

Toxicity and Bioaccumulation of Copper to Black Bindweed (*Fallopia convolvulus*) in Relation to Bioavailability and the Age of Soil Contamination

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Abstract. The use of ecotoxicity test results obtained in the laboratory for prediction of effects of potentially toxic concentrations of chemicals in the field is hampered by several factors differing between the laboratory and the field situations. One important factor is the binding of test chemicals to soil, which is affected by the age of the contamination and soil type. The present study investigated the effect of contamination age by introducing an aging period of 1 to 12 weeks between mixing the test substance, copper sulfate, in with soil and introducing the test plant, *Fallopia convolvulus* (L.) A. Löve. Copper accumulation, emergence of cotyledons, and growth were assessed and related to total (boiling with HNO_3) and 0.01 M CaCl_2 - and DTPA-extractable soil copper concentrations. Aging of the copper-contaminated soil had only small effects on bioaccumulation of copper, copper toxicity, and extractable soil copper fractions. Soil copper had no effect on emergence of cotyledons. Estimated EC_{50} values for shoot and root growth averaged 280 mg Cu/kg. Effects on growth in these laboratory-treated soils were much more severe than in a study performed in soil from an old copper-contaminated field site. Neither CaCl_2 - nor DTPA-extractable copper fractions could explain all of the differences in effects between aged spiked soil and field soil. The accumulation pattern for roots and shoots of *F. convolvulus* indicated that excessive copper was accumulated and adsorbed mainly by the fine roots, whereby the copper concentrations of other plant parts were kept low until the plant was no longer able to maintain this regulation. An internal threshold for effects on growth of about 20 mg Cu/kg shoot dry weight was estimated, coinciding with a soil copper concentration of approximately 200 mg/kg.

The ultimate goal of laboratory toxicity tests is to predict the possible negative effects of potentially toxic substances on field ecosystems. Toxic effects are a function of exposure to the substance in question, and in general, the degree of exposure of soil-living organisms to contaminants in soil depends on the life strategy of the organisms, the properties of the soil, and the

persistence and the binding characteristics of the substance. Important soil characteristics affecting the distribution of copper and other metals over the various phases in the soil matrix are clay content and type, organic matter content and type, and pH (e.g., Adriano 1986; Kabata-Pendias and Pendias 1984), all of which are parameters that may differ between laboratory tests and field conditions.

There is also a temporal aspect of bioavailability because the binding of chemicals to soil binding sites tends to increase with time (Alexander 1995; Smit and Van Gestel 1998). Bioavailability in laboratory experiments with spiked soil may therefore be unrealistically high compared to the field situation (Van Straalen and Denneman 1989), as also found in the study of Kjær *et al.* (1998) on the effects of copper on *Fallopia convolvulus*. Furthermore, the addition of metals as metal salts to soil may result in a different copper speciation than in the field and in toxic effects of the counterion added together with the metal ion.

Generally, soil heavy metal concentrations are determined with, e.g., atomic absorption spectrometry (AAS) after bringing (part of) the metal into solution. Different extractants have been proposed to simulate the fraction experienced by different organisms; even for different plant species the bioavailable fraction seems to differ (e.g., Robson and Reuter 1981; Taylor *et al.* 1992). DTPA and CaCl_2 are among the substances proposed for extracting the bioavailable part of metals from soils. DTPA is a chelating agent developed as a method for estimating available micronutrients in soils with low nutrient concentrations (Lindsay and Norvell 1978) but has also been applied to estimate metal uptake under other circumstances (e.g., Singh and Narwal 1984). Extraction with 0.01 M CaCl_2 has been recommended for assessment of bioavailable fractions of various nutrients and micronutrients, because, among other virtues, it has a salinity close to the soil solution and has no influence on the pH of the extract (Houba *et al.* 1996).

Plants are exposed to heavy metals mainly through root uptake (contaminated soil in general, including aerial deposition) or in some cases through uptake over the above-ground biomass (aerial deposition). At low levels, Cu is a plant micronutrient, essential in several enzyme systems with minimum requirements generally of 1–5 mg/kg in plant tissue, while at concentrations higher than 20–30 mg/kg, depending on plant species, it may cause toxicity (Marschner 1995). Toxicity

symptoms include chlorosis, reduced growth, and root abnormalities. Various mechanisms for dealing with elevated copper levels are found in plants (Ernst *et al.* 1992; Turner 1994; De Knecht *et al.* 1995; Murphy *et al.* 1999). Excretion of metal-binding compound may complex the free metal ions near the roots and thereby lower metal availability. Once the metal has entered the plant, metal-complexing peptides (phytochelatins) may be induced, which reduce the concentration of free metal ions in the cytosol. A similar function may be exerted by metal-storing vacuoles. Control of the distribution of metals to different plant parts may keep metals away from the more sensitive parts. Translocation to old leaves offers the opportunity of getting rid of excess metal load when the leaves are shed. However, these regulatory mechanisms are only effective up to a certain copper level, often named the critical value (Macnicol and Beckett 1985).

In the present study, several aspects related to bioavailability were investigated in a greenhouse experiment on interactions of copper sulfate with black bindweed, *F. convolvulus*, and the soil in which it was grown. The key questions were:

- How does aging of copper contamination affect copper accumulation and effects in *F. convolvulus*?
- How is the accumulated copper distributed within the plant (roots versus shoots)?
- Is the Cu content of *F. convolvulus* correlated with measures of total or available copper?
- Is it possible to correlate toxic parameters, such as growth, with measures of Cu uptake in the plant and/or measures of available Cu in the soil?

