Journal of Plant Nutrition, 29: 2179–2198, 2006

ISSN: 0190-4167 print / 1532-4087 online
DOI: 10.1080/01904160600972845

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Effect of Excess Copper on Tomato Plants: Growth Parameters, Enzyme Activities, Chlorophyll, and Mineral Content

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ABSTRACT

In this work, the effect of excess copper (Cu) on tomato plants grown in hydroponic solutions for up to 15 days was studied. The solutions contained different Cu concentrations ranging from 0.05, 0.15, 0.20, and 0.35 mM Cu. Dry mass, root length, and foliar area decreased with time and Cu solution concentration. Copper accumulated more heavily in roots than in leaves. Mineral uptake was affected by increasing Cu concentrations in solution with calcium (Ca), iron (Fe), and zinc (Zn) contents in leaves decreasing. Except for Ca, the concentrations of these elements in the roots also decreased, which indicated that root uptake was affected and the translocation to upper plant parts was disrupted. Iron leaf deficiency was the probable cause of the observed chlorosis and decrease in chlorophyll levels. The activities of three enzymes studied in leaves (guaiacol peroxidases (GPOD), catalase (CAT), and polyphenol oxidase (PPO)) increased transiently, probably as an early response mechanism against Cu induced oxidative stress. At higher Cu concentrations, this defense mechanism broke down and the activities of the enzymes decreased accordingly.

Keywords: catalase, copper, *Lycopersicon esculentum*, mineral content, peroxidase, phytotoxicity, polyphenol oxidase, tomato

Received 9 June 2005; accepted 15 March 2006.

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INTRODUCTION

Copper (Cu) is a plant micronutrient and an important component of several enzymes and coenzymes involved in metabolic pathways of plants. However, at high concentrations, Cu can become phytotoxic affecting plant development due to direct or indirect interference with numerous physiological processes (Maksymiec, 1997; Vangronsveld and Clijsters, 1994). Excess Cu may affect species differently and it can cause various effects depending on the plant growth stage at which the metal was applied, the concentration of Cu, and the duration of action. Copper accumulation in soils can be the result of natural soil properties, agricultural practices, like the use of Cu-containing fertilizers, organic residues, sewage sludges, fungicides and bactericides (Kaplan, 1999), or other anthropogenic activities (mining, waste disposal, etc.) (Rhoads, Olson, and Manning, 1989; Brun, Le Corff, and Maillet, 2003).

Phytotoxic effects of Cu can be observed at tissue concentrations slightly higher than its optimal levels (Mazhoudi et al., 1997) and references have been made of excess Cu in plants affecting changes in growth parameters, mineral and chlorophyll contents, and several enzyme activities (Fernandes and Henriques, 1991; Wallace, Wallace, and Cha, 1992; Mocquot et al., 1996; Maksymiec, 1997; Mazhoudi et al., 1997; Ouzounidou et al., 1998; Chen, Lin, and Kao, 2000; Brun et al., 2003).

Copper can catalyze the formation of harmful free radicals, such as the hydroxyl and superoxide radicals (Weckx and Clijters, 1996; Shaw, Sahu, and Mishra, 2004). Thus, the main effect of phytotoxic amounts of Cu is the induction of oxidative stress, which can cause changes in metabolic pathways as a defense mechanism that results in differential responses of enzymes in plant parts (Mazhoudi et al., 1997; Van Assche and Clijsters, 1990; Clijsters, Cuypers, and Vangronsveld, 1999). Peroxidases have been reported to increase as a response to the oxidative stress induced by excess Cu (Mocquot et al., 1996). Induction of catalases by excess Cu has also been detected (Weckx and Clijsters, 1996).

In this study we analyzed the effect of excess Cu on morphology, development, and metabolic changes on roots and leaves of tomato plants (*Lycopersicon esculentum* Mill. cv. Hynema) as a function of both elevated concentrations of Cu and of time. The following parameters were studied: foliar area, root length, dry weight, mineral content (uptake/accumulation of nutrients), and chlorophyll content. We also analyzed the effect of excess Cu on the enzyme activity in leaves of two enzymes directly involved in the removal of excess H_2O_2 produced as result of plant oxidative stress defense mechanisms, guaiacol peroxidase (GPOD; EC 1.11.1.7) and catalase (CAT; EC 1.11.1.6). A third enzyme, polyphenol oxidase (PPO, EC 1.10.3.1), was studied.

Polyphenol oxidase enzymes are Cu-containing enzymes that catalyze the conversion of catechol (or substituted catechols) to quinones. Polyphenol oxidase enzymes have reportedly been involved in plant defense mechanisms against fungal or bacterial infections, herbivore feeding, mechanical damage

etc. (Stewart et al., 2001; Maksymiec, 1997) and have been used as a measure of Cu nutrition status of tomato plants (Onyezili and Harris, 1993) while there have been also reports that they can have catalase-like activity (Gerdemann et al., 2001).

