

The effect of soil pH on copper toxicity to forage maize grown under field conditions

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Summary

Copper toxicity to forage maize was studied in a factorial field experiment comprising four Cu and pH levels. Results are presented for crop yield and mineral composition. Raising the soil pH reduced the toxic effect of Cu to a smaller extent than would be predicted from the reduction in the Cu^{2+} ion activity. Apparently an increase in pH increased the toxicity of Cu^{2+} ions in solution.

Introduction

The addition of copper sulphate to the rations for fattening pigs, so as to improve their growth performance and feed conversion rate, entails the production of manure with a high Cu content. When such manure is used to fertilize agricultural land at rates recommended for the major nutrients, significant amounts of Cu are added to the soil. Because losses from the soil, via crop uptake or leaching to the subsoil, are relatively small, repeated use of pig manure results in an accumulation of Cu in the top soil layers.

Concern has arisen that the Cu content of soils eventually may reach levels which are toxic to crops. With a view to the appraisal of this risk, a research program was undertaken to study the behaviour of Cu in soils, and its effects on plant growth and mineral composition. As a part of this program a field experiment was carried out to determine at what Cu level toxicity occurs to maize grown for silage. Maize was selected as a test crop, because it has become the most important arable crop in those Dutch regions where pig feeding farms have concentrated.

Copper toxicity is known to be intensified by acidifying the soil (Westgate, 1952; Reuther et al., 1953; Drouineau & Mazoyer, 1953, 1962; Patterson, 1971; and others). This result has been attributed to an increase in the concentration of cupric ions in the soil solution. It therefore seemed desirable to include soil pH

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Table 1. Soil test values, August 1977.

	Mean value	pH level			
		A	B	C	D
pH-KCl		3.6	3.8	4.2	4.7
Cu-HNO ₃	8.1				
Zn-HNO ₃		5.5	7.1	8.1	8.6
K-HCl	12				
Mg-NaCl		13	22	34	50
P-AL	70				

Cu-HNO₃, Zn-HNO₃ = Cu and Zn extractable with 0.43 M HNO₃, expressed in mg metal per kg soil.
 K-HCl = exchangeable K (0.1 M HCl) expressed in mg K₂O per 100 kg soil.
 Mg-NaCl = exchangeable Mg (0.5 M NaCl) expressed in mg MgO per kg soil.
 P-AL = P extractable with ammonium lactate-acetic acid expressed in mg P₂O₅ per 100 g soil.

as a main variable and to quantify the effect of pH on Cu²⁺ in solution, in order to compare this specific effect of pH with its overall effect on Cu toxicity.

Materials and methods

The field used for the experiment is situated at Wageningen, on a slightly loamy, medium coarse textured, gravelly sand. The soil has been classified as a 'Loopodzolgrond' (mapping unit gcY30) with a deep water-table. The agricultural possibilities of this soil type are somewhat limited on account of its susceptibility to drought (Buringh, 1951; Soil Survey Institute, 1973). The topsoil contains 1.6 % organic matter, as determined from loss on ignition, corrected for the loss of structural water. Its organic carbon content is 0.8 % (dichromate oxidation), and its potential cation exchange capacity is 3.6 meq/100 g (neutral ammonium acetate). The trial field consists of four blocks of sixteen plots each and is surrounded by a border at least 4 m wide. The plot dimensions are 4 m × 5 m. Four pH levels had already been established in 1972.

Soil samples were taken in August 1977 to characterize the initial situation with respect to pH, Cu, Zn, K, Mg and P. The results are given in Table 1. The P status of the soil was high, the levels of Cu, Zn and K were considered adequate. The Mg status depended on pH, ranging from very low at the lowest pH level to sufficient at the higher levels. Four Cu levels were introduced by applying CuSO₄ at rates of 0, 100, 200 and 300 kg Cu ha⁻¹. The pH levels were adjusted by means of application of pulverized CaCO₃ to cover a wider range. Half of the amounts of Cu and lime required was applied in October 1977 and worked in with a rotary tiller. In January 1978 the field was ploughed and in March the remaining half was applied and again worked in with a rotary tiller. Maize (*Zea mays* cv. Capella, also known as Caldera 535) was sown on 5 May at a depth of 5 cm, a row distance of 75 cm and a density of 107 500 seeds ha⁻¹. Weeds were controlled by a single

Table 2. Weather conditions during the growing period.

Decade (10-day period)		Precipitation (mm)	Contribution to the accumulated temperature (Ontario Units) ¹	Average daily global radiation (MJ m ⁻²)
May	I	25.9	115	12.67
	II	6.8	80	16.38
	III	0.4	163	16.55
June	I	16.4	210	18.63
	II	4.9	147	20.63
	III	40.3	130	11.46
July	I	47.0	134	10.95
	II	8.4	146	16.70
	III	1.3	223	18.43
August	I	9.1	183	11.04
	II	2.3	178	16.09
	III	19.6	162	13.27
September	I	5.7	152	11.03
	II	9.8	131	8.69
	III	53.9	124	5.34

¹ Ontario Units are calculated from daily maximum (T_{\max}) and minimum (T_{\min}) temperatures, expressed in °C. The contribution Y_{\max} from T_{\max} is:

$$Y_{\max} = 3.33 (T_{\max} - 10) - 0.084 (T_{\max} - 10)^2.$$

When $T_{\max} < 10$, $Y_{\max} = 0$. The contribution Y_{\min} from T_{\min} is:

$$Y_{\min} = 1.8 (T_{\min} - 4.4). \text{ When } T_{\min} < 4.4, Y_{\min} = 0.$$

The daily contribution in Ontario Units is $0.5 (Y_{\max} + Y_{\min})$.

application, on 10 May, of atrazine at a rate of 1.25 kg a.i. ha⁻¹. Fertilization included broadcast applications of a mixed P-K fertilizer (60 kg P₂O₅ and 120 kg K₂O per hectare) and kieserite (75 kg MgO ha⁻¹), on 10 May and of ammonium nitrate limestone (150 kg N ha⁻¹) on 22 May. On 26 July a side dress application was made of ammonium nitrate limestone at a rate of 100 kg N ha⁻¹.

The weather conditions during the growing season, as recorded at Wageningen, are indicated in Table 2. Daily maximum and minimum temperatures were used to calculate the decadal contributions to the accumulated temperature, expressed in Ontario Units (Carr & Hough, 1978).

At harvest (27 September) living plants from the centre of each plot (3 × 3 m row length) were collected and weighed. After chopping, a subsample of 1 kg was taken and dried for 48 hours in a forced draught oven at 70 °C. The dried material was weighed and ground in a Peppink stainless steel mill to pass a 1-mm sieve. On 3 October soil samples were taken to a depth of 20 cm. The soil was air dried and passed through a 2-mm sieve.

Plant material was analysed for total N, P, K, Ca, Mg, Na, Cl, NO₃ and SO₄ by the routine methods adopted by the Department. Cu, Zn, and Mn were determined

after digesting 1 g of plant material in 10 cm³ of a mixture containing concentrated HNO₃, HClO₄ and H₂SO₄ in a ratio of 40:4:1 by volume. Cu was measured colorimetrically with zinc dibenzyl-dithiocarbamate, Zn by atomic absorption spectrophotometry (AAS) and Mn colorimetrically with formaldoxim. All results were expressed on the basis of oven-dried material.

pH-KCl was measured potentiometrically after shaking 20 g of soil with 50 cm³ 1 M KCl for 2 hours and leaving the suspension to settle. pH-CaCl₂ was measured in a suspension of 20 g soil in 50 cm³ 0.01 M CaCl₂ after shaking for 18 hours. Copper extractable by dilute nitric acid (Westerhoff, 1955) was obtained by shaking 10 g of soil with 100 cm³ 0.43 M HNO₃ for 2 hours, filtering through a hard ash-free filter and measuring by AAS. In order to assess the efficiency of this extraction procedure, total Cu was determined in a number of samples. For this purpose 25 g of soil was powdered in a tungsten carbide disc mill and sub-samples of 1 g digested with HNO₃, HClO₄ and H₂SO₄ in a ratio of 40:10:1 by volume.

