Journal of Plant Biology, March 2005, 48(1) : 64-84

Morpho-Anatomical Responses of *Trigonella foenum graecum* Linn. to Induced Cadmium and Lead Stress

Syed Hilal Ahmad[2], Zafar Reshi[2], Javed Ahmad[1], and Muhammad Iqbal[1]
[1]Department of Botany, Jamia Hamdard, Hamdard Nagar, New Delhi-110062, India
[2]Department of Botany, University of Kashmir, Srinagar-190006, J&K, India

Effect of different concentrations of cadmium (0, 5, 15, 30, 50 μg/g of soil) and lead (0, 25, 50, 100, 200 μg/g of soil) on morphological and anatomical features of *Trigonella foenum graecum* Linn. was studied at pre-flowering, flowering and post flowering stages. Morphological attributes, like number of leaves per plant, total leaf area of the plant and single leaf area, dry mass of stem, root and leaf, length of shoot, root and plant, size of stomata and stomatal pore, and the density of stomata on both epidermises were significantly reduced under metal stress at all the developmental stages. Trichome length on both epidermises increased while their density decreased under metal stress. Under cadmium stress, proportion of pith and vasculature decreased but cortex increased in the stem. Under lead stress, proportion of pith and vasculature increased but cortex decreased in the stem. In the root, proportion of vasculature and pith increased and cortex decreased in response to both cadmium and lead stresses. Dimensions of vessel element and xylem fibre in the wood of stem and root decreased under the cadmium and lead stresses. Decrease in density of vessel element in the stem and root with advancement of age was more pronounced in plants grown under cadmium and lead stresses.

Keywords: anatomical changes, cadmium, lead, morphological changes, *Trigonella foenum graecum*

Increasing environmental pollution with heavy metals has sharpened focus on their impact on various organisms including plants. Among non-nutrient heavy metals, Cd and Pb are the most widespread that result from human economic activities, like burning liquid and solid fuels, smelting and foundry works, sewage high in Pd and Cd, and soil-applied chemicals including fertilizers (Sanita di Toppi and Gabbrielli, 1999). When bound on the cell surface and also within the cell, Cd and Pb ions interact with the functional groups of proteins, nucleic acids and polysaccharides and substitute other metal ions already bound to these functional groups that can lead to various metabolic disorders and reduction in growth (Costa and Spitz, 1997; Skorzynska-Polit and Baszynski, 1997; Seregin and Ivanov, 2001).

Trigonella foenum graecum Linn. (Leguminosaceae), an important plant in traditional medicine, is grown in temperate regions of India usually on marginal lands which are contaminated to varying extents with heavy metals due to industrial activities. In view of adverse effects of heavy metals on growth and development of plants, present studies were carried out to investigate the influence of cadmium and lead on morpho-anatomical features of the species at different stages of development.

MATERIALS AND METHODS

Healthy seeds of *T. foenum graecum* were procured from Indian Agricultural Research Institute (IARI), New Delhi, and were sown in earthen pots containing 10 kg of sterilized soil. Sludge and farm compost (2 kg per pot) were used as manure and mixed thoroughly with soil at the time of sowing. Plants were treated at the seedling stage after one month since sowing with different concentrations of cadmium chloride (5, 15, 30, 50 μg/g of soil) and lead acetate (25, 50, 100, 200 μg/g of soil). Untreated plants served as control. The seeds were sown in August when the monthly mean temperature ranged from 24 to 33°C. Replicate plant samples were collected at the pre-flowering (two and half months after sowing), flowering (after four months of sowing) and post-flowering (five and half months after sowing). Samples were oven dried at 80°C for 48 h to measure dry weights. Height, shoot length, number of leaves and number of branches were recorded in 10 replicate plants in each treatment. Total foliar and single leaf area was estimated with digital Leaf Area Meter (3000A, LICOR, USA).

*Corresponding author; fax 91-194-2433091
e-mail hilu1999@yahoo.co.in

For anatomical studies, samples were fixed in FAA (formyl aceticalcohol) and preserved in 70% ethanol. Transverse sections were obtained with a sliding microtome (Reicherts, USA). After dehydration with ethanol series, the sections were stained with safranin and haematoxylin 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. For measuring the dimensions of xylem fibres and vessel elements, tissues were macerated by treating with hot HNO_3 (Ghouse and Yunus, 1972). The epidermal peels were also obtained using hot HNO_3 (Ghouse and Yunus, 1972). The peels were then processed in the customary ethanol series for dehydration, stained with iron-aluminium haemotaxylin or Bismarck brown (Sass, 1958), and mounted in Canada balsm. Various dimensions of stomata and trichomes were measured under a binocular microscope using a calibrated ocular-micrometer scale. Concentration of cadmium and lead in different plant parts was determined with an Atomic Absorption Spectrometer (Thermo Jarrel Ash, USA). Significance level was determined using Student's t test.

RESULTS

Although the magnitude of increase was significantly lower with the increasing concentration of the metals, increase in the shoot length, root length and total plant height was also observed with increasing age of the plant (Table 1).

The data summarized in Table 2 present a comparative account of the foliar features, such as number of leaves, average leaf area and number of branches per plant, in the plants grown under control and metal stressed conditions. All the above parameters registered increase with the age of the plants under control conditions. Under the metal stress, magnitude of increase was significantly lower compared to the untreated control plants. Decline in all the above mentioned became more pronounced with increasing concentration of the metals. Similar trend was observed in the dry weight (Table 3).

Data on foliar epidermal traits at various developmental stages are summarized in Tables 4-7. Stomatal density, length and width declined under metal stress at all the stages of development. Trichome dimensions (Tables 4-7) also showed variation under the influence of cadmium and lead, however, trichome length in adaxial and abaxial epidermis increased even under the metal stress conditions but the magnitude of increase was significantly lower. Trichome density in both epidermal layers decreased with increasing age of the plant under the control condition and the stress conditions. The decline was most pronounced at the highest metal concentration.

Increase in the proportion of vascular tissue and decrease in the proportion of the other two tissues were apparent with increasing age of the plant. However, the vasculature decreased during pre-flowering and post-flowering stages except at 5 μg cadmium and 25 μg lead treatments. Thereafter, continuous increase during post-flowering stage at all the concentrations of these metals, except at 200 μg of lead, was observed (Table 8).

Decrease in the proportion of pith was apparent with the age of the plants in control and treated conditions. Maximum decline was recorded at 50 μg cadmium and 200 μg lead during pre-flowering and post-flowering stages and 200 μg lead during flowering stage.

Decrease in the cortex was apparent with the age of the plants in control and the treated plants. Proportion of cortex was higher in the metal treated plants compared to the control. Maximum variation was observed at 50 μg cadmium and 200 μg lead.

Cell lengths in the third internodes of stem from the base of plants are presented in Tables 10 and 11. Under untreated conditions, length and width of xylem fibres consistently increased with the age of the plants. In the treated plants, however, increase in the length and width of fibres was relatively lesser as compared to control. Length of vessel elements increased as the developmental stage advanced in control plants. The same pattern persisted under the metal stress conditions, but the length of vessel elements was less in the metal-treated plants. Decline in the length of vessel elements became more pronounced with increasing concentration of the metals used. Width and area of the vessel elements increased with the age of the plants under controlled conditions. Though similar trend was recorded under the metal stress condition, the increase was markedly lower than the control plants. Decline in the vessel width was enhanced with increase in the metal concentration. Vessel density in the stem wood decreased with the age of the plants in the controlled condition. The same pattern was also observed under metal stress but vessel density was significantly lower in the treated plants compared to that of control plants. Reduction in the vessel density was more pronounced with increasing concentrations of the metals.

Effect of heavy metal stress on the extent of root

Table 1. Length of different plant parts (cm) at different developmental stages of *T. foenum graecum* Linn. under Cd and Pb stress. Mean ± S.D. is based on ten replicates. Parentheses include percent variation.

Parameters/Developmental stage	Cadmium chloride (μg/g of soil)				
	0	5	15	30	50
Shoot length					
Pre-flowering	26.78 ± 1.37	20.90 ± 1.29 (21.96)**	15.28 ± 1.46 (42.94)**	14.37 ± 1.42 (46.34)**	11.00 ± 1.00 (58.92)**
Flowering	42.00 ± 4.63	35.00 ± 1.00 (16.67)*	28.40 ± 5.85 (32.38)**	21.80 ± 4.08 (48.09)**	18.60 ± 2.40 (55.71)**
Post-flowering	59.60 ± 1.14	54.40 ± 1.14 (8.72)**	44.00 ± 1.58 (26.17)**	43.40 ± 2.07 (27.18)**	38.00 ± 1.58 (36.24)**
Root length					
Pre-flowering	16.40 ± 1.14	9.16 ± 0.24 (44.15)**	6.50 ± 0.50 (60.37)**	4.66 ± 0.60 (71.59)**	2.80 ± 0.83 (83.93)**
Flowering	17.30 ± 2.58	10.00 ± 2.07 (42.20)**	6.60 ± 0.54 (61.85)**	4.60 ± 0.54 (73.41)**	2.80 ± 1.83 (83.82)**
Post-flowering	17.60 ± 1.14	10.60 ± 1.14 (39.77)**	9.20 ± 1.30 (47.73)**	7.40 ± 1.14 (57.95)**	4.04 ± 1.14 (75.00)**
Plant length					
Pre-flowering	43.18 ± 3.22	30.06 ± 3.28 (30.38)**	21.78 ± 2.26 (49.56)**	19.00 ± 2.26 (60.00)**	13.80 ± 2.84 (68.04)**
Flowering	54.20 ± 5.76	44.00 ± 2.65 (18.82)*	35.00 ± 5.96 (35.42)**	26.40 ± 4.34 (51.29)**	21.40 ± 2.30 (60.52)**
Post-flowering	76.80 ± 2.86	64.80 ± 2.84 (15.63)**	53.20 ± 2.30 (30.73)**	50.80 ± 2.79 (33.85)**	42.40 ± 2.51 (44.79)**
	Lead acetate (μg/g of soil)				
	0	25	50	100	200
Shoot length					
Pre-flowering	26.78 ± 1.37	24.04 ± 1.26 (10.23)**	19.06 ± 1.24 (28.83)**	17.02 ± 1.63 (36.45)**	13.04 ± [illegible].71 (51.31)**
Flowering	42.00 ± 4.63	37.60 ± 2.07 (10.48)^{NS}	33.00 ± 1.41 (21.43)**	32.40 ± 1.14 (22.86)**	21.80 ± [illegible].30 (48.10)**
Post-flowering	59.60 ± 1.14	59.20 ± 2.48 (0.67)^{NS}	52.60 ± 1.34 (11.74)**	46.00 ± 2.23 (22.82)**	43.00 ± [illegible].58 (27.85)**
Root length					
Pre-flowering	16.40 ± 1.14	12.46 ± 0.27 (-24.02)**	7.12 ± 0.66 (56.59)**	6.28 ± 0.19 (61.71)**	2.96 ± 0.16 (81.95)**
Flowering	17.30 ± 2.58	11.60 ± 0.54 (32.95)**	10.20 ± 0.28 (41.04)**	9.40 ± 0.54 (45.66)**	5.60 ± 0.54 (67.63)**
Post-flowering	17.60 ± 1.14	16.40 ± 1.10 (6.82)^{NS}	14.11 ± 1.67 (19.82)*	13.80 ± 1.48 (21.59)**	11.60 ± 1.14 (34.09)**
Plant length					
Pre-flowering	43.18 ± 3.22	36.00 ± 3.22 (16.63)*	26.18 ± 3.57 (39.37)**	23.30 ± 2.57 (46.04)**	16.00 ± 2.80 (62.95)**
Flowering	54.20 ± 5.76	49.00 ± 3.73 (9.60)^{NS}	43.20 ± 2.64 (20.30)**	41.80 ± 2.64 (22.88)**	27.80 ± 2.30 (48.71)**
Post-flowering	76.80 ± 2.86	75.20 ± 3.03 (2.08)^{NS}	67.80 ± 2.68 (11.72)**	59.80 ± 3.42 (22.14)**	54.60 ± 2.51 (28.91)**

**Significant at 1% level; *Significant at 5% level; NS, non-significant.

Table 2. Yield attributes at different developmental stages of *T. foenum graecum* Linn. grown under Cd and Pb stress. Mean ± S.D. is based on twenty five replicates. Parentheses include percent variation.