MATERIALS AND METHODS

Plant Material and Copper Treatment

Tomato seeds (*Lycopersicon esculentum* Mill., cv. Hynema) were germinated at 20°C, in a 5 cm vermiculite layer, soaked with deionized water for 7 days. After germination, the seedlings were grown in half-strength Hoagland's solution (De Rijck and Schrevens, 1998). Two-week-old seedlings were planted on perforated polystyrene plates, floating on an aerated Hoagland nutrient solution, which was renewed every 5 days. The plants were grown for 4 weeks in a growth chamber at 18–24°C and 65 % humidity, with 14/10 h dark/light periods. Subsequently, tomato plants were treated with nutrient solutions containing different Cu concentrations: 0.0001 (control), 0.05, 0.10, 0.15, 0.20, and 0.35 mM, supplied as $CuSO_4$.

Tomato plants were grown under these conditions and samples were harvested for analysis after 2, 6, 10, and 15 days, for each of the Cu treatments. Both leaves and roots of plants were used as samples, and all the determinations were repeated at least 3 times. The early response of the enzyme activities were analyzed only in leaves after 1,2,3,5 and 6 days.

Growth Parameters and Mineral Content

Leaf and root samples were oven dried at 105°C, until they reached a constant weight. At least four plants were analyzed for each Cu treatment, aerial and root parts were analyzed separately.

For mineral composition determination [calcium (Ca), Cu, iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), and zinc (Zn)], samples of dried plant material were ashed at 480°C in a muffle furnace, twice digested in 10 mL of 3 *M* HCl at 90°C, and analyzed by flame atomic absorption spectrophotometry (Unicam Solaar M). Phosphorus (P) was determined by the molybdovanadate colorimetric method (using an Hitachi U-2000 UV/Vis Spectrophotometer).

Foliar area was determined by collecting all leaves from each plant, scanning them, and measuring the dark area using computer software (Delta-T scan 2.03), which gave the results in arbitrary units of area. Root length was obtained by measuring the maximum length of the root of each plant, using four plants for each Cu concentration and time.

Chlorophyll Content

Chlorophyll content was determined by a non-destructive method using the Minolta SPAD-502 apparatus. Results were expressed in arbitrary units (SPAD units) that are proportional to chlorophyll content (Madeira et al., 2000). Determinations were performed in at least four plants for each time and Cu treatment.

Enzyme Assays

A crude extract was obtained by maceration of 0.2 g of leaves in the presence of 2% (w/w) insoluble polyvinylpolypyrollidone (PVP), with 1 mL of 100 mM Tris-HCl buffer (pH 7.8), containing 3 mM dithiothreithol (DTT) and 1 mM ethylenediamine-tetracetic acid (EDTA). The homogenate was centrifuged for 30 min at 10 000 g and filtered through 0.2 micron membranes. All procedures were performed at a temperature below 4°C. Peroxidase activity was determined according to Adams (1978), catalase activity according to Aebi (1983), and polyphenol oxidase activity according to Oktay (1995). Soluble protein content was determined by the Lowry method (Lowry et al., 1951) with bovine serum albumin as standard. All enzyme activities and soluble protein determinations were measured using spectrophotometric methods (Hitachi U-2000 UV/Vis Spectrophotometer).

Statistical Analysis

The statistical analysis was performed using the Statistica software '99 Edition (StatSof, Inc, 1999). The results were subjected to a one-way ANOVA, using the Tukey test to check significant differences between means ($P \leq 0.05$).

RESULTS AND DISCUSSION

Growth Parameters, Root Length, and Foliar Area

Plant growth was visibly reduced both as a function of time and Cu concentration. For example, after 10 days growing in a Cu concentration of 0.150 mM, fresh weights were reduced to 73% of the weights of the plants grown in the control. As can be seen in Figure 1, there was an increase in percentage leaf dry weight with time, which was more evident for the higher Cu concentrations and for the longer times of exposure to excess Cu, compared to the control plants. Results for the roots were very similar. High Cu concentrations may have a

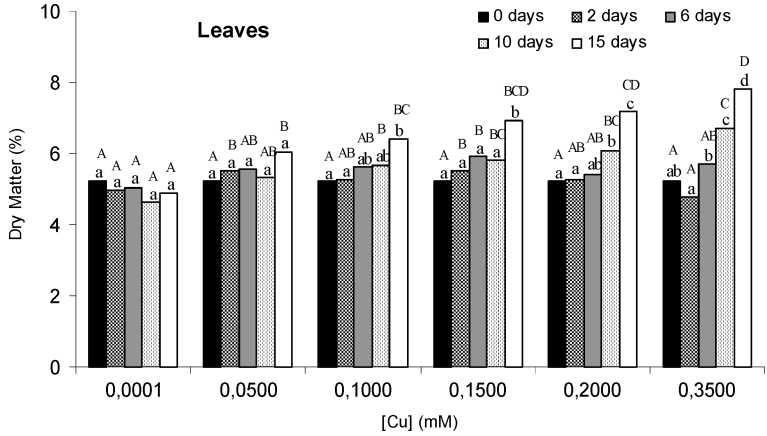

Figure 1. Effect of copper concentration in nutrient solution and time on leaf dry matter percentage. Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

negative effect on plant growth by influencing root development, which may cause a lower absorption of water.

