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# Structural response of oregano stems to excess of soil copper

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Oregano plants (*Origanum vulgare* subsp. *hirtum*) were grown in soils containing increasing concentrations of Cu. High Cu concentrations exerted on plants a toxic effect which morphometrically, morphologically and anatomically comprised: a. reduction of the number of annual stems per plant. b. reduction of the stem length. c. thickening of the stem. d. decrease of the internode length. e. increase of the density of the stem non-glandular hairs. f. development of a large cavity in the center of the stem. g. disorganization of the stem cortex (chlorenchyma and collenchyma) and h. increase of the number of vessels in the xylem of the stem. Increased soil Cu concentration further resulted in a parallel increase of Cu, Fe, Mn, Mg and Ca bioaccumulation in the oregano stem. K bioaccumulation became greatly reduced.

**Key words:** *Origanum vulgare*, copper toxicity, stem, structure.

## INTRODUCTION

Heavy metals at high concentration in the substrate when taken up by the plants develop a toxic character, which becomes expressed with morphological and anatomical alterations or even malformations. In oregano, Cu toxicity resulted in significantly smaller leaves, chlorotic, covered by a great number of hairs (glandular and non-glandular) and stomata (Panou-Filotheou *et al*., 2001). Chloroplasts of mesophyll cells declined dramatically in number and volume resulting in reduced leaf chlorophyll content. Copper toxicity in oregano had further a negative effect on root length and total root volume, while simultaneously roots underwent extensive malformations (Panou-Filotheou & Bosabalidis, 2004). The root epidermis became destroyed and in the cortex the amyloplasts turned into leucoplasts containing dark globular inclusions and translucent droplets.

In the present work, the effects of high Cu-concentrations in the substrate on the stem of oregano (morphology, anatomy, morphometry and element analysis) were studied in order to determine the structural features of Cu toxicity in the organ.

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## MATERIALS AND METHODS

Oregano plants (*Origanum vulgare* subsp. *hirtum*) were grown in soils containing increasing concentrations of Cu (0.3, 13.0, 17.0, 19.0, 22.0, 24.5, 25.5 $\mu M\ g^{-1}$ of soil). For each concentration, there were six pots of plants in four replications. Observations were made after 2 months of growth, so that new stems had developed during the Cu treatment.

Small pieces of stems were prefixed with 5% glutaraldehyde in 0.05 M phosphate buffer, washed, and then postfixed with 2% osmium tetroxide similarly buffered. Samples were dehydrated in an alcohol series and finally embedded in Spurr's (1969) resin. Semi-thin sections for light microscopy were cut on a Reichert Om $U_2$ ultramicrotome, stained with 1% toluidine blue and observed with a Zeiss III photomicroscope.

For the morphometric assessment of the relative volumes of the stem histological components, a square lattice of point arrays, 10 mm apart, was laid over light micrographs of stem cross sections (× 120). The point-counting analysis technique was then applied (Steer, 1981).

The determination of elements was performed on dried stem material, which was ground and wet-digested in a nitric-perchloric acid (4:1 v/v) solution. Total concentrations of Cu, Fe, Mn, Mg, Ca and K were measured by atomic absorption spectrometry

(Perkin Elmer 2380).

For statistics, the experimental plant parameters were compared using regression analysis. The slope (*b*), intercept (*a*), and coefficient of determination (*r*) were estimated. To check the values which were significantly different from 0 and the slopes which differed from 1, confidence limits were determined for *t* and *n-2* degrees of freedom and for *a* and *b*, with 95% probability. The z-criterion was used for variables expressed as a percentage. Tables of correlation were created to investigate interactions between variables.

## RESULTS

Annual stems of control oregano plants are green, fleshy and flexible, with an average thickness of 2 mm. They bear scattered long non-glandular hairs, each composed of a line of cells with the terminal one having an acute apex (Fig. 1A). Among non-glandular hairs, glandular hairs producing an essential oil also occur. Annual stems are roughly 40 cm long and have about 10 distinguished nodes (Fig. 3 A, D).

