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STUDIES ON THE MANGANESE UPTAKE OF LETTUCE ON STEAM-STERILISED GLASSHOUSE SOILS

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SUMMARY

During steam sterilisation of glasshouse soils appreciable amounts of easily reducible manganese are converted into exchangeable manganese. The reverse process takes place much more gradually. As a result, manganese toxicity occurs in several crops on newly steamed soils. In the Netherlands, lettuce has been found to be particularly prone to manganese toxicity. An investigation was carried out to obtain more information about the manganese status of steamed glasshouse soils in which lettuce was used as the test crop. The following results were noted.

The uptake of exchangeable manganese is easier in the lighter soils than in heavy soils. Application of iron to the soil inhibits manganese uptake by the plant, but the iron must be applied in the form of chelate. The pH has a profound effect on manganese uptake on steamed as well as on unsteamed soils. However, the relationship between the pH and the manganese content of the crop on steamed soils is different from that found on unsteamed soils.

The slow rate of oxidation of manganese in steamed glasshouse soil may be explained by the fact that the oxidising bacteria are killed during the steam sterilisation process. The fixation of manganese can be accelerated appreciably by inoculating the steamed soil with manganese-oxidising bacteria.

The effect on manganese uptake of five soil desinfection chemicals used in the investigation proved to be very small.

The lettuce varieties used in the Netherlands show a wide variation in susceptibility to manganese toxicity. This cannot be explained by different rates of manganese uptake. It is more likely that the varietal differences are based on different levels of resistance to manganese present in the plants.

INTRODUCTION

On many Dutch nurseries steam sterilisation of glasshouse soils is undertaken as an annual measure to control pathogenic soil organisms. The method has some unfavourable side effects with the result that the development of some crops on newly steamed soils leaves a lot to be desired. In the Netherlands this is the case for instance with lettuce. Growth disturbances may occur in this crop on newly steamed soil, particularly under poor light conditions. The prime cause is excessive manganese uptake by the crop. A previous publication dealt with the subject in detail [19].

The results described in the above publication show that during steam sterilisation large amounts of easily reducible manganese in the soil are converted into exchangeable manganese. The degree to which this conversion takes place depends on the length of time during which the soil is heated and on the temperature used. After prolonged steaming of soils with a high content of easily reducible manganese, high levels of exchangeable manganese are found. In the Netherlands this is particularly the case in soils with a high clay content.

Most of the exchangeable manganese found in the soil consits of the element in its simplest form, the bivalent manganese ion, which is readily taken up by the plant. Under normal cropping conditions the exchangeable manganese in the soil is largely converted by oxidation into manganese oxides which are not directly available to the plants. In steam-sterilised soils manganese oxidation takes place very slowly. In many cases manganese which has become available during steaming is still not completely fixed six to nine month later.

The next stage of the investigation was concerned partly with finding the reasons for the slow manganese oxidation and exploring the possibilities of accelerating the process. Several other factors were checked out at the same time. Amongst them were the pH, the iron content and the bacterial life of the soil, the varieties grown, the soil type and the method of soil sterilisation. Lettuce served as the test crop and the most important results obtained are discussed in this publication.

METHODS

Soil analysis

The levels of exchangeable manganese and exchangeable iron were determined in the way generally employed for glasshouse soils in the Netherlands, by extracting the soil sample with Morgan's solution. This is a buffer solution of acetic acid and sodium acetate (pH 4.8). The proportion of extraction was 1 part soil to 2½ parts Morgan's solution.

Leaf analysis

Crop samples taken consisted of whole heads of lettuce. Where this resulted in excessively large samples only part of the heads was used with a representative proportion of older and younger leaves. After drying, the samples were analysed for manganese. In one experiment the older and younger leaves were analysed separately.

Pot experiments

The lettuce was grown in ten litre pots with, as a rule, two plants per pot, unless otherwise stated.

Manganese-oxidising bacteria

Manganese-oxidising bacteria were cultured on petri dishes containing 1% $MnCO_3$ and 2% agar in demineralised water. Colonies were isolated from these petri dishes and cultured on the medium described by Gerretsen [8]. The pure cultures were multiplied on the same medium and applied to the soil as a suspension.

Soil sterilisation

The soil was steam sterilised for about ten hours with steam at 100°C or with a steam–air mixture at 70°C. Chemical treatments of the soil were carried out by the application of 15 ml chloropicrin, 25 g methylbromide, 20 ml DD, 20 ml of a solution of 10 volumes per cent EDB and 30 ml of a solution of 40 volume per cent methamsodium, all per 100 litres of soil.

