PROEFSTATION VOOR TUINBOUW ONDER GLAS TE NAALDWIJK

Water, DTPA and HNO_3 extracts for assessing the availability of Cu in peat substrates.

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January, 1991.

Intern verslag nr 2

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1. INTRODUCTION

The aim of the experiment described here was to determine if water extract can be used for assessing Cu content in peat substrates in comparison with DTPA and HNO_3 extraction. DTPA extract is widely used for peat substrates, and HNO_3 for soils in situ. Cu in the three extracts, at different Cu-levels in the peat substrate, was related to Cu-content in plant material to search for the best relationship.

2. METHODS

2.1. Trial

Two substrates were used:
A. 20% white peat, 55% coarse sieved peat, 25% peat lumps
B. 40% black peat, 20% white peat, 40% peat moss.

The initial addition of copper was for the four Cu levels 0, 1.75, 3.50 and 7.0 g Cu per m^3 substrate, and during the trial weekly fertilizations (six in total) of 0, 0.08, 0.16 and 0.32 g Cu per m^3 substrate, respectively for Cu levels 1 - 4 mentioned before.
To substrate A 1.0 and 2.0 kg $CaCO_3$ per m^3 substrate was added, respectively for low and high pH, for substrate B this was 1.5 and 3.0 $kg.m^{-3}$. The addition of the other nutrients was in $g.m^{-3}$ N 110; P 35; K 158; Mg 29; S 37; B 0.3; Mn 1.6; Mo 2.0; Zn 0.4 and Fe as EDTA 0.9.
The substrate was filled into plastic containers, with upper diameter 17 cm and height 17 cm, each of which contained about 2 litres of substrate. The trial was conducted in duplicate and a plot consisted of 20 pots.
Cucumber, cv 'Mustang', was sown in a sandlayer on March, 9, 1990 and 4 days later the young plants were put into the substrate.
The trial was ended May, 2, 1990. Then the height of the plants was about 170 cm. The greenhouse air temperature was between 20 and 35° C.
The pots of one plot were in a gulley so that during watering no leachate or nutrient solution was lost. Pots were covered with plastic foil to prevent a transpiration from the pot. During growth, nutrient solution, made from high-quality rainwater, was given to the plant, according to the transpiration, from the top or from the bottom of the pot with the following composition in mM: NO_3 10.6; NH_4 1.1; K 5.5; Ca 3.0; Mg 0.75; H_2PO_4 1.5; SO_4 1.0 and in uM: Fe 15; Mn 5; B 10; Mo 0.5 and Zn 7. The electrical conductivity was 1.4 $dS.m^{-1}$ and pH 5.0. No Cu was added to the nutrient solution; the Cu-content was lower than 0.5 uM.

2.2. Water content of substrates at pF 1

Cylinders with innerdiameter 10 cm were filled for 7 cm height without compaction. After filling a weight of 10 kPa was put on the sample. The cylinder was flooded from beneath on a sandlayer for 3 hours. During 3 hours the water level in the sandbox was maintained at 10 cm below the middle of the cylinder, so the mean pressure head was -10 cm (pF 1). Water content was determined by drying the substrate at 105° C, for 17 hours. Water content was expressed as gram water per gram fresh substrate.

2.3. Sampling and extraction of substrate

Of the two substrates water content at pF 1 was determined (see par. 2.3). At the beginning, halfway through (April, 18) and at the end of the trial, samples were taken from the substrate.
Of a subsample the water content was determined and it was calculated how much extractant has to be added to 100 gram of substrate to bring the water content at pF 1.
The extractants used were: water, DTPA (adjusted to pH 5,5 with NaOH) and HNO_3. The concentrations of the DTPA and HNO_3 differed from sample to sample depending on the initial water content and the water content at pF 1, in such a way that at pF 1 the concentrations in the soil solutions became 0.005 M DTPA and 0.4 N HNO_3. For equilibration the substrates were kept for 17 hours at room temperature. With pressure of 5000 kPa 50 to 100 cm^3 of soil solution was gathered. The extracts were centrifuged for 30 minutes at 5000 revolutions per minute. The extracts were divided into two parts: one part for EC (only water extracts) and pH measurement; the other part was filtered over 0,45 um membrane filter. To the water extracts 0.1 cm^3 of concentrated HNO_3 was added. In the extract Cu, Fe, Mn and Zn were determined with atomic absorption spectrophotometry, and B spectrophotometrically with azomethine complex. B-determination was not done in DTPA-extracts due to the possible interference with DTPA.

2.4. Sampling and determination of Cu in plant material

Halfway through and at the end of the trial leaf blades (without petioles) of young fully developed and old leaves were taken. Leaves were washed with deionized water with 0.1% Teepol, dried at 80° C, ground and 2.5 g of dry material was predigested with 2*5 cm^3 HNO_3 (65%) and digested with 15 cm^3 of a HNO_3 (65%): H_2SO_4 (96%): $HClO_4$ (70%) mixture 32:8:1 (vol/vol), according to the Schäumlöffel-modified method. Cu was determined with atomic absorption spectrophotometry.

3. RESULTS

3.1. Water content at pF 1

The water contents of substrates A and B were 0.89 and 0.85 g water per g fresh material respectively.

3.2. EC and pH in water and DTPA extract

The Cu-level had no influence on EC and pH. In table 1 the average values for the four Cu-levels are given. In DTPA-extract the pH was on average 0.3 pH-value lower than in water-extract. At the low pH level the pH in water was 5.4 and at the high level 6.0.

Table 1. EC and pH in water and DTPA extract at the beginning, halfway through and the end of the experiment.