The Cu²⁺ activity in 0.01 M CaCl₂ was determined by a resin method, so as to avoid interferences of soluble Cu complexes. Soil samples of 20 g were shaken for 18 hours together with 50 cm³ 0.01 M CaCl₂ and 1 g of Dowex 50W-X4 strongly acidic cation exchange resin in the Ca form. The resin was contained in a double polyester bag: a coarse-meshed inner bag to retain the resin and a fine-meshed outer bag to exclude coarse soil particles and root fragments that would be difficult to remove from the resin. After shaking, the resin bag was withdrawn from the soil suspension, the outer bag removed and the resin washed free from soil under a jet of demineralized water. The pH of the resin contacted soil suspension was recorded (pH-RES). Cu was stripped off the resin by repeated shaking with 0.01 M (NH₄)₄EDTA and determined colorimetrically. Using a calibration curve, the Cu content of the resin was converted into the corresponding pCu value (pCu being the negative logarithm of the Cu²⁺ activity in solution, expressed in M). This resin method and its calibration will be treated in full detail in a forthcoming paper.

Results and discussion

Soil analysis

The Cu-HNO₃ and pH levels established by the soil treatments are shown in Table 3, together with their standard deviations. Cu-HNO₃ increases linearly with added Cu, indicating a constant efficiency of this extraction method over the concentration range employed. The efficiency is very high: 94 % of the added Cu is recovered (Fig. 1). Soil pH has no effect on the results, which is not surprising in view of the large excess of H⁺ ions added with the extractant.

Cu²⁺ activity. Both the Cu content of the soil and its pH have a very pronounced effect on the Cu²⁺ activity in 0.01 M CaCl₂ (Fig. 2), activity increasing with in-

Table 3. Cu-HNO₃ and pH levels, October 1978.

Rate of Cu application (kg Cu/ha)	Cu-HNO ₃ (mg Cu/kg)	pH level	pH-KCl	pH-CaCl ₂
0	10.3 ± 1.3	A	3.58 ± 0.06	3.86 ± 0.07
100	42.2 ± 5.1	B	4.40 ± 0.23	4.96 ± 0.23
200	71.0 ± 7.3	C	5.22 ± 0.31	5.70 ± 0.30
300	102.1 ± 9.2	D	5.89 ± 0.20	6.23 ± 0.17

creasing Cu content and decreasing pH. The effect of pH appears to be independent of the Cu content of the soil. Evidently it is much smaller than the often quoted approximation by Norvell & Lindsay (1969), who suggested a 100-fold increase in activity (i.e. a decrease in pCu by two units) for each decrease in pH by one unit. The increase in activity with increasing Cu content of the soil points at adsorption as the activity controlling process, which is a common observation. Adsorption of Cu onto soil constituents has been studied often, and organic matter in particular has a high affinity for Cu, as was shown by Wei (1958), McLaren & Crawford (1973), McLaren et al. (1978) and others. The relation between the Cu content of the solid phase and the Cu²⁺ activity in solution is given by the adsorption isotherm. For heterogeneous systems like soils, where one may expect a decreasing heat of adsorption with increasing site coverage, the empirical Freundlich equation often gives a fairly good description of adsorption isotherms for heavy metals like Cu (Harmsen, 1977). Taking into account the effect of pH

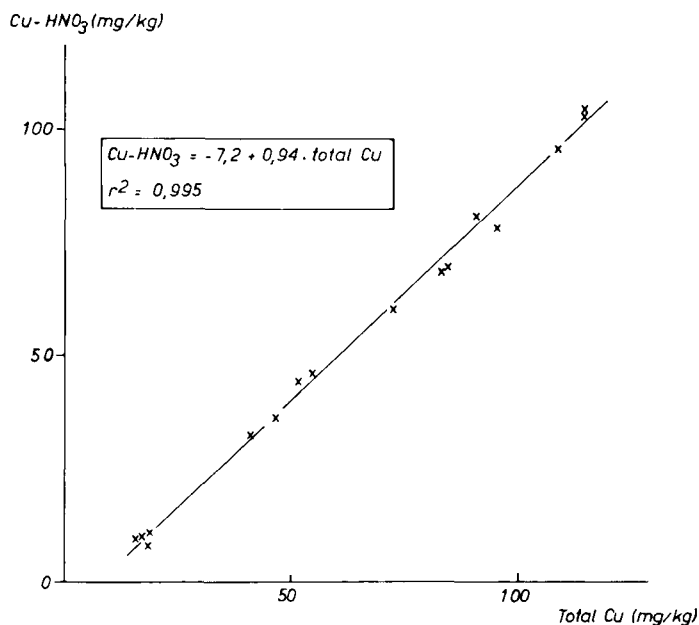


Fig. 1. Recovery of added Cu by extraction with dilute nitric acid.

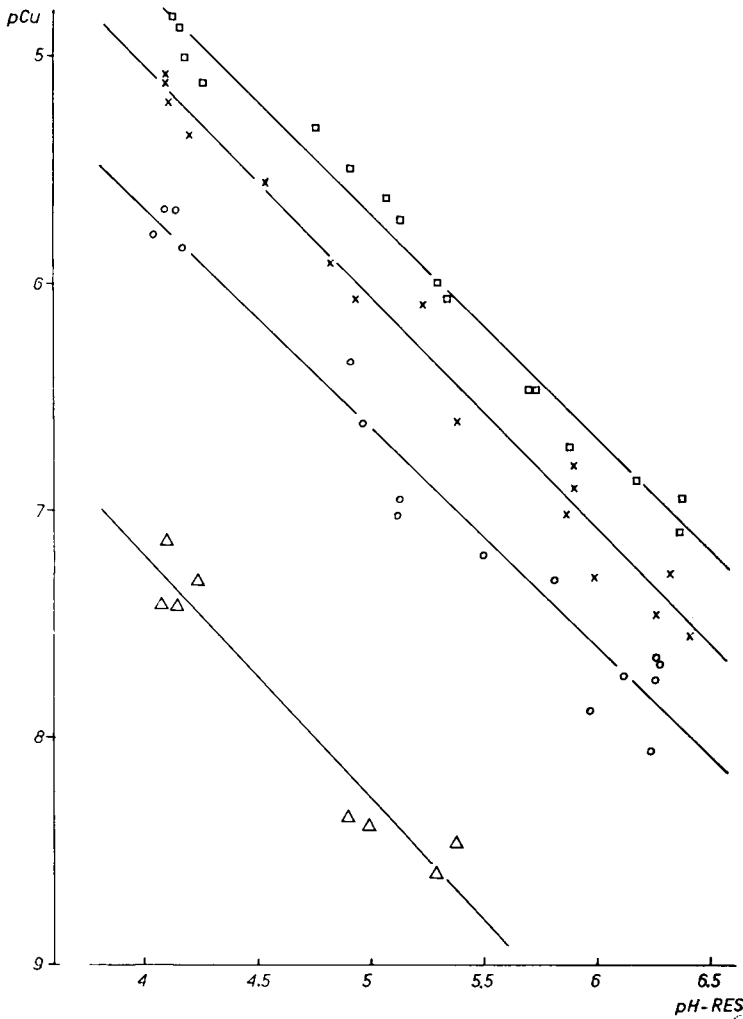


Fig. 2. Cu^{2+} activity (0.01 M CaCl_2) as a function of pH at four soil copper levels.