RESULTS

Rate of manganese uptake

Manganese is taken up rapidly by the crop. This is demonstrated by the results obtained in a pot experiment in which young lettuce plants, raised in soil blocks, were grown on in soil to which varying quantities of manganese sulphate had been added. Twelve plants were planted out per pot and every week two plants were analysed for manganese. The results are given in Figure 1. Already at the end of one week the manganese content of the plants corresponded with the different manganese levels in the soil. During the final weeks of the experiment the manganese contents in the crop declined which was probably caused by the fact that the levels of exchangeable manganese in the soil were reduced by oxidation.

At the end of the experiment old and young leaves were analysed

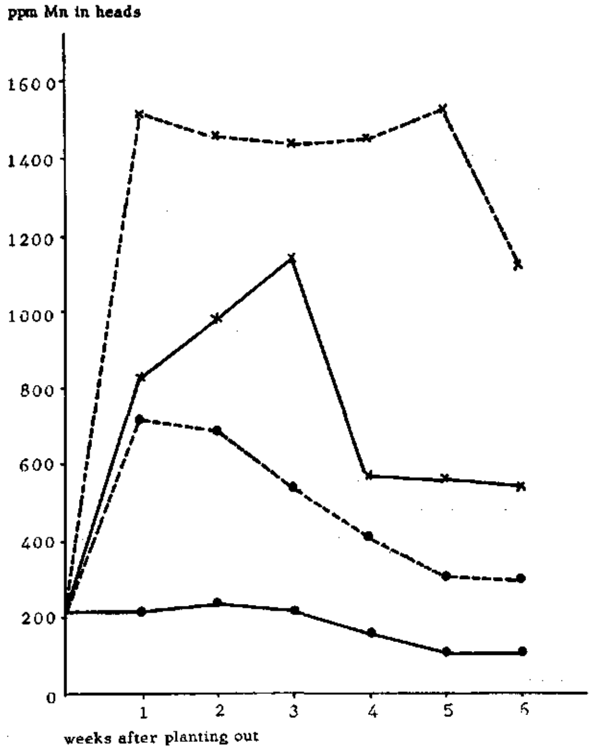

Fig. 1. The course of the manganese content of lettuce heads grown on soil to which different quantities of manganese sulphate were added: ●——● no, ●---● 200, ×——× 400 and ×----× 800 mg $MnSO_4.H_2O$ per l of soil.

separately. The results are given in Table 1. In the old leaves the manganese content was on average twice as high as in the young leaves. This agrees with published data for other crops [3 7 24 26].

pH

In pot experiments, using several types of steam sterlised soil, a study was made of the relationship between the pH of the soil and the manganese content of the crop. The different pH levels were achieved by applying calciumhydroxide. Table 2 shows the content of exchangeable manganese in the soils used and Figure 2 shows the relationship between the pH of the soil and the manganese content of the crop.

TABLE 1

The manganese content (Mn, ppm of dry matter) of young and old leaves of lettuce

mg $MnSO_4 . H_2O$ per l soil	Young leaves	Old leaves
0	70	116
200	240	333
400	317	697
800	573	1223

TABLE 2

Exchangeable manganese content (ppm of Morgan's extract) of the soils used in pot experiments with different pH levels

Soil no	Soil type	Exchangeable Mn
1	River loam	83
2	Peaty clay	59
3	Loomy sand	20
4	Peaty clay	44
5	Clay-peat	20

The relationship mentioned is not linear. It resembles the relationship found on unsteamed soil by Page [17] between the pH and the content of water-soluble manganese in the soil. Page calculated the following equation for this relationship:

$$pMn = 0.5\ pH - 3$$

in which pMn represents the negative logarithm of the content of water-soluble manganese in the soil.

The strong resemblance between the relationship shown in Figure 2 and that found by Page is understandable because there is a linear relationship between the content of water-soluble mangenese in the soil and the manganese content of the crop [15]. The following equation might therefore be expected for the relationship between the pH of the soil and the manganese content of the crop:

