# Morphological and Anatomical Variations of Cajanus cajan (Linn.) Huth Raised in Cadmium-Rich Soil 

Tarannum Khudsar ${ }^{\mathbf{1}}$, Mahmooduzzafar ${ }^{1}$, Woong Young Soh ${ }^{\mathbf{2}}$, and Muhammad Iqbal ${ }^{1 *}$<br>${ }^{1}$ Department of Botany, Hamdard University, Hamdard Nagar, New Delhi 110 062, India<br>${ }^{2}$ Department of Biological Sciences, Chonbuk National University, Chonju 560-756, Korea


#### Abstract

Different concentrations of cadmium in the growing media affected morphological parameters of Cajanus cajan. Over time, the amount of increase in shoot and root lengths, number of branches and leaves per plant, single and total leaf areas, and dry mass of leaves, was significantly lower for treated plants compared with controls. The root-shoot length ratio, which varied little over time, was relatively low for the treated plants. Although dry mass of both stems and roots increased, the rates were considerably low under Cd stress. The root-shoot dry mass ratio in the controls was highest during flowering and lowest in the post-flowering stage, but continually declined over time for the stressed plants. Compared with the controls, treated plants had fewer pods, with the number decreasing as the Cd concentration increased. Cd content was greater in roots than in stems or leaves, and leaves had greater amounts than did stems at higher doses. For all plants, the width and density of vessel elements and the length of fibers in the wood of stems and roots increased with plant age. However, the rate of increase was generally lower in the treated plants, the difference being more pronounced with higher doses of Cd. This indicated a reduced ascent of sap and, hence, less available water for tissues in treated plants.


Keywords: Biomass, cadmium, Cajanus cajan, growth, xylem cells, yield

In regions that experience high pollution emissions, such as in mining areas and industrialized zones, or where agricultural soils are contaminated by phosphoric fertilizers and/or sewage sludge, toxic trace metals affect plant development. For example, cadmium ions $\left(\mathrm{Cd}^{2+}\right)$ are readily absorbed by roots and translocated into aerial portions in several species (Kabata-Pendias and Pendias, 1984). High levels of metal accumulation cause phytotoxicity, which can disturb physiological processes and reduce growth (Barceló et al., 1988a, 1988b). Metal ions may interact with sulfhydryl groups and inactivate plant proteins (Assche and Clijsters, 1990), thereby inhibiting seed germination and reducing growth in the length and mass of roots and shoots (Moya et al., 1993; Moral et al., 1994). They also retard photosynthesis (Weigel, 1985a, 1985b; Ali et al., 1998; Mehindirata et al., 1999) and respiration (Reese and Roberts, 1985).

Cajanus cajan (Linn.) Huth, in the Papilionaceae family, is a pulse crop that is a significant constituent of the human diet in the Indian subcontinent and adjoining countries. It is grown througho't India, sometimes in fields located near industrial plants that

[^0]emit Cd as part of their waste. Because many physiological processes, including photosynthesis, are retarded, overall growth and morphology of the plant are affected. Performance of crops growing under the influence of Cd should be evaluated. Therefore, the objective of this study was to investigate the morphological and anatomical variations caused by cadmium at different stages of development in $C$. cajan plants.

## MATERIALS AND METHODS

Healthy seeds of C. cajan (Linn.) Huth from the Indian Agricultural Research Institute, New Delhi, were sown in pots containing 10 kg of sterilized, cad-mium-free soil. Sludge and farm compost ( 3 kg per pot) were used as manure. This was mixed thoroughly with the soil at the time of sowing. After a month of seed germination, individual pots were randomly treated with one of five concentrations of $\mathrm{CdCl}_{2}(5,10,15,25$, or $50 \mu \mathrm{~g}$ per g soil). Untreated plants were the controls. Plants were sampled at 3 months (pre-flowering phase), 5 months (flowering stage) and 6.5 months (post-flowering phase). The seeds had been sown in August, when the mean monthly temperature ranged from $25^{\circ} \mathrm{C}$ (minimum)
to $32^{\circ} \mathrm{C}$ (maximum); sampling occurred in November $\left(13^{\circ} \mathrm{C}\right.$ and $\left.25^{\circ} \mathrm{C}\right)$, January ( $6^{\circ} \mathrm{C}$ and $20^{\circ} \mathrm{C}$ ), and March $\left(14^{\circ} \mathrm{C}\right.$ and $27^{\circ} \mathrm{C}$ ).

Root, shoot, and leaf samples were separated and
oven-dried at $80^{\circ} \mathrm{C}$ for 48 h to determine dry masses. Individual- as well as total foliar area on single plants were estimated with a digital LICOR 3000A Leaf Area Meter (LI-COR, Lincoln, NE, USA).

Table 1. Growth parameters for various plant parts at different developmental stages of C. cajan plants grown as control and on various Cd concentrations. Mean $\pm \mathrm{SD}$ are based on five replicates. Parentheses include percent variation.