△	$10.4 \pm 1.1\text{ mg Cu-HNO}_3\text{ kg}^{-1}$	$\text{pCu} = 2.90 + 1.07\text{ pH-RES}$	$r^2 = 0.94$
○	$42.2 \pm 5.1\text{ mg Cu-HNO}_3\text{ kg}^{-1}$	$\text{pCu} = 1.78 + 0.97\text{ pH-RES}$	$r^2 = 0.96$
x	$71.0 \pm 7.3\text{ mg Cu-HNO}_3\text{ kg}^{-1}$	$\text{pCu} = 0.96 + 1.02\text{ pH-RES}$	$r^2 = 0.98$
□	$102.1 \pm 9.2\text{ mg Cu-HNO}_3\text{ kg}^{-1}$	$\text{pCu} = 0.77 + 0.99\text{ pH-RES}$	$r^2 = 0.98$

on the Cu^{2+} activity by including a pH term in the Freundlich equation, the relation between Cu^{2+} activity and Cu content of the solid phase (represented by Cu-HNO_3^* , i.e. Cu-HNO_3 minus the quantities of Cu transferred from the soil to the solution and the resin in the course of the extraction) was calculated to be:

$$\text{pCu} = 5.08 - 2.38 \log \text{Cu-HNO}_3^* + 1.07\text{ pH-RES} \quad R^2 = 0.989 \quad (1)$$

The introduction of a $\log \text{Cu-HNO}_3^* \times \text{pH-RES}$ interaction term in the regres-

sion equation did not significantly increase the correlation, thus confirming that the effect of pH on the Cu^{2+} activity is indeed independent of the Cu content of the soil, at least in the range studied.

It is important to realize that a good approximation of Cu^{2+} activity in situ requires minimal changes to take place in the system under study. There will, of course, always be some transfer of Cu from the solid phase to resin and liquid phase and the amount transferred will increase with increasing activity. The difference between $\log \text{Cu-HNO}_3$ and $\log \text{Cu-HNO}_3^*$ ranged from 0.001 for the 0 Cu treatments to 0.045 for treatment A 300. In addition, the presence of the resin caused an upward shift of pH of 0.28 unit at the lowest pH level and of 0.06 unit at pH level B. For the higher pH levels no such effect could be detected. The most likely explanation for this observation is that Al^{3+} ions from the soil have exchanged for Ca^{2+} from the resin. To eliminate these consequences of the determination, values for Cu-HNO_3 and pH-CaCl_2 were substituted in Eq. 1 to obtain an approximation of the Cu^{2+} activity in the soil solution, to which the plant roots have been exposed during their growth and development.

Crop response

Field observations. Seedling emergence was delayed by treatments A200 and A300. These plants had serious difficulty in unfolding their leaves, obviously by lack of turgor, and many of them died. The leaves of the surviving plants had a bluish green appearance; they curled upwards during periods of sunshine. Within a few weeks at treatments A200, A300 and B300, stem and leaves showed a definite purpling, which persisted during the entire growing season. Gradually it became clear that growth was inhibited at the lowest pH when 0 and 100 kg Cu ha^{-1} had been applied. The lower and middle leaves at treatment A0 and especially at A100, began to exhibit small brown spots between the veins; the lower leaves of these plants only yellowed and withered by early September. According to Bergmann (1968) these symptoms point at manganese toxicity, the occurrence of which is indeed not improbable under these very acid conditions. Iron chlorosis has not been observed at any stage of growth. At harvest, root development was found to be reduced to various degrees at treatments A100, A200, A300 and B300. As far as damage by pests is concerned, a slight attack by frit fly was noted early in the growing period, but it is unlikely to have reduced crop yield significantly.

Crop yield and mineral composition. Table 4 presents yield, dry matter content and the results of plant analysis. It does not include the Na, NO_3 and SO_4 contents, because their values were low and unaffected by the treatments. The lowest pH level has considerably depressed yield at the 0 Cu level and the addition of Cu at this pH reduces yield to practically zero. This pH level was considered as a class by itself and was, therefore, excluded from the analysis of variance, the results of which have been added to Table 4. The weather conditions have a profound effect

Table 4. Yield and mineral composition of forage maize as affected by Cu and pH.

Treatment		Yield (tonnes DM ha ⁻¹)	DM con- Content (mmol kg ⁻¹ DM)					Content (mg kg ⁻¹ DM)				
pH	Cu		tent (%)	N _{tot}	P	K	Ca	Mg	Cl	Cu	Zn	Mn
A	0	6.85	23.3	981	63	285	46	57	77	4.7	71	339
	100	2.40	20.1	1067	72	277	56	60	56	10.0	83	373
	200	0.30	16.3	1309	76	318	50	52	52	14.8	75	252
	300	0.14	17.5	1378	60	276	55	55	46	25.2	77	262
B	0	11.18	23.1	886	61	299	78	67	76	3.8	41	67
	100	11.73	24.1	861	51	259	77	65	71	4.5	60	62
	200	6.53	21.9	948	57	274	81	75	83	5.8	53	104
	300	2.82	22.6	977	62	285	80	68	78	7.7	63	114
C	0	12.09	23.7	892	59	294	79	74	90	3.9	34	39
	100	12.06	22.9	848	58	274	77	70	88	4.2	37	40
	200	10.22	22.5	838	54	264	85	75	85	4.7	39	43
	300	8.08	22.5	881	57	268	90	86	92	5.1	49	46
D	0	12.35	24.1	853	63	269	77	72	74	4.1	31	27
	100	13.01	23.8	832	59	258	78	75	77	4.4	34	26
	200	11.12	23.7	849	56	272	91	94	87	4.9	34	32
	300	9.73	22.5	911	57	263	93	88	83	5.4	30	36

F ratios in the analysis of variance, computed for pH levels B, C and D only:

Source													
Cu	61.96**	4.20*	3.36*	2.50	3.84*	4.31*	<1	22.13**	3.58*	7.01**			
pH	42.74**	2.13	4.64*	<1	1.62	7.11**	4.33*	8.68**	22.10**	65.43**			
Cu × pH	7.36**	1.28	1.01	1.79	<1	1.23	<1	3.53**	1.49	3.05*			
C.V. (%)	11.0	4.3	6.7	8.0	8.1	11.3	14.3	14.3	22.8	27.9			

* P < 0.05.

** P < 0.01.

on maize production. The 1978 growing season was characterized by rather dry, cool and dull weather and therefore not very suitable to grow maize (Table 2). In the period from sowing until harvesting only 200 mm of precipitation were recorded, as against a normal amount of 340 mm. In view of the relatively small water holding capacity of the soil, it seems likely that the amount of available water has limited maximum crop yields. Temperature largely controls the rate of development of maize. Crop maturity is expressed in the dry matter content, which is an important parameter for the crop's quality, because profitable ensiling requires at least 25 % DM. This value had not yet been reached in the present experiment. The accumulated temperature calculated for the period from sowing until harvesting amounted to 2210 Ontario Units (OU); in a normal year this would have been about 2700 OU. According to British data (Carr & Hough, 1978) a relatively late maturing cultivar like Capella requires some 2400 OU to reach 25 % DM.

The amounts of nutrients accumulated by the crop are slightly, but not exceptionally low (Pain, 1978). The side dress application of nitrogen made by the end of July has probably remained ineffective, because precipitation was insufficient to mediate N transport to the plant roots.