Time	pH-level	EC, mS/cm		pH			
		substrate A	substrate B	substrate A		substrate B	
		water	water	water	DTPA	water	DTPA
Begin	low	2.5	1.9	4.5	4.3	4.3	4.4
	high	2.5	2.0	6.0	5.8	5.5	5.4
Halfway	low	1.0	1.0	6.0	5.3	6.0	5.1
	high	1.0	1.1	6.4	5.7	6.1	5.7
End	low	1.5	1.3	5.9	5.5	5.5	5.0
	high	1.6	1.4	6.0	5.8	6.0	5.6

3.3. Mn-, Fe-, Zn- and B-contents in extracts

Mn-, Fe-, Zn- and B-concentrations in water, DTPA and HNO_3-extract are given in Appendix 1. The Cu-levels had no influence on the concentrations of these elements.
At the beginning substrate A had higher Mn-, Fe-, Zn- and B-content in water extract than substrate B; the opposite was found for iron in DTPA and HNO_3. At the beginning the low pH-level gave higher Mn-, Fe- and B-content and lower Zn-content in water extract than high pH-level. In DTPA and HNO_3 there were no great differences between low and high pH.
Halfway through the trial substrate A gave higher Mn-, and lower Fe-content in water extract than substrate B. Substrate A gave lower Fe-, and Zn-content both in DTPA and HNO_3 than substrate B.
The low pH gave higher Fe-, and lower Zn-content in water extract than high pH.

At the end of the trial substrate A gave higher Mn- and Zn-content in water-extract than substrate B. Substrate A gave lower Fe- and Zn-content both in DTPA and HNO_3 than substrate B.
In table 2 the relations are given. Low correlations were found between the contents in water and the other two extracts, except for B. Between DTPA and HNO_3 high correlations were found, special for Zn ($r = 0.96$).

Table 2. Relation between Mn-, Fe-, Zn- and B-content in extracts. Number of values is 48.

Element	x - value extract	min. uM	max. uM	y - value extract	min. uM	max. uM	Correlation coeff.	Correlation
Mn	H_2O	1.1	17.0	DTPA	56	108	0.44	y = 77 + 1.2 x
Mn	H_2O	1.1	17.0	HNO_3	55	120	0.61	y = 78 + 1.9 x
Mn	DTPA	56	108	HNO_3	55	120	0.87	y = 7 + 1.0 x
Fe	H_2O	12	174	DTPA	310	1117	0.22	-
Fe	H_2O	12	174	HNO_3	352	1009	0.16	-
Fe	DTPA	310	1117	HNO_3	352	1009	0.89	y = 221 + 0.7 x
Zn	H_2O	1	197	DTPA	35	238	0.68	y = 79 + 1.0 x
Zn	H_2O	1	197	HNO_3	43	204	0.68	y = 80 + 0.9 x
Zn	DTPA	35	238	HNO_3	43	204	0.96	y = 12 + 0.9 x
B	H_2O	9	47	HNO_3	36	112	0.78	y = 34 + 1.6 x

3.4. Cu-content in extracts

Cu-contents in extractants are given in Appendix 2 and table 3. The relations are given in figures 2 - 4 and in table 4.
pH-level had no influence on Cu-contents in extracts. Cu levels were reflected in Cu contents in extracts. Substrate A gave higher Cu-contents than substrate B. There were significant ($p < 0.001$) correlations between Cu-contents in the three extracts. The correlation coefficients from table 3 were transformed according to Fisher. These transformed values have a standard normal distribution and were tested on differences. There were no significant differences between $r = 0.849$ and $r = 0.817$, but there was a significant ($p = 0.05$) difference between $r = 0.849$ and $r = 0.932$ and a significant ($p = 0.02$) difference between $r = 0.817$ and $r = 0.932$.

Table 3. Cu-content in H_2O-, DTPA- and HNO_3-extract, averaged over time and pH.

Cu-level	Cu in H_2O		Cu in DTPA		Cu in HNO_3	
	substrate	substrate	substrate	substrate	substrate	substrate
	A	B	A	B	A	B
B	uM	uM	uM	uM	uM	uM
1	2.1	3.4	7.5	9.3	18.8	16.3
2	7.8	5.5	48.0	55.6	51.0	35.6
3	16.4	7.9	109.0	113.1	91.9	77.6
4	31.1	18.6	220.9	161.2	242.2	146.8
average	14.6	8.9	92.1	80.0	101.0	69.1

Table 4. Relations between Cu-contents in H_2O, DTPA- and HNO_3-extracts.

Cu-content,	uM	Number of values	Correlation coeffic.	Correlation
x	y	n	r	
H_2O	HNO_3	47	0.849	$y = 27.16 + 4.993\ x$
H_2O	DTPA	42	0.817	$y = 36.55 + 4.523\ x$
DTPA	HNO_3	43	0.932	$y = -4.87 + 0.9984\ x$

3.5. Cu in plant visible Cu-deficiency, and dry matter content

Cu-content in leaves without petioles is given in table 5.

Table 5. Cu-content in young and old leaves; t2 = halfway through, t3 = end of experiment.

Substrate	pH	Cu	Cu in young leaves t2	Cu in young leaves t3	Cu in old leaves t2	Cu in old leaves t3
			mg / kg		DM	
A	low	1	2.5	1.9	3.2	1.9
		2	8.9	8.9	5.7	5.1
		3	8.3	8.3	6.4	6.4
		4	10.8	13.4	8.9	9.5
	high	1	2.5	2.5	3.2	2.5
		2	7.0	8.9	3.8	5.1
		3	8.9	10.8	6.4	7.0
		4	8.3	11.4	5.7	7.6
B	low	1	2.5	1.9	3.2	2.5
		2	5.1	5.1	2.5	2.5
		3	8.3	8.3	3.8	3.8
		4	9.5	10.8	4.5	5.7
	high	1	2.5	2.5	3.2	1.9
		2	3.8	5.1	3.2	2.5
		3	5.7	7.6	3.2	3.8
		4	8.3	9.5	4.5	5.1

In the beginning of the experiment Cu-deficiency was visible in old leaves as a chlorosis between the veins and later on a necrosis of the entire leaf. In table 6 results are given of a visible determination of the chlorosis and necrosis. Later on, new formed leaves no longer showed any deficiency symptoms.

Table 6. Visible Cu-deficiency on 18 April 1990.
0 = no deficiency 10 = very severe deficiency.

