work of Krampitz, Woods & Werkman (1943) is germane to this point.

It is unfortunately not possible for me to continue this work at the moment. The results are presented as provisional ones that might provide some useful pointers in future investigations on the biochemistry of nitrogen immobilization by soil.

SUMMARY

1. The immobilization of nitrogen in soils by various organic materials has been studied by a soil-percolation technique.

2. The amount of nitrogen immobilization, and the amount of denitrification, induced by an organic compound differs from compound to compound.

3. The effects of enzyme poisons and of carbon dioxide on the immobilization process suggest that some heavy-metal enzyme and some carbon dioxide-assimilative process are involved.

Most of this work was done at the Agricultural Research Council Unit of Soil Metabolism. I should like to acknowledge help and advice from Dr J. H. Quastel and to thank the A.R.C. for permission to publish these results.

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The Effects of Zinc and Copper on Soil Nitrification

By H. LEES,* *Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts*

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Although many papers have been published dealing with the effects of trace elements on plants, and on soil processes in the field, there appears to have been no exhaustive study of the effect of trace elements on the microbial processes of the soil. As these processes are an essential part of agriculture, the effect of trace elements is of practical as well as theoretical interest. Hence the work here reported was begun.

METHODS

The soil-percolation technique devised by Lees & Quastel (1946*a*) for the study of soil metabolism has been used throughout. The soil-percolation apparatus used was the simplified and improved form (Lees, 1947). The influence of either zinc or copper on soil nitrification was assessed by comparing the course of nitrification in a soil percolated with $(NH_4)_2SO_4$ solution with the course of nitrification in a soil percolated with a similar $(NH_4)_2SO_4$ solution to which a known concentration of either $ZnSO_4$ or $CuSO_4$ had been added.

Mineral nitrogen analyses were performed from time to time on samples of the percolates by methods similar to those already described (Lees & Quastel, 1946*a*). As it was found early in the work that the conversion of ammonium N into nitrate N was always reasonably quantitative, ammonium-N estimations are not included in the results. Nitrite N was never found in quantities greater than a few μg./ml.; nitrite N is therefore bulked with nitrate in the reported nitrate-N figures. Percolate analyses have been converted into 'μg. N/g. soil' by the methods already described (Lees & Quastel, 1946*a*).

Copper was estimated in the percolates by the method of Sherman & McHargue (1942). Zinc was estimated spectrophotometrically.

Soils were stored damp in earthenware containers and turned from time to time. Samples of soil were taken from these containers, and air-dried and sieved (4·0–1·0 mm. mesh) before use.

All experiments were conducted with 10 g. soil percolated at 18–21° with 100 ml. of $2{\cdot}5 \times 10^{-3}$M-$(NH_4)_2SO_4$, with or without the addition of $ZnSO_4$ or $CuSO_4$. Three soils were used: (1) an allotment soil (0·4% organic N, pH 6·8); (2) a Lincolnshire Fen soil (2·5% organic N, pH 7·0); (3) a Romney Marsh soil (0·2% organic N, pH 7·8).

Stimulated soils (Lees & Quastel, 1946*b*) were prepared as follows. The soils were first percolated with 100 ml. of $2{\cdot}5 \times 10^{-3}$M-$(NH_4)_2SO_4$ until nitrification was complete, washed out two or three times with 50 ml. lots of distilled water, and finally percolated with 100 ml. distilled water for 6 hr. This wash percolate was then discarded and the soils were ready for the experiment proper.

* Now at Imperial College of Tropical Agriculture, Trinidad.

Poisoning of a soil by diethyldithiocarbamate was carried out as follows. Ten ml. of 4×10^{-3}M-sodium diethyldithiocarbamate were poured on to the stimulated but unwashed soil. The liquid that dripped through was recovered and again poured on to the soil. This process was thrice repeated

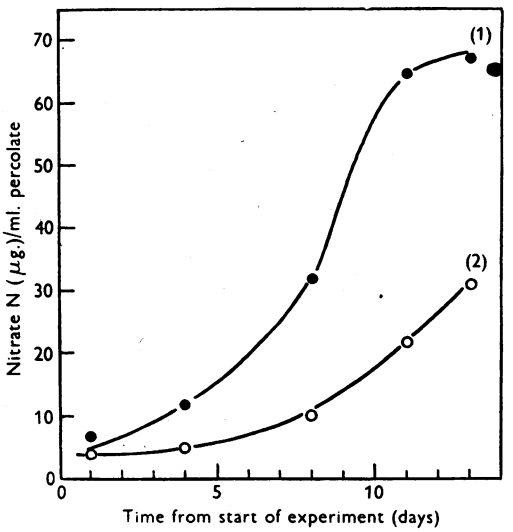

Fig. 1. The effect of zinc on nitrification in fresh (unstimulated) allotment soil. Soil (10 g.) was percolated with 100 ml. of (curve 1) $2{\cdot}5 \times 10^{-3}$M-$(NH_4)_2SO_4$ or (curve 2) $2{\cdot}5 \times 10^{-3}$M-$(NH_4)_2SO_4$ plus 4×10^{-3}M-$ZnSO_4$.

