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INVESTIGATIONS ON TRACE ELEMENTS IN THE NETHERLANDS*

E. G. MULDER**

Introduction.

The beneficial effect of trace elements on plant growth in the Netherlands was shown for the first time by Sjollema and Hudig in experiments on a disease of oats on neutral and slightly alkaline peaty soils (14). Although in those days Hudig was of the opinion that this effect of manganese had to be attributed to the correction of some unfavourable condition of the organic matter, it was shown by further investigations that the oats disease was caused by lack of available manganese. Söhngen (16) and particularly Gerretsen (6) demonstrated the effect of microorganisms in rendering soluble manganese compounds in soil unavailable for the plants.

In 1924 it was found by Hudig and Meyer (7) that the so-called reclamation disease which was found on newly reclaimed peaty and sandy soils in the Netherlands may be cured by adding copper sulphate to the soil. In the same way as with manganese, the beneficial influence of the heavy metal was attributed to some mysterious effect on the soil organic matter. Other investigators were of the opinion that the disease was caused by microorganisms (2). Copper should have a disinfecting effect. After the discovery of the indispensability of copper for plant growth (Sommer (15), Brandenburg (3)), it was shown by the author (10) that the reclamation disease is caused by copper deficiency of the plants.

In 1933 Sjollema published a report on copper deficiency in cattle (licking disease) which was found in areas where the plants suffered from the reclamation disease (13).

In addition to manganese and copper a beneficial effect of iron, zinc, boron and molybdenum on plant growth has been found in the Netherlands.*** More details concerning these elements will be given below.

Occurrence of trace element deficiencies in the Netherlands.

Iron deficiency is found only sporadically in agricultural crops in the Netherlands. In horticulture this element is of more importance.

Manganese deficiency may be found on sandy and peaty soils

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*—Presented by Dr. Geo. A. Thornton, Asso. Microbiologist, College of Agriculture, Univ. of Florida, Gainesville.

**—Prof. of Soil Microbiology, Agricultural Experiment Station and Institute for soil Research T.N.O., Groningen, Holland.

***—Magnesium is not listed to the trace elements by the author. Therefore the investigations carried out on this element will not be recorded in this lecture.

where the reaction is approximately neutral. After the extensive investigations carried out by Dutch workers in the past, this fact is well-known by the farmers and therefore the reaction on this type of soil is maintained mostly between pH 5.0 and 5.6. At these values no manganese deficiency of the plants will be found.

On some clay soils containing a certain amount of organic matter and with a high content of calcium carbonate, where manganese deficiency also may be found, this adjustment of pH is not feasible. This is particularly the case on the light clay and sandy soils in the young polders of the Zuiderzee, which may contain more than 10 percent of calcium carbonate. Here applications of manganese salts have to be carried out every year. In some cases spraying of the crops with a 1.0-1.5 percent solution of manganese sulphate appeared to be more successful than application of the salt on the soil.

According to Gerretsen of this Experiment Station the insolubilization of manganese in neutral soils containing a certain amount of organic matter has to be attributed to the activity of microorganisms (6).

In liming experiments on a lowmoor peat containing a high amount of clay, carried out by the author, no manganese deficiency was observed within a period of 10 years after liming of the soil. It is unknown whether this result was due to the absence of manganese-oxidizing microorganisms or to the particular condition of the soil organic matter.

Zinc deficiency is found on apple, pear and cherry trees growing on soil very rich in phosphate (9).*

Copper deficiency (reclamation disease) may be found on sandy and peat soils, particularly shortly after reclamation of these soils. It is well-known by the farmers that under these circumstances they have to apply 50-100 kg of copper sulphate per ha. Therefore heavy symptoms of copper deficiency are found only sporadically in agricultural crops. A slight increase in yield after a copper dressing may be obtained rather frequently. More recently copper deficiency has been observed in orchards by Dr. D. Mulder, Laboratorium van Zeelands Proeftuin, Goes.