Substrate	pH	Cu-level	Visible deficiency
A	low	1	8
		2	0
		3	0
		4	0
	high	1	10
		2	3
		3	0
		4	0
B	low	1	6
		2	5
		3	1
		4	0
	high	1	5
		2	4
		3	2
		4	1

In figure 1 relations are given between the Cu-content in leaves and the visible Cu-deficiency. No Cu-deficiency was found at Cu-contents equal to or higher than 9 mg/kg DM in young leaves and 5 mg/kg DM in old leaves.

The treatments, time of sampling and age of leaves had no influence on dry matter content of leaf blades, (average 13.3%, minimum 10.4%, maximum 16.0%) except at the end of the experiment when old leaves were taken, which were necrotic at low Cu-levels; dry matter contents of these leaves were 16.5 - 25.0%.

3.5. Relation between Cu in extracts and in plant

In Table 7 and in Figures 5 - 7 relations between Cu in extracts and in plant are given. The logarithmic relations fit better than the linear relations. The correlation coefficients were transformed according to Fisher. These transformed values have a standard normal distribution and were tested on differences. There were no significant differences.

Table 7. Relations between Cu in extracts and Cu in plant.

Cu in extract x uM	Cu in plant y mg/kg DM	Number of values n	Correlation coefficient r	Relation
H_2O	young leaves	31	0.881	y = -0.331 + 3.027 ln x
DTPA	young leaves	30	0.865	y = -2.38 + 2.253 ln x
HNO_3	young leaves	32	0.888	y = -6.01 + 3.084 ln x
H_2O	old leaves	31	0.784	y = 0.641 + 1.624 ln x
DTPA	old leaves	30	0.663	y = 0.159 + 1.059 ln x
HNO_3	old leaves	32	0.780	y = 2.31 + 1.634 ln x

3.6. Variation between duplicates

Between the duplicates the variation, standard deviation devided by the mean, was calculated (table 8).

Table 8. Mean and mean standard coefficient calculated from the duplicates.

Element	Mean			Variation coefficent		
	Water uM	DTPA uM	HNO_3 uM	Water %	DTPA %	HNO_3 %
Mn	5,1	82,5	86,1	19,6	7,0	8,9
Fe	68,1	669,0	694,3	18,4	9,2	7,0
Zn	46,5	128,1	128,4	17,4	10,1	10,3
Cu	12,6	100,5	93,2	35,5	38,6	37,2
B	16,8	--	58,0	15,6	--	14,8

For Cu all the three extracts gave a high variation. For the other elements water extract gave higher variation than DTPA and HNO_3 extract.

4. DISCUSSION AND CONCLUSIONS

In the beginning of the trial the two pH-levels differed 1.5 (substrate A) and 1.2 (substrate B) pH-unit. Halfway through and at the end of the trial the difference in pH was very small and almost negligible being 0.1 - 0.6 pH unit. The pH-difference in the beginning was achieved by differences in $CaCO_3$ addition and these differences were expected to continue with the same nutrition during the experiment, but this did not occur: at the low pH-level pH raised. The pH-level had almost no influence on Mn-, Zn-, Fe-, B- and Cu-contents in peat-extracts, which is realistic, with respect to the small differences in pH.

Boron-determination was not possible in DTPA-extract caused by interference of the colour of the extract with the photometric determination. EC and pH measurements were possible in water and DTPA-extracts, not in HNO_3. For Mn, Fe and Zn significant correlations were found between DTPA and HNO_3-extract. For these elements the correlations with water and other two extracts was less than the above mentioned correlations. For Cu also the correlations between DTPA and HNO_3 was higher ($r = 0.932$) than the correlations between H_2O and HNO_3 ($r = 0.849$) and H_2O and DTPA ($r = 0.817$), but the correlations did not differ significantly.

Substrate A gave higher Mn- and Zn-contents in water-extract and Cu-contents in water-, DTPA- and HNO_3-extracts caused by a lower adsorption of these elements in substrate A than in B.

Substrate B gave higher Fe-contents in water-, DTPA and HNO_3-extract and higher Zn-contents in DTPA- and HNO_3-extract due to higher content of raw material of substrate B than A.

Visible Cu-deficiency showed high correlation with Cu-content in leaves. Visible deficiency occurred at Cu-contents lower than 9 mg/kg DM for young leaves and lower than 5 mg/kg DM in old leaves.

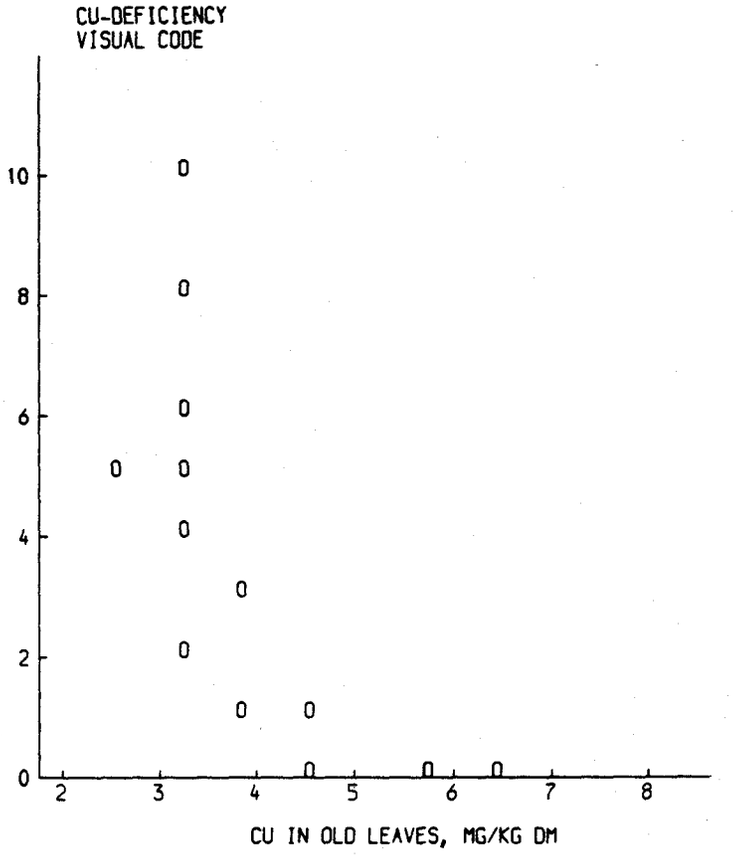
Correlations between Cu-content in plant and Cu content in the three extracts were significant ($p < 0.001$). The correlations did not differ significantly, so it is possible to use the three extracts for assessing the Cu-content of plant material. In water extract it is possible, beside Cu, also to determine all other micro-elements, the macro-elements, EC and pH. Therefore from a practical point of view water as an extract of peat substrate is recommended.

