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The effect of pH on copper toxicity to hydroponically grown maize

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Summary

The effect of pH on copper toxicity to maize was studied in three solution culture experiments of different design. Raising the pH intensified the toxic effect of Cu, which presented itself first of all by a reduction in root growth and by a reduction in uptake of phosphate and iron. This result was explained from an enhanced association of Cu^{2+} ions with physiologically essential sites in the roots, when competition from protons was lowered.

Introduction

The toxic effect of copper to soil-grown plants is alleviated by raising the pH and, consequently, liming is usually considered the corrective treatment to be applied to Cu-polluted soils. It has been suggested that an increase in pH adds to the soil's power to bind Cu, thus reducing the concentration in solution of the Cu^{2+} ion, which is generally assumed to be the biologically active form of Cu. The development of Cu^{2+} specific electrodes carries with it the possibility to check the correctness of this hypothesis. Cavallaro & McBride (1980), McBride & Blasiak (1979) and Lexmond (1980) have confirmed the decrease in Cu^{2+} ion activity with increasing soil pH. Lexmond (1980), however, introduced a new aspect by showing that the decrease in Cu^{2+} ion activity exceeded the reduction in Cu toxicity to maize. Apparently, Cu^{2+} ions in solution became more toxic at higher pH.

Hunter (1975) studied the effect of pH on Cu toxicity to maize roots by exposing the roots for 1 hour to nutrient solutions containing 126 μM Cu at varying pH and measuring subsequent growth in a water-saturated atmosphere during 24 hours. Increasing the pH of the treatment solution enhanced Cu uptake by the roots and decreased subsequent growth.

The experiments described here were designed to investigate a possible effect of pH on Cu toxicity to maize under conditions of a prolonged exposure to much lower concentrations, which are more likely to prevail in Cu-polluted soils.

Materials and methods

All experiments were done with *Zea mays* cv. Capella, also known as Caldera 535. Seeds were planted to quartz sand moistened with demineralized water and allowed to germinate in a greenhouse. Seedlings were selected for uniformity from at least twice the number required, before they were transferred to the nutrient solutions.

Experiment 1. Seven to eight days after sowing, seedlings were transplanted and grown for another ten days in 60-litre PVC containers with nutrient solutions of different Cu concentration and pH, with either NO_3 or NH_4 as the source of nitrogen. The composition of the solutions is given in Table 1. The solutions were continuously circulated by an electric waterpump and renewed every fourth day. pH was controlled by automatic titration with KOH or HNO_3 as required. Full details of the equipment used were given by Breteler (1973). Copper was added as a solution of $CuSO_4$ and measured at frequent intervals to check if the intended concentration levels were maintained. No attempt was made to inhibit nitrification in the NH_4 solutions. The experiment consisted of 4 trials. In each trial 2 treatments were compared, viz. a low (0.16 *µM*) and a high (3.1 *µM*) Cu concentration, using 40 seedlings per treatment. pH and N source varied between trials. The experiment was done in a growth cabinet kept at 20 °C (night and day), a relative humidity of ca. 80 %, a daylength of 16 h and a light intensity of 20 klx.

Experiment 2. Eight days after sowing, seedlings were transplanted and grown for sixteen days in polyethylene-lined, 6-litre pots which were continuously flushed with aerated nutrient solution at a rate of 20 litres per day. The pots and nutrient solution reservoirs were arranged on a ca. 40 m^2 rotating disk in an unconditioned greenhouse. The disk made 10 revolutions per hour. The

Table 1. Composition of the nutrient solutions used in Exp. 1.

NO_3 solution	NH_4 solution
2.50 m*M* $Ca(NO_3)_2$	1.25 m*M* $(NH_4)_2SO_4$
2.50 m*M* KNO_3	1.67 m*M* KCl
0.25 m*M* KH_2PO_4	0.25 m*M* KH_2PO_4
1.00 m*M* $MgSO_4$	1.00 m*M* $MgSO_4$
	1.67 m*M* $CaCl_2$

0.10 m*M* Na_2SiO_3; 0.20 m*M* HCl; 1 *µM* $MnSO_4$; 1 *µM* $znCl_2$; 10 *µM* H_3BO_3; 0.1 *µM* $(NH_4)_6Mo_7O_{24}$. Iron was supplied daily as $FeSO_4$ at a level of 10 *µM*.

pots were placed along the edge of the disk to ensure uniform climatological conditions for all plants. The experiment comprised 9 treatments, 3 Cu and 3 pH levels in a factorial design, each in 3 replications carrying 7 plants. Copper was supplied at levels of 0.16, 1.6 and 3.1 μM. pH was adjusted to 4.0, 4.8 and 5.8 by adding HNO_3 or KOH to the NO_3 solution used in Exp. 1. Moreover, $(NH_4)_2SO_4$ was added at rates of 5, 1 and 0 % of total N in order to maintain pH at the levels aimed at. The effectiveness of this method of pH control was checked by measuring pH every afternoon.

Experiment 3. Four days after sowing seedlings were transplanted and grown for another seven to ten days in polyethylene lined, 6-litre pots, continuously flushed with aerated nutrient solution at a rate of 30 litres per day. Each pot carried eighteen seedlings. The pots were arranged as in Exp. 2. Copper was varied at nine levels within each trial and there were no replications. pH was varied between trials by adding KOH or HCl to the NO_3 solution described in Table 1, but used at half strength and slightly modified. Metasilicate in combination with HCl was omitted in this experiment, KH_2PO_4 was supplied in a concentration of 50 μM only and NaCl was included at the level of 50 μM. Iron was not supplied as it was felt that the seed reserves would suffice in these short lasting trials with a low level of dry matter production. In this experiment seedlings were accommodated in the perforated covers in such a position that the seed was on top of the cover, thus preventing adventitious roots from growing into the solution. In this way the response of the primary roots was studied without interference of adventitious root growth.

At the termination of each experiment plants were separated into shoots (portion above the root crown), and roots. The seeds were discarded. Roots were washed thoroughly with demineralized water. Plant material was dried, weighed, ground if necessary, digested and analysed by the routine methods in use in the Department (Slangen & Hoogendijk, 1970). In Exp. 1 four plants per treatment were chosen at random for the determination of the root surface area. For this purpose roots were counted and their average length and diameter measured. The surface area was calculated under the assumption that roots behave like ideal cylinders.