Parameters/Developmental stage	Cadmium chloride (µg/g of soil)				
	Control	0	5	15	30
Number of leaves/plant					
Pre-flowering	92.60 ± 9.21	82.20 ± 8.77 (11.23)^{NS}	44.60 ± 7.67 (51.84)**	41.60 ± 8.05 (55.08)**	35.60 ± 9.14 (61.56)**
Flowering	151.60 ± 11.14	144.00 ± 11.91 (5.01)^{NS}	132.40 ± 12.07 (12.66)*	115.40 ± 14.51 (23.88)**	107.00 ± 11.00 (29.42)**
Post-flowering	277.60 ± 15.14	203.80 ± 11.42 (26.59)**	146.40 ± 12.07 (47.26)**	142.20 ± 11.92 (48.78)**	130.40 ± 11.15 (53.03)**
Average leaf area (Cm-^{2})					
Pre-flowering	250.62 ± 13.10	195.66 ± 13.21 (21.93)**	79.62 ± 13.07 (68.23)**	48.73 ± 13.03 (80.56)**	32.87 ± 13.05 (86.88)**
Flowering	356.11 ± 13.84	187.47 ± 14.39 47.36)**	167.17 ± 13.82 (53.06)**	147.56 ± 13.62 (58.56)**	73.69 ± 13.79 (79.31)**
Post-flowering	2183.26 ± 36.63	1889.22 ± 34.70 (13.47)**	1576.76 ± 34.34 (27.78)**	654.08 ± 34.79 (70.04)**	456.99 ± 34.84 (78.66)**
Number of branches/plant					
Pre-flowering	5.20 ± 1.30	4.80 ± 0.84 (7.69)^{NS}	4.60 ± 0.55 (11.54)^{NS}	4.40 ± 1.14 (15.38)^{NS}	3.00 ± 1.00 (42.31)*
Flowering	5.80 ± 0.84	4.80 ± 0.84 (17.24)^{NS}	4.60 ± 0.45 (20.69)*	4.40 ± 1.14 (24.14)*	3.60 ± 1.14 (37.93)*
Post-flowering	8.40 ± 1.14	5.40 ± 1.14 (35.71)**	5.40 ± 1.14 (35.71)**	4.80 ± 0.84 (42.86)**	4.60 ± 0.55 (45.24)**
	Lead acetate (µg/g of soil)				
	0	25	50	100	200
Number of leaves/plant					
Pre-flowering	92.60 ± 9.21	64.40 ± 8.36 (30.45)**	40.20 ± 8.27 (56.59)**	34.20 ± 9.92 (63.07)**	27.60 ± 9.78 (70.19)**
Flowering	151.60 ± 11.14	145.60 ± 15.12 (3.96)^{NS}	114.05 ± 4.05 (24.67)**	103.40 ± 13.67 (31.79)**	8.00 ± 13.98 (47.23)**
Post-flowering	277.60 ± 15.14	151.00 ± 12.58 (45.61)**	132.00 ± 12.74 (52.45)**	126.30 ± 12.58 (54.50)**	122.00 ± 13.43 (56.05)**
Average leaf area (Cm-^{2})					
Pre-flowering	250.62 ± 13.10	171.60 ± 12.65 (31.53)**	72.29 ± 12.52 (71.16)**	42.87 ± 13.08 (82.89)**	23.64 ± 12.13 (90.57)**
Flowering	356.11 ± 13.84	185.24 ± 12.49 (47.98)**	108.70 ± 13.50 (69.48)**	67.33 ± 12.45 (81.09)**	33.02 ± 12.52 (90.73)**
Post-flowering	2183.26 ± 36.62	1655.65 ± 37.47 (24.17)**	1215.90 ± 35.25 (44.31)**	610.70 ± 34.92 (72.03)**	328.40 ± 33.38 (84.96)**
Number of branches/plant					
Pre-flowering	5.20 ± 1.30	5.00 ± 1.00 (3.85)^{NS}	3.80 ± 0.83 (26.92)*	3.40 ± 0.74 (34.62)*	3.40 ± 0.94 (34.62)*
Flowering	5.80 ± 0.84	5.60 ± 0.96 (3.45)^{NS}	5.25 ± 1.67 (9.48)^{NS}	5.00 ± 1.63 (13.79)^{NS}	3.60 ± 1.14 (37.93)*
Post-flowering	8.40 ± 1.14	7.230 ± 1.30 (14.29)^{NS}	6.20 ± 0.84 (26.19)*	5.20 ± 0.84 (38.10)**	3.60 ± 0.84 (57.14)**

**Significant at 1% level; *Significant at 5% level; NS, non-significant.

Table 3. Dry weight of different plant parts (g) at different developmental stages of *T. foenum graecum* Linn. grown under Cd and Pb stress. Mean ± S.D. is based on ten replicates. Parentheses include percent variation.

Parameters/Developmental stage	Cadmium chloride (µg/g of soil)				
	0	5	15	30	50
Shoot dry weight					
Pre-flowering	0.50 ± 0.08	0.42 ± 0.01 (16.00)**	0.26 ± 0.03 (48.00)**	0.13 ± 0.02 (74.00)**	0.08 ± 0.01 (84.00)**
Flowering	0.85 ± 0.04	0.37 ± 0.01 (56.47)**	0.34 ± 0.02 (60.00)**	0.31 ± 0.01 (63.53)**	0.22 ± 0.01 (74.12)**
Post-flowering	4.07 ± 0.11	2.62 ± 0.64 (35.63)**	1.60 ± 0.80 (60.69)**	1.54 ± 0.15 (62.16)**	1.86 ± 0.13 (73.96)**
Root dry weight					
Pre-flowering	0.08 ± 0.01	0.05 ± 0.02 (37.50)**	0.04 ± 0.01 (50.00)**	0.03 ± 0.01 (62.50)**	0.01 ± 0.05 (87.50)**
Flowering	0.11 ± 0.02	0.09 ± 0.01 (18.18)^{NS}	0.04 ± 0.02 (63.64)*	0.03 ± 0.01 (72.73)*	0.02 ± 0.07 (81.81)*
Post-flowering	0.71 ± 0.04	0.32 ± 0.02 (54.93)**	0.15 ± 0.02 (78.89)**	0.12 ± 0.02 (83.10)**	0.09 ± 0.01 (88.32)**
Plant dry weight					
Pre-flowering	5.26 ± 1.30	0.95 ± 0.02 (24.60)**	0.55 ± 0.02 (56.35)**	0.33 ± 0.08 (73.81)**	0.19 ± 0.03 (84.92)**
Flowering	5.80 ± 0.84	0.95 ± 0.15 (41.36)**	0.889 ± 0.11 (45.68)**	0.78 ± 0.13 (51.85)**	0.56 ± 0.12 (65.43)**
Post-flowering	8.06 ± 1.14	4.83 ± 0.46 (40.07)**	2.87 ± 0.42 (64.39)**	2.58 ± 0.48 (67.99)**	1.71 ± 0.46 (78.78)**
	Lead acetate (µg/g of soil)				
	0	25	50	100	200
Shoot dry weight					
Pre-flowering	0.50 ± 0.08	0.47 ± 0.01 (6.00)^{NS}	0.33 ± 0.02 (34.00)^{NS}	0.29 ± 0.01 (42.00)**	0.13 ± 0.01 (74.00)**
Flowering	0.85 ± 0.04	0.45 ± 0.01 (47.06)**	0.34 ± 0.01 (60.00)**	0.33 ± 0.01 (61.18)**	0.29 ± 0.01 (65.88)**
Post-flowering	4.07 ± 0.11	3.19 ± 0.15 (21.62)**	2.63 ± 0.11 (35.38)**	2.50 ± 0.11 (38.57)**	1.37 ± 0.13 (66.34)**
Root dry weight					
Pre-flowering	0.08 ± 0.01	0.06 ± 0.01 (25.00)*	0.05 ± 0.01 (37.50)**	0.05 ± 0.01 (37.50)**	0.03 ± 0.01 (62.50)**
Flowering	0.13 ± 0.04	0.10 ± 0.08 (23.08)**	0.09 ± 0.01 (30.77)**	0.08 ± 0.01 (38.46)**	0.04 ± 0.02 (69.23)**
Post-flowering	0.71 ± 0.04	0.33 ± 0.02 (53.52)**	0.17 ± 0.01 (76.06)**	0.13 ± 0.02 (81.69)**	0.11 ± 0.01 (84.51)**
Plant dry weight					
Pre-flowering	5.20 ± 1.30	5.00 ± 1.00 (3.85)^{NS}	3.80 ± 0.83 (26.92)*	3.40 ± 0.74 (34.62)*	3.40 ± 0.94 (34.62)*
Flowering	5.80 ± 0.84	5.60 ± 0.96 (3.45)^{NS}	5.25 ± 1.67 (9.48)^{NS}	5.00 ± 1.63 (13.79)^{NS}	3.60 ± 1.14 (37.93)*
Post-flowering	8.40 ± 1.14	7.20 ± 1.30 (14.29)^{NS}	6.20 ± 0.84 (26.19)*	5.20 ± 0.84 (38.10)**	3.60 ± 0.84 (57.14)*

**Significant at 1% level; *Significant at 5% level; NS, non-significant.

Table 4. Epidermal traits (lower epidermis) at different developmental stages of *T. foenum graecum* Linn. grown under Cd stress. Mean ± S.D. is based on twenty five replicates. Parentheses include percent variation.

Parameters/Developmental stage	Cadmium chloride (μg/g of soil)				
	0	5	15	30	50
Length of the stomata (μm)					
Pre-flowering	58.24 ± 6.01	54.36 ± 5.08 (6.66)*	49.36 ± 2.06 (15.25)**	44.38 ± 3.04 (23.80)**	39.41 ± 2.09 (32.33)**
Flowering	60.26 ± 6.05	54.22 ± 7.03 (10.02)**	50.28 ± 5.03 (16.56)**	46.28 ± 3.06 (23.20)**	41.26 ± 4.06 (31.53)**
Post-flowering	61.22 ± 8.02	55.10 ± 3.03 (9.99)**	51.09 ± 4.08 (16.55)**	47.11 ± 7.02 (23.05)**	43.15 ± 6.05 (29.52)**
Width of stomata (μm)					
Pre-flowering	45.26 ± 5.63	40.65 ± 7.47 (18.66)**	37.10 ± 5.25 (17.01)**	32.70 ± 4.92 (27.75)**	28.40 ± 3.38 (37.25)**
Flowering	43.12 ± 6.84	39.24 ± 5.49 (8.98)*	36.70 ± 3.50 (14.87)**	37.33 ± 4.45 (27.33)**	27.02 ± 2.52 (37.32)**
Post-flowering	41.62 ± 6.10	38.60 ± 4.65 (7.26)**	34.29 ± 3.52 (17.61)**	29.87 ± 5.08 (25.23)**	26.64 ± 4.13 (35.99)**
Length of stomata (μm)					
Pre-flowering	45.10 ± 6.23	42.14 ± 4.02 (6.56)*	40.19 ± 5.02 (10.89)**	38.32 ± 3.01 (15.03)**	36.65 ± 2.03 (18.74)**
Flowering	44.16 ± 5.12	41.10 ± 4.01 (6.93)*	39.12 ± 3.01 (11.41)**	37.31 ± 1.05 (15.51)**	35.49 ± 2.02 (19.63)**
Post-flowering	43.20 ± 4.23	40.07 ± 5.01 (7.25)*	38.11 ± 4.02 (11.78)**	36.21 ± 3.05 (16.18)**	34.36 ± 2.03 (20.46)**
Stomatal density (per microscopic field)					
Pre-flowering	33.20 ± 3.60	29.21 ± 2.18 (11.48)**	27.20 ± 2.02 (17.58)**	25.19 ± 1.06 (23.67)**	22.17 ± 2.05 (32.82)**
Flowering	34.40 ± 23.00	30.18 ± 1.04 (12.27)**	28.18 ± 2.04 (23.65)**	26.19 ± 1.05 (39.27)**	23.18 ± 2..03 (32.08)**
Post-flowering	35.60 ± 5.50	31.17 ± 2.03 (12.44)**	27.18 ± 2.07 (23.65)**	25.18 ± 1.05 (24.27)**	24.18 ± 2.05 (32.08)**
Length of trichome (μm)					
Pre-flowering	201.70 ± 5.48	202.58 ± 519 (-0.41)^{NS}	232.96 ± 4.56 (-15.56)**	263.49 ± 7.85 (-30.63)**	275.60 ± 6.76 (-36.67)**
Flowering	213.29 ± 5.02	223.78 ± 6.12 (-4.92)**	54.01 ± 4.83 (-19.09)**	274.61 ± 5.14 (-26.18)**	290.11 ± 4.40 (-32.78)**
Post-flowering	223.48 ± 8.30	235.59 ± 9.79 (-5.42)**	226.61 ± 4.89 (-19.30)**	218.99 ± 5.64 (-26.18)**	296.74 ± 6.72 (-32.78)**
Trichome density (per microscopic field)					
Pre-flowering	10.07 ± 1.35	9.92 ± 2.01 (1.49)^{NS}	8.80 ± 1.56 (12.61)**	7.80 ± 1.05 (22.54)**	6.20 ± 1.25 (38.43)**
Flowering	9.99 ± 2.20	8.80 ± 2.42 (11.91)^{NS}	7.60 ± 2.96 (23.92)**	6.20 ± 1.05 (37.94)**	5.20 ± 2.00 (47.95)**
Post-flowering	8.97 ± 2.35	8.01 ± 2.43 (10.70)^{NS}	7.01 ± 2.42 (21.85)**	5.25 ± 1.30 (41.47)**	4.80 ± 1.50 (46.49)**