|  | Control | Cadmium concentration [ $\mu \mathrm{g}\left(\mathrm{CdCl}_{2}\right) \mathrm{g}^{-1}$ (soil d.m.)] |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 5 | 10 | 15 | 25 | 50 |
| Root length (cm) |  |  |  |  |  |  |
| Pre-flowering | $8.00 \pm 0.41$ | $\begin{aligned} & 6.62 \pm 0.33 \\ & (17.25)^{* *} \end{aligned}$ | $\begin{aligned} & 6.00 \pm 0.81 \\ & (25.00)^{* *} \end{aligned}$ | $\begin{aligned} & 5.50 \pm 0.29 \\ & (31.25)^{* *} \end{aligned}$ | $\begin{aligned} & 4.75 \pm 0.95 \\ & (40.62)^{* *} \end{aligned}$ | $\begin{aligned} & 3.25 \pm 0.75 \\ & (59.37)^{* *} \end{aligned}$ |
| Flowering | $14.25 \pm 0.78$ | $\begin{aligned} & 11.27 \pm 0.65 \\ & 100911^{* *} \end{aligned}$ | $\begin{aligned} & 10.50 \pm 0.45 \\ & (26.31)^{* *} \end{aligned}$ | $\begin{aligned} & 9.50 \pm 0.55 \\ & (33.33)^{* *} \end{aligned}$ | $\begin{aligned} & 8.35 \pm 0.46 \\ & (41.40)^{* *} \end{aligned}$ | $\begin{aligned} & 7.25 \pm 0.70 \\ & (49.12)^{* *} \end{aligned}$ |
| Post-flowering | $22.25 \pm 1.70$ | $\begin{aligned} & 13.75 \pm 1.89 \\ & (38.20)^{* *} \end{aligned}$ | $\begin{aligned} & 13.00 \pm 1.44 \\ & (41.57)^{* *} \end{aligned}$ | $\begin{aligned} & 12.5 \pm 01.35 \\ & (43.33)^{* *} \end{aligned}$ | $\begin{aligned} & 10.87 \pm 1.85 \\ & (51.14)^{* *} \end{aligned}$ | $\begin{aligned} & 9.75 \pm 1.75 \\ & (56.17)^{* *} \end{aligned}$ |
| Root dry mass (g) |  |  |  |  |  |  |
| Pre-flowering | $0.56 \pm 0.32$ | $\begin{aligned} & 0.40 \pm 0.07 \\ & (28.57)^{\mathrm{NS}} \end{aligned}$ | $\begin{aligned} & 0.36 \pm 0.11 \\ & (35.71)^{\mathrm{NS}} \end{aligned}$ | $\begin{aligned} & 0.32 \pm 0.09 \\ & (42.85)^{\mathrm{NS}} \end{aligned}$ | $\begin{aligned} & 0.28 \pm 0.01 \\ & (50.00)^{*} \end{aligned}$ | $\begin{aligned} & 0.23 \pm 0.13 \\ & (58.92)^{*} \end{aligned}$ |
| Flowering | $1.09 \pm 0.25$ | $\begin{aligned} & 0.51 \pm 0.19 \\ & (53.21)^{* *} \end{aligned}$ | $\begin{aligned} & 0.42 \pm 0.08 \\ & (64.22)^{* *} \end{aligned}$ | $\begin{aligned} & 0.39 \pm 0.10 \\ & (64.22)^{* *} \end{aligned}$ | $\begin{aligned} & 0.35 \pm 0.17 \\ & (67.88)^{* *} \end{aligned}$ | $\begin{aligned} & 0.32 \pm 0.14 \\ & (72.47)^{* *} \end{aligned}$ |
| Post-lowering | $1.90 \pm 0.13$ | $\begin{aligned} & 0.60 \pm 0.26 \\ & (68.42)^{* *} \end{aligned}$ | $\begin{aligned} & 0.48 \pm 0.08 \\ & (74.73)^{* *} \end{aligned}$ | $\begin{aligned} & 0.43 \pm 0.03 \\ & (77.36)^{* *} \end{aligned}$ | $\begin{aligned} & 0.39 \pm 0.17 \\ & (79.47)^{* *} \end{aligned}$ | $\begin{aligned} & 0.33 \pm 0.11 \\ & (82.63)^{* *} \end{aligned}$ |
| Total leaf area ( $\mathbf{c m}^{\mathbf{2}}$ ) |  |  |  |  |  |  |
| Pre-flowering | $459.66 \pm 89.48$ | $\begin{aligned} & 144.74 \pm 16.05 \\ & (68.51)^{* *} \end{aligned}$ | $\begin{aligned} & 100.0628 .63 \\ & (78.23)^{* *} \end{aligned}$ | $\begin{aligned} & 86.68 \pm 23.35 \\ & (81.14)^{* *} \end{aligned}$ | $\begin{aligned} & 70.56 \pm 24.76 \\ & (84.64)^{* *} \end{aligned}$ | $\begin{aligned} & 65.74 \pm 15.20 \\ & (85.69)^{* *} \end{aligned}$ |
| Flowering | $604.75 \pm 89.48$ | $\begin{aligned} & 315.15 \pm 82.89 \\ & (47.88)^{* *} \end{aligned}$ | $\begin{aligned} & 246.4744 .68 \\ & (59.24)^{* *} \end{aligned}$ | $\begin{aligned} & 206.90 \pm 72.49 \\ & (65.78)^{* *} \end{aligned}$ | $\begin{aligned} & 90.32 \pm 51.20 \\ & (68.52)^{* *} \end{aligned}$ | $\begin{aligned} & 148.24 \pm 37.27 \\ & (75.48)^{* *} \end{aligned}$ |
| Post-flowering | $965.341 \pm 25.02$ | $\begin{aligned} & 2601.44 \pm 121.45 \\ & (37.69)^{* *} \end{aligned}$ | $\begin{aligned} & 415.11132 .40 \\ & (59.99)^{* *} \end{aligned}$ | $\begin{aligned} & 338.46 \pm 119.98 \\ & (64.93)^{* *} \end{aligned}$ | $\begin{aligned} & 221.02 \pm 85.63 \\ & (77.10)^{* *} \end{aligned}$ | $\begin{aligned} & 171.36 \pm 59.65 \\ & (82.24)^{* *} \end{aligned}$ |
| Total leaf dry mass (g) |  |  |  |  |  |  |
