INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2006/08–1–123–128
http://www.fspublishers.org

Changes Induced by Copper and Cadmium Stress in the Anatomy and Grain Yield of *Sorghum bicolor* (L.) Moench

WEDAD A. KASIM
Botany Department, Faculty of Science, Tanta University, Tanta, Egypt
E-mail: wedkasim@yahoo.com

ABSTRACT

Sorghum bicolor plants were grown in sandy soil for two months and irrigated with half-strength Hoagland solution supplemented with 10^{-5} M $CuSO_4$, 10^{-6} M $CdSO_4$ or a mixture of both (1:1, v/v). Copper or cadmium applied alone or in combination caused significant reduction in root diameter, width and thickness of leaf midrib, diameter of xylem vessels of all seedling organs, parenchyma cell area in the stem, leaf midrib and pith and cortex of root, dimensions of stem vascular bundles, number of xylem arms in root, and frequency of stomata on abaxial leaf surface. Total number of grains/plant, number of filled grains/plant, weight of 1000 grains and the percentage of filled grains were severely reduced in response to different treatments. The effect was more pronounced when Cu and Cd were applied together. Such reduction in grain yield might be due to the heavy metal-induced changes in the structure and consequently the function of the vascular and stomatal apparatus.

Key Words: Anatomy; Cadmium; Copper; Grain yield; *Sorghum bicolor*

INTRODUCTION

Despite their potential physiological and economical significance, anatomical alterations induced by heavy metals in plants have so far been grossly overlooked. Furthermore, few studies are carried out to date to investigate the impact of heavy metals on one or a few anatomical parameters of the plant and therefore a coordinated investigation on a broad scale to asses the impact of heavy metal stress on the structure of the plant vegetative body seems crucial (Khudsar *et al*., 2001; Panou-Filotheou & Bosabalidis, 2004). Similarly, the impact of heavy metals on the yield of economically important crop plants was unduly neglected (Khan *et al*., 2003; Wu *et al*., 2003).

Plants are seldom exposed in nature to the effect of a single heavy metal since excess metal ions exist mostly in mixtures in variously polluted soils and irrigation water worldwide (Souza & Rauser, 2003). However, only a few studies dealing with the interactive effects of a combination of heavy metals on plants are so far on record (Zeid, 2001; Yang *et al*., 2004).

The present study was undertaken to investigate the changes induced by Cu^{2+} (as an essential micronutrient) and Cd^{2+} (as a non-essential heavy metal), either singly or in a combination, in the anatomy of the stem, leaves and root as well as in the grain yield and fertility of the economically important grain crop *Sorghum bicolor* (L.) Moench.

MATERIALS AND METHODS

Grains of *Sorghum bicolor* were supplied by the Ministry of Agriculture, Giza, Egypt. The grains were kept at room temperature (24 ± 2°C). They were washed in distilled water, sown in plastic pots (10 grains per pot) containing acid washed sandy soil and irrigated with distilled water for one week until full germination at 25ºC ± 1, under 16 h photoperiod and 62% ± 2 relative humidity. The seedlings were then irrigated every other day for two months with half-strength Hoagland solution (Hoagland & Arnon, 1950) supplemented with either 10^{-5} M copper sulphate, 10^{-6} M cadmium sulphate, or a combination of both solutions (1:1, v/v). The two concentrations of heavy metals were chosen after a preliminary experiment in which they caused moderate inhibition of germination. Control plants were irrigated with half-strength Hoagland solution only. Three plastic pots were prepared for each treatment. After two months, one plant of each pot was harvested for anatomical study. The seedlings were then left to grow under the same growth conditions until flowering and fruiting. The grains of 10 plants from the three pots of each treatment were collected after 3.5 months of treatments in order to determine the grain yield.