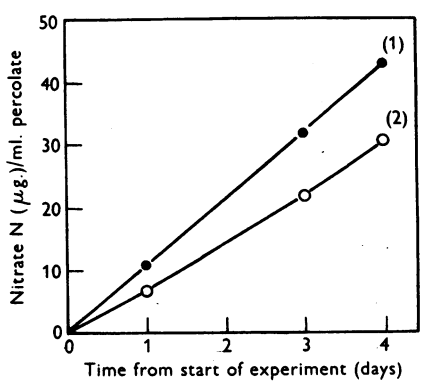

Fig. 2. The effect of zinc on nitrification in stimulated allotment soil. Soil (10 g.) was percolated with 100 ml. of (curve 1) $2{\cdot}5 \times 10^{-3}$M-$(NH_4)_2SO_4$ or (curve 2) $2{\cdot}5 \times 10^{-3}$M-$(NH_4)_2SO_4$ plus 4×10^{-3}M-$ZnSO_4$.

in order to distribute the poison evenly through the soil, which was then left untouched for 1 hr. Excess of the poison was removed from the soil by three 50 ml. washings with distilled water followed by percolation for 6 hr. with 100 ml. distilled water. This wash percolate was then discarded and the soil was ready for the experiment.

Experimental procedure. The toxic effect of zinc on soil nitrification may be demonstrated either as a flattening of the sigmoid curve characteristic of nitrification in a fresh soil (Fig. 1), or as a reduction of the slope of the straight line characteristic of nitrification in a stimulated soil (Fig. 2).

The inhibition due to zinc in either case may be measured as

$$100 \times \frac{C-T}{T},$$

where C = nitrate formation in absence of zinc, and T = nitrate formation in presence of zinc. This value would be constant throughout the control nitrification in ideal experiments with stimulated soils (Fig. 2), but it is less obviously so in experiments with unstimulated soils (Fig. 1).

In experiments with unstimulated soils the inhibition of nitrification due to zinc has therefore been measured at two roughly defined points during the experiment: (1) when control nitrification was half completed, and (2) when control nitrification was 90% completed. In practice the inhibition measured at point (1) has been found to agree well with that measured at point (2). But because the use of stimulated soils excludes spurious effects of zinc on the initial proliferation rate of the nitrifying organisms and minimizes the possibility of their adapting their activities to the presence of zinc, stimulated soils rather than unstimulated soils have been used in most of the work reported here.

The above explanation is based on the effect of zinc on soil nitrification; it should be understood to apply equally well to the effect of copper on the same process.

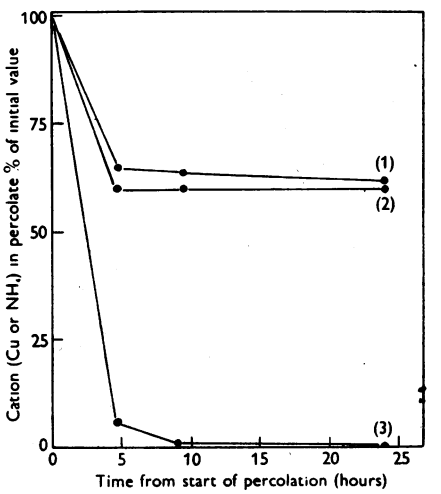

Fig. 3. The removal of ammonium and copper ions from the percolates of 10 g. Marsh soil percolated with 100 ml. of either 5×10^{-3}M-$(NH_4)_2SO_4$ or 5×10^{-3}M-$(NH_4)_2SO_4$ plus 5×10^{-3}M-$CuSO_4$. Curve 1, ammonium ion concentration in presence of copper; curve 2, ammonium ion concentration in absence of copper; curve 3, copper ion concentration.

