Plant and Soil **34**, 133–144 (1971) Ms. 1518

MANGANESE TOXICITY TO PEANUTS IN AUTOCLAVED SOIL *

by H. W. BOYD

Department of Plant Pathology, University of Georgia College of Agriculture Experiment Stations, Georgia Station, Experiment, Georgia

SUMMARY

The interveinal leaf chlorosis occurring in Argentine peanuts growing in autoclaved soil is the result of manganese toxicity. The toxic level of manganese results from two mechanisms: the direct release of manganese complexed with the organic fraction of the soil, and the killing of microorganisms that normally transform available manganese into higher oxides.

Propylene oxide treatment of soil resulted in a gradual increase in available manganese but selectively blocked microbial conversion of divalent Mn to a nonavailable state. Methyl bromide had no measureable effect.

INTRODUCTION

There are many reports of increased available manganese following soil sterilization [4 8 9 16]. The mechanism is not agreed upon and is thought to be both biological [12 13] or nonbiological [5 9].

Manganese availability to plants is related to oxidative conditions favoring the formation of higher oxides (unavailable to plants), and reductive conditions resulting in divalent manganese (available to plants) [14 19]. The organic matter fraction of the soil is involved, but in what way is not clear [16]. Manganese may form complexes with organic molecules [1 2], but there are conflicting reports as to the availability to plants of this complexed manganese [1 2 10 11 19 20]. The addition of specific organic amendments has been shown to both increase [7 15 18] and decrease [21] available manganese.

The present work resulted from greenhouse observations of an unidentified disorder in peanuts. An interveinal leaf chlorosis was

* Journal Series Paper No. **813**, University of Georgia College of Agriculture Experiment Stations, Georgia Station, Experiment, Georgia 30212.

the prominent symptom, and was related to treatments employing autoclaved soil. Manganese toxicity was suspected to be the cause, and possible mechanisms of the manganese release were investigated.

MATERIALS AND METHODS

Glasshouse test

Observations of symptoms were made on Spanish type peanuts (*Arachis hypogaea* L., variety Argentine) planted five seeds per 4-in pot and growing at a temperature range of 21 to 28°C. Symptom severity was rated as follows: 0 = no symptoms; 1 = slight symptoms; 2 = moderate symptoms; 3 = severe symptoms. A symptom index was obtained by multiplying the per cent plants affected in each pot times the severity rating divided by 10. Figures presented in the tables represent the mean of 8–10 individual pots (40–50 plants).

The two soils used were Davidson clay loam and Davidson loam, the latter employed unless otherwise indicated. Soil was treated in 4-in clay pots for glasshouse tests and in 50 g samples in petri plates for laboratory tests. Autoclaved soil was exposed to 122°C at 16 psi for two one-hour periods interrupted by a 24 hour interval at room temperature. Soil was treated with propylene oxide by exposing six plates of soil to 10 ml propylene oxide per liter volume in a sealed container for 24 hours; and to methyl bromide by exposing the same to 1 lb methyl bromide in a 52 gal drum for 24 hours. Dry heated soil was placed for the times indicated in an oven preheated to 122°C. To test the effect of small quantities of autoclaved soil, weighed amounts of soil were layered one inch below the surface in clay pots filled with washed, autoclaved sand. Manganese was added in the form of $MnSO_4$ calculated in ppm on a soil dry-weight basis, mixed throughout the soil, and left standing two weeks before analysis.

Manganese analysis

In preliminary tests leaf necrosis correlated well with the quantity of manganese extractable with ammonium acetate [6]. Reference to manganese in the text is to this fraction. The single exception is referred to as easily reducible manganese (Table 3), which was obtained by adding 0.2% hydroquinone to the extracting solution. Manganese concentrations were measured colorimetrically.

Source of available manganese

Samples of soil treated with methyl bromide were plated in various dilutions on Difco nutrient agar to which 100 ppm Mn had been added. Developing colonies were largely actinomycetes. One week old cultures growing on Difco nutrient broth were blended for 1 min and 20 ml added to each petri plate containing 50 g soil. The covered plates were incubated for the times indicated at 25°C until analyzed for manganese.

To study the effects of an added energy source upon manganese, 15 ml nutrient broth and 20 ml 1% H_2O_2 were added to the soil plates described above. In other tests attempts were made to remove the soil organic fraction by extracting 720 g soil in 1800 ml 0.5 *N* NaOH and then lowering the pH with 0.1 *N* H_2SO_3.

