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ACTIVITY OF DEHYDROGENASES, CATALASE AND UREASE IN COPPER POLLUTED SOIL*

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Abstract

Copper is a life essential element. However, in excess it can be destructive to metabolism of microbial, plant, animal and human cells. Thus, an understanding of all conditions associated with the effect produced by copper on natural environment is vital.

The purpose of the present study has been to evaluate the effect of soil contamination with copper on the activity of dehydrogenases, catalase and urease as well as to determine the tolerance of these enzymes to excessive amounts of copper in soil.

The variable factors of the experiment consisted of:

1) soil type: loamy sand and sandy loam;

2) copper pollution rate in mg kg^{-1} d.m. of soil: 0, 150, 450;

3) soil use: unseeded and seeded soil;

4) crop species: barley, spring oilseed rape and yellow lupine;

5) dates of enzymatic analyses: 25 and 50 day.

The results have revealed that copper pollution, within the rates of 150 to 450 mg kg^{-1} d.m. of soil, significantly inhibits the activity of dehydrogeanses, urease and catalase, with catalase being the most tolerant to excessive copper, unlike dehydrogeneases, which were the most sensitive enzymes. Urease was found to be intermediate in the response to copper. Dehydrogenases, urease and catalase are the least tolerant to the inhibitory effect of copper in soil under spring oilseed rape, being the most tolerant to the pollution in soil under oats. Copper produces stronger inhibitory effect on soil enzymes in unseeded than in seeded soil. The negative effect of excess copper in soil persists and, instead of diminishing, the longer copper remains in soil, the stronger effect it yields. Dehydrognases and catalase are less tolerant to copper in sandy loam than in loamy sand, unlike urease, which was more tolerant to the pollutant in loamy sand than in sandy loam. Tolerance of plants to soil contamination with copper is a species-specific trait. Among the tested crops, yellow

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lupine was the least tolerant whereas spring oilseed rape was the most tolerant to copper contamination.

Key words: copper, enzymatic activity, tolerance index, vulnerability index, soil contamination with copper.

AKTYWNOŚĆ DEHYDROGENAZ, KATALAZY I UREAZY W GLEBACH ZANIECZYSZCZONYCH MIEDZIĄ

Abstrakt

Miedź jest pierwiastkiem niezbędnym do prawidłowego funkcjonowania wszystkich organizmów, jednakże jej nadmiar w środowisku może działać destrukcyjnie na metabolizm komórek drobnoustrojów, roślin i zwierząt oraz ludzi. Zatem poznanie wszystkich uwarunkowań oddziaływania miedzi na środowisko przyrodnicze jest ze wszech miar uzasadnione.

Celem badań było określenie wpływu zanieczyszczenia gleby miedzią na aktywność dehydrogenaz, katalazy i ureazy oraz określenie oporności tych enzymów na nadmiar miedzi w glebie.

W doświadczeniu czynnikami zmiennymi były:

1) gatunek gleby: piasek gliniasty i glina piaszczysta;

2) stopień zanieczyszczenia miedzią w $mg \cdot kg^{-1}$ s.m. gleby: 0, 150 , 450;

3) sposób użytkowania gleby: gleba nieobsiana i obsiana roślinami;

4) gatunek uprawianej rośliny: owies, rzepak jary i łubin żółty;

5) termin analiz enzymatycznych: 25. dzień i 50. dzień.

W wyniku badań stwierdzono, że zanieczyszczenie miedzią, w zakresie od 150 mg do 450 $mg \cdot kg^{-1}$ gleby, hamuje istotnie aktywność dehydrogenaz, ureazy i katalazy. Przy czym najbardziej odporna na nadmiar miedzi jest katalaza, a najmniej dehydrogenazy. Pośrednie miejsce zajmuje ureaza. Dehydrogenazy, ureaza i katalaza są najbardziej oporne na inhibicyjne działanie miedzi w glebie pod uprawą rzepaku jarego, a najmniej pod uprawą owsa. Miedź silniej hamuje aktywność enzymów w glebie nieobsianej roślinami niż w glebie obsianej. Negatywne działanie nadmiaru tego pierwiastka w glebie ma charakter trwały i zamiast ustępować nasila się wraz z czasem jego zalegania w glebie. Dehydrogenazy i katalaza są bardziej oporne na działanie miedzi w glinie piaszczystej niż w piasku gliniastym, a ureaza odwrotnie – bardziej oporna w piasku gliniastym niż w glinie piaszczystej. Wrażliwość roślin na zanieczyszczenie miedzią jest cechą gatunkową. Spośród badanych roślin najbardziej wrażliwy jest łubin żółty, a najmniej rzepak jary.