Materials and Methods

Plants

F. convolvulus is an annual weed common in agricultural fields and disturbed areas and intensively studied by Kjær (1994), Kjær and Elmgaard (1996), and Kjær *et al.* (1998). Approximately 25 seeds of equal size (5–7 mg) were sown in each experimental pot, after seed dormancy was broken by 3 months' storage at 100% humidity and 5°C (Justice 1941; Timson 1966). Seeds from uncontaminated plants were sown in soil to which copper had been added at different dates, resulting in copper contamination of different age. Emergence of cotyledons and seedling survival were noted, and then all plants but one per pot were cut away. Thereafter the plants were left to grow until harvest, where dry weight of roots and shoots was determined.

Soil

The test medium was an uncontaminated field soil collected at the Hygum field site, Denmark (Bruus Pedersen *et al.* 1999). The characteristics of the soil were assumed to be identical to those determined for the contaminated part of the field site, with a clay content of 13.8%, a organic matter content of 4.5%, a water holding capacity of 38%, and a pH of 6.7. The copper content of the uncontaminated soil was about 22 mg/kg. The soil was dried for 24 h at 80°C and sieved through a 2-mm mesh.

Test Chemicals

Copper was added as a CuSO₄ solution, since in a pilot project the sulfate counterion proved less toxic than nitrate and chloride. CuSO₄ was mixed with soil 1 week, 5 weeks, and 12 weeks before the start of the experiment, respectively (aging period). The soil was kept at 20°C in closed plastic pots.

The nominal copper concentrations were 0, 100, 200, 300, 400, 500, and 600 mg/kg, with 10 replicates, *i.e.*, 70 pots per aging period, resulting in 210 pots. In addition, two extra pots without plants were prepared per aging period and treatment for determination of soil copper. Half a kilogram of dry soil was used per pot. CuSO₄ was dissolved in 100 ml water and mixed thoroughly in with the soil. The resulting moisture content was about half the water holding capacity of the soil. At the time of sowing (*i.e.*, start of the experiment) the aged soil was mixed thoroughly again, and any evaporated water was replaced.

Experimental Conditions

The plants were kept in a greenhouse at a day temperature of minimum 18°C and a night temperature of minimum 10°C. When light conditions (16 h) were below 5 klux (corresponding to approximately 90 µE/m²/s of photosynthesis-active light, assuming daylight conditions), artificial light of about 300 µE/m²/s was supplied, and when light intensity increased to more than 25 klux (corresponding to approximately 450 µE/m²/s), the artificial light was turned off. Three times per week, water and fertilizers were added from above as 50 or 100 ml standard fertilizer solution per pot (approximately 107 mg NO₃-N/L, 38 mg NH₄-N/L, 32 mg P/L, 231 mg K/L, 3 mg Mg/L, 200 µg B/L, 100 µg Cu/L, 142 µg Fe/L, 540 µg Mn/L, 30 µg Mo/L, and 100 µg Zn/L; pH adjusted to 6 with H₂SO₄), depending on soil humidity as judged visually. No water leached out of the pots. Pots without plants were treated the same way.

Determination of Emergence Success

Three times per week, the numbers of seeds germinating successfully (fully expanded cotyledons) and plants with true leaves were recorded for every pot. After 24 days, all were cut away, except for one plant of similar size per pot.

Plant Growth

Five weeks after sowing, the surviving plants were harvested. Total above-ground and root biomass (dry) was determined, the latter after gently washing off the soil with demineralized water, which does not remove copper adsorbed on the surface of the roots (Harrison *et al.* 1979). Dead plants were not included.

Copper in Soil

At sowing and at harvest, soil samples were taken for determination of total copper, CaCl₂-extractable copper, and DTPA-extractable copper in the pots without plants. At each occasion, the soil was thoroughly mixed before taking out two subsamples for each of the following analyses:

1. Dissolution in nitric acid as an estimate of the total extractable amount: About 0.3 g soil was boiled with concentrated (65%)

HNO₃, starting at 80°C, until all organic material had been digested. The samples were then dried at 135°C, redissolved in 0.1 M HNO₃, and analyzed with flame atomic absorption spectrometry (AAS; Perkin Elmer 4100).

The following extractions were performed to give estimates of the bioavailable fraction:

2. 0.01 M CaCl₂ (Novozamsky *et al.* 1993); 20 ml + 2 g dry soil were shaken in an end-over-end shaker for 20 h and then centrifuged. The supernatant was used for Cu analysis with AAS.
3. DTPA (Lindsay and Norvell 1978): 0.005 M diethylenetriaminepentaacetic acid (DTPA), 0.01 M CaCl₂, 0.1 M triethanolamine, pH 7.3; 20 ml solution + 2 g soil were shaken for 20 h and then centrifuged. The supernatant was analysed with AAS.

The measurements of total soil copper were checked by analysis of two certified reference materials (Institute of Environmental Chemistry Academia Sinica, China, GBW 07403, and Water Quality Institute, Denmark, VKI-J1). Recovery was 87 ± 5% and 105 ± 3% of the certified value.

Copper in Plants

The harvested plants (shoot and root) were dried, comminuted, and approximately 10 mg (or less) was digested in 65% Suprapur® nitric acid (Merck). After dissolving the pellet in 0.1 M HNO₃, the total copper content was determined using graphite furnace AAS. For some of the plants (5-week aging period), a distinction was made between fine and coarse roots, fine roots defined as the outer, very thin root parts that would easily break off when touched.

