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EFFECTS OF ADDITION OF COPPER, MANGANESE, ZINC AND CHROMIUM COMPOUNDS ON AMMONIFICATION AND NITRIFICATION DURING INCUBATION OF SOIL

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INTRODUCTION

Most of the work which has been done on trace elements in soils has been in relation to deficiency or toxicity effects on plants. Although a certain amount of work has been reported on the effects of trace elements on ammonification and nitrification in soils[1 2 3 4 5 6] the results obtained are conflicting. Trace elements may be introduced into soil where pesticide sprays *e.g.* copper fungicides are used, or by application of sewage materials or town composts. In addition some soils may be fairly high in one or more trace elements due to the geology of the soil or through the application of mining wastes. In these ways the trace elements in soils may be increased to such an extent as to be toxic to plant growth and soil microbiological processes.

This paper reports on the effects of adding copper, manganese, zinc and chromium as sulphates and copper and zinc as carbonates on ammonification and nitrification on subsequent incubation of soil.

EXPERIMENTAL

The soil used was an alluvial sandy loam top soil from a cultivated area. The soil was air dried and ground to pass a 2-mm sieve. Some characteristics of the soil are as follows: 53% sand, 24% silt, 17% clay, 0.16% total nitrogen,

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2.0% organic carbon and pH 7.1. The soil contained 33 ppm copper, 57 ppm zinc, 224 ppm manganese and less than 0.2 ppm chromium as determined by extraction with boiling 6 *N* HCl.

The following three experiments were carried out:

A: Copper, manganese, zinc, chromium and calcium were mixed as finely ground sulphates at levels of 100, 1,000 and 10,000 ppm of the cations on the air-dry soil basis. The calcium sulphate was included as a treatment to allow for the effects of sulphates of the mineralisation processes. Ten-gram portions in duplicate of treated and control soils were weighed into flat-bottom vials and water added to 50% of the maximum water holding capacity (m.w.h.c.). The barium peroxide method [7] was used for aeration and absorption of carbon dioxide during incubation of the stoppered tubes. After three weeks of incubation at 30°C 10 ml of water were added to each soil tube and the pH was determined. Then 10 ml of 0.2 *N* Na EDTA (pH 4.0) were added, the contents shaken for two minutes and after filtration a suitable aliquot of the extract was analysed for ammonium-N and nitrite + nitrate-N by the micro-diffusion method [8]. The filtrate was also analysed for nitrite-N using a colorimetric alpha-naphthylamine-sulphanilic acid method.

B: The procedure was the same as in Experiment A, except that water was added to 100% m.w.h.c. to give an anaerobic moisture content, and the barium peroxide aeration treatment was not included.

C: The procedure was as in Experiment A, except that only copper, zinc and calcium as carbonates were added.

0.1 *N* Na-EDTA (pH 4.0) was used for extraction since the extracts were also to be analysed for the added trace elements (the results of which will be reported in a later paper). Preliminary experiments showed that the EDTA reagent was suitable for extracting mineral forms of nitrogen.

RESULTS AND DISCUSSION

The results of Experiments A and B are given in Fig. 1 and of experiment C in Fig. 2. The results are accumulation values (those obtained after three weeks of incubation minus those present initially) for ammonium-N (broken line) and nitrate-N (solid line) in ppm on the dry-soil basis, and are means of duplicate values. The effects of the treatments on soil pH after three weeks of incubation are shown in Table 1 for all experiments.

Expt. A. *Aerobic incubation; copper, manganese, zinc, chromium and calcium added as sulphates* (Fig. 1a).

The LSD at $P < 0.05$ was 3.2 ppm for ammonium-N and 7.6 ppm for nitrate-N. There was no accumulation of nitrite-N in any of the

incubated soils. The addition of 10,000 ppm of Ca^{2+} (as sulphate) significantly increased nitrate accumulation, whilst the lower levels had no significant effect. The results of the effects of different levels of the trace elements will be compared with the respective levels of calcium.

The only treatment which increased ammonification was 100 ppm Cu^{2+}. The 1,000 ppm Cu^{2+} level had no effect, whilst 10,000 ppm level reduced ammonification. With all levels of Cu^{2+} mineral-N accumulated entirely as nitrate. Mineral nitrogen accumulation, which was accounted for entirely as nitrate, was not affected by 100 ppm Cr^{3+}, but was reduced by the two higher levels to about the same extent. Nitrification was partially reduced by 1,000 ppm and was prevented by 10,000 ppm Cr^{3+}. Nitrogen mineralisation was reduced to about the same extent by 100 and 1,000 ppm levels of Mn^{2+} and mineral-N accumulated entirely as nitrate. 10,000 ppm Mn^{2+} slightly reduced ammonification and completely prevented nitrification. Ammonification was reduced by 100 and 10,000 ppm Zn^{2+}; at the lower level mineral-N accumulated entirely as nitrate and at the higher level entirely as ammonium. The 1,000 ppm Zn^{2+} level did not affect ammonification but partially reduced nitrification.