Plants grown in increasing concentrations of soil Cu, exhibit a dramatic raise in the density of non-glandular hairs ascribing to the stem a heavily pubescent appearance (Fig. 1B). Stems simultaneously become inflexible and obtain a brownish col-

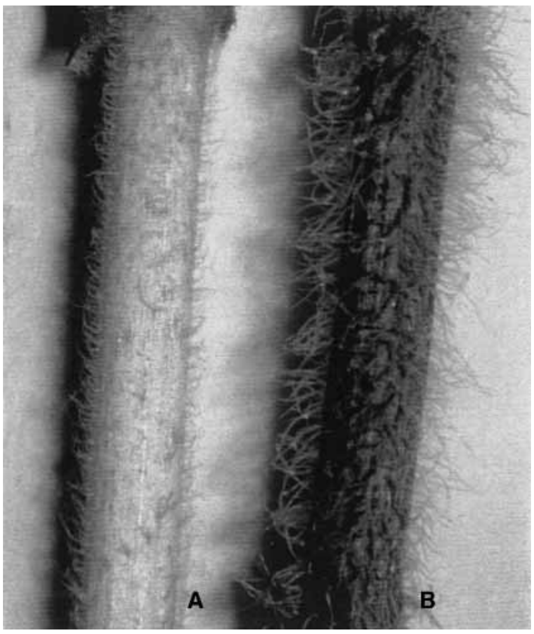


FIG. 1. Portions of annual stems of oregano. A = control, B = treated with increased concentration of Cu (24.5 μM g-1). × 5.5.

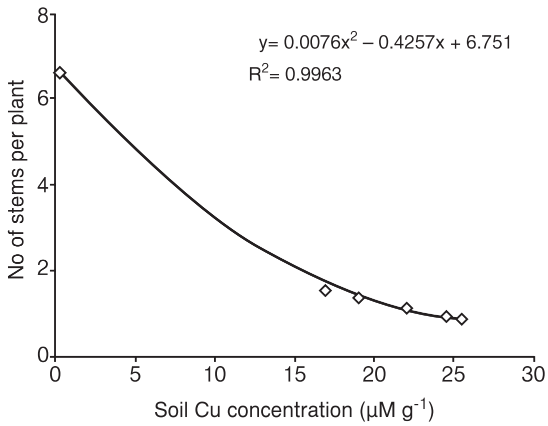


FIG. 2. Effects of soil Cu concentrations on number of stems per plant.

oration. In the period of the experiment (2 months), control plants developed an average of 6.6 stems each, while plants treated with 13.0 μM Cu developed 2.7 stems, those with 19.0 μM Cu, 1.7 stems, and the ones with 25.5 μM Cu only one short stem (Fig. 2). The correlation coefficient between soil Cu-content and number of stems per plant is high (0.97) which confirms the toxic effect of Cu on oregano. Under Cu stress, the stem length becomes decreased, while in parallel the stem thickness significantly increases (Fig. 3A, B). The number of nodes per stem does not remarkably alter (Fig. 3D), a fact revealing that stem shortening is principally due to shortening of internodes (Fig. 3C).

Anatomical studies on oregano control plants showed that the stem in cross-section exhibits a square profile, typical for the members of the Lamiaceae family (Fig. 4A). The epidermal cells are small, closely arranged, and many of them are differentiated into glandular – and non-glandular hairs. At the four stem corners, a collenchyma pole is developed in the hypodermal area. In the cortex, apart from collenchyma, several layers of chlorenchyma cells (giving to the stem a green coloration) also occur. The conductive tissue is also located at the four stem corners and has a well-developed xylem. The pith is compact and is composed of parenchyma cells with varying size. The larger pith cells are centrally cited, while the smaller ones are distributed to the periphery.