Copper determinations were checked by analysis of reference material (Institute of Environmental Chemistry Academia Sinica, China, GBW 08501), and recovery was 89 ± 8% of the certified value.

Statistics

The influence of aging period on the various soil copper extracts (HNO₃, DTPA, and CaCl₂) estimated both at the start and at the end of the experiment was evaluated by analysis of variance including an assessment of interactions, and comparisons of means were made with a Tukey *t* test. Likewise, possible aging period effects on copper accumulation in shoots, on emergence of cotyledons, and on growth of shoots and roots were tested with analysis of variance and Tukey comparisons.

Dose-response relationships for shoot and root dry weight as a function of copper were established by SAS procedure NLIN, Gauss-Newton method, applying the log-logistic model, $y = k/(1 + ([Cu]/EC_{50})^c)$, where *y* is the response parameter, [Cu] is the copper concentration, *k* is the response in controls, and *c* is the slope of the dose-response curve. For determination of both EC₁₀ and EC₅₀, the model was rewritten to

$$y = \frac{k}{1 + e^{\ln 9 \left(\frac{\ln[Cu] - \ln EC_{50}}{\ln EC_{50} - \ln EC_{10}} \right)}}$$

(Van Brummelen *et al.* 1996).

Linear regressions were performed with interactive data analysis in SAS, including automatic checks of assumptions.

All tests were evaluated at the α = 0.05 level. The phrase “tendency” is used when mean values display a clear pattern but are not necessarily significantly different.

Results

Copper in Soil

For all calculations, the measured total soil copper concentrations were used rather than the nominal values, but nominal values are referred to in order to specify treatments. Linear regression of measured total copper versus nominal concentrations gave R² values of 0.97 both at the start and at the end of the experiment (intercepts of 12 and 15 mg Cu/kg, slopes of 0.93 and 0.91 at start and end).

CaCl₂-extractable copper increased with increasing total soil copper concentrations (Figure 1), and constituted an increasing fraction of total soil copper at increasing total copper concentration but never exceeded 2%. Results for control soil are omitted because they were below detection limit (0.1 mg/kg). Values from the start the experiment were significantly lower than values from the end up to nominal soil copper concentrations of 300 mg/kg, but similar at the higher copper concentrations, except that the values for 600 mg/kg differ for the 1-week aging period (Figure 1). No clear trend in differences existed between soils aged for different periods, even though significant differences were detected at nominal concentrations up to 400 mg/kg. The pH of CaCl₂ extracts varied from 5.4 to 6.9 at the start of the experiment, and from 5.4 to 6.7 at the end of the experiment, with the highest values in controls and the lowest values at the highest soil copper concentrations.

DTPA-extractable copper also increased as a fraction of total soil copper with increasing total copper levels (Figure 2). Maximum values were close to 100% of total copper for both start and end. DTPA-extractable copper was generally unaffected by the aging period at the lower nominal concentrations, up to 400 mg/kg, whereas an effect was seen at the higher concentrations (Figure 2). Significant differences between extractions at the start and at the end of the experiment occurred at low and high soil copper concentrations. There was a significant interaction between copper and aging period (p = 0.0001), indicating that the effect of aging was stronger for the higher copper levels.

In the following, copper concentrations measured at the end of the experiments are used.

Emergence of Cotyledons

Emergence success ranged between 15 and 35%. There was no general tendency of effects of aging period on emergence of cotyledons, and emergence at elevated soil copper concentrations never differed significantly from emergence in controls.

Plant Growth

Both above-ground biomass and root biomass (dry weight) were affected by soil copper, decreasing when total soil Cu exceeded 200 mg/kg (Figures 3 and 4). The picture was the same irrespective of aging period. The fraction shoot bio-

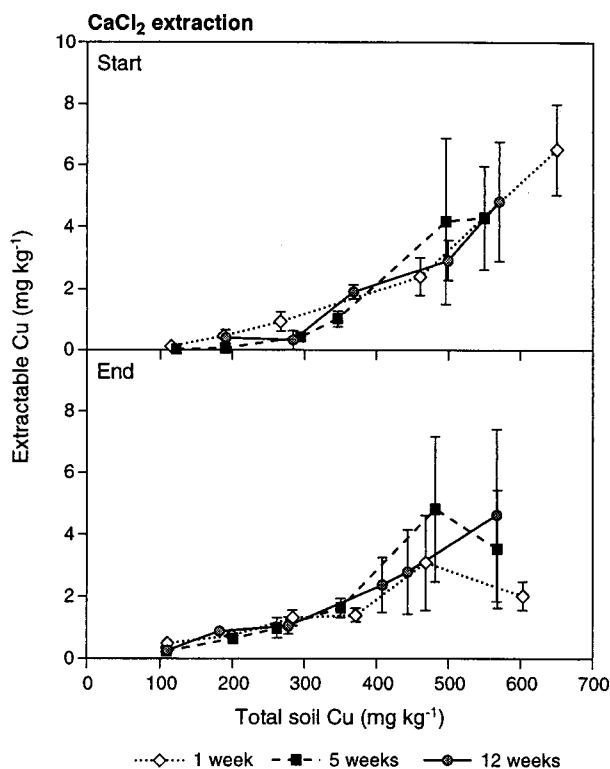


Fig. 1. Copper concentrations extractable with 0.01 M CaCl_2 at the start and at the end of a 5-week toxicity experiment with *Fallopia convolvulus*. The copper-contaminated soils were aged 1, 5, and 12 weeks before the start of the experiment. Points indicate means of four subsamples from two pots for each copper and aging treatment, vertical lines are standard deviations