RESULTS

Glasshouse tests

Growth irregularities in plants of Argentine peanuts were correlated with those treatments containing autoclaved soil. The most obvious symptom was an interveinal chlorosis present at emergence and in subsequent new growth (Plate 1). In later stages a marginal leaf necrosis developed, and some stunting occurred in severe cases. Chlorosis and stunting were also evident in corn, cotton, and soybeans planted in the same autoclaved soil with a leaf crinkle noted in the latter two crops.

When mixed with nontreated soil, as little as 25% autoclaved soil resulted in considerable symptoms (Table 1). Soil treated with propylene oxide did not reduce the symptom index to the same extent

TABLE 1

Effect of mixtures of treated and nontreated (25–100%) soil on interveinal leaf chlorosis in peanuts

Soil composition (%)			
Autoclaved	Non-treated	Propylene oxide treated	Index*
100	—	—	18
75	25	—	18
75	—	25	18
50	50	—	13
50	—	50	16
25	75	—	4
25	—	75	15
—	25	75	2
—	50	50	0
—	100	—	0
—	—	100	6

* See Materials and Methods section.

TABLE 2

Effect of small amount of autoclaved soil on interveinal leaf chlorosis in peanuts

Quantity of soil added (g)		Symptom index*
Autoclaved	Nonautoclaved	
50	—	30
—	50	14
40	—	26
—	40	5
30	—	26
—	30	2
20	—	28
—	20	4
10	—	16
—	10	10
5	—	17
—	5	10
Autoclaved sand		10
Nonautoclaved sand		7

* See Materials and Methods Section.

as nontreated soil (Table 1), indicating that a biological entity was involved in the etiology of symptom development. Only 5 g of autoclaved soil added as a layer of soil to a pot of sand was sufficient to increase symptom severity (Table 2). This suggested a toxicity which was seen at first to contradict a biological mechanism for the effect.

Manganese toxicity

Manganese toxicity was suspected and confirmed as the cause of foliar chlorosis. Symptoms induced in peanuts by adding manganese sulfate were very similar to those induced by autoclaved soil (Plate 2). The symptoms were expressed only with large quantities of added manganese, but analysis indicated that the soil had the capacity to oxidize or otherwise render unavailable much of the divalent manganese added (Table 3). Analysis of the soil indicated large increases in extractable manganese in autoclaved soil with little change in pH (Table 4). This high manganese level was not appreciably lower even after 16 weeks (Fig. 1). The potassium and phosphorous levels were

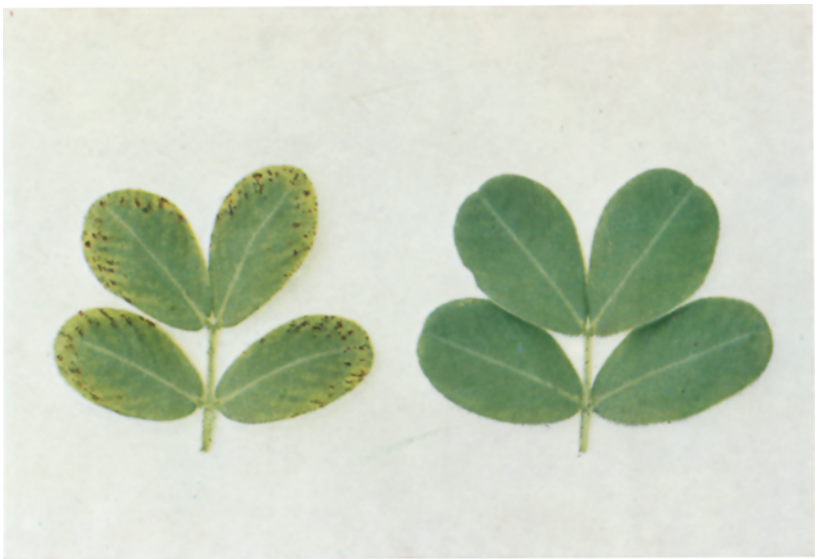


Plate 1. Symptoms (*left*) on peanuts planted in autoclaved soil.

Plate 2. A comparison of symptoms in peanuts induced by adding $MnSO_4$ and autoclaving soil.

TABLE 3

Exchangeable manganese in soil after autoclaving and adding manganese ($MnSO_4$)

Soil autoclaved	Manganese (ppm)		pH*
	Added	Measured*	
+	0	51	6.6
−	0	2	6.4
+	4	49	6.6
−	4	2	6.5
+	40	56	6.6
−	40	2	6.2
+	400	104	6.5
−	400	13	5.9

* After two weeks.