The stem of oregano growing in excess of copper exhibits anatomically a number of differences compared to the control stem (Fig. 4B). Thus, the stem profile in cross section is much larger, a fact principally due to the increase of the volume of pith. The

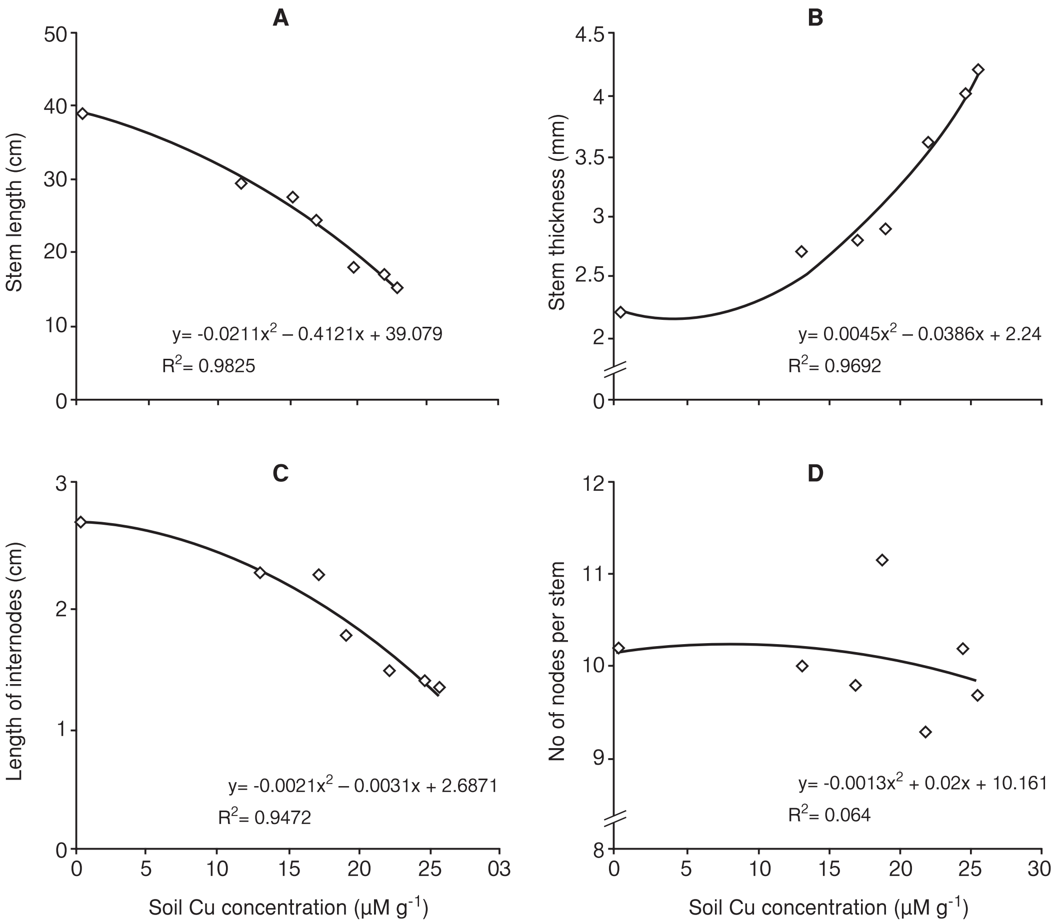


FIG. 3. Effects of soil Cu concentrations on plant morphometric parameters.