This disadvantage of water is the high variation between the duplicates.

5. SUMMARY

In an experiment with cucumber in two peat substrates and 4 Cu-levels the aim was to determine if water could be used as an extract for assessing the availability of Cu in peat substrate. Water was compared with 0.005 M DTPA and 0.4 M HNO_3. One of the substrates was selected for low adsorption (without black peat) and the other for high adsorption (with black peat). Extract solution was added to the substrate, so that it had a water content of pF 1 and then it was pressed.
Cu-contents in these extracts were correlated to Cu-contents in leaves. For young leaves correlation coefficients were 0.88; 0.87 and 0.89 for water, DTPA and HNO_3, all significant at $p < 0.001$. The three correlation coefficients did not differ significantly from each other, so it is possible to use these three extracts for assessing the availability of Cu in peat substrates. From a practical point of view water is recommended.

Figure 1. Visible Cu-deficiency and Cu in plant. (0 = no deficiency, 10 = very severe deficiency).



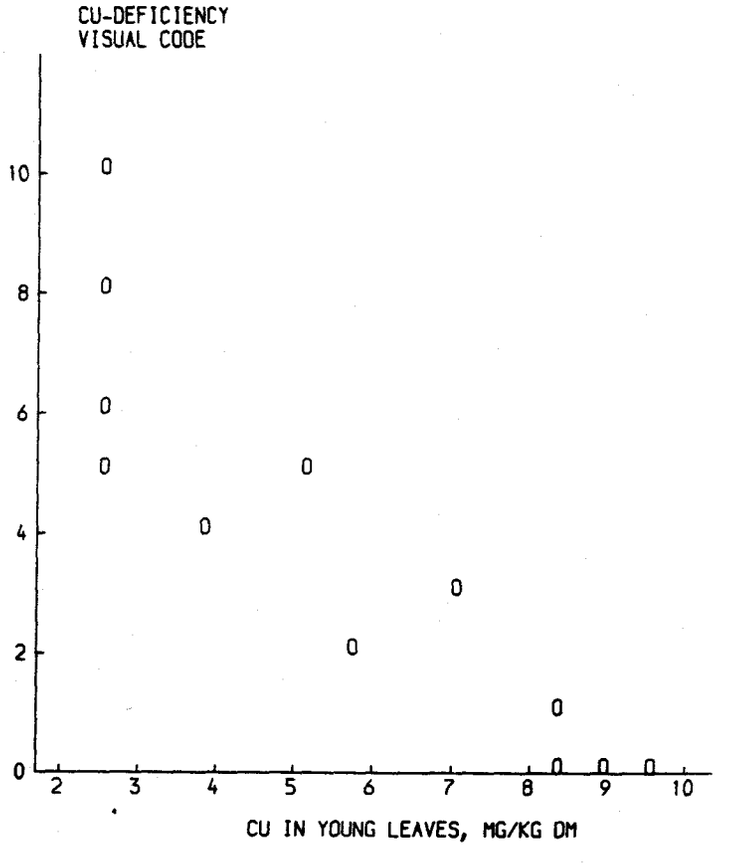


Figure 2. Relation between Cu in H_2O and in HNO_3 extract.