Where nitrogen, mineralised during incubation, accumulates entirely as nitrate, this indicates that nitrification is limited by the rate of ammonification, if it is assumed that nitrate is being produced only by the normal chemoautotophic process of oxidation of ammonium. If this is accepted then only the 10,000 ppm Cu^{2+} level reduced ammonification, whilst the two lower levels of Cu^{2+} and the 100 ppm level of Cr^{3+} had no effect on this process. It is only where either ammonium or ammonium + nitrate accumulate during incubation that it can be stated with certainity that nitrification is being reduced. These effects occurred with the highest level of Mn^{2+} and the two higher levels of Zn^{2+} and Cr^{3+}.

Although ammonification is not much affected by pH [9 10 11] nitrification rate tends to fall off rapidly as the pH falls below 6 [12 13 14]. The 100 and 1,000 ppm levels of all the trace element salts and the 10,000 ppm level of Mn^{2+} did not reduce the pH below 6, but the 10,000 ppm level of other cations did so (Table 1). Thus Mn^{2+} is the only cation where any reduction of nitrification can be ascribed to toxic effect and not to pH. Only the 10,000 ppm level of

Mn^{2+} prevented nitrification, but had no effect on ammonification. The two lower levels of Mn^{2+} depressed ammonification but had little effect on nitrification. These results differ from those reported by Brown [1] who found both ammonification and nitrification were increased in aerobic soil by levels of Mn^{2+} similar to those used here. The depressing effect of 1,000 ppm of Zn^{2+} on nitrification cannot be due to pH, although the inhibitory effect of 10,000 ppm Zn^{2+} may

TABLE 1

Effects of addition of copper, manganese, zinc, chromium and calcium as sulphates (Expt. A and B) and copper, zinc and calcium as carbonates (Expt. C) on soil pH after 3 weeks of incubation. Initial soil pH was 7.1

Element added	Level used ppm	Expt. A.	Expt. B.	Expt. C.
Control.	0	7.2	7.3	7.1
Copper.	100	7.2	7.3	7.5
Copper.	1000	6.4	6.4	7.4
Copper.	10000	4.6	4.8	7.7
Manganese.	100	7.2	7.3	—
Manganese.	1000	6.6	7.0	—
Manganese.	10000	6.2	6.4	—
Zinc.	100	7.0	7.3	7.2
Zinc.	1000	6.2	6.8	7.4
Zinc.	10000	5.6	5.7	8.5
Chromium.	100	7.2	7.3	—
Chromium.	1000	6.2	6.8	—
Chromium.	10000	2.9	3.0	—
Calcium.	100	7.0	7.3	7.5
Calcium.	1000	7.0	7.2	7.7
Calcium.	10000	6.9	7.2	7.3

be due to both pH and its toxic effect. Sedova [2] found that even 1 ppm Cr^{3+} supressed nitrification in soil, but the present study shows that even 100 ppm Cr^{3+} had no effect on nitrification. 1,000 ppm Cr^{3+} reduced nitrification and this was probably not due to pH reduction, whilst 10,000 ppm Cr^{3+} prevented nitrification, but this may have been due to reduction in pH. The literature shows that variable results have been obtained with respect to effects of copper. Lipman and Burgess [3] showed that 500 ppm Cu^{2+} markedly stimu-

lated nitrification, whilst Lipman and Gericke[4] showed that Cu^{2+} in excess of 100 ppm inhibited nitrification. The present study showed that even 1,000 ppm Cu^{2+} did not reduce nitrification.

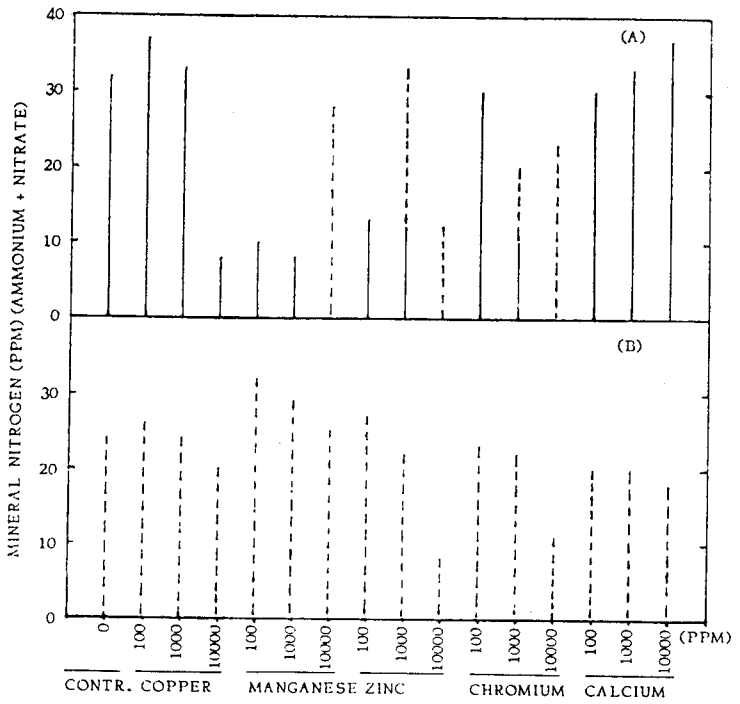

Fig. 1. Mineral nitrogen (total line), ammonium (broken line) and nitrate (solid line) accumulation during three weeks incubation (30°C) of the soil treated with different levels of copper, manganese, zinc, chromium and calcium as sulphates; (**a** aerobic incubation, **b** anaerobic incubation.

