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Characterization of Phytoavailable Copper in Compost–Peat Substrates and Determination of a Toxicity Level

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Abstract. Heavy metal-sensitive 'Express Orchid' petunias (*Petunia* ×*hybrida* Hort Vilm.-Andr. 'Express Orchid') were grown in substrates of 2 green yard waste compost : 3 peat (v/v) with target Cu contents of 100 and 200 mg·kg^{-1} at varying pH. Iron supply was also varied. Copper contents of the substrate were determined by H_2O, NH_4NO_3, NH_4OAc, $CaCl_2$, $CaCl_2$–DTPA, and aqua regia extraction. Plant Cu concentration increased with increasing Cu supply and decreasing pH, indicating that Cu phytoavailability depended on substrate pH. Extraction of fresh substrates with $CaCl_2$–DTPA provided a good prediction of plant Cu concentration and reflected well the influence of pH on Cu phytoavailability. The percentage of $CaCl_2$–DTPA extractable Cu increased with decreasing pH. Extractions of Cu with NH_4NO_3, H_2O, NH_4OAc, and $CaCl_2$ resulted in very low extractable amounts and hence were not suitable. Plants showed Cu toxicity induced iron-like deficiency chlorosis, which was alleviated by additional Fe supply. This Fe supply did not seem to affect total Fe concentration of petunias, but reduced Cu concentration of the shoots. Since yield reduction was not observed, the occurrence of chlorosis during the culture period was chosen as the toxicity parameter, resulting in a Cu threshold toxicity level of 12.3 mg·kg^{-1} plant dry weight. From this, a threshold toxicity level for $CaCl_2$–DTPA extractable Cu in compost–peat substrates of 3 mg·L^{-1} substrate was determined. Chemical name used: diethylenetriamine–pentaacetic acid (DTPA).

Composts of green yard and organic wastes often contain large amounts of Cu, Zn, Pb, Cd, and Mn, depending on their origin. These heavy metals can be toxic to plants (Kehres, 1991) when the composts are used as components of substrates for ornamental and container plants. Plants exhibit metabolic abnormalities and growth inhibition as well as chlorosis with Cu concentrations in tissues only slightly higher than normal levels (Fernandes and Henriques, 1991). Levels of Cu at 3 to 15 mg·kg^{-1} plant dry weight (DW) are regarded as normal for a variety of plants, but toxicity may begin at Cu levels of 15 to 20 mg·kg^{-1} plant DW (Sauerbeck and Harms, 1992). Yield reduction is often observed at ≈15 to 20 mg·kg^{-1} plant DW (Macnicol and Beckett, 1985). Copper induces Fe deficiency chlorosis (Amberger et al., 1982), since it inhibits Fe^{3+} reduction on the root surface and inside the plant (Olsen et al., 1982). Copper competes with Fe for sensitive metabolic sites inside leaves, indicating an antagonism between Cu and Fe (Greipsson, 1994).

Copper phytoavailability depends on several factors, e.g., soil pH and organic matter content (Herms, 1989). Minimum Cu solubility occurs between pH 5 and 7 (Herms, 1982). Copper mobility increases below pH 4.5 (Hornburg and Brümmer, 1993) due to decreased specific and unspecific sorption of Cu (Herms, 1982). On the other hand, Cu mobility increases at higher pH beginning at pH 6.5 to 7.5, since stability and solubility of organic Cu complexes increases, resulting in higher Cu contents in the soil solution (Hornburg and Brümmer, 1993). Nevertheless, it is not known which and to what extent soluble organic Cu complexes are phytoavailable, but a lower biological effectiveness is supposed compared to free M^{2+} species (Herms and Brümmer, 1984). Plants can only absorb Cu from the liquid phase (Brümmer et al., 1986). Thus, the pool of potentially available metals comprises those fractions which can deliver metals from solid phases to the soil solution within a relatively short time, e.g., one vegetation period (Brümmer et al., 1986). Since Cu mobility and phytoavailability depend on pH, it is important to consider pH in extraction of phytoavailable Cu.

Methods using water, neutral salt solutions, and complexing agents as extractants for estimation of phytoavailable heavy metals reported in the literature were developed for use with soils, except the $CaCl_2$–DTPA method (Alt and Peters, 1992) which was designed for horticultural substrates. However, little information is available about the suitability of these methods in relation to their ability to extract phytoavailable heavy metals of compost–peat substrates. Köster and Merkel (1982) reported that Cu extraction with neutral salts failed, since the extracted amounts were below the level of detection. On the other hand, a weak correlation between Cu extracted with acids or organic complexing agents and plant Cu concentration was observed (Köster and Merkel, 1982). Robson and Reuter (1981) as well as Jarvis (1981) describe DTPA containing extraction solutions as useful for inclusion of organically bound Cu. Furthermore, extraction of fresh, moist substrates might better reflect the actual situation of heavy metal phytoavailability than extraction of dried substrates. Therefore, the aim of the research was to identify a suitable method for characterization of Cu phytoavailability in compost–peat substrates and to determine a toxicity level for Cu in petunias, and for phytoavailable Cu in compost–peat substrates.