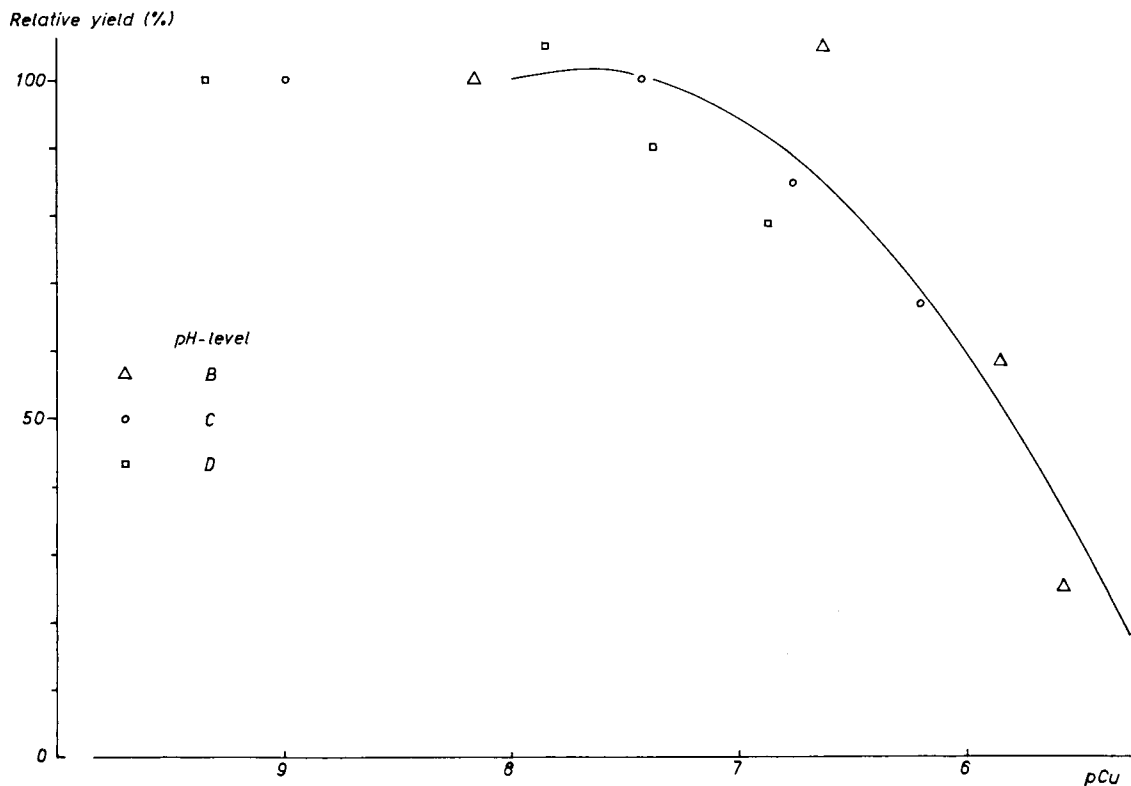


Fig. 3. Relative yield as a function of Cu^{2+} activity.

Both decreasing pH and increasing Cu reduce the yield. As expected, the Cu induced yield depressions increase with decreasing pH. The DM content is reduced by Cu at all pH levels.

Total N, which is approximately equal to organic N, increases with added Cu and with decreasing pH. The effect of Cu on the P content is non-linear: an initial decrease is followed by an increase, but at the lowest pH level this course is reversed. K is not affected by either pH or Cu. An increase in concentration implies that the rate of nutrient uptake is less affected than the rate of dry matter production. The effect of Cu on the rate of uptake increases in the order $N < K < P$, which is the order of decreasing mobility in the soil. This is in agreement with observations that Cu exerts its toxic action primarily by a reduction in root growth (Hunter, 1975) and, therefore, would be expected to affect the uptake of immobile nutrients in particular. The initial increase in the P content at the lowest pH level cannot be explained in this way.

Ca and Mg show an increase with increasing Cu, especially at the higher pH levels. The effect of pH on Mg might in part be due to a residual effect of the pH dependent differences in the initial Mg status of the soil (Table 1). At the lowest pH level Ca is much lower than at the levels B to D. No explanation can be offered for the higher Cl concentrations at pH level C.

The Cu content of the crop increases with increasing Cu content of the soil, but the rate of increase depends on soil pH as well. The effect of soil acidity on the availability of Mn and Zn is clearly reflected in their concentrations in the crop. Both Mn and Zn are increased by Cu applications. When Cu is added to the soil it may displace Mn^{2+} and Zn^{2+} ions from binding sites, thereby increasing the activity of these ions in the soil solution. On the other hand, the simultaneous increase in Cu^{2+} , Mn^{2+} and Zn^{2+} activities may give rise to competition between these ions at the absorption sites in the plant roots or in the process of translocation to the tops. Such a competition might be responsible for the decrease of the Mn content at the lowest pH level and of the Zn content at pH level D. Because of the yield reductions that have occurred, only the comparison of the Zn uptake at treatments B0 and B100 shows a significant increase ($P < 0.01$).

Cu²⁺ activity and reduction of yield

Fig. 3 presents relative yield as a function of the calculated pCu values. Relative yield is defined here as the ratio of yield (Y) to the yield of the 0 Cu treatment at the same pH level (Y_0). The lowest pH level has been left out of consideration. The descending part of the curve can be described by a quadratic function:

$$Y/Y_0 = -8.35 + 2.46 (pCu) - 0.16 (pCu)^2 \quad R^2 = 0.845 \quad (2)$$

It is, however, quite obvious that the relation between relative yield and the Cu^{2+} activity is dependent on pH, the yield decrease being more pronounced at higher pH levels. In this respect it is interesting to note that Hunter (1975), when studying the effect of Cu^{2+} on the elongation of maize roots, found that increasing the pH

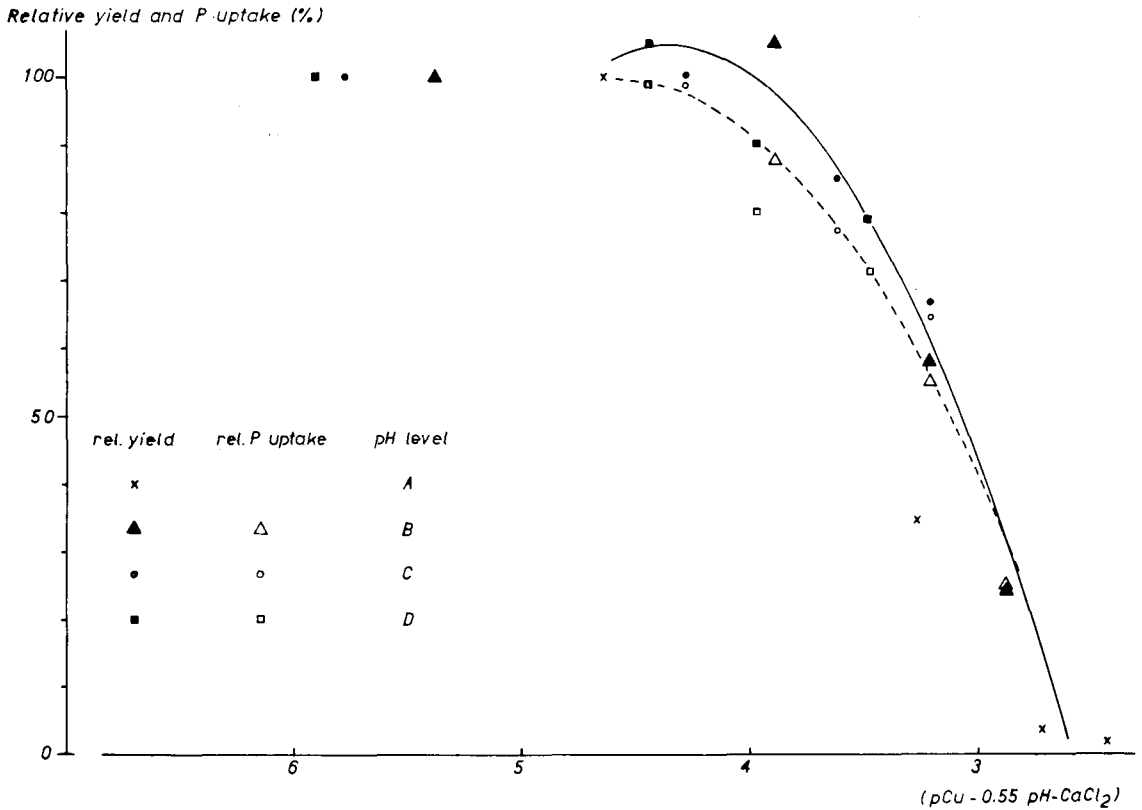


Fig. 4. Relative yield and P uptake as a function of the 'toxicity index'

of the Cu treatment solution increased Cu adsorption onto the roots and decreased subsequent growth. Comparable observations were made by Lexmond & van der Vorm (not yet published) when growing maize seedlings in continuous flow cultures. It therefore seems that adsorption onto the roots is the key process in the toxic action of Cu. It does not appear unrealistic to describe this adsorption process, in analogy to the Cu adsorption onto the soil solid phase, by a Freundlich equation modified to include a pH term (cf. Eq. 1). Thus, a linear combination of pCu and pH might be a better parameter to describe the toxic effect of Cu. In the regression analysis the optimum combination of pCu and pH that might serve as a 'toxicity index', was found to be $(pCu - 0.55 pH - CaCl_2)$. The resulting regression equation reads:

$$Y/Y_0 = -5.85 + 3.23 (pCu - 0.55 pH - CaCl_2) - 0.38 (pCu - 0.55 pH - CaCl_2)^2$$

$$R^2 = 0.955$$

This relationship is presented in Fig. 4. As Cu decreased the P content of the crop and, consequently, had a stronger effect on P uptake than on growth, the relation