RESULTS

When a solution containing zinc or copper is percolated through a soil, nearly all the zinc or copper is, within a few hours, transferred from the percolate to the soil. Fig. 3 illustrates the removal of copper

from copper sulphate percolated through Marsh soil, but it should be understood to typify the result obtained when a solution of either zinc or copper is percolated through any soil. The mechanism responsible for the transfer of zinc or copper from percolate to soil is not the normal soil base-exchange system; the normal base-exchange equilibrium reached by ammonium ions percolated at the same time through the same soil is unaffected by the concomitant transfer of zinc or copper to the soil. The zinc and copper are probably taken up by the organic matter of the soil (Bremner, Mann, Heintze & Lees, 1946; Brun, 1945); therefore the percolate concentrations of zinc and copper given in the tables are initial ones. Within 24 hr. from the start of any experiment reported the percolate concentration of either zinc or copper was certainly less than 0·5 μg./ml., but no exact determination of this value was carried out.

Complete protocols of experiments on the effects of zinc and copper on nitrification in unstimulated Romney Marsh soil are given in Table 1. The results from parallel experiments with stimulated Romney Marsh soil are given in Table 2.

As these two tables adequately illustrate the type of experimental data obtained, the results from experiments on stimulated allotment soil (Table 3) and stimulated Fen soil (Table 4) are given simply as mean values from replicate experiments. The results given in Tables 1–4 agree in showing that zinc was more inhibitory of soil nitrification than was copper. Low concentrations of copper appeared

Table 1. *The effects of zinc and copper sulphates on nitrification in unstimulated Romney Marsh soil*

(Soil (10 g.) was percolated with 100 ml. $2·5 \times 10^{-3}$ M-$(NH_4)_2SO_4$, with or without $ZnSO_4$ or $CuSO_4$. Results are given (1) at half completion of control nitrification; (2) at 90% completion of control nitrification.)

	Metal in percolate (M)	Nitrate N formed/g. soil in different experiments (μg.)							Inhibition of nitrification (%)
(1)	Nil	260	280	300	320	330	330	370	—
	$ZnSO_4$, 4×10^{-3}	—	—	0	90	110	—	150	74
	$ZnSO_4$, 2×10^{-3}	250	—	200	250	160	160	100	41
	$ZnSO_4$, 1×10^{-3}	110	230	250	170	320	200	230	31
	$ZnSO_4$, 5×10^{-4}	—	280	300	300	—	—	—	2
	Nil	390	370	340	340	—	—	—	—
	$CuSO_4$, 4×10^{-3}	260	160	200	—	—	—	—	44
	$CuSO_4$, 2×10^{-3}	—	260	260	200	—	—	—	31
	$CuSO_4$, 1×10^{-3}	390	350	250	260	—	—	—	13
	$CuSO_4$, 5×10^{-4}	400	350	—	—	—	—	—	1
(2)	Nil	670	590	600	700	700	700	700	—
	$ZnSO_4$, 4×10^{-3}	—	—	100	240	160	—	350	69
	$ZnSO_4$, 2×10^{-3}	500	—	550	440	500	360	270	36
	$ZnSO_4$, 1×10^{-3}	160	530	500	700	680	690	550	18
	$ZnSO_4$, 5×10^{-4}	—	590	600	700	—	—	—	0
	Nil	650	730	690	690	—	—	—	—
	$CuSO_4$, 4×10^{-3}	550	440	350	—	—	—	—	35
	$CuSO_4$, 2×10^{-3}	—	600	570	500	—	—	—	21
	$CuSO_4$, 1×10^{-3}	650	680	690	700	—	—	—	1
	$CuSO_4$, 5×10^{-4}	670	710	—	—	—	—	—	0

Table 2. *The effects of zinc and copper sulphates on nitrification in stimulated Romney Marsh soil*

(Soil (10 g.) was percolated with 100 ml. $2·5 \times 10^{-3}$ M-$(NH_4)_2SO_4$, with or without $ZnSO_4$ or $CuSO_4$. A negative inhibition means that nitrification was stimulated.)

Metal in percolate (M)	Nitrate N formed/g. soil in different experiments after		Inhibition of nitrification after	
	3 days (μg.)	5 days (μg.)	3 days (%)	5 days (%)
Nil	250, 270, 520, 500, 310, 310	730, 680, 700, 720, 710, 710	—	—
$ZnSO_4$, 4×10^{-3}	0, 30, 0, 100, 30, 0	0, 140, 150, 200, 0, 0	93	88
$ZnSO_4$, 2×10^{-3}	170, 140, 400, 150, 200, 140	430, 450, 500, 580, 340, 370	44	37
$ZnSO_4$, 1×10^{-3}	120, 70, 250, 370, 140, 240	400, 200, 470, 700, 580, 450	45	34
$ZnSO_4$, 5×10^{-4}	220, 240, 300, 350, 310, 300	580, 670, 720, 700, 600, 470	20	12
Nil	290, 270, 430, 410	750, 680, 600, 690	—	—
$CuSO_4$, 4×10^{-3}	250, 160, 270, 270	300, 300, 370, 440	32	48
$CuSO_4$, 2×10^{-3}	290, 140, 370, 490	740, 550, 450, 600	8	14
$CuSO_4$, 1×10^{-3}	320, 270, 410, 400	750, 620, 580, 630	0	5
$CuSO_4$, 5×10^{-4}	250, 340, 370, 480	740, 670, 590, 700	−3	1