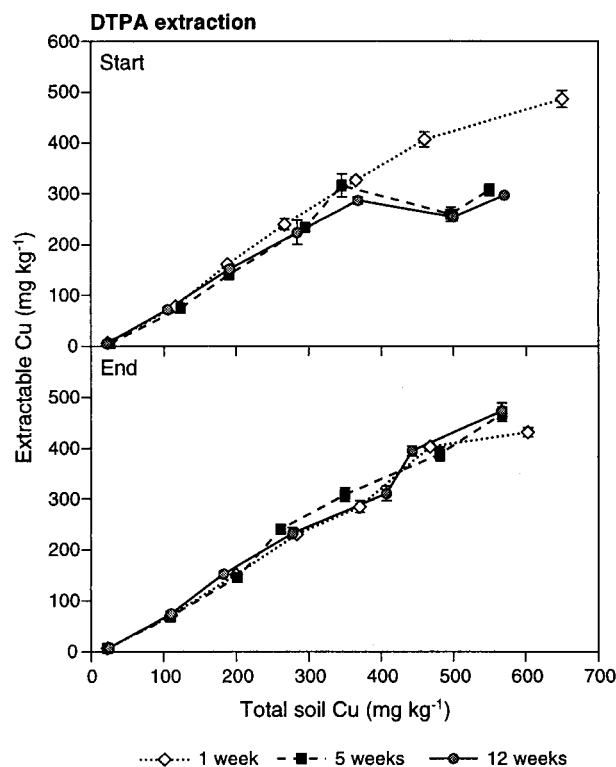


Fig. 2. Copper concentrations extractable with DTPA at the start and at the end of a 5-week toxicity experiment with *Fallopia convolvulus*. The copper-contaminated soils were aged 1, 5, and 12 weeks before the start of the experiment. Points indicate means of four subsamples from two pots for each copper and aging treatment, vertical lines are standard deviations

mass:root biomass varied around 9, with a tendency to decrease slightly above 200 mg Cu/kg soil and a slight tendency to increase again at 600 mg/kg. Some significant differences between 1 and 12 weeks aging period were detected for shoot growth, but there was no clear trend concerning aging period for neither shoot nor root biomass (Figures 3 and 4). The picture was the same when growth was expressed as function of CaCl_2 - or DTPA-extractable copper (only shown for shoot growth versus CaCl_2 -extractable copper, Figure 3).

EC_{50} values were generally very similar for the different aging periods, around 270 mg Cu/kg soil for shoot dry weight, and around 290 mg/kg for root dry weight (Table 1). EC_{10} values ranged between 177 and 257 mg Cu/kg soil for shoot dry weight, and between 227 and 259 mg/kg for root dry weight. There was no general tendency concerning aging period.

EC_{50} values for effects on shoot and root biomass were quite similar (Table 1). The same was seen for EC_{50} values based on CaCl_2 - and DTPA-extractable copper concentrations. EC_{10} values based on CaCl_2 -extractable copper ranged between 0.88 and 1.19 mg Cu/kg soil for shoot dry weight, and between 0.82 and 1.21 mg/kg for root dry weight. The corresponding ranges based on DTPA-extractable copper were 155–198 and 187–200 mg Cu/kg soil.

Copper in Plants

Accumulation of copper in shoots increased up to total soil copper concentrations of 300–400 mg/kg, and then stabilized at higher soil copper concentrations (Figure 5). Copper accumulation in shoot may be divided in three parts (Figure 5): up to about 200 mg Cu/kg soil, a moderate increase was seen; from 200 to 300 mg/kg, accumulation rate increased; and at soil copper concentrations above 300 mg/kg, accumulation stagnated. The copper concentration of shoots was always lower than the total soil copper concentration. Shoots of plants grown in soil aged for 12 weeks generally accumulated less copper than plants grown in soil aged for a shorter period, and for some copper concentrations (100, 400, and 500 mg/kg soil), this difference was significant. Copper accumulation in shoots expressed as a function of DTPA-extractable copper displayed a similar picture, whereas for shoot copper accumulation as a function of CaCl_2 -extractable copper the differences between aging periods were less clear (Figure 5).

For copper contents of roots, only results for 5 weeks' aging are shown and only for total soil copper concentrations up to 400 mg/kg, since hardly any fine roots were found at higher soil copper concentrations. For both coarse and fine root parts, copper content increased linearly with soil copper concentrations; this pattern was the same for all three extraction methods (Figure 6). The magnitude of accumulation in shoots and

