INCUBATION STUDY OF NITROGEN MINERALISATION AND NITRIFICATION IN RELATION TO SOIL pH AND LEVEL OF COPPER(II) ADDITION

M. S. I. QURAISHI & A. H. CORNFIELD

Chemistry Department, Imperial College, London, SW7 2AZ, Great Britain

ABSTRACT

The effects of adding 100 and 1000 ppm Cu, as sulphate, to a sandy loam previously adjusted to three pH levels on nitrogen mineralisation and nitrification during aerobic incubation for three weeks at 30°C were studied. 100 ppm Cu had no effect on nitrogen mineralisation at pH 5·1 and 5·9, but decreased it slightly at pH 7·3. 1000 ppm Cu stopped nitrogen mineralisation at pH 5·1, but was less toxic with increasing pH. At pH 5·1 and 5·9 nitrification was not affected by 100 ppm Cu, but was stopped by 1000 ppm Cu. At pH 7·3 the accumulation of mineralised nitrogen entirely as nitrate with both added Cu levels suggested that neither level affected nitrification. Results are discussed in relation to exchangeable and complexed forms of Cu present in the soils.

INTRODUCTION

The copper content of soils may be increased by the application of mining, industrial and sewage wastes or by the use of copper-containing pesticides. Variable results have been reported in incubation studies on the effects of addition of copper compounds to soils on nitrogen mineralisation and nitrification. Gyulakhmedov (1960) found that addition of 0-5 ppm Cu(II) to calcareous chestnut soils enhanced both processes, but that higher levels were toxic. Turk (1939) found that 40 ppm Cu(II) had no effect on, or inhibited both processes in, acid or limed muck soils. Quraishi & Cornfield (1971) found that 100 and 1000 ppm copper (as CuO or CuHPO₄) added to a slightly calcareous sandy loam receiving dried blood stimulated both processes, and even 10,000 ppm Cu was not toxic. Variations among soils in their responses to copper additions are probably due to differences in properties such as pH, organic matter content and texture. The present paper reports...
on the effects of adding two levels of copper, as sulphate, on nitrogen mineralisation and nitrification during incubation of a soil previously adjusted to three pH levels.

MATERIALS AND METHODS

The soil used was an alluvial sandy loam (16% clay, 24% silt) from a cultivated area, which after air-drying and sieving (2 mm) had a pH (in water) of 5.1 and contained 0.16% total nitrogen and 2.0% organic carbon. The soil contained 33 ppm total copper, determined by boiling with 6N-hydrochloric acid followed by analysis of the extract by atomic absorption spectroscopy (Premi & Cornfield, 1968). On the basis of previous lime requirement determinations, 500 g portions of soil were mixed with 0.3% and 0.7% finely-ground calcium carbonate. These soils, as well as 500 g of original soil, were wetted to 50% saturation in glass pots and held at room temperature for two weeks, water being added, when necessary, to maintain moisture content between 40% and 50% saturation. After air-drying and rubbing through a 2 mm sieve the soils had pH values of 5.1, 5.9 and 7.3 and no free carbonate could be detected in any of them. 150 g samples of soil of each pH were mixed with finely-ground copper sulphate pentahydrate to supply 100 and 1000 ppm added copper on the dry soil basis. For incubation 10 g samples of soils from each of the three treatments (0, 100 and 1000 ppm Cu) were weighed into 10 x 2.5 cm tubes and water was added to bring the moisture content to 50% saturation. This moisture content was known to be optimum for both nitrogen mineralisation and nitrification in this soil. Sufficient replicates of each treatment at each pH were prepared to allow for determination, in duplicate, of pH and analysis for mineral nitrogen and extractable forms of Cu initially and after incubation. The barium peroxide method (Cornfield, 1961) was used for aeration and absorption of carbon dioxide during incubation of the tubes, which were closed with rubber bungs, for three weeks at 30°C. For analysis, 10 ml of water was added to each tube and the pH measured after shaking. Then 10 ml of N-sodium acetate was added, and after 2 min shaking the tubes were centrifuged. Suitable aliquots of the supernatant liquid were analysed for ammonium- and nitrate-nitrogen by a microdiffusion method (Bremner & Shaw, 1955), for nitrite-nitrogen by a diazotisation and coupling method (Barnes & Folkard, 1951), and for Cu by atomic absorption spectroscopy. Other replicates were shaken with 20 ml of 0.05 M EDTA (pH 4.0) and analysed for Cu after filtration.

RESULTS AND DISCUSSION

Table 1 shows the extent of accumulation of mineral-nitrogen (ammonium- and nitrate-nitrogen) after incubation, in ppm on dry soil basis, obtained by subtracting initial values from those after incubation. Nitrite could not be detected initially or after incubation in any of the samples. The table also shows the levels of Cu
extracted by the sodium acetate and EDTA reagents. Incubation and the copper sulphate treatments tended to decrease pH, but not by more than 0.3 units.

In the control soils (no Cu treatment) the increasing nitrogen mineralisation and nitrification with pH followed the normal trend.

Addition of 100 ppm Cu had no significant effect on either process in any soil except at pH 7.3, where nitrogen mineralisation was decreased slightly. 1000 ppm Cu stopped nitrification at pH 5.1 and 5.9 and apparently decreased it to a moderate extent at pH 7.3. However, since mineralised nitrogen accumulated entirely as nitrate at pH 7.3 with both added levels of Cu, whilst some ammonium-nitrogen accumulated in the control soil, this suggests that not only was nitrification limited by nitrogen mineralisation, but that both Cu treatments stimulated nitrification. This is confirmed by a previous study (Quraishi & Cornfield, 1971) with this soil, pretreated with sufficient calcium carbonate to make it slightly calcareous and given dried blood before incubation, to ensure that nitrification was not limited by nitrogen mineralisation, where 100 and 1000 ppm Cu stimulated nitrification.