Materials and Methods

The test plant was the heavy metal-sensitive 'Express Orchid' petunia (*Petunia* ×*hybrida* 'Express Orchid') (Bucher and Schenk, 1997).

Treatments. Mixtures of 2 green yard waste compost : 3 white peat (v/v) were used as substrates. Copper sulfate addition was made before composting, to achieve similar bond types as in the compost of polluted raw material. The addition was varied to match contents occurring in commercial composts. Composting lasted for 14.5 to 19 weeks till maturity of the compost. Three experiments were conducted to assess the methods with different composts. Composts were prepared separately for each experiment. Table 1 shows the Cu treatments involved in each experiment. The target Cu values of compost–peat substrates (100 and 200 mg·kg^{-1}) were not met exactly, as can be deduced from Table 1.

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Table 1. Target and aqua regia soluble Cu content in the compost–peat substrates.

Target Cu content	Aqua regia soluble Cu content ($mg \cdot kg^{-1}$ substrate dry wt)[z]		
($mg \cdot kg^{-1}$ substrate dry wt)	Expt. 1	Expt. 2	Expt. 3
Basic load[y]	12	---[x]	30
100	85	85	136
200	197	176	---

[z]Average of all treatments with different pH.
[y]Natural Cu content of compost prepared of unamended raw material.
[x]Data not available.

The pH was adjusted to 4, 5, 6, or 7 according to buffer curves by using H_2SO_4 or CaO. Those target values were reached on average with an accuracy of +0.1 and –0.33 pH units. Compared to the start of the trial, pH was increased on average by ≈0.35 units at the time of harvest. The bulk density of the fresh substrates varied between 0.508 and 0.624 $kg \cdot L^{-1}$ and between 0.157 and 0.186 $kg \cdot L^{-1}$ for the dried substrates. Basic N fertilization was provided as 200 $mg \cdot L^{-1}$ substrate in the form of NH_4NO_3. No further nutrients were necessary as basic fertilization, since they were present in the compost. Plants were fertigated during the culture period with a nutrient solution containing N, P, K, Fe, B, Cu, Mn, Mo, and Zn at 75, 15.26, 91, 0.6, 0.15, 0.01, 0.25, 0.025, and 0.05 $mg \cdot L^{-1}$, respectively. In addition to fertigation, 1 mg Fe as Fe–EDDHA (ethylenediamine di(o-hydroxyphenylacetic acid) per plant, dissolved in 50 mL H_2O, was applied to the substrate 1 week after transplanting as is usual in horticultural practice (+Fe treatments). Further treatments were included which did not receive the additional Fe supply (–Fe treatments).

Growing conditions. Seeds were sown and seedlings pricked out in standard potting mixture. Seedlings were potted in compost–peat substrates in 250 mL plastic pots. They were grown in a greenhouse at days/nights of 14 to 16/12 to 14 °C, ventilation temperature being 2 °C higher. Experiments 1 and 3 were conducted in March to May 1994 and 1995. Experiment 2 was conducted in October to December 1994, and supplemental light [photosynthetically active radiation (*PAR*) 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 12 h] was provided. The growing period (potting to harvest) was 45 d for Expt. 1, 56 d for Expt. 2, and 38 d for Expt. 3. The pH 4 ($CaCl_2$) treatments received water without carbonate hardness, to maintain the low pH during the culture period.

Chemical analysis and observation of plants. The pH was determined in all substrates at the start of culture and at harvest using a 0.01 mol $CaCl_2$ solution with a pH electrode. Substrate samples for Cu determination were taken before potting the plants. After digestion of dried, ground substrates with aqua regia (2.5 g substrate, 10.5 mL HCl + 3.5 mL HNO_3, 150 °C for 2 h), copper was detected by flame atomic absorption spectrophotometry (AAS) (Hoffmann, 1991). Extractable Cu contents in substrates were determined in moist, sieved (2 mm mesh, stainless steel) as well as in oven-dried (at 70 °C until constant weight; sieved after drying) substrate samples by five extraction methods, but each method was not used in every experiment. The extraction ratio is given in grams substrate per milliliter extraction solution (weight/volume); no compensation of water contents was made for the moist substrates.

H_2O method: double distilled water, dried substrates, extraction ratio 1:50, shaking 1 h, (according to van der Paauw, 1971); used for Expt. 3.