As to the occurrence of copper deficiency in animals the situation is more complicated. On soils poor in available copper, cattle may show symptoms of copper deficiency (licking disease). In some cases, however, a beneficial effect of feeding small amounts of copper to cattle has been observed notwithstanding the herbage which was fed had a normal copper content. This result is obtained particularly in the reclaimed North West polder of the Zuiderzee. Apparently uptake of copper from the food may be affected by some unknown factor. Although it is sometimes supposed that excess of molybdenum in the herbage may

*—Dr Gerretsen at this Station has worked out a microbiological method to estimate available zinc in soil.

be the cause of the high copper requirement, more evidence is needed to confirm this hypothesis.

Boron deficiency may be found rather sporadically on sandy soils in the South of the country and on river-clay soils. Besides beets and turnips, leguminous crops may respond sometimes to small amounts of this element. In experiments with peas the author has shown that on boron deficient soils nitrogen fixation may be badly affected as a result of which the plants become deficient in nitrogen and die at an early date (11).

Recent investigations on trace elements carried out in the Netherlands.

During the last 15 years much work has been done by the author on the effect of copper and molybdenum on plant growth and on some physiological phenomena in both higher plants and microorganisms. These investigations started in 1935 when an extensive study was undertaken to elucidate the role of copper in preventing the socalled reclamation disease in plants. This disease which may occur on sandy and peaty soils particularly when they are newly reclaimed, occurs in many areas of Western Europe as well as in the U.S.A. and Australia.

To prove that the reclamation disease has to be attributed to a lack of plant-available copper the following three sets of experiments were undertaken.

a) *A comparison was made of the symptoms of copper deficiency in culture solutions with those of the reclamation disease.*

These symptoms appeared to be quite similar. This was not only true of the pronounced cases in which dead white tips occur at the youngest leaves of cereals and no ears emerge, but also of the light cases in which the ears emerge normally but the grain production is reduced. Plant species less susceptible to the reclamation disease (potatoes, rye) required less copper than highly susceptible plants like wheat, barley and oats. In addition the former have a greater absorption capacity for less available soil copper than the latter. This was concluded from the fact that the difference in copper requirement between both groups of plants growing in soil is much greater than when growing in nutrient solutions.

A crop very susceptible to copper deficiency is canary grass (*Phalaris canariensis*) which was successfully used by the author in pot tests as an indicator plant for copper deficiency.

When a comparison is made of the amounts of copper required for normal plant growth in nutrient solution and in peaty or sandy soil, it will be found that in the former case an amount of 50 γ* of copper per 2 l of nutrient solution, added only once, is enough to secure normal plant growth. When ap-

* γ = *gamma*, here and elsewhere below.

plied to the soil 5 mg. of copper per 2 kg of soil are required. This discrepancy is caused by the fixation of copper by soil organic matter. This was demonstrated by employing the root separation technique. One half of a cylinder was filled with "diseased" soil, the other half, separated by a glass plate, with quartz sand or with a nutrient solution. Plants were grown with part of their roots in the soil and the other part in the sand or the nutrient medium. When the copper was added to the nutrient-solution half, amounts similar to those required in the culture solution experiments were able to give normal plants. When added to the soil half, twenty times greater amounts of copper were unable to give normal plants.

b) *Copper determinations* were carried out in plants grown on normal and "diseased soils." In the latter case much lower copper values were obtained than in cereals grown on "healthy" soils.

c) *Plant available copper was determined in the soil by using a microbiological assay.* This method is based on the fact that the fungus *Aspergillus niger* requires small amounts of copper for the development of normal black spores. In a nutrient solution purified from copper this fungus develops a white sterile mycelium. With 0.2*γ* of copper in 40 cc of nutrient solution yellow spores are formed, with 0.4*γ* the colour of the spores is yellowish brown, with 1.0*γ* grey-brown, with 1.5*γ* grey-black, while 2.5*γ* of available copper and higher amounts give black spores. For the estimation of available copper in soil one gram of air-dried soil is added to 40 cc of a purified nutrient solution in 1 l Erlenmeyer flasks. This medium is inoculated with a suspension of *Aspergillus* spores and after 4 days of incubation at 30° C the colour of the mycelia is compared with the colour scale of a set of standard cultures to which different amounts of copper have been added.

