

HEAVY METAL INDUCED CHANGES IN THE EPIDERMAL PATTERN OF SAFFLOWER

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ABSTRACT:

During the present study, some accessions of safflower were evaluated for assessing the extent and type of lead and arsenic induced variation in the foliar epidermal pattern. The analyses were conducted for both abaxial and adaxial epidermis using the parameters, guard cell index (GCI), pore area index (PAI) total pore area (TPA). The source of lead (Pb) was lead acetate and for arsenic (As) was sodium arsenate and for testing the effects of lead and arsenic 10^{-3} M solution was used. Control sets were raised in Hoagland's solution lacking lead and arsenic showed the presence of normal stomata while in the leaves of lead and arsenic treated sets, several stomatal anomalies were observed.

INTRODUCTION:

The soil pollution across the globe is caused by the heavy metals. The cultivable lands are also affected by the heavy metal contaminations such as copper (Cu), Lead (Pb), arsenic (As), cadmium (Cd) and mercury (Hg) etc. The heavy metals are not only carcinogenic to human beings but also have the adverse effects on plant morphology, metabolic activities and cause cellular anomaly. The accumulation of heavy metals causes cellular injuries by forming reactive oxygen species (ROS). However, there some plant species which can grow and sustain heavy metals toxicity by adapting themselves even at high concentrations and one of the plant species is safflower.

Safflower (*Carthamus tinctorius* L.) is a medicinal plant and getting its consideration as a potential oilseed crop of industrial interest. As far as literature concern very few studies were conducted towards heavy metal tolerance of safflower and anomaly behaviour at cellular level. Therefore to study the heavy metals induced anomalies is necessary and beneficial in bioremediation processes.

A maximum portion of the aerial component of the terrestrial plants is occupied by leaves. Unquestionably, these serve as interaction area of the shoot with the atmosphere. Foliar epidermis, the outer most skin of the leaves immediately in contact with the atmosphere, accomplishes consequential responsibility in gaseous exchange and water evaporation. Because of this persisting referral with the atmosphere, it is prone to riposte the air pollution in various ways. The stomata present on these layers operate, as inlet and outlet for gases, like oxygen and carbon dioxide and water vapor. Consequently, induced modifications in structural components of the stomata are liable to affect gaseous exchange as well as transpirational loss of water. Several workers (Sen *et al.*, 1972; Jafri *et al.*, 1979; Yunus and Ahmed, 1979; Yunus *et al.*, 1979, 1982; Debnath, 1980; Garg and Varshney, 1980; Hall and Schulza, 1980; Bhiravamurthy and Kumar, 1983; Bhiravamurthy *et al.*, 1985; Krishnamurthy and Rajachidambaram, 1986; Ramanathan and Kanabiran, 1989; Farouki and Singh, 1990; Salgare and Acharekar, 1991; Robinson *et al.*, 1998), while analyzing the response of foliar epidermis to different types and levels of air pollutants noticed the alterations in the epidermal traits including stomata.

The present study is focused on the changes in epidermal pattern of safflower when subjected to concentration of heavy metals like- lead (Pb) and arsenic (As).

MATERIALS AND METHODS:

To analyze the lead Pb and arsenic As induced variations in the foliar epidermal pattern, mature expanded leaves of the treated and control plants were fixed in FAA (formalin: acetic acid: alcohol). The middle portions of the mature leaves of about one square centimeter were only analyzed for various epidermal parameters. The abaxial and adaxial epidermises were peeled out by gently scrapping the tissues from the

opposite side, respectively, with the help of a razor blade. The peels were stained in Delafield's haematoxylin and mounted in glycerin. Photomicrographs were taken from these temporary preparations.

Following parameters were analyzed for evaluating the variations induced in epidermal pattern:

1. Number of epidermal cells / mm²,
 2. Number of stomata / mm²,
 3. Ratio between number of stomata and epidermal cells (S/E),
 4. Guard cell index (GCI),
 5. Types and frequency of structural abnormalities induced in stomata,
 6. Total percent anomalies of stomata,
 7. Pore area index (PAI), and
 8. Total pore area per mm² (TPA). This is defined as the „percent of the total area of the pores per mm²“.
- Guard cell indexes (GCI), pore area index (PAI) and total pore area (TPA) were calculated using the formulae:

$$\text{GCI} = \frac{\text{Number of guard cells in stomata per mm}^2}{(\text{Number of guard cells} + \text{epidermal cells}) \text{ per mm}^2} \times 100$$

$$\text{PAI} = \frac{\text{Pore area (}\text{③m}^2\text{)}}{\text{Length} \times \text{breadth of stomata (}\text{③m}^2\text{)}} \times 100$$

$$\text{TPA} = \frac{\text{Pore area (}\text{③m}^2\text{)} \times \text{Number of stomata per mm}^2}{10^6 (1000 \times 1000 \text{ ③m}^2)} \times 100$$