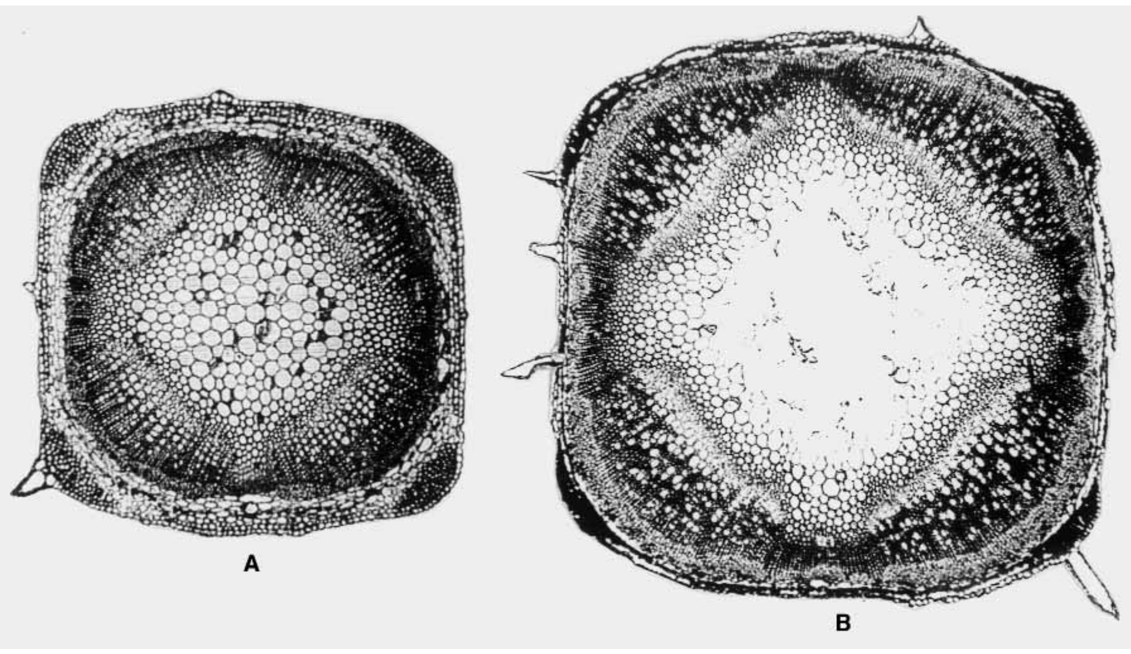


FIG. 4. Anatomy of oregano stems in control (A) and Cu-treated (B) plants. × 50.

pith does not remain any longer compact, but its central region becomes disorganized resulting in an open cavity. In the xylem, the number of vessels is remarkably increased. The cortical cells (collenchyma and chlorenchyma) are disintegrated forming a dark zone. The epidermal cells, however, appear in good shape and a great number of them differentiate into non-glandular hairs.

Atomic absorption analyses showed that the bioaccumulation of Cu, Fe, Mn, Mg and Ca in the stem increases parallel to the increase of soil Cu-concentration (Fig. 5). Increased soil Cu-concentration further resulted in a high decrease of K bioaccumulation, particularly at the upper part of the stem. In the cases of Cu, Fe, Mn and Mg, bioaccumulation was somehow higher in the lower part of the stem