Anatomical measurements. Permanent cross-sections of stems, roots and leaves of two-month-old plants were prepared. Sectioning was carried out in the stem and root at two cm above and below the transition zone, respectively. Leaf sections were cut in the middle part of the midrib of the third leaf above the soil surface. The mean cell area (in μm^2) of parenchyma in stem ground issue, mesophyll of leaf midrib, root cortex and root pith was measured by counting the number of cells in accurately specified areas in the cross sections. The number of replicates for each of the four parenchyma tissues is 15 (five measurements x three replicate plants). To test the accuracy of this method, photographs of cross sections were scanned into the computer, and mean cell areas were measured directly on the monitor from other randomly chosen areas of the four parenchyma tissues using the Adobe Photoshop 7.0 ME program. Results of both methods coincided remarkably. The diameter of metaxylem vessels was measured in the largest ten vascular bundles of the stem, and the mean of 60 measurements (ten bundles x two vessels per bundle x three replicate plants) was recorded. Similarly, the mean of 18 measurements made from the leaf midrib (three large bundles x two vessels per bundle x three plants) was recorded, and the mean of 24 measurements (the largest eight xylem arms x one vessel per arm x three replicate plants) was recorded from the roots. Width and radial length of the largest ten vascular bundles in cross section of the stem were recorded. Similarly, the dimensions of the median and the largest two lateral vascular bundles in the leaf midrib were measured. The stomatal frequency was calculated (as the number of stomata mm^{-2}) in strips of the lower (abaxial) epidermis of the third leaf above the soil surface (five counts x three replicate plants). All measurements were made using a calibrated ocular micrometer.

Grain yield. Mean grain yield of 3.5 month-old plants was expressed as the weight of 1000 intact and chaffed grains, the total number of grains per plant (A) and the number of filled grains per plant (B). The percentage of filled grains was calculated as the ratio of (B) over (A).

Statistical analysis. All data were subjected to one-way ANOVA analysis to calculate the least significant difference (LSD) using the SPSS v. 10 package.

RESULTS

The applied heavy metal concentrations induced a number of measurable alterations in the anatomy of the vegetative organs as well as in the grain yield of sorghum plants. The basic anatomical features of *Sorghum bicolor* were described in detail by Clifford and Watson (1977), Gould and Shaw (1983) and Salih *et al.* (1999).

Anatomical changes. While neither Cu nor Cd had an effect on the thickness of the stem except when in combination, they induced significant reduction in the root diameter, more so with Cu + Cd (Fig. 1A). Width, total thickness and the thickness of mesophyll parenchyma of the leaf midrib were significantly diminished by the different treatments, Cu and Cd in combination caused the greatest effect (Fig. 1B). Most of the reduction in midrib total thickness (Fig. 1B) and in root diameter (Fig. 1A) was due to a clear decrease in the thickness of their parenchyma tissues.

Dimensions of vascular bundles in stem (Fig. 1C) and in leaf midrib (Figs. 1D & 1E) were reduced considerably by the different treatments. The reduction was greater in vascular bundles of the stem than in those of the leaf midrib. The effect of individual treatments was in the order: Cu + Cd > Cu > Cd, indicating an additive effect between Cu and Cd.

Table I. The percentage reduction caused by 10^{-5} M Cu, 10^{-6} M Cd or Cu + Cd in parenchyma cell area (PCA) of stem ground tissue, mesophyll of leaf midrib, root cortex and root pith, diameter of metaxylem vessels (DMX) in stem, leaf midrib and large xylem arms of root, and in grain yield of *Sorghum bicolor.*

Criteria	Tissue/Organ	% reduction		
		Cu	Cd	Cu + Cd
PCA	Stem ground tissue	21.13	23.17	29.67
	Mesophyll of leaf midrib	23.87	31.22	37.68
	Root cortex	35.59	39.81	41.63
	Root pith	33.25	38.17	42.50
DMX	Vascular bundles of midrib	12.80	11.54	10.15
	Vascular bundles of stem	18.25	14.76	15.95
Number	Large xylem arms in root	20.31	17.95	20.94
Number/plant	All grains	55.31	24.78	23.45
	Filled grains	62.94	40.00	53.48
Weight	1000 intact grains	2.21	5.22	5.99
	1000 chaffed grains	4.40	11.12	15.81

The mean total number of xylem arms and the mean number of large arms in root were reduced by the heavy metal treatments (Fig. 1F), the reduction in the total number was in the order: Cu + Cd > Cu > Cd. However, the effect on the number of large xylem arms was in the order: Cu + Cd > Cd > Cu. This is yet another indication of the additive effect of both metals.