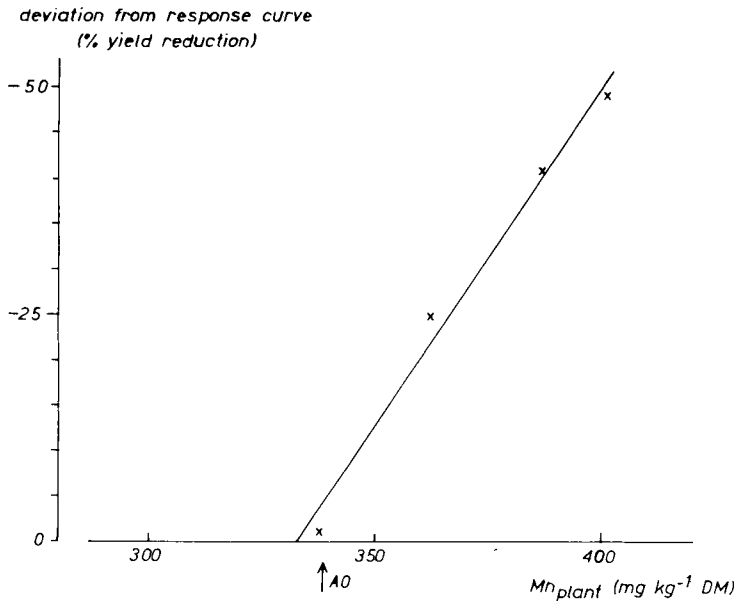


Fig. 5. Treatment A100. Relation between deviations from the yield response curve (Fig. 4) and the Mn content of the crop. The mean Mn content of treatment A0 has been indicated for reference.

between the relative P uptake and the 'toxicity index' is also shown in Fig. 4.

For reasons of comparison the observations made at the lowest pH level have been plotted in Fig. 4. The actual yield reduction found in treatment A100 is greater than expected. As already indicated, this treatment showed intenser symptoms of Mn toxicity than the 0 Cu treatment at this pH level, and the Mn content of the crop was higher (Table 4). The deviations from the yield response curve were calculated for the individual observations and plotted as a function of the Mn content of the crop (Fig. 5). The highly significant correlation between these quantities suggests that the very strong effect of Cu observed in this treatment is in part to be considered as an intensification of the Mn toxicity, induced by Cu application.

Cu²⁺ activity and the Cu content of the crop

In Fig. 6 the Cu content of the crop has been plotted as a function of pCu. It is compared with the N content, as it has been observed that Cu in plant tissues occurs in combination with organic N compounds (Rasheed & Seeley, 1966; Reilly, 1972). The inhibition of growth at low pCu values is associated with an accumulation of both Cu and organic N in the shoot DM.

Beyme (1971) observed that the Cu and N contents of oat plants changed considerably with the stage of growth, but that their ratio remained essentially constant.

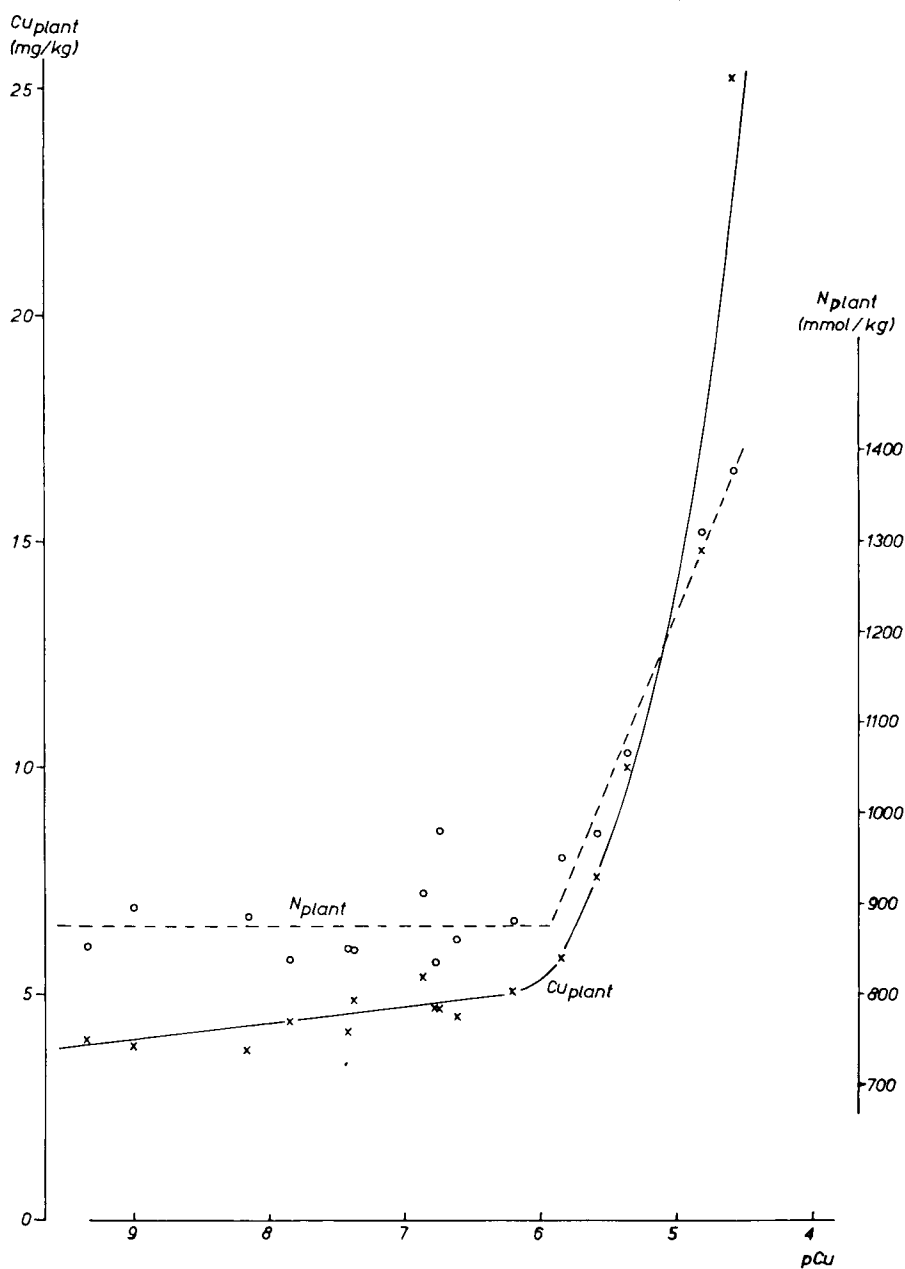


Fig. 6. Cu and N content of the crop in relation to Cu^{2+} activity.

