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A VIABLE ALTERNATIVE MECHANISM IN ADAPTING THE PLANTS TO HEAVY METAL ENVIRONMENT

RAFIA AZMAT[1*], SABA HAIDER[2], HAJRA NASREEN[2], FARHA AZIZ[3] AND MARINA RIAZ[1]

[1]*Department of Chemistry,* [2]*Department of Botany and* [3]*Department of Biochemistry*
Jinnah University for Women, Nazimabad, Karachi, Pakistan
E-mail: rafiasaeed200@yahoo.com

Abstract

This study was undertaken to test the hypothesis that variations in leaf anatomy and morphology reflect their adaptability to the environmental stress. A self defense mechanism system related with the trichomes on the surface of leaves for the detoxification of Pb was observed in *Phaseolus mungo* and *Lens culinaris* through light microscopy in leaves. The presence of trichomes and increase in number of stomata in the adaxial (upper) leaf surface of both species seems to constitute an important morphological mechanism for survival that allows this species to maintain good photosystem II efficiency during the stress. Foliar morphological variability in *Phaseolus mungo* and *Lens culinaris* may be considered an adaptive advantage that enables leaves to develop and function in habitats marked by strong variations of Pb toxicity with solar radiation, air temperature and humidity. These hairs may constitute a shield against Pb pollution and act as a physical barrier for the protection of plants and can act as a biomonitor of environmental contamination and biogeochemical indicator of Pb. Enhancement in the hairs on leaves at high dose of Pb may be related with reducing evaporation of water from the surface of leaves in stress condition. The protection provided by the trichomes could afford advantages under stress conditions, especially during leaf development. The importance of the increase in the number of stomata in relation with the absorption of CO_2 with increase in CK enzymatic activity, creatine, glucose and reducing sugars ($p<.001$) for both species under the metal stress were examined.

Introduction

Plants exposed to stressing agents such as drought, salinity, excess of heavy metals, air pollutants, or pathogens have developed strategic defense mechanisms that vary between species and the nature of stressing agent (Sharma & Dubey. 2005). Lead usually enters plants by similar pathways as micro- and macronutrients. However, the metabolic pathways underwent by this element within plant cells are not fully understood (Patra *et al*., 2004). Most ecological studies suggest that plant growing under stress tend to possess leaves that have more hairs than similar or related plants from normal condition. Several possibilities exist for the function of leaf pubescence in plants from arid habitats. These include i) reduction of light absorption during conditions of high temperature and drought, ii) hindrance of the diffusion of gasses across the leaf air – air interface. iii) reduction of predation by insects and larger herbivores. The function of leaf pubescence may differ among various plant species, but it would not be surprising to discover that in some cases pubescence served several function (Tomar *et al*., 2000).

Pb-induced changes in the leaf epidermis structure involved a reduction in the cell size, more abundant wax coating and an increase in the number of stomata and trichomes per unit area with simultaneous reduction in the size of the guard cells (Weryszko-Chmielewska & Chwil, 2005). The effects of leaf hairs on photosynthesis, transpiration, and leaf energy balance were measured on the desert shrub *Encelia farinosa* in order to determine the adaptive significance of the hairs (Ehleringer & Moone 1978). The pubescence reduces leaf absorption resulting in a reduced heat load and as a consequence lower leaf temperatures and lower transpiration rates.

Stiborova *et al*., (1987) and Verma & Dubey (2001) reported the increase in sugar and glucose contents under metal stress with reduced carbon metabolism, which results in the disorder of light reactions of photosynthesis leading to growth inhibition. Lanaras *et al*., (2006) and Farres *et al*., (2002) observed that creatine kinase a key enzyme in cellular energy homeostasis of vertebrates offers the promise of engineering plants with enhanced stress tolerance. Creatinine works by binding itself to phosphates which provide recharging energy and help in recovery in drought condition.

In the present report the adverse effect of Pb on two species of the leguminous family *viz*., *Phaseolus mungo* and *Lens culinaris* is presented to investigate the adaptation mechanisms that occur in two plants. The importance of the increase in the number of stomata and trichomes for plants under the metal stress were examined. The level of glucose, reducing sugars, creatinine and CK enzyme activity explain the survival of plants in stress condition since no studies have been reported yet with these two members of the family.

Material and Methods

The seedlings of the *Phaseolus mungo* and *Lens culinaris* were investigated by subjecting them to water culture for a period of 15 d. To the Hoagland nutrient solution, Pb was added as $PbCl_2$ at concentrations *viz*., 0, 50, 100, 150, 200 and 250 mg dm^{-3}. Observations of foliar architecture, blade and petiole epidermal and number of stomata in *Phaseolus mungo* and *Lens culinaris* leaves tissues were examined using light microscopy by clearing the leaves using 10% KOH solution. All stomata and leaf pubescence within the eyepiece grid (SC) were counted and compared with the leaves of control plant.

After 15 d of exposure to the treatments, the leaves were harvested, homogenized, and centrifuged and the supernatants were analyzed for sugar, glucose, creatinine and creatine kinase activity determined by using randox kits available in the laboratory (Rao & Deshpande 2007). All determinations were performed in triplicate.

Statistics: The values reported are the average of three replicates ± standard error obtained from the one-way analysis of variance followed by Tukey's honestly significant difference analysis performed with the statistical package SPSS Version 12.0 (SPSS, Chicago, IL, USA.).

Results and Discussion

Growth rate of both species *viz*., *Phaseolus mungo* and *Lens culinaris* are presented in Table 1. It was observed that growth of both species were reduced under Pb stress reflecting the reduction in length weight ratio of both species (Table 1). Leaf areas of both species were reduced with the increased concentration of Pb as observed earlier by Klich (2000). The length weight ratio of both plant species showed that Pb is toxic specially in the seedling stage and the of value of R^2 suggesting the inverse relation with the applied dose of Pb and growth of plants (Figs. 1&2).

Table 1. Length weight ratio of *Phaseolus mungo* and *Lens culinaris* under Pb stress.

Pb (ppm)	Av. length of plant (cm)	Av. weight of plant (cm)	Length/ weight
Phaseolus mungo			
0	27.4 ± 10.