Results

Experiment 1. The high Cu treatment brought about visible symptoms at pH 4.7, but not at pH 4.0, irrespective of the N source. Root morphology was modified and the leaves showed a chlorosis which resembled iron deficiency. Results of yield determinations, root measurements and chemical analyses have been listed in Table 2. Copper had no effect on root and shoot yield, but root surface area was reduced by 3.1 μM Cu at pH 4.7. Both number and length of secondary and , more in particular, tertiary roots were reduced. The same treatment also lowered the PO_4 content of roots and shoots and the Fe content of the shoots, but had little effect on the levels of other nutrients (data not

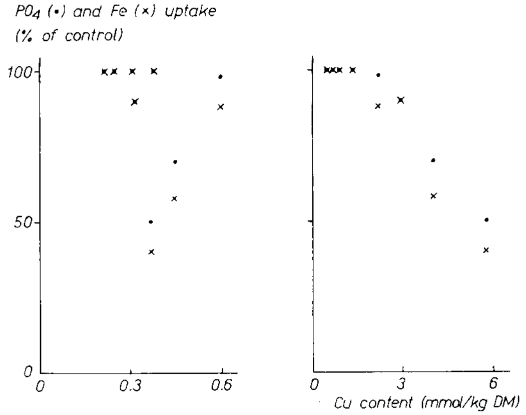

Fig. 1. Copper-induced reduction in PO_4 and Fe accumulation by maize shoots in relation to the Cu content of shoots (left) and roots (right). Experiment 1.

shown). The Cu content of both roots and shoots was increased by an increase in Cu supply, the effect on root Cu being more pronounced. The size of the reduction in PO_4 and Fe accumulation by the shoots appeared to be related to root Cu only (Fig. 1). The effects of Cu on PO_4 and Fe uptake and on the root Cu content were somewhat larger in the NO_3 solutions than in the NH_4 solutions, but the effect of N source was small in comparison to the effect of pH.

When carrying out this experiment, several disadvantages were recognized in the technique chosen. The limited availability of titration sets prohibited replication of the trials. The frequent renewal did not prevent the accumulation of organic compounds in the nutrient solutions. This was detected in the determination of Cu in the nutrient solution, since layer separation following the liquid/liquid extraction became more difficult with increasing age of the solution. Organic compounds may complex Cu and thus reduce Cu^{2+} ion activity.

Table 2. Effects of increased Cu supply on growth and chemical composition of shoots and roots of maize grown at varying pH and N source (Exp. 1).

Treatment			Yield (mg DM/plant)		Root surface area (cm^2/plant)	Content (mmol/kg DM)					
N source	pH	Cu (μM)	shoot	root		PO_4		Fe		Cu	
						shoot	root	shoot	root	shoot	root
NO_3	4.0	0.16	264	161	236 ± 50	657	367	2.13	108	0.22	0.52
		3.1	272	167	232 ± 77	576	377	1.86	155	0.32	2.94
	4.7	0.16	357	203	245 ± 100	527	419	2.33	205	0.25	1.33
		3.1	334	209	*103 ± 19*	*284*	*215*	*1.00*	184	0.37	5.76
NH_4	4.0	0.16	214	164	319 ± 26	737	478	2.06	103	0.31	0.68
		3.1	199	159	268 ± 71	776	474	1.95	127	0.60	2.20
	4.7	0.16	301	177	349 ± 78	683	432	2.61	99	0.38	0.91
		3.1	305	178	*154 ± 84*	*469*	*256*	*1.50*	81	0.45	4.00

Another problem was the disappearance of Cu from solution at higher pH levels tested (5.4 and 6.1), possibly as the result of coprecipitation with added Fe. Exp. 2 was designed to overcome these difficulties. Continuous renewal of the culture solution checks the build up of organic compounds which may affect Cu^{2+} ion activity and a higher solution/plant ratio reduces the changes in Cu concentration as the result of uptake and coprecipitation with Fe.

Experiment 2. Compared to Exp. 1 the technique used in this experiment has the inherent disadvantage of a less rigid control of pH, but measurements showed the actual deviations to be limited. With 5 % NH_4-N in the nutrient solution pH remained 4.0, but with 1 % NH_4-N it decreased slightly to 4.7 at all Cu levels. The 100 % NO_3-N solution showed the largest deviations: at 0.16 μM Cu pH rose steadily to 6.1 and at 3.1 μM Cu it fell to 5.6 after the plants had become chlorotic. At the intermediate Cu level pH remained essentially constant at 5.8.

Plates 1 and 2 show the plants on the day before harvest. Root morphology was affected by 3.1 μM Cu at the higher pH levels and by 1.6 μM Cu at the highest pH only. Chlorosis was severe in plants grown at the highest pH when supplied with 3.1 μM Cu and somewhat milder at 1.6 μM Cu. Plants grown at the intermediate pH level with 3.1 μM Cu showed incipient chlorosis. At low pH no visible effects of Cu toxicity became manifest.

Table 3. Main effects of Cu on yield and element accumulation by maize (Exp. 2). Figures represent the mean values for the two levels of increased Cu supply.

	% of control		
pH	4.0	4.7	5.6 – 6.1
% NH_4-N	5	1	0
Shoot			
yield	105.8	98.7	75.5
N_{org}	106.9	92.7	64.7
NO_3-N	102.0	98.0	62.8
PO_4	102.3	85.3	46.2
K	101.8	94.5	66.2
Ca	106.7	106.5	87.9
Mg	104.5	106.0	79.2
Cl	109.8	126.0	89.2
SO_4	109.3	121.6	108.0
Fe	97.8	75.6	48.9
Cu	233	189	100.7
Root			
yield	103.5	89.2	82.1
PO_4	126.4	87.7	56.5
Fe	126.3	75.1	43.7
Cu	577	658	475

