIX

1. Effects of pH of 0.1N EDTA-Na on extractable levels of Cu, Zn, Fe and Mn from soils of different pH.

METHODS

500g samples of Silwood soil were adjusted to various pH levels as described in Chapter II. After air drying and sieving through a 2mm sieve, soils of pH 4.8, 6.0, and 7.4 were selected for study. Each soil was mixed with $CuSO_4.5H_2O$ to give an added rate of Cu of 100 ppm on the dry soil basis. The soils were kept moist (50% mwhc) for 2 weeks and were then air dried and ground to pass a 2mm sieve. 0.1N EDTA solutions of pH 4.0, 5.5, 7.0, and 8.0 were prepared by using varying levels of sodium hydroxide and starting with H_4-EDTA. 10g portions of soil of each pH were shaken for 2 minutes with 20 ml portion of 0.1N EDTA of different pH (EDTA-Na) and after centrifuging and filtering, the extracts were analysed for Cu, Zn, Fe and Mn.

RESULTS

Results for extractable Cu, Zn, Fe and Mn, on the dry soil basis are shown in Table 21-A.

At any pH of EDTA, extractable Cu was little affected by

Table 21-A

Levels of Cu, Zn, Fe and Mn in soils of different pH (4.8, 6.0 and 7.4) as extracted by 0.1N EDTA-Na of different pH (4.0, 5.5, 7.0 and 8.0).

(Results are given in ppm on dry soil basis)

pH of EDTA-Na	Soil pH 4.8	6.0	7.4	Soil pH 4.8	6.0	7.4
		Cu			Zn	
4.0	110	120	120	31	80	31
5.5	112	115	115	27	61	30
7.0	105	108	115	22	33	22
8.0	100	100	103	20	30	17
		Fe			Mn	
4.0	345	183	222	56	61	65
5.5	258	128	165	48	40	42
7.0	253	110	137	43	27	22
8.0	225	91	119	37	16	14
L.S.D at P <0.05	for Cu = 8.5, Zn = 6.2, Fe = 15 and Mn = 6.5					

soil pH, although there was a slight trend for it to increase with soil pH. At any soil pH there was a trend for extractable Cu to decrease with increasing pH of EDTA. However, the magnitude of the decrease was small, ranging from 1.1- to 1.2-fold.

At any pH of EDTA, Extractable Zn was higher from soil of pH 6.0 than from soils of pH 4.8 and 7.4, between which there were only slight differences. At any soil pH, there was a distinct trend for extractable Zn to decrease with increasing pH of EDTA. The decrease ranged from 1.5- to 2.7-fold.

At any pH of EDTA, extractable Fe was highest from soil of pH 4.8, intermediate from soil of pH 7.4 and lowest from soil of pH 6.0. At any soil pH, extractable Fe decreased with increasing pH of EDTA. The decrease ranged from 1.5- to 2.0-fold.

With EDTA of pH 4.0 and 5.5 there was little difference in extractable Mn due to soil pH. However, with EDTA of pH 7.0 and 8.0 there was a distinct trend for extractable Mn to decrease with increasing soil pH. At any soil pH, extractable Mn decreased with increasing pH of EDTA. The decrease was 1.5-, 3.9-, and 4.6-fold for soils of pH 4.0, 6.0, and 7.4 respectively.

DISCUSSION

The main effect to be noted in this experiment was the fact that neither soil pH nor EDTA pH had much effect on the levels of EDTA extractable Cu, whilst there were fair difference due to both variables on levels of extractable Zn, Fe, and Mn. EDTA extracted 81-97% of the total Cu present in the soils (24 ppm native Cu plus 100 ppm added Cu).

The main purpose of doing this experiment was to see if soil pH and EDTA pH had any effect on the amounts of extractable Cu. The results obtained indicate that neither of these factors influenced EDTA-extractable Cu very much. The original proposal (178) for using 0.1N EDTA of pH 4.0 for assessing the Cu status of soils does not take into account the possible ability of soils of high pH to increase the pH of EDTA (which is not well-buffered at 0.1N concentration). This experiment has shown, however, that the pH of EDTA had relatively little effects on levels of extractable Cu. However, since many of the experiments in this study used soils of high pH it was decided that with such soils EDTA of pH 7.0 should be used.