A great number of "diseased" and normal soils from different parts of the Netherlands were tested for available copper according to the above method. Part of the results is given in Table 1.

From these figures it will be seen that soils on which the plants show symptoms of the reclamation disease have a very low content of available copper. Soils on which the plant growth is normal have a considerable higher copper content.[1]

The results of these experiments clearly show that the reclamation disease is brought about by a lack of available copper in the soil.

[1]—The microbiological assay is employed by the Soil Testing Laboratory at Groningen to test soil samples for available copper. The method is modified by Dr. Gerretsen of this Experiment Station in order to be able to estimate amounts of available copper in the range —15*γ* per gram of soil. This is possible by doubling the concentration of nutrients in the solution.

TABLE 1.—PLANT GROWTH AND AVAILABLE-COPPER CONTENT

Soil	Plant growth	*Aspergillus*-available copper per 1 g of air-dried soil, *r*
Sandy soil	Normal wheat	> 2.5
Sandy soil	Normal white oats	> 2.5
Sandy soil	White oats, severely diseased	0.1
Sandy soil	White oats, moderately diseased	1.1
Sandy soil	White oats, normal	1.5
Sandy soil	White oats, normal	2.5
Sandy soil	White oats, diseased	0.3
Peaty soil	Wheat, severely diseased, rye normal	0.2
Sandy soil	Normal wheat	> 2.5
Sandy soil	Normal canary grass	> 2.5
Peaty soil	Normal wheat	> 2.5
Peaty soil	Normal wheat	> 2.5
Sandy soil	Normal wheat	2.5
Sandy soil	Wheat, slightly diseased	1.-
Sandy soil	White oats, normal	1.8
Sandy soil	White oats, diseased	0.4
Sandy soil	White oats, severely diseased	0.2
Peaty soil	White oats, severely diseased	0.1
Peaty soil	Wheat, slightly diseased	1.-
Sandy soil	White oats, severely diseased	0.25
Same field	Slightly diseased area	0.80
Same field	Normal area	1.70
Same field	Plants cured by copper sulfate	> 2.50
Peaty soil	Wheat, severely diseased	0.20
Same field	Normal area	2...-2.5

*—*r* = gamma

The low content of available copper is often a result of the presence in the soil of black humus. This was shown in experiments with *Aspergillus niger* and also in percolation experiments. In the former copper sulfate was added in different amounts to the black humus from a healthy soil very poor in available copper. The mixture was incubated for 24 hours at room temperature, sterilized at 110° C for 10 minutes and thereafter added to a copper-free nutrient solution of *Aspergillus niger*. The following results were obtained.

	Fungus available copper
2 g black heath humus (total copper 2.6*r*)	0.2*r*
2 g black heath humus + 3*r* Cu, as sulphate, added	0.6*r*
2 g black heath humus + 5*r* Cu, as sulphate, added	0.8*r*
2 g black heath humus + 10*r* Cu, as sulphate, added	1.0*r*
2 g black heath humus + 20*r* Cu, as sulphate, added	2.0*r*

It will be seen that this black humus fixed added copper to a considerable degree.

In a subsequent experiment a sandy soil very poor in plant-available copper was percolated with a 0.25 percent copper sulphate solution for 8-10 hours with a speed of 100 cc per hour. Then the soil was washed with distilled water until a practically

negative reaction on copper by carbamate reagent was obtained. Subsequently the soil was percolated with a solution of 1 percent calcium nitrate until no more than 2γ of copper per 10 cc of liquid was washed out. Then the exchanged and still bound copper were determined. The following figures were obtained:

	Exchanged by $Ca(NO_3)_2$ (per 1 g. of org. matter).	Retained in soil (per 1 g. of org. matter).
Soil with severe copper deficiency	46.7 mg Cu	24.3 mg Cu
Normal soil	37.8 mg Cu	5.1 mg Cu

These data show that in the copper-deficient soil the total amount of copper which can be retained after treatment with distilled water is considerably higher than in the normal soil. Of this copper 34 percent was not liberated after treatment with calcium nitrate. In the normal soil only 11.5 percent was retained after treatment with calcium nitrate.