Słowa kluczowe: miedź, aktywność enzymów, indeks oporności, indeks wrażliwości, zanieczyszczenie gleby miedzią.

INTRODUCTION

One of the side effects of civilization progress is excessive accumulation of toxic substances in soil environment, including such chemicals as heavy metals, which are among the most dangerous causes of degradation of natural environment. Accumulation of toxic compounds in soil is ecologically hazardous because of the risk that their remobilization may be delayed

(De Brouwere et al. 2007, Mertens et al. 2007, Oliveira, Pampulha 2006). Heavy metals cause disorders in soil metabolism (Wyszkowska et al. 2008). They depress soil fertility and activity of soil enzymes (Renella et al. 2005, Mikanova et al. 2001). They can also affect negatively the growth and development of plants (Shumaker, Begonia 2005).

Copper is classified as one of the most hazardous heavy metals, although it poses risk only when its quantities exceed natural background. It is so because copper is also a micronutrient, without which no living organism could function. On the other hand, its excess in natural environment may cause malfunctions of ecosystems (Wyszkowska et al. 2005, Wyszkowska et al. 2005a). Thus, an understanding of all conditions involved in the effects produced by copper on natural environment is important, both for expanding our knowledge and for practical purposes. Regarding soils, measurements of soil enzymatic activity is a good index of soil condition (Bielińska 2005).

The aim of the present study has been to determine the effect of soil pollution with copper on the activity of dehydrogenases, catalase and urease as well as to establish the tolerance of these enzymes on excess copper in soil. The study has been performed as part of own research project No N N305 2258 33, financed by the Ministry for Science and Higher Education.

MATERIALS AND METHODS

The experiments were conducted in a greenhouse, in polyethylene pots, with five replications. The trials were set up on soil material collected from the arable humus horizon of proper brown soils. The soils belonged to loamy sand (pH_{KCl} – 6.7, content of (in g kg^{-1}) C_{org} – 11.0, N_{og} – 0.97) and sandy loam (pH_{KCl} – 7.1, content of (in g kg^{-1}) C_{org} – 12.7, N_{og} – 1.16). The granulometric composition of the soils is presented in Table 1.

Table 1

Granulometric composition of soil

Type of soil	Percentage of fractions (d)		
	sand 2.00 ≥ d > 0.05 mm	dust 0.05 ≥ d > 0.002 mm	clay d ≤ 0.002 mm
Loamy sand	75.56	22.92	1.52
Sandy loam	47.92	48.71	3.37

The following were the variable factors of the experiments:

1) soil type: loamy sand and sandy loam;

2) soil pollution with $CuCl_2 \cdot 2H_2O$ in mg Cu kg^{-1} d.m. of soil : 0, 150, 450;

3) soil use: unseeded and seeded soil;

4) crop species: barley, spring oilseed rape and yellow lupine;

5) dates of enzymatic analyses: 25 and 50 day.

Prior to placing soil in pots (3 kg per pot), it was mixed in a polyethylene container with macronutrients and, in some objects, with copper chloride. Once in pots, the soil moisture was brought to the level of 60% capillary water capacity and in some pots, following the variable factors listed as points 3 and 4, crops were sown: cv. Kasztan oats, cv. Huzar spring oilseed rape and cv. Mister yellow lupine. After emergence, the plants were thinned and left in the pots for the following number of days: 12 for oats, 8 for rape and 5 for yellow lupine.

All the objects received identical fertilization in mg kg^{-1} soil: N × 100 (yellow lupine was not fertilized with nitrogen), P – 35, K – 100, Mg – 20. Nitrogen was applied as $CO(NH_2)_2$, phosphorus as KH_2PO_4, potassium as KH_2PO_4 + KCl and magnesium as $MgSO_4 \cdot 7H_2O$.