Table 3. *The effects of zinc and copper sulphates on nitrification in stimulated allotment soil*

(Soil (10 g.) was percolated with 100 ml. $2{\cdot}5 \times 10^{-3}$ M-$(NH_4)_2SO_4$, with or without $ZnSO_4$ or $CuSO_4$. Each figure is the mean value from four separate experiments. A negative inhibition means that nitrification was stimulated.)

Metal in percolate (M)	Nitrate N formed/g. soil after			Mean inhibition of nitrification after		
	1 day (μg.)	3 days (μg.)	4 days (μg.)	1 day (%)	3 days (%)	4 days (%)
Nil	120	320	440	—	—	—
$ZnSO_4$, 4×10^{-3}	40	40	90	67	87	80
$ZnSO_4$, 2×10^{-3}	80	210	270	33	34	39
$ZnSO_4$, 1×10^{-3}	80	250	320	33	22	27
$ZnSO_4$, 5×10^{-4}	95	250	350	21	22	21
$ZnSO_4$, $2{\cdot}5 \times 10^{-4}$	110	250	405	8	22	8
Nil	120	340	450	—	—	—
$CuSO_4$, 4×10^{-3}	30	80	140	75	76	69
$CuSO_4$, 2×10^{-3}	75	260	360	37	24	20
$CuSO_4$, 1×10^{-3}	100	270	380	17	21	16
$CuSO_4$, 5×10^{-4}	160	310	435	−33	9	3
$CuSO_4$, $2{\cdot}5 \times 10^{-4}$	140	370	470	−17	−9	−4

to stimulate nitrification a little, but the stimulation was not significant. Nevertheless the implied suggestion that copper may play some part in soil nitrification is supported by the results of experiments in which Fen soils were treated with sodium diethyldithiocarbamate (Table 5). This treatment,

Table 4. *The effects of zinc and copper sulphates on nitrification in stimulated Fen soil*

(Soil (10 g.) was percolated with 100 ml. $2{\cdot}5 \times 10^{-3}$ M-$(NH_4)_2SO_4$, with or without $ZnSO_4$ or $CuSO_4$. Each figure is the mean value from three separate experiments. A negative inhibition means that nitrification was stimulated.)

Metal in percolate (M)	Nitrate N formed/g. soil after		Mean inhibition of nitrification after	
	2 days (μg.)	4 days (μg.)	2 days (%)	4 days (%)
Nil	240	520	—	—
$ZnSO_4$, 4×10^{-3}	150	410	37	21
$ZnSO_4$, 2×10^{-3}	180	340	25	35
$ZnSO_4$, 1×10^{-3}	180	500	25	4
$ZnSO_4$, 5×10^{-4}	230	430	4	17
$ZnSO_4$, $2{\cdot}5 \times 10^{-4}$	220	510	8	2
Nil	230	420	—	—
$CuSO_4$, 4×10^{-3}	230	340	0	19
$CuSO_4$, 2×10^{-3}	200	360	13	14
$CuSO_4$, 1×10^{-3}	230	400	0	5
$CuSO_4$, 5×10^{-4}	220	440	4	−5
$CuSO_4$, $2{\cdot}5 \times 10^{-4}$	240	440	−4	−5

which removed 13 μg. Cu/g. soil (about 30 times the exchangeable copper of the soil), effectively inhibited soil nitrification. The inhibition could be reversed completely by percolating copper sulphate through the soils, partially by percolating manganese sulphate, but not at all by percolating ferrous sulphate, zinc sulphate, or cadmium chloride.

Table 5. *The effects of various metals in reversing diethyldithiocarbamate poisoning of nitrification in Fen soil*

(Diethyldithiocarbamate-treated soils (10 g.) were percolated with 100 ml. $2{\cdot}5 \times 10^{-3}$ M-$(NH_4)_2SO_4$ plus metals as indicated. Control soil was not treated with diethyldithiocarbamate.)