NH_4NO_3 method: 1 mol NH_4NO_3, dried substrates, extraction ratio 1:8, shaking 2 h, (modified from Prüeß, 1992); used for Expt. 3.

NH_4 acetate method: 1 mol NH_4OAc, extraction ratio 1:20 for dried (used for Expts. 1, 2, and 3) and 1:8 for moist (used for Expts. 1, 2, and 3) substrates, shaking 2 h, (modified from Dües, 1989).

$CaCl_2$ method: 0.1 mol $CaCl_2$, extraction ratio 1:10 for dried (used for Expts. 1, 2, and 3) and 1:8 for moist (used for Expts. 1, and 2) substrates, shaking 1 h, (modified from Köster and Merkel, 1982).

$CaCl_2$–DTPA method (CAT method): 0.01 mol $CaCl_2$ + 0.002 mol DTPA (diethylenetriamine–pentaacetic acid), extraction ratio 1:8 for fresh (used for Expts. 1, 2, and 3) and dried (used for Expts. 1, 2, and 3) substrates, shaking 1 h, (modified from Alt and Peters, 1992).

All results of the extractions are expressed as milligrams Cu per liter substrate.

Plants were harvested at the end of the culture (on average after 48 d) for analysis and dry matter determination. Dried ground plant tissue was digested under pressure in polytetrafluorethylene vessels (teflon) with nitric acid. Detection of Cu and Fe was carried out using flame AAS and inductively coupled plasma spectrophotometry (ICP). Chlorosis of the plants was monitored weekly in all trials and is presented as a percentage of chlorotic plants, not considering the severity of chlorosis.

Experimental design and statistical analysis. A completely randomized block design with three 10 plant replicates of each treatment was utilized for each experiment. Statistical analysis was

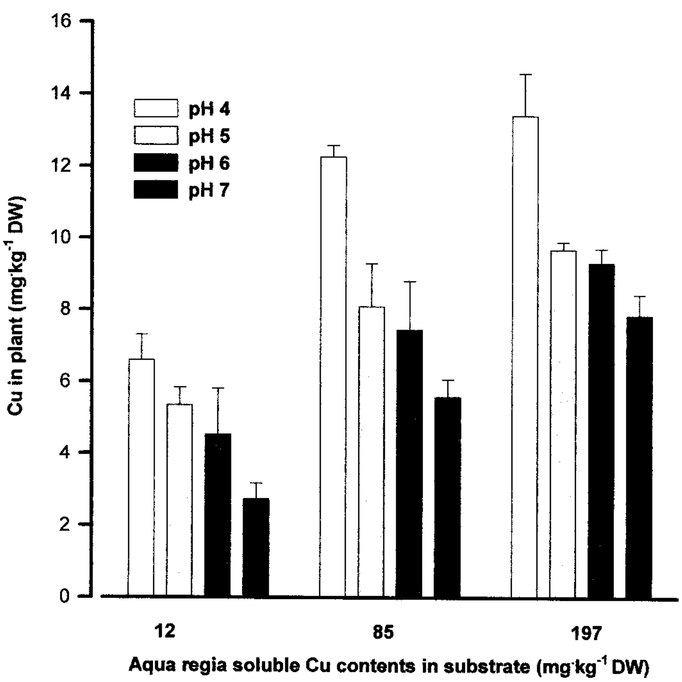


Fig. 1. Cu concentration in 'Express orchid' petunia dry matter as affected by Cu content and pH of the substrate (Expt. 1). Vertical bars = SD.

Table 2. Dry matter yield of petunias as influenced by Cu supply.

Cu supply ($mg \cdot kg^{-1}$ substrate	Dry matter yield (g/plant)			
dry wt)	pH 4	pH 5	pH 6	pH 7
Basic load	3.11 a[z]	3.30 a	3.07 a	2.99 a
100	3.30 a	3.36 a	3.10 a	3.02 a
200	3.14 a	3.45 a	2.97 a	2.80 a

[z]Mean separation within columns by Tukey studentized range test ($P < 0.05$).

Table 3. Range of extracted substrate Cu contents and coefficients of determination for the regression between extractable Cu and plant Cu concentration for Expt. 1 and for all experiments together.

Method	Substrate preparation	Range of Cu concns in Expt. 1 ($mg \cdot L^{-1}$ substrate)	Expt. 1 (r^2)[z]	All Expts. (r^2)[y]
CaCl2 + DTPA	Dried	0.04–5.28	0.67***	0.66***
CaCl2 + DTPA	Fresh	0.11–5.96	0.74***	0.73***
Aqua regia	Dried	1.64–34.61	0.43*	0.32***

[z]n = 12.
[y]n = 28.
*,***Significant at $P < 0.05$ or 0.001, respectively.

carried out with the statistical package 'SAS for Windows' (SAS Institute, Inc.). Data were subjected to analysis of variance, and multiple linear regression analysis. Means were separated using Tukey's studentized range test.