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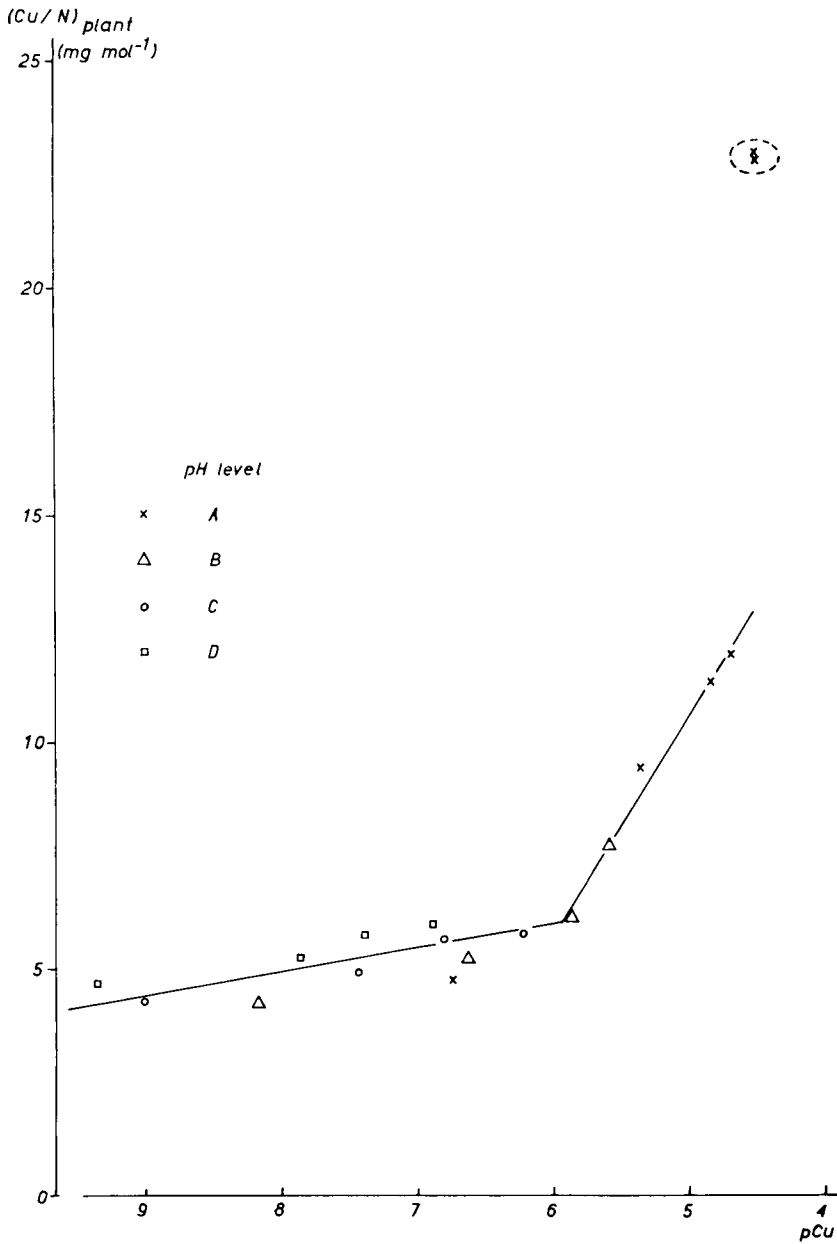


Fig. 7. The Cu/N ratio as a function of Cu^{2+} activity.

He therefore considered the Cu/N ratio a better parameter to reflect the availability of Cu in the soil than the Cu content in itself. Tiffin (1972) found Cu in xylem exudate and root sap of several plant species to be present as an anionic complex and not as the free Cu^{2+} ion. Although he did not identify the chemical nature of the complexants, he assumed that amino acids were involved, because of the high affinity of the Cu^{2+} ion for the N atom of amino carboxylic acids. Recently, Goodman & Linehan (1979) reported on the formation of a soluble Cu complex in wheat roots. This complex showed an electron paramagnetic resonance spectrum similar to that of Cu amino acid complexes. The existence and the nature of the relation between Cu and N seem well established by these results.

In order to take into account the differences in growth dilution and maturity encountered in the present experiment, Beyme's suggestion was followed and Cu was expressed on the basis of N instead of DM. The course of Cu/N as a function of pCu exhibits two linear regions with a transition near pCu 6 (Fig. 7). Partial regression analysis was applied to the two classes of observations thus formed. The positive influence of N_{plant} on Cu_{plant} was highly significant in both classes ($P < 0.01$).

At relatively low Cu^{2+} activities (pCu > 6), where the effects on yield are relatively small, both pCu and pH have a highly significant ($P < 0.01$) effect on the Cu/N ratio:

$$\text{Cu/N} = 6.16 - 0.57 \text{ pCu} + 0.58 \text{ pH-CaCl}_2 \quad R^2 = 0.911 \quad (4)$$

with Cu/N expressed in mg mol^{-1} .

Combining this equation with Eq. 1, which describes the effect of pH on Cu^{2+} activity, results in:

$$\text{Cu/N} = 3.26 + 1.36 \log \text{Cu-HNO}_3 - 0.03 \text{ pH-CaCl}_2 \quad (5)$$

which means that the net contribution of soil pH to Cu/N is practically negligible, thus implying that in this activity range $\log \text{Cu-HNO}_3$ is as good a predictor for the Cu/N ratio as the combination of pCu and pH. This was verified by computing the regression of Cu/N on $\log \text{Cu-HNO}_3$:

$$\text{Cu/N} = 3.12 + 1.34 \log \text{Cu-HNO}_3 \quad r^2 = 0.910 \quad (6)$$

Apparently the maize plant possesses a pH dependent uptake mechanism that enables it to maintain a certain rate of Cu uptake when the Cu^{2+} activity is decreased by increasing the soil pH. Experiments related to the effects of soil pH on Cu uptake have invariably shown that this effect, if detectable at all, is much smaller than the expected effect on Cu^{2+} activity (Blevins & Massey, 1959; van Luit & Henkens, 1967; Lutz et al., 1972).

For pCu < 6 , where yield was depressed considerably, the situation was quite different. In this range the relation between Cu/N and pCu was independent on pH and the sensitivity to changes in pCu much more pronounced:

$$\text{Cu/N} = 34.75 - 4.84 \text{ pCu} \quad r^2 = 0.977 \quad (7)$$

Two observations from treatment A300 were excluded from this calculation, because they showed substantially divergent values of Cu/N (Fig. 7). In search of an explanation, the amino acid composition was determined of two A300 samples, one with a very high, the other with a lower Cu/N ratio. Several differences were observed. In the sample with the high Cu/N ratio, the mole fraction of asparagine + aspartic acid was 100 % higher (21.7 as against 10.9 mole % N); lysine was 30 % higher, whereas leucine, proline and glutamine + glutamic acid were all 30 to 40 % lower. The results obtained indicate that the Cu/N ratio might be a valuable concept in the evaluation of Cu accumulation in maize, provided that no important changes occur in the amino acid composition of the crop. It seems likely that the property of the crude protein fraction to complex Cu is dependent on its composition. Dragun et al. (1976) studied Cu accumulation by maize seedlings grown for four weeks in solution cultures of varying pCu. A low (1) and a high (2) Cu accumulating hybrid were compared in that experiment. The relation between the Cu with a transition near pCu 6.8. No data were presented for the organic N contents, thus preventing a direct comparison with the results obtained in the present experiment. Only if it is assumed that the N content has remained unchanged in their experiment the slopes of the $Cu_{\text{plant}} - pCu$ relations can be compared with the regression coefficients of equations (4) and (7). Because no effects on yield or uptake of other elements had occurred up to pCu 6.1, there was no reason to suspect N accumulation in these treatments. In the range $13.0 \geq pCu \geq 6.8$ the increase of Cu_{plant} was 0.7 and 1.2 mg kg⁻¹ DM per unit decrease in pCu for hybrids 1 and 2, respectively. In the range $6.8 \geq pCu \geq 6.1$ values of 3.8 and 10.6 were obtained. In spite of the differences in experimental technique, the results of the experiments seem to be in fair agreement with each other.

The results of the present experiment suggest that the relations between pCu and yield on the one hand and pCu and Cu/N on the other are not identical. In addition they are both modified by pH, but in different ways. It therefore seems that Cu toxicity and translocation of Cu from roots to tops are essentially unrelated phenomena. For this reason and because of the involvement of org. N it is unlikely that critical levels can be established for leaf Cu. As was mentioned before, the toxic action of Cu²⁺ ions is supposed to be located in the roots, possibly resulting from damage to the plasmalemma of root cells (Wainwright & Woolhouse, 1977). Root analysis for Cu might then offer a better prospect, as is demonstrated by the results of Mitchell et al. (1978) that have been listed in Table 5. These data originate from an experiment in which wheat was grown on an acid and a calcareous soil amended with sewage sludge, that had been treated with variable amounts of CuSO₄. There is a striking agreement between root Cu and effects on yield, but the relation between leaf Cu and yield is far from clear. No data were presented on Cu²⁺ activities in the soil solution.