The results obtained for the root length, as a function of time and Cu concentration, are shown in Figure 2. As expected, root length decreased with

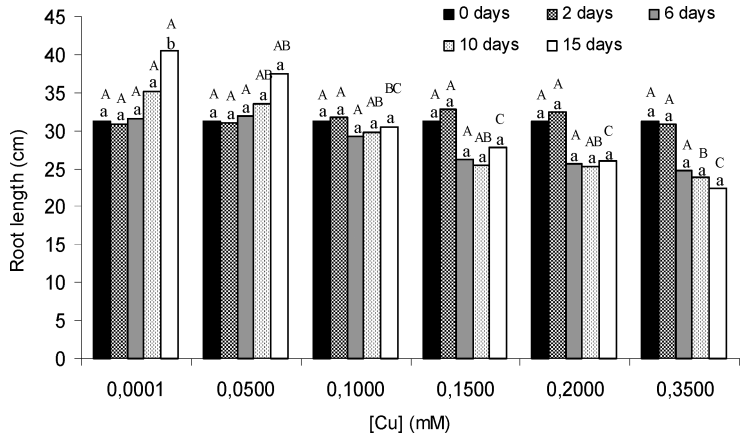

Figure 2. Effect of copper concentration in nutrient solution and time on root length. Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

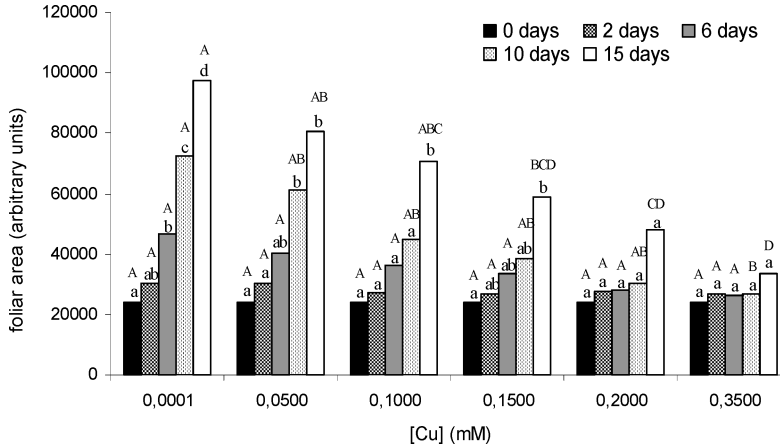

Figure 3. Effect of copper concentration in nutrient solution and time on foliar area. Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

time and with Cu concentration; however, due to the high dispersion of the results there are only significant differences at the highest Cu concentrations and times. It appears that roots stopped growing in Cu concentrations above 0.10 mM, compared to the control. The effect of excess Cu on roots was evident for the highest Cu concentrations, the roots became dark and dehydrated, which is in agreement with the other plant changes detected, such as dry matter.

Figure 3 shows the variation of the foliar area (expressed in arbitrary area units), as a function of time and Cu concentration. As expected, the foliar area shows a general increase with time for each Cu concentration but a significant decrease with Cu concentration after 10 and 15 days. Although there is an apparent decrease in foliar area after 2 days, the differences are only significantly different after 10 days. Cuypers et al. (2000) studied the effect of excess Cu on *Phaseolus vulgaris* and found that the leaf area was significantly reduced but the effect on shoot growth was less pronounced.

As can be seen in these three figures, excess Cu in nutrient solutions affects plant growth, even at the lowest Cu concentration, with the effect becoming more pronounced with time and with increases in Cu levels. After 2 days, we found that the first growth factor affected by Cu is root fresh mass, as it decreased more pronouncedly than leaf fresh mass, down to 63% of the control value for the highest Cu concentration compared to a decrease to 70% of the control value for leaf fresh weight. This can be attributed to Cu accumulation in the roots, rather than in the shoots, even though a percentage of this Cu can be expected to be found in the root apoplast. Due to the higher dispersion of root

length measurements, in Figure 2, only a general trend of root growth inhibition can be established.

According to Blaya and Garcia (2000) the main symptoms of excess Cu can be detected in roots by a decrease in root growth, along with darkening. It has been reported that in cucumber plants, the preferential Cu effect is elongation inhibition rather than biomass reduction (Alaoui-Sossé et al., 2004). Ouzounidou et al. (1995) also showed that excess Cu in maize roots affected both root growth and ultrastructure. In fact, the primary toxic action of Cu in plants grown on a Cu-contaminated substrate takes place in the roots, by alteration of the cell membrane properties (De Vos et al., 1991), although it also affects the metabolism of leaves (Vangronsveld and Clijsters, 1994).

Mazhoudi et al. (1997) found a decrease in growth, more pronounced in leaves and in stems than in roots of tomato plants. Chen et al. (2000) observed a progressively decreasing root length after increasing concentrations of Cu from 20 to 50 μM, but not in shoot length. Chen et al. (2000) concluded that Cu-induced inhibition in root growth of rice seedlings is likely due to cell wall stiffening, and this can be an explanation to the Cu-excess effect on roots influencing plant growth.