Results

Experiment 1 was used to study the effect of Cu and pH level on extractable Cu content and plant growth in detail. The other experiments were conducted for validation of the results of Expt. 1.

Copper in plants. The Cu concentration in petunia increased significantly ($P = 0.01$) with increasing Cu content in the substrate from 12 to 197 $mg \cdot kg^{-1}$ DW. A significant ($P = 0.01$) decrease in plant Cu concentration was observed with increasing pH from 4 to 7 in the compost–peat substrates (Fig. 1).

Variation of Cu supply in the substrate did not affect plant DW yield of petunias (Table 2). Substrate pH affected DW yield only slightly regarding the wide range of variation.

Phytoavailable cu in compost–peat substrates. Five extraction solutions were tested with regard to their ability to characterize the pH dependent phytoavailability of Cu. The amounts of Cu extracted with NH_4NO_3, H_2O, NH_4OAc, and $CaCl_2$ were very low and the concentration in the extract was close to the detection level. Results of extraction with $CaCl_2$–DTPA and digestion with aqua regia are presented in Table 3. For better comparability, aqua regia soluble Cu contents were expressed also in $mg \cdot L^{-1}$ substrate as usual for extractable contents, which took into account bulk density of the substrates.

As expected, aqua regia was the strongest extractant, because it dissolved almost all the Cu. The amounts extracted with $CaCl_2$–DTPA from dried and moist substrates were in the same range.

Coefficients of determination for the regressions between extractable Cu and plant Cu concentration for Expt. 1 increased in the following order: aqua regia, $CaCl_2$–DTPA (dried substrate), $CaCl_2$–DTPA (fresh substrate).

Reflection of the influence of pH on Cu phytoavailability by $CaCl_2$–DTPA (fresh substrate) is illustrated in Fig. 2. Copper content extracted with $CaCl_2$–DTPA decreased with increasing pH at every level of Cu supply resembling the amount absorbed by the plant. However, Cu phytoavailability at pH 4 was underestimated using $CaCl_2$–DTPA as the extractant.

Results of Expt. 1 were confirmed by including the data of Expts. 2 and 3 in the computations (Table 3). The strong correlation between $CaCl_2$–DTPA extractable Cu of fresh substrates and plant Cu concentration was also confirmed by data distribution for all experiments, as shown in Fig. 3.

Calcium chloride–DTPA extractable Cu contents as a percentage of aqua regia soluble Cu were similar in all experiments, depending on pH and Cu level. The percentage increased with decreasing pH from ≈11% at pH 7 to 22% at pH 4 with a target Cu supply of 100 $mg \cdot kg^{-1}$ substrate DW and from 8% at basic load to 38% at target Cu of 200 $mg \cdot kg^{-1}$ substrate DW (pH 4).

Influence of cu supply on plant appearance. Interveinal chlorosis of the younger leaves resembling Cu induced iron deficiency chlorosis was visible in all experiments during the culture period. Chlorosis was a transient symptom as illustrated in Fig. 4. Chlorosis increased between weeks 2 and 3 after start of Cu treatment and then decreased. Chlorosis was more severe at pH 4 (Fig. 4A) than at pH 6 (Fig. 4B), because of a 1.5 fold higher $CaCl_2$–DTPA extractable Cu content at the lower pH. A supply of 1 mg Fe per plant as Fe–EDDHA reduced chlorosis to the level of chlorosis at basic Cu load at pH 4 and to zero at pH 6.

Influence of fe supply on plant cu and fe content. No significant influence of the additional Fe supply on plant Fe concentration was observed (Table 4), although it reduced Cu toxicity induced iron-like deficiency chlorosis. In addition, plant Fe concentration was not influenced by substrate pH. In contrast, Cu concentration of petunias was significantly ($P < 0.05$) reduced by the additional Fe supply at all levels of pH (Table 4). Concentration of Fe in plant dry matter varied much more than Cu concentration,