All three heavy metal treatments caused significant reduction in parenchyma cell area of stem ground tissue, mesophyll of leaf midrib, root cortex and root pith (Figs. 2A & 2B). Enlargement of parenchyma cells was clearly hampered by Cu and Cd whether administered separately or in a combination. The effect was consistently greater on parenchyma cells in the two tissues of the root than on those of the shoot (Table I). In all four tissues, the effect of the different treatments was consistently in the order: Cu + Cd > Cd > Cu. Fig. 2C showed that the reduction in the diameter of metaxylem vessels in stem, leaf midrib and roots was highly significant in response to heavy metal treatments. The percentage reduction in the three organs relative to the control was in the order: root > stem > leaf midrib, the greatest percentage reduction in metaxylem vessel diameter was attained by Cu + Cd in the root (Table I). The stomatal frequency was reduced significantly by heavy metal treatments (Fig. 2D).

Grain yield changes. The greatest reduction in grain yield was obtained in the average number of grains/plant and the average number of filled grains/plant in response to Cu, although it hardly affected the weight of 1000 grains (Figs. 3A & 3C, Table I). The percentage of filled grains was also severely reduced from about 76% (control) to less than 46% by Cu + Cd (Fig. 3B). While the weight of 1000 chaffed grains was reduced by the three heavy metal treatments, the weight of 1000 intact grains was reduced significantly only by Cd and Cu + Cd (Fig. 3C, Table I). Weight reduction

Fig. 1. Effect of 10^{-5} M Cu, 10^{-6} M Cd, or Cu + Cd on the leaf, stem and root anatomy of *Sorghum bicolor*. All measurements were in µm. Each bar value is the mean of three replicates. Vertical bars are the S.D. Similar letters on tops of corresponding bars indicate statistically non-significant differences at P ≤ 0.01. Tot Thick = total thickness, Par Thick = parenchyma thickness, L = length, W = width, VB = vascular bundle, MVB = median vascular bundle, LVB = lateral vascular bundles.