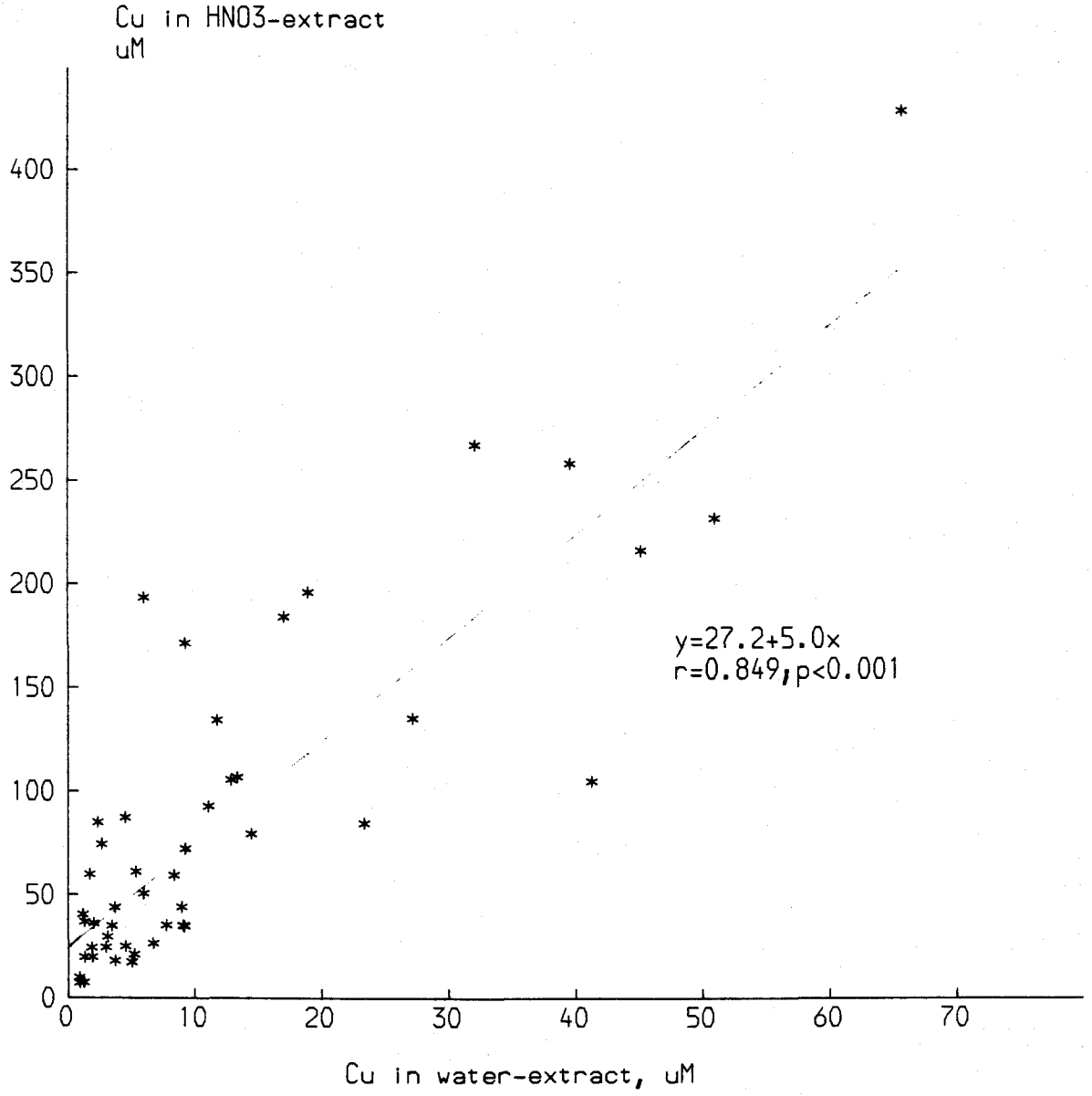


Figure 3. Relation between Cu in H_2O and in DTPA extract.

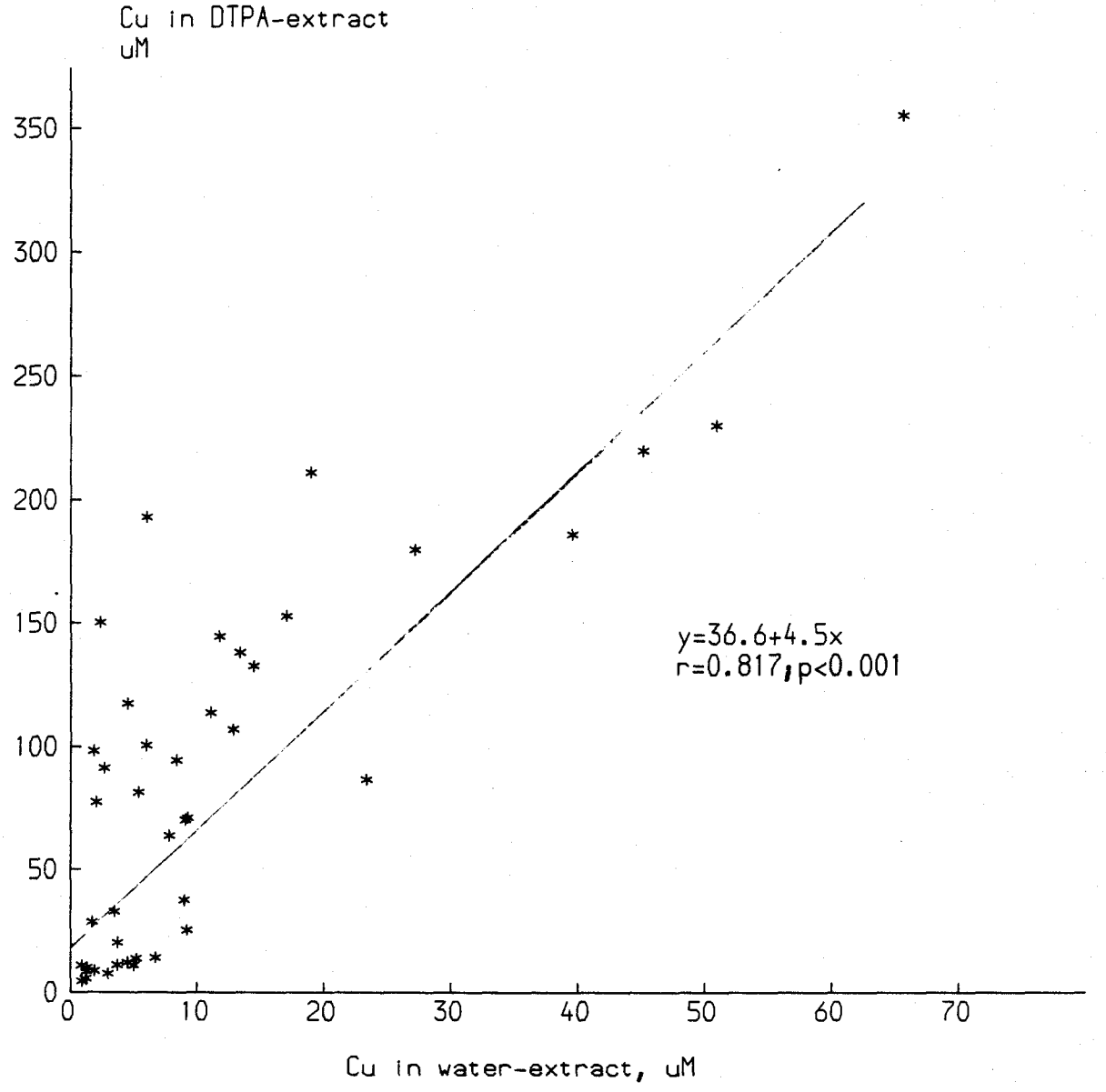


Figure 4. Relation between Cu in DTPA and HNO_3 extract.