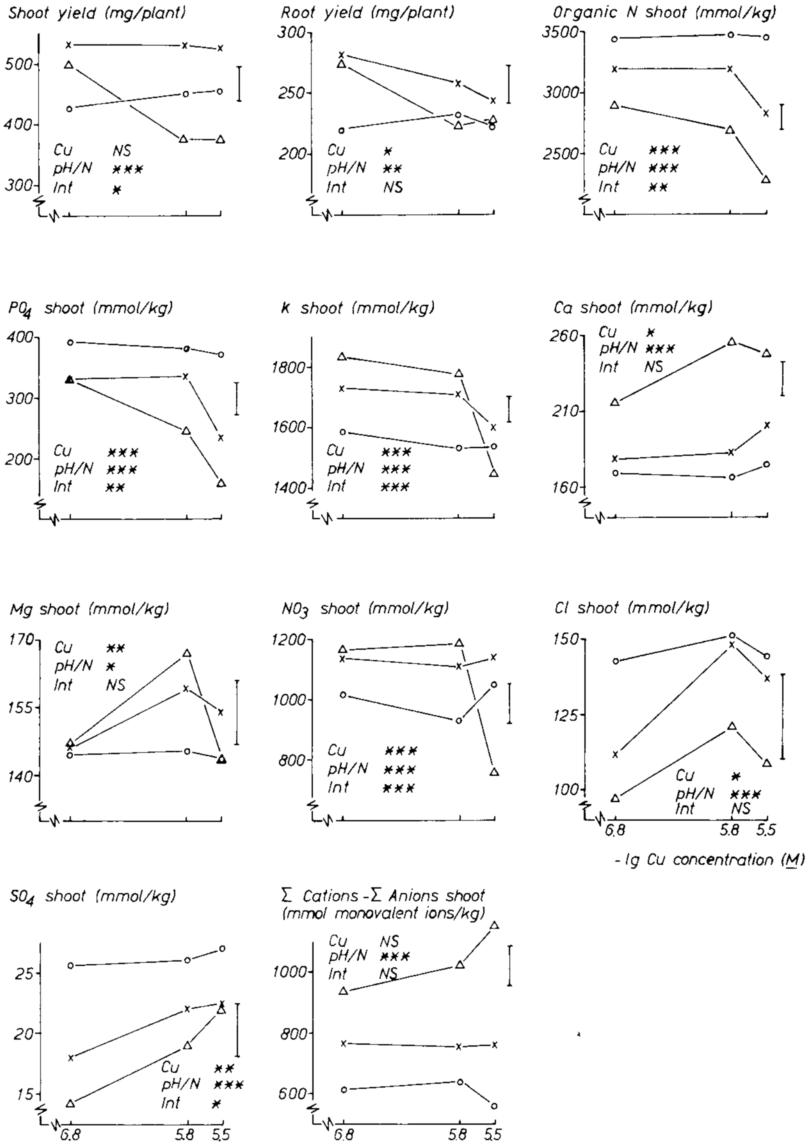

Fig. 2. Yield and composition of maize shoots and roots as affected by increasing Cu concentrations at three pH levels which were maintained by inclusion of varying quantities of NH_4-N in the nutrient solution. Experiment 2.
Asterisks indicate the degree of significance in the effects of copper (Cu), pH and N source (pH/N) and their interaction (Int). Bars indicate the least significant difference ($P < 0.05$) between individual treat-

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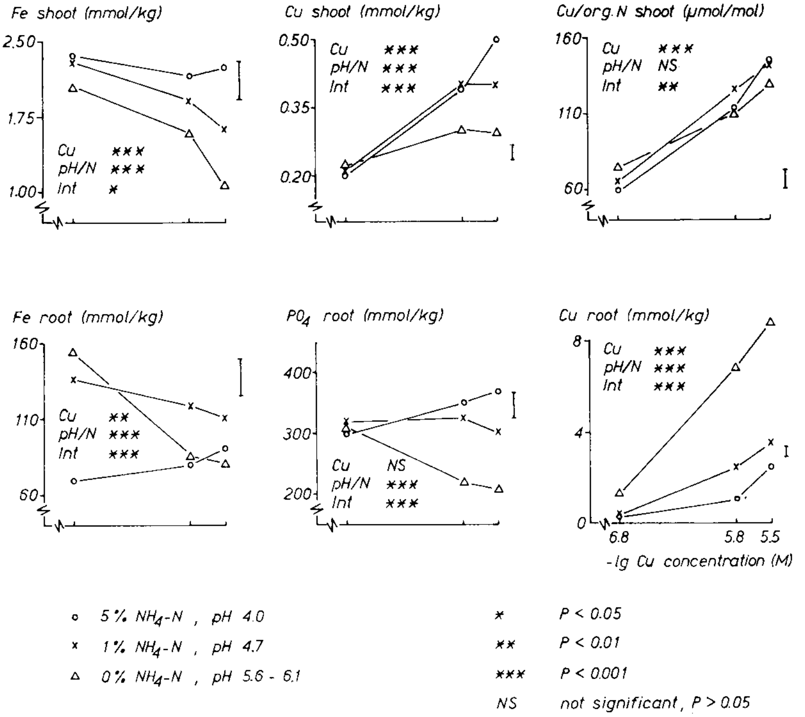

Data on yield and chemical composition are presented in Fig. 2, although the main effects of Cu are read more conveniently from Table 3. At pH 4.0 Cu did not affect yield or element accumulation in the shoots, except for an expected increase in Cu uptake. At pH 5.6-6.1 Cu reduced shoot yield and markedly inhibited uptake of PO_4 and Fe. Somewhat less pronounced, but still substantial, were the reduction in N and K uptake and the inhibition of the synthesis of organic nitrogen compounds. The uptake of Ca, Mg and Cl was affected less than the dry matter production. The PO_4, Fe, K and org. N contents of the shoot were decreased by 3.1 μM Cu at pH 4.7, but 1.6 μM Cu apparently had no effect.

Root yield was reduced by 3.1 μM at the higher pH levels and by 1.6 μM Cu at the highest pH level. Roots were analysed for PO_4, Fe and Cu only. At the highest pH level Cu depressed the PO_4 and Fe contents. At low pH there was a tendency for the PO_4 content of the roots to increase with increasing Cu.

The course of the shoot Cu content was remarkable: whereas it rose continuously at low pH, it flattened at the higher pH levels. The Cu/N ratio however increased continuously with increasing Cu supply (Fig. 2). The rate of increase in the Cu/N ratio was higher for plants grown in NH_4-containing nutrient solutions than for plants grown without NH_4. The linear regression of Cu/N (μmol/mol) on lg Cu in solution (M) yielded the following regression

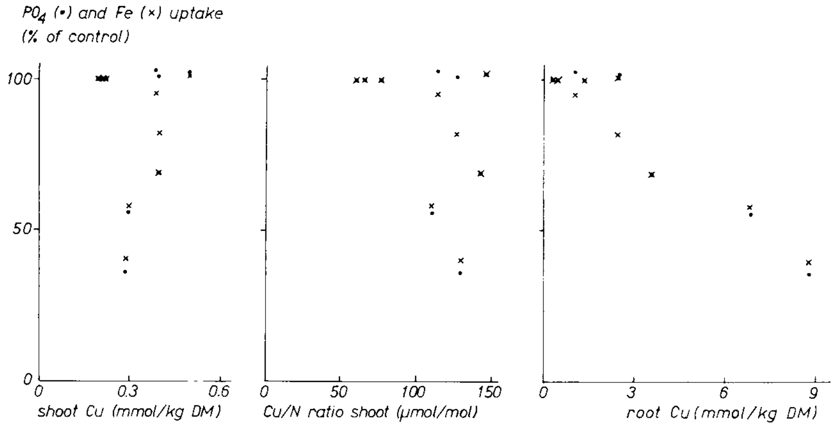

Fig. 3. Copper-induced reduction in PO_4 and Fe accumulation by maize shoots in relation to the Cu content of shoots (left) and roots (right) and to the Cu/N ratio of shoots (middle). Experiment 2.

coefficients and 95 % confidence intervals: 39.1 ± 6.1 for no NH_4, 59.3 ± 2.3 for 1 % NH_4-N and 62.7 ± 4.7 for 5 % NH_4-N.

The Cu content of the roots behaved quite differently. At all Cu levels root Cu increased with increasing pH. As is shown in Fig. 3 the effect of Cu on PO_4 and Fe uptake seemed to be related to root Cu rather than to shoot Cu or to the Cu/N ratio in the shoots.

