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Concentrations of Cu, Growth, and Chlorophyll Content of Field-Cultivated Wheat Growing in Naturally Enriched Cu Soil

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The Serbo-Macedonian massif of northern Greece is notable for the occurrence of numerous small areas of sulphide mineralisation (Reeves et al. 1986). Varying degrees of porphyry copper mineralisation, associated with post-Miocene volcanic rocks of rhyolitic composition, can be encountered in agricultural fields which are used mainly for wheat production. Although Cu is a trace element essential to plant nutrition, in excess, it is phytotoxic causing stunted growth, chlorosis and root malformation (Kabata-Pendias and Pendias 1984; Van Assche and Clijsters 1990; Punz and Sieghardt 1993). Previous studies on wheat growing in these naturally enriched Cu soils have shown that plants have reduced growth, chlorosis and chloroplast ultrastructural changes (Eleftheriou and Karataglis 1989) and a reduced efficiency of the photochemistry of photosystem II (PSII) (Lanaras et al. 1993). The objective of this study was to examine the relationships between the Cu concentration of the soil and the plant tissue Cu concentrations, growth and chlorophyll content of field-cultivated wheat growing in soils with varying degrees of porphyry copper mineralisation.

MATERIALS AND METHODS

Wheat plants *(Triticum aestivum* L. cv. Vergina) and soil samples from around their roots were collected along a naturally occurring Cu gradient in a cultivated field, located near the village of Gerakario (41° 07'N, 22° 55' E), Province of Kilkis, Northern Greece in June 1993. Plant samples were transferred within 2 h to the laboratory for analysis. Taller plants had reached early anthesis.

Leaf discs (1 cm^2) were cut from one of the pair of youngest fully expanded leaves, approximately one third of the distance from the base of the leaf. Individual discs were pulverised in liquid nitrogen and chlorophyll was extracted in 90% acetone for 30 min at 4 °C. After centrifugation of extracts at 10 000 g for 3 min, total chlorophyll was determined from the values of absorbance at 664 nm and 647 nm using the extinction coefficients given by Jeffrey and Humphrey (1975).

Leaf area was measured using a Mk2 Area Meter (Delta-T Devices Ltd, UK) connected to a TC7000 Series Camera (Burle Industries Inc, USA). Plant height refers to the length of the stem.

Soil and plant tissue (roots, stems, leaves) were oven-dried at 80 °C to constant

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weight. Samples (0.5 g) were digested with a nitric-perchloric acid solution (4:1 v/v) for 5 h at 150 °C. The concentration of Cu was determined in an atomic absorption spectrophotometer (Perkin Elmer 2380) using SpectrosoL (BDH Chemicals Ltd, Poole, England) as a standard solution. All concentrations measured for the samples were within the linear range (0-5.0 mg L^{-1}) specified by the manufacturer for Cu. The detection limit (mg L^{-1}) of the absorption spectrophotometer operated with a flow spoiler and using an air-acetylene flame was 0.002 for Cu.

Differences between means were assessed using one way analysis of variance (ANOVA) followed by a multiple comparison test (LSD) (Sokal and Rohlf 1981). Single regression analysis, followed by analysis of covariance (Sokal and Rohlf 1981) was used for testing the relationships between the Cu concentration in the soil and the Cu concentration in the plant tissue and chlorophyll concentration.

RESULTS AND DISCUSSION

The soil Cu concentration ranged from 106-419 (mg kg^{-1} DW) along a naturally occurring Cu gradient in the field. There were significant differences (ANOVA $P < 0.001$) between the highest and lowest soil Cu concentrations. The mean concentrations of Cu in non-contaminated soils range from 6-60 mg kg^{-1} DW and total concentrations of Cu in soils considered to be phytotoxic are between 60-125 mg kg^{-1} DW (Kabata-Pendias and Pendias 1984). The soil Cu concentrations measured in the study area (106-419 mg kg^{-1} DW) indicate that the soils were Cu-contaminated and potentially phytotoxic even at the lowest soil Cu concentrations.

The Cu concentration in the roots, stem and leaves ranged from 57-410, 4-14 and 9-27 (mg kg^{-1} DW), respectively. Natural Cu concentrations in various plants growing in unpolluted regions range from 1-10 mg kg^{-1} DW, while excessive or toxic levels range from 20-100 mg kg^{-1} DW. The normal concentration of Cu in mature leaf tissue ranges from 5-30 mg kg^{-1} DW (Kabata-Pendias and Pendias 1984). Wheat tissue Cu concentrations were therefore potentially phytotoxic.