Metal addition to percolate (M)	Nitrate N formed/g. soil after		
	1 day (μg.)	3 days (μg.)	5 days (μg.)
Nil (control)	160	350	620
Nil	30	80	130
$CuSO_4$, 4×10^{-3}	110	360	490
$CuSO_4$, 2×10^{-3}	100	300	580
$CuSO_4$, 1×10^{-3}	80	320	580
$CuSO_4$, 5×10^{-4}	40	250	590
$ZnSO_4$, 4×10^{-3}	30	30	100
$ZnSO_4$, 2×10^{-3}	20	60	100
$CdCl_2$, 4×10^{-3}	30	40	110
$CdCl_2$, 2×10^{-3}	30	60	110
$FeSO_4$, 4×10^{-3}	20	40	140
$FeSO_4$, 2×10^{-3}	30	80	150
$MnSO_4$, 4×10^{-3}	20	120	210
$MnSO_4$, 2×10^{-3}	30	120	200

DISCUSSION

The results indicate that it should be possible to study quantitatively by the percolation technique the effect of any trace element on the microfloral activities of soil. Zinc has been found to be more inhibitory to soil nitrification than copper, but the inhibition due to either metal is less on an organic soil than on a mineral soil. This accords with the agronomic finding that trace-element deficiencies are more usually associated with organic, rather than mineral, soils. In an organic soil the availability of a trace element (and therefore its toxicity, if it is toxic) tends to be less than in a mineral soil.

It would be unwise to conclude either from the present, or from the previous results (Lees, 1946), that copper is essential to the nitrifying organisms. The balance, as well as the amounts, of trace elements in a nitrifying culture may profoundly affect the speed of nitrification therein (Meiklejohn & Lees, in preparation). The inhibition of soil nitrification that ensues when copper is removed from a soil may therefore be due, not directly to a copper deficiency, but to a resultant imbalance of the remaining available trace elements. Such a theory would perhaps explain the partial reversal of diethyldithiocarbamate poisoning by manganese. Lucas (1945) has shown by plant growth experiments that there is some interaction between soil copper and soil manganese.

The results of the present work suggest a possible basis for a direct biological test for trace element deficiencies in soil. A diethyldithiocarbamate-treated soil is copper-deficient; as a result of this deficiency the nitrifying power of the soil is lowered, but may be raised again if copper is added to the soil. Copper deficiency in soil is therefore biologically detectable by the fact that addition of copper to the soil increases the nitrifying power. The possibility of measuring natural trace-element deficiencies by this sort of technique, which is an extension of the culture test elaborated by Mülder (1940), is obvious.

SUMMARY

1. It has been found possible quantitatively to study the effects of zinc and copper on soil nitrification by the percolation technique.

2. The toxic effect of either element is less on an organic than on a mineral soil.

3. Sodium diethyldithiocarbamate poisons soil nitrification. The poisoning is reversible by copper and partially reversible by manganese.

4. The possible importance of copper in nitrification is discussed.

5. It is suggested that the results might form the basis of a direct soil test for trace-element deficiencies.

I am indebted to Mr W. J. Redmond for accurate and skilled assistance throughout the work, and to Dr J. B. Hale for spectrophotometric determinations of zinc.

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Studies in Detoxication

16. THE METABOLISM OF ACETANILIDE IN THE RABBIT

By J. N. SMITH and R. T. WILLIAMS, *Department of Biochemistry, University of Liverpool*

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It is now sixty years since Cahn & Hepp (1887) introduced acetanilide (antifebrin) as an analgesic and antipyretic. Nevertheless, its fate in the body has not been studied quantitatively until recently (Greenberg & Lester, 1946). Furthermore, its metabolites are not exactly known. Early work (Müller, 1887; Kumagawa, 1888; Gregoire & Hendrick, 1904, etc.) established that acetanilide was oxidized *in vivo*, and that by suitable treatment of the urine *p*-aminophenol could be isolated. Jaffé & Hilbert (1888) found that the metabolites of acetanilide in the dog were different from those in the rabbit. From dog urine the main compound isolated was benzoxazolone together with small amounts of *p*-aminophenol, whereas from rabbit urine only *p*-aminophenol was isolated. It is likely that the benzoxazolone of Jaffé & Hilbert (1888) is an artefact derived from *o*-aminophenol and urea. It appears therefore that in dogs acetanilide is oxidized mainly in the *o*-position and in rabbits in the *p*-position. None of the above-mentioned work gives any clue whether acetanilide is deacetylated or not.

In 1889 Mörner, carefully avoiding hydrolytic procedures, worked up urine from a subject to whom acetanilide had been administered, and isolated potassium *p*-acetamidophenylsulphate as a double