Experiment 3. The effect of Cu on root growth, which was clearly visible in Exp. 1 and 2, was not or only slightly reflected in root yield (Table 2, Fig. 2). Root surface area appeared to be a more sensitive parameter (Table 2), but unfortunately this parameter is not easily quantified. The growth of coarse adventitious roots, arising from the root crown, remained relatively unaffected compared with the growth of secondary and tertiary roots. These adventitious roots do not contribute much to root surface area, but they do so to root weight. It was thought, therefore, that prevention of adventitious root growth might allow use of the reduction in root yield as a sensitive and easily measurable parameter of Cu toxicity.

Fig. 4 shows the results of this experiment. Copper induced a very marked reduction in the root/shoot ratio, but the concentration at which this effect became apparent was strongly dependent on pH. The decrease in the root/shoot ratio resulted mainly from a decrease in root yield (Plate 2b), although there seemed to be a slight increase in shoot yield as well. Treatments bringing about less than 10 % change in the root/shoot ratio when compared to the treatment not receiving copper, resulted in a shoot yield of 102.2 ± 4.0 % of the control values. Treatments giving a 10 to 50 % reduction in root/shoot ratio had a shoot yield of 108.0 ± 4.4 %. When treatments reduced the root/shoot ratio by more than 50 %, shoot yield was still at the high level of 98.1 ± 6.3 %. These results leave no doubt that the toxic effect of Cu manifested itself

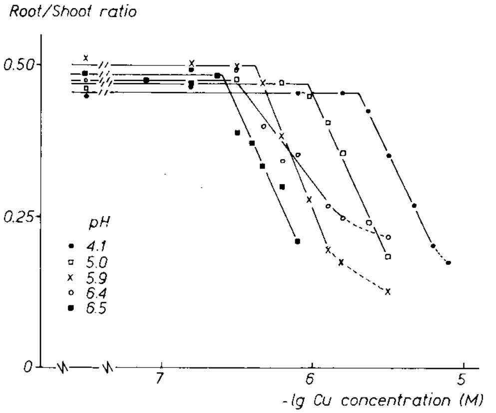

Fig. 4. Effect of Cu on the root/shoot ratio of maize at different pH levels. Experiment 3.

primarily by a reduction in root growth. The slope of the descending part of the curves was somewhat variable. This probably resulted from differences in the duration of the trials, as the trial at pH 6.4 lasted for 7 days only, whereas the other trials lasted for 9 or 10 days. Consequently, the shoot dry matter production at pH 6.4 was some 35 % lower than in the other trials (59 against 91 mg DM/plant). Copper also affected PO_4 uptake (Table 4). In some cases of severe toxicity the midribs on the underside of the leaves became purple (Plate 2b). Copper did not hasten the occurrence of Fe-deficiency chlorosis. When

Table 4. Effect of Cu on the PO_4 accumulation (μmol/plant) by maize shoots grown in nutrient solutions of varying pH (Exp. 3).

Cu (μM)	pH 4.1	pH 5.0	pH 5.9	pH 6.4	pH 6.5
—	26.3	31.8	32.6	26.0	30.4
0.08					31.9
0.16	27.7	28.9	34.0	23.8	32.6
0.24					30.4
0.31		28.8	29.6	22.4	*32.3*
0.39					*32.8*
0.47			30.6	*24.9*	*30.5*
0.63		31.1	*28.8*	*18.9*	*27.3*
0.79	25.5			*19.4*	*25.6*
0.94		30.7	*21.1*		
1.3		31.9	*17.8*	*14.2*	
1.6	25.4	*29.0*	*16.8*	*11.4*	
2.4	23.7	*24.1*			
3.1	*20.7**	*17.3*	*15.4*	*10.8*	
4.7	*17.1*				
6.3	*15.1*				
7.9	*15.2*				

* Italicized values refer to treatments that reduced the root/shoot ratio by more than 10 %.

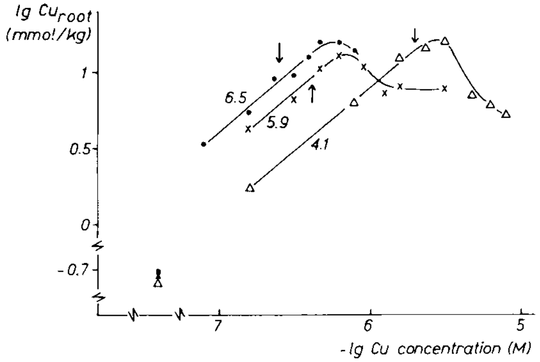

Fig. 5. Copper content of maize roots as a function of the Cu concentration at three pH levels. Experiment 3. Arrows correspond to the Cu concentration at which the root/shoot ratio begins to decrease (cf. Fig. 4).

plants were maintained on the solutions for some extra time, chlorosis developed first of all in the low Cu treatments.

The Cu content of the shoots was always higher in plants supplied with Cu than in plants grown without, but there was no clear relationship between shoot Cu and the level of Cu supply. The values averaged over the five experiments were 0.15 ± 0.04 mmol Cu/kg DM for the no Cu treatment and 0.38 ± 0.06 for all treatments receiving Cu.

The Cu content of the roots did not show a continuous increase with increasing Cu in solution as in Exp. 2, but rather followed a course in which three phases could be distinguished. As illustrated by Fig. 5, the rapid initial increase levelled off beyond the point where the root/shoot ratio was reduced and eventually turned into a decrease in treatments that were severly toxic.

Discussion

Copper toxicity to maize

The immediate effect of an increased supply of Cu is an inhibition of root growth (Exp. 3). The growth of secondary and tertiary roots appears to be more sensitive to excess Cu than the growth of coarse adventitious roots. Copper toxicity, therefore, results in a coarser root system with a reduced surface area, but not necessarily with a reduced weight. The use of dry matter production as the measure of root growth can thus obscure an inhibition of growth. In Exp. 1 Cu brought about a considerable decrease in root surface area, without affecting yield (Table 2). In particular for soil-grown plants, the capacity of the root system to take up nutrients with a relatively low mobility (PO_4 , Fe) can be expected to vary more directly with its surface area than with its weight.