The relationships between the soil Cu concentration (S) and the plant tissue Cu concentration (Pt) were linear (Fig. la, c). The relationships for roots and leaves were biphasic with break points corresponding to soil Cu concentrations of 284 and 273 mg kg^{-1} DW and tissue Cu concentrations of 134 and 10 mg kg^{-1} DW, respectively (Fig. 1a,c). The models best describing the relationships were of the form $Pt = a + bS$, where a is the intercept and b the slope of the regression line, and good fits exist between the models and the data. The equations of the fitted models are given in the legend to Fig. 1. Wheat plants initially excluded Cu from the leaves and had a low Cu uptake in the roots. Above the breakpoint the plant tissues had a higher Cu uptake with increasing soil Cu concentration, with maximum concentrations of 410 and 27 mg kg^{-1} DW in the roots and leaves, respectively in the plants studied. The wheat stems had the lowest Cu content. The stem Cu content demonstrated a slight increase, which was not biphasic, over the range of soil Cu concentrations encountered (Fig. la). This is consistent with the role of the stem as a conducting element, rather than a sink for mineral accumulation. The [leaf]/[root] partition ratio was less than one and decreased with increasing soil Cu concentration, tending to a lower limit of about 0.06 (Fig. lc, inset).

The concentration of Cu in plant tissues is generally a function of its level in the nutrient solution or soil, but the pattern of the relationship differs among plant species and plant parts. The majority of plant species accumulate higher