| Pre-flowering | $1.56 \pm 0.58$ | $\begin{aligned} & 0.86 \pm 0.16 \\ & (48.80)^{*} \end{aligned}$ | $\begin{aligned} & 0.58 \pm 0.12 \\ & (62.51)^{* *} \end{aligned}$ | $\begin{aligned} & 0.56 \pm 0.18 \\ & (64.11)^{* *} \end{aligned}$ | $\begin{aligned} & 0.45 \pm 0.11 \\ & (71.13)^{* *} \end{aligned}$ | $\begin{aligned} & 0.20 \pm 0.07 \\ & (87.08)^{* *} \end{aligned}$ |
| Flowering | $1.71 \pm 0.27$ | $\begin{aligned} & 1.31 \pm 0.20 \\ & (22.39)^{*} \end{aligned}$ | $\begin{aligned} & 0.93 \pm 0.22 \\ & (45.61)^{* *} \end{aligned}$ | $\begin{aligned} & 0.91 \pm 0.23 \\ & (46.78)^{* *} \end{aligned}$ | $\begin{aligned} & 0.88 \pm 0.15 \\ & (48.53)^{* *} \end{aligned}$ | $\begin{aligned} & 0.82 \pm 0.27 \\ & (52.04)^{* *} \end{aligned}$ |
| Post-flowering | $1.96 \pm 0.27$ | $\begin{aligned} & 1.74 \pm 0.33 \\ & (12.86)^{\mathrm{NS}} \end{aligned}$ | $\begin{aligned} & 1.250 \pm .26 \\ & (36.22)^{* *} \end{aligned}$ | $\begin{aligned} & 1.04 \pm 0.22 \\ & (53.80)^{* *} \end{aligned}$ | $\begin{aligned} & 1.04 \pm 0.21 \\ & (53.80)^{* *} \end{aligned}$ | $\begin{aligned} & 0.96 \pm 0.30 \\ & (51.02)^{* *} \end{aligned}$ |
| Stem length (cm) |  |  |  |  |  |  |
| Pre-flowering | $33.75 \pm 4.21$ | $\begin{aligned} & 32.00 \pm 4.69 \\ & (5.18)^{\text {NS }} \end{aligned}$ | $\begin{aligned} & 29.00 \pm 2.16 \\ & (14.07)^{*} \end{aligned}$ | $\begin{aligned} & 25.75 \pm 4.99 \\ & (23.70)^{*} \end{aligned}$ | $\begin{aligned} & 23.25 \pm 4.85 \\ & (31.11)^{*} \end{aligned}$ | $\begin{aligned} & 20.87 \pm 5.86 \\ & (38.16)^{* *} \end{aligned}$ |
| Flowering | $80.50 \pm 14.20$ | $\begin{aligned} & 62.75 \pm 11.80 \\ & (22.04)^{N S} \end{aligned}$ | $\begin{aligned} & 52.62 \pm 7.76 \\ & (34.63)^{* *} \end{aligned}$ | $\begin{aligned} & 48.37 \pm 11.12 \\ & (36.18)^{* *} \end{aligned}$ | $\begin{aligned} & 45.37 \pm 12.61 \\ & (39.91)^{* *} \end{aligned}$ | $\begin{aligned} & 40.37 \pm 5.72 \\ & (49.85)^{* *} \end{aligned}$ |
| Post-flowering | $109.75 \pm 25.32$ | $\begin{aligned} & 76.25 \pm 9.24 \\ & (30.52)^{*} \end{aligned}$ | $\begin{aligned} & 74.00 \pm 12.86 \\ & (32.57)^{*} \end{aligned}$ | $\begin{aligned} & 61.37 \pm 8.80 \\ & (44.08)^{* *} \end{aligned}$ | $\begin{aligned} & 50.25 \pm 6.39 \\ & (54.21)^{* *} \end{aligned}$ | $\begin{aligned} & 43.12 \pm 4.36 \\ & (60.70)^{* *} \end{aligned}$ |
| Stem dry mass (g) |  |  |  |  |  |  |
| Pre-flowering | $2.25 \pm 0.65$ | $\begin{aligned} & 1.09 \pm 0.45 \\ & (51.55)^{* *} \end{aligned}$ | $\begin{aligned} & 0.98 \pm 0.15 \\ & (56.44)^{* *} \end{aligned}$ | $\begin{aligned} & 0.83 \pm 0.10 \\ & (63.11)^{* *} \end{aligned}$ | $\begin{aligned} & 0.68 \pm 0.15 \\ & (69.77)^{* *} \end{aligned}$ | $\begin{aligned} & 0.55 \pm 0.15 \\ & (75.55)^{* *} \end{aligned}$ |
| Flowering | $4.13 \pm 0.62$ | $\begin{aligned} & 2.26 \pm 0.65 \\ & (45.27)^{* *} \end{aligned}$ | $\begin{aligned} & 1.47 \pm 0.30 \\ & (64.40)^{* *} \end{aligned}$ | $\begin{aligned} & 1.35 \pm 0.21 \\ & (67.31)^{* *} \end{aligned}$ | $\begin{aligned} & 1.30 \pm 0.14 \\ & (68.52)^{* *} \end{aligned}$ | $\begin{aligned} & 1.00 \pm 0.09 \\ & (75.78)^{* *} \end{aligned}$ |
| Post-flowering | $8.56 \pm 0.70$ | $\begin{aligned} & 5.85 \pm 0.95 \\ & (31.65)^{* *} \end{aligned}$ | $\begin{aligned} & 4.81 \pm 0.65 \\ & (43.80)^{* *} \end{aligned}$ | $\begin{aligned} & 3.90 \pm 0.71 \\ & (54.43)^{* *} \end{aligned}$ | $\begin{aligned} & 2.50 \pm 0.60 \\ & (70.79)^{* *} \end{aligned}$ | $\begin{aligned} & 1.80 \pm 0.21 \\ & (78.97)^{* *} \end{aligned}$ |
| Pods per plant |  |  |  |  |  |  |
| Post flowering | $63.07 \pm 17.35$ | $\begin{aligned} & 52.05 \pm 6.50 \\ & (17.47)^{* *} \end{aligned}$ | $\begin{aligned} & 45.02 \pm 5.50 \\ & (28.61)^{* *} \end{aligned}$ | $\begin{aligned} & 37.01 \pm 4.65 \\ & (41.31)^{* *} \end{aligned}$ | $\begin{aligned} & 30.01 \pm 5.65 \\ & (52.41)^{* *} \end{aligned}$ | $\begin{aligned} & 25.02 \pm 7.25 \\ & (60.32)^{* *} \end{aligned}$ |