The inhibition of shoot growth lags behind the effect on the roots (Exp. 3) and can therefore be considered an indirect effect of Cu toxicity. When roots

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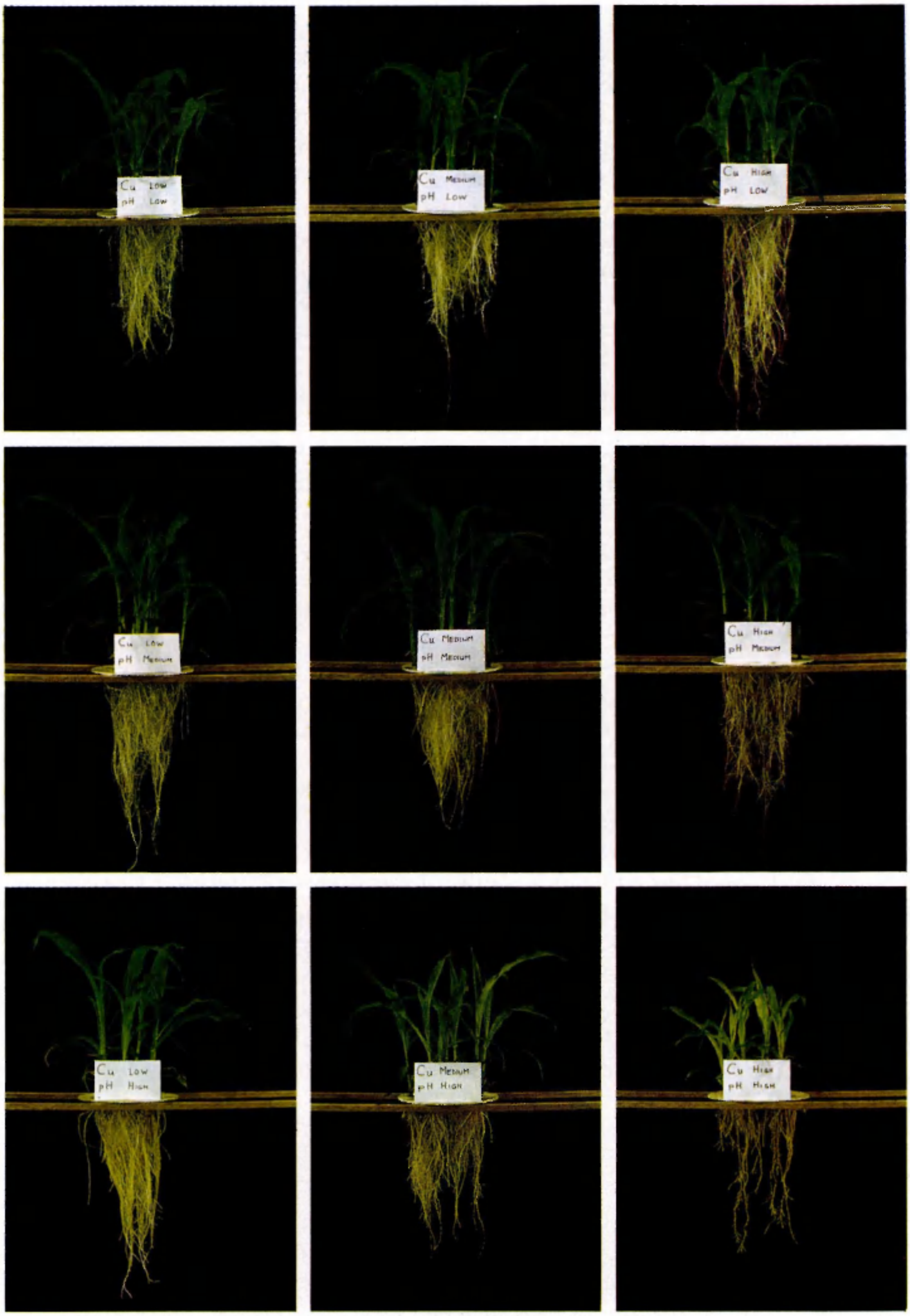
Plate 1. Maize grown in nutrient solutions with varying amounts of Cu at three pH levels maintained by varying NH_4/NO_3 ratios. Experiment 2.


Cu Low
pH Low
Cu Medium
pH Low
Cu High
pH Low
Cu Low
pH Medium
Cu Medium
pH Medium
Cu High
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Cu High
pH High

have been affected to the extent that nutrient absorption and translocation to the shoots are inhibited, imbalances may arise in the metabolism of the shoots, which eventually may lead to a reduced rate of growth. Some shoot growth must occur however, before such reductions in growth rate can become apparent. In short-lasting experiments shoot growth still depends on nutrient supply from the seed reserves and it remains essentially constant when root growth is already inhibited. Consequently, the root/shoot ratio decreases (Exp. 3). When such experiments are continued, shoot growth will become dependent on the functioning of the roots. The variation in the root/shoot ratio can, therefore, be expected to decrease with time and eventually to disappear completely.

Copper appears to interfere first of all with the uptake of iron and phosphate (Table 2, Fig. 2). Iron deficiency chlorosis is a generally observed symptom of Cu toxicity in solution culture experiments (Reuther & Labanauskas, 1966), although its occurrence in soil-grown plants is considerably less consistent. The results of Exp. 1 and 2 indicate that the Fe-deficiency is directly caused by an insufficient supply of Fe to the shoots, which agrees with the early observations of Smith & Specht (1953). Lingle et al. (1963) demonstrated that Cu interferes with Fe absorption and translocation in short term uptake experiments.

Far less attention has been given to the effect of Cu on PO_4 uptake in solution culture experiments. Adriano et al. (1971) observed that increasing the Cu concentration from 0.5 to 5.0 μM at pH 5.0 reduced the PO_4 and Fe content of maize shoots by circa 50 and 30 % respectively, without having substantial effects on shoot or root yield. Dragun et al. (1976), also using maize, found a decrease of over 50 % in the PO_4 content of the shoots when the Cu concentration was increased from 1.3 to 2.5 μM (solution pH not given). At the latter Cu level root and shoot growth were mildly depressed and the plants exhibited PO_4 deficiency symptoms. Dragun et al. (1976) used a split root system, in which part of the roots was growing in a small volume of soil supplied with Fe-EDTA. Iron could therefore be absorbed by roots that were unaffected by Cu. Under those conditions shoot growth was more depressed than Fe uptake and the Fe content of the shoots increased to high levels. Apparently, it depends on the experimental conditions what nutrient first becomes growth-limiting in the shoots.