OBSERVATIONS AND DISCUSSIONS:

During the present study, the plants were grown in culture tubes for a week hydroponically in 10⁻³M solution of lead acetate and sodium arsenate, thereafter, mature leaves were collected, fixed and then analyzed for foliar epidermal pattern. The leaves of *C. tinctorius*, were amphistomatic. The walls of the epidermal cells of the abaxial epidermis were chiefly wavy (fig.5 n) while those of adaxial epidermis were relatively straight (fig.5 o). Data related to the ratio between the abaxial and adaxial epidermal cells, stomata per mm², guard cell indices are given in tables 1, 2 and data for pore area, pore area index, and total pore area given in tables 3, 4.

Table 1. Ratio between abaxial and adaxial EF, SF, and GCI in lead treated leaves.

Acc.	T-20		T-87		T-98		T-104		T-111		T-113		T-140		T-178		T-185		T-186		T-223	
	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr
EF	1.23	1.09	1.16	1.11	0.98	1.06	1.08	1.13	1.04	1.15	1.05	1.02	1.06	1.06	1.15	1.34	1.11	1.12	1.19	1.06	1.16	1.21
SF	1.43	1.46	1.25	1.23	1.32	1.30	1.44	1.53	1.34	1.32	1.24	1.07	1.27	1.65	1.09	1.33	1.47	1.48	1.28	1.32	1.41	1.21
GCI	1.08	1.19	1.04	1.06	1.17	1.11	1.18	1.20	1.14	1.08	1.10	1.03	1.11	1.31	0.98	1.00	1.17	1.16	1.04	1.13	1.11	1.00

Table 2. Ratio between abaxial and adaxial EF, SF, and GCI in arsenic treated leaves.

Acc.	T-20		T-46		T-87		T-93		T-98		T-104		T-178		T-180		T-185		T-186		T-210	
	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr	Co	Tr
EF	1.23	1.11	1.11	1.21	1.16	1.16	0.98	1.21	1.08	1.23	1.23	1.28	1.20	1.27	1.27	1.27	1.15	1.25	1.19	1.23	1.19	1.24
SF	1.43	1.24	1.43	1.25	1.25	1.30	1.32	1.25	1.44	1.42	1.48	1.19	1.25	1.02	1.35	1.39	1.09	1.47	1.47	1.28	1.28	1.36
GCI	1.08	1.07	1.15	1.02	1.04	1.07	1.17	1.01	1.18	1.10	1.10	0.96	1.02	0.86	1.03	1.05	0.98	1.10	1.12	1.02	1.04	1.06

EF = frequency of epidermal cell, SF= frequency of stomata, GCI= guard cell index.

Table 3. Response coefficient values for epidermal characteristics of lead treated leaves

Code/RC			T-20	T-87	T-98	T-104	T-111	T-113	T-140	T-178	T-185	T-186	T-223
Co	Ad	PA	0.12	0.16	0.05	0.05	0.04	0.03	0.03	0.06	0.01	0.15	0.00
		GCI	-	-	-	-	-	-	-	-	-	-	-
		PAI	0.32	0.23	0.13	0.21	0.13	0.16	0.16	0.14	0.08	0.25	0.08
		TPA	0.30	0.33	0.40	0.32	0.33	0.22	0.23	0.04	0.26	0.22	0.34
	Ab	PA	0.24	0.22	0.27	0.16	0.32	0.27	0.26	0.13	0.27	0.18	0.25
		GCI	0.26	0.27	0.27	0.26	0.34	0.34	0.23	0.17	0.17	0.14	0.24
		PAI	0.21	0.14	0.08	0.15	0.09	0.09	0.10	0.08	0.04	0.15	0.05
		TPA	0.39	0.67	0.83	0.34	0.65	0.35	0.34	0.05	0.60	0.38	0.64
Tr	Ad	PA	0.44	0.35	0.39	0.40	0.44	0.43	0.36	0.04	0.24	0.27	0.29
		GCI	0.01	0.11	0.03	0.10	0.07	0.00	0.02	0.09	0.02	0.03	0.04
		PAI	0.24	0.11	0.18	0.14	0.17	0.24	0.07	0.03	0.06	0.13	0.21
		TPA	0.23	0.20	0.20	0.22	0.21	0.26	0.10	0.10	0.07	0.14	0.26
	Ab	PA	0.44	0.27	0.40	0.15	0.38	0.29	0.24	0.27	0.21	0.27	0.33
		GCI	0.16	0.06	0.20	0.07	0.36	0.32	0.14	0.24	0.26	0.23	0.12
		PAI	0.33	0.09	0.30	0.37	0.20	0.23	0.13	0.20	0.10	0.18	0.25
		TPA	0.14	0.12	0.13	0.13	0.13	0.15	0.06	0.06	0.05	0.08	0.14