The plants were harvested at the flowering stage. Their yields were determined as well as the index of sensitivity to copper contamination using the formula:

$$I_K = 1 - \frac{Y_c}{Y_{nc}}$$

where:

Y_c – is the yield of crops growing on contaminated soil,
Y_{nc} – the yield of crops growing on uncontaminated soil,
I_K – assumes values from +1 to -1,
+1 – 100% inhibition of development,
-1 – 100% stimulation of development.

On two occasions during the experiment (on days 25 and 50), soil samples from each replication in three consecutive replications were taken to determine the activity of soil enzymes: dehydrogenases 9 (EC 1.1), urease (EC 3.5.1.5) and catalase (EC 1.11.1.6). Dehydrogenases were determined with the method suggested by Öhlinger (1996), whereas urease and catalase were determined according to the procedures described by Alef & Nannpieri (1998). Additionally, resistance of enzymes to soil contamination with copper was calculated as suggested by Orwin & Wardle (2004).

The results of the experiments were processed statistically using Duncan's multiple range test. The tables show results of interaction between the following factors: crop species and soil contamination with copper, soil use and copper pollution, type of soil and copper pollution, date of analysis and soil pollution with copper. The statistical analysis was run using the software Statistica (StatSoft, Inc....2006).

RESULTS AND DISCUSSION

The activity of enzymes in soil depended on a crop species and degree of soil contamination with copper (Table 2). The crop species produced significant influence on the activity of dehydrogenases and urease in uncontaminated soils, but had no effect on the activity of catalase. In uncontaminated soil, the highest activity of dehydrogenases was observed under oats and the lowest one – under spring oilseed rape. In turn, the highest activity of urease was determined in soil under yellow lupine and the lowest one – under spring oilseed rape. The response of soil enzymes to copper pollution was evidently negative, with excess copper inhibiting most strongly the activity of dehydrogenases, followed by urease and, most weakly, catalase. The inhibition of the activity of soil enzymes was more severe as the degree of soil pollution with this metal went up. However, all the soil enzymes

Table 2

Effect of soil pollution with copper and crop species on activity of soil enzymes

Cu dose mg kg^{-1} of soil	Crop species		
	oats	spring oilseed rape	yellow lupine
Dehydrogenases , mmol TFF · kg d.m. of soil · h^{-1}			
0	19.121 ± 0.705	12.397 ± 0.547	12.657 ± 0.824
150	3.757 ± 0.045	4.387 ± 0.308	2.996 ± 0.110
450	0.650 ± 0.155	1.207 ± 0.555	1.134 ± 0.237
Average	7.842	5.997	5.596
LSD	a – 0.161; b – 0.161; $a \cdot b$ – 0.282		
Catalase, mol O_2 · kg^{-1} d.m. of soil · h^{-1}			
0	0.157 ± 0.004	0.153 ± 0.002	0.154 ± 0.005
150	0.127 ± 0.003	0.151 ± 0.004	0.140 ± 0.003
450	0.080 ± 0.003	0.126 ± 0.003	0.125 ± 0.005
Average	0.121	0.143	0.140
LSD	a – 0.002; b – 0.002; $a \cdot b$ – 0.003		
Urease, mmol $N\text{-}NH_4$ · kg^{-1} d.m. of soil · h^{-1}			
0	1.975 ± 0.021	1.759 ± 0.020	2.157 ± 0.057
150	0.696 ± 0.034	0.806 ± 0.017	0.909 ± 0.010
450	0.301 ± 0.014	0.693 ± 0.060	0.590 ± 0.013
Average	0.990	1.086	1.219
LSD	a – 0.020; b – 0.020; a · b – 0.034		

LSD for: a – copper rate, b – crop species

examined proved to be more tolerant to the inhibitory influence of copper in soil under spring oilseed rape. Their tolerance was the weakest in soil under oats (Table 3). Yellow lupine cultivation acted intermediately compared to rape an oats.