[^1]Transverse sections ( $15 \mu \mathrm{~m}$ thick) of roots and stems were made with a Reicherts sliding microtome. After dehydration in an ethanol series, they were stained with safranin and hematoxylin solutions and mounted in Canada balsam on glass slides. Relative proportions of various tissue zones (cortex, vasculature, and pith) were calculated from these sections under a compound light microscope. Tissues were macerated by treatingthem with hot $\mathrm{HNO}_{3}$ (Ghouse and Yunus, 1972). The vessel elements and xylem fibers were measured from the macerated tissue, using a calibrated ocular micrometer scale.
Cd content in various plant parts was determined with an Atomic Absorption Spectrometer (Video 11, Thermo Jarrel Ash Corporation, Franklin, TN, USA). The data were analyzed for statistical significance, using the Student's t-test.

## OBSERVATIONS

Growth data for leaves, stems, and roots are shown in Table 1. For both treated and control plants, the number of leaves per plant, and single and total leaf areas increased with age. However, the rate of increase was significantly lower under the influence of Cd . The maximum retardation in growth (by about $33 \%$ in leaf number, $79 \%$ in single leaf area, and $86 \%$ in total foliage area) was detected for a $50-\mu \mathrm{g} \mathrm{Cd}$ dose during the pre-flowering stage. Leaf dry mass also showed a similar trend.
Shoot length and root length also increased with age, although the rate of increase, again, was significantly lower in the treated plants. The same applied
to the number of branches per plant and total plant height (data not given). Root-shoot length ratios for treated plants showed only slight ontogenetic variations, remaining relatively low throughout the growing period. This reduction was significant in the preflowering and post-flowering stages (data not given). Dry masses of both stems and roots in the controls increased with plant age. This was also true for those plants under Cd stress, but at a considerably lower magnitude of increase. The reduction in root dry mass was insignificant during the pre-flowering stage, except at the $25-$ and $50-\mu \mathrm{g}$ levels. The root-shoot dry mass ratios in the control plants were lowest $(0.22)$ at the post-flowering stage. For plants under Cd stress, ratios were higher than in the controls at the pre-flowering stage, slightly altered during flowering, and distinctly lower in the post-flowering stage. The number of pods per plant was significantly less under Cd treatment, steadily declining with increased concentrations (Table 1).
Cd was not detected in the leaves and stems of control plants, but their roots did have trace amounts, which increased over time. However, Cd was present in the leaves, stems, and roots of treated plants; this became increasingly prominent with age as well as with degree of treatment for all three tissue types. Roots had the greatest levels of Cd. The maximum mean Cd contents in leaves, stems, and roots were $0.40 \mathrm{ppm}, 0.26 \mathrm{ppm}$, and 0.86 ppm , respectively. These high levels were recorded during the post-flowering stage in plants receiving a $30-\mu \mathrm{g}$ dose (Table 2).
Relative proportions of tissue comprising the vasculature, core, and pith zones varied with plant age and level of Cd stress. Over time, an increase in vascular

Table 2. Cadmium content (ppm) of leaves, stems, and roots in different developmental stages of $C$. cajan plants grown as control and on various concentrations of $\mathrm{CdCl}_{2}$.

|  | Control | Cadmium concentration ( $\mu \mathrm{g} / \mathrm{g}$ soil d.m.) |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 5 | 10 | 15 | 25 | 50 |
| Leaves |  |  |  |  |  |  |
| Pre-flowering |  | $0.02 \pm 0.005$ | $0.04 \pm 0.001$ | $0.10 \pm 0.005$ | $0.18 \pm 0.02$ | $0.26 \pm 0.01$ |
| Flowering |  | $0.03 \pm 0.001$ | $0.05 \pm 0.01$ | $0.13 \pm 0.02$ | $0.20 \pm 0.01$ | $0.33 \pm 0.02$ |
| Post-flowering |  | $0.04 \pm 0.005$ | $0.06 \pm 0.01$ | $0.16 \pm 0.02$ | $0.23 \pm 0.02$ | $0.40 \pm 0.005$ |
| Stem |  |  |  |  |  |  |
| Pre-flowering | $0.00 \pm 0.00$ | $0.03 \pm 0.001$ | $0.05 \pm 0.002$ | $0.09 \pm 0.004$ | $0.13 \pm 0.01$ | $0.20 \pm 0.02$ |
| Flowering | $0.00 \pm 0.00$ | $0.06 \pm 0.002$ | $0.07 \pm 0.002$ | $0.10 \pm 0.01$ | $0.15 \pm 0.01$ | $0.23 \pm 0.02$ |
| Post-flowering | $0.00 \pm 0.00$ | $0.08 \pm 0.01$ | $0.11 \pm 0.02$ | $0.13 \pm 0.01$ | $0.16 \pm 0.02$ | $0.26 \pm 0.05$ |
| Root |  |  |  |  |  |  |
| Pre-flowering | $0.00 \pm 0.001$ | $0.13 \pm 0.01$ | $0.30 \pm 0.03$ | $0.40 \pm 0.05$ | $0.53 \pm 0.05$ | $0.60 \pm 0.04$ |
| Flowering | $0.02 \pm 0.002$ | $0.16 \pm 0.01$ | $0.36 \pm 0.02$ | $0.46 \pm 0.04$ | $0.60 \pm 0.05$ | $0.80 \pm 0.05$ |
| Post-flowering | $0.03 \pm 0.002$ | $0.20 \pm 0.04$ | $0.43 \pm 0.01$ | $0.53 \pm 0.02$ | $0.63 \pm 0.02$ | $0.86 \pm 0.03$ |