In a subsequent experiment the same copper-deficient soil was compared with a peaty soil on which plants grew normally. In this case the total amount of copper which was retained by the copper-deficient soil after removal of the excess copper sulphate with distilled water was also nearly twice as high as the amount retained by the normal soil. Upon treatment with ½ N hydrochloric acid practically all of the copper was liberated from both soils.

Fixation of copper by hydrogen-sulphide producing bacteria.

In experiments with *Aspergillus niger* and cereals it was found that copper precipitated by hydrogen-sulphide forming bacteria was unavailable. Since copper precipitated chemically by hydrogen sulphide is readily absorbed by *Aspergillus* as well as by higher plants it must be assumed that the copper compound formed by the microorganisms is either copper sulphide, present inside the bacteria cell, so that it is protected from being oxidized, or is not copper sulphide.

In experiments with sterile cultures of barley and oats similar symptoms of copper deficiency were obtained as in solutions which were not sterilized.

Interaction of copper and other nutrients.

Copper-Manganese. Application of copper sulphate to copper-deficient soils sometimes may result in the appearance of manganese deficiency in the plants grown on these soils (8). Although the possibility that such soils are deficient in available manganese as well as in available copper may not be excluded, evidence is available that copper may catalyze the oxidation of

available manganese to less soluble compounds. Although it is unknown to what extent the insolubilization of manganese in soil has to be attributed to microbial activity, it is rather simple to demonstrate the oxidation of manganese compounds to manganic oxide by microorganisms (Gerretsen (6), Sohngen (16), Beyerinck (1). The author isolated a manganese-oxidizing fungus and studied the effect of copper on its capacity to oxidize manganous to manganic compounds. It appeared that traces of copper stimulated the formation of black manganic oxides to a considerable extent (10). Although it is unknown whether in copper-deficient soil a similar stimulation may occur after application of a copper salt, it is likely that under certain circumstances this may be true.

In an experiment with rye in nutrient solutions a clear effect of copper on manganese deficiency of the plants was observed. In this experiment copper was added in amounts of 0, 2, 10 and 50 *r* per culture. When the plants were about one month old, heavy symptoms of manganese deficiency were observed in the solutions supplied with 50 *r* of Cu. Those with 10 *r* were free from manganese deficiency. Some vessels were supplied with 3 mg of $MnSO_4$; they recovered within a few days (Table 2). It is unknown whether in this experiment copper has stimulated the oxidation of manganese in the nutrient solution (the nutrient medium was not sterile) or that precipitation has taken place in the plant tissue.

TABLE 2.—Effect of Copper Supply on Manganese Deficiency in Rye.

Copper, *r*/pot	0.2 mg $MnSO_4 . 4 H_2O$		3 mg $MnSO_4 . 4 H_2O$	
	Grain, g	Straw, g	Grain, g	Straw, g
0	0	1.17	0	0.61
2	0	3.18	0	5.08
10	0.2	9.06	1.04	6.43
50	0	1.18	0.15	5.03

In barley, oats and wheat no effect of copper on the manganese supply was observed.

Copper-Zinc, Copper-Iron.

In culture solution experiments with different amounts of copper, zinc and iron no interaction between copper and zinc and copper and iron was observed (barley).

Copper-Cadmium.

In experiments with *Aspergillis niger* a clear interaction of cadmium and copper was observed. With increased amounts of cadmium more copper had to be supplied to induce black spores (Table 3). In barley no effect was observed.

TABLE 3.—Effect of Cadmium Sulphate on Colour of Aspergillus Spores Supplied With Different Amounts of Copper.