**Significant at 1% level; *Significant at 5% level; NS, non-significant.

Table 5. Epidermal traits (lower epidermis) at different developmental stages of *T. foenum graecum* Linn. grown under Pb stress. Mean ± S.D. is based on twenty five replicates. Parentheses include percent variation.

Parameters/Developmental stage	Lead acetate (μg/g of soil)				
	0	25	50	100	200
Length of stomata (μm)					
Pre-flowering	58.24 ± 6.01	54.00 ± 4.50	50.75 ± 3.50	46.00 ± 2.20	40.50 ± 2.10
		(7.28)**	(12.86)**	(21.02)**	(30.46)**
Flowering	60.26 ± 6.05	56.25 ± 5.50	52.50 ± 4.00	48.00 ± 3.25	41.50 ± 2.50
		(6.65)*	(12.88)**	(20.35)**	(31.13)**
Post-flowering	61.22 ± 8.02	57.00 ± 4.50	55.25 ± 5.75	53.50 ± 3.25	42.00 ± 2.20
		(6.89)*	(9.75)**	(12.61)**	(31.39)**
Width of stomata (μm)					
Pre-flowering	45.26 ± 5.63	41.52 ± 6.48	38.27 ± 5.36	36.78 ± 3.38	32.75 ± 2.48
		(8.26)*	(15.44)**	(18.74)**	(27.64)**
Flowering	43.11 ± 6.48	40.27 ± 3.26	37.01 ± 4.23	35.98 ± 50.26	31.74 ± 6.26
		(6.59)^{NS}	(14.15)**	(16.54)**	(26.37)**
Post-flowering	41.62 ± 6.10	38.20 ± 4.21	36.51 ± 4.21	34.48 ± 3.25	30.86 ± 6.26
		(5.18)*	(12.28)**	(17.16)**	(25.85)**
Length of stomata (μm)					
Pre-flowering	45.10 ± 6.23	41.40 ± 4.03	39.59 ± 3.04	36.98 ± 4.04	32.60 ± 5.04
		(8.20)*	(12.22)**	(18.00)**	(27.72)**
Flowering	44.16 ± 5.21	40.30 ± 6.02	38.37 ± 3.03	35.95 ± 4.05	31.96 ± 6.05
		(8.74)*	(13.11)**	(18.59)**	(27.63)**
Post-flowering	43.20 ± 4.23	40.21 ± 5.01	37.34 ± 5.02	34.65 ± 3.58	30.44 ± 3.03
		(6.92)*	(13.56)**	(149.79)**	(29.54)**
Stomatal density (per microscopic field)					
Pre-flowering	33.20 ± 3.20	28.80 ± 2.86	26.40 ± 3.46	24.20 ± 1.88	22.60 ± 2.76
		(12.73)**	(20.00)**	(26.67)**	(31.52)**
Flowering	34.40 ± 3.00	30.40 ± 20.64	28.80 ± 1.49	26.60 ± 3.46	23.52 ± 2.80
		(11.63)**	(16.28)**	(22.67)**	(31.63)**
Post-flowering	35.60 ± 5.50	32.00 ± 2.08	30.00 ± 4.87	28.60 ± 3.85	25.20 ± 2.15
		(10.11)**	(15.73)**	(19.66)**	(29.21)**
Length of trichome (μm)					
Pre-flowering	201.70 ± 5.48	235.56 ± 6.33	253.33 ± 4.15	270.72 ± 5.36	289.66 ± 8.48
		(-16.79)**	(-25.60)**	(-34.22)**	(-43.61)**
Flowering	213.29 ± 5.02	249.27 ± 4.53	268.65 ± 6.02	286.49 ± 7.32	304.75 ± 7.83
		(-16.87)**	(-25.96)**	(-34.32)**	(-42.88)**
Post-flowering	223.48 ± 8.30	260.56 ± 7.77	279.58 ± 7.01	297.58 ± 7.01	315.34 ± 8.02
		(-16.59)**	(-25.10)**	(-32.93)**	(-14.10)**
Trichome density (per microscopic field)					
Pre-flowering	10.07 ± 1.35	9.65 ± 1.50	8.62 ± 2.50	7.21 ± 1.65	6.31 ± 2.65
		(4.17)^{NS}	(14.40)*	(28.40)**	(37.34)**
Flowering	9.99 ± 2.02	9.02 ± 1.99	8.10 ± 1.60	6.99 ± 2.01	5.92 ± 2.02
		(9.71)^{NS}	(18.92)**	(30.03)**	(40.74)**
Post-flowering	8.97 ± 2.35	8.02 ± 2.35	7.91 ± 1.25	6.12 ± 1.01	5.20 ± 1.98
		(10.59)^{NS}	(11.82)^{NS}	(31.77)**	(42.03)**

**Significant at 1% level; *Significant at 5% level; NS, non-significant.

Table 6. Epidermal traits (upper epidermis) at different developmental stages of *T. foenum graecum* Linn. grown under Pb stress. Mean ± S.D. is based on twenty five replicates. Parentheses include percent variation.

Parameters/Developmental stage	Lead acetate (µg/g of soil)				
	0	25	50	100	200
Length of the stomata (µm)					
Pre-flowering	55.23 ± 5.05	50.20 ± 4.04	44.18 ± 3.03	39.18 ± 6.02	35.16 ± 5.05
		(9.11)**	(20.01)**	(29.06)**	(36.34)**
Flowering	58.17 ± 7.05	52.16 ± 5.20	47.15 ± 3.04	42.16 ± 4.02	38.16 ± 6.19
		(10.33)	(18.94)**	(27.52)**	(34.40)**
Post-flowering	59.20 ± 6.04	54.13 ± 7.01	50.15 ± 6.01	46.15 ± 5.03	40.14 ± 4.02
		(8.56)*	(15.29)**	(22.04)**	(32.20)**
Width of stomata (µm)					
Pre-flowering	26.80 ± 2.86	21.80 ± 2.84	17.20 ± 2.30	15.80 ± 2.79	13.40 ± 2.51
		(18.66)**	(35.82)**	(41.04)**	(50.00)**
Flowering	264.20 ± 5.76	19.00 ±2.65	16.00 ± 5.96	14.40 ± 4.34	12.40 ± 2.30
		(21.49)**	(33.88)**	(40.50)**	(48.76)**
Post-flowering	23.18 ± 3.22	18.06 ± 3.25	15.78 ± 2.26	13.00 ± 2.62	11.80 ± 2.84
		(22.08)**	(31.92)**	(43.92)**	(49.09)**
Length of stomata (µm)					
Pre-flowering	36.56 ± 5.70	32.85 ± 4.95	28.81 ± 3.65	25.90 ± 2.71	22.50 ± 3.60
		(10.15)*	(21.20)**	(29.16)**	(30.25)**
Flowering	34.25 ± 4.65	31.26 ± 2.65	27.47 ± 3.30	24.35 ± 2.10	20.68 ± 2.15
		(8.41)*	(19.51)**	(28.66)**	(37.59)**
Post-flowering	32.25 ± 4.65	29.09 ± 5.45	26.98 ± 3.15	23.93 ± 2.10	20.68 ± 2.15
		(9.80)*	(16.34)**	(26.11)**	(35.88)**
Stomatal density (per microscopic field)					
Pre-flowering	28.15 ± 2.23	25.60 ± 3.24	20.80 ± 2.76	17.60 ± 1.35	15.20 ± 2.40
		(9.06)**	(26.11)**	(37.48)**	(46.00)**
Flowering	30.32 ± 2.62	27.60 ± 1.99	22.00 ± 2.94	18.20 ± 1.57	16.20 ± 2.49
		(8.97)**	(27.44)**	(39.97)**	(46.57)**
Post-flowering	31.50 ± 2.79	28.20 ± 2.29	24.80 ± 2.00	20.80 ± 3.94	17.80 ± 2.10
		(10.48)**	(21.27)**	(33.97)**	(43.49)**
Length of trichome (µm)					
Pre-flowering	196.56 ± 3.76	206.68 ± 4.35	230.06 ± 24.80	254.74 ± 6.05	296.40 ± 7.70
		(-5.15)**	(-17.84)**	(-29.60)**	(-50.79)**
Flowering	201.32 ± 31.20	216.90 ±4.49	256.47 ± 6.68	285.15 ±5.49	228..60±8.89
		(-7.74)**	(-27.39)**	(-14.64)**	(-63.22)**
Post-flowering	221.02 ± 4.63	238.46 ± 6.98	265.11 ±5.40	301.44 ± 6.45	332.00± 5.95
		(-7.90)**	(-19.95)**	(-36.39)**	(-50.21)**
Trichome density (per microscopic field)					
Pre-flowering	15.75 ± 2.27	13.25 ± 1.20	12.50 ± 1.70	10.75 ± 2.25	9.00 ± 2.15
		(15.87)**	(20.63)**	(31.75)**	(42.86)**
Flowering	14.75 ± 2.30	12.85 ± 1.62	11.62 ± 2.50	9.00 ± 1.00	8.37 ± 2.56
		(12.88)**	(21.22)**	(38.98)**	(43.25)**
Post-flowering	13.00 ± 1.11	11.50 ± 2.33	10.50 ± 1.84	8.37 ± 2.02	7.12 ± 1.50
		(11.54)**	(19.23)**	(35.62)**	(45.23)**

**Significant at 1% level; *Significant at 5% level; NS, non-significant.

Table 7. Epidermal traits (upper epidermis) at different developmental stages of *T. foenum graecum* Linn. grown under Cd stress. Mean ± S.D. is based on twenty five replicates. Parentheses include percent variation.