$$p\,ay = 0.5\,pH - 3$$

in which a is the coefficient for the relationship between the manga-

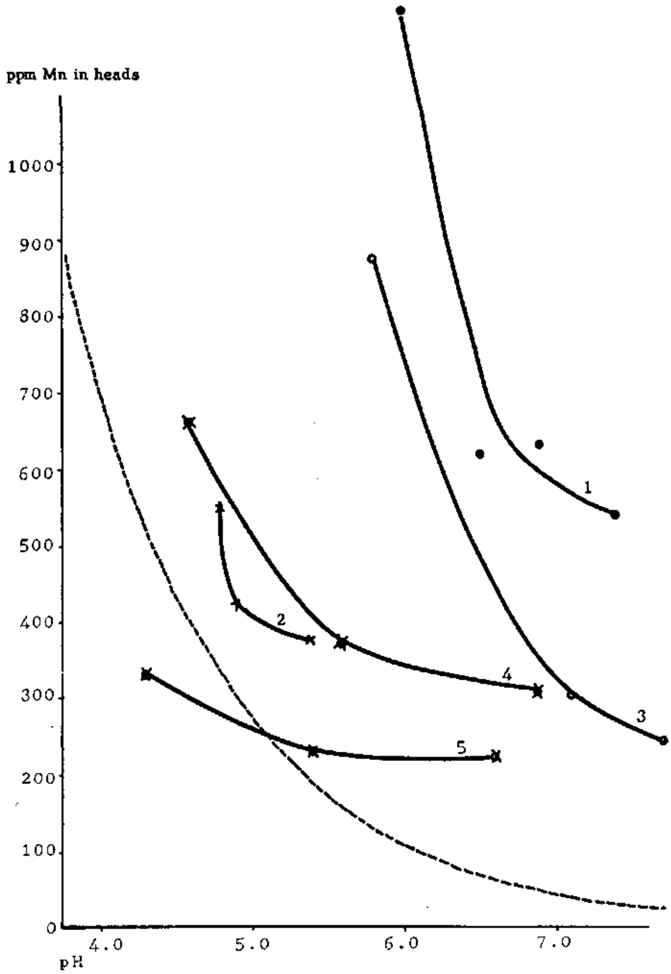

Fig. 2. The relationship between pH of the soil and the manganese content of lettuce heads on five steam sterilised soils (see Table 2). The broken line gives the relationship found on unsteamed soils in a previous investigation.

nese content of the crop and the soil and y represents the manganese content of the crop.

This function may be converted to:

$$\log y = -0.5 \text{ pH} + 3 - \log a$$

The value of a is generally between 0.1 and 0.01 [15 22]. Therefore the function may be reduced to:

$$\log y = -0.5 \text{ pH} + C$$

in which C will have a value of between 4 and 5. This equation compares favourably with that found for unsteamed soil in an earlier investigation [19], *i.e*

$$\log y = -0.403 \text{ pH} + 4.451$$

Figure 2 shows that manganese uptake on steamed soil was significantly greater than after last mentioned equation. This may be explained by the fact that the content of exchangeable manganese in steamed soil is much greater than in unsteamed soil. The relationship found by Page is therefore not valid for steamed soil.

Soil type

As mentioned in the previous paragraph, the manganese content of the crop shows a linear relationship with the content of exchangeable manganese in the soil. The regression coefficient for the relationship between the factors mentioned varies greatly for different soil types. The more clay and organic matter the soil contains, the smaller the regression coefficient will be. This may be explained by the fact that the greater the adsorption complex of the soil, the more strongly it will fix exchangeable manganese.

These effects showed up clearly in a pot experiment in which the manganese uptake of lettuce was compared in a sandy and a clay soil. Figure 3 demonstrated the manganese uptake relative to the manganese content of these soils. The regression coefficient of the equation for this relationship is about four times as great for the sandy soil as it is for the clay soil.

Iron

The effect of iron on the manganese uptake was investigated in a pot experiment. Different levels of both iron and manganese were applied. Of manganese, 0, 400, or 800 mg $MnSO_4.H_2O$ were applied per litre of soil and of iron, 0, 50 or 100 mg Fe-EDDHA. Table 3 shows the manganese contents of the crop.

It appears that the application of iron chelate reduces the manganese uptake. In the treatments in which no manganese was given, the application of iron chelate even led to manganese deficiency.