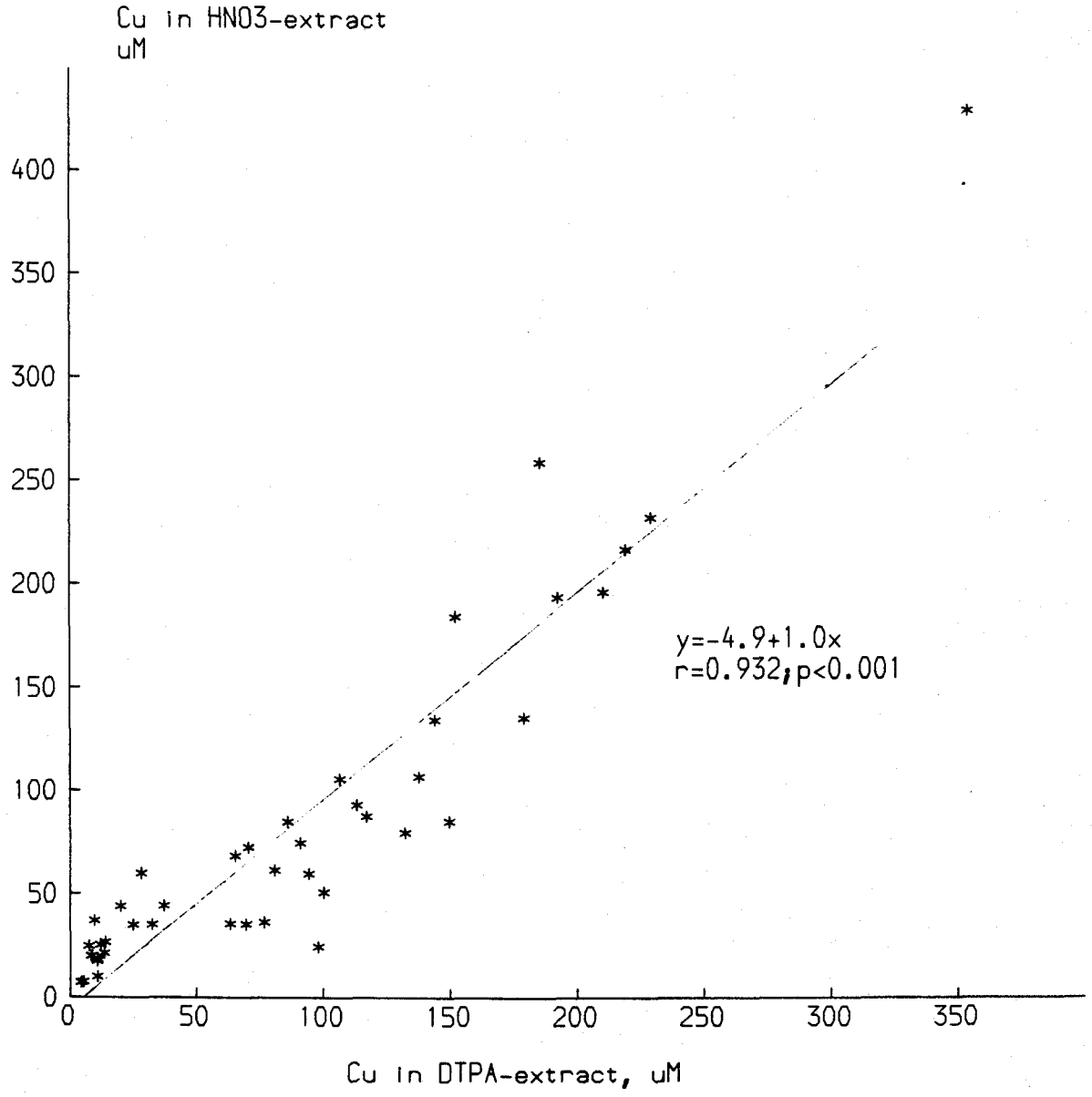


Figure 5. Relation between Cu in H_2O extract and Cu in young leaves.