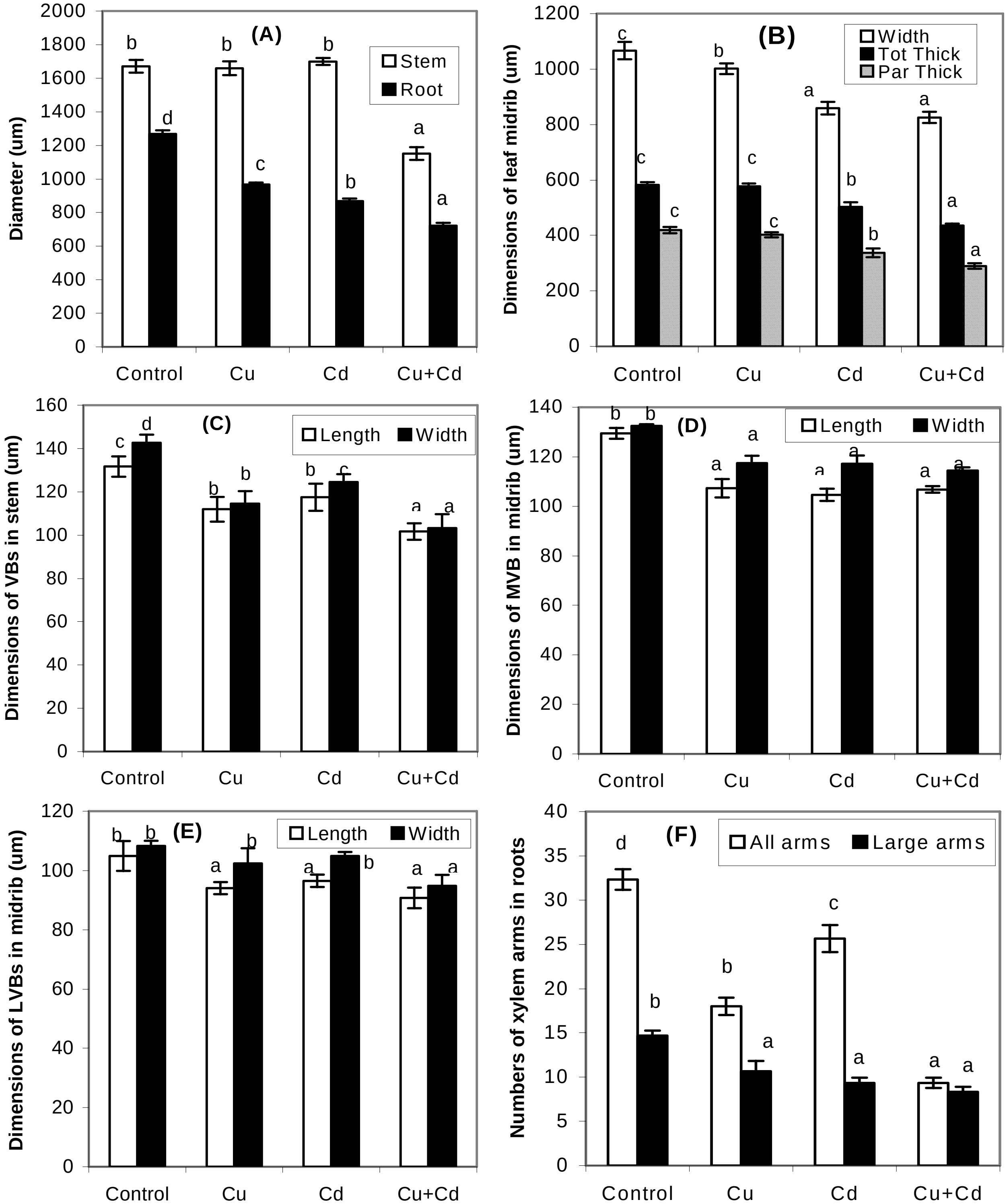