3 Cd SO_4 . 8 H_2O r	No copper supplied	2r Cu	6r Cu	20r Cu
0	bright yellow	black	black	black
25	bright yellow	black	black	black
50	bright yellow	brown-black	black	black
100	bright yellow	brown-black	black	black
200	no spores	gray-brown	brown-black	black
500	no spores	yellow	brown-black	brown-black
1000	no spores	yellow	brown	brown-black
2000	no spores	yellow	yellow-brown	brown

Copper-Nitrogen.

In pot experiments with wheat growing in a copper-deficient soil supplied with different amounts of copper and nitrogen (as ammonium nitrate), an interaction between copper and nitrogen was observed. In the absence of supplied nitrogen and copper, small but entirely normal plants developed which were able to produce normal seeds. Supplied with a small amount of nitrogen, heavy symptoms of copper deficiency appeared and no grains were produced. As will be seen from Fig. 1, increasing amounts of added ammonium nitrate required increasing amounts of copper to obtain normal plants.

Physiological effect of copper.

Copper plays a role as the prosthetic group of oxidizing enzymes (tyrosinase, laccase, ascorbinase etc.). Apparently due to this function the effect of copper on a number of oxydation reactions, studied by the author, may be explained. These reactions are: a) blackening of *Aspergillus niger* spores, b) blackening of aging cultures of *Azotobacter chroococcum*, c) oxidation of manganous compounds to manganic oxid by fungi, d) transformation of aethyl alcohol to acetic acid by *Acetobacter aceti* (10).

More recently the effect of copper on tyrosinase activity in potato tubers was investigated in the author's laboratory. In an extensive study on the blackening of potassium-deficient potato tubers it was found that this phenomenon is due to the oxidation of tyrosine to red and then black oxidation products by tyrosinase. This reaction can proceed only when the cells are injured, so that tyrosine is subjected to tyrosinase activity. Potassium-deficient tubers are much more liable to injury than those with a normal potassium supply. In addition their tyrosine content is much higher (12). Both factors are responsible for the blackening.

When potatoes are cultivated on soils poor in available copper and poor in available potassium, blackening of bruised tubers

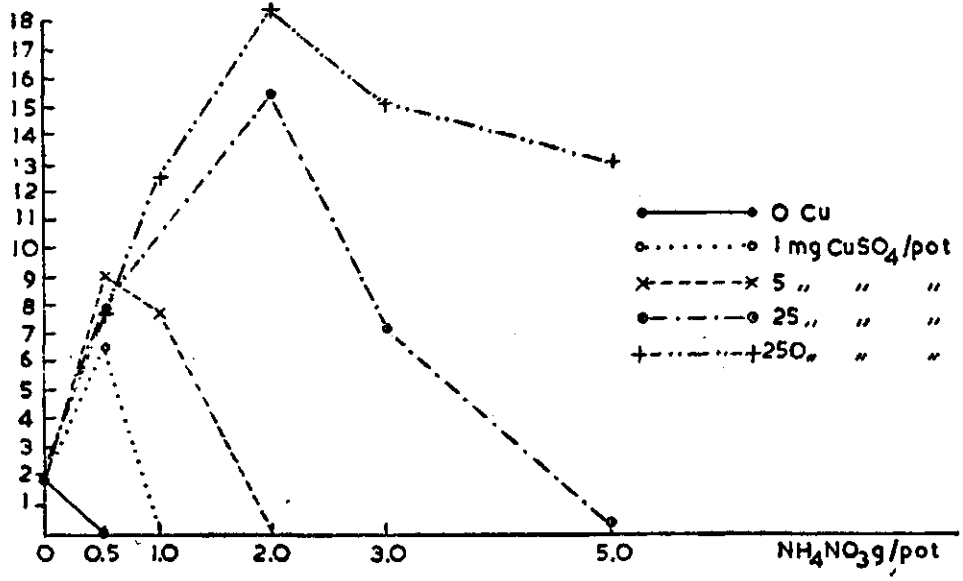

Figure 1.—Interaction of copper and nitrogen (Pot experiment with wheat) Yield on ordinate is grams grain per pot.