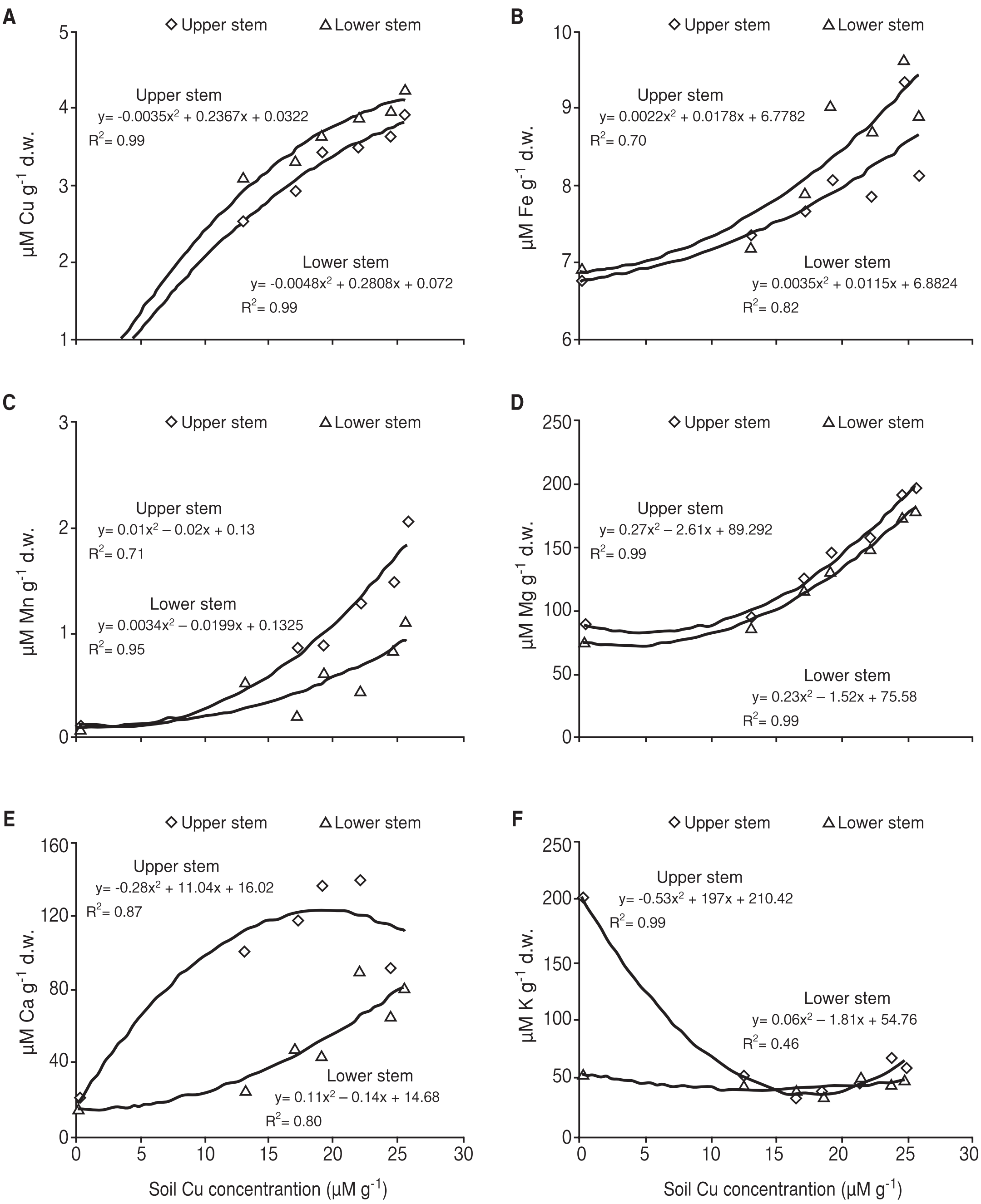


FIG. 5. Effects of soil Cu concentrations on stem bioaccumulation of Cu (A), Fe (B), Mn (C), Mg (D), Ca (E) and K (F). Upper stem = above the 5th node from the stem basis; Lower stem = below the 5th node.

TABLE 1. Relative volumes (%) of the histological components of the stem (cross section) (± SD, n = 12, * significantly different from the control, $p < 0.05$)

| | Cu concentration (μM g$^{-1}$ of soil) | | |
|---|---|---|---|
| | 0.3 (control) | 13.0 | 25.5 |
| Epidermis | 5.27±0.65 | 5.46±0.54 | 5.79±0.51 |
| Cortex | 28.67±2.73 | 20.90±2.03 | 11.83±2.02* |
| Phloem | 9.75±2.85 | 9.46±1.66 | 9.01±0.87 |
| Xylem | 27.15±4.12 | 31.01±2.81 | 35.48±3.08* |
| Pith | 29.16±2.10 | 33.17±2.73 | 37.89±3.60* |

(below the 5$^{th}$ node) compared to the upper part (above the 5$^{th}$ node). For Ca and K, just the opposite held true.