Chlorophyll Content

Figure 4 presents the variation of the SPAD units (as a measure of the chlorophyll content), for several periods of time of exposure to different Cu solution

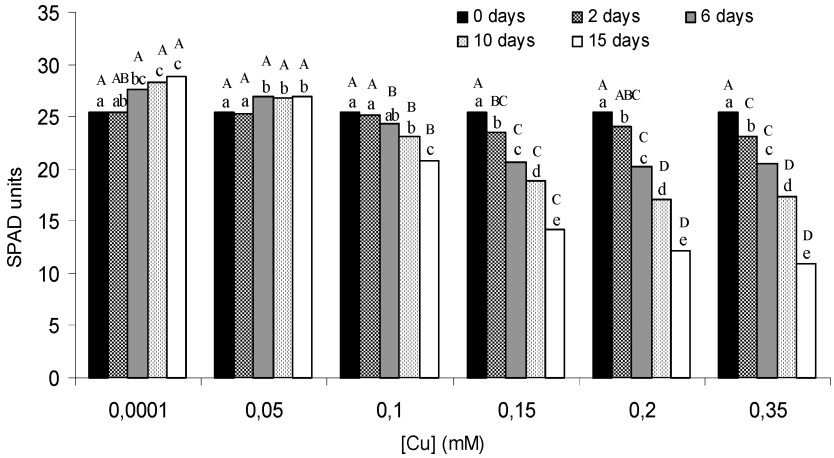

Figure 4. Effect of copper concentration in nutrient solution and time on SPAD units (as a measure of chlorophyll content). Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

concentrations. It was found that, for all the different treatments, SPAD values decreased both with time and with Cu concentration, with the effect being more pronounced at the higher Cu concentrations, showing that chlorophyll content was severely affected by the induced Cu toxicity. There were significant differences in the measured SPAD values after 2 days for the nutrient solutions containing 0.15 mM Cu or higher. In fact, visible symptoms of excess Cu on tomato plants were detected after 2 days at the two highest Cu concentrations, 0.20, and 0.35 mM. After four days, these plants had new leaves smaller than the control, and after 6 days, the apical zone of the plants presented clear chlorosis symptoms, more intense near the leaf midrib and in younger leaves. After 6 days, plants growing on Cu concentration of 0.15 mM also showed some of these visible effects, but they were more evident for the higher Cu concentrations of 0.20 and 0.35 mM.

Chen and Kao (1999) reported that chlorophyll levels decreased with increases of Cu in solution, indicating that excess Cu induces the loss of chlorophyll in detached rice leaves, although the opposite effect has also been reported (Myśliwa-Kurdziel and Strzalka, 2002). We have observed the same results as Chen and Kao (1999), as indicated by our decrease in SPAD units, which were proportional to chlorophyll content. The decreased chlorophyll levels may be a direct consequence of Cu on the photosynthetic system (Macksymiec, 1997); but on the other hand, there was also a decrease in Fe content (see below), both in the roots and in the leaves. This showed that Fe uptake by the roots is inhibited and translocation to the shoots is also affected, consequently affecting chlorophyll formation, probably as a result of Fe deficiency due to competition with Cu. This antagonistic interaction has been proposed by Pätsikkä et al. (2002), as they showed that increasing Fe content in nutrient solutions, to a certain level, decreased the amount of Cu in leaves. According to the same authors, the main effect of excess Cu in leaves is Fe deficiency that leads to reduced chlorophyll concentration, which leads in turn to an increased susceptibility to photoinhibition. Welsh et al. (1993) proposed that the root reductase system, involved in the reduction of Fe(III) and Fe(II) absorption by dicotyledons, might also be involved in Cu absorption, and our results seem to agree with this proposition.

Mineral Composition

The following elements were analyzed, both in leaves and in roots: Na, Ca, Mg, K, P, Mn, Fe, Cu, and Zn. The results for these elements as a function of time and Cu concentration in solutions are presented in Figures 5 to 11 (only results where significant differences occur were shown, as stated in the text). As can be seen in Figures 5 and 6, Cu content increased both in roots and in leaves with time and Cu solution concentration. After 15 days at the highest Cu concentration, the Cu levels in the leaves increased to more than 5.8 times the

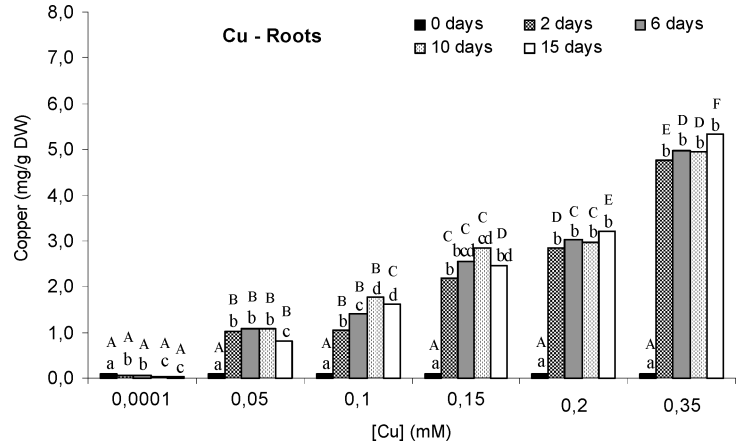

Figure 5. Effect of copper concentration in nutrient solution and time on copper contents in roots. Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

initial control value, while in roots it increased more than 62 times the initial control values. Copper content in leaves and roots increased linearly with Cu concentrations in the nutrient solution. Fitting a straight line through the data yielded correlation coefficients between 0.97 and 0.99.