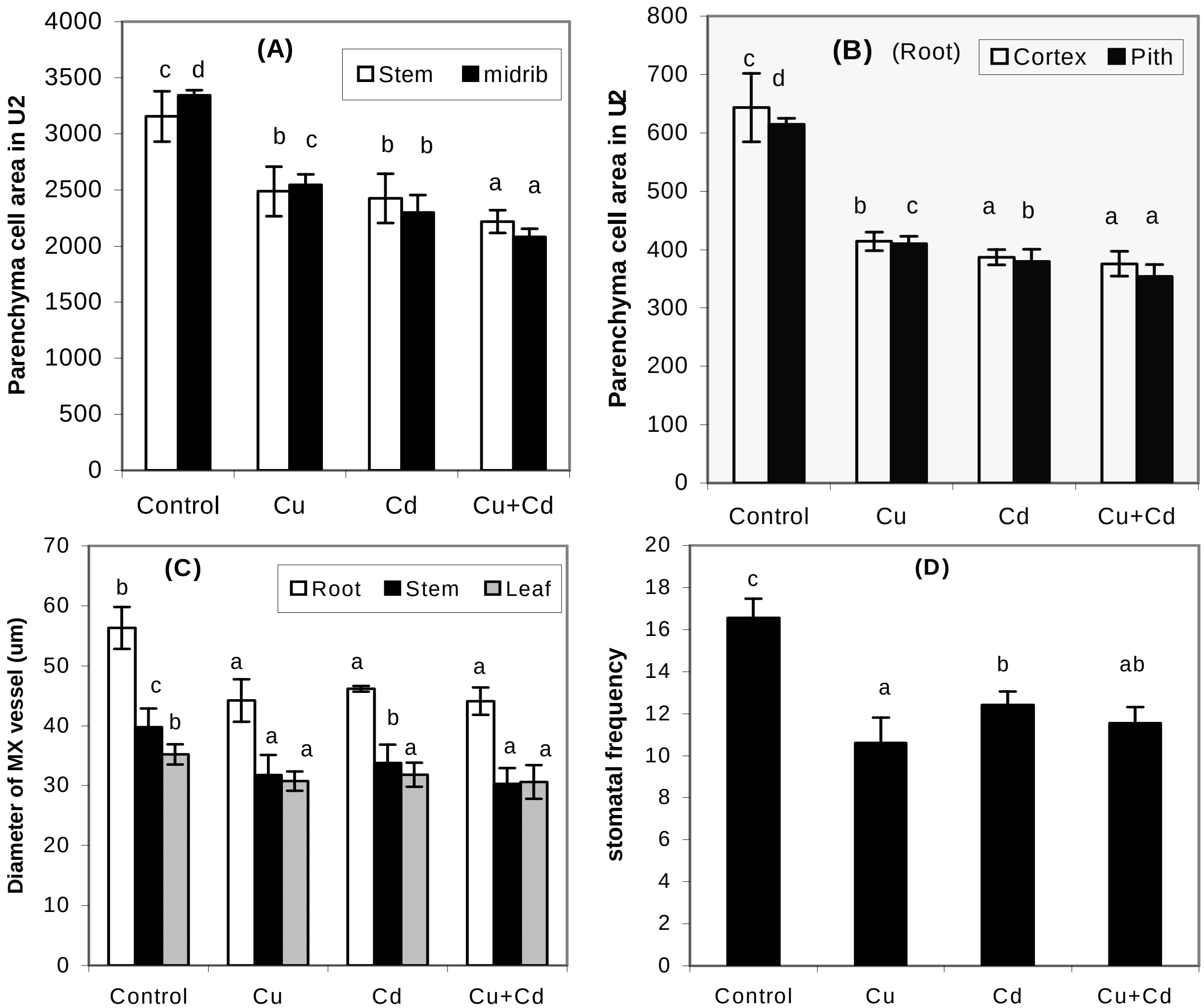