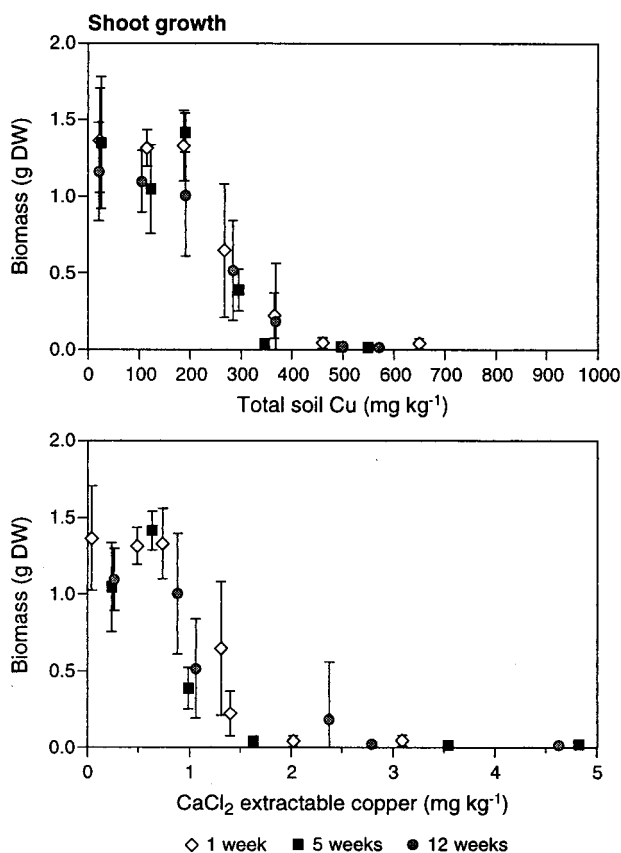


Fig. 3. Shoot biomass of *Fallopia convolvulus* harvested 35 days after sowing in copper-contaminated soil aged for 1, 5, and 12 weeks. In the upper part, growth is shown as a function of measured total soil copper, in the lower part as a function of 0.01 M CaCl₂-extractable soil copper. Points indicate means of 10 plants, and vertical lines are the standard deviations

coarse roots was comparable, with an average copper content of 0.4 times the soil copper concentration in coarse roots (slope of line in Figure 6). Fine root parts contained far more copper than coarse root parts and contained copper concentrations well exceeding (on average) about twice the soil copper concentrations (slope of line in Figure 6).

Relationships Between Effects and Accumulation

Shoot growth decreased with increased copper accumulation in shoots (Figure 7). EC₅₀ values tended to decrease with increasing aging period, from 43 mg/kg dry weight shoot to 30 mg/kg, whereas EC₁₀ values were more similar for the three aging periods, 19–25 mg/kg dry weight shoot (Table 2).

Discussion

The observed differences in copper accumulation patterns for shoots, coarse roots, and fine roots of *F. convolvulus* are probably partly due to adsorbed copper on especially the fine

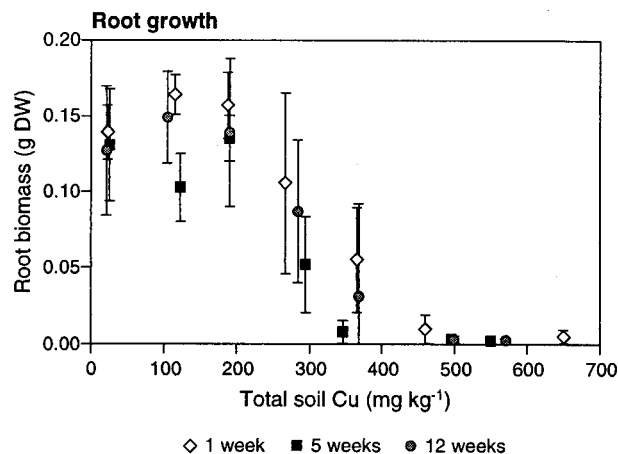


Fig. 4. Root biomass of *Fallopia convolvulus* harvested 35 days after sowing in copper-contaminated soil aged for 1, 5, and 12 weeks as a function of measured total soil copper. Points indicate means of 10 plants, and vertical lines are the standard deviations

Table 1. EC₅₀ values (mg Cu/kg dry soil) based on the logistic dose-response model for shoot dry weight and root dry weight of *Fallopia convolvulus* grown in soil treated with copper 1, 5, and 12 weeks before the start of the experiment

Extraction	Aging Period (weeks)	EC ₅₀ Shoot DW	EC ₅₀ Root DW
0.01 M CaCl ₂	1	1.31 [1.28–1.33]	1.35 [1.32–1.38]
	5	0.96 [0.95–0.98]	0.98 [0.97–0.99]
	12	1.04 [1.00–1.09]	1.08 [1.00–1.15]
DTPA	1	228 [214–242]	260 [243–276]
	5	228 [188–268]	234 [221–247]
	12	218 [198–238]	241 [227–255]
Total Cu	1	284 [266–302]	329 [305–353]
	5	258 [256–261]	260 [258–263]
	12	259 [234–284]	291 [270–312]

The effect values are based on three different soil copper measures from the end of the experiment (extraction); 95% confidence limits are given between brackets.

root parts (Harrison *et al.* 1979). However, since the copper concentrations in fine roots were always about twice as high as total soil copper concentrations, as opposed to the lower copper concentrations in coarse root parts and in shoots (Figures 5 and 6), part of this difference may be interpreted in terms of a regulation of copper accumulation and further distribution inside the plant: Most of the copper was accumulated in or on the outside of fine roots and thereby prevented from entering further into the plant, until copper concentrations became so high (approximately 200 mg/kg soil) that the regulation system, keeping copper concentrations in shoots low, broke down, and copper concentrations in shoot parts increased rapidly (Figure 5). The pattern of copper accumulation in shoots of *F. convolvulus* was reflected in the effects on growth (Figures 3 and 4). A similar accumulation pattern has been found in several other species, as reviewed by Baker and Walker (1990), Loneragan (1981), and Jarvis (1981), with moderate accumu-