Figure 1. Pie diagrams showing the relative proportion of vasculature, pith, and cortex in the stem of the control and the cad-mium-treated plants of $C$. cajan at different stages of plant development.
tissue in the stems was paralleled by a concomitant reduction in the other two zones. The amount of vascular tissue was generally greater in treated plants than in controls. The pith and cortex were larger during early growth than in the later stages (Fig. 1).
Xylem-fiber length and the length and width of vessel elements in the stem increased with age, although this increase was much lower for the treated plants. In fact, fibers of treated plants were up to $25 \%$ shorter, with the maximum differences occurring with the highest Cd dose as well as at the post-flowering stage at any dose. Vessel-element length was most affected (up to 33\%) at the highest dose but only in the earlier stages of plant development. However, no obvious trends were seen for the variations in vessel width over time for treated plants. Nonetheless, vessel density in stems declined with plant age, and was significantly lower in treated plants (Table 3).
The proportion of vascular tissue increased in the roots over time (Fig. 2), while it decreased in stems and leaves. For plants at all levels of Cd treatment, the proportion of vascular tissue was greater during the pre-flowering and flowering stages but lower in the last phase, compared with the controls. The proportions of pith and cortex declined ontogenetically. Pith areas were consistently smaller for treated plants,
whereas the amount of cortical area differed from the controls rather consistently.
In the roots of control plants, the lengths of xylem fibers and vessel elements, as well as the width and density of vessels, increased with plant age; the extent of these increases was significantly less in the treated plants (Table 4). Fiber length was reduced up to $28 \%$ under the influence of Cd, while the length and width of vessel elements was up to $29 \%$ and $35 \%$ less, respectively. Patterns of variation according to plant age and Cd dose were quite similar to those seen in the stem.

## DISCUSSION

$\mathrm{Cd}^{2+}$ treatment significantly decreased the amount of growth in C. cajan plants. The number of leaves, single leaf area, and total leaf area were significantly lower throughout the development of the treated plants. The amount by which leaf-area expansion is reduced normally is linearly correlated with Cd concentration in the medium (Skorzynska-Polit and Baszynski, 1997), perhaps because $\mathrm{Cd}^{2+}$ may affect cell division and differentiation during shoot growth (see Iqbal and Khudsar, 2000). In our study, stem-

Table 3. Fiber length, vessel width, and density in the stem wood at different developmental stages of $C$. cajan plants grown as control and on various concentrations of Cd . Mean $\pm \mathrm{SD}$ are based on 100 readings. Parentheses include percent variation. Differences are significant at $1 \%$ level.

|  | Control | Cadmium concentration $\left[\mu \mathrm{g}\left(\mathrm{CdCl}_{2}\right) \mathrm{g}^{-1}\right.$ (soil d.m.)] |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 5 | 10 | 15 | 25 | 50 |
| Fiber length ( $\mu \mathrm{m}$ ) |  |  |  |  |  |  |
| Pre-flowering | $331.00 \pm 34.20$ | $\begin{aligned} & 302.60 \pm 33.24 \\ & (8.56) \end{aligned}$ | $\begin{aligned} & 287.80 \pm 51.76 \\ & (13.05) \end{aligned}$ | $\begin{aligned} & 280.60 \pm 46.35 \\ & (15.22) \end{aligned}$ | $\begin{aligned} & 271.20 \pm 49.40 \\ & (18.06) \end{aligned}$ | $\begin{aligned} & 253.60 \pm 47.92 \\ & (23.38) \end{aligned}$ |
| Flowering | $333.40 \pm 33.00$ | $\begin{aligned} & 316.00 \pm 43.99 \\ & (5.21) \end{aligned}$ | $\begin{aligned} & 300.00 \pm 39.94 \\ & (10.01) \end{aligned}$ | $\begin{aligned} & 290.20 \pm 64.57 \\ & (12.95) \end{aligned}$ | $\begin{aligned} & 272.20 \pm 49.49 \\ & (18.35) \end{aligned}$ | $\begin{aligned} & 269.20 \pm 46.54 \\ & (19.25) \end{aligned}$ |
| Post-flowering | $388.60 \pm 57.50$ | $\begin{aligned} & 323.20 \pm 48.29 \\ & (16.82) \end{aligned}$ | $\begin{aligned} & 318.80 \pm 50.00 \\ & (17.96) \end{aligned}$ | $\begin{aligned} & 311.80 \pm 46.94 \\ & (19.86) \end{aligned}$ | $\begin{aligned} & 301.80 \pm 66.10 \\ & (22.39) \end{aligned}$ | $\begin{aligned} & 291.70 \pm 35.31 \\ & (24.93) \end{aligned}$ |
| Vessel width ( $\mu \mathrm{m}$ ) |  |  |  |  |  |  |
| Pre-flowering | $83.20 \pm 8.90$ | $\begin{aligned} & 77.20 \pm 10.35 \\ & (7.21) \end{aligned}$ | $\begin{aligned} & 76.50 \pm 9.10 \\ & (8.50) \end{aligned}$ | $\begin{aligned} & 72.39 \pm 12.79 \\ & (12.99) \end{aligned}$ | $\begin{aligned} & 68.50 \pm 11.43 \\ & (17.66) \end{aligned}$ | $\begin{aligned} & 41.40 \pm 6.52 \\ & (18.02) \end{aligned}$ |
| Flowering | $92.80 \pm 2.50$ | $\begin{aligned} & 87.20 \pm 12.46 \\ & (6.03) \end{aligned}$ | $\begin{aligned} & 83.20 \pm 10.08 \\ & (10.34) \end{aligned}$ | $\begin{aligned} & 83.20 \pm 11.10 \\ & (10.34) \end{aligned}$ | $\begin{aligned} & 82.90 \pm 9.26 \\ & (10.66) \end{aligned}$ | $\begin{aligned} & 74.60 \pm 8.07 \\ & (19.61) \end{aligned}$ |
| Post-flowering | $101.00 \pm 11.29$ | $\begin{aligned} & 89.10 \pm 7.73 \\ & (11.78) \end{aligned}$ | $\begin{aligned} & 88.00 \pm 10.20 \\ & (12.87) \end{aligned}$ | $\begin{aligned} & 86.50 \pm 11.66 \\ & (16.76) \end{aligned}$ | $\begin{aligned} & 85.70 \pm 10.10 \\ & (15.14) \end{aligned}$ | $\begin{aligned} & 85.70 \pm 8.01 \\ & (15.14) \end{aligned}$ |
| Vessel density ( $\mathrm{cm}^{\mathbf{- 2}}$ ) |  |  |  |  |  |  |
| Pre-flowering | $96.32 \pm 9.56$ | $\begin{aligned} & 68.56 \pm 17.28 \\ & (28.82) \end{aligned}$ | $\begin{aligned} & 68.16 \pm 17.28 \\ & (29.23) \end{aligned}$ | $\begin{aligned} & 62.00 \pm 13.60 \\ & (35.63) \end{aligned}$ | $\begin{aligned} & 61.28 \pm 13.36 \\ & (36.37) \end{aligned}$ | $\begin{aligned} & 59.44 \pm 12.88 \\ & (38.28) \end{aligned}$ |
| Flowering | $84.241 \pm 3.60$ | $\begin{aligned} & 61.92 \pm 15.76 \\ & (28.49) \end{aligned}$ | $\begin{aligned} & 61.52 \pm 11.84 \\ & (28.97) \end{aligned}$ | $\begin{aligned} & 61.44 \pm 11.56 \\ & (27.06) \end{aligned}$ | $\begin{aligned} & 59.60 \pm 14.36 \\ & (29.24) \end{aligned}$ | $\begin{aligned} & 55.28 \pm 10.60 \\ & (34.37) \end{aligned}$ |
| Post-flowering | $74.88 \pm 6.28$ | $\begin{aligned} & 49.60 \pm 10.56 \\ & (33.76) \end{aligned}$ | $\begin{aligned} & 45.48 \pm 8.96 \\ & (39.23) \end{aligned}$ | $\begin{aligned} & 42.08 \pm 8.48 \\ & (43.80) \end{aligned}$ | $\begin{aligned} & 40.96 \pm 8.60 \\ & (45.29) \end{aligned}$ | $\begin{aligned} & 40.32 \pm 8.80 \\ & (46.15) \end{aligned}$ |