Parameters/Developmental stage	Cadmium chloride (µg/g of soil)				
	0	5	15	30	50
Length of stomatal (µm)					
Pre-flowering	55.23 ± 5.05	51.27 ± 4.08 (7.17)**	45.27 ± 3.04 (18.03)**	38.24 ± 2.02 (30.76)**	32.23 ± 5.11 (41.64)**
Flowering	58.17 ± 7.05	52.20 ± 4.04 (10.26)**	46.17 ± 3.08 (20.63)**	39.18 ± 5.05 (32.65)**	36.20 ± 7.05 (37.77)**
Post-flowering	59.20 ± 6.04	54.13 ± 6.03 (8.56)**	48.12 ± 5.02 (18.72)**	42.14 ± 4.02 (28.82)**	38.15 ± 6.07 (35.56)**
Width of stomata (µm)					
Pre-flowering	26.80 ± 2.86	22.20 ± 3.03 (17.16)**	20.80 ± 2.86 (22.39)**	17.80 ± 3.42 (33.58)**	15.60 ± 2.51 (41.79)**
Flowering	24.20 ± 5.76	21.00 ± 3.73 (13.22)**	19.20 ± 2.64 (20.66)**	16.80 ± 2.64 (30.58)**	14.80 ± 2.30 (38.48)**
Post-flowering	23.18 ± 3.22	20.00 ± 3.22 (13.72)**	18.18 ± 3.57 (21.57)**	15.30 ± 2.57 (33.99)**	13.00 ± 2.80 (43.92)**
Length of stomata (µm)					
Pre-flowering	36.56 ± 5.70	31.60 ± 4.30 (13.57)**	28.60 ± 4.92 (21.77)**	27.80 ± 4.38 (23.96)**	23.60 ± 5.51 (35.45)**
Flowering	34.13 ± 4.62	30.20 ± 2.92 (11.51)**	27.20 ± 2.28 (20.30)**	26.80 ± 2.92 (21.48)**	22.20 ± 2.92 (34.95)**
Post-flowering	32.25 ± 4.65	29.40 ± 2.14 (8.84)*	26.20 ± 3.89 (18.76)**	24.60 ± 2.07 (23.72)**	20.00 ± 4.18 (37.98)**
Stomatal density (per microscopic field)					
Pre-flowering	28.15 ± 2.23	25.97 ± 2.33 (7.74)**	21.93 ± 3.32 (22.10)**	18.30 ± 2.35 (34.99)**	15.12 ± 3.29 (46.29)**
Flowering	30.32 ± 2.62	27.73 ± 3.59 (8.54)**	24.03 ± 2.09 (2.75)**	20.66 ± 1.56 (31.86)**	18.51 ± 2.79 (38.95)**
Post-flowering	31.50 ± 2.79	28.74 ± 2.65 (8.76)**	25.08 ± 3.48 (20.38)**	22.39 ± 2.54 (28.92)**	19.14 ± 3.69 (39.24)**
Length of trichome (µm)					
Pre-flowering	196.56 ± 3.76	219.20 ± 5.56 (-11.50)**	224.00 ± 2.80 (-13.96)**	247.20 ± 3.08 (-33.23)**	269.40 ± 6.39 (-25.76)**
Flowering	201.32 ± 3.20	222.20 ± 5.65 (-10.37)**	234.40 ± 8.03 (-16.43)**	259.40 ± 4.60 (-28.65)**	259.00 ± 4.87 (-46.53)**
Post-flowering	221.02 ± 4.63	243.00 ± 5.80 (-9.94)**	265.80 ± 4.77 (-20.26)**	219.40 ± 8.25 (-31.84)**	306.00 ± 6.78) (-38.45)**
Trichome density (per microscopic field)					
Pre-flowering	15.75 ± 2.27	13.62 ± 2.32 (13.52)**	11.00 ± 1.02 (30.16)**	9.50 ± 1.02 (39.68)**	8.75 ± 2.50 (44.44)**
Flowering	14.75 ± 2.30	12.27 ± 1.57 (6.81)**	10.25 ± 2.05 (30.51)**	8.50 ± 1.25 (44.07)**	7.22 ± 2.75 (51.05)**
Post-flowering	13.00 ± 1.11	11.12 ± 2.60 (14.46)**	9.00 ± 2.25 (30.77)**	7.00 ± 1.75 (46.15)**	6.12 ± 2.85 (52.92)**

**Significant at 1% level; *Significant at 5% level; NS, non-significant.

Table 8. Relative proportion of vasculature, pith and cortex (%) of stem at different developmental stages of *T. foenum graecum* Linn. grown under Cd and Pb stress. Parentheses include percent variation.

Developmental stage	**Cadmium chloride (µg/g of soil)**				
	0	5	15	30	50
Pre-flowering	C = 0.84 V = 93.30 P = 93.00	C = 0.89(-5.95) V = 5.60(-5.66) P = 93.50(0.37)	C = 1.38(-64.29) V = 4.99(5.85) P = 93.62(0.25)	C = 2.11(-151.19) V = 4.29(19.06) P = 93.59(0.28)	C = 2.74(-226.19) V = 4.50(23.58) P = 93.20(0.69)
Flowering	C = 0.69 V = 5.66 P = 93.64	C = 0.77(-11.59) V = 6.03(-6.37) P = 93.10(0.58)	C = 1.23(-78.26) V = 5.35(5.48) P = 93.41(0.25)	C = 1.98(-186.96) V = 4.75(16.08) P = 93.26(0.41)	C = 2.77(-301.44) V = 4.55(19.61) P = 92.67(1.04)
Post-flowering	C = 0.56 V = 6.37 P = 93.06	C = 0.70(-25.00) V = 6.85(-7.54) P = 92.44(0.67)	C = 1.05(-87.50) V = 6.24(1.98) P = 92.70(0.39)	C = 2.42(-332.14) V = 5.87(7.85) P = 91.70(1.46)	C = 2,83(-405.36) V = 5.84(8.32) P = 91.32(1.87)
	Lead acetate (µg/g of soil)				
	0	25	50	100	200
Pre-flowering	C = 0.84 V = 5.30 P = 93.00	C = 0.80(4.76) V = 5.44 (-2.64) P = 93.75(0.11)	C = 1.48(-76.19) V = 5.05(4.72) P = 93.46(39.00)	C = 2.79(-232.14) V = 5.23(1.32) P = 91.97(2.00)	C = 3.18(-278.57) V = 4.72(10.94) P = 92.09(1.88)
Flowering	C = 0.69 V = 5.66 P = 93.64	C = 0.66(4.35) V = 5.86(-3.53) P = 93.47(0.18)	C = 0.96(-39.13) V = 5.86(0.71) P = 93.41(0.24)	C = 1.91(-176.81) V = 5.22(7.77) P = 92.86(0.83)	C = 2.44(-253.62) V = 5.18(8.40) P = 92.37(1.36)
Post-flowering	C = 0.56 V = 6.37 P = 93.06	C = 0.97(-73.21) V = 7.62(-19.62) P = 91.40(1.78)	C = 1.07(91.56) V = 7.73(-21.35) P = 91.22(1.98)	C = 1.33(-137.50) V = 7.50(-17.74) P = 91.16(2.04)	C = 1.45(-155.36) V = 7.31(-14.76) P = 91.25(1.94)

C, cortex; V, vasculature; P, pith.

Table 9. Relative proportion of vasculature, pith and cortex (%) of root at different developmental stages of *T. foenum graecum* Linn. grown under Cd and Pb stress. Mean ± S.D. is based on ten replicates.

Developmental stage	**Cadmium chloride (µg/g of soil)**				
	0	5	15	30	50
Pre-flowering	C = 6.92 V = 90.30 P = 2.68	C = 5.78 (10.69) V = 91.08(-0.86) P = 2.73 (-1.87)	C = 4.36 (36.99) V = 92.66(-2.61) P = 2.96 (-10.44)	C = 4.13 (40.32) V = 95.09(-2.72) P = 3.10(-15.67)	C = 3.68(46.82) V = 93.16(-3.17) P = 3.15(-17.54)
Flowering	C = 5.30 V = 92.59 P = 2.10	C = 5.02(5.28) V = 92.69(-0.11) P = 2.38(-8.57)	C = 2.85(46.23) V = 94.79(-2.38) P = 2.35(-11.90)	C = 2.78(47.55) V = 94.81(-2.39) P = 2.40(-14.29)	C = 2.73(48.49) V = 95.07(-2.68) P = 2.56(21.90)
Post-flowering	C = 4.53 V = 93.47 P = 1.99	C = 3.72(17.88) V = 94.14(-0.72) P = 2.13(-7.04)	C = 2.83(37.53) V = 94.90(-1.53) P = 2.26(-13.57)	C = 1.90(58.05) V = 95.81(-2.50) P = 2.30(15.58)	C = 1.61(64.46) V = 96.03(-2.74) P = 2.35(-18.67)
	Lead acetate (µg/g of soil)				
	0	25	50	100	200
Pre-flowering	C = 6.92 V = 90.30 P = 2.68	C = 5.49 (20.66) V = 91.75 (-1.61) P = 2.76 (-2.99)	C = 4.84(30.06) V = 92.16(-2.06) P = 2.99(-11.57)	C = 4.58(33.82) V = 92.28(-2.19) P = 3.13(-16.79)	C = 4.09(40.90) V = 92.68(-2.64) P = 3.22(-20.15)
Flowering	C = 5.30 V = 92.59 P = 2.10	C = 3.73(29.62) V = 93.95(-1.47) P = 2.31(-10.10)	C = 3.62(31.70) V = 93.96(-1.48) P = 2.41(-14.76)	C = 3.54(33.21) V = 93.97(-1.49) P = 2.49(-18.57)	C = 2.94(44.53) V = 94.49(-2.52) P = 2.56(21.90)
Post-flowering	C = 4.53 V = 93.47 P = 1.99	C = 3.60(20.53) V = 94.24(-0.82) P = 2.15(-8.04)	C = 2.70(40.40) V = 94.99(-1.63) P = 2.30(-15.58)	C = 1.78(60.71) V = 95.80(-2.49) P = 2.41(-21.11)	C = 1.40(69.09) V = 96.10(-2.81) P = 2.50(-25.63)

C, cortex; V, vasculature; P, pith.

Table 10. Stem anatomical features at different developmental stages of *T. foenum graecum* Linn. grown under Pb stress. Mean ± S.D. is based on twenty five replicates. Parentheses include percent variation.