Table 3

Index of resistance of enzymes to soil pollution with copper depending on crop species*

Cu dose mg kg^{-1} of soil	Crop species		
	oats	spring oilseed rape	yellow lupine
Dehydrogenases			
150	0.115 *c*	0.244 *a*	0.146 *b*
450	0.018 *f*	0.055 *d*	0.049 *e*
Average	0.066 *z*	0.149 *x*	0.098 *y*
Catalase			
150	0.672 *d*	0.899 *a*	0.722 *b*
450	0.372 *f*	0.685 *c*	0.666 *e*
Average	0.522 *z*	0.792 *x*	0.694 *y*
Urease			
150	0.307 *c*	0.415 *a*	0.348 *b*
450	0.073 f	0.318 *d*	0.226 *e*
Average	0.190 *z*	0.367 *x*	0.287 *y*

* homogenous group designated with the same letter

Leaving aside crop species, the activity of dehydrogenases was higher in cropped than in unseeded soil, regardless the degree of soil contamination with copper. In contrast, the activity of catalase and urease was higher in unseeded soil (Tabela 4). In unseeded soil, the enzymes were more tolerant to the inhibitory effect of copper (Tabela 5). The average resistance index was 0.250 for dehydrogenases, 0.697 for catalase and 0.457 for urease. In the cropped soil, the respective indices reached: 0.104, 0.669 and 0.281.

Aside the crop species, land use or the degree of copper pollution, the activity of soil enzymes was influenced by the type of soil (Tabela 6). Irrespective of the degree of copper contamination, it was found that dehydrogenases, catalase and urease were more active in sandy loam than in loamy sand. However, the resistance of the enzymes to the inhibitory effect of copper in the two types of soil was not always so unambiguous. Higher values of resistance index of dehydrogenases, by an average of 0.14, and catalase, 0.03 higher on average, were observed in sandy loam than in loamy sand (Tabela 7). On the other hand, for urease, the average resistance index was by 0.225 higher in loamy sand than in sandy loam.

Table 4

Effect of soil pollution with copper and land use on activity of soil eznymes

Cu dose mg kg^{-1} of soil	Land use	
	oats	yellow lupine
Dehydrogenases, mmol TFF · kg d.m. of soil · h^{-1}		
0	7.665 ± 0.220	14.725 ± 0.692
150	2.702 ± 0.153	3.713 ± 0.154
450	0.801 ± 0.103	0.997 ± 0.116
Average	3.723	6.478
LSD	*a* – 0.161; *b* – 0.131; a · *b* – 0.227	
Catalase, mol O_2 · kg^{-1} d.m. of soil · h^{-1}		
0	0.170 ± 0.003	0.153 ± 0.004
150	0.152 ± 0.003	0.139 ± 0.003
450	0.123 ± 0.005	0.111 ± 0.004
Average	0.148	0.134
LSD	*a* – 0.003; *b* – 0.02 ; *a* · *b* – 0.004	
Urease, mmol N-NH_4 · kg^{-1} d.m. of soil · h^{-1}		
0	2.242 ± 0.027	1.964 ± 0.032
150	1.157 ± 0.024	0.804 ± 0.020
450	0.637 ± 0.017	0.528 ± 0.021
Average	1.345	1.098
LSD	*a* – 0.020; *b* – 0.016; *a* · *b* – 0.028	

LSD for: *a* – copper rate, *b* – land use

The activity of soil enzymes varied throughout the experiment (Tabela 8). In the unpolluted soil, the activity of dehydrogenases and catalase was significantly greater on day 50 than on day 25. For urease, it was opposite – the enzyme was more active on day 25 than on day 50. The inhibitory effect of copper on these enzymes was persistent and increased as the experiment continued (Table 9). The average indices of resistance for dehydrogenases, catalase and urease were higher on day 25 than on day 50 of the trials.

Copper pollution of soil had negative influence not only on the soil enzymes but also on the test plants (Tabela 10). The crops were significantly more sensitive to higher soil pollution rates and this was a species-specific trait. Yellow lupine proved to be the most sensitive to copper pollution, unlike spring oilseed rape, which was the most tolerant species, especially when it was grown on more compact soil, i.e. on sandy loam. Oats proved to possess intermediate resistance to copper pollution, irrespective of the type of soil on which it grew.