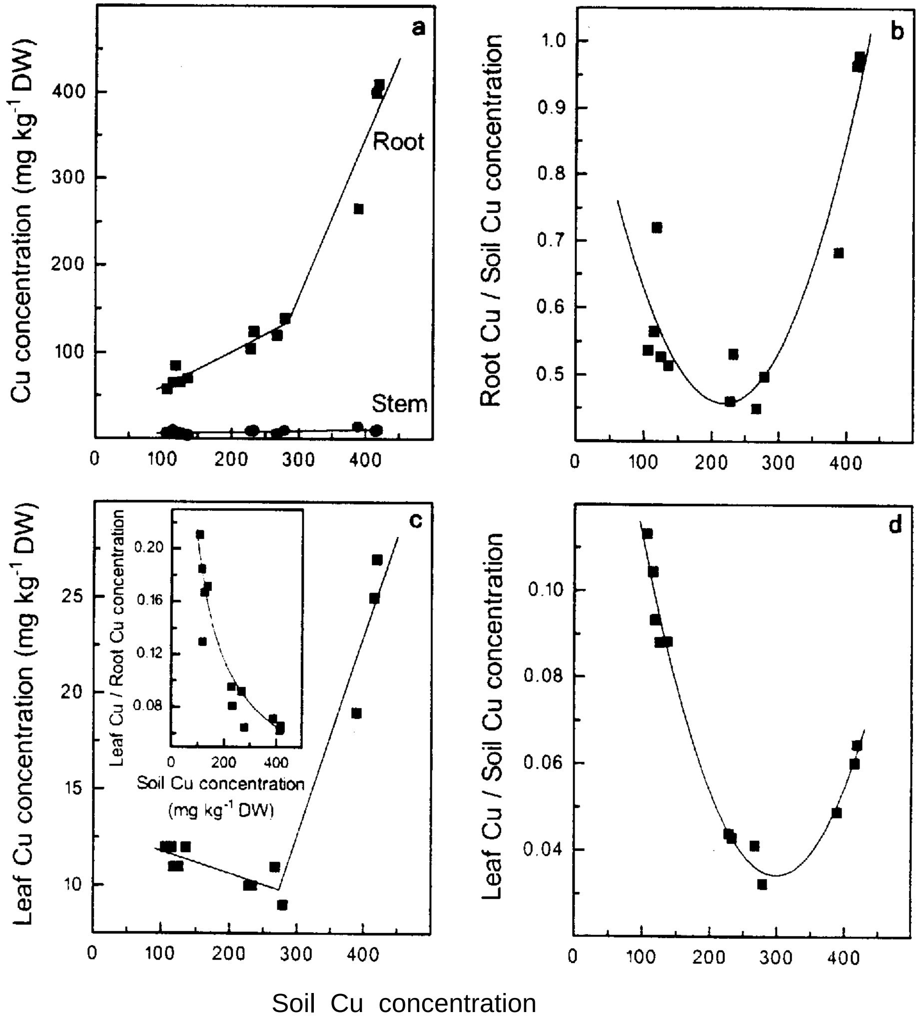

Figure 1. The relationship between the Cu concentration in the roots and stem (a). leaves (c). the [leaf]/[root] partition ratio (c, inset) and the soil Cu concentration, and between the ratio of the plant tissue Cu concentration to the soil Cu concentration (Pt/S) against the soil Cu concentration for the roots (b) and leaves (d) for field-cultivated wheat growing on varying soil Cu concentrations. The regression models best describing the relationships given in the figure, with determination coefficient (R-) and significance level (P) are as follows: (a. roots) for $x = 0\text{-}284$, $y = 20.030 + 0.405x$, $R^2 = 0.914$, $P < 0.001$, for $x > 284$, $y = -386.880 + 1.839x$, $R^2 = 0.926$, $P < 0.05$; (a, stem) $y = 4.479 + 0.015x$, $R^2 = 0.464$, $P < 0.05$; (b) $y = 1.20\ 10^{-5}x^2 - 0.005x + 1.03$, $R^2 = 0.858$, $P < 0.05$; (c) for $x = 0\text{-}273$, $y = 12.996 - 0.012x$, $R^2 = 0.642$, $P < 0.05$, for $x > 273$, $y = -18.678 + 0.104x$, $R^2 = 0.932$, $P < 0.05$; (c, inset) $y = 9.224x^{-0.826}$, $R^2 = 0.791$, $P < 0.001$; (d) $y = 1.97\ 10^{-6}x^2 - 0.001x + 0.211$, $R^2 = 0.982$, $P < 0.05$.

concentrations of Cu in the roots (Kabata-Pendias and Pendias 1984; Baker and Walker 1990; Punz and Sieghardt 1993) and the Cu concentration in the wheat roots was an order of magnitude greater than that in the leaves. The biphasic

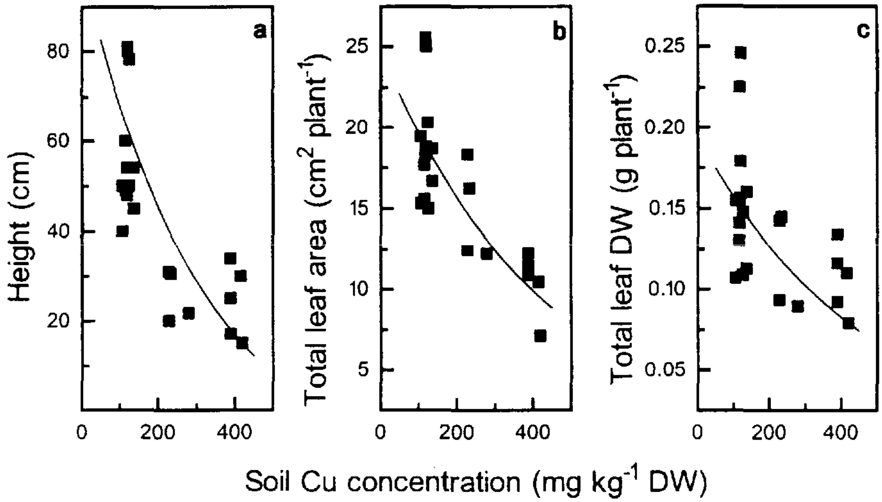

Figure 2. The relationship between the height (a), total plant leaf area (b) and total plant leaf DW (c) of field-cultivated wheat and soil Cu concentrations. The regression models best describing the relationships given in the figure. with determination coefficient (R^2) and significance level (P) are as follows: (a) $y=\exp(4.337-3.2\ 10^{-3}x)$, $R^2=0.670$, $P<0.001$; (b) $y=\exp(3.213-2.3\ 10^{-3}x)$, $R^2=0.704$, $P<0.001$; (c) $y=\exp(-1.639-2.14\ 10^{-3}x)$, $R^2=0.510$, $P<0.001$.

plant-soil relationship exhibited by wheat is characteristic of plants which have “excluder” strategy (Baker 1981). Shoot metal concentrations are maintained at a constant low level until a critical soil concentration is reached. Toxicity then ensues and unrestricted metal transport occurs. Critical soil Cu concentrations were in the range of 250-300 mg kg^{-1} DW for wheat. Cu uptake by rice roots in solution culture also consists of two distinct accumulation phases (Lidon and Henriques 1992). However, the Cu tolerant species *Rumex acetosella, Thymus sibthorpii, Minuartia verna,* which grow on porphyry Cu and sulphide mineralisation in northern Greece, have linear plant-soil Cu relationships for soil Cu concentrations ranging from 15-10000 mg kg^{-1} DW, enabling these plants to be used as indicators in biogeochemical mineral prospecting (Reeves et al. 1980).