Parameters/Developmental stage	Lead acetate (µg/g of soil)				
	0	25	50	100	200
Length of vessel element (µm)					
Pre-flowering	17.65 ± 2.58	17.34 ± 1.28	14.36 ± 2.25	12.64 ± 2.50	11.73 ± 2.90
		(1.76)^{NS}	(18.64)**	(28.39)**	(33.54)**
Flowering	19.50 ± 2.41	18.98 ± 2.71	17.48 ± 1.06	16.95 ± 2.58	13.25 ± 1.26
		(2.67)^{NS}	(10.36)*	(13.08)*	(32.05)**
Post-flowering	19.90 ± 2.12	19.00 ± 2.52	18.57 ± 1.58	18.55 ± 1.09	17.48 ± 1.85
		(4.52)^{NS}	(6.73)^{NS}	(6.78)^{NS}	(12.16)*
Width of stem vessel element (µm)					
Pre-flowering	16.50 ± 2.77	16.35 ± 2.49	14.21 ± 1.12	14.06 ± 1.33	12.02 ± 1.76
		(0.91)^{NS}	(13.88)*	(14.79)**	(27.15)**
Flowering	19.28 ± 2.95	16.67 ± 1.49	15.69 ± 2.51	14.23 ± 1.54	14.32 ± 1.32
		(13.54)*	(18.62)**	(26.19)**	(25.73)**
Post-flowering	21.10 ± 1.66	17.63 ± 1.87	16.17 ± 1.61	16.50 ± 1.45	14.34 ± 1.23
		(16.45)**	(20.81)**	(21.81)**	(32.04)**
Area of stem vessel element (µm)					
Pre-flowering	18.63 ± 1.37	17.48 ± 1.88	16.30 ± 1.14	16.23 ± 1.14	14.48 ± 1.14
		(6.17)^{NS}	(12.51)**	(12.88)**	(22.28)**
Flowering	20.45 ± 2.12	19.42 ± 2.34	19.13 ± 2.51	19.08 ± 2.64	15.22 ± 2.28
		(5.04)^{NS}	(6.45)^{NS}	(6.70)^{NS}	(25.57)**
Post-flowering	20.74 ± 1.06	19.59 ± 1.56	19.22 ± 1.11	19.12 ± 2.12	17.13 ± 2.23
		(5.54)^{NS}	(7.33)*	(7.81)*	(17.41)**
Density of stem vessel element (per microscopic field)					
Pre-flowering	79.81 ± 10.33	72.27 ± 9.82	61.83 ± 7.75	58.66 ± 9.61	56.80 ± 5.60
		(9.45)^{NS}	(22.53)**	(20.50)*	(23.83)**
Flowering	66.80 ± 13.97	65.66 ± 13.74	59.70 ± 13.67	58.10 ± 17.55	54.40 ± 11.49
		(1.71)^{NS}	(10.63)^{NS}	(13.02)^{NS}	(18.56)*
Post-flowering	50.55 ± 8.87	45.26 ± 9.20	43.50 ± 8.07	43.23 ± 10.45	39.32 ± 10.23
		(10.46)^{NS}	(13.95)^{NS}	(14.48)^{NS}	(22.22)*
Length of xylem fiber (µm)					
Pre-flowering	343.00 ± 72.92	306.50 ± 71.92	289.14 ± 52.04	270.30 ± 26.61	234.34 ± 29.70
		(10.46)^{NS}	(15.70)^{NS}	(21.20)*	(31.68)**
Flowering	371.63 ± 56.47	311.08 ± 41.91	303.18 ± 41.20	288.78 ± 52.91	261.23 ± 53.29
		(16.08)*	(18.42)*	(22.29)*	(29.71)**
Post-flowering	447.25 ± 62.60	353.34 ± 37.83	317.47 ± 36.97	299.02 ± 38.51	269.44 ± 46.19
		(21.00)**	(29.02)**	(33.14)**	(39.76)**
Width of vessel elements (µm)					
Pre-flowering	22.49 ± 2.21	21.32 ± 1.93	21.13 ± 2.72	20.04 ± 1.64	18.81 ± 2.71
		(5.20)^{NS}	(6.05)^{NS}	(10.89)*	(16.36)**
Flowering	24.62 ± 2.22	22.09 ± 1.71	21.67 ± 1.73	21.24 ± 1.68	21.16 ± 1.18
		(10.28)*	(11.98)**	(13.73)**	(14.05)**
Post-flowering	43.45 ± 5.00	38.38 ± 1.00	25.68 ± 4.20	25.53 ± 3.39	22.96 ± 1.96
		(11.67)**	(40.90)**	(41.24)**	(47.16)**

**Significant at 1% level; *Significant at 5% level; NS, non-significant.

Table 11. Stem anatomical features at different developmental stages of *T. foenum graecum* Linn. grown under Cd stress. Mean ± S.D. is based on twenty five replicates. Parentheses include percent variation.

Parameters/Developmental stage	Cadmium chloride (μg/g of soil)				
	0	5	15	30	50
Length of vessel element (μm)					
Pre-flowering	17.65 ± 2.58	15.44 ± 1.60 (12.52)*	15.08 ± 1.50 (14.56)*	15.02 ± 1.59 (14.90)*	14.03 ± 1.29 (20.51)**
Flowering	19.50 ± 2.41	17.72 ± 1.64 (9.13)^{NS}	16.47 ± 1.42 (15.54)**	15.55 ± 1.93 (20.26)**	14.62 ± 1.39 (25.03)**
Post-flowering	19.90 ± 2.12	19.05 ± 1.94 (2.01)^{NS}	17.08 ± 1.85 (14.17)*	16.15 ± 1.40 (18.84)**	15.37 ± 2.91 (22.76)**
Width of vessel element (μm)					
Pre-flowering	16.50 ± 2.77	15.63 ± 2.57 (5.27)^{NS}	13.55 ± 1.05 (17.88)**	10.85 ± 1.01 (34.24)**	10.32 ± 1.37 (37.45)**
Flowering	19.28 ± 2.95	17.76 ± 1.19 (7.88)^{NS}	17.65 ± 1.37 (8.45)**	14.90 ± 1.03 (22.72)**	14.35 ± 2.00 (25.57)**
Post-flowering	21.10 ± 1.66	20.02 ± 1.90 (5.12)^{NS}	18.45 ± 1.40 (12.56)**	17.50 ± 1.05 (17.06)**	14.77 ± 1.15 (30.00)**
Area of stem vessel element (μm)					
Pre-flowering	18.63 ± 1.37	16.26 ± 1.99 (12.72)*	15.59 ± 1.01 (16.32)**	15.22 ± 1.41 (18.30)**	12.23 ± 1.23 (34.35)**
Flowering	20.45 ± 2.12	17.87 ± 2.72 (12.62)*	15.72 ± 1.57 (23.13)**	15.40 ± 1.80 (24.69)**	13.29 ± 1.64 (35.01)**
Post-flowering	20.74 ± 1.06	17.89 ± 1.11 (13.74)**	16.04 ± 2.00 (22.66)**	16.97 ± 2.80 (18.18)**	13.92 ± 1.18 (32.88)**
Density of vessel element (per microscopic field)					
Pre-flowering	135.60 ± 4.90	128.90 ± 5.84 (4.94)**	110.90 ± 5.68 (18.22)**	106.20 ± 3.01 (21.68)**	99.58 ± 4.35 (26.56)**
Flowering	125.56 ± 19.48	110.63 ± 8.60 (11.89)*	107.83 ± 7.10 (14.12)*	105.80 ± 14.61 (15.74)*	99.13 ± 11.97 (21.05)*
Post-flowering	123.00 ± 20.86	111.40 ± 13.72 (9.43)^{NS}	107.20 ± 9.81 (12.85)*	105.50 ± 14.88 (14.23)*	95.30 ± 18.61 (22.52)**
Length of xylem fiber (μm)					
Pre-flowering	343.00 ± 72.92	334.51 ± 85.24 (2.48)^{NS}	311.36 ± 51.27 (9.22)^{NS}	295.68 ± 35.48 (13.80)^{NS}	204.88 ± 63.35 (40.27)**
Flowering	371.63 ± 56.47	342.32 ± 50.78 (7.89)^{NS}	340.21 ± 60.08 (8.45)^{NS}	325.22 ± 54.79 (12.48)^{NS}	301.33 ± 56.73 (18.92)**
Post-flowering	447.25 ± 62.60	360.54 ± 47.10 (19.39)*	345.45 ± 49.07 (22.76)**	335.38 ± 71.60 (25.01)*	305.48 ± 72.61 (31.70)**
Width of vessel elements (μm)					
Pre-flowering	22.39 ± 4.21	21.06 ± 4.77 (6.36)^{NS}	20.97 ± 4.18 (6.76)^{NS}	20.04 ± 5.04 (10.89)^{NS}	18.78 ± 3.64 (16.50)*
Flowering	24.62 ± 4.22	22.32 ± 4.93 (9.34)^{NS}	21.12 ± 2.72 (14.22)*	20.08 ± 4.63 (18.44)*	18.81 ± 2.71 (23.60)*
Post-flowering	43.45 ± 5.00	25.24 ± 7.15 (41.91)**	25.20 ± 4.22 (42.00)**	24.64 ± 3.16 (43.29)**	21.64 ± 3.23 (50.20)**

**Significant at 1% level; *Significant at 5% level; NS, non-significant.

vasculature and other histological zones is presented in Table 9. Increase in the vascular tissue and decrease in the pith and cortex with age was noticed under both control and treated conditions. However, proportion of vascular tissue reduced at all the concentrations of cadmium and lead. Proportion of pith decreased with increasing age of the plants in either with or without metal stress. But the proportion of pith at all the metal concentrations was higher than the one in the controls. The maximum proportion of pith was obtained at 50 μg cadmium and 200 μg lead. The proportion of cortex also registered a decline with the age of plants under both control and metal treatments. Overall, the proportion of cortex was lesser in the metal-treated plants compared to the control.

Reduction in the length and width of xylem fibres was also noticed under metal stress (Tables 12 and 13). The reduction became more pronounced as the metal concentration increased. Width and area of vessel elements in the roots increased gradually with age in both control and metal-treated plants, but the magnitude of increase in the metal-treated plants was significantly lower at all the developmental stages. Density of vessels in the root wood decreased as the plant grew. In the metal-treated plants, the vessel density decreased more significantly compared to the control. Reduction in vessel density was getting larger as the metal concentration increased.

Concentrations of cadmium and lead in different plant parts increased as the concentration of the metal in the medium increased in all the developmental stages assayed. Maximum concentrations of Cd^{2+} in the leaf (21.76 μg), stem (23.21 μg) and root (48.70 μg) were recorded during the post-flowering stage at 50 μg $CdCl_2$ in the medium. For Pb^{2+}, the maximum levels in the leaf (87.10 μg), stem (93.10 μg) and root (195.00 μg) were detected during the post-flowering stage at 200 μg $PbCH_3COO^-$ in the medium (Table 14).

DISCUSSION

Data on various growth attributes tabulated in Tables 1-3 are indicative of significant impact of both cadmium and lead at all the stages of growth in general, and during pre-flowering stage, in particular. In comparison to control, all the metal treatments did reduce plant length, root and shoot length, dry weight of root and shoot, leaf area and number of leaves and branches per plant but the impact was more pronounced at highest concentrations of metals used. Cadmium treatment of 50 μg/g of soil had much higher negative influence on shoot and root length than lead at 200 μg/g of soil. However, lead at its highest concentration had comparatively more deleterious impact on average leaf area, number of leaves and branches per plant. Root and shoot dry weight of the presently investigated species exhibited marked decline under highest cadmium treatment of 50 μg/g of soil in comparison to highest lead treatment of 200 μg/g of soil. These findings are in conformity with similar findings of Sudha et al. (2001), who also recorded increased growth inhibition at higher concentrations of heavy metals. Such inhibition has also been shown to occur even at very low concentrations of heavy metals (Allaway, 1986) as has been recorded in the present study as well. Growth inhibition has been attributed to a variety of causes viz., partial root damage (Turner, 1973), injury to enzyme systems (Page et al., 1972), reduction of cell water content and/or cell wall elasticity (Lane et al., 1978; Barcelo and Poschenrieder, 1990), smaller size of plant cells and intercellular spaces (Barcelo et al., 1988), alteration in the nutrient uptake (Greger and Bertell, 1992) and inhibited photosynthesis and CO_2 assimilation leading to reduced carbohydrate content of cells (Greger and Bertell, 1992). Dry matter production vis-à-vis heavy metal pollution has been used, by some workers, as a key attribute to evaluate the response of the crops to such stress because fundamental metabolic processes of photosynthesis and respiration have been shown to result in adverse influences on plant biomass (Page et al., 1972; van Assche and Clijsters, 1993; Tabassum et al., 2001). Moreover, the impact of metal stress was more pronounced during the pre-flowering stage (seedling stage) which is in accordance with the findings of Rauno et al. (1988). Decline in leaf area under both cadmium and lead treatments during the present study needs to be emphasized as it has implications for related processes of photosynthesis and transpiration and thereby growth and yield. Decreased leaf area with increased metal concentration draws support from similar observations in runner bean (Skorzynska-Polit and Baszynski, 1997), radish (Khan and Frankland, 1983). *Phaseolus vulgaris* (Polson and Adams, 1970), and mung bean (Singh et al., 1994). Bharadwaj and Mascarenhas (1989) also observed marked leaf area reduction with increase in concentration of the heavy metal in the medium. Besides leaf area, significant decrease was observed in the number of leaves, single leaf area and total leaf area of the presently investigated species at all its developmental stages, which draws support from similar observa-

Table 12. Root anatomical features at different developmental stages of *T. foenum graecum* Linn. grown under Pb stress . Mean ± S.D. is based on twenty five replicates. Parentheses include percent variation.