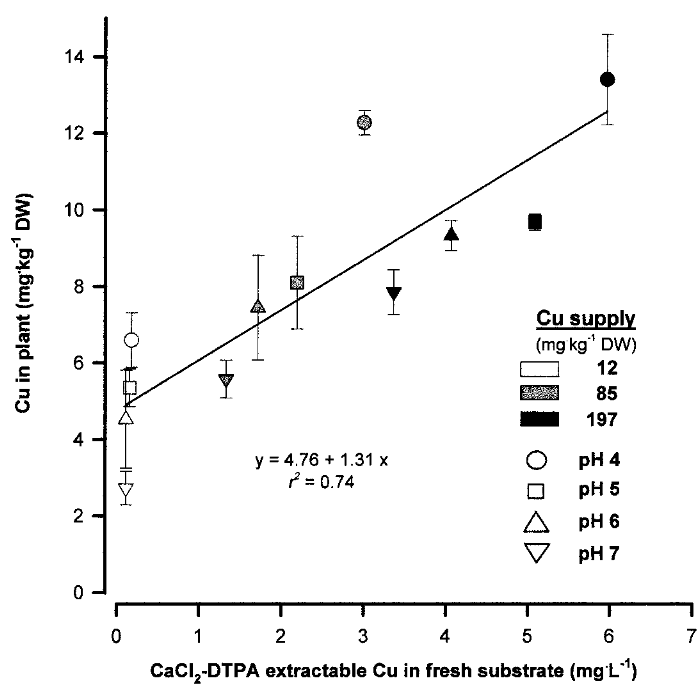


Fig. 2. Relationship between Cu concentration of 'Express orchid' petunia and $CaCl_2$–DTPA extractable Cu content in fresh substrate with varied Cu supply and pH (Expt. 1). Vertical bars ±SD.

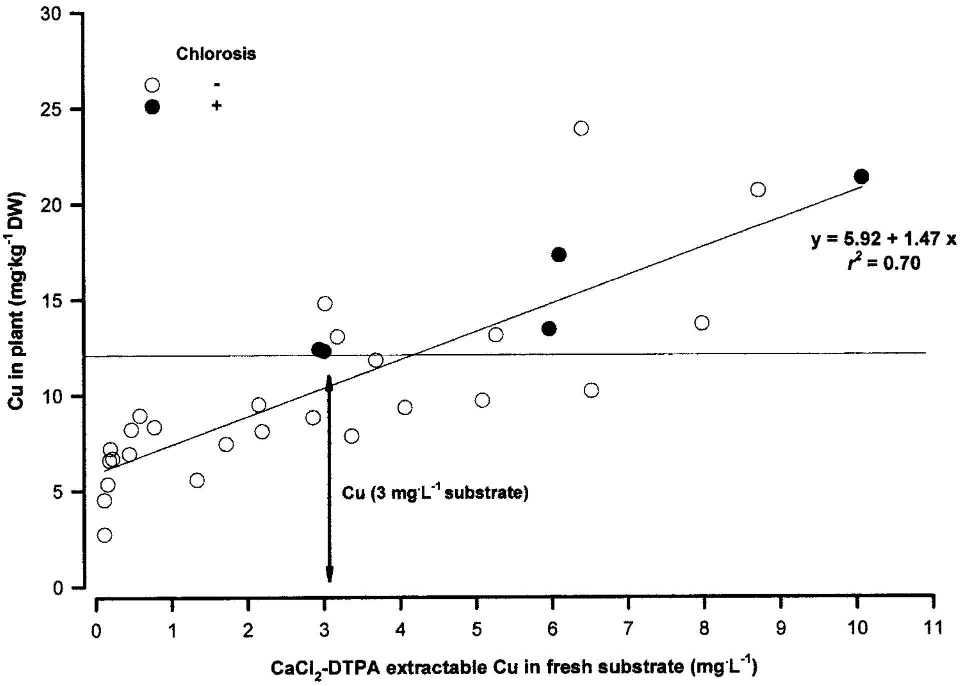


Fig. 3. Threshold toxicity level for $CaCl_2$–DTPA extractable Cu in the compost–peat substrates related to the plant Cu concentration and to the occurrence of chlorosis of 'Express orchid' petunias (Expts. 1–3).

resulting in a high standard deviation. Thus, changes in Fe concentration were not significant.

Threshold toxicity level for Cu in petunias and for phytoavailable Cu in compost–peat substrates. Since plant dry matter yield was not influenced by Cu supply, the occurrence of Cu toxicity induced iron-like deficiency chlorosis was chosen as the toxicity parameter. Any Cu level inducing chlorosis at any time of the culture period was considered. Results of Expts. 1 to 3 are summarized in Fig. 5. Chlorosis of petunias during the culture period was first observed with a plant Cu concentration of 12.3 mg·kg^{-1} plant DW. Nevertheless nonchlorotic plants were observed at higher plant Cu concentration.

Taking into account the threshold Cu toxicity level of 12.3 mg·kg^{-1} plant DW, a threshold toxicity level for $CaCl_2$–DTPA extractable Cu of ≈3 mg·L^{-1} substrate was ascertained (Fig. 3) at which chlorosis of petunias occurred.

Discussion

Copper in plants. Copper phytoavailability in compost–peat substrates depended on pH of the substrate, since plant Cu concentrations increased significantly with decreasing pH. Hence, a suitable extraction solution for phytoavailable Cu has to consider the significant influence of pH on Cu phytoavailability.