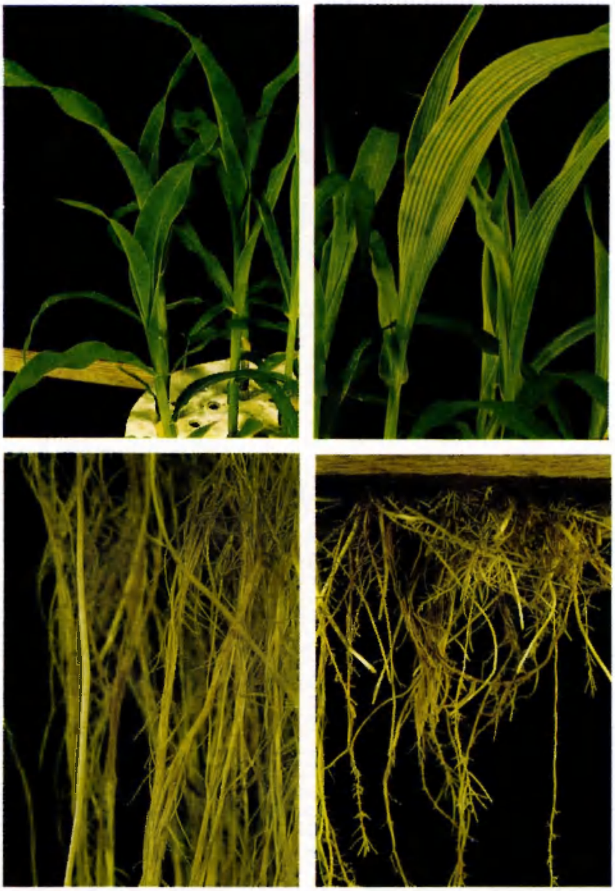
The results regarding PO_4 uptake obtained in solution culture experiments can by no means be explained from precipitation of copper phosphates. The quantity of PO_4 in solution exceeds the amount of Cu by far and, therefore, Cu cannot exert any appreciable effect on the availability of PO_4. The possibility of copper phosphates being precipitated on or in the roots causing translocation to be impeded can be rejected on the principle that toxic Cu concentrations reduced the PO_4 content of the roots (Exp. 1 and 2). The capacity of plants to take up PO_4 from soil is related to the root surface area, but is does not seem likely that the Cu-induced reduction in root surface area by itself has specifically limited PO_4 uptake in the well mixed solutions used in the present experiments. It seems more likely that Cu has impaired the mechanism that is responsible for PO_4 uptake, just like the effect Cu has on Fe uptake (Lingle et al., 1963). Both PO_4 and Fe are taken up actively by the roots. In connection with the theory of carrier-mediated uptake one may visualize such an effect of Cu as the result of an association with some component of the carrier system, leading to partial or complete inactivation.

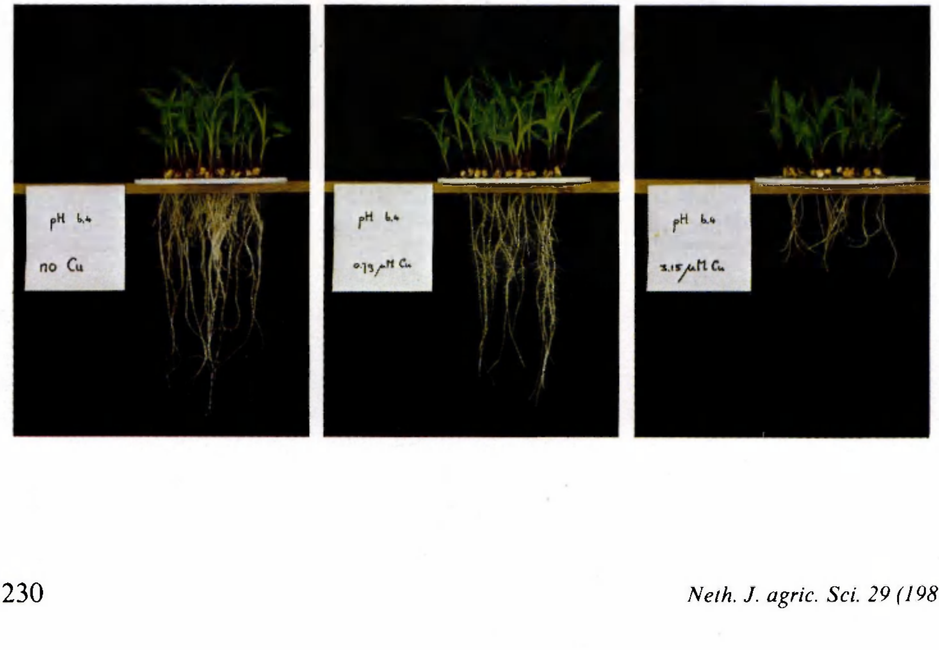
Other changes in mineral composition brought about by Cu are less outspoken and the possibility cannot be excluded that they are only an indirect result of Cu toxicity, related to the Cu-induced Fe deficiency. Considering the results of Exp. 2 obtained with the 100 % NO_3-N solution, it becomes evident that Cu has a significant effect on the pattern of ion uptake. The difference between inorganic cation and anion contents of the shoot (ΣC-ΣA, Fig. 2) increases, but since the content of org. N derived from NO_3 taken up decreases, the real difference between the effects Cu has on cation and on anion uptake is quite marked. A larger reduction in anion uptake than in cation uptake, a decrease in org. N production without accumulation of free NO_3, a shift in cation uptake in favour of divalent ions and a lowering of the pH of the nutrient solution have all been associated with iron stress (van Egmond & Aktas, 1977).

Distribution of copper within the plant

An increase in Cu supply affects the Cu content of shoots and roots in dissimilar ways. The distribution coefficient (defined as the ratio of the shoot and root Cu content) decreases with increasing Cu concentration (Exp. 1 and 2), showing all with all a 20-fold variation (Fig. 6). At toxic levels of Cu a further increase in supply does not lead to a higher rate of translocation, since no further rise in shoot Cu is observed (Fig. 2). This result cannot be taken to mean that the plants are actively excluding Cu from their shoots. It rather seems to reflect an impairment of the translocation mechanism. Copper does not move within the plant in the free cationic form, but in a complexed anionic form (Tiffin, 1972). Recent work of Goodman & Linehan (1979) and Goodman et al. (1979) has reinforced earlier suggestions that amino acids be involved in the translocation of Cu. One may therefore expect this process to be dependent on the availability of amino acids for the sequestration of Cu. As toxic Cu treatments inhibit the synthesis of organic N compounds (Fig. 2), the availability of amino acids may be reduced and Cu may thus have affected its own mobility in the plant.

From results obtained under field conditions, Lexmond (1980) concluded that the level of organic N has a significant effect on shoot Cu values in maize. Following a suggestion by Beyme (1971), he therefore used the Cu/N ratio rather than the Cu content in the dry matter to evaluate the effects of soil treatments on Cu accumulation in the shoot. The present results appear to stress the advantages of this approach (Fig. 2), but nevertheless it is evident from Fig. 3 that no relation exists between the occurrence of toxicity and Cu/N ratios in the shoot. One can therefore not count upon critical values to




pH 6.4
no Cu
pH 6.4
0.79 µM Cu
pH 6.4
3.15 µM Cu

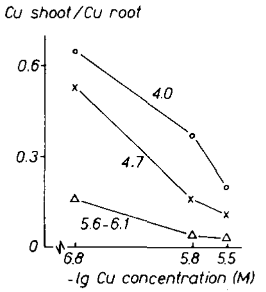


Fig. 6. The ratio of the shoot Cu content to the root Cu content as affected by the Cu concentration at three levels of pH/N source. Experiment 2.

be established for the Cu/N ratio in shoots.

Copper accumulated in roots is essentially non-exchangeable, as Ca^{2+}, Mg^{2+} and Ba^{2+} ions are ineffective in replacing the bulk of the Cu taken up (Larsen, 1966; Harrison et al., 1979). One would expect so, because if Cu were bound by purely electrostatic forces, the binding would be non-preferential and Ca^{2+} and Cu^{2+} would have to be present in the roots in approximately the same proportion as in solution. From the results of an experiment comparable to Exp. 2 the Ca content to maize roots can be estimated at 140 mmol/kg DM. The results of Exp. 2 show that the Cu content of the root is circa 5 mmol/kg (average value) at a Cu concentration of 3.1 μM and a Ca concentration of 2.50 mM. Even if it is assumed that Ca in the roots is completely exchangeable, exchangeable Cu cannot account for more than 3-4 % of total Cu in the roots.