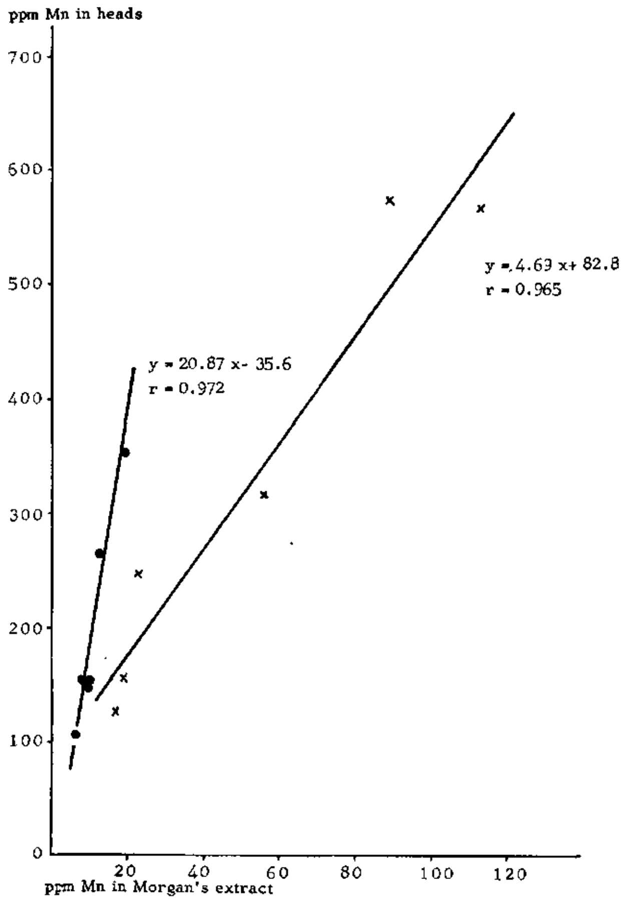

Fig. 3. The relationship between content of exchangeable manganese of the soil and the manganese content of lettuce heads on a sandy soil (●) and on a clay soil (×).

However, manganese toxicity was not significantly decreased by iron chelate in treatments where manganese sulphate was applied.

In the next experiment the application of larger quantities of iron was investigated. The soil used in this experiment was steam sterilised and iron was applied in the form of ferric sulphate, ferrous sulphate and Fe-EDDHA. Two iron levels were included, equivalent to 10 and 100 mg Fe per litre of soil. The contents of exchangeable iron and manganese and the manganese contents of the crop are shown

TABLE 3

The manganese content of lettuce heads (ppm of dry matter) in a pot experiment with different quantities of manganese and iron applied to the soil

mg $MnSO_4 . H_2O$ per l soil	mg Fe-EDDHA per l soil 0	50	100	Average
0	17	14	6	12
400	225	186	147	186
800	504	426	450	460
Average	249	209	201	219

TABLE 4

The content of exchangeable iron (ppm of Morgan's extract), two days after addition of the iron compounds to the soil, and the content of exchangeable manganese (ppm of Morgan's extract) and the manganese content of lettuce heads (ppm of dry matter) at harvest

Iron compound	Quantity mg Fe/l soil	Fe in soil	Mn in soil	Mn in heads
Fe $SO_4 . 7H_2O$	10	2.4	42	476
	100	2.6	40	527
$Fe_2(SO_4)_3 . 6H_2O$	10	2.3	42	596
	100	2.6	39	508
Fe-EDDHA	10	5.0	40	221
	100	49.0	44	139
Control	—	2.2	42	628

in Table 4. The levels of exchangeable iron were determined two days after application of the iron compounds and the contents of exchangeable manganese and the manganese contents of the crop were determined at the end of the cropping period.

The effects of the ferric and the ferrous sulphate treatments on the manganese content of the crop proved to be very slight. This is understandable as the iron in these compounds is fixed very rapidly by the soil. Two days after application it could not be traced any more in the Morgan's extract. However, Fe-EDDHA persisted and showed a clear effect on the manganese uptake of lettuce.

Varieties

The current glasshouse lettuce varieties used in Holland vary a good deal in their susceptibility to manganese toxicity. Several pot experiments were carried out to determine whether this is the result of variations in manganese uptake.

In the first experiment comparisons were made of the manganese toxicity symptoms and the manganese uptake of six lettuce varieties grown in steamed soil. The varieties showed great differences in the degree of manganese toxicity – as is shown in Table 5 – but differences in the manganese content of the plants were only slight, and they did not reflect the degree of toxicity. The variations in the manganese content of the crop were not statistically significant.

In a further experiment a comparison was made of two varieties grown in three successive crops (Table 6). In this case the number of replications was much greater than in the previous experiment and the variations found in the manganese contents of the crops were therefore statistically significant. The confidence limits in the successive crops were 0.03, 0.12, and 0.04 respectively. The differences found between the two varieties corresponded with those recorded for the same varieties in the previous experiment. There is no relationship between the manganese content of the crop and the degree of manganese toxicity. Therefore, the variations in susceptibility are not the result of differences in manganese uptake, but of different levels of resistance to manganese present in the plant. Messing [14] also recorded clear differences in susceptibility to manganese toxicity in a number of English lettuce varieties.