Plant Cu concentrations between 2.7 mg·kg^{-1} DW at basic load and 21.3 mg·kg^{-1} DW at a Cu supply of 200 mg·kg^{-1} substrate DW were obtained in the experiments. According to Sauerbeck and Harms (1992) Cu toxicity may begin at 15 to 20 mg·kg^{-1} DW, whereas 3 to 15 mg·kg^{-1} DW are regarded as normal Cu concentrations in plant DW, depending on the species. Although plant Cu concentrations seemed to be in the toxicity range, growth reduction due to Cu treatment was not observed in our trials. In other research, yield reduction was observed at 18 mg·kg^{-1} for oats (*Avena sativa* L.), red clover (*Trifolium pratense* L.) (Hodenberg and Finck, 1975), and barley (*Hordeum vulgare* L.) (Beckett and Davies, 1977). Levels of 20 mg·kg^{-1} DW were defined as toxic for barley, bean (*Phaseolus* L. sp.), and tobacco (*Nicotiana tabacum* L.) (Robson and Reuter, 1981).

High Cu supply resulted in chlorotic leaves of petunias resembling Fe deficiency chlorosis, which was in most cases a transient symptom during the culture period. Such an induction of iron-like deficiency chlorosis by Cu is reported for such species as *Trifolium pratense*, *Phaseolus vulgaris* L., winter radish (*Raphanus sativus* L.) (Amberger et al., 1982), and cucumber (*Cucumis sativus* L.) (Swiader, 1985).

Supply of additional Fe reduced the observed chlorosis of petunias indicating possible Fe deficiency chlorosis,

Fig. 4 (**below**). Occurrence of chlorosis during the culture of 'Express orchid' petunia as influenced by the Cu content of the compost–peat substrates and Fe supply at (**A**) pH 4 and (**B**) pH 6 (Expt. 1). Vertical bars ±SD.

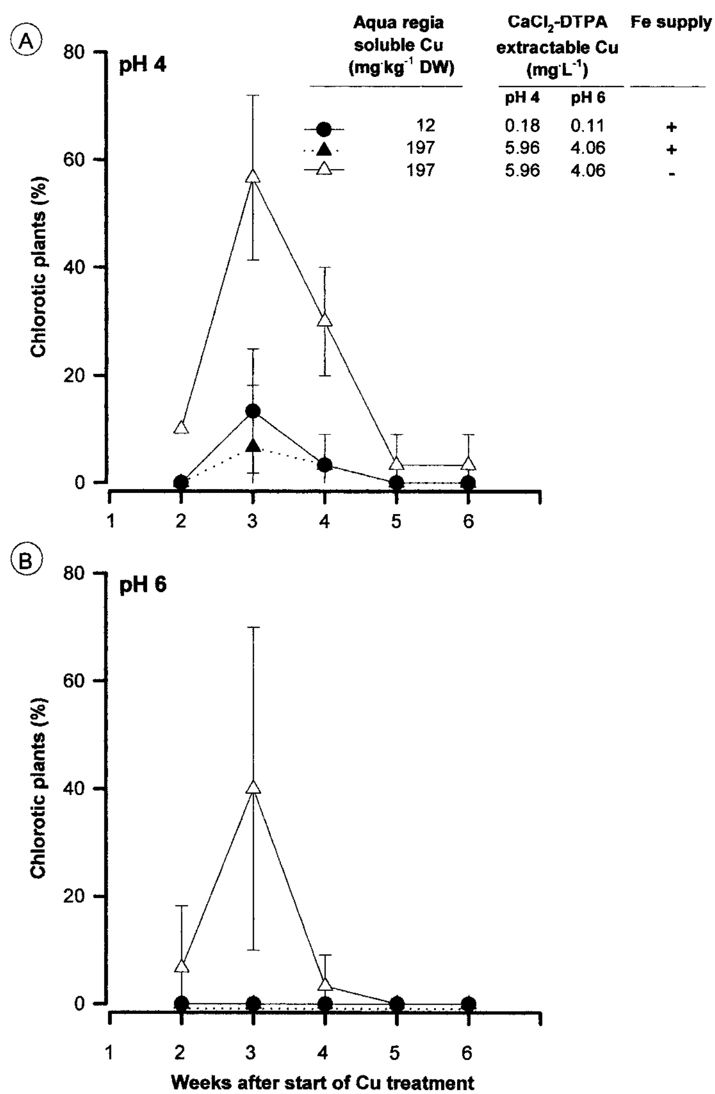


Table 4. Influence of Fe supply on Fe and Cu concentration of petunia after 6 weeks treatment with Cu at 197 mg·kg^{-1} substrate dry weight (aqua regia soluble) at varied pH (Expt. 1).