Lead ions, however, are quite effective in replacing Cu from roots (Larsen, 1966; Harrison et al., 1979) and in preventing Cu from associating with roots (Goren & Wanner, 1971). Other heavy metals are intermediate in their ability to displace Cu (Larsen, 1966); Harrison et al., 1979). These observations suggest that the bonding of Cu in the roots is covalent by nature. Protons can therefore be expected to have a strong effect on the association of Cu with the roots. Brams & Fiskell (1971) observed that 0.1 M HCl rapidly removed 95 % of the Cu taken up by citrus roots. The observation by Hunter (1975) and the results of the present study (Table 2, Fig. 2, Fig. 5) which show Cu uptake by the roots of maize to increase with increasing pH, can be accounted for by the covalent character of the association. Accordingly, one would also expect uptake by roots of other heavy metals to be pH dependent. The results of Rasmussen & Henry (1963) show this to be the case for Pb uptake by citrus roots, but few studies appear to have been made on this subject.

Plate 2a. Typical symptoms of Cu toxicity to maize in solution culture are Fe deficiency chlorosis in the leaves and a profound change in root morphology (right). Plants low in Cu are shown to the left. Experiment 2.

Plate 2b. Copper toxicity results in a reduced root growth before the growth of shoots is affected. Consequently the root/shoot ratio decreases, in this trial from from 0.48 (left) via 0.34 (centre) to 0.22 (right). Experiment 3.

Veltrup (1976) has made a detailed study of Cu uptake by root tips of barley as a function of the Cu concentration in solution. He obtained linear double logarithmic plots over a wide range of concentrations (1.6-79 μM) in short-term experiments lasting up to 24 h. The results of Exp. 3 (Fig. 5), however, show marked deviations from linearity at lower Cu concentrations than used by Veltrup. In this connection it is of interest that Cu has been found to accumulate primarily in the tips of roots (Hunter, 1975). Since the growth of roots is reduced considerably by toxic Cu treatments, the proportion of root tips in the root dry matter is decreased and this may explain the relationship between root Cu and Cu in solution presented in Fig. 5.

A comparison of the root Cu levels obtained in the different experiments at approximately equal Cu concentrations and pH values (Table 2, Figs 2 and 5), shows higher root Cu values for Exp. 3 than for Exps. 1 and 2. Several factors might be responsible for this result. The withholding of Fe from the nutrient solution used in Exp. 3 could have enabled Cu to occupy sites which otherwise would have been occupied by Fe. It is also possible that the roots in Exp. 3 had a higher capacity per unit weight to retain Cu, because of the absence of relatively inactive adventitious roots or because of the presence of a greater portion of root tips.

Effects of nitrogen source on the toxicity of copper

The results of Exp. 1 (Table 2) show the effect of N source on Cu toxicity to be small in comparison to the effect of pH. Still there appears to be a somewhat stronger effect of Cu on PO_4 and Fe uptake and on the Cu content of the roots in the NO_3 solutions. This difference could not be accounted for by the higher SO_4 concentration in the NH_4 solution although it allowed a more extensive formation of the $CuSO_4^0$ complex and thus slightly reduced the Cu^{2+} ion activity. The difference may, however, be related to changes in pH rather than be the direct result of variation in N source. Maize supplied with NO_3 increases the solution pH, whereas NH_4-supplied plants bring about a decrease in pH. It is conceivable that, due to these differential patterns of ion uptake, pH close to the roots and in the root free space is higher in the NO_3 treatments, although the bulk pH of the solution is kept at the same level. As an increase in pH is accompanied by an increase in toxicity, this would explain the somewhat larger effects of Cu observed in the NO_3 solutions.

The direct effect of N source on Cu toxicity can be considered limited. This is in agreement with the results of Smith & Specht (1953), but at variance with results of Moore et al. (1957), who compared the effect of increasing Cu concentrations on the growth of lettuce in a NO_3 solution and a solution containing 25 % NH_4-N and 75 % NO_3-N. High Cu concentrations reduced yield and the Fe content of the plant tops in both solutions, but the effects were most pronounced in the NO_3 solution. Moore et al. (1957) did not present any information concerning the pH values of their treatment solutions, and probably did not control pH. The inclusion of NH_4-N can be expected to have lowered the pH by inducing a shift in the pattern of ion uptake by the lettuce. In view of the results of the present study the results obtained by Moore et al. can be readily explained from such differences in pH.

Effects of pH on copper toxicity

The results obtained in the present study prove Cu to be more toxic to maize at higher pH levels and thus confirm the results of Hunter (1975), but under conditions of prolonged exposure to relatively low concentrations, more likely to prevail in soil. Before discussing this effect, it is of interest to note that the dependency on pH appears to be a general feature of Cu toxicity, since it has been observed in an array of organisms. Increased toxicity of Cu at higher pH (or, vice versa, increased tolerance at low pH) has been detected in algae (*Chlorella pyrenoidosa;* Steemann Nielsen et al., 1969) and *Hormidium rivulare* (Hargreaves & Whitton, 1976)), fungi (*Aureobasidium pullulans* (Gadd & Griffiths, 1980) and *Scytalidium* sp. (Starkey, 1973)), fungal spores (*Alternaria tenuis;* Biedermann & Müller, 1952), crustaceans (*Daphnia magna;* Andrew et al., 1977) and fishes (*Salmo gairdneri;* Howarth & Sprague, 1978), suggesting similarities in the mechanism of the toxicity.

Hunter (1975) made a detailed study of Cu toxicity to roots of maize after short exposure to very high Cu concentrations. He could distinguish between two phases: a first phase of several hours during which toxicity was reversible and a second phase during which irreversible changes took place. The initial phase during and immediately following uptake of Cu was characterized by inhibition of growth, loss of K in amounts which could not be accounted for by regular ion exchange with Cu, and an inhibition of cell division, although cells dividing at the beginning of the Cu treatment completed the process. Rinsing the roots with EDTA and histidine solutions for ½ to 1 h during this phase removed a major part of the Cu taken up and allowed the roots to resume normal growth. The second phase was characterized by continued growth inhibition, browning of the roots and initiation of lateral root primordia. Rinsing with EDTA during this phase did not reverse the toxicity.

The characteristics of the first phase suggest that Cu can inhibit the growth of roots when it has not yet penetrated into the symplasm. The release of cell K points at an association of Cu with the plasmalemma, resulting in a change in retentive properties. The inhibition of cell division does not necessarily imply that Cu has penetrated into the cell and has directly affected the cell nucleus. An interaction of Cu with the cell membrane which leads to an inhibition of growth might indirectly control cell division.