Expt. B. Anaerobic incubation; copper, manganese, zinc, chromium and calcium added as sulphates (Fig. 1b)

The LSD at $P < 0.05$ was 2.9 ppm for ammonium-N. There was no accumulation of nitrite or nitrate in any of the incubated soils. All levels of Mn^{2+} increased ammonification to a fair extent when compared with calcium sulphate treatments. The two lower levels of Cu^{2+} and the 100 ppm level of Zn^{2+} and Cr^{3+} had a significant, but only small effect, in increasing ammonification. 10,000 ppm Zn^{2+} and Cr^{3+} depressed ammonification, but 10,000 ppm Cu^{2+} had no effect. The general effect of the treatments was to reduce the pH

(Table 1) although the extent of reduction was usually not quite as great as occurred under aerobic incubation.

The absence of any nitrite or nitrate is not surprising in view of the anaerobic conditions prevailing during incubation. The generally lower effect of trace elements in reducing ammonification under anaerobic than under aerobic conditions indicates that those organisms (facultative and obligate anarobes) responsible for ammonification under anaerobic conditions are generally less susceptible to toxic effects of the trace elements studied. The exceptions were the highest levels of Zn^{2+} and Cr^{3+}, which reduced ammonification to a greater extent under anaerobic than under aerobic conditions. The increased ammonification under anaerobic conditions due to lower rates of application of all the trace elements and all the rates of Mn^{2+} indicates a stimulating effect on the activity of some of the many types of organisms (bacteria, fungi, actinomycetes etc.) responsible for the process.

Expt. C. Aerobic incubation; copper, zinc and calcium added as carbonates (Fig. 2).

The LSD at $P < 0.05$ was 10.9 ppm for nitrate-N. There was no accumulation of nitrite-N or ammonium-N due to any of the treat-

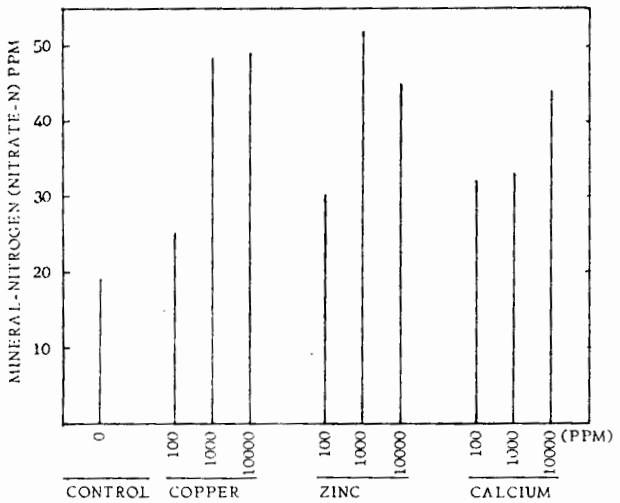

Fig. 2. Mineral nitrogen (nitrate) accumulation during 3 weeks incubation (30°C) of the soil treated with different levels of copper, zinc, and calcium as carbonates.

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ments. Except for 100 ppm Cu^{2+} and Zn^{2+} all treatments significantly increased nitrate-N accumulation in comparison with the control. When compared with the corresponding levels of Ca^{2+} the only levels of Cu^{2+} and Zn^{2+} which affected nitrification were the 1,000 ppm levels, which increased nitrate-N accumulation by about 50 per cent. 10,000 ppm Zn^{2+} increased soil pH by 1.2 units compared with the corresponding level of Ca^{2+}. The other Cu^{2+} and Zn^{2+} treatments did not alter pH by more than 0.4 units compared with the corresponding levels of Ca^{2+}.

This experiment has shown that even the 10,000 ppm level of Cu^{2+} and Zn^{2+} added as carbonate to a neutral soil did not reduce ammonification or nitrification. The stimulating effect of 1,000 ppm of Cu^{2+} and Zn^{2+} on nitrogen mineralisation indicates that the activity of both Cu^{2+} and Zn^{2+} was sufficiently high to stimulate this process but not high enough to effect nitrification. The very low solubility of Cu^{2+} and Zn^{2+} in the slightly alkaline condition in this soil probably explains the lack of the effect of these elements in reducing either ammonification or nitrification even where applied at the highest rate.

SUMMARY

The effects of adding 100, 1,000, and 10,000 ppm of copper, manganese, zinc and chromium as sulphates and of copper and zinc as carbonates on ammonification and nitrification during incubation (3 weeks) of an initially neutral soil under aerobic and anaerobic conditions were studied. Some stimulating but more usually inhibitory effects of trace elements on both processes were found. These effects varied considerably depending on the level and type of the cation added, whether the conditions were aerobic or anaerobic, and on the extent to which pH was altered by the treatments.

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