	Fe	Concn in plant dry matter (mg·kg^{-1})			
Element	supply	pH 4	SD	pH 6	SD
Fe	+	151.3 a^{z}	13.6	121.0 a	11.8
Fe	–	201.0 a	75.4	146.7 a	30.7
Cu	+	13.4 a	1.2	9.3 a	0.4
Cu	–	18.0 b	3.1	12.6 b	2.1

^{z}Mean separation within columns by Tukey studentized range test (P < 0.05).

which is in accordance with the literature. Iron supply was reported effective as Fe chelate in nutrient solution (Daniels et al., 1973) as was application of a solution of inorganic Fe^{2+} salts to the leaves (Olsen et al., 1982). In our study the effect of Fe supply was not due to increased Fe content in the plant but decreased Cu content (Table 4). Similar effects of Fe supply have been observed for Cu in tomato (*Lycopersicon esculentum* Mill.) (Pich et al., 1994) and for Zn in petunias (Bucher and Schenk, 1999). This might be associated with findings that Fe reduces Cu absorption from soil solutions (Kabata-Pendias and Pendias, 1992).

Although the leaves were chlorotic, plant Fe concentrations were in the range of normal contents, i.e., 60 to 300 mg·kg^{-1} plant DW (Vose, 1982). This is in accordance with findings of Amberger et al. (1982), who observed Cu induced Fe deficiency chlorosis although total Fe concentration was 1100 mg·kg^{-1} in *Raphanus sativus* dry matter. Since the total Fe concentration of plants was not affected by Cu supply, the interaction between Cu and Fe cannot be due to impaired Fe uptake. It is quite possible it is caused by interactions within the plant, since Cu competes with Fe for metabolic sites inside leaves (Greipsson, 1994) and inhibits reduction of Fe^{3+} to Fe^{2+}, which is required for incorporation of Fe in enzyme systems (Olsen et al., 1982).

Occurrence of Cu induced chlorosis during the culture period was chosen as the toxicity parameter since it was the most sensitive parameter, and moreover, ornamental plants cannot be sold with chlorotic leaves. In our experiments chlorosis was observed with Cu at 12.3 mg·kg^{-1} DW. Toxicity levels for Cu in plant dry matter, mentioned in the literature, are always based on yield reduction; nevertheless they are only slightly higher than the one determined for occurrence of chlorosis of petunias.

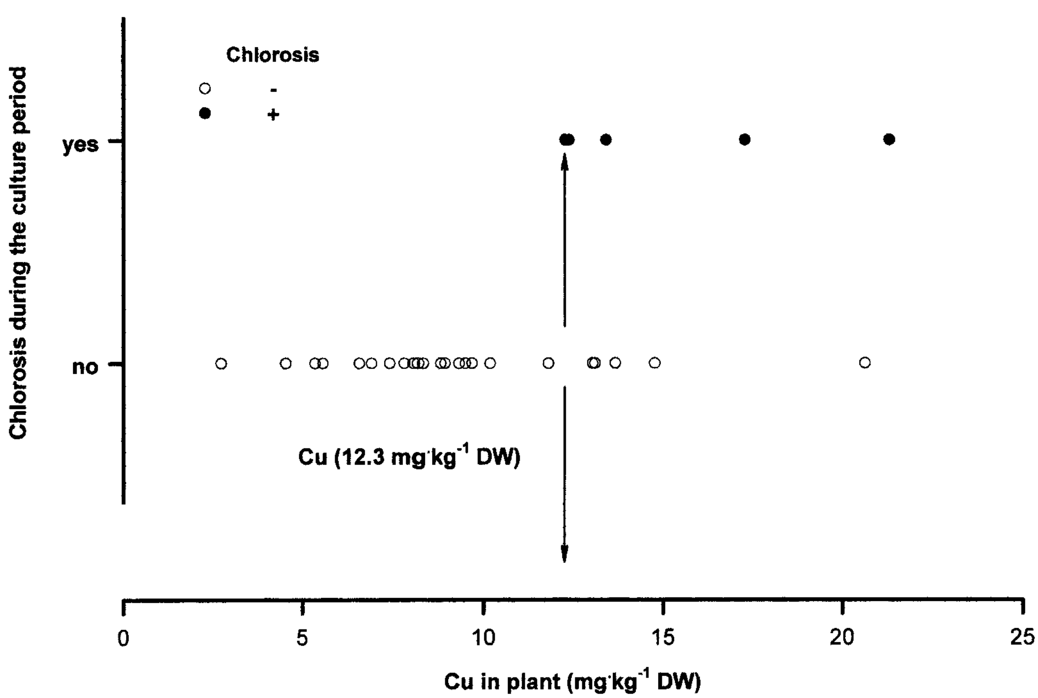
Phytoavailable Cu in compost–peat substrates. Increasing pH resulted in decreasing Cu concentrations in plant dry matter, because Cu phytoavailability decreased (Albasel and Cottenie, 1985). A suitable extraction method should reflect this characteristic. Water, NH_4NO_3, NH_4OAc, and $CaCl_2$ procedures extracted very low levels of Cu and concentrations in the extract were below or at the level of analytical detection, thus they were not suitable. Use of $CaCl_2$–DTPA for extraction of fresh substrates resulted in a good correlation with the plant Cu concentration and reflected well the influence of pH (Fig. 2). Of the methods tested, $CaCl_2$–DTPA extraction resulted in the best reflection of pH influence, nevertheless it was not perfect. The correlation might be improved by altering the DTPA and/or salt concentration of the extraction solution. The method is sufficient in the pH range of 5 to 7, which is usual for horticultural practice, whereas plant Cu concentrations are underestimated at pH 4, which is only used for specific crops in the Ericaceae.