TABLE 4

Analysis of two soils after autoclaving

Autoclaved	pH	P*	K*	Manganese (ppm)	
				Exchangeable	Easily Reducible
Davidson clay loam					
−	6.0	032	245	4	−
+	6.2	003	112	49	−
Davidson loam					
−	5.5	040	217	2	43
+	5.9	002	070	150	40

* Pounds/acre.

significantly lower indicating that other disturbances occurred with autoclaving (Table 4). Foliar manganese levels were high in plants growing in autoclaved soil and symptom expression correlated closely with relative amounts of extractable manganese present in the soil (Table 5).

TABLE 5

Foliar manganese and interveinal leaf chlorosis in peanuts grown in autoclaved soil

Treatment	Manganese (ppm)		Symptoms
	Soil	Foliar	
Check	3	6	None
Added Mn*	7	46	Light
Autoclaved	47	66	Severe

* 400 ppm.

TABLE 6

The effect of autoclaving soil on manganese levels after adding an oxidant

Treatment	pH	Mn (ppm)
H_2O_2–autoclaved	6.1	99
Autoclaved only	6.0	117
Autoclaved–H_2O_2	6.2	135
Check	5.3	3
Check–H_2O_2	5.8	11

Source of available manganese

The evidence is negative that autoclaving reduces higher oxides of manganese. There was no proportional decrease in the easily-reducible fraction (which should contain these oxides) accompanying the increase in exchangeable manganese (Table 3). Also the addition of an oxidant did not appreciably alter the effects of autoclaving (Table 6). When the organic fraction was removed by digestion with NaOH, the increase in extractable manganese resulting from autoclaving was smaller (Table 7). The increase in exchangeable manganese when the pH was lowered after extraction has not been explained (Table 7).

The increase in exchangeable manganese resulting from treatment with propylene oxide was more gradual than that caused by autoclaving, and methyl bromide had no measurable effect (Table 8).

TABLE 7

The effect of autoclaving soil on extractable manganese after removing the organic fraction with NaOH

Treatment	pH	Mn (ppm)*
Check	5.3	2
Autoclaved only	5.9	138
NaOH	10.0	1
NaOH & autoclaved	10.0	11
NaOH**	7.6	210
NaOH & autoclaved**	7.6	276

* Analyzed after 3 days incubation at 25°C.
** The pH lowered with H_2SO_3.

TABLE 8

The effect of three sterilants upon extractable manganese

	Analysis after:			
	24 hours		2 weeks	
Treatment	pH	Mn (ppm)	pH	Mn (ppm)
Propylene oxide	6.6	4	6.8	59
Methyl bromide	6.1	2	6.2	1
Autoclaved	6.3	45	6.6	51
Check	6.0	2	6.0	1

Because symptoms were not expressed in plants growing in soil fumigated with methyl bromide, this compound was used as a screening agent for microorganisms capable of oxidizing divalent manganese. The resulting isolates were added to pots of autoclaved soil and incubated at 19, 22, or 25°C. None of the isolates alleviated symptoms of plants subsequently planted in autoclaved soil, nor did they reduce the level of manganese in 16 weeks time (Fig. 1). The broth added with the isolates unexpectedly increased the level of exchangeable manganese in the nonautoclaved treatments included as checks, and after about 6 weeks, 5 out of 12 isolates reduced this elevated level of manganese to approximately that of the original soil (Fig. 2).

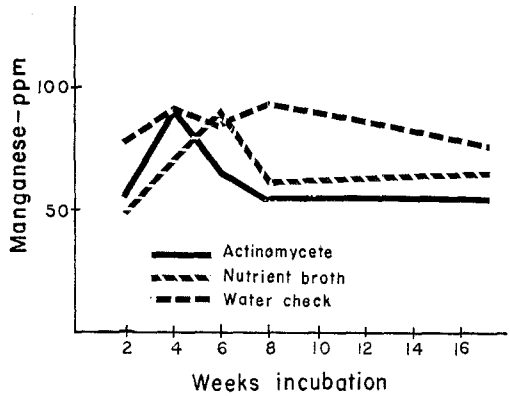


Fig. 1. Extractable manganese in autoclaved soil after incorporation of an actinomycete isolate.

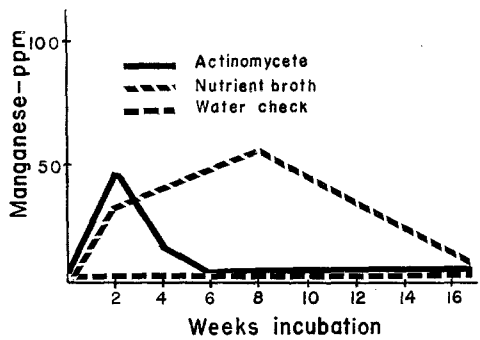


Fig. 2. Extractable manganese in nonautoclaved soil after incorporation of one of the four actinomycete isolates effective in reducing the level of manganese.