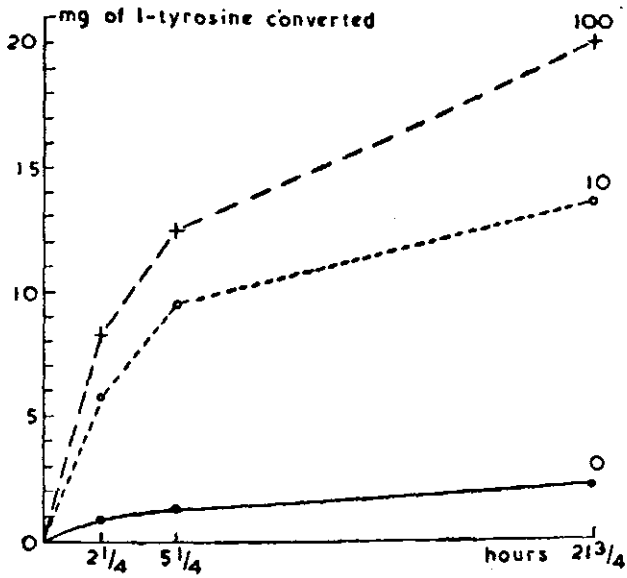

Figure 2.—Effect of the supply of copper to potatoes on the tyrosinase activity in the tubers. On the Chart:
0 no copper supplied
10 copper sulfate, 10 kg./ha.
100 copper sulfate, 100 kg./ha.

occurs only to a small extent. This is due to the low tyrosinase activity of potatoes poor in copper (Fig. 2).

The question may be put whether or not tyrosinase plays the part of a terminal oxidase in the respiration of potato tubers. Although some authors are of the opinion that this is true, little evidence can be found in literature.

In the author's experiments no difference in respiration rate of copper-deficient and normal tubers was observed. Infiltration of tuber slices with the copper reagent diaethyl-dithio-carbamate gave a considerable decrease of carbon dioxide output, however, indicating that copper may be of considerable interest in respiration. These investigations are being continued.

Effect of molybdenum on plant growth and nitrogen metabolism of plants and microorganisms.

A second trace element which has been studied extensively by the author in the last few years is molybdenum. In comparison with copper it is required by higher plants and microorganisms in considerably lower amounts.

Some years ago the effect of molybdenum on growth and nitrogen metabolism of a number of higher plants and micro-

organisms was studied. From these investigations which are published in Plant and Soil 1, 94, 1948, the following conclusions may be drawn:

a) Molybdenum is an *essential element* for the normal development of green plants and a number of microorganisms.

b) In the green plant as well as in the cells of bacteria and fungi, molybdenum is required for the *assimilation of nitrate* nitrogen. This was shown in experiments with tomato and barley plants and with denitrifying bacteria and the fungus *Aspergillus niger*.

c) Molybdenum is essential for the *fixation of nitrogen* by free-living bacteria as well as by symbiotic bacteria (*Rhizobium*). In experiments with peas many nodules developed on the roots in nutrient solution without supplied molybdenum but the nitrogen fixation of these nodules was insignificant so that the plants became nitrogen-deficient and died at an early stage.

Experiments with soil.

In order to investigate whether or not molybdenum deficiency might occur in Dutch soils, pot and field experiments were carried with peas on soils where the pea growth was poor. No effect of molybdenum application was observed so that it was concluded that molybdenum deficiency did not occur in the Netherlands.

After the war some pot experiments were carried out with molybdenum-deficient soil received from Australia by courtesy of Professor Prescott, Waite Institute, Adelaide. A clear response to molybdenum was found in white clover and subterranean clover but not in pea (Table 4). Since the Australian soils used in these experiments were rich in ironstone and rather acid, pot experiments were undertaken with an acid lowmoor peat soil, rich in ironstone, from the province of Groningen (organic matter 60 percent, pH 5.0).

TABLE 4.—Effect of Molybdenum on Yield and Nitrogen Fixation of Clover, Grown in Australian Soils.