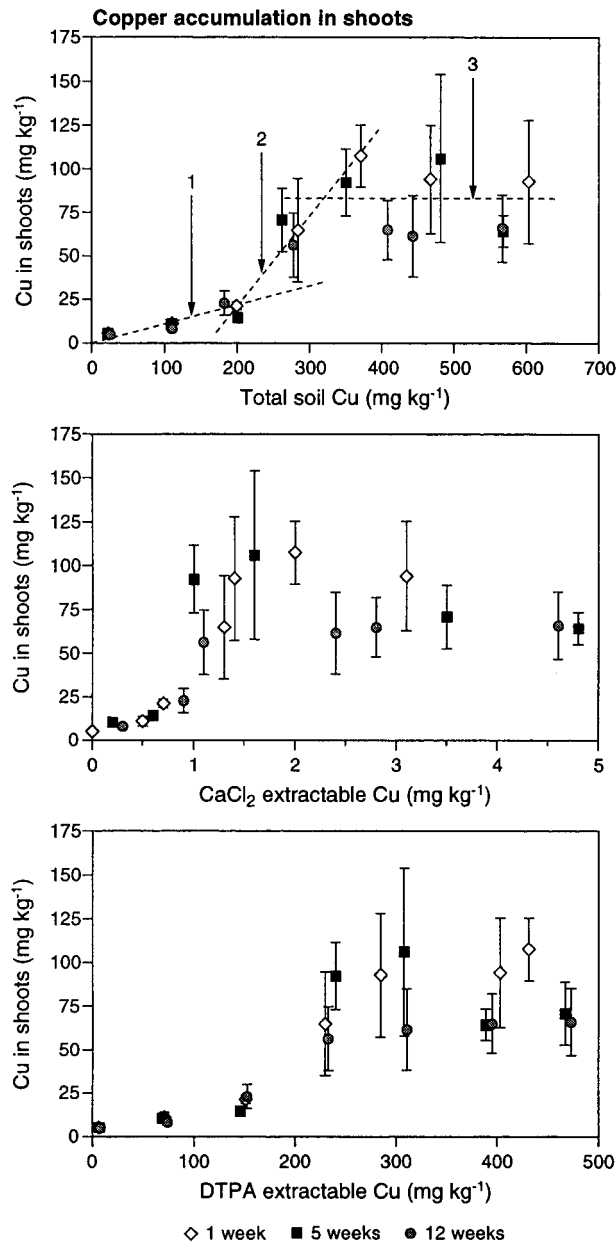


Fig. 5. Copper accumulation in shoots of *Fallopia convolvulus* grown in soil treated with copper 1, 5, and 12 weeks before the start of exposure, as a function of the three copper estimates, viz. total, 0.01 M CaCl_2 -extractable and DTPA-extractable. Points indicate means of 10 plants for the different soil copper concentrations and aging periods, vertical lines show standard deviations. Arrows and dotted lines indicate three phases of copper accumulation in shoots

lation up to a certain level, where regulation breaks down and distribution of copper within the plant is no longer restricted. Other species have different accumulation patterns, some reflecting the environmental soil copper concentrations quite precisely and some accumulating metal concentrations far above soil copper concentrations (Baker and Walker 1990).

In the present study, elevated soil copper did not significantly affect the emergence of *F. convolvulus*. Kjær *et al.*