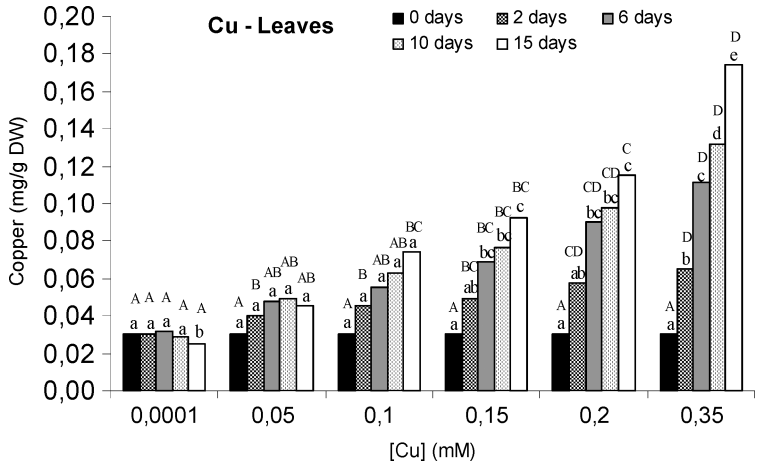

Figure 6. Effect of copper concentration in nutrient solution and time on copper contents in leaves. Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

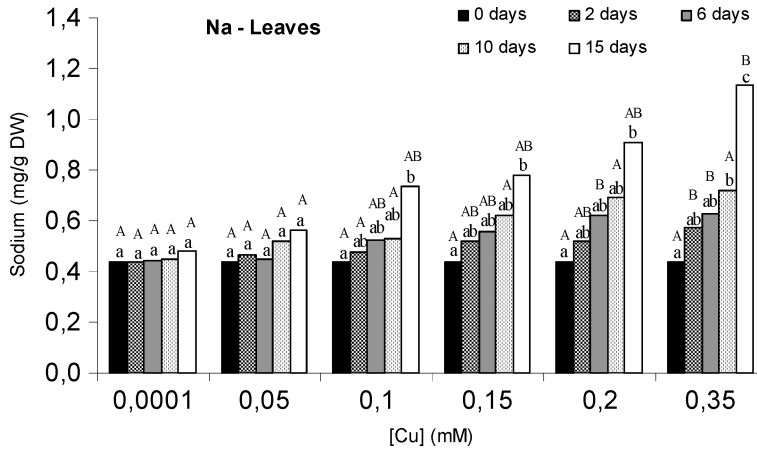

Figure 7. Effect of copper concentration in nutrient solution and time on sodium contents in leaves. Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

As expected, Cu accumulated heavily in roots, and its concentration both in leaves and in roots increased with increasing Cu levels in the nutrient solution. The prevention of translocation of phytotoxic amounts of heavy metals from the roots to the shoots is one mechanism of Cu tolerance that plants may use (Fernandes and Henriques, 1991). However, with the tomato plants growing in relatively heavy concentrations of Cu, although Cu translocation to the leaves may have been affected, it did not eliminate Cu toxicity in the leaves, as can be seen by the affected leaf area and chlorophyll contents. The concentration of Cu in the roots increased more rapidly than that in the leaves. Similar results have also been obtained by Liao et al. (2000) for excess Cu in tomato plants.

The uptake of excessive amounts of Cu also disrupts the regular uptake and flow of other nutrients. Sodium levels in leaves increased (Figure 7) and Ca levels in roots remained constant but decreased in leaves (Figure 8). In roots, both Na and Ca levels remained constant (results not shown). While there was a slight decrease in leaf Ca content, it was only significant for 2 and 10 days. This demonstrated that although the uptake of Ca^{2+} by the roots was not affected, the translocation of Ca to upper plant parts was disrupted. Similar results have been reported for cucumber (Alaoui-Sossé et al., 2004), Lotus purshianus (Lin and Wu, 1994), tomato (Rhoads et al., 1989), and runner bean plants (Maksymiec and Baszyński, 1998) while Mocquot et al. (1996) reported an increase of Ca content in maize leaves. As leaf area showed a correspondent decrease, this could be explained either by direct Cu action over cell walls or from displacing Ca from the cell wall. Ouzounidou et al. (1995) reported a decrease in Ca content

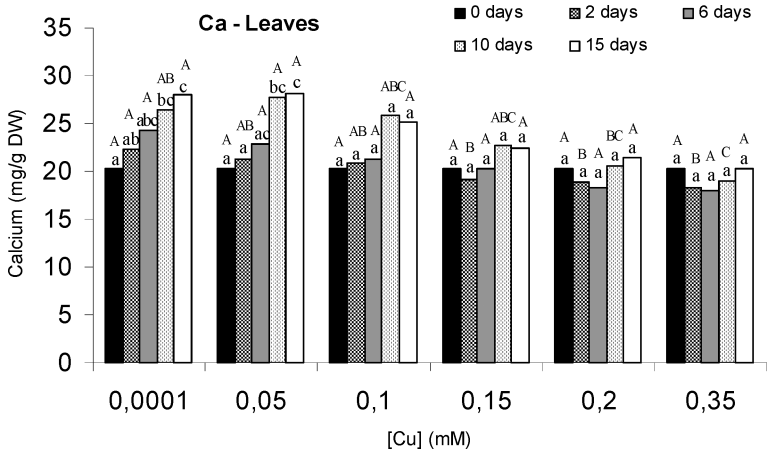

Figure 8. Effect of copper concentration in nutrient solution and time on calcium contents in leaves. Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

of 37 and 76% for leaves and roots, respectively, when *Silene compacta* was subjected to a Cu treatment of 160 μM for 15 days.