Figure 2. Pie diagrams showing the relative proportion of vasculature, pith, and cortex in the root of the control and the cad-mium-treated plants of $C$. cajan at different stages of plant development..

Table 4. Fiber length, vessel width, and vessel density in the root in different developmental stages of $C$. cajan plants grown as control and on various concentrations of Cd. Mean $\pm$ SD are based on 100 readings. Parentheses include percent variation. Differences are significant at $1 \%$ level.

|  | Control | Cadmium concentration [ $\mu \mathrm{g}\left(\mathrm{CdCl}_{2}\right) \mathrm{g}^{-1}$ (soil d.m.)] |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 5 | 10 | 15 | 25 | 50 |
| Fiber length ( $\mu \mathrm{m}$ ) |  |  |  |  |  |  |
| Pre-flowering | $343.70 \pm 35.40$ | $\begin{aligned} & 312.40 \pm 52.35 \\ & (9.10) \end{aligned}$ | $\begin{aligned} & 304.00 \pm 45.89 \\ & (11.55) \end{aligned}$ | $\begin{aligned} & 287.20 \pm 68.24 \\ & (16.43) \end{aligned}$ | $\begin{aligned} & 287.21 \pm 40.63 \\ & (22.25) \end{aligned}$ | $\begin{aligned} & 256.60 \pm 42.88 \\ & (25.34) \end{aligned}$ |
| Flowering | $374.00 \pm 54.18$ | $\begin{aligned} & 315.80 \pm 73.57 \\ & (15.56) \end{aligned}$ | $\begin{aligned} & 304.20 \pm 58.03 \\ & (18.66) \end{aligned}$ | $\begin{aligned} & 292.00 \pm 43.04 \\ & (21.92) \end{aligned}$ | $\begin{aligned} & 285.30 \pm 58.21 \\ & (23.63) \end{aligned}$ | $\begin{aligned} & 270.19 \pm 46.79 \\ & (27.75) \end{aligned}$ |
| Post-flowering | $406.20 \pm 82.97$ | $\begin{aligned} & 328.60 \pm 60.87 \\ & (19.10) \end{aligned}$ | $\begin{aligned} & 314.80 \pm 54.59 \\ & (22.50) \end{aligned}$ | $\begin{aligned} & 313.10 \pm 47.47 \\ & (22.91) \end{aligned}$ | $\begin{aligned} & 300.70 \pm 45.84 \\ & (25.97) \end{aligned}$ | $\begin{aligned} & 295.00 \\ & (27.37) \end{aligned}$ |
| Vessel width ( $\mu \mathrm{m}$ ) |  |  |  |  |  |  |
| Pre-flowering | $63.20 \pm 7.26$ | $\begin{aligned} & 49.30 \pm 6.70 \\ & (21.99) \end{aligned}$ | $\begin{aligned} & 49.00 \pm 7.21 \\ & (22.46) \end{aligned}$ | $\begin{aligned} & 42.80 \pm 7.01 \\ & (23.27) \end{aligned}$ | $\begin{aligned} & 41.40 \pm 6.62 \\ & (34.49) \end{aligned}$ | $\begin{aligned} & 40.90 \pm 6.82 \\ & (35.28) \end{aligned}$ |
| Flowering | $65.60 \pm 3.29$ | $\begin{aligned} & 53.30 \pm 6.43 \\ & (18.75) \end{aligned}$ | $\begin{aligned} & 52.70 \pm 9.26 \\ & (19.66) \end{aligned}$ | $\begin{aligned} & 51.70 \pm 5.94 \\ & (21.18) \end{aligned}$ | $\begin{aligned} & 51.60 \pm 6.95 \\ & (21.34) \end{aligned}$ | $\begin{aligned} & 51.40 \pm 5.44 \\ & (21.64) \end{aligned}$ |
| Post-flowering | $75.60 \pm 9.72$ | $\begin{aligned} & 57.40 \pm 4.76 \\ & (24.07) \end{aligned}$ | $\begin{aligned} & 56.40 \pm 6.42 \\ & (25.39) \end{aligned}$ | $\begin{aligned} & 54.60 \pm 6.40 \\ & (27.77) \end{aligned}$ | $\begin{aligned} & 53.20 \pm 7.09 \\ & (29.62) \end{aligned}$ | $\begin{aligned} & 52.60 \pm 8.09 \\ & (30.42) \end{aligned}$ |
| Vessel density ( $\mathrm{cm}^{\mathbf{- 2}}$ ) |  |  |  |  |  |  |
| Pre-flowering | $123.04 \pm 7.68$ | $\begin{aligned} & 96.96 \pm 17.44 \\ & (21.19) \end{aligned}$ | $\begin{aligned} & 90.72 \pm 9.44 \\ & (26.26) \end{aligned}$ | $\begin{aligned} & 88.32 \pm 7.72 \\ & (23.21) \end{aligned}$ | $\begin{aligned} & 87.28 \pm 7.60 \\ & (29.06) \end{aligned}$ | $\begin{aligned} & 85.28 \pm 7.64 \\ & (30.68) \end{aligned}$ |
| Flowering | $103.60 \pm 15.16$ | $\begin{aligned} & 93.60 \pm 8.84 \\ & (9.65) \end{aligned}$ | $\begin{aligned} & 89.12 \pm 16.60 \\ & (13.97) \end{aligned}$ | $\begin{aligned} & 84.56 \pm 11.68 \\ & (18.37) \end{aligned}$ | $\begin{aligned} & 80.88 \pm 6.28 \\ & (21.93) \end{aligned}$ | $\begin{aligned} & 79.28 \pm 8.24 \\ & (23.47) \end{aligned}$ |
| Post-flowering | $99.84 \pm 9.84$ | $\begin{aligned} & 89.92 \pm 8.04 \\ & (9.93) \end{aligned}$ | $\begin{aligned} & 85.44 \pm 6.28 \\ & (14.42) \end{aligned}$ | $\begin{aligned} & 84.32 \pm 6.32 \\ & (15.54) \end{aligned}$ | $\begin{aligned} & 76.96 \pm 10.36 \\ & 22.91) \end{aligned}$ | $\begin{aligned} & 76.80 \pm 9.76 \\ & (23.07) \end{aligned}$ |