Soil desinfectants

The effects of several soil desinfection chemicals on the manganese uptake of lettuce was investigated using a clay soil rich in re-

TABLE 5

Index figures for manganese toxicity and manganese content of lettuce heads (ppm of dry matter) for different varieties. Index for manganese toxicity: 1–3 light, 4–6 moderate and 7–10 severe symptoms

Variety	Manganese toxicity	Mn in heads
Blackpool	9.0	781
Rapide	8.0	774
Noran	7.2	672
Deciso	6.0	659
Deci-Minor	4.0	727
Plenos	0.2	799

TABLE 6

Index figures for manganese toxicity and manganese content of lettuce heads (ppm of dry matter) of three successive crops. For the index of manganese toxicity, see Table 5

Variety	Crop	Manganese toxicity	Mn in heads
Blackpool	1	3,6	580
Deciso	1	1,8	489
Noran	2	5,3	356
Plenos	2	2.5	459
Noran	3	1,0	372
Plenos	3	0,5	456

ducible manganese. The soil was analysed for exchangeable manganese a fortnight after treatment. The crop was sampled at harvest and analysed for manganese content. The results are shown in Table 7. Steam sterilisation of the soil at 100°C clearly has an effect on the level of exchangeable manganese in the soil and the manganese content of the crop. The steam–air mixture also has some effect, but the effect of soil desinfectants is slight.

In another experiment – also on clay soil – the effects of steaming and desinfection with DD were compared. The effect of manganese which was mixed with the soil a few days before the lettuce was planted was also investigated in this experiment. Several analyses were made of the exchangeable manganese content in the course of cropping. The manganese content of the crop was determined at harvest. The results are given in Table 8.

The results of the soil analyses show that steam sterilisation caused a sharp increase in the exchangeable manganese content. The increase in the exchangeable manganese content, as a result of manganese sulphate applications, was also greatest in the steam-sterilised soil. In the untreated and the DD-desinfected soil part of the manganese sulphate was already fixed after five days. A second and third sampling confirmed that the manganese applied is rapidly fixed in untreated and DD-treated soil. On the other hand, there was practically no fixation of manganese in the steam-sterilised soil. This is caused by the fact that the bacteria which promote manganese oxidation in the soil were killed by the steam sterilisation. This probably did not happen in the DD-treated soil in which manganese oxidation took place normally. The manganese contents in the crop show-

TABLE 7

The content of exchangeable manganese (ppm of Morgan's extract) and the manganese content of lettuce heads (ppm of dry matter) on a clay soil treated with different desinfectants

Treatment	Mn in soil	Mn in heads
Control	18	30
Steam (100°C)	56	740
Steam-air (70°C)	26	78
Chloropicrin	17	53
Methylbromide	16	45
DD	14	36
EDB	14	34
Methamsodium	13	38

TABLE 8

The effects of soil sterilisation and manganese applications to the soil on the content of exchangeable manganese (ppm of Morgan's extract) and the manganese content of lettuce heads (ppm of dry matter)

Soil sterilisation	mg $MnSO_4 . H_2O$ per l soil	Mn in soil, Days after application: 5	50	75	Mn in heads
None	0	16	14	15	52
	500	70	30	18	131
DD	0	16	14	17	77
	500	67	25	20	126
Steam	0	62	59	60	441
	500	143	124	124	756

ed a close relationship with the exchangeable manganese levels in the soil'

Fixation by bacteria

Most of the soil bacteria are destroyed by steam sterilisation. It was suggested in the previous paragraph that this would have an effect on manganese oxidation, because certain species of bacteria are necessary for rapid oxidation [4 8 9]. If this assumption is correct it should be possible to accelerate oxidation by inoculating steam sterilised soil with manganese-oxidising bacteria. This was tested in some incubation experiments.