2. Effects of various levels of Cu compounds on Morgan-extractable phosphate during incubation of soils.

In two experiments (experiments 2 and 3 in Chapter V) in which the effects of Cu compounds on N changes were studied

the opportunity was taken, where the Morgan reagent was used to extract mineral-N, also to determine phosphate in this extract by the 1-2-4-aminonaphtholsulphonic acid method (196). The Morgan reagent has been widely used for indicating the potential availability of soil phosphate to plants.

(a) Effects of 100-10,000 ppm Cu (as CuO) on Morgan-extractable phosphate after 1 and 12 weeks of incubation (30°C, 50% mwhc) of Silwood soil (pH 5.4) and Harlington soil (pH 6.2) treated initially with 100 ppm N as KNO_3 and 0.2% sucrose.

Full details of the methods used are described in experiment 2, Chapter V.

Results are shown in Table 22-A, for Morgan-extractable phosphate-P on the dry soil basis initially and after 1 and 12 weeks of incubation.

In the Silwood soil 100 ppm Cu had no effect, whilst 1000 and 10,000 ppm Cu decreased extractable phosphate-P during the immobilisation phase (1 week of incubation). After 12 weeks there was little further change in extractable phosphate except for a slight non-significant trend for extractable phosphate to increase with all levels of Cu.

In the Harlington soil after 1 week of incubation extractable phosphate was not significantly affected by any level of Cu, in spite of a trend for the value to increase with level

Table 22-A

Levels of Morgan-extractable phosphate initially (zero week) and after 1 and 12 weeks of incubation of Silwood and Harlington soils treated with 100-10,000 ppm Cu (as CuO), 100 ppm N as KNO_3 and 0.2% sucrose.

(Results are given in ppm on dry soil basis)

Soil type	Added Cu	Initial value PO_4-P	After incubation PO_4-P	
			1 wk	12 wks
Silwood	0	15	17	17
	100	14	15	16
	1000	14	11	13
	10,000	14	12	14
Harlington	0	31	27	29
	100	32	28	33
	1000	33	29	34
	10,000	35	31	50

L.S.D. at $P < 0.05 = 4.1$

of added Cu. After 12 weeks of incubation 100 and 1000 ppm Cu increased extractable phosphate to a small but significant extent compared with the levels after 1 week, but not compared with initial levels of phosphate. 1000 ppm Cu increased extractable phosphate to a fair extent compared with the initial and 1-week values.

(b) Effects of 1000 ppm Cu (as CuO) on Morgan-extractable phosphate after incubation (30°C, 50% mwhc) for 3, 6, 12 and 18 weeks of Harlington soil (pH 6.3) treated initially with 100 ppm N as KNO_3 and 0.5% grass or straw.

The details are described in experiment 3, Chapter V.

Results are shown in Table 23-A for extractable phosphate-P on the dry soil basis.

In spite of some differences in extractable phosphate-P levels due to the Cu treatments it was, in fact, found that there were no significant differences after any stage of incubation when resuls for grass- and straw-treated soils were compared with appropriate controls.

DISCUSSION

It was shown in experiment 2, Chapter IV that nitrogen changes due to Cu additions were no different between adding

Table 23-A

Levels of Morgan-extractable phosphate initially (zero week) and after 3, 6, 12 and 18 weeks of incubation of Harlington soil treated initially with 100 ppm N as KNO_3 and 0.5% grass or straw and without and with added 1000 ppm Cu (as CuO).