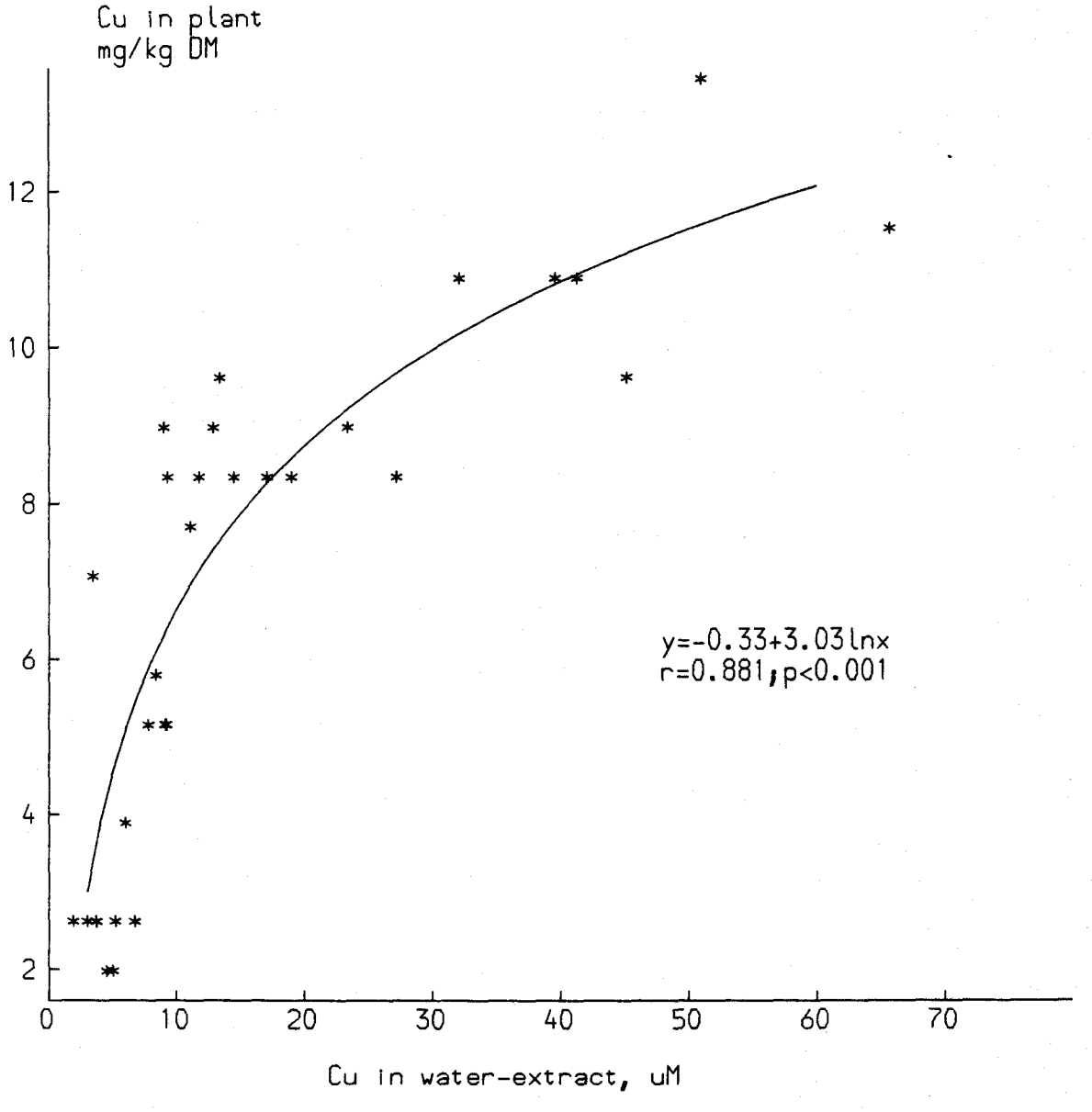


Figure 6. Relation between Cu in DTPA extract and Cu in young leaves.

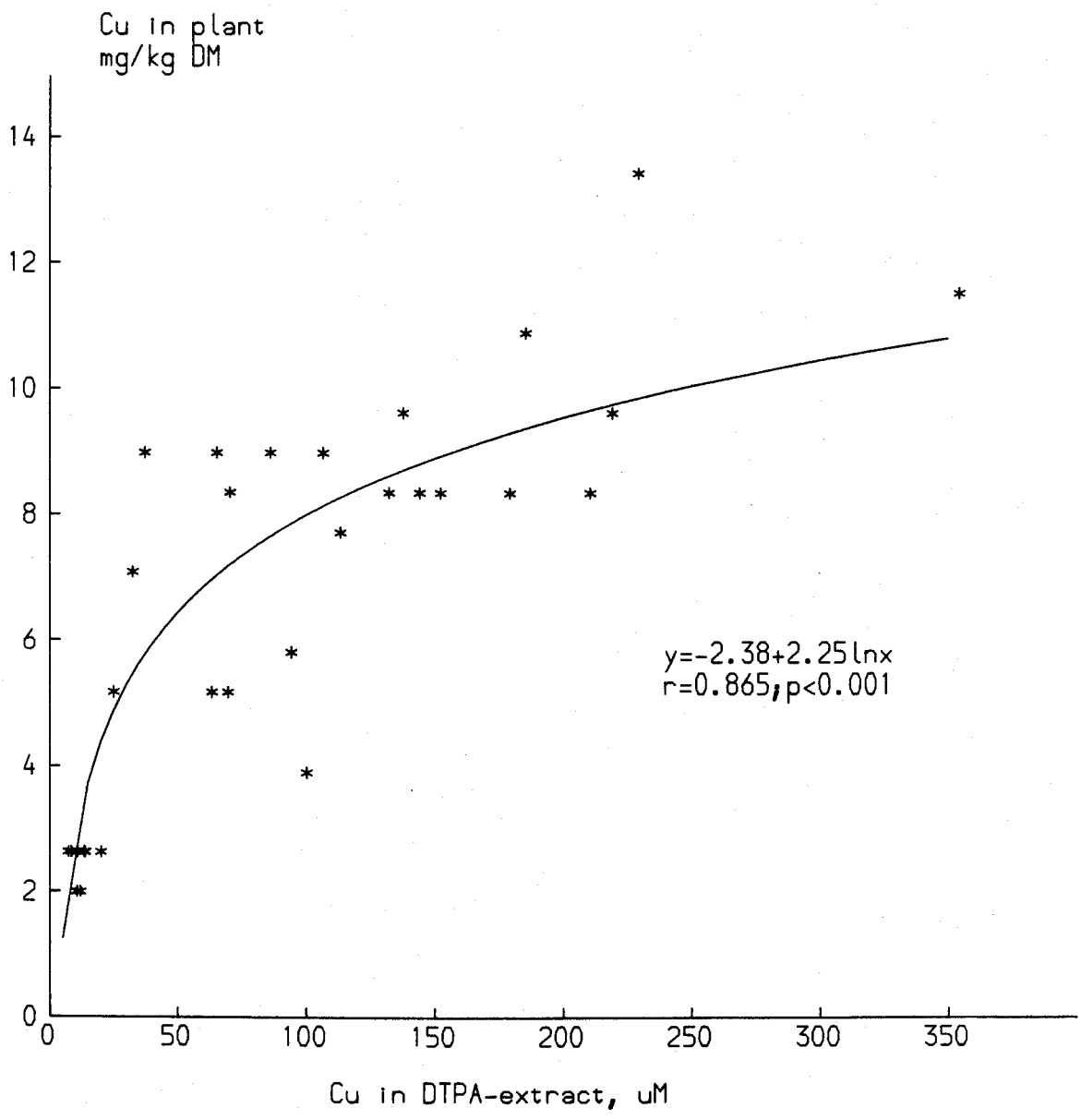
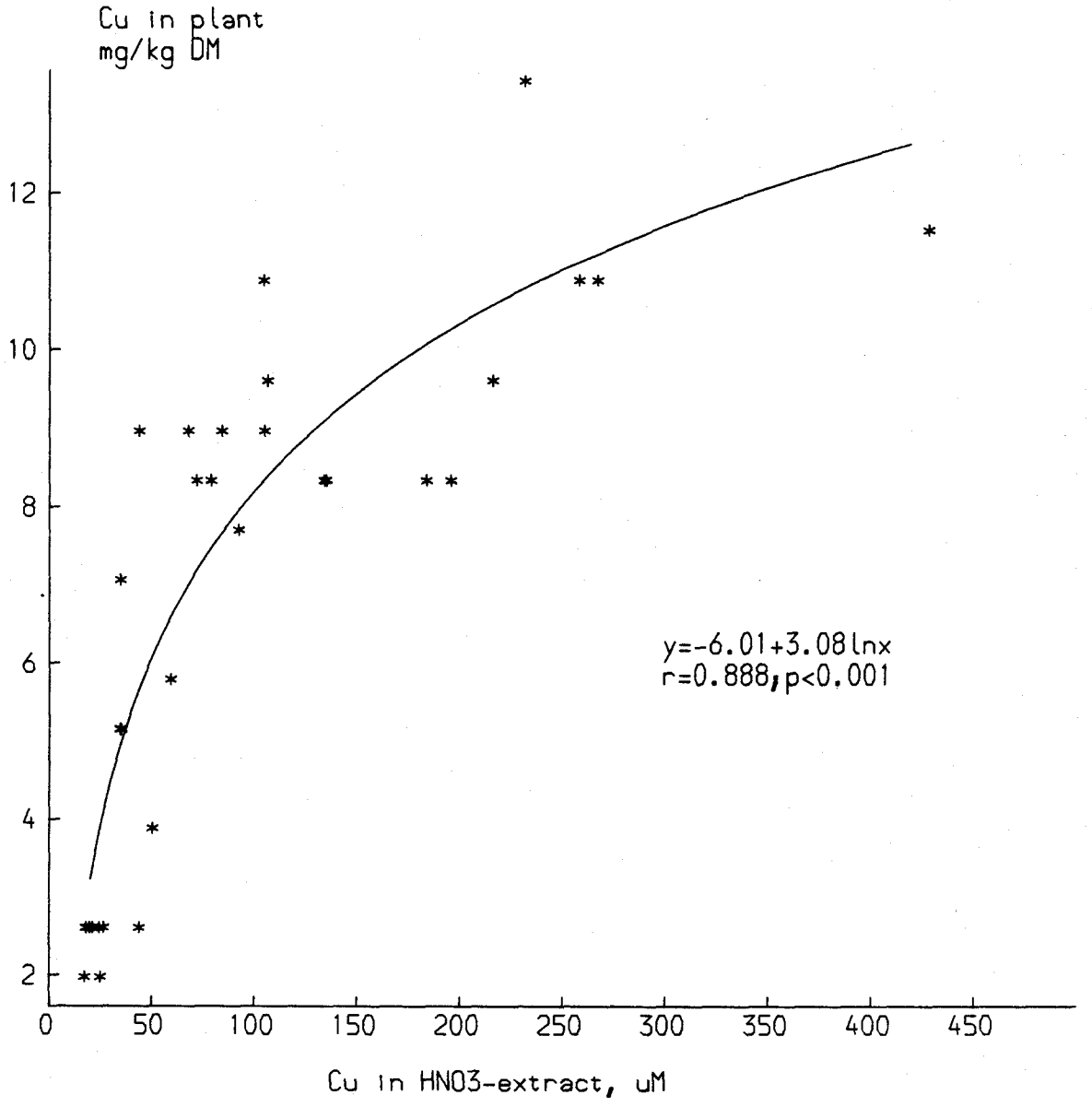