The quantities of Cu taken up by the crop are only slightly affected by Cu additions to the soil. Application of 100 kg Cu ha⁻¹ has increased Cu uptake at pH levels B, C and D, but higher doses reduce uptake because of their effect on yield. The greatest increase was found at pH level B, where Cu uptake rose from

42 g ha⁻¹ at the 0 Cu level to 53 g ha⁻¹ at treatment B1000. Indeed, growing maize is not a very efficient way to extract Cu from the soil.

Acceptable Cu levels in soils

It is realized that a single field experiment does not provide conclusive answers as to the amounts of Cu that can be tolerated in soils. The organic matter content of the soil has a strong influence on its Cu binding capacity and soils show much variation in this respect. In fact, the soil used in this study is lower in organic matter (OM) than most arable sandy soils in the Netherlands. Other soil components like the hydrous oxides and oxides of Fe, Al and Mn and to a lesser extent clay minerals may also contribute to the soil's buffering capacity towards Cu²⁺ ions (Grimme, 1968; McLaren & Crawford, 1973; Kinniburgh et al., 1976; McBride, 1978).

Cu was added as a soluble salt and not, as will be the case in practice, in the partly organic matrix of a pig manure slurry. It is unknown to what extent the adsorption characteristics of the soil will undergo changes as a result of this simultaneous addition of OM. Sewage sludge certainly raises the OM content of the soil, but pig slurry might be less effective in this respect.

It is also uncertain to what extent the Cu²⁺ activity will decrease with time as a result of slow fixation processes. This 'reversion' (Leeper, 1978) has been described for several heavy metals added to soils at low rates, but no convincing evidence is available for Cu added at higher rates. Walsh et al. (1972) found no reduction of Cu toxicity in the second year of their field trial.

The resin method used in this study is expected to take into account these effects of soil composition and time on the Cu²⁺ activity, but its rather laborious nature might prevent its use in the practice of soil testing. Therefore a translation of the results into conventional soil test values seems useful.

From Fig. 4 and Eq. 1 the critical soil Cu level associated with 10 % yield reduction was obtained in terms of Cu-HNO₃, as a function of pH-KCl (Fig. 8). The same procedure was used to calculate critical Cu-HNO₃ levels which are expected to reduce P uptake by 10 %, thus indicating a depression of root activity.

Table 5. Relative grain yield and Cu concentrations in wheat leaves and roots in relation to soil Cu addition. Compilation of data from Mitchell et al. (1978).

Relative grain yield (%)	Cu content (mg kg ⁻¹)					
	soil		root		leaf	
	acid*	calc.*	acid	calc.	acid	calc.
100	5	5	13	11	10.5	11.5
circa 90	85	165	115	142	10.7	13.9
circa 75	165	325	275	268	11.8	15.4
circa 55	325	645	801	630	13.9	21.0

* Acid and calc. indicate acid and calcareous soil respectively.

SOIL pH AND COPPER TOXICITY TO FORAGE MAIZE

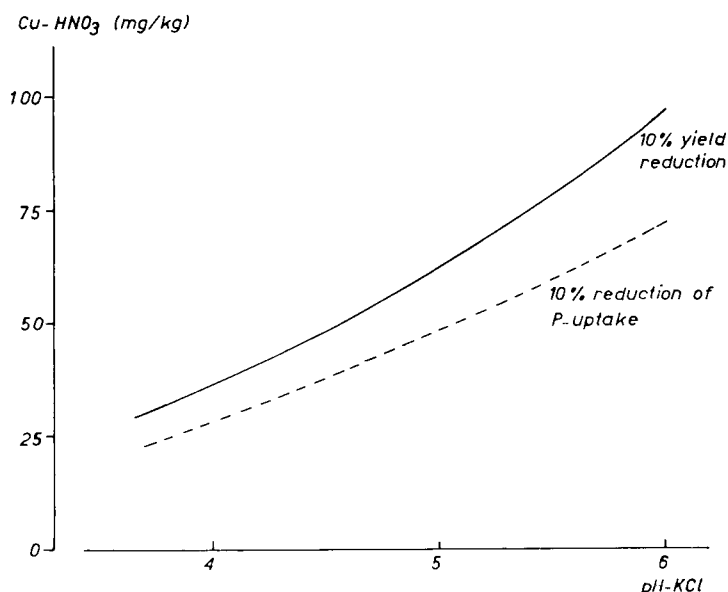


Fig. 8. Critical Cu-HNO₃ levels as a function of pH-KCl.

Kloke (1977) considers additions up to 100 mg kg⁻¹ as tolerable¹, but this value is evidently too high for this soil type, because yield depressions can be expected at all pH values of practical interest. The guidelines of the USA Environmental Protection Agency relate the amount of Cu that can be safely added to a soil to its cation exchange capacity. For this soil 125 kg ha⁻¹ is considered acceptable,¹ which would correspond to a Cu-HNO₃ content of 48 mg kg⁻¹. In the Netherlands, Henkens (1975) and the Institute for Soil Fertility (1978) have proposed not to exceed 50 mg kg⁻¹. At this Cu level yield depressions can be expected up to pH-KCl 4.6 and effects on P uptake up to pH-KCl 5.1. Other crops than maize however may be less tolerant of Cu. Results by Purves (1977) and Lexmond (unpublished) indicate that yield reductions tend to be stronger with adequate moisture than with a limited moisture supply. Because in this study maximum crop yield was limited by the amount of available water, the critical Cu levels obtained may be somewhat too high. Finally, virtually nothing is known about interactions of high Cu and Zn levels. As both Cu and Zn are present in pig manure in rather high concentrations, it is imperative to study if and to what extent the toxic effects of these ions are intensified by each other's presence. Because of these uncertainties it seems necessary to include a safety factor between the critical and the acceptable Cu level in order to ensure protection of the productive capacity of the soil.

¹ Provided soil pH is maintained above 6.5.

One way to introduce such a factor is to reduce the pH dependent critical Cu level by a chosen factor, thus obtaining an acceptable Cu level as a function of soil pH. Another way is to base the acceptable Cu level on a rather low soil pH value, thus making use of the safety margin offered by the favourable effect of an increase in pH. In view of the variability of soil pH, the latter approach might be safer. The results of this experiment suggest that it seems unwise to permit the Cu-HNO₃ content of the soil used to exceed 30 mg kg⁻¹. This value would correspond to a safety factor of two for the Cu-HNO₃ content at a pH-KCl value of 5.5, which, for the soil used in this experiment, may be considered as the optimum pH for a crop rotation comprising maize, grains and potatoes. It would also allow pH-KCl to drop by more than one unit before adverse effects are to be expected. Without doubt, however, higher Cu levels can be tolerated in soils with a higher OM content.

Conclusions

The solution activity of cupric ions in an acid sandy soil increased with increasing Cu content of the soil and with decreasing soil pH, thus indicating that the activity was governed by a pH dependent adsorption process. A Freundlich isotherm, modified to include a pH term was successfully used to describe the relationship between the Cu content of the soil, as measured by a dilute nitric acid extraction, soil pH and Cu²⁺ activity in 0.01 M CaCl₂.

Cu toxicity to maize reduced yield and the uptake of major nutrients in the order N < K < P, indicating that especially root growth was inhibited. The concentrations of Zn and Mn in the crop increased, possibly as the result of displacement reactions in the soil, which increased the activities of these ions. At the lowest pH level this may have caused a Cu induced intensification of Mn toxicity.

Raising the soil pH reduced the occurrence and the intensity of Cu toxicity but the effect of pH on Cu²⁺ activity exceeded its effect on Cu toxicity. This discrepancy was accounted for by the observation that Cu adsorption onto the plant roots, which might be the key process in the toxic action of Cu, increases with increasing pH. The net result of these opposite effects is an alleviation of Cu toxicity, but to a smaller extent than would be expected from the reduction in Cu²⁺ activity. A high, positive correlation was found between Cu and organic N in the crop and therefore Cu accumulation was evaluated in terms of the Cu/N ratio. At relatively low Cu²⁺ activities (pCu > 6), Cu appeared to be taken up according to a pH dependent mechanism; at higher activities no such effect of pH could be detected. The sensitivity of the Cu/N ratio to changes in Cu²⁺ activity also differed for pCu values > 6 and < 6. A very high Cu/N ratio was observed in association with an accumulation of asparagine + aspartic acid.