Table 4. Response coefficient values for epidermal characteristics of arsenic treated leaves

Code/RC			T-20	T-46	T-87	T-93	T-98	T-104	T-178	T-180	T-185	T-186	T-210
Co	Ad	PA	0.05	0.03	0.02	0.19	0.05	0.05	0.01	0.02	0.06	0.02	0.01
		GCI	0.17	0.10	0.23	0.22	0.23	0.16	0.23	0.04	0.37	0.13	0.13
		PAI	0.21	0.09	0.25	0.07	0.18	0.11	0.20	0.02	0.32	0.11	0.12
		TPA	0.26	0.36	0.29	0.33	0.26	0.37	0.31	0.24	0.04	0.16	0.22
	Ab	PA	0.21	0.18	0.18	0.27	0.16	0.22	0.30	0.09	0.01	0.13	0.18
		GCI	0.28	0.20	0.14	0.24	0.19	0.23	0.18	0.08	0.02	0.17	0.14
		PAI	0.13	0.03	0.15	0.03	0.12	0.07	0.13	0.01	0.19	0.07	0.07
		TPA	0.25	0.67	0.75	0.73	0.37	0.74	0.88	0.34	0.08	0.19	0.38
Tr	Ad	PA	0.41	0.28	0.33	0.40	0.38	0.36	0.37	0.12	0.34	0.29	0.26
		GCI	0.05	0.05	0.02	0.00	0.07	0.02	0.05	0.01	0.02	0.02	0.03
		PAI	0.28	0.22	0.20	0.27	0.25	0.33	0.37	0.01	0.14	0.24	0.08
		TPA	0.24	0.26	0.22	0.27	0.29	0.30	0.40	0.00	0.16	0.25	0.10
	Ab	PA	0.41	0.45	0.18	0.35	0.10	0.34	0.24	0.11	0.33	0.23	0.27
		GCI	0.12	0.38	0.06	0.20	0.04	0.30	0.22	0.07	0.24	0.22	0.23
		PAI	0.27	0.30	0.09	0.23	0.19	0.20	0.12	0.04	0.20	0.10	0.18
		TPA	0.14	0.15	0.12	0.17	0.18	0.19	0.27	0.00	0.09	0.15	0.06

PA= pore area in mm²; GCI= guard cell index; PAI= pore area index; TPA= total pore area,

Co= control, Tr= treated, Ad= adaxial, Ab= abaxial.

In case of lead and arsenic treatments, the frequencies of epidermal cells, stomata, guard cell indices, pore area, pore area indices and total pore area, decreased in most of the accessions. In the treated leaves following structural stomatal anomalies were noticed:

1. Degeneration of single guard cell,
2. Degeneration of both guard cells,
3. Degeneration of stomata,
4. Presence of common subsidiary cell,
5. Presence of contiguous stomata,
6. Presence of three guard cells,
7. Presence of more than three guard cells, and
8. Variability in stomatal size.

During this study, it was observed that not only the frequencies of epidermal cells and stomata changed in the epidermis of treated sets but also the proportions between these of abaxial and adaxial also got modified. Lower value for S/E and GCI in treated sets, in relation to control, indicated inhibition of the frequency of stomata and/or stimulation to the frequency of epidermal cells, while higher values for these indicated opposite effects. Number of cells per unit area confides upon the rate of numerical increase of cells and degree of cell expansion. A higher rate of numerical increase of cells per unit area along with the slower rate of cell expansion increases the number of cells per unit area, while higher amount of cell expansion can lead to decrease in the number of cells per unit area. Lead and arsenic treatments incited several types of organizational changes either because of the disintegration of guard cells and subsidiary cells or due to inaccuracy committed during the development of the stomata. Disintegration of the guard cells was available only in mature stomata. It is possible that lead and arsenic might induce the synthesis of lysogenic enzymes in some guard cells and/or subsidiary cells, finally leading to the disintegration of these cells. As already stated, the control leaves showed the presence of normal stomata, however, in the lead and arsenic treated leaves several stomatal anomalies were observed. All the development anomalies in the organization of stomata resulted from the division and/or elongation of the subsidiary cells in various directions. (Fryns-Claessens and Cotthem, 1973; Palevitz, 1982).