Figure 7. Relation between Cu in HNO_3 extract and Cu in young leaves.



Appendix 1. Mn-, Fe-, Zn and B-content in extracts, average of four Cu-levels.

Time	Substrate	pH level	Mn, uM			Fe uM			Zn, uM			B, uM	
			water	DTPA	HNO_3	water	DTPA	HNO_3	water	DTPA	HNO_3	water	HNO_3
Begin	A	low	15.2	100	109	31.7	457	462	5.0	45	47	44	100
		high	8.8	91	101	16.8	361	491	10.1	42	49	20	80
	B	low	9.8	86	97	21.4	1082	873	2.0	55	53	20	74
		high	4.3	91	91	15.1	948	913	4.9	50	52	13	63
Halfway	A	low	1.9	71	74	56.5	358	419	33.6	121	131	15	49
		high	2.3	86	91	21.9	350	479	64.2	130	131	13	56
	B	low	1.5	81	83	98.2	883	870	26.9	172	160	12	50
		high	1.5	94	95	64.0	744	942	55.0	154	164	11	49
End	A	low	3.5	66	68	91.8	441	460	72.0	151	138	13	56
		high	7.5	82	89	121.4	446	518	141.1	173	168	17	58
	B	low	2.4	73	78	152.0	997	822	42.9	196	176	11	45
		high	3.9	82	81	115.2	785	842	92.3	188	174	14	52

Appendix 2. Cu-content in H_2O-, DTPA- and HNO_3 extracts; t1 = begin, t2 = halfway through, t3 = end of experiment
* duplicate vary too much or value was strongly different.

soil	pH	Cu	Cu in water, uM			Cu in DTPA, uM			Cu in HNO3, uM		
			t1	t2	t3	t1	t2	t3	t1	t2	t3
A	low	1	0.8	2.6	4.1	3.2	5.3	9.9	4.9	22.0	22.5
		2	1.3	*	8.6	26.3	63.5	35.3	57.3	65.6	41.6
		3	4.2	8.9	26.8	115.3	68.6	177.4	84.7	69.7	132.9
		4	5.7	39.2	50.6	190.7	183.6	227.5	191.0	256.1	229.5
	high	1	0.5	1.5	3.3	2.3	6.4	18.0	4.7	17.3	41.4
		2	2.8	3.1	23.0	*	30.6	84.3	27.1	32.4	82.0
		3	5.0	12.5	40.9	79.0	104.7	*	58.6	102.9	102.4
		4	8.9	16.7	65.3	*	150.4	352.4	169.0	181.6	426.2
B	low	1	0.9	4.8	4.6	6.2	11.4	8.6	17.2	18.6	14.9
		2	0.9	7.4	8.7	7.7	61.5	67.8	34.3	32.8	32.6
		3	0.8	11.4	14.1	*	142.3	130.4	37.8	131.8	76.8
		4	2.0	13.0	31.7	148.0	135.8	*	82.4	104.1	265.0
	high	1	0.5	3.3	6.3	8.7	8.8	11.8	7.4	15.6	24.0
		2	1.7	5.6	8.8	75.0	98.3	23.1	33.5	48.0	32.3
		3	2.3	8.0	10.7	89.1	92.3	111.5	71.8	56.9	90.2
		4	1.5	18.6	44.8	96.1	208.6	217.3	21.8	193.5	214.0