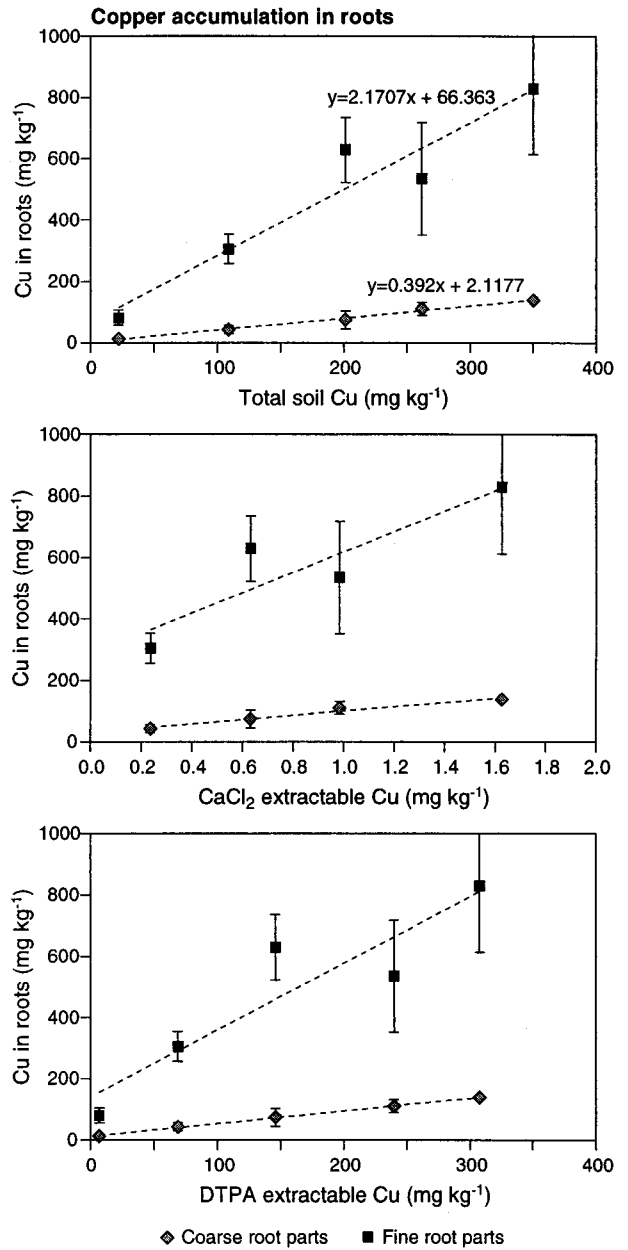


Fig. 6. Copper accumulation in fine and coarse root parts of *Fallopia convolvulus* as a functions of the three soil copper estimates, viz. total, 0.01 M CaCl_2 -extractable and DTPA-extractable. The contaminated soil was aged for 5 weeks before the start of exposure. Points indicate means of 10 plants, and standard deviations are shown as vertical lines. For total soil copper, the best linear equations are shown ($R^2 = 0.74$ and 0.86 , $p < 0.0001$). For CaCl_2 - and DTPA-extractable copper, linear relationships are also significant ($R^2 = 0.53$ – 0.87 , $p < 0.0001$)

(1998) found effects of copper on emergence of cotyledons at the copper levels studied here. However, their study was performed in a soil with less clay and organic matter, and consequently a higher fraction of CaCl_2 - and DTPA-extractable copper than the soil used in the present study, indicating a higher availability of copper. In addition, Kjær *et al.* (1998) added copper sulfate on top of the soil, which resulted in higher

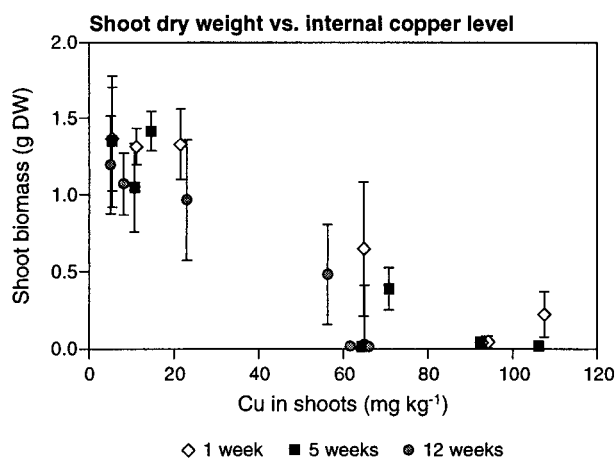


Fig. 7. Mean shoot dry weight of *Fallopia convolvulus* as a function of copper concentrations in shoots of plants grown in copper-contaminated soil aged 1, 5, and 12 weeks before the start of exposure. Points are means of 10 plants, and standard deviations are shown as vertical lines

Table 2. EC₁₀ and EC₅₀ values for shoot dry weight of *Fallopia convolvulus* grown in soil treated with copper 1, 5, and 12 weeks before the start of the experiment

Aging (weeks)	EC ₁₀ (mg Cu/kg DW)	EC ₅₀ (mg Cu/kg DW)
1	24 [17–33]	43 [37–49]
5	25 [12–50]	39 [28–55]
12	19 [15–25]	30 [26–34]

The effect values are based on accumulated copper in shoots; 95% confidence limits are given between brackets.

copper concentrations in the uppermost soil layer, where the seeds were sown, than in the rest of the soil. In the present study, EC₅₀ values for reduced growth were approximately 270 mg Cu/kg soil, which is quite consistent with the values found for *F. convolvulus* by Kjær and Elmgaard (1996) in a different soil type (less clay and less organic matter) and with a different application method (irrigation with copper solution before transplant of seedlings).

EC₅₀ values for growth based on accumulation of copper in the plant tended to differ for the shortest and longest aging periods (Table 2), with the highest EC₅₀ value for the shortest aging period, which is the opposite of what may be expected. On the other hand, EC₁₀ values for the three aging periods showed overlapping confidence intervals. If EC₁₀ is considered the concentration where effects start, an internal threshold value for copper accumulated in shoots may tentatively be set at 20 mg Cu/kg dry weight. As seen from Figure 5, 20 mg/kg dry shoot biomass coincided with the point where the accumulation rate shifted from moderate to high, at 200 mg Cu/kg soil. At accumulated copper concentrations of 60–100 mg Cu/kg, accumulation in shoots stopped (Figures 5 and 7), corresponding to the soil copper concentration (about 400 mg/kg) at which growth ceased (Figure 3). De Vos *et al.* (1991) found the same relationship between accumulation and effects for *Silene cucubalus*. The threshold value of 20 mg Cu/kg shoot for effects

on growth is equal to the critical level found by Davis *et al.* (1978) for spring barley. In a large review, Macnicol and Beckett (1985) reported critical level between 5 and > 64 mg Cu/kg dry plant for a number of crop species and estimated that as a general rule tissue concentrations below 10 mg/kg dry weight will not cause yield reduction. However, for some plant species such low tissue concentrations may cause copper deficiency (Marschner 1995).

Concerning the influence of the age of contamination on bioavailability, the time span used in this study may be too narrow to reveal any clear effects of aging period. No distinct effects of aging the freshly spiked soil were seen on extractability of copper with CaCl₂ and DTPA (Figures 1 and 2), and growth even tended to be negatively affected by aging of the contaminated soil. The length of the aging period had no clear influence on accumulation in shoots (Figure 5). Copper accumulation and effects expressed as a function of CaCl₂- or DTPA-extractable soil copper concentrations revealed the same pattern as seen for total soil copper (Figures 3 and 5).