Fig. 2. Effect of 10^{-5} M Cu, 10^{-6} M Cd, or Cu + Cd on cell enlargement and stomatal frequency in *Sorghum bicolor*. Each bar value is the mean of 15, 60, 24, 18 and 15 for measurements of parenchyma, metaxylem vessels of stem, metaxylem vessels of root, metaxylem vessels of leaf midrib and stomatal frequency, respectively. Vertical bars are the S.D. Similar letters on tops of corresponding bars indicate statistically non-significant differences at P ≤ 0.01.



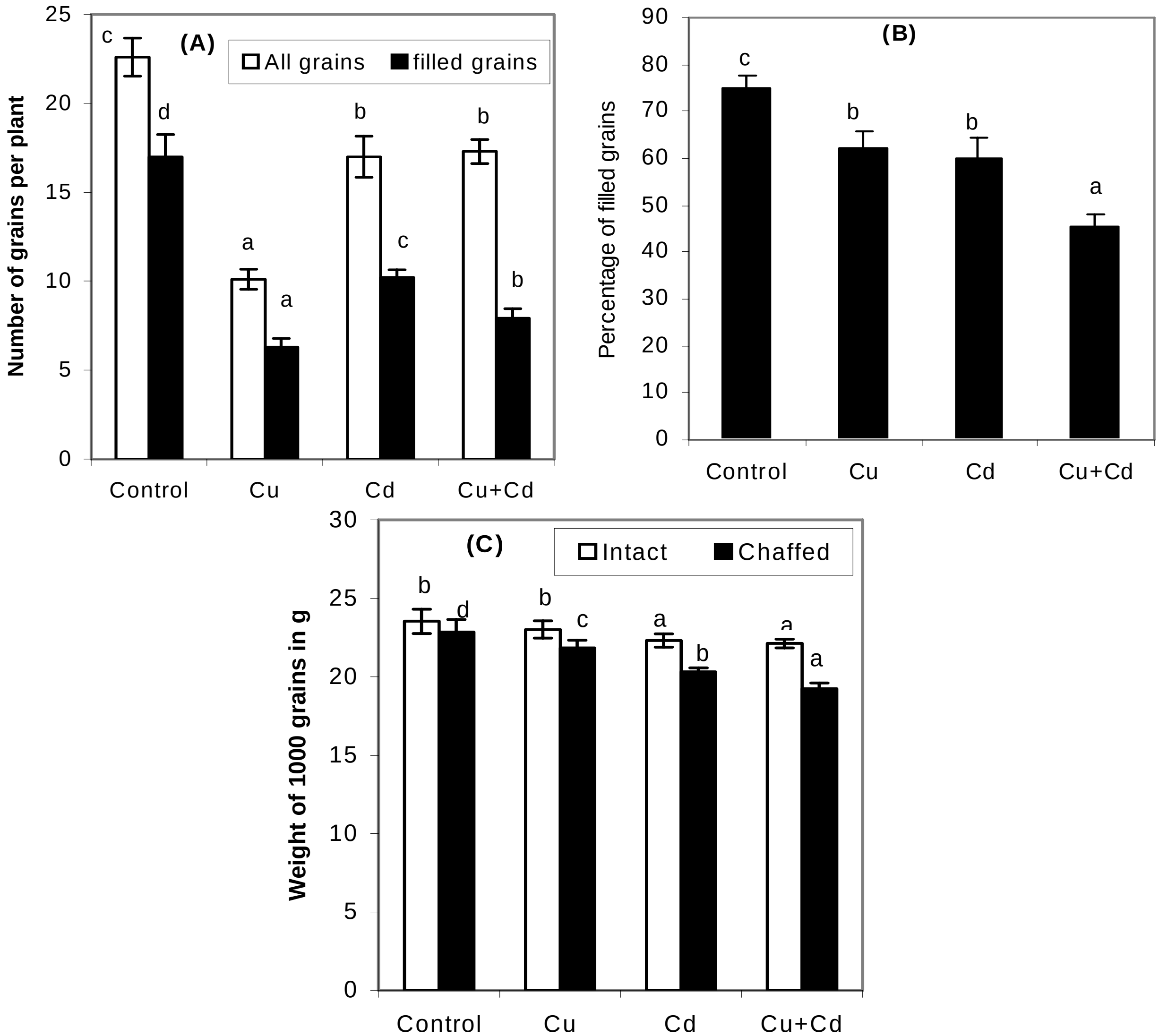
was greater in the grains than in their chaffs. The percentage reduction in grain weight caused by either Cu or Cd was nearly twice that in the chaff weight, and it was increased to about 3-fold in response to Cu + Cd treatment.

DISCUSSION

Heavy metal stress by Cu or Cd not only affects the growth criteria of *Sorghum bicolor* reported previously (Kasim, 2001), but it also had an adverse effect on its anatomical structure (this study), especially when the plant was subjected to both metals simultaneously. Grain yield was also drastically reduced. Similar alterations in anatomical parameters and grain yield of different crop plants have been previously reported (Setia & Bala, 1994; Kovačević *et al*., 1999; Shalini *et al*., 1999; Khudsar *et al*., 2001; Papadakis *et al*., 2004).