Parameters/Developmental stage	Lead acetate (µg/g of soil)				
	0	25	50	100	200
Length of vessel element (µm)					
Pre-flowering	21.77 ± 2.14	16.95 ± 2.39 (22.14)**	15.38 ± 1.53 (29.35)**	12.13 ± 2.16 (44.28)**	10.23 ± 3.96 (53.01)**
Flowering	25.40 ± 3.58	17.38 ± 2.54 (31.57)**	15.51 ± 3.18 (38.94)**	12.32 ± 2.43 (51.50)**	11.12 ± 2.15 (56.22)**
Post-flowering	25.67 ± 3.41	20.23 ± 2.50 (21.19)**	18.80 ± 2.03 (26.76)**	18.55 ± 2.01 (27.74)**	15.11 ± 1.00 (41.44)**
Width of vessel element (µm)					
Pre-flowering	22.74 ± 2.86	20.51 ± 1.41 (9.81)*	17.97 ± 1.43 (20.98)**	15.15 ± 2.98 (33.38)**	13.24 ± 1.32 (41.78)**
Flowering	21.84 ± 2.43	18.25 ± 2.39 (16.44)**	15.05 ± 3.84 (31.09)**	13.00 ± 2.50 (40.48)**	11.03 ± 2.46 (49.50)**
Post-flowering	16.79 ± 1.12	15.18 ± 1.76 (9.59)*	12.83 ± 2.25 (23.59)*	11.61 ± 1.40 (30.85)**	10.62 ± 1.13 (36.75)**
Area of stem vessel element (µm)					
Pre-flowering	18.42 ± 2.90	17.28 ± 1.12 $(6.19)^{NS}$	16.57 ± 2.03 $(10.04)^{NS}$	14.71 ± 1.91 (20.14)*	12.27 ± 1.78 (39.82)**
Flowering	22.98 ± 3.92	22.01 ± 2.70 $(4.22)^{NS}$	19.04 ± 2.61 (17.15)*	15.03 ± 1.20 (34.60)**	13.83 ± 1.78 (39.82)**
Post-flowering	34.18 ± 4.33	22.16 ± 2.74 (35.17)**	20.13 ± 3.85 (41.11)*	16.51 ± 1.50 (51.70)**	14.43 ± 1.67 (57.78)**
Density of vessel element (per microscopic field)					
Pre-flowering	347.93 ± 24.25	346.06 ± 29.14 $(0.54)^{NS}$	321.63 ± 17.93 (7.56)*	286.59 ± 26.47 (17.63)**	166.43 ± 9.71 (52.17)**
Flowering	296.36 ± 12.11	233.70 ± 35.66 (21.14)**	177.46 ± 34.00 (40.12)**	167.00 ± 12.39 (43.65)**	156.90 ± 17.53 (47.06)**
Post-flowering	206.46 ± 12.73	192.07 ± 9.69 (6.97)*	164.61 ± 24.92 (20.27)**	152.23 ± 28.44 (26.27)**	142.24 ± 29.21 (31.11)**
Length of xylem fiber (µm)					
Pre-flowering	211.66 ± 42.02	208.40 ± 66.84 $(1.54)^{NS}$	202.02 ± 44.66 $(4.56)^{NS}$	171.58 ± 23.47 (18.94)*	154.62 ± 29.24 (26.95)*
Flowering	232.20 ± 101.91	228.40 ± 55.21 $(1.64)^{NS}$	208.85 ± 38.36 $(10.06)^{NS}$	185.66 ± 36.42 $(20.04)^{NS}$	163.73 ± 25.24 (29.49)*
Post-flowering	294.93 ± 94.18	248.65 ± 89.84 $(15.69)^{NS}$	220.96 ± 57.85 (25.08)*	200.78 ± 38.38 (31.92)*	140.89 ± 33.23 (52.23)**
Width of vessel elements (µm)					
Pre-flowering	25.05 ± 3.37	22.59 ± 2.25 $(9.82)^{NS}$	18.06 ± 2.95 (27.90)**	14.11 ± 3.78 (43.67)**	12.03 ± 2.57 (51.98)**
Flowering	24.42 ± 2.23	24.58 ± 3.40 (13.51)*	20.39 ± 2.95 (28.25)**	17.14 ± 4.09 (39.69)**	14.59 ± 2.50 (48.66)**
Post-flowering	32.54 ± 5.28	28.78 ± 4.56 $(11.28)^{NS}$	25.38 ± 4.50 (22.00)**	20.52 ± 3.50 (36.94)*	16.43 ± 5.44 (49.51)**

**Significant at 1% level; * Significant at 5% level; NS, non-significant.

Table 13. Root anatomical features at different developmental stages of *T. foenum graecum* Linn. grown under Cd stress. Mean ± S.D. is based on twenty five replicates. Parentheses include percent variation.

Parameters/Developmental stage	Cadmium chloride (μg/g of soil)				
	0	5	15	30	50
Length of vessel element (μm)					
Pre-flowering	21.77 ± 2.14	16.76 ± 2.19	16.69 ± 1.79	18.60 ± 2.78	13.00 ± 1.62
		(23.01)**	(23.33)**	(37.53)**	(40.28)**
Flowering	25.40 ± 3.58	22.20 ± 2.71	18.89 ± 4.94	15.70 ± 2.05	14.03 ± 2.92
		(12.60)*	(25.63)**	(38.19)**	(43.70)**
Post-flowering	25.67 ± 3.14	23.77 ± 2.08	20.08 ± 3.68	19.78 ± 2.36	15.09 ± 2.72
		(7.40)^{NS}	(21.78)**	(22.95)**	(41.22)**
Width of vessel element (μm)					
Pre-flowering	22.74 ± 2.86	20.30 ± 2.24	18.13 ± 2.64	17.72 ± 1.47	12.30 ± 1.47
		(10.73)*	(20.27)*	(22.08)**	(22.08)**
Flowering	21.84 ± 2.43	17.90 ± 2.93	17.32 ± 1.32	15.02 ± 1.52	10.62 ± 1.35
		(18.04)*	(20.70)**	(31.23)**	(51.37)**
Post-flowering	16.79 ± 1.12	15.17 ± 1.75	12.83 ± 1.25	12.60 ± 2.39	10.50 ± 1.29
		(9.65)**	(23.59**	(24.96)**	(37.46)**
Area of stem vessel element (μm)					
Pre-flowering	18.42 ± 2.90	14.28 ± 1.48	14.20 ± 1.08	11.77 ± 1.93	9.23 ± 1.29
		(22.48)**	(22.91)**	(36.10)**	(49.89)**
Flowering	22.98 ± 3.92	18.75 ± 1.02	15.45 ± 2.23	12.66 ± 1.49	10.21 ± 1.23
		(18.41)*	(32.77)**	(44.91)**	(55.57)**
Post-flowering	34.18 ± 4.33	21.37 ± 2.96	18.09 ± 2.13	17.27 ± 2.66	16.34 ± 2.65
		(37.48)**	(47.07)**	(49.47)**	(52.19)**
Density of vessel element (per microscopic field)					
Pre-flowering	347.93 ± 24.25	314.63 ± 18.01	234.93 ± 35.85	212.25 ± 23.01	184.30 ± 24.08
		(9.55)*	(32.48)**	(38.97)**	(47.03)**
Flowering	296.36 ± 12.11	274.56 ± 33.00	192.90 ± 17.25	184.22 ± 33.79	161.23 ± 32.92
		(7.36)^{NS}	(34.91)**	(37.84)**	(45.60)**
Post-flowering	206.46 ± 12.73	194.27 ± 17.22	188.27 ± 23.10	174.36 ± 17.09	160.54 ± 23.28
		(5.90)^{NS}	(8.81)*	(15.55)**	(22.24)**
Length of xylem fiber (μm)					
Pre-flowering	211.66 ± 42.02	156.09 ± 37.65	151.94 ± 36.42	142.44 ± 26.00	112.74 ± 29.09
		(26.25)*	(28.22)*	(32.70)**	(46.74)**
Flowering	232.20 ± 101.91	206.82 ± 52.03	172.08 ± 21.37	139.69 ± 29.01	112.74 ± 32.19
		(10.93)^{NS}	(25.89)^{NS}	(39.84)*	(51.45)*
Post-flowering	294.93 ± 94.81	256.50 ± 52.63	219.31 ± 84.89	162.40 ± 23.97	141.23 ± 24.89
		(13.03)^{NS}	(25.64)^{NS}	(44.94)**	(52.11)**
Width of vessel elements (μm)					
Pre-flowering	25.05 ± 3.37	21.43 ± 3.62	81.71 ± 2.32	16.80 ± 6.69	13.42 ± 3.59
		(14.45)*	(25.31)**	(32.93)**	(46.43)**
Flowering	28.42 ± 2.23	24.98 ± 2.49	20.00 ± 4.27	18.51 ± 3.21	14.65 ± 3.41
		(12.10)**	(29.63)**	(34.87)**	(48.45)**
Post-flowering	32.54 ± 5.28	29.96 ± 5.47	26.30 ± 4.41	24.72 ± 3.17	22.84 ± 5.62
		(7.93)^{NS}	(19.18)**	(24.03)**	(29.81)**

**Significant at 1% level; *Significant at 5% level; NS, non-significant.

Table 14. Concentrations of cadmium and lead (µg/g dry weight) in different plant parts at different developmental stages of *T. foenum graecum* Linn. The values represent mean ± S.D. is based on three replicates

Parameters/Developmental stage	Cadmium chloride (µg/g of soil)				
	0	5	15	30	50
Leaf					
Pre-flowering	0.00 ± 0.00	0.72± 0.12	3.01 ± 1.09	6.72 ± 1.10	12.76 ± 1.40
Flowering	0.00 ± 0.00	1.51 ± 0.11	5.25 ± 1.12	10.50 ± .10	18.00 ± 2.19
Post-flowering	0.00 ± 0.00	2.30 ± 0.10	6.76 ± 1.14	17.25 ± 2.11	21.76 ± 1.65
Stem					
Pre-flowering	0.00 ± 0.00	0.76 ± 0.13	3.74 ± 2.00	6.00 ± 2.09	13.56 ± 2.40
Flowering	0.00 ± 0.00	2.22 ± 0.09	6.12 ± 1.18	9.00 ± 2.16	13.14 ± 1.92
Post-flowering	0.00 ± 0.00	3.71 ± 0.16	7.51 ± 1.01	16.50 ± 2.15	23.21 ± 3.10
Root					
Pre-flowering	0.00 ± 0.00	5.20 ± 0.17	8.25 ± 2.05	15.76 ± 3.50	27.28 ± 2.01
Flowering	0.72 ± 0.06	7.26 ± 0.12	9.76 ± 0.81	23.21 ± 3.40	36.76 ± 1.78
Post-flowering	0.73 ± 0.08	10.60 ± 0.13	14.26 ±2.11	24.20 ± 3.01	48.70 ± 3.10
	Lead acetate (µg/g of soil)				
	0	25	50	100	200
Leaf					
Pre-flowering	0.00 ± 0.00	5.29 ± 1.12	9.72 ± 1.20	24.00 ± 2.10	51.02 ± 3.39
Flowering	0.00 ± 0.00	7.75 ± 0.92	15.79 ± 3.16	33.75 ± 3.29	72.00 ± 2.41
Post-flowering	0.00 ± 0.00	9.75 ± 1.86	21.00 ± 2.15	48.01 ± 1.96	87.10 ± 3.16
Stem					
Pre-flowering	0.00 ± 0.00	6.00 ± 2.02	12.00 ± 1.72	21.00 ± 1.66	54.00 ± 3.91
Flowering	0.00 ± 0.00	9.05 ± 2.72	19.50 ± 1.69	28.50 ± 2.99	54.75 ± 4.01
Post-flowering	0.00 ± 0.00	12.01 ± 1.96	23.29 ± 2.92	51.00 ± 3.92	93.10 ± 3.05
Root					
Pre-flowering	0.00 ± 0.00	15.75 ± 2.51	25.49 ± 2.22	48.76 ± 3.96	108.01±3.29
Flowering	0.00 ± 0.00	22.50 ± 2.39	27.76 ± 1.96	71.26 ± 4.29	147.00± 4.06
Post-flowering	0.00 ± 0.00	30.00 ± 3.16	44.25 ± 3.01	73.50 ± 4.22	195.00± 4.25

tions made in *Cajanus cajan* under Cd stress (Khudsar et al., 2000).