Soil received from	pH	$Na_2MoO_4 . 2 H_2O$ per pot (2 kg soil) mg.	White clover Yield, g Dry-wt.	White clover Nitrogen mg.	Subterranean clover Yield, g Dry-wt.	Subterranean clover Nitrogen mg.
Tasmania	6.2	0	4.2	110.5	12.9	272.0
Tasmania	6.2	5	12.2	382.0	16.8	428.5
S. Australia	5.4	0	4.3	93.4	11.1	243.0
S. Australia	5.4	5	5.9	170.0	17.3	395.0

In agreement with the results obtained with the Australian soils a big response to molybdenum was observed in white and red clover. Without application of molybdenum many nodules had developed in both plant species. These nodules were smaller

than those of plants supplied with molybdenum, whereas the colour was not pinkish as usual but yellow or brown-gray. Nitrogen fixation was quite inadequate, as a result of which the plants became pale green and grew much poorer than those supplied with traces of molybdenum. With improved molybdenum supply the number of nodules decreased but their nitrogen fixing capacity increased considerably (Table 5).

TABLE 5.—Effect of Applications of Molybdenum on Nitrogen Fixation and Yield of White Clover Grown on an Acid Lowmoor Peat Soil Rich in Ironstone.

Na_2MoO_4. 2 H_2O applied per pot[1] r	Dry weight g. per pot[2]	Nitrogen in plants, per pot, mg.[2]	Number of nodules per 173 cm² bottom of glass cylinder[2]	Molybdenum in plants mg. per kg of dry matter[2]
0	2.1	51.6	456	< 1
10	2.7	71.7	368	1.8
50	4.1	131.0	254	2.4
100	4.7	152.0	151	3.7
500	4.8	158.7	87	4.3
1000	4.8	155.0	116	3.3
2500	4.7	150.0	123	6.5
5000	4.6	147.9	116	13.7
10000	4.8	151.0	107	25.4
20000	3.8	127.0	109	80.0
50000	4.4	147.0	70	183.1

[1]—Each pot contained about 500 g of soil.
[2]—Averages of duplicate values

As will be seen from these results, optimal nitrogen fixation of white clover was attained already at 100 r Na_2MoO_4. 2 H_2O per pot (about 200 g per ha). With higher rates, nitrogen fixation was only slightly changed. The molybdenum content of the plant tissue rose considerably, however. Since it is a well-known fact that a molybdenum content of the herbage of 20 mg per kg of dry matter may cause cattle-poisoning, care should be taken that these values will not be reached.

With red clover similar results were obtained. Peas and beans did not respond to molybdenum.

In a subsequent pot experiment with 33 soils from different parts of the Netherlands the response of white clover to molybdenum was studied. These soils were mostly rich in iron. On about 20 of these soils big responses to molybdenum were obtained. Amongst the latter soils there were two clay soils.

In another experiment responses to molybdenum in white clover were observed on acid sandy soils with pH-values varying

from 4.2-4.9. The difficulty here was that independently of molybdenum supply, nodulation was much depressed by an acid soil reaction. Once nodules had developed, a clear stimulation of nitrogen fixation was observed when molybdenum had been supplied to the plants.

From these results it may be concluded that molybdenum deficiency is of much more general importance in the Netherlands than was originally believed. No doubt in other countries of Western Europe and perhaps in the U.S.A. similar results may be found. Further research is in progress to study the effect of molybdenum on white clover under field conditions and to see whether other leguminous crops, particularly alfalfa, respond to molybdenum similarly to white clover.

Another problem which is being studied by the author is the interaction copper-molybdenum. It is a well-known fact that on pastures high in molybdenum cattle will be poisoned by a high molybdenum content in the herbage (teart pastures of Somerset (5)). Under such circumstances copper shows a beneficial effect on the health of the cattle. Burema and Wieringa (4) described an interaction between copper and molybdenum in *Azotobacter* some years ago. Although in nitrogen fixing white clover in some cases a clear effect of copper on molybdenum supply has been observed by the author, more research has to be done before conclusions can be drawn.

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