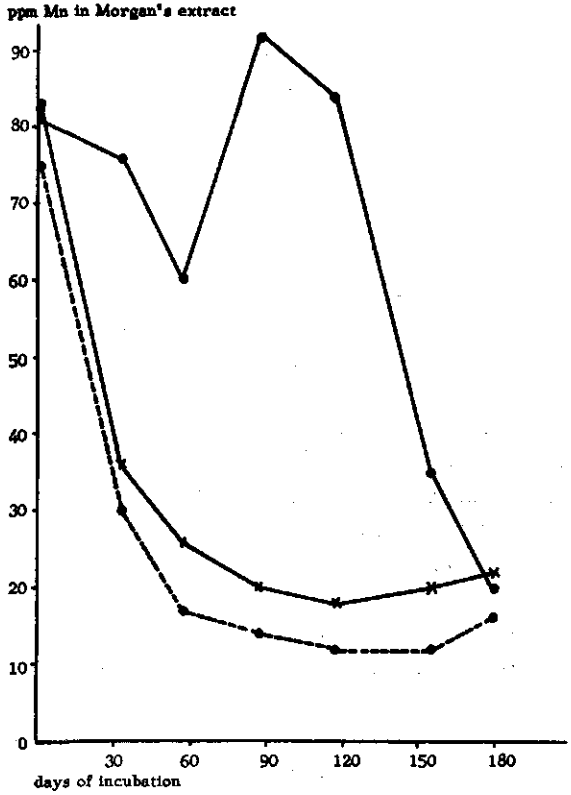

Fig. 4. The course of the content of exchangeable manganese on a steam sterilised clay soil after incubation: ●------● mixed with 5% unsteamed soil, ×———× mixed with a suspension of bacteria, ●———● control.

In these experiments a steam-sterilised clay soil was stored for several months, partly in sterile condition and partly after inoculation with manganese-oxidising bacteria. Inoculation was carried out in two ways, by the addition of 5% unsteamed soil and by the application of a bacterial suspension. The suspension was prepared with cultured bacteria. The exchangeable manganese levels were determined at regular intervals during incubation. The results of one of the experiments are given in Figure 4. Without bacterial inoculation manganese oxidation did not take place for 120 days. Bacterial inoculation on the other hand resulted in rapid oxidation. In other experiments the application of unsteamed soil also produced rapid oxidation of manganese which was not always the case where bacterial suspensions were applied. It is possible that cultured bacteria need time to adapt themselves to the soil environment. This aspect will be investigated in another research project.

DISCUSSION

On many types of soil so much manganese is released during steam sterilisation that toxicity symptoms may occur in crops planted out shortly after steaming. The occurrence of manganese toxicity does not depend only on the amount of available manganese in the soil and the quantity taken up by the crop, but also on the susceptibility of the crop to the manganese taken up. Susceptible crops like lettuce [12 15 19], beans [10] and roses [20] may show toxicity symptoms at manganese levels as low as 200 to 400 ppm of the dry-matter content. Less susceptible crops like cucumbers [13] and carnations [16] show symptoms at manganese levels above 600 to 800 ppm, whilst the least susceptible crops like tomatoes do not show toxicity symptoms unless the manganese levels exceed 1,500 to 2,000 ppm [6 12].

Dutch lettuce growers try to avoid manganese toxicity by steam sterilising the soil as long before planting the crop as possible. They also aerate the soil well after steaming and the pH level is checked to ensure that it is at least 6.5 [19]. These techniques are also recommended in other growing areas [2]. However, the amounts of manganese released during steaming of Dutch glasshouse soils are so large that these measures are not adequate to prevent manganese toxicity.

Manganese uptake may also be limited by the application of iron to the soil. This was shown in the work under discussion and has also been found by other research workers [23 25]. However, there is little practical prospect in iron applications as the quantities required are large and iron must be applied in the form of chelates. The price of these iron compounds is too high for large scale applications. An aspect related to the use of chelates is that organic compounds are released during steaming which may have properties similar to chelates in that they influence the uptake of iron and manganese. However, this aspect has not yet been included in our research.

Promotion of manganese oxidation by inoculation of the soil with manganese-oxidising bacteria has given promising results in experiments. However, the scope for large scale applications of the technique needs to be studied in greater detail.

The occurrence of manganese toxicity may also be prevented by using soil desinfection methods other than steam sterilisation. The results of this work show that the soil desinfectants used in the Netherlands have little effect on the manganese uptake of lettuce. This is contrary to the findings of other research workers [1 11 18] who have reported strong stimulation of manganese uptake by chemical soil desinfectants. An extreme stimulation was found in avocado [11]. Besides treatment with chemical soil desinfectants, pasteurisation of the soil with steam–air mixtures at temperatures below 100°C has also given promising results [5 21].

For the Dutch lettuce crops grown on steamed soils, probably the best results may be expected from the right choice of varieties. There are quite large differences in the susceptibility of Dutch lettuce varieties to manganese toxicity. Further development of the range of resistant varieties by breeding may well solve most of the problems.

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