(Results are given in ppm on dry soil basis)

Treatments org. matt.	Cu	Initial value	3 wks PO_4-P	6 wks PO_4-P	12 wks PO_4-P	18 wks PO_4-P
Cont	0	47	27	30	32	28
Grass	0	61	38	39	44	38
Straw	0	59	36	39	42	34
Cont	1000	56	28	32	34	29
Grass	1000	64	41	43	42	40
Straw	1000	65	33	43	44	43

L.S.D. at $P < 0.05 = 6.0$

CuO and $CuHPO_4$. This indicated that phosphate did not influence the effects of Cu on N changes. The experiment with grass and straw just described above indicated that the addition of Cu to soils had little effect on changes in extractability of phosphate. In the presence of added Cu and decomposing organic materials it is likely that many processes involving phosphate are occurring simultaneously such as (a) microbial immobilisation of inorganic phosphate, (b) mobilisation of Ca and Fe phosphates under the influence of the CO_2 released from the decomposing organic materials, (c) copper affecting microbial assimilation and mineralisation of organic phosphorus compounds, (d) the direct effect of Cu on extractable inorganic phosphate and (e) the ability of grass and straw to affect the extent of fixation of PO_4 by soil. If two or more of these effects occur concurrently, and some would have opposing effects on PO_4 levels, it is perhaps not surprising that the overall effects of Cu on the extractable PO_4 were not significant in presence of decomposing grass and straw.

Only with a very high level of added Cu (10,000 ppm in experiment (a) in the absence of any added organic material) was there an increase in extractable PO_4 of any magnitude, but then only after 12 weeks of incubation and in only one of the two soils studied.

3. Effects of addition of sewage sludge on mineral nitrogen levels during incubation of soil treated with 100 ppm N as KNO_3 and 0.2% sucrose.

Sewage sludge is now commonly used as the cheap source of organic manure. Most sewage works now treat sewage by the activated sludge process. This involves an initial aerobic digestion in channels with air bubbled through the moving sewage. After allowing the sludge to settle the supernatent liquid is discharged into the river or sea. The settled sludge is then digested anaerobically to bring about further decomposition of organic materials. The residue is then treated by rotary vacuum filtration or drainage on sand beds to eliminate most of the water. The material is sold after being flash-dried, pulverised and bagged.

Sewage sludge from works handling sewage from industrial areas are usually contaminated with fairly high levels of potentially toxic trace elements e.g. Ni, Pb and Cr. A study was made of the effects of adding varying levels of such sewage sludge to a soil on subsequent N changes during incubation. Effects on both immobilisation and mineralisation of N were studied in a single experiment by addition of sucrose plus KNO_3, initially and giving a long incubation period to allow time for initially immobilised N to be re-mobilised.

METHODS

The sewage sludge used was obtained from the Mogden Sewage Works, Isleworth, London. The material is processed as described above and is sold in dried pulverised form in 56 lb bags. A sample of this material was ground in Christie and Norris Mill and analysed. It contained on the dry soil basis, 3.2% total N, 28% organic C and the following (determined after extraction with boiling 6N HCl):- 3.2% Ca, 0.29% Mg, 1.13% Fe, 821 ppm Cu, 2175 ppm Zn, 1135 ppm Cr, 343 ppm Ni and 562 ppm Pb. Sedge Peat was found to contain 14 ppm Cu, 22 ppm Zn, 3.4 ppm Cr, 6 ppm Ni and 12 ppm Pb.

500g samples of Silwood soil (pH 5.4) were mixed with 0, 0.1, 0.5 and 2.5% by weight of the dried ground sewage. In addition, since the sludge contained an amount of carbonate equivalent to 1.1% $CaCO_3$, appropriate amount of extra $CaCO_3$ were added to the soils receiving the lower levels of sludge and also to a control soil, receiving no sludge, in order to equalise $CaCO_3$ addition so as to eliminate effects of pH. The four soils were then placed in glass pots with free drainage and wetted to 50% mwhc. The weighed pots containing the soils were held at room temperature for 6 months, water being added when necessary to maintain the moisture content. In addition, after 2, 4, and 6 months each pot was given four inches of water in order to leach out soluble salts. After the final

leaching the soils were air dried and ground to pass a ∧2mm sieve.