length growth rates decreased significantly at all Cd concentrations, except for the $5-\mu \mathrm{g}$ dose. Root-length growth also declined at each concentration of Cd . Overall, growth became more and more stunted with increasing Cd concentration; the root-shoot ratio was significantly lower than for the controls.
All heavy metals inhibit plant growth at higher concentrations, but the effect may also appear at very low concentrations. This could be partially due to root damage, which may involve injury to enzyme systems (Page et al., 1972), reductions in cell-water content and/or cell-wall elasticity (Becerril et al., 1989; Poschenrieder et al., 1989; Barceló and Poschenrieder, 1990), a reduction in the size of cells and intercellular spaces (Barceló et al., 1988a, 1988b), and/or a reduced carbohydrate content in cells (Greger and Bertell, 1992). Inhibition of growth and growth processes due to cadmium stress has been reported for many cereals and leguminous crops. For example, roots take up considerably larger amounts of heavy metals than do leaves (Kovacěvić et al., 1999). An accumulation of $\mathrm{Cd}^{2+}$ causes the calcium content to decrease, which may indirectly affect root growth (Creger and Bertell, 1992). In our study, Cd contents that were lower in the shoots than in the roots may have indicated that either 1) the detoxifica-
tion processes started soon after the initial accumulation of Cd in root tissue, thereby minimizing the amount of residual Cd for uptake by the shoots; or 2) the means for detoxification were stronger in the aerial parts, especially in the stem. This inhibitory effect of $\mathrm{Cd}^{2+}$ on the increase in root and shoot lengths is not uncommon (Iqbal and Khudsar, 2000).
When Cd toxicity inhibits growth, the amount of dry matter is reduced Jalil et al., 1994; Kovacěvić et al., 1999). Symptoms of $\mathrm{Cd}^{2+}$ stress in economically important crops include yellowing of leaves and reductions in grain yield and seedling mass (Skorzyn-ska-Polit and Baszynski, 1997; Kovacěvić et al., 1999). In our study, the amount of dry mass in leaves was significantly and consistently lower under Cd stress, except at the lowest concentration during the post-flowering stage. For treated plants, stem and root dry masses were significantly lower at each stage, although these differences were relatively small at low Cd doses during the early phase of development. Root-shoot dry mass ratios remained relatively and significantly lower throughout lives of the treated plants. The exceptions to this statistical significance were for plants at the pre-flowering stage growing under stress levels of 5,10 , or $15 \mu \mathrm{~g} \mathrm{CdCl}_{2}$.
Relative production of vascular tissues, pith, and
cortex also is affected by metal pollutants. The number and size of vascular bundles may be reduced, as Kovacěvić et al. (1999) observed in wheat leaves. In the current study, the overall proportion of vascular tissue increased in the C. cajan stem. In the roots, however, the ontogenetic increase in their proportions was not as rapid as that seen in the control.
Gaseous pollutants can reduce incremental xylem development (Gilbert, 1983). However, the amount of vasculature may also increase under pollution stress, thereby serving as an adaptive response. A sizeable increase in xylem development, during the flowering stage and afterward, may help ensure that water uptake is maintained so that plants can withstand the continuous pollution load during flowering and fruiting. Our observations were consistent with those by Iqbal et al. (1987a, 1987b) for Cassia occidentalis, Cassia tora, and Lantana camara. A sequence of important physiological events leads to decreased wood production in stems and roots. This involves the inhibition of photosynthesis and the synthesis of hormonal growth regulators, followed by a decrease in the amounts of carbohydrates and hormones being transported to the lower part of the stem and, then, to the root system (Kozlowski and Constantinidou, 1986).

Smaller shoot and root dimensions under pollution stress may result from the suppressed growth in component cells. Fibers in the stems and roots of $C$. cajan were significantly shorter in Cd-treated plants than in the controls. Length and width of vessel elements also were significantly and consistently less in the stressed samples. Cd caused a greater reduction in vesseldiameter growth in wheat leaves than did either Ni or Pb (Kovacěvić et al., 1999). In addition, Cd reduced the relative water conductivity in the excised stem sections of treated silver maple (Lamoreaux and Chaney, 1977). This was because less xylem tissue was available for water conductivity, vessels and tracheids were smaller than normal, and the xylem elements were partially blocked. Shorter vessels have been reported in a number of dicotyledonous trees and weeds developing under stress conditions (Pozgaj et al., 1996).
Here we showed that, at each stage of plant development, vessel density was markedly lower in cad-mium-treated C. cajan stems and roots compared with the controls. This agrees with the observations of Ghouse and Yunus (1972), who showed that air pollution caused a decrease in vessel abundance in several angiosperm herbs and shrubs (for review, see Pozgaj et al., 1996).