The toxic effect of 1000 ppm Cu on nitrogen mineralisation decreased with increasing pH, but was still apparent even at pH 7.3. The absence of any accumulation of nitrite indicates that where Cu decreased nitrification its effect was no greater on the organism (Nitrobacter) responsible for the second stage than that (Nitrosomonas) responsible for the first stage of the two-stage chemoautotrophic nitrifying process.

Decreased nitrogen mineralisation and nitrification could not have been due to the sulphate residue of even the 1000 ppm Cu treatment, since it had been shown

### TABLE 1

**EFFECT OF SOIL pH AND LEVEL OF COPPER ADDITION ON MINERAL NITROGEN ACCUMULATION* AND EXTRACTABLE COPPER AFTER THREE WEEKS OF INCUBATION**

(Result on dry soil basis)

<table>
<thead>
<tr>
<th>Soil pH</th>
<th>Cu added†</th>
<th>NH₄–N ppm</th>
<th>NO₃–N ppm</th>
<th>min–N ppm</th>
<th>0.5N sodium acetate extr. Cu ppm</th>
<th>0.05M EDTA extr. Cu ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>0</td>
<td>11.0</td>
<td>3.1</td>
<td>14.1</td>
<td>&lt;0.1‡</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>9.3</td>
<td>1.9</td>
<td>11.2</td>
<td>0.8</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>60.0</td>
<td>907</td>
</tr>
<tr>
<td>5.9</td>
<td>0</td>
<td>7.2</td>
<td>15.8</td>
<td>23.0</td>
<td>&lt;0.1</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7.4</td>
<td>19.0</td>
<td>26.4</td>
<td>&lt;0.1</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>5.7</td>
<td>0.0</td>
<td>5.7</td>
<td>2.5</td>
<td>718</td>
</tr>
<tr>
<td>7.3</td>
<td>0</td>
<td>3.7</td>
<td>41.6</td>
<td>45.3</td>
<td>&lt;0.1</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.0</td>
<td>38.5</td>
<td>38.5</td>
<td>&lt;0.1</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.0</td>
<td>38.5</td>
<td>38.5</td>
<td>&lt;0.1</td>
<td>749</td>
</tr>
<tr>
<td>L.S.S.</td>
<td>P &lt; 0.05</td>
<td>3.1</td>
<td>3.3</td>
<td>4.1</td>
<td>2.1</td>
<td>23</td>
</tr>
</tbody>
</table>

* Initial levels subtracted from those after incubation
† as copper sulphate
‡ Lower limit of detection
for this soil (Sindhu & Cornfield, 1967) that addition of sulphate, as calcium sulphate, at five times the level used in this study had no effect on either process. It is also known for this soil (Ekpete, 1963) that even the maximum reduction (0·3 units) in pH due to the sulphate residue of the added Cu salt could not have significantly affected either process. Thus, any changes in nitrogen mineralisation and nitrification due to the addition of copper sulphate can be ascribed to the effect of the added Cu.

0·5 N-sodium acetate extracts exchangeable, including water-soluble, Cu, whilst EDTA extracts these forms as well as complexed forms of Cu. Cu levels obtained by each extractant before incubation were very similar to those obtained after incubation, indicating little change from one form to another due to incubation. Except for the soil of pH 5·1 receiving 1000 ppm Cu, only a very small proportion of the added Cu was found in exchangeable form in any of the soils. On the other hand, most of the Cu, including that originally present in the soil, was recovered by EDTA extraction. Where 1000 ppm Cu was added there was a critical pH between 5·1 and 5·9 where a considerable decrease in exchangeable Cu and a moderate decrease in complexed Cu occurred. Between pH 5·9 and 7·3 there were negligible differences in both extractable forms of Cu at any level of added Cu.

An EDTA extractant similar to that used here was shown by Pizer et al. (1966) to be a suitable indicator for the availability of Cu to cereals. In plateau sands Cu deficiency was apparent when EDTA-extractable soil Cu was less than 2 ppm. No information is available on the toxic effects of high Cu levels in soils of varying pH on nitrogen biodynamics in relation to extractable forms of Cu. In the present study the complete inhibition of nitrogen mineralisation by 1000 ppm Cu at pH 5·1 is probably due to the relatively high level of exchangeable Cu. At the two higher pH levels there were no consistent relationships between the extent of toxicity of 1000 ppm Cu and the Cu levels extracted by either reagent. Both processes were stopped or greatly decreased at pH 5·9, whilst nitrogen mineralisation was decreased to a lesser extent at pH 7·1 than at pH 5·9, yet each extractable form of Cu was very similar at both pH levels.

The high proportion of added Cu present in complexed form suggests that, where nitrogen mineralisation and nitrification were inhibited, this was probably due to the ability of Cu to compete with other essential mineral elements for the cation-complexing sites of the enzymes involved in both processes. The results obtained in this study suggest that the toxicity of high levels of Cu to nitrogen mineralisation and nitrification in acid soils may be eliminated or decreased by raising soil pH.

ACKNOWLEDGEMENT

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REFERENCES