While Mg levels in leaves remained constant a significant decrease in root Mg concentrations occurred (Figure 9). Magnesium concentration in the roots

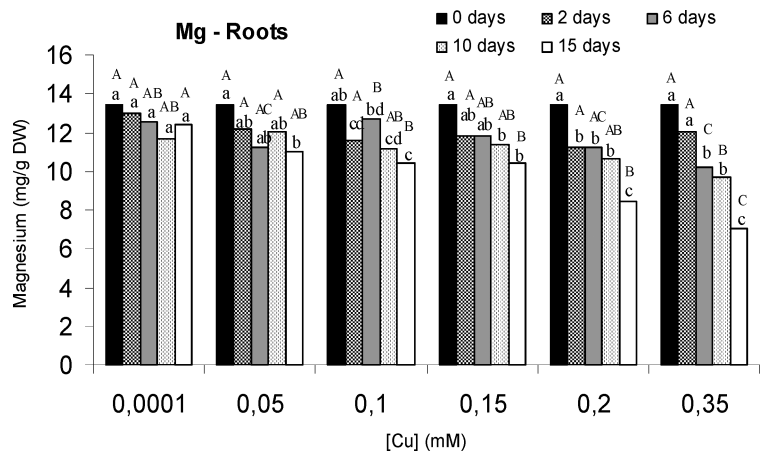

Figure 9. Effect of copper concentration in nutrient solution and time on magnesium contents in roots. Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

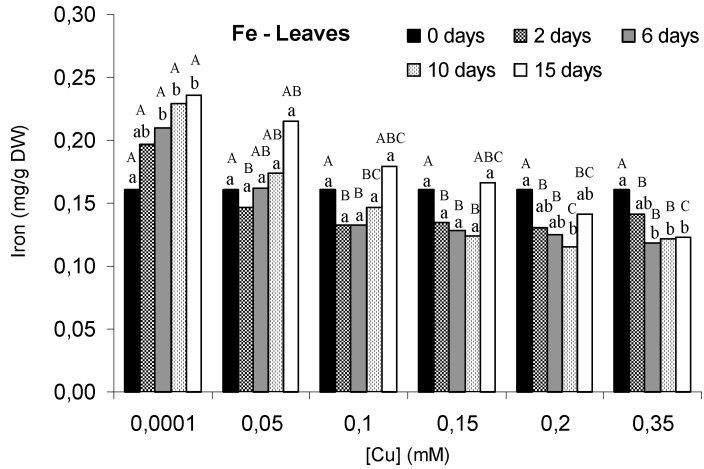

Figure 10. Effect of copper concentration in nutrient solution and time on iron contents in leaves. Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

showed a slight decrease, which could indicate Cu interference in the uptake of this element by the roots. In the leaves, the contents of both Mg and K were not significantly different. So, although Mg plays a key role in photosynthetic metabolism, no conclusive relation could be established between a deficiency of this element and the observed disruption of plant metabolism.

Iron and Zn levels decreased both in leaves and in roots (Figures 10 and 11; only the graphics for leaves are shown, but for roots they follow a similar pattern). A correlation between root growth inhibition and Fe concentration in the roots was determined, which showed that the uptake of this element was restricted by the Cu-induced toxicity at the root level. Iron deficiency was one of the main observed consequences of this study as the translocation of Fe to the leaves was also affected as shown in Figure 10. This effect was more pronounced for the longer times and higher Cu concentrations. Naturally, this has severe consequences at several metabolic levels leading to leaf chlorosis and to a decrease in the capacity of some plant antioxidative defense mechanisms, as several of the enzymes involved in this process (like catalase and peroxidase) require Fe structurally.

Zinc was also affected by Cu concentrations. Figure 11 showed a general decrease in Zn content with time and Cu concentrations in leaves and roots. Similar results have been reported by Lin and Wu (1994) for Lotus purshianus, when subjected to 24 days at a maximum Cu concentration of 10 μM. Zinc, together with Ca, was involved in the plasma membrane stabilization and so a deficiency in these elements could lead to membrane damage and increased

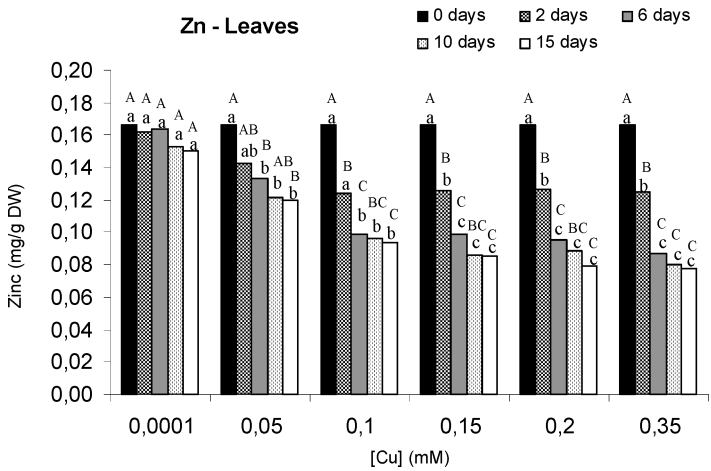

Figure 11. Effect of copper concentration in nutrient solution and time on zinc contents in leaves. Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

leakage. Lidon and Henriques (1992) have also reported that excess Cu affects Mn and Fe translocation rates in rice.