Sufficient 10g samples of each soil were treated with 0.2% sucrose and 100 ppm N as KNO_3 to allow for duplicate analysis initially and after 1 and 12 weeks of incubation ($30^{\circ}C$, 50% mwhc). The Morgan extract was used to determine ammonium- and nitrate-N initially and after each incubation period.

RESULTS

Results are shown in Fig. 17-A and Table 24-A. It is seen that during the immobilisation phase (1 week of incubation) the extent of decrease in mineral-N was not affected by any level of sewage sludge. There was a slight accumulation of ammonia-N in all soils with no significant effects due to levels of sludge. During the subsequent re-mobilisation phase (1-12 weeks) the extent of re-mobilisation of N was not affected where 0.1 or 0.5% sludge, but was increased slightly where 2.5% sludge was added. However, a comparison of the 12 week values with initial values showed that mineral-N levels after 12 weeks were not significantly different from those present initially. During re-mobilisation mineral-N accumulated almost entirely as nitrate in all soils except where 0.5% sludge had been added, where it accumulated entirely as nitrate.

Fig:- 17-A

Effects of sewage sludge on ammonia- and nitrate-N levels of Silwood soil treated with 0.2% sucrose and 100 ppm N as KNO_3.

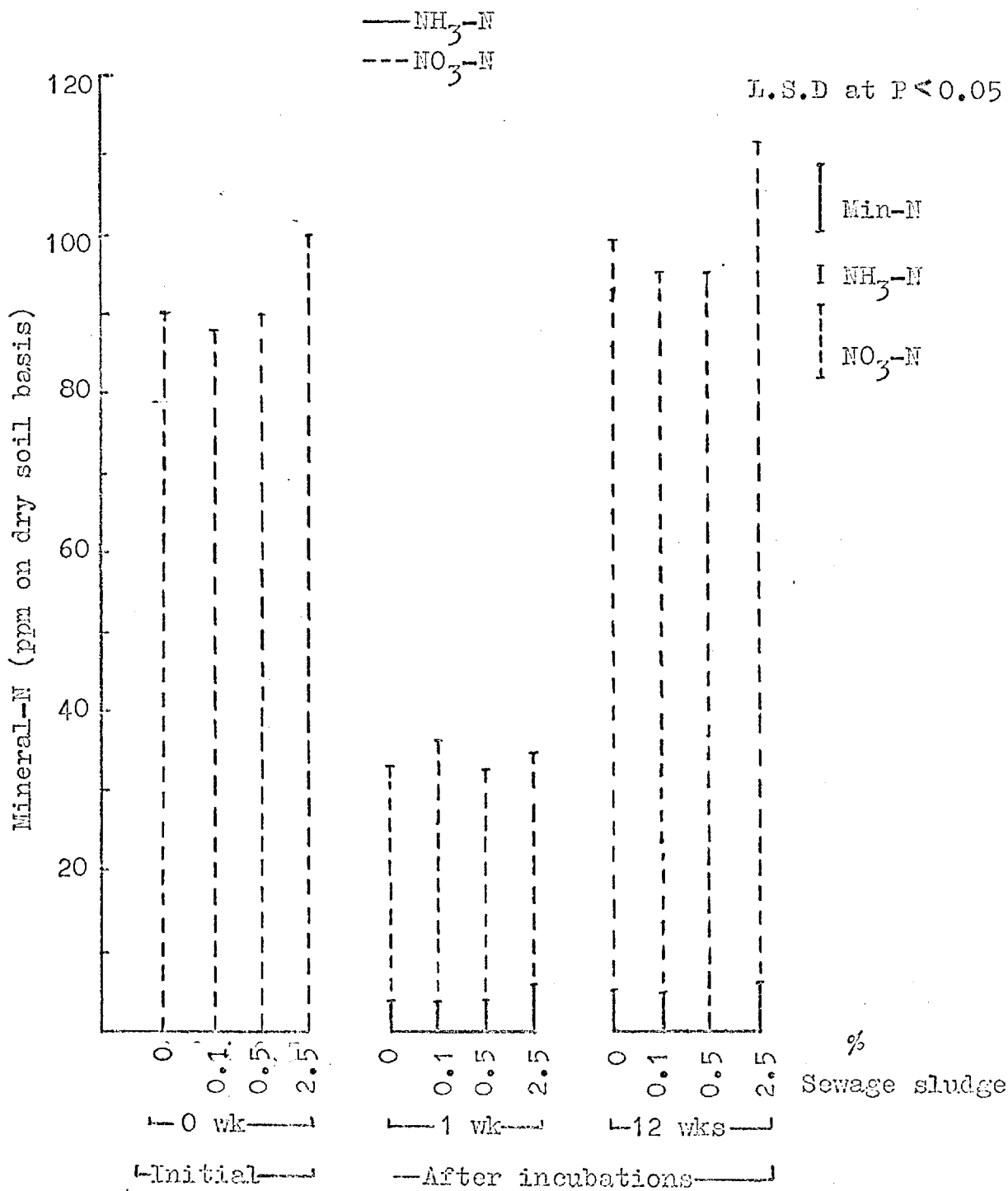


Table 24-A

Levels of ammonia- and nitrate-N initially and after 1 and 12 weeks of incubation of Silwood soil treated with different levels of sewage sludge, 100 ppm N as KNO_3 and 0.2% sucrose.

(Results are given in ppm on dry soil basis)

Treatments % S.S.	Initial values NO_3 N	NH_3 N	Min N	After incubations NO_3-N 1 wk	12 wks	NH_3-N 1 wk	12 wks	Min-N 1 wk	12 wks
Cont	90	0	90	33	94	4	5	37	99
0.1%	88	0	88	37	90	4	5	41	95
0.5%	90	0	90	33	95	4	0	37	95
2.5%	100	0	100	35	105	6	6	41	111

L.S.D at $P < 0.05$ for Min-N = 7.7, NH_3-N = 2.0 and NO_3-N = 8.0

DISCUSSION