The up-take of heavy metals by the root seems to trigger a series of structural alterations with potential functional consequences in the plant. A marked decrease in cell size might be the result of a decrease in the elasticity of cell walls of the root as was previously demonstrated (Sieghardt, 1984; Barceló *et al*., 1986). Results of the present study indicated that the Cu- and Cd- induced reduction in the cell size include all root cells whether they will remain parenchymatous in the cortex and pith or will develop into xylem vessels. The net outcome of this reduction in the cell size is the root diameter shrinkage.

Fig. 3. Effects of 10^{-5} M Cu, 10^{-6} M Cd, or Cu + Cd on grain yield of *Sorghum bicolor*. Each bar value is the mean of weights or counts of grains of 10 plants. Vertical bars are the S.D. Similar letters on tops of corresponding bars indicate statistically non-significant differences at P ≤ 0.01.



The reduction in the diameter of metaxylem vessels was the most remarkable result as it is one of the factors affecting their capacity as translocation conduits (Poschenrieder & Barceló, 1999). Movement of water and minerals from root to shoot through xylem might be, therefore, diminished. Consequently, plants might need to decrease water loss through transpiration as was indicated by Greger and Johansson (1992) in sugar beet. This seems to be in agreement with the observed decrease in stomatal frequency induced by heavy metals in this study. Limitation in water supply (leading to stomatal closure) and stomatal frequency might impinge on gas exchange and also the rate of photosynthetic electron transport (Mediavilla *et al*., 2002), which ultimately may be reflected on the reduced accumulation of dry matter (Kasim, 2001) and on the diminished grain yield observed here.

The molecular-physiological mechanism responsible for observed changes in anatomy is not yet clear. According to Pasternak *et al*. (2005), architectural changes imposed by Cu and other abiotic stresses on roots of *Arabidopsis thaliana* may be attributed to alteration in phytohormone metabolism and local auxin accumulation near the root pericycle. They also found that Cu impinges primarily on cell elongation and not so much on cell division. Reduction in the width of the root cortical parenchyma and the mesophyll parenchyma of the leaf midrib observed in this study was mainly the result of a decrease in the mean cell size and not in the number of cell layers. The reduced width of metaxylem vessels in the root, stem and leaves of *Sorghum bicolor* is yet another example of the effect of heavy metals on cell enlargement. However, since the chemistry and architecture of the cell walls of grasses and the changes which take place in them during cell elongation are different from those of all other flowering plants (Carpita, 1996), the role of auxins in mediating heavy metal-induced alterations in the anatomy of *Sorghum bicolor* is yet to be tested.

Acknowledgements. I am most grateful to Prof. Marcel A.K. Jansen (The University College Cork, Ireland) for illuminating discussion and useful suggestions and Prof. M.A. Elhaak (Tanta University) for help with the statistical analysis.

REFERENCES

Barceló, J., Ch. Poschenrieder, I. Andreu and B. Gunse, 1986. Cadmium-induced decrease of water stress resistance in bush bean plants (*Phaseolus vulgaris* L. cv. Contender). I. Effects of Cd on water potential, relative water content, and cell wall elasticity. *J. Pl. Physiol*., 125: 17–25

Carpita, N.C., 1996. Structure and biogenesis of the cell walls of grasses. *Annu. Rev. Pl. Physiol. Pl. Mol. Biol.,* 47: 445–76

Clifford, H.T. and L. Watson, 1977. *Identifying Grasses: Data, Methods and Illustrations*. University of Queensland Press, St. Lucia, Queensland, Australia

Gould, F.W. and R.B. Shaw, 1983. *Grass Systematics. 2nd ed*. Texas A and M University Press, Texas, USA

Greger, M. and M. Johansson, 1992. Cadmium effects on leaf transpiration of sugar beet (*Beta vulgaris*). *Physiol. Pl.,* 86: 465–73

Hoagland, D.R. and I.I. Arnon, 1950. The water culture methods for growing plants without soil. *California Agric. Expt. Stat. Circular,* 347

Kasim, W.A., 2001. Effect of copper and cadmium on some growth criteria and physiological aspects of *Sorghum bicolor*. *Egypt J. Biotechnol*., 9: 298–310