Present investigations also revealed significant reduction in stomatal length and width under induced metal stress (Tables 4-7). Irrespective of the metal used, reduction was more pronounced in adaxial epidermis at highest metal concentration. However, reduction did not vary significantly at different stages of development. Such results have been reported by Puckett et al. (1977) and Khudsar et al. (2000). Density of stomata in the presently investigated species also declined under heavy metal stress. Such response has been suggested to be a common mechanism adopted by the plants to cope with the pollution load (Khudsar et al., 2000). Reduction in leaf size in the treated plants, as discussed earlier, indicates not only retarded growth but also results in reduction in green surface area and thereby accommodation of lesser number of stomata. Sharma and Tyree (1973) and Palaniswamy et al. (1995) showed similar trend in the stomatal response. In addition, reduced stomatal density has been reported to occur under other types of stresses as well, such as elevated CO_2 concentration, particularly, in expanding leaves of upper portion of poplar clones (Ceulemans et al., 1995). Length of stomatal pore in the presently investigated species was also significantly reduced at all the three developmental stages under Cd^{2+} and Pb^{2+} stress and such response was concentration dependent. Partial stomatal closure on exposure to Cd^{2+} (Bazzaz et al., 1974) and Pb^{2+} (van Assche et al., 1980) has also been reported earlier with possible impairment of leaf transpiration and CO_2 fixation. A direct effect on *in vivo* stomatal regulation has been postulated for Cd^{2+}, Ni^{+2}, and Pb^{2+} by Carlson et al. (1975). The stomatal closure and reduced stomatal pore dimensions have been related to decrease in net photosynthesis and inhibition of transpiration (Carlson et al., 1975; Sheoran et al., 1990a, b). The mechanism of stomatal closure is also related to abscisic acid (ABA) accumulation whose consequent effects on water relations of expanding bean leaves has been suggested to result in the stomatal closure under heavy metal treatment (Poschenrieder et al., 1989). In contrast, the studies conducted by Rauser and Dumbroff (1981) indicated that excess Ni^{+2}, CO^{2+} and Zn^{2+}, though may increase ABA content but without any adverse effect on plant-water relations and hence the stomatal closure. Thus, it has been suggested that inhibition of stomatal opening in plants exposed to Cd^{2+} may depend on metal concentration, exposure time and also on the degree of toxicity suffered by plants (Barcelo et al., 1988). Apart from stomatal closure, reduction in stomatal opening under metal stress, as observed during the present studies, is also not uncommon (Garg and Varshney, 1980; Varshney, 1985). Rauser and Dumbroff (1981) indicated that in *P. vulgaris* the direct effect of heavy metals on stomatal opening might be caused by the alteration of K^+ fluxes through the membrane. The changes in the inter anticlinal walls of the guard cells have been shown to be induced by Pb stress; in that these walls which line the stomatal aperture become more thickened in the leaves of Pb treated plants than in the control plants. Notwithstanding the varying underlying causes of reduced stomatal pore dimensions under metal stress as observed during the present investigation, it has been regarded as a protective measure against the pollutants (Gupta and Ghouse, 1987) and decrease in size of stomatal aperture or the stomatal closure resulting from an inhibitory action of a pollutant may, in fact, represent an avoidance mechanism (Kimmerer and Kozlowski, 1981; Iqbal et al., 1996)

In cadmium and lead treated plants of *T. foenum graecum*, length of trichomes increased where as density of trichomes in both leaf epidermal surfaces decreased significantly at all the developmental stages. Decrease intensified with increasing concentration of the metal. Decline in trichome density in response to air pollution is well on record (Palaniswamy et al., 1995). Lower trichome density on both epidermises has been reported in *Peristrophe bicalyculata*, *Ruellia tuberosa*, and *Trymphetta rhombipodea* (Nighat, 1998) under pollution stress.

The present observations of reduction in xylem tissue are consistent with the earlier findings of Iqbal et al. (1987a, b) on *Cassia occidentalis, Cassia tora,* and *Lantana camara*. It has been suggested that a series of important physiological events such as inhibition of photosynthesis and synthesis of hormonal growth regulators, followed by decrease in the amount of carbohydrates and transport of hormones to the lower part of the stem and then to the root system (Kozlowski and Constantinidou, 1986), lead to such decreased wood production in stem and root. Furthermore, during the present investigation the proportion of vascular tissue did not increase in roots with growing age of the plant when exposed to cadmium and lead as it did under control conditions. Similar observations have been made in *Croton bonplandianum* (Ghouse et al., 1986a) and *Cajanus cajan* (Iqbal et al., 1986a, b). Though not much literature exists on the effect of heavy metals on anatomical and histological features of plants but plausible conclusions can be drawn on the basis of studies conducted to study the influence of other pollutants on such features. A highly significant correlation between the amount of xylem increment in trees and the degree of coal firing in brick production complex indicated that SO_2 and fluorine inhibit the cambial growth (Gilbert, 1983). Fluorine dust and effluents from a copper smelter also reduced the xylem production in *Pinus sylvestris* and *Pinus monophylla,* respectively (Thompson, 1981). The pollutants, besides reducing xylem increment in *Picea abies*. *Abies* sp., *P. sylvestris*. *Populus tremuloides* and *Betula pendula*, may also alter wood structure (Eckstein et al., 1981; Kozlowski and Constantinidou, 1986). On the contrary, increase in the area occupied by vasculature under the stress of environmental pollution has also been reported and has been regarded as an adaptive response to ensure the maintenance of water uptake particularly during the flowering and fruiting stages of *Sida spinosa* (Mahmooduzzafar et al., 1986), *Cleome viscosa* (Ahmad et al., 1987), *Lantana camara*, *C. tora*, *C. occidentalis* (Iqbal et al., 1987a, b), *Amaranthus viridis*, *Euphorbia hirta* (Usmani, 1990), and *C. cajan* (Khudsar et al., 2000).

The distortion of cell shape in root cortex, observed during the present investigations in lead treated plants, might have resulted from disorientation of microfibrillar arrangement in the cell walls. The cellulose microfibrils arise from some granular material situated on outside of the plasma membrane. As Pb^{2+} is reported to bind with membranes, it probably causes separation of the granular material from the plasma membrane, thus, inhibiting growth of polysaccharide chains that ultimately lead to disorientation of cellulose microfibrils. Cadmium ions are also reported to attack various cellular components, including cell walls and membranes (Yu, 1998), resulting in differential alterations that ultimately lead to their disorganisation. Structural changes in the plant axis have also been correlated to disfunctioning of root system due to insufficient supply of essential nutrients (Prasad and Hagemeyer, 1999) and hormones from the roots (Davies, 1992). Thus, it can be safely concluded that heavy metals affect both cell division and cell elongation in plants (Setia and Bala, 1994), which is bound to cause considerable decrease in the girth and length of root and stem.

In the present study, stem and root fibres of *T. foenum graecum* were significantly shorter in the metal exposed plants than in plants growing without any stress. This finding is in conformity with the observations made in *Croton bonplandianum* (Ghouse et al., 1986b), *Datura innoxia* (Iqbal et al., 1986a), and *Psorelea corylifolia* (Ali, 1998) under the impact of coal smoke pollution in which lead is an important constituent. Vessel element length and width decreased significantly and consistently in the stem and root of the metal treated samples of *Trigonella*. Development of short vessels in plants growing under stressed conditions has also been reported in *Polygonum glabrum* (Khan et al., 1984), *Calotropis gigentia* (Iqbal et al., 1986b), *Euphorbia hirta* (Usmani, 1990), *Lagerstroemia reginae,* and *Alstonia scholaris* (Jabeen and Abraham, 1998), and *Peristrophe* and *Ruellia* (Nighat, 1998; Khudsar et al., 2000). Such variations can have severe physiological and metabolic implications for the plants as Lamoreaux and Chaney (1977) reported reduced relative water conductivity in excised stem sections of Cd-treated silver maple. The reduction was due to decrease of xylem tissue available for water conductivity, reduced size of vessel and tracheids and a partial blockage of xylem elements. Cd^{2+} disturbance of water balance has been attributed to its influence on stomatal function and on the size and number of tracheary elements (Barcelo et al., 1988).

Present observation of marked reduction in vessel density draws support from similar findings recorded by Khudsar et al. (2000) in stem and root of *C. cajan.* Other stresses like those caused by air pollutants also lead to substantial decrease in the number of vessels per unit area of xylem as has been reported in *C. bonplandianum* (Ghouse et al., 1986a), *Sida spinosa* (Mahmooduzzafar et al., 1986), *C. tora* and *C. occidentalis* (Iqbal et al., 1987a), and *Psoralea corylifolia* (Ali, 1998). Though pollutants in general reduce incremental xylem development (Gilbert, 1983), but in some cases, the amount of vasculature also increases under pollution stress, thereby serving as an adaptive response, particularly, during the flowering stage and beyond when it may help in uptake of water so that plants can withstand the continuous pollution load during these critical phases of life history.

In the present investigation, heavy metal (Cd^{2+} and Pb^{2+}) content in different plant parts (root, stem and leaf) of *T. foenum graecum* at different developmental stages increased with the enhanced metal levels in the soil. The accumulation of metal was higher in root than in shoot. In the previous studies, the Cd^{2+} concentration in roots and shoots showed positive correlation (Costa and Spitz, 1997; Hernandez et al., 1998) both in long term as well as in short term experiments, although the rate of metal accumulation in plants has been reported to vary between species (Rolfe, 1973) and between different soils (John and van Laerhoven, 1972; Miller, 1974). The uptake of Cd^{2+} and Pb^{2+} by plants has been reported to increase with their increasing concentration either in soil or nutrient solution (Piotrowska and Dudka, 1994).

The present study revealed that the translocation of Cd^{2+} and Pb^{2+} in *T. foenum graecum* to stem and leaves was much less resulting in higher concentration in roots as has also been observed in previous studies conducted by Rolfe (1973). Cd^{2+} is less readily immobilized in soil and absorbed passively (Cutler and Rains, 1974) due to which is more readily available to the plant (Miller et al., 1974). In comparison, availability and translocation of Pb^{2+} to the plant primarily depends upon the soil type and its pH (Miller et al., 1975a, b). However, in general, roots accumulate more lead than leaves as observed in the present study also. The higher accumulation of Cd^{2+} and Pb^{2+} in the roots of *T. foenum graecum* as compared to the shoots might be suggestive of the avoidance strategy employed by these plants. Such avoidance mechanisms are not uncommon and have been seen in other plants by Lozano-Rodrigerez et al. (1997). Others have argued it to be a tolerance mechanism (Vogeli-Lange and Wagner, 1990; Fett et al., 1994), being associated with the cross-linking of Cd^{2+} and Pb^{2+} to carboxyl groups of cell wall proteins (Barcelo and Poschenrieder, 1990) and/or because of interaction with the thiol group of soluble proteins and non-protein thiols.

The present study is thus indicative of significant changes in morphological as well as anatomical features of *T. foenum graecum* due to Cd^{2+} and Pb^{2+} stress that are, in fact, manifestations of altered physiological and metabolic functioning of the plant.

ACKNOWLDGEMENTS

The authors are greatly thankful to the Departments of Botany of Jamia Hamdard and University of Kashmir for providing laboratory facilities.

Received September 20, 2004; accepted December 27, 2004.