Table 5

Index of resistance of enzymes to soil pollution with copper depending on land use*

Cu dose mg kg^{-1} of soil	Land use	
	unseeded soil	seeded soil
Dehydrogenases		
150	0.238 *b*	0.168 *c*
450	0.263 *a*	0.040 *d*
Average	0.250 *x*	0.104 *y*
Catalase		
150	0.819 *a*	0.764 *b*
450	0.576 *c*	0.574 *c*
Average	0.697 *x*	0.669 *y*
Urease		
150	0.546 *a*	0.357 *c*
450	0.369 *b*	0.206 *d*
Average	0.457 *x*	0.281 *y*

* homogenous group designated with the same letter

Table 6

Effect of soil pollution with copper and soil type on soil enzymatic activityes

Cu dose mg kg^{-1} of soil	Type of soil	
	loamy sand	sandy loam
Dehydrogenases, mmol TFF· kg d.m. of soil· h^{-1}		
0	12.789 ± 0.537	13.130 ± 0.610
150	2.645 ± 0.255	4.275 ± 0.152
450	0.290 ± 0.079	1.606 ± 0.048
Average	5.242	6.337
LSD	a – 0.161; b – 0.131; $a \cdot b$ – 0.227	
Catalase, mol $O_2 \cdot kg^{-1}$ d.m. of soil· h^{-1}		
0	0.115 ± 0.003	0.200 ± 0.004
150	0.103 ± 0.003	0.182 ± 0.003
450	0.080 ± 0.003	0.148 ± 0.005
Average	0.099	0.265
LSD	a – 0.003; b – 0.02 ; $a \cdot b$ – 0.004	
Urease, mmol $N\text{-}NH_4 \cdot kg^{-1}$ d.m. of soil· h^{-1}		
0	1.049 ± 0.028	3.017 ± 0.035
150	0.610 ± 0.018	1.174 ± 0.024
450	0.408 ± 0.012	0.703 ± 0.031
Average	0.689	1.631
LSD	a – 0.020; b – 0.016; $a \cdot b$ 0.028	

LSD for: a – copper rate, b – soil type

Table 7

Index of resistance of enzymes to soil pollution with copper depending on type of soil*

Cu dose mg kg^{-1} of soil	Type of soil	
	loamy sand	sandy loam
Dehydrogenases		
150	0.130 *c*	0.242 *a*
450	0.012 *d*	0.180 *b*
Average	0.071 *y*	0.211 *x*
Catalase		
150	0.773 *b*	0.783 *a*
450	0.549 *d*	0.599 *c*
Average	0.661 *y*	0.691 *x*
Urease		
150	0.539 *a*	0.268 *c*
450	0.336 *b*	0.157 *d*
Average	0.438 *x*	0.213 *y*

* homogenous group designated with the same letter

Table 8

Effect of soil pollution with copper and date of analysis on activity of soil enzymes

Cu dose mg kg^{-1} of soil	Time of analysis, days	
	25	50
Dehydrogenases , mmol TFF· d.m. of soil· h^{-1}		
0	12.133 ± 0.671	13.786 ± 0.477
150	2.950 ± 0.243	3.971 ± 0.165
450	0.978 ± 0.045	0.918 ± 0.039
Average	5.354	6.225
LSD	*a* – 0.161; *b* – 0.131; *a* · *b* – 0.227	
Catalase, mol O_2 · kg^{-1} d.m. of soil · h^{-1}		
0	0.154 ± 0.004	0.160 ± 0.004
150	0.150 ± 0.003	0.135 ± 0.003
450	0.122 ± 0.004	0.106 ± 0.004
Average	0.142	0.134
LSD	*a* – 0.003; *b* – 0.02 ; *a* · *b* – 0.004	
Urease, mmol $N\text{-}NH_4$ · kg^{-1} d.m. of soil · h^{-1}		
0	2.087 ± 0.023	1.979 ± 0.040
150	1.054 ± 0.020	0.729 ± 0.022
450	0.609 ± 0.013	0.502 ± 0.030
Average	1.250	1.070
LSD	*a* – 0.020; *b* – 0.016; *a* · *b* 0.028	

LSD for: *a* – copper rate, *b* – date of analysis

Table 9

Index of resistance of enzymes to copper pollution depending on the date of analysis*

Cu dose mg kg^{-1} of soil	Time of analysis, days	
	25	50
Dehydrogenases		
150	0.153 *b*	0.205 *a*
450	0.146 *c*	0.046 *d*
Average	0.149 *x*	0.125 *y*
Catalase		
150	0.852 *a*	0.704 *b*
450	0.652 *c*	0.496 *d*
Average	0.752 *x*	0.600 *y*
Urease		
150	0.486 *a*	0.322 *b*
450	0.289 c	0.204 *d*
Average	0.388 *x*	0.263 *y*