This study was not concerned with the extent of mineralisation of organic N in sewage sludge. Premi (103) found that the organic N in sewage sludge was very resistant to mineralisation during incubation with soil. He found that only 4% or less of the organic N was mineralised after 6 weeks of aerobic incubation. If any mineralisation had occurred during the 6 months moist pre-incubation period any nitrate formed would have been removed by the leachings given during this period. This is confirmed by the fact that the nitrate-N values at zero week incubation were around 100 ppm, and this would be accounted for by the nitrate added as KNO_3.

The purpose of this study was to check whether the various mineral elements which were present in sewage sludge in high concentrations would have any effect on N changes during incubation. The results show, in fact, that even the 2.5% level of added sludge (equivalent to an application of 25 tons per acre) had no significant effect on immobilisation of added nitrate and subsequent re-mineralisation of N. In addition, the fact that re-mineralised N appeared virtually entirely as nitrate showed that the sludge treatment did not inhibit nitrification.

It seems unlikely that any of the trace elements present in the added sludge were removed by leaching during the

6 months pre-incubation period, since water extraction of soils have shown that trace elements cannot be removed in this way. In addition, the trace elements in the sludge would be held mainly in chelated form (103) and this would prevent any loss by leaching with water. Thus all the trace elements present in the sludge would be present in the soil during incubation. Although their concentrations in the soil would be much decreased due to dilution with the soil, the soil concentrations where 2.5% sludge was added would be 22 ppm for Cu, 54 ppm for Zn, 28 ppm for Cr, 9 ppm for Ni and 14 ppm for Pb. The lack of any effect of these levels of these trace elements in combination is most likely due to the fact that they are held by the sludge in chelated form or possibly even in stronger combination so that they were not available to the microorganisms concerned with immobilisation and mineralisation of nitrogen and nitrification.

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