Another study has been performed on the same soil type (Kjær *et al.* 1998) but with soil from a 70-year-old copper contamination stemming from a timber preservation plant. In the study of Kjær *et al.* (1998), no effects were seen on the growth of *F. convolvulus* at soil copper concentrations up to 928 mg/kg. The CaCl₂- and DTPA-extractable copper concentrations from the study by Kjær *et al.* (1998) may be compared to the corresponding fractions in the present study (Figure 8). There are consistent differences in the extractability of copper between these soils, but up to 500 mg/kg the differences are small. Therefore, effects on growth as a function of extractable copper show nearly the same discrepancy between field soil and spiked soil as effects based on total soil concentrations. This means that other parameters determine soil copper toxicity to *F. convolvulus*. An obvious suggestion would be the counterion sulfate, which was originally present in both kinds of copper-contaminated soil but now is probably almost unavailable in the field soil, due to leaching or binding to, *e.g.*, calcium. Studies of Schrader *et al.* (1998) and Smit and Van Gestel (1998) on arthropods indicated that the toxic action of counterions may explain part of the difference between laboratory spiked soil and field soil. Kjær and Elmgaard (1996) studied the effect of Na₂SO₄ on black bindweed compared to copper sulfate and found hardly any effects on growth but some effect on reproductive biomass. Unpublished data on conductivity of CuSO₄-spiked soil (2,000 mg Cu/kg soil), obtained by mixing 25 g soil and 100 ml demineralized water, gave conductivity values of less than 1 mS, which should not be harmful to plants at all (Schierup and Jensen 1981). All in all, the sulfate counterion probably does not account for the differences in toxicity between spiked soil and field soil.

Returning to the key questions asked in the introduction to this paper:

- Aging of copper-contaminated soil for 1 to 12 weeks had no clear impact on accumulation and effects of copper on *F. convolvulus*, and the chosen aging periods were not long enough to simulate the differences in effects seen when *F. convolvulus* was exposed to copper in soil from an old contaminated field site and in laboratory-spiked soil.
- Fine root parts apparently contained more copper and were more affected by copper than the rest of the plant, but part of

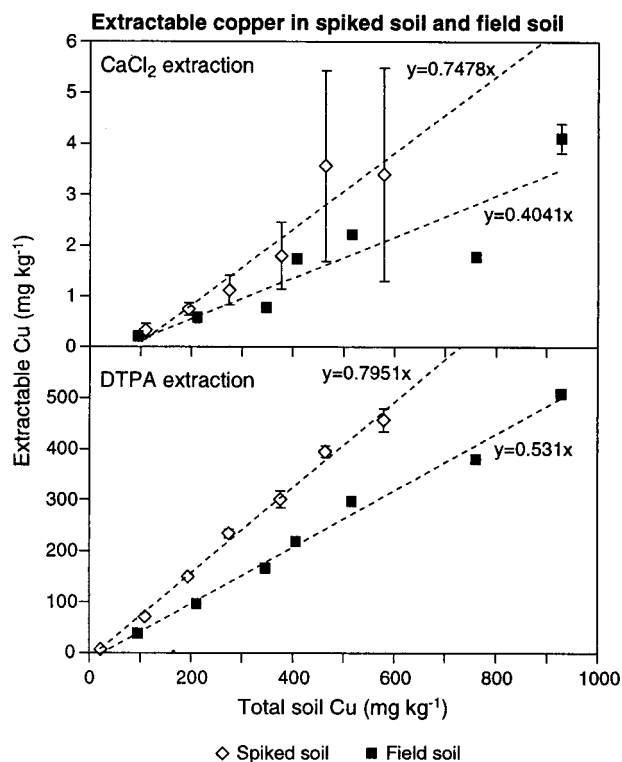


Fig. 8. Comparison of 0.01 M CaCl_2 - and DTPA-extractable soil copper concentrations for spiked soil aged 1–12 weeks (this study) and soil from a 70-year-old copper contaminated field site (data from Kjær *et al.* 1998). Each point is the mean of four subsamples from two pots or field samples, and vertical lines indicate standard deviations. Equations of best linear fits are included ($R^2 = 0.48\text{--}0.95$, $p < 0.0001$)

the copper content of fine roots may be ascribed to copper adsorption on the outside of the roots. Coarse root parts and shoots accumulated comparable copper concentrations.

- Copper accumulation in shoots could be divided in three phases. It seems likely that *F. convolvulus* copes with elevated soil copper concentrations by immobilizing excessive copper on the outside of or in the fine root parts, thereby preventing a large proportion of the copper from entering the coarse root parts and the shoots. However, there also seems to be a limit to this regulatory mechanism at about 200 mg Cu/kg soil and 20 mg Cu/kg shoot biomass, above which accumulation in shoots increases rapidly until copper concentrations get so high that growth and uptake stops.
- Copper effects on growth follows the pattern of copper accumulation in shoots. Expressing copper effects as a function of the soil copper concentrations extracted by the chosen extractants, CaCl_2 and DTPA, only removed a small part of the differences in copper effects seen between spiked soil and soil from a contaminated field site compared to total soil copper concentrations.

Thus, for *F. convolvulus* it does not seem feasible to overcome the differences in copper toxicity of spiked soil and field soil merely by aging the contaminated soil and/or by estimating the differences in availability of copper by measurements of extractable copper. Since the same is probably true for other

species and for (some of) the other parameters differing between laboratory and field exposure, field studies seem an indispensable supplement to laboratory toxicity studies. In some cases, measurements of shoot copper concentrations may be applicable, *e.g.*, for screening and comparison of different soil types or field sites.

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