* homogenous group designated with the same letter

Whereas the inhibitory influence of soil contamination with copper was to be expected even before the experiment, it seemed more important to prove that species of crops could also modify the resistance of soil enzymes to the pollutant. The fact that spring oilseed rape and yellow lupine, unlike oats, could alleviate the negative effect of copper on soil enzymes may be linked to a more favourable effect produced by these two crops, in contrast to cereal plants, on physical and chemical properties of soil (KARLEN et al. 2003). It is interesting to find out that enzymes of the same class, i.e. dehydrogenases and catalase, respond rather differently to copper pollution of soil. Catalase is less sensitive to this pollutant than dehydrogenases, which may be connected with the specificity of dehydrogenases.

It is not quite clear why enzymes in unseeded soil were more resistant to copper pollution than those in cropped soil. What makes it even more difficult to clarify is that root secretions would typically have positive influence on soil microorganisms (DIJKSTRA et al. 2006), which is directly connected with the activity of soil enzymes.

The enzymes did not respond unambiguously to copper pollution in the test soils. It could be expected that enzymes would be more tolerant to excess copper in sandy loam, a more buffered soil, rather than in loamy sand (KARLEN et al. 2003, SCHOENHOLTZ et al. 2000, WYSZKOWSKA et al. 2005). And this dependence *did* occur in the case of dehydrogenases and catalase,

Table 10

Index of sensitivity of crops to soil pollution with copper*

Cu dose mg kg^{-1} of soil	Type of soil					
	loamy sand			loamy sand		
	crop species					
	oats	spring oilseed rape	yellow lupine	oats	spring oilseed rape	yellow lupine
150	0.104 *c*	0.019 *b*	0.275 *e*	0.015 *b*	-0.171 *a*	0.515 *f*
450	0.578 *g*	0.608 *h*	0.776 *j*	0.653 *i*	0.239 *d*	0.890 *k*
Average	0.341 *n*	0.314 *p*	0.525 *m*	0.334 *o*	0.034 *r*	0.702 *l*

* homogenous group designated with the same letter

whereas urease behaved differently. It was more resistant to copper contamination in loamy sand.

The negative effect of copper on the activity of the tested soil enzymes, like that produced by other heavy metals (WYSZKOWSKA et al. 2008, WYSZKOWSKA et al. 2005a), persisted and did not decrease as the experiment progressed. Contrary to that, the negative influence of copper grew stronger. Such an outcome is due to the character of the experiment (pot trials), when nutrients do not migrate outside the reach of the root system.

The most important test verifying the state of soil environment is a plant test. It is only partly correlated with the activity of soil enzymes. Resistance of dehydrogenases, catalase and urease to copper was higher in soil cropped with spring oilseed rape, the crop which likewise proved to be the least sensitive to copper, with the rape plants growing on a more compact soil, i.e. sandy loam, showing minimum sensitivity to this soil pollutant. No such correlation was found between resistance of soil enzymes in soil under yellow lupine versus the sensitivity of this crop to excess copper.

CONCLUSIONS

1. Copper contamination of soil from 150 to 450 mg·kg^{-1} significantly inhibit the activity of dehydrogenases, urease and catalase. Catalase is the most tolerant to excess copper, in contrast to dehydrogenases, which are the most sensitive soil enzymes. Urease is intermediately resistant to copper.

2. Dehydrogenases, urease and catalase are the least resistant to the inhibitory effect of copper in soil under spring oilseed rape. In turn, they are the most sensitive in soil cropped with oats.

3. Copper inhibited the activity of the enzymes more strongly in unseeded soil than in cropped soil. The negative effect of excessive quantities of this metal in soil is persistent and instead of disappearing gradually, it intensifies the longer the pollutant remains in soil.

4. Dehydrogenases and catalase are the more resistant to the effect of copper in sandy loam than in loamy sand, in contrast to urease, which is more tolerant to copper in loamy sand than in sandy loam.

5. Tolerance of crops to copper contamination in soil is a species-specific trait. Among the three tested crops, yellow lupine was the most sensitive whereas spring oilseed rape was the most tolerant to soil pollution with copper.

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