The increase in exchangeable manganese resulting from adding the broth to the soil was more gradual than that caused by autoclaving (Fig. 3), and was accompanied by a corresponding increase in pH (Fig. 4); two indications of biological activity. The broth itself contained less than 1 ppm manganese, and because no similar increase in manganese occurred when it was added to soil that had been treated with propylene oxide (Table 9), it is concluded that broth affected the manganese level by acting as an energy source for the growth of microorganisms, somehow resulting in more available manganese.

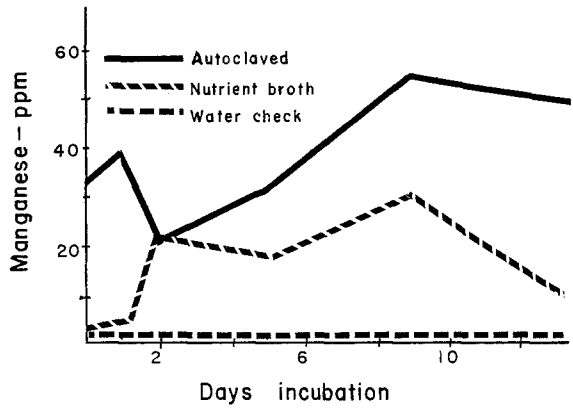


Fig. 3. Extractable manganese in autoclaved soil, nonautoclaved soil, and soil with nutrient broth added.

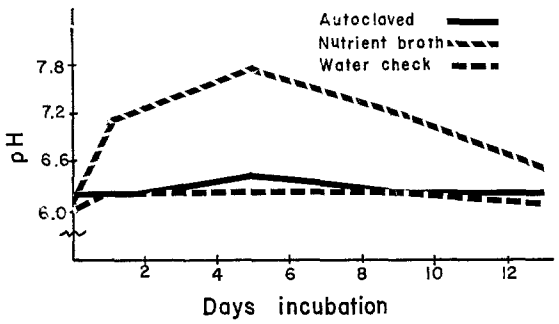


Fig. 4. The pH of autoclaved soil, nonautoclaved soil, and soil with nutrient broth added.

TABLE 9

The effect of nutrient broth on extractable manganese in propylene oxide-treated and nontreated soil

		Analysis after:			
		3 days		2 weeks	
Soil treatment	Broth	pH	Mn (ppm)	pH	Mn (ppm)
Propylene oxide	+	6.6	6	7.1	69
Propylene oxide	–	6.5	5	6.8	108
Check	+	7.6	23	7.1	54
Check	–	6.2	2	6.2	0

DISCUSSION

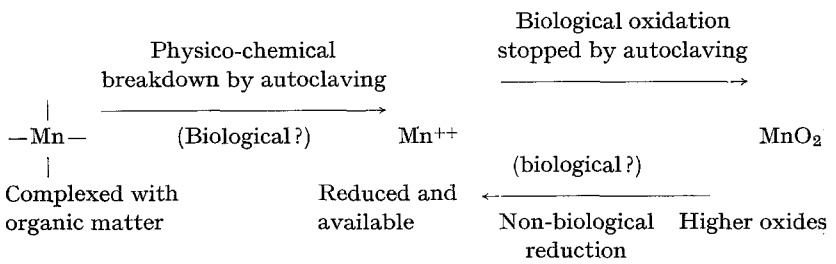
Most of the symptoms observed in plants growing in autoclaved Davidson soil can be accounted for as manganese toxicity. However, with a number of physico-chemical changes occurring when soil is autoclaved several complexing factors may be involved. The marginal leaf necrosis, for example, may be due to an actual potassium deficiency or perhaps to a Mn induced K deficiency.

It is proposed that the transformation of manganese is at the same time both biological and nonbiological. The immediate flush of available manganese after autoclaving is apparently nonbiological, perhaps due to the breakdown of manganese-organic molecule complexes, since the data indicate that higher oxides were not reduced. At the same time the organisms that normally oxidize manganese to some higher nonavailable oxide are killed so that the manganese remains available at levels toxic to plants. It is suggested that propylene oxide acts only in the latter way allowing the reduction processes to continue without the normal compensating oxidation; while methyl bromide, at the concentration used, does not affect the organisms important in the manganese cycle. Further research into the specific action of soil fumigants may be fruitful.