## ACKNOWLEDGEMENT

The first author is indebted to the Muslim Association for the Advancement of Science (MAAS), Aligarh, India, for providing scholarship during this study.

Received July 5, 2000; accepted September 15, 2000.

## LITERATURE CITED

Ali G, Iqbal M, Srivastava PS (1998) Interactive effect of Cd and Zn on the morphogenic potential of Bacopa monniera (L) Wettst. Plant Tiss Cult Biotech 4: 159-164
Assche VF, Clijsters H (1990) Effects of metal on enzyme activity in plants. Plant Cell Environ 13: 195-206
Barceló J, Poschenrieder C (1990) Plant water relations as affected by heavy metal stress: A review. J Plant Nutr 13: 1-37
Barceló J, Vazquez MD, Poschenrieder C (1988a) Cadmium induced structural and ultrastructural changes in the vascular system of bush bean stems. Bot Acta 101: 254-261
Barceló J, Vazquez MD, Poschenrieder C (1988b) Structural and uitrastructural disorders in cadmium-treated bush bean plants (Phaseolus vulgaris L). New Phytol 108: 37-49
Becerril JM, Gonzalez-Murua C, Munez-Rueda A, de Felipe MR (1989) Changes induced by cadmium and lead in gas exchange and water relations of clover and Lucerne. Plant Physiol Biochem 27: 913-918
Ghouse AKM, Yunus M (1972) Preparation of epidermal peels from leaves of gymnosperms by treatment with hot $60 \% \mathrm{HNO}_{3}$. Stain Technol 47: 322-324
Gilbert OL (1983) The growth of planted trees subject to fumes from brickworks. Environ Pollut 31: 301-310
Greger M, Bertell G (1992) Effects of $\mathrm{Ca}^{2+}$ and $\mathrm{Cd}^{2+}$ on the carbohydrate metabolism in sugar beet (Beta vulgaris). J Exp Bot 43: 167-173
Iqbal M, Khudsar T (2000) Heavy metal stress and forest cover: Plant performance as affected by cadmium toxicity, In RK Kohli, HP Singh, SP Vij, KK Dhir, DR Batish, DK Khurana, eds, Man and Forests, DNES, IUFRO, ISTS, Punjab University, Chandigarh, India, pp 85-112
Iqbal M, Mahmooduzzafar, Ghouse AKM (1987a) Impact of air pollution on the anatomy of Cassia occidentalis L . and Cassia tora L. Indian J Appl Pure Biol 2: 23-26
Iqbal M, Mahmooduzzafter, Kabeer I, Kaleemullah, Ahmad Z (1987b) The effect of air pollution on the stem anatomy of Lantana camara L. J Sci Res 9: 121-122
Jalil A, Selles F, Clarace JM (1994) Effects of cadmium on growth and uptake of cadmium and other elements by durum wheat. J Plant Nutr 17: 1839-1858
Kabata-Pendias A, Pendias H (1984) Trace Elements in Soil and Plants. CRC Press, Boca Raton, Florida
Kovacěvić G, Kastori R, Merkulov MJ (1999) Dry matter and leaf structure in young wheat plants as affected by
cadmium, lead, and nickel. Biol Plant 42: 119-123
Kozlowski TT, Constantinidou HA (1986) Response of wood plants to environmental pollution. I. Source and types of pollutants and plant responses. Forestry Abstr 47: 5-51
Lamoreaux RJ, Chaney WR (1977) Growth and water movement in silver maple seedlings affected by cadmium. J Environ Qual 6: 201-205
Mehindirata S, Ali ST, Mahmooduzzafar, Siddiqi TO, Iqbal $M$ (1999) Cadmium-induced changes in foliar responses of Solanum melongena L. Phytomorphol 49: 295-302
Moral R, Gomez I, Navarro-Pedreno J, Mataixe J (1994) Effects of cadmium on nutrient distribution, yield and growth of tomato grown in soil-less culture. J Plant Nutr 17: 953-962
Moya JL, Ros R, Picazo I (1993) Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. Photosynth Res 36: 75-80
Page AL, Bingham FT, Nelson C (1972) Cadmium absorption and growth of various plant species as influenced by solution cadmium concentrations. J Environ Qual 1: 288-291
Poschenrieder C, Gunse B, Barceló J (1989) Influence of
cadmium on water relations, stomatal resistance and abscisic acid content in expanding bean leaves. Plant Physiol 90: 1365-1371
Pozgaj A, Iqbal M, Kucera LJ (1996) Development, structure and properties of wood from trees affected by air pollution, In M Yunus, $M$ lqbal, eds, Plant Response to Air Pollution, John Wiley \& Sons, Chichester, UK, pp 395-424
Reese RN, Roberts LW (1985) Effects of Cd on whole cell and mitochondrial respiration in tobacco cell suspension culture (Nicotiana tabaccum L. var. Xanthi). Plant Physiol 120: 123-140
Skorzynska-Polit E, Baszynski T (1997) Differences in sensitivity of the photosynthetic apparatus in Cd-stressed runner bean plants in relation to their age. Plant Sci 128: 11-21
Weigel HJ (1985a) The effect of $\mathrm{Cd}^{2+}$ on photosynthetic reactions of mesophyll protoplasts. Physiol Plant 63: 192-200
Weigel HJ (1985b) Inhibition of photosynthetic reactions of isolated chlorophasts by cadmium. J Plant Physiol 119: 179-189


[^0]:    *Corresponding author; fax +91-11-608-8874
    e-mail root@hamduni.ren.nic.in

[^1]:    **, Significant at $1 \%$ level; *, Significant at $5 \%$ level; NS, Non-significant.