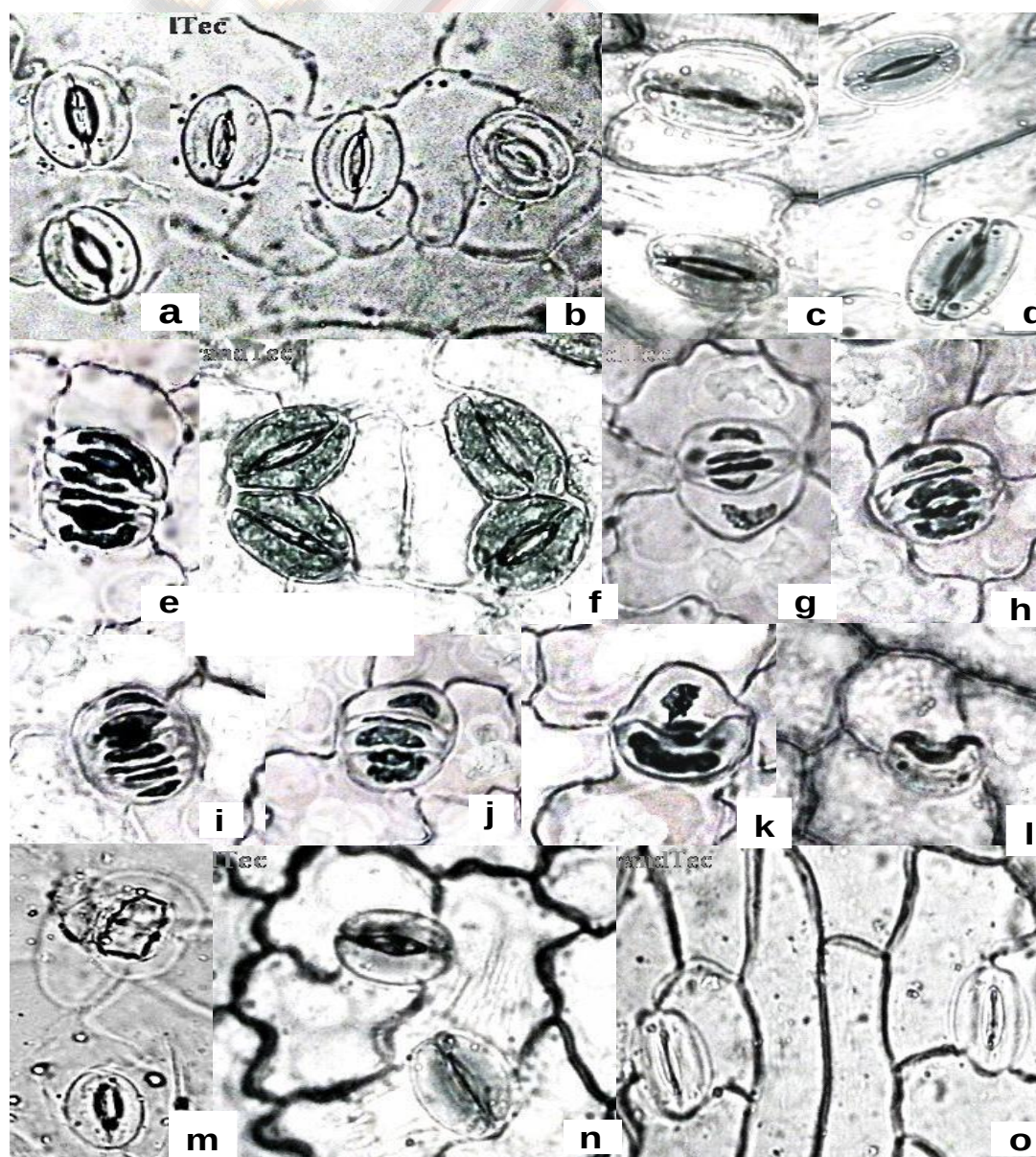


Fig.5 a, b. Common subsidiary cell between stomata, c, d. stomata showing size variability, e, f. contiguous stomata, g, h. presence of three guard cells, i, j. presence of more than three guard cells, k, l. degeneration of single guard cell, m. degeneration of stomata, n. wavy walls of epidermal cells on abaxial surface, o. straight walls of epidermal cells on adaxial surface.

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