The most plausible inference from Hunter's work is that Cu acts upon the plasmalemma, thereby altering its retentive properties and inhibiting cell growth. Results obtained by Wainwright & Woolhouse (1977) regarding the effect of Cu on root cell elongation and K leakage in *Agrostis tenuis* agree with this hypothetical mechanism.

Uptake of Cu increases proportionally to the increase in external pH, not only in maize roots, but also in algae (Steeman Nielsen et al., 1969; Mierle & Stokes, 1976) and fungi (Gadd & Griffiths, 1980). The extent to which Cu associates with physiological essential sites in the plasmalemma can accordingly be expected to increase with increasing pH. Such a pH dependent association of Cu with biological membranes might explain the similar effect of pH on Cu toxicity to widely different organisms.

Up till now the chemical form of Cu which is actually toxic has been left undiscussed. The effect of pH on Cu toxicity might be explained from a competition between Cu^{2+} and H^+ ions, but also from toxicity of one of the hydrolysis products of Cu, which increase in activity when pH increases, due to the reactions:

$$\begin{aligned} Cu^{2+} + H_2O &\rightleftharpoons CuOH^+ + H^+, \\ CuOH^+ + H_2O &\rightleftharpoons Cu(OH)_2^0 + H^+, \\ 2Cu^{2+} + 2H_2O &\rightleftharpoons Cu_2(OH)_2^{2+} + 2H^+. \end{aligned}$$

Most work on toxicity of Cu in relation to its aquatic chemistry has been carried out with freshwater organisms. These studies are quite complicated because of extensive hydrolysis of Cu^{2+} and complexation by carbonate ions at the high pH and alkalinity levels common to freshwater systems. A number of studies aimed at the identification of the chemical species that is actually toxic. The approach followed therein is based on the assumption that if anyone of the species is responsible for the toxicity, its critical concentrations should be nearly constant in different conditions. Pagenkopf et al. (1974) concluded from chemical equilibrium calculations on data obtained from bioassays with fishes (*Salmo gairdneri* and *Pimephales promelas*) that Cu^{2+} is the major toxic species, but that $CuOH^+$ might also be toxic. The did not, however, allow for the protective effect of increasing hardness (Ca and Mg concentrations), which has been firmly established (for a recent review see Hodson et al., 1979). When the differences in hardness are taken into account, the critical concentrations of Cu^{2+} and $CuOH^+$ appear to decrease considerably when pH increases from 7.2 to 8.2. Andrew et al. (1977) investigated Cu toxicity to the crustacea *Daphnia magna* at constant hardness. They demonstrated conclusively that complexing by carbonate ions reduced toxicity. Toxicity was related only to the Cu^{2+}, $CuOH^+$ and $Cu_2(OH)_2^{2+}$ ion activities. As pH was maintained constant within each test, the relative toxicities of these ions were not directly determined. A comparison of the different experiments, however, showed that increasing pH from 7.4 to 8.0 decreased the critical concentrations of Cu^{2+} and $CuOH^+$. Further work with *Salmo gairdneri* was described by Howarth & Sprague (1978), who measured Cu lethality in an experiment in which hardness and pH were varied systematically. Cu^{2+}, $CuOH^+$ and $Cu_2(OH)_2^{2+}$ were considered the toxic species since they yielded the smoothest response surface with the best fit to the observed median lethal concentrations. At each level of hardness the critical Cu^{2+} concentration decreased with increasing pH. The critical concentrations of $CuOH^+$ and $Cu_2(OH)_2^{2+}$ increased when pH rose from 5.0 to 7.0, but decreased upon a further increase in pH to 9.0. The critical concentration of $Cu(OH)_2^0$, which was considered a toxic species by Chakou-

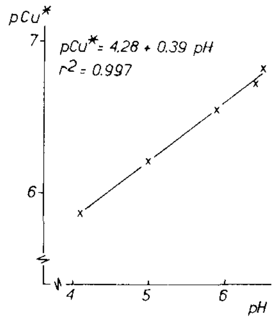


Fig. 7. The critical Cu^{2+} ion activity (pCu*) as a function of pH. Experiment 3.

makos et al. (1979) and Wagemann & Barica (1979), increased when pH increased from 5.0 to 8.0, but decreased when pH increased from 8.0 to 9.0.

It is evident from this discussion that the results obtained cannot be explained straightforwardly from toxicity of one chemical species only. If one of the hydrolysis products were the active species, its toxicity would have to be extremely high at low pH levels, where Cu^{2+} dominates and hydroxides are nearly absent, and at very high levels, where $Cu(CO_3)_2^{2-}$ is the dominant species. In the intermediate range, where the hydroxides are present in substantial amounts their toxicity would have to be relatively small. Such a relationship is difficult to imagine. If Cu^{2+} were the active species, its toxicity would have to increase steadily with increasing pH. The effect of pH might in that case be visualized as the result of a competition between Cu^{2+} and H^+ ions: the higher pH, the smaller the power of protons to keep Cu^{2+} ions from associating with physiologically essential sites.

The size of the effect of pH on Cu toxicity to maize can be estimated directly from the results of Exp. 3. In Fig. 7 the critical values of pCu have been plotted as a function of pH. These pCu* values were obtained from the Cu concentrations at which the root/shoot ratio began to decrease (cf. Fig. 4), by

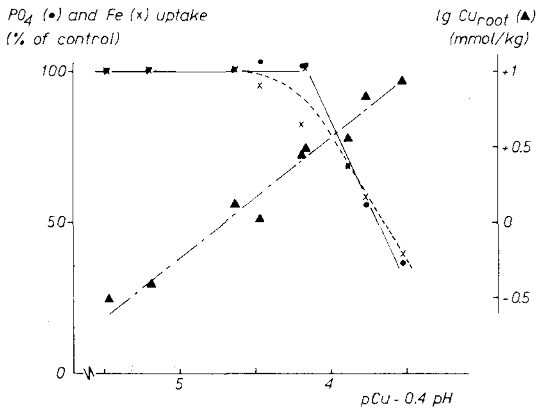


Fig. 8. Copper-induced reduction in PO_4 and Fe uptake by maize shoots and the Cu content of maize roots as a function of pCu and pH. Experiment 2.

calculating the speciation of Cu with the computer program MINEQL (Westall et al., 1976) and converting concentrations to activities. Equilibrium constants were taken from Lindsay (1979) and Mattigod & Sposito (1977). In the pH range of the experiment (4.1 to 6.5), Cu^{2+} is the dominating species, accounting for 95-90 % of total Cu in solution. The slope of the graph obtained implies that the critical Cu^{2+} ion activity decreases by a factor 2.5 when pH increases by 1 unit. This is slightly less than the factor 3.5 estimated by Lexmond (1980) from the results of a field experiment. Fig. 8 demonstrates that the results of Exp. 2 fit quite well to a linear combination of pCu and pH, which may therefore serve as an index of the stress imposed by excess Cu.

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