The amounts of Cu extracted with $CaCl_2$–DTPA were in the same range for both fresh (moist) and dried substrates. However, the coefficient of determination was slightly higher for fresh substrates. Drying of the substrate might change solubility of the organic matter and of Cu complexed with the organic matter (Bartlett and James, 1980; Khan and Soltanpour, 1978; Legett and Argyle, 1983).

Although the molarity of $CaCl_2$ was 10-fold higher with the $CaCl_2$ method than with the $CaCl_2$–DTPA method, the latter extracted 20-fold higher amounts of Cu. Thus, the higher extraction strength of $CaCl_2$–DTPA must have been due to the complexing agent DTPA, because in addition to the exchangeable fraction, it extracts organically bound Cu (Brümmer et al., 1986).

As expected, aqua regia extraction did not estimate the phytoavailable Cu content of compost–peat substrates, since almost all the Cu was extracted and the influence of substrate pH was not reflected. This was also indicated by the increase in r^2 resulting from including pH in the multiple regression of the relationship between plant Cu concentration, aqua regia soluble Cu (x_1) and pH (x_2) ($y = 15.17 + 0.04\ x_1 - 1.66\ x_2$), which resulted in an r^2 of 0.50. But aqua regia soluble Cu plus consideration of pH is not intended to give a suitable estimation of the potentially phytoavailable Cu content of compost–peat substrates, because phytoavailability of Cu is influenced by

Fig. 5. Occurrence of chlorosis during the culture period of 'Express orchid' petunias as a function of the plant Cu concentration (Expts. 1–3).



additional factors apart from total Cu content and pH. These additional factors might be taken into account by $CaCl_2$–DTPA extraction. For example, Cu is complexed by organic matter, which differs in quantity and quality between the composts. These differences might be detected by $CaCl_2$–DTPA extraction, whereas aqua regia (+ pH) cannot reflect Cu organic matter bonding strengths, since it dissolves almost all the Cu. However, in our experiments different Cu bonding strength on organic matter did not seem to be significant, since the percentage of $CaCl_2$–DTPA extractable Cu did not vary with the trials.

An increase of Cu solubility due to increased stability and solubility of organic Cu complexes with increasing pH as described by Hornburg and Brümmer (1993) for soils was not observed at the target pH of 7. This might be due to the fact that during composting, heavy metals tend to form increasingly less soluble forms, for example with humified organic matter. If compost is matured correctly, an increase in high molecular weight organic compounds such as humic acids is observed (Canarutto et al., 1991), which function as sinks for heavy metals, in contrast to low molecular weight organic compounds (fulvic acids), which tend to form water soluble complexes with heavy metals (Takács, 1994).

Threshold toxicity level for phytoavailable Cu in compost–peat substrates. A threshold toxicity level for phytoavailable Cu in compost–peat substrates was determined as the $CaCl_2$–DTPA extractable Cu content resulting in chlorosis of petunias at any time during the culture period. This occurred with Cu at ≈3 $mg \cdot L^{-1}$ substrate. Alt and Peters (1992) observed normal growth with Cu up to 40 $mg \cdot L^{-1}$ substrate in chrysanthemum (*Dendranthema grandiflorum* hybrids Des Moul). The toxicity level obtained in our experiments is lower, which might be due to consideration of chlorosis at any time during the culture period and not only at harvesting time. Furthermore, it has to be considered that a heavy metal sensitive test plant was used. Nevertheless, the threshold toxicity level obtained for $CaCl_2$–DTPA extractable Cu in substrates seemed to be plausible, since it was based on plant Cu concentrations resulting in chlorosis, which was in the range of toxicity levels for plant Cu concentrations mentioned in the literature.

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