Translocation of Cu from roots to shoots and Cu toxicity were found to be essentially unrelated phenomena. The use of leaf analysis in defining critical Cu levels was therefore considered limited; root analysis offers a better prospect.

The translocation potential of Cu in maize is low: the addition of 100 kg Cu

ha⁻¹ caused a maximum increase in Cu uptake from 42 to 53 g ha⁻¹. Larger Cu additions decreased Cu uptake because of a reduction in yield.

It was proposed that Cu-HNO₃ should not exceed 30 mg kg⁻¹ in the soil used for the experiment in order to ensure protection of its productive capacity.

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References

- Bergmann, W., 1968. Die Bedeutung der Mikronährstoffe in der Landwirtschaft. *Fortschrittsberichte für die Landwirtschaft* 6 (Heft 2/3), 121 pp.
- Beyme, B., 1971. Beziehungen zwischen Zink- und Kupfergehalt in Haferpflanzen und Böden. *Zeitschrift für Pflanzenernährung und Bodenkunde* 130: 256-270.
- Blevins, R. L. & H. F. Massey, 1959. Evaluation of two methods of measuring available soil copper and the effects of soil pH and extractable aluminum on copper uptake by plants. *Soil Science Society of America Proceedings* 23: 296-298.
- Buringh, P., 1951. Over de bodemgesteldheid rondom Wageningen. *Verslagen van Landbouwkundige Onderzoekingen* 57.4, 113 pp.
- Carr, M. K. V. & M. N. Hough, 1978. The influence of climate on maize production in north-western Europe. In: Forage maize, production and utilisation, pp. 15-55. Agricultural Research Council, London.
- Drouineau, G. & R. Mazoyer, 1953. Toxicité du cuivre et évolution des sols sous l'influence des antiparasitaires. *Comptes Rendus hebdomadaires des séances de l'Académie d'Agriculture de France* 39: 390-392.
- Drouineau, G. & R. Mazoyer, 1962. Contribution à l'étude de la toxicité du cuivre dans les sols. *Annales agronomiques* 13: 31-53.
- Dragun, J., D. E. Baker & M. L. Risius, 1976. Growth and element accumulation by two single-cross corn hybrids as affected by copper in solution. *Agronomy Journal* 68: 466-470.
- Goodman, B. A. & D. J. Linehan, 1979. An electron paramagnetic resonance study of the uptake of Mn (II) and Cu (II) by wheat roots. In: J. L. Harley & R. Scott Russell (Eds.), *The soil-root interface*, pp. 67-82. Academic Press, London.
- Grimme, H., 1968. Die Adsorption von Mn, Co, Cu und Zn durch Goethit aus verdünnten Lösungen. *Zeitschrift für Pflanzenernährung, Düngung und Bodenkunde* 121: 58-65.
- Harmsen, K., 1977. Behaviour of heavy metals in soils. *Agricultural Research Reports* 866, 171 pp.
- Henkens, Ch. H., 1975. Zuiveringsslib in de landbouw. *Bedrijfsontwikkeling* 6: 98-103.
- Hunter, R. B., 1975. Copper toxicity to roots of corn (*Zea mays*). Ph. D. Thesis, Utah State University, 102 pp.
- Institute for Soil Fertility, 1978. De mest- en gierverspreiding op landbouwgrond in de E.G., deel 1. Wetenschappelijke basis voor het beperken van de verspreiding en criteria voor regulerende maatregelen. *Informatie over Landbouw* 47, 154 pp. Commissie van de Europese Gemeenschappen, Luxemburg.
- Kinniburgh, D. G., M. L. Jackson & J. K. Syers, 1976. Adsorption of alkaline earth, transition and heavy metal cations by hydrous oxide gels of iron and aluminum. *Soil Science Society of America Journal* 40: 796-799.
- Kloke, A., 1977. Orientierungsdaten für tolerierbare Gesamtgehalte einiger Elemente in Kulturböden. *Mitteilung Verband Deutschen Landwirtschaftliche Untersuchungs- und Forschungsanstalten*, Heft 2.

- Leeper, G. W., 1978. Managing the heavy metals on the land. Marcel Dekker, New York, 121 pp.
- Lutz, J. A., C. F. Genter & G. W. Hawkins, 1972. Effect of soil pH on element concentration and uptake by maize: II. Cu, B, Zn, Mn, Mo, Al and Fe. *Agronomy Journal* 64: 583-585.
- McBride, M. B., 1978. Retention of Cu^{2+} , Ca^{2+} , Mg^{2+} , and Mn^{2+} by amorphous alumina. *Soil Science Society of America Journal* 42: 27-31.
- McLaren, R. G. & D. V. Crawford, 1973. Studies on soil copper II. The specific adsorption of copper by soils. *Journal of Soil Science* 24: 443-452.
- McLaren, R. G., R. S. Swift & J. G. Williams, 1978. The adsorption of copper by soil materials at low solution copper concentrations. 11th Congress of the International Society of Soil Science, Edmonton.
- Mitchell, G. A., F. T. Bingham & A. L. Page, 1978. Yield and metal composition of lettuce and wheat grown on soils amended with sewage sludge enriched with cadmium, copper, nickel and zinc. *Journal of Environmental Quality* 7: 165-171.
- Norvell, W. A. & W. L. Lindsay, 1969. Reactions of EDTA complexes of Fe, Zn, Mn, and Cu with soils. *Soil Science Society of America Proceedings* 33: 86-91.
- Pain, B. F., 1978. Nutritional requirements of forage maize. In: Forage maize, production and utilisation, pp. 87-116. Agricultural Research Council, London.
- Patterson, J. B. E., 1971. Metal toxicities arising from industry. In: Trace elements in soils and crops. *Technical Bulletin* 21: 193-207. The Great Britain Ministry of Agriculture, Fisheries and Food.
- Purves, D., 1977. Trace-element contamination of the environment. Elsevier Scientific Publishing Company, Amsterdam, 260 pp.
- Rasheed, M. A. & R. C. Seeley, 1966. Relationship between the protein and copper contents of some plants. *Nature*, London 212: 644-645.
- Reilly, C., 1972. Amino acids and amino acid-copper complexes in water-soluble extracts of copper-tolerant and non-tolerant *Becium homblei*. *Zeitschrift für Pflanzenphysiologie* 66: 294-296.
- Reuther, W., P. F. Smith & G. K. Scudder, 1953. Relation of pH and soil type to toxicity of copper to citrus seedlings. *Florida State Horticultural Society Proceedings* 66: 73-80.
- Soil Survey Institute, 1973. Bodemkaart van Nederland, schaal 1:50.000. Blad 39 Oost, Rhenen.
- Tiffin, L. O., 1972. Translocation of micronutrients in plants. In: J. J. Mortvedt, P. M. Giordano & W. L. Lindsay (Eds.), Micronutrients in agriculture, pp. 199-229. Soil Science Society of America, Madison.
- Van Luit, B. & Ch. H. Henkens, 1967. Invloed van de kopertoestand van de grond op het kopergehalte van gras en klaver. *Verslagen van Landbouwkundige Onderzoekingen* 695, 33 pp.
- Wainwright, S. J. & H. W. Woolhouse, 1977. Some physiological aspects of copper and zinc tolerance in *Agrostis tenuis* Sibth: Cell elongation and membrane damage. *Journal of Experimental Botany* 28: 1029-1036.
- Walsh, L. M., W. H. Erhardt & H. D. Seibel, 1972. Copper toxicity in snapbeans (*Phaseolus vulgaris* L.). *Journal of Environmental Quality* 1: 197-200.
- Wei, L-S., 1958. The chemistry of soil copper. Ph. D. Thesis, University of Illinois, 77 pp.
- Westerhoff, H., 1955. Beitrage zur Kupferbestimmung im Boden, *Landwirtschaftliche Forschung* 7: 190-192.
- Westgate, P. J., 1952. Preliminary report on copper toxicity and iron chlorosis in old vegetable fields. *Florida State Horticultural Society Proceedings* 65: 143-146. (*Horticultural Abstracts* 23 (1953) 3015).