Phosphorous concentrations decreased only slightly in leaves and remained constant in roots (results not shown).

The disruption of mineral nutrient uptake by the roots seems to be strongly dependant on the plant type as there are reports on both the presence and absence of this effect (Alaoui-Sossé et al., 2004). For example, Mocquot et al. (1996), reported no changes in maize root levels for P, K, Ca, Mg, Fe, Mn, and Zn, although lower Cu concentrations were used (up to 10 μM).

Enzyme Activities

In Figures 12 to 14, the results obtained for the relative enzyme activities of peroxidase, polyphenoloxidase, and catalase in tomato leaves, as a function of Cu concentration in nutrient solution were presented. Measurements were made after 1, 2, 3, 5, and 6 days of Cu application to determine the feasibility of using these enzymes as early indicators of Cu phytotoxicity. All the enzyme activities were expressed in relation to the control value at the same time.

As can be seen in Figure 12, it was found that after 1 day of Cu supply, peroxidase activity in tomato leaves increased for all Cu concentrations higher than 0.05 mM. The highest peroxidase activity value was obtained with Cu solution concentrations of 0.10 mM after 3 days. Peroxidase activity increased significantly after only 2 days at the lowest Cu concentration of 0.05 mM, while

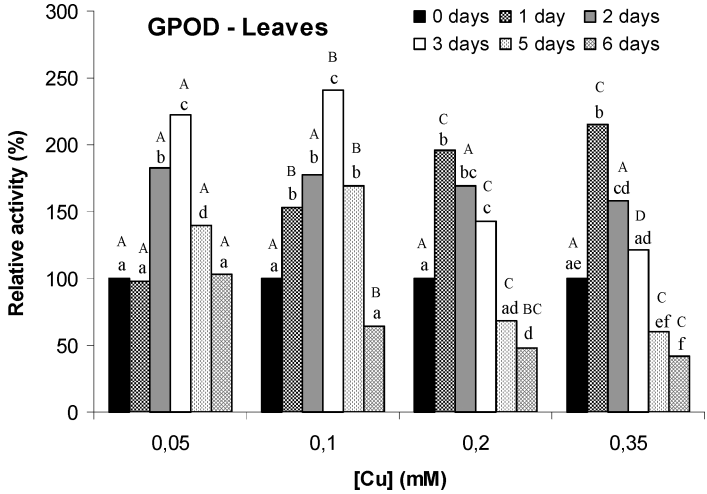

Figure 12. Effect of copper concentration in nutrient solution and time on the activity of peroxidase (GPOD) in leaves (results expressed as percentage of the control value at each time). Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

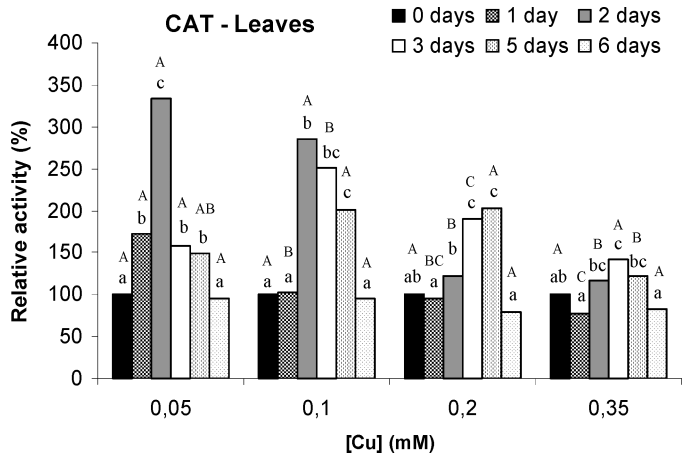

Figure 13. Effect of copper concentration in nutrient solution and time on the activity of catalase (CAT) in leaves (results expressed as percentage of the control value at each time). Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

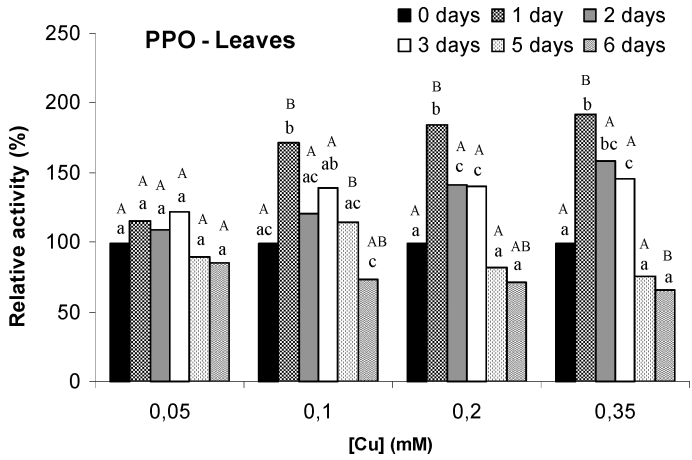

Figure 14. Effect of copper concentration in nutrient solution and time on the activity of polyphenol oxidase (PPO) in leaves (results expressed as percentage of the control value at each time). Above each bar, different capital letters indicate significant differences at each time for different copper concentrations, while different small letters indicate significant differences at each copper concentration for different times (Tukey test, $P < 0.05$).

dry matter and most other parameters had not yet significantly changed (except Cu levels, see Figure 5); although some symptoms, like leaf area reduction, were already appearing.