Kasim, W.A., 2005. The correlation between physiological and structural alterations induced by copper and cadmium stress in broad beans (*Vicia faba* L.). *Egypt J. Biol.*, 7: (in press)

Khan, H.R., G.K. McDonald and Z. Rengel, 2003. Zn fertilization improves water use efficiency, grain yield and seed Zn content in chickpea. *Pl. Soil,* 249: 389–400

Khudsar, T., Mahmooduzzafar and M. Iqbal, 2001. Cadmium-induced changes in leaf epidermis, photosynthetic rate and pigment concentrations in *Cajanus cajan. Biol. Pl*., 44: 59–64

Kovačević, G., R. Kastori and I.J. Merkulov, 1999. Dry matter and leaf structure in young wheat plants as affected by cadmium, lead and nickel. *Biol. Pl.,* 42: 119–23

Mediavilla, S., H. Santiago and A. Escudero, 2002. Stomatal and mesophyll limitations to photosynthesis in one ever-green and one deciduous Mediterranean oak species. *Photosynthetica*, 40: 553–9

Panou-Filotheou, H. and A.M. Bosabalidis, 2004. Root structural aspects associated with copper toxicity in oregano (*Origanum vulgare* subsp. *hirtum*). *Pl. Sci.,* 166: 1497–504

Papadakis, I.E., K.N. Dimassi, A.M. Bosabalidis, I.N. Therios, A. Patakas and A. Giannakoula, 2004. Effects of B excess on some physiological and anatomical parameters of 'Navalina' orange plants grafted on two rootstocks. *Environ. Exp. Bot*., 51: 247–57

Pasternak, T., V. Rudas, G. Potters and M.A.K. Jansen, 2005. Morphogenic effects of abiotic stress: reorientation of growth in *Arabidopsis thaliana* seedlings. *Environ. Exp. Bot*., 53: 299–314

Poschenrieder, C. and J. Barceló, 1999. Water relations in heavy metal stressed plants, *In*: Prasad, M.N.V. and J. Hagemeyer (eds.), *Heavy Metal Stress in Plants*. pp: 207–29. The Springer, Berlin

Salih, A.A., I.A. Ali, A. Lux, M. Luxova, Y. Cohen, Y. Sugimoto and S. Inanaga, 1999. Rooting, water up-take, and xylem structure adaptation to drought of two sorghum cultivars. *Crop Sci*., 39: 168–73

Sieghardt, H., 1984. Eine anatomisch-histochemische Studie zur Bleiverteilung in Primärwurzeln von *Pisum sativum* L. *Mikroskopie,* 41: 125–33

Setia, R.C. and R. Bala, 1994. Anatomical changes in root and stem of wheat (*Triticum aestivum* L.) in response to different heavy metals. *Phytomorph.,* 44: 95–104

Shalini, M., S.T. Ali, M.T.O. Siddiqi and M. Iqbal, 1999. Cadmium-induced changes in foliar responses of *Solanum melongena* L. *Phytomorph.*, 49: 295–302

Souza, J.F. and W.E. Rauser, 2003. Maize and radish sequester excess cadmium and zinc in different ways. *Pl. Sci.*, 165: 1009–22

Wu, F., H. Wu, G. Zhang and D.M.L Bachir, 2004. Differences in growth and yield in response to cadmium toxicity in cotton genotypes. *J. Pl. Nutr. Soil Sci.,* 167: 85–90

Yang, X.E., X.X. Long, H.B. Ye, Z.L. He, D.V. Calvert and P.J. Stoffella, 2004. Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (*Sedum alfredii* Hance). *Pl. Soil*, 259: 181–9

Zeid, I.M., 2001. Responses of *Phaseolus vulgaris* to chromium and cobalt treatments. *Biol. Pl.*, 44: 111–5

(Received 10 November 2005; Accepted 16 December 2005)