The microorganisms tested did not lower the level of available manganese in autoclaved soil, which may indicate the necessity for the presence of two organisms [3], or may be due to some physico-chemical changes in the soil that prevent successful recolonization. The latter explanation is supported by the fact that some isolates were able to lower the level of available manganese in nonautoclaved soil (Fig. 2). The rather stable level of available manganese maintained in autoclaved soil even after 16 weeks (Fig. 1), indicates that manganese-oxidizing organisms do not readily recolonize autoclaved soil.

Addition of nutrient broth to soil increased manganese availability indirectly apparently by providing an energy source for microorganisms. Whether these organisms reduce higher oxides of manganese or uncouple manganese complexed with organic molecules is not known.

The following diagram is suggested as one that represents the data presented:



ACKNOWLEDGEMENTS

I am grateful to A. T. Alexander of this department for conducting the manganese analyses, and to R. M. Harrison and O. E. Anderson of the Department of Agronomy – Soils Section for helpful advice.

Received June 12, 1970

REFERENCES

1 Beckwith, R. S., Metal complexes in soils. Australian J. Agr. Research **6**, 685–698 (1954).
2 Bremner, J. M., Heintze, S. G., Mann, P. J. G., and Lees, H., Metallo-organic complexes in soil. Nature **158**, 790–791 (1946).
3 Bromfield, S. M., and Skerman, V. B. D., Biological oxidation of manganese in soils. Soil Sci. **69**, 337–348 (1951).
4 Conner, S. D., Factors affecting manganese availability in soils. J. Am. Soc. Agron. **24**, 726–733 (1932).
5 Eno, C. F., and Popenoe, H., Gamma radiation compared with steam and methyl bromide as a soil sterilizing agent. Soil Sci. Soc. Am. Proc. **28**, 533–535 (1964).
6 Fujimoto, C. K., and Sherman, G. D., The effect of drying, heating, and wetting on the levels of exchangeable manganese in Hawaiian soils. Soil Sci. Soc. Am. Proc. **10**, 107–112 (1945).
7 Fujimoto, C. K., Sherman, G. D., Behavior of manganese in the soil and the manganese cycle. Soil Sci. **66**, 131–145 (1948).
8 Fujimoto, C. K., and Sherman, G. D., Manganese availability as influenced by steam sterilization of soils. J. Am. Soc. Agron. **40**, 527–534 (1954).
9 Gerretsen, F. C., Manganese deficiency of oats and its relation to soil bacteria. Ann. Botany **1**, 207–230 (1937).
10 Heintze, S. G., and Mann, P. J. G., Studies on soil manganese. J. Agr. Sci. **39**, 80–95 (1949).
11 Heintze, S. G., and Mann, P. J. G., A study of various fractions of the manganese of neutral and alkaline organic soils. J. Soil Sci. **2**, 234–242 (1951).
12 Hurwitz, C., Effect of temperature of incubation of amended soil on exchangeable manganese. Soil Sci. **66**, 267–272 (1948).
13 Jones, L. H. P., The effect of liming a neutral soil on the uptake of manganese by plants. Plant and Soil **8**, 301–304 (1957).

14 Leeper, G. W., The forms and reactions of manganese in the soil. Soil Sci. **63**, 79–94 (1947).
15 Mann, P. J. G., and Quastel, J. H., Manganese metabolism in soils. Nature **158**, 154–156 (1946).
16 McCool, M. M., Effect of various factors on the soluble manganese in soils. Contrib. Boyce Thompson Inst. **6**, 147–164 (1934).
17 Mulder, E. G., and Gerretsen, F. C., Soil manganese in relation to plant growth. Adv. Agron. **4**, 221–277 (1952).
18 Passioura, J. B., and Leeper, G. W., Available manganese and the X hypothesis. Agrochimica **8**, 81–90 (1963).
19 Pipei, C. S., The availability of manganese in the soil. J. Agr. Sci. **21**, 762–779 (1931).
20 Quastel, J. H., Hewitt, E. J., and Nicholas, D. J. D., The control of manganese deficiency in soils. I. The effects of sulphur and thiosulphates on crops growing on manganese-deficient soils. J. Agr. Sci. **38**, 315–322 (1948).
21 Russell, E. W., The manganese compounds. *In*: Soil Conditions and Plant Growth, pp 516–517, Longmans, Green, London (1961).
22 Takkar, P. N., Effect of organic matter on soil iron and manganese. Soil Sci. **108**, 108–112 (1969).