The relative accumulation coefficient, Pt/S, decreased with increasing soil Cu concentration with a lower limit at soil Cu concentrations of 220 and 300 mg kg^{-1} DW for roots and leaves, respectively (Fig. 1b,d). This phase corresponds to the low uptake of Cu in the roots and shoot. Above these soil Cu concentrations the ratio increased indicating that the plant tissues, particularly the roots, take up increasing amounts of Cu in relation to the soil Cu concentration. This fact probably reflects the breakdown of an exclusion mechanism which involves increased membrane permeability. The model that best described these relationships was $Pt/S = a(S)^2 + bS + c$. Parameters derived from the regression model are given in the legend to Fig. 1.

Although the relative accumulation of Cu by plant tissues was higher at soil Cu concentrations greater than 300 mg kg^{-1} DW, and tissue concentrations increased, the [leaf]/[root] partition ratio decreased and stabilised (Fig. 1c, inset) indicating that a strong exclusion mechanism was still operating to prevent Cu from reaching the leaves. This would suggest that the primary exclusion mechanism involved is the restriction of internal metal transport from the root to the shoot. Although Cu can pass freely into the apoplast, restricted uptake at the endodermis can occur since it is subject to metabolic control (Baker 1990). [Shoot]/[root] partition ratios have been used as a measure of the restriction of metal transport with ratios of less than one for "excluders" and greater than one for "accumulators" (Baker and Walker 1990). Reilly and Reilly (1973a) reported [leaf]/[root] copper ratios ranging from 0.003-2.61 for a range of species, with the lowest values being typical of grasses and the highest of trees. Values for wheat ranged from 0.063-0.211, similar to those reported for grasses and are consistent with wheat being an excluder of Cu.

Plant height, total leaf area and total leaf DW per plant all decreased with increasing soil Cu concentrations (Fig. 2). There were significant differences between plant height (ANOVA $P < 0.001$), total leaf area (ANOVA $P < 0.001$) and total leaf DW (ANOVA $P < 0.05$). The regression models best describing the relationships between plant morphometric characteristic (y) and soil Cu concentration (S) were of the form $y = \exp(a + bS)$, where a is the intercept and b the slope of the regression line. The equations of the fitted models are given in the legend to Fig. 2. The ratios of plant height to total leaf area and total leaf area to total leaf DW decreased slightly with increasing soil Cu concentration however, the decreases were not statistically significant.

The total chlorophyll concentration of wheat decreased with increasing soil Cu concentrations and was significantly lower (ANOVA $P < 0.05$) at the highest soil Cu concentrations. The model best describing the relationship between chlorophyll concentration and soil Cu concentration was $Chl = \exp(a+bS)$ (Table 1). Chlorophyll a and b also decreased exponentially with increasing soil Cu concentrations (Table 1). The slopes of the regression lines were significantly greater than zero (analysis of covariance; $P < 0.05$) and there were no significant differences between them (analysis of covariance; $P > 0.05$) indicating that the ratio of chlorophyll a/b remained unchanged.

Table 1. Parameters of the regression model $Chl = \exp(a + bS)$ where Chl is the chlorophyll concentration (µg cm^{-2}), either total, a or b, S the soil Cu concentration (mg kg^{-1} DW), a the intercept, b the slope, R^2 the determination coefficient and P the significance level.

Parameter	Total Chl	Chl a	Chl b
a	4.268	3.939	2.299
b	-0.004	-0.004	-0.005
R^2	0.418	0.407	0.443
P	<0.001	<0.001	<0.001

Heavy metal accumulation in leaves can effect pigment content, stomatal function, chloroplast structure and metabolism, and photosynthetic enzymes leading to reductions in net photosynthetic rate and growth (Clijsters and Van Assche 1985; Van Assche and Clijsters 1990). Heavy metals in excess generally cause a decrease in total chlorophyll content (Clijsters and Van Assche 1985) and chlorosis can result from heavy metal-induced Fe- or Zn-deficiency. (Reilly and Reilly 1973b; Kabata-Pendias and Pendias 1984).

In this study, the levels of Cu encountered in the fields were phytotoxic to wheat causing growth inhibition and chlorosis, with plant tissue Cu concentrations increasing with increasing soil Cu concentrations. Cu was primarily accumulated in the root and excluded from the shoot, indicating restricted transport of Cu across the endodermis. At soil concentrations greater than 270 mg kg^{-1} DW Cu uptake markedly increased in the root and shoot, though transport of Cu to the shoot continued to be restricted.

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