## DISCUSSION

The principal morphological alterations associated with copper toxicity in oregano were a reduction of the number of annual stems per plant and a shortening of the stem length. Both parameters reflect a reduction of the plant aerial biomass, which suggests an inhibitory effect of Cu on meristem activity (Eleftheriou & Karataglis, 1989). The reduced photosynthetic rate attributed to the excess of Cu (Lidon & Henriques, 1993, Moustakas *et al*., 1994) may additionally contribute to the restricted growth of oregano. The fact that stems become shorter by decreasing the length of their internodes (and not the number of nodes) indicates that Cu has a negative effect not only on cell division, but also on cell elongation. Shortening of stem and decrease of internode length have been reported for other heavy metals (Cd, Zn, Pb, Ni, Cr, Hg) occurring in increasing concentrations in the substrate (Heumann, 1987; Karataglis *et al*., 1988).

The anatomical and morphometric studies on stem cross sections of oregano revealed that the histological components which appeared significantly affected by Cu toxicity were the cortex, pith and xylem. The relative volume of the cortex became reduced from 28.67% (control) to 11.83% (Cu-treated), because of the disorganization of the parenchyma and collenchyma tissues. The volume of the pith region increased by 29.94% through the development of a large central cavity formed by tearing apart of the pith cells. The great restriction of the parenchymatic tissue (cortical and medullar) under Cu stress implies that stem becomes mostly composed of vascular tissue, a fact entailing hardening and stiffening of the organ.

Of significance is the observation that Cu stress in oregano resulted in an increase of the number of vessels in the xylem of the stem (the increase in xylem volume principally reflects an increase of the number of vessels, since vessel diameter does not remarkably alter). The higher number of vessels may facilitate the movement of water (absorbed in low amounts, because of the structural damage of the root; Panou-Filotheou & Bosabalidis, 2004) and also constitute a water depository mechanism. According to Aloni (1987), the presence of stress factors (toxic Cd concentrations) results in stems with more numerous vessels (increasing levels of auxin). In severely Cu deficient plants, Rahimi (1972) has found a reduced number of xylem elements, a feature also observed in plants treated with toxic levels of Cd, Zn, Al and Cr (Barceló & Boschenrieder, 1990). The formation of a dense pubescence in the stems of oregano plants grown in high Cu-concentrations is rather attributed to the stress conditions developed by Cu toxicity, as in other relevant cases (Barceló *et al*., 1988; Panou-Filotheou *et al*., 2001).

The concentration of Cu in the substrate influences the uptake and bioaccumulation in the stem of other elements. Bioaccumulation in stems of toxic metals does not seem to follow a common pattern in the various species. Thus, in *Origanum vulgare*, increasing concentrations of Cu in the substrate affected positively stem bioaccumulation of Fe, Mn, Mg, and Ca and negatively that of K. In *Thlaspi ochroleucum*, high concentrations of Cu seemed to inhibit Ca, Mg, K and Fe bioaccumulation (Ouzounidou *et al*., 1992), while in *Oryza sativa*, Ca, Mg, K, Na and Al concentrations in stem tissues did not reveal any correlation with increasing Cu level (Lidon & Henriques, 1993). Copper bioaccumulation is generally higher in the stems (entire) of young seedlings and becomes decreased towards maturity (Loneragan *et al*., 1980). When Cu exists in the substrate in adequate amounts, plants accumulate more Cu in the stem apical part rather than in the basal part. By contrast, Cu deficiency implies higher Cu-accumulation in the basal stem part than in the apical one (Loneragan, 1981). In oregano, increasing Cu-concentrations in the substrate resulted that Cu bioaccumulation in the apical and basal parts of the stem did not significantly differ. This refers again to the functional particularity of plant species in general.

Considering the specific alterations (under Cu stress) in oregano stem morphology and structure and their evaluation by morphometric assessments, it could be suggested that increasing Cu concentrations have a toxic effect on stem. This effect becomes anatomically expressed by a disorganization of a great amount of parenchymatic tissue in the stem cortex and pith. Additional physiological studies (endogenous gibberellic acid and other phytohormones, phenoloxidase and other enzymes, saps of vessels and sieve tubes, etc.) would strengthen structural data and provide grounded interpretations as to the manner of toxic action of Cu.

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