LITERATURE CITED

Ahmad S, Ismail F, Majeed J (1987) Effect of atmospheric pollution on chlorophyll and protein contents of some plants growing in Karachi, Pakistan. Pak J Sci Res 29: 464-467

Ali, ST (1998) Sulphur dioxide induced changes in the growth pattern of *Psoralea corylifolia* Linn. at different stages of development. Ph.D. thesis, Jamia Hamdard, New Delhi

Allaway WH (1986) Agronomic controls over environmental cycling of trace elements. Adv Agron 20: 235-274

Barcelo J, Poschenrieder C (1990) Plant water relations as affected by heavy metal stress: A review. J Plant Nutr 13: 1-37

Barcelo J, Vazquez MD, Poschenrieder C (1988) Cadmium induced structural and ultrastructural changes in the vascular system of bush bean stems. Bot Acta 101: 254-261

Bazzaz FA, Rolfe GL, Carlson RW (1974) Effect of cadmium on photosynthesis and transpiration of excised leaves of corn and sunflower. Plant Physiol 32: 373-376

Bharadwaj R, Mascarenhas C (1989) Cadmium induced inhibition of photosynthesis *in vitro* during development of chloroplast in *Triticum aestivum* L. Plant Physiol Biochem 16: 40-48

Carlson RW, Bazzaz FA, Rolfe GL (1975) The effects of heavy metals on plants. II. Net photosynthesis and transpiration of whole corn and sunflower plants treated with Pb, Cd, Ni and TL. Environ Res 10: 113-120

Ceulemans R, Vanpraet L, Jiang XN (1995) Effects of CO_2 enrichment, leaf position and clone on stomatal index and epidermal cell density in poplar (*Populus*). New Phytol 131: 99-107

Costa G, Spitz E (1997) Influence of cadmium on soluble carbohydrates, free amino acids, protein content of in vitro cultured *Lupinus albus*. Plant Sci 128: 131-140

Cutler JM and Rains DW (1974) Characterization cadmium uptake by plant tissue. Volume and proximity highways. Environ Sci Technol 4: 318-322

Davies BE (1992) Trace metals in environment; retrospect and prospect. *In* DC Adriano, ed, Biochemistry of Trace Metals. Lewis Publishers, Baca Raton, pp 1-18

Eckstein D, Grieve U, Fruhwald A (1981) Anatomische and mechanischtechnologische untersuchungen am holtz einer SO_2 geschadigten fitche and tanne. Holzalsroh werkstoff 39: 477-487

Fett JP, Cambria J, Oliva MA, Joradao CP (1994) Absorption and distribution of cadmium in water hyacinth plants. J Plant Nutr 17: 1219-1230

Garg KK, Varshney CK (1980) Effects of air pollution on leaf epidermis at the submicroscope level. Experientia 36: 1364-1368

Ghouse AKM, Ahmad Z, Saquib M, Khan MS (1986a) Air pollution and wood formation in *Mangifera indica* Linn. Indian J Appl Pure Biol 1: 37-39

Ghouse AKM, Khair S, Usmani NR (1986b) Micromorhological variations in *Croton bonplandianum* L Bail as induced by pollutants resulting from fossil fuel firing. J Plant Environ 3: 93-95

Ghouse AKM, Younus M (1972) Preparation of epidermal peels from leaves of gymnosperms by treatment with hot 60% HNO_3. Stain Technol 47: 322-331

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 Ca^{2+} and Cd^{2+} on the carbohydrate metabolism in sugar beet (*Beta vulgaris*). J Exp Bot 43: 167-173

Gupta MC, Ghouse AKM (1987) The effects of coal smoke pollutants on the growth, yield and leaf epidermis features of *Abelmoschus esculentus* Moench. Environ Poll 43: 263-270

Hernandez LE, Lozano-Rodrigruez E, Garate A, Carpena-Ruiz R (1998) Influence of cadmium on the uptake, tissue accumulation and subcellular distribution of manganese in pea seedlings. Plant Sci 132: 139-151

Iqbal M, Abdin MZ, Mahmooduzzafar Yunus M, Agarwal M (1996) Resistance mechanisms in plants against air pollution. *In* M Yunus, M Iqbal, eds, Plant Response to Air Pollution. John Wiley and Sons, Chichester, pp 195-240

Iqbal M, Ahmad Z, Kabeer I, Mahmooduzzafar, Kaleemullah (1986a) Stem anatomy of *Datura innoxia* Mill in relation to coal smoke pollution. J Sci Res 8: 103-105

Iqbal M, Mahmooduzzafar K, Ahmad Z (1986b) A plant grows under the stress of environmental pollution. Res J Plant Environ 3: 5-7

Iqbal M, Mahmooduzzafar K, Ghouse AKM (1987a) Impact of air pollution on the anatomy of *Cassia occidentalis* L. and *Cassia tora* L. Indian J Apple Pure Boil 2: 23-26

Iqbal M, Mahmooduzzafar K, Kabeer L, Ahmad Z 1987b) The effect of air pollution on the stem anatomy of *Lantana camara* L. J Sci Res 9: 121-122

Jabeen C, Abraham S (1998) Histological changes in *Lagerstroemia reginae* and *Alstonia scholaris* exposed to air pollutants. J Environ Biol 19: 79-82

John MK, van Laerhoven CJ (1972) Lead uptake by lettuce and oats as affected by lime, nitrogen, and sources of lead. J Environ Qual 1: 169-71

Khan DH, Frankland B (1983) Effects of cadmium and lead on radish plants with particular reference to movement of metals through soil profile and plant. Plant Soil 20: 335-345

Khan FA, Khair A, Usmani NR, Sulaiman IM (1984) A note on the anatomical responses of *Polygonum glabrum* to air pollutants arising out of coal burning. IBC A 127-128

Khudsar T, Mahmooduzzafar, Woung YS, Iqbal M (2000) Morphological and anatomical variations of *Cajanus cajan* (Linn.) Hirth raised in cadmium rich soil. J Plant

Biol 4: 149-157

Kimmerer TW, Kozlowski TT (1981) Stomatal conductance and sulphur uptake of five clones of *Populus tremuloides* exposed to sulphur dioxide. Plant Physiol 67: 990-995

Kozlowski TT, Constantinidou HA (1986) Responses 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

Lane SD, Martin ES, Garrot JF (1978) Lead toxicity effects on indole-3-acetic acid induced cell elongation. Planta 144: 79-84

Lozano-Rodrigerez E, Hernandez LE, Boney P, Carpena-Ruiz RO (1997) Distribution of cadmium in shoot and root tissue of maize and pea plants: Physiological disturbance. J Exp Bot 306: 123-128

Mahmooduzzafar, Iqbal M, Kalimullah AZ(1986) Anatomical variation in *Sida spinosa* L due to coal smoke pollution. Res J Plant Envir 3: 9-11

Miller JE, Hassett JJ, Koeppe DE (1975a) The effects of soil properties and extractable lead levels on lead uptake by soybeans. Comm Soil Sci Plant Anal 6: 339-347

Miller JE, Hassett JJ, Koeppe DE (1975b) The effects of soil lead absorption capacity on the uptake of lead by corn. Comm Soil Sci Plant Anal 6: 349-358

Miller JE, Hassett JJ, Koeppe DE, Rolfe GL, Wheeler GL (1974) Effect of soil properties on Pb uptake by corn and effects of Pb and Cd on corn. Proc Ann NSF Trace Contaminants Conf, Asilomar

Nighat F (1998) Growth performance of certain annuals in relation to coal smokes pollution. Ph.D. thesis, Jamia Hamdard, New Delhi

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

Palaniswamy M, Gunamani T, Swaminathan K (1995) Effects of air pollution caused by automobile exhaust gases on crop plants. Proc Acad Environ Biol 4: 255-260

Piotrowska M, Dudka S (1994) Estimation of maximum permissible levels of cadmium in a light soil by using cereal plants. Water Air Soil Pollut 73: 179-188

Polson DE, Adams MW (1970) Differential response of beans (*Phaseolus vulgaris* L) to zinc I. Differential growth and elemental composition at excess Zn levels. J Agron 62: 557-560

Poschenrieder C, Gunse B, Barcelo J (1989) Influence of cadmium on water relation, stomatal resistance and abscisic acid content in expanding bean leaves. Plant Physiol 90: 1365-1371

Prasad MNV, Hagemeyer J (1999) Heavy Metal Stress in plants: From Molecules to Ecosystems. Springer-Verlag, Berlin, Heildelberg

Puckett KJ, Tomassinin FD, Nieboer E, Richardson DHS (1977) Potassium efflux by lichen thalli following exposure to aqueous sulphur dioxide. New Phytol 179:135-145.

Rauno A, Barcelo J, Poschenrieder CH (1988) Zinc toxicity-induced variation of mineral element composition in hydroponically grown bush bean. J Plant Nutr 10: 373-384

Rauser WE, Dumbroff EB (1981) Effect of excess cobalt, nickel and zinc on the water relations of *Phaseolus vulgaris* L. Environ Exp Bot 21: 249-255

Rolfe GL (1973) Lead uptake by selected tree seedlings. J Environ Qual 2: 153-7

Salt DE, Prince RC, Pickering IJ, Raskin I (1995) Mechanism of cadmium mobility and accumulation in Indian mustard. Plant Physiol 109: 1427-1433

Sanita di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41: 105-130

Sass JE (1958) Botanical Microtechnique. Oxford and IBH Pub, Calcutta, London

Seregin IV, Ivanov VB (2001) Physiological aspects of cadmium and lead toxic effects on higher plants. Russian J Plant Physiol 48: 606-630

Setia RC, Bala R (1994) Anatomical changes in root and stem of Wheat (*Triticum aestivum* L.) in response to different heavy metals. Phytomorphology 44: 95-104

Sharma GK, Tyree J (1973) Geographic leaf cuticular and gross morphological variations in *Liquidambar styraciflua* L and their possible relationship to environmental pollution. Bot Gaz 134: 179-184

Sheoran IS, Singhal HR, Singh R (1990a) Effect of cadmium and nickel on photosynthesis and enzymes of photosynthetic carbon reduction in pigeon pea. Photosynth Res 23: 345-351

Sheoran IS, Singhal HR, Singh R (1990b) Effects of cadmium and nickel on photosynthesis and enzymes of the photosynthetic carbon reduction cycle in pigeon pea *(Cajanus cajan L)*. Photosynth Res 23: 345-351

Singh RP, Bharti N, Kumar G (1994) Differential toxicity of heavy metals to growth and nitrate reductase activity of *Sesamum indicum* seedlings. Phytochem 35: 1153-1156

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

Sudha MR, Ganesh R, Bosen KK (2001) Physiological and biochemical tolerance of bean to heavy metal stress. Polln Res 20: 135-138

Tabassum S, Dube BK, Sinha P, Chatterjee C (2001) Studies on the growth, yield and metabolism of brinjal (*Solanum melongena* L.) coupled with uptake of cobalt and cadmium. J Environ Poll 8: 159-164

Thompson MA (1981) Tree rings and air pollution: A case study of *Pinus monophylla* growing in east central Nevada. Environ Poll 26: 251-266

Turner MA (1973) Effect of cadmium treatment on cadmium and zinc uptake by selected vegetables species. J Environ Qual 2: 118-119

Usmani NR (1990) Anatomical responses of *Amaranthus* *viridis* and *Euphorbia hirta* to coal smoke pollution. Ph.D. thesis, Aligarh Muslim University, Aligarh

van Assche F, Ceulemans R, Clijsters H (1980) Zinc mediated effects on leaf CO_2 diffusion conductance and net photosynthesis in *Phaseolus vulgaris* L. Photosynth Res 1: 171-180

van Assche F, Clijsters H (1993) Multiple effects of heavy metal toxicity on photosynthesis. *In* R Marcelle, H Clijsters, eds, Effects of stress on Photosynthesis. Nijhoff, The Hague, pp 371-382

Varshney CK (1985) Role of plants in indicating, monitoring and mitigating air pollution. *In* GV Subramanyam, DN Rao, CK Varshney, DK Biswas, eds, Air Pollution and Plants: A state of the art report. Ministry of Environment and Forests, Department of Environment, Govt. of India, New Delhi, pp 146-170

Vogeli-Lange R, Wagner GJ (1990) Subcellular localization of cadmium and cadmium-binding peptides (phytochelatins) in leaves of intact tobacco seedlings. Plant Sci B 114: 11-18

Yu SW (1998) Plant resistance to SO_2 injury. *In* DN Rao, KJ Ahmad, M Yunus, SW Singh, eds, Perspectives in Environmental Botany. Today and Tomorrow Pub, New Delhi, pp 251-282