After 5 days of growing in solutions containing Cu concentrations greater than 0.20 mM, plants started to develop visible symptoms of toxicity. Thus, the activity levels also decreased, because excess Cu was affecting the plant's growth and metabolism, and the enzyme proteins were probably being affected by excessive amounts of reactive oxygen species or their synthesis was inhibited (Mazhoudi et al., 1997). As is widely accepted, Cu may induce oxidative stress in plants, leading to the production of excessive amounts of H_2O_2. The observed increase in peroxidase activity is thus indicative of the early activation of antioxidative defense mechanisms. These results are in agreement with other authors that reported increased GPOD activity under Cu stress (Cuypers, Vangronsveld, and Clijsters, 2002; Mocquot et al., 1996; Van Assche and Clijsters, 1990).

As we have seen, Cu mainly accumulates in roots and Cuypers, Vangronsveld, and Clijsters (2000) refers that in primary leaves of *Phaseolus vulgaris* an increase of the Cu content in the leaves was only observed 48 h after the start of the Cu supply. However, an increase of metabolites indicative of stress occurred immediately following Cu application. A significant increase of peroxidase activity was reported in young maize plants (Mocquot et al., 1996) in all investigated plant organs in relation to their Cu content, while, as referred

above, mineral composition in roots did not change. Cuypers, Vangronsveld, and Clijsters (1999, 2000) reported that a toxic concentration of Cu (50 μM) induced oxidative stress and differential responses of antioxidant enzymes in different plant parts. Mukherji and Das Gupta (1972) have also reported peroxidase induction in relation to toxic Cu levels. In tomato plants, Mazhoudi et al. (1997) reported that the activity levels of GPOD increased in response to oxidative damage, while that of ascorbate peroxidase remained constant.

Catalase activities (Figure 13) increased after only one day at the lowest Cu concentration. For the higher Cu concentrations, the activity was much lower. These results for catalase are similar to those obtained for GPOD, with an observed transient increase in its activity for the lowest Cu concentrations and times followed by a general activity decrease with time and Cu concentration. So it seems that for moderate concentrations of Cu, both GPOD and CAT are efficient in reducing the induced H_2O_2 levels in the plant tissues; but afterwards, this defence mechanism started to break down. According to Mazhoudi et al. (1997) the activity level of catalase, in tomato plants, was not modified in leaves and in stems, but it decreased in roots.

Polyphenoloxidase activity levels (Figure 14) increased significantly in the first day, at Cu nutrient solution concentrations of 0.1 mM, with a higher increase for a higher Cu concentration. This could be due to a putative catalase-like activity (Gerdemann et al., 2001), with this enzyme thus helping in the removal of H_2O_2. However, after the second day the activity levels for the higher Cu concentrations started to decrease, and after 5 days a relative decrease in activity occurred, probably due to general metabolism disruption caused by Cu toxicity.

Other authors have shown that excess Cu increases the activities of antioxidative enzymes such as peroxidase (Cuypers et al., 2002), catalase (Rama Devi and Prasad, 1998), superoxide dismutase (Rama Devi and Prasad, 1998), and enzymes of the ascorbate-gluthatione cycle (Gupta et al., 1999). Weckx and Clijsters (1996) showed that toxic Cu concentrations induced oxidative stress in primary leaves of Phaseolus vulgaris, and they induced an efficient defense mechanism against oxidative stress by increasing the capacity of ascorbate peroxidase and catalase, without any effect on total superoxide dismutase activity.

CONCLUSIONS

In vivo uptake of phytotoxic amounts of Cu by higher plants can interfere with plant development affecting numerous physiological processes. In this work, the effect of excess Cu in roots and leaves of tomato plants was studied. Visible symptoms of toxicity and a decrease in dry mass, root length, and foliar area with time and Cu concentrations were observed. The uptake of several elements was also affected, with Ca, Mn, K, Fe, and Zn contents in leaves decreasing. For the roots, the results were very similar (with the exception of Ca) and it

was verified that Cu accumulated more heavily in roots than in leaves. Thus, not only nutrient root uptake was affected but its translocation to upper plant parts also was disrupted. Visible leaf chlorosis and a decrease in chlorophyll levels were also detected, which could be due to a measured decrease in leaf Fe levels.

As was shown by these results, peroxidase, catalase, and polyphenoloxidase activities appear to have potential to be used as an early indicator of Cu stress in tomato plants and as diagnostic criteria to evaluate the potential phytotoxicity of metal-contaminated soils, as their activities increased transiently early in the experiment.

However, further work is still needed to clarify some results that have been reported, mainly at lower Cu concentrations. The activity of other enzymes (like superoxide dismutase) and the appearance of different isoenzymes (and changes in isoenzyme profiles) may also be induced by Cu toxicity.

ACKNOWLEDGMENTS

This work was funded by FCT project POCTI/AGG/44895/2002. The authors acknowledge the technical assistance of Fernando Varela, Rute Malvarez, and Marta Carneiro in some of the determinations and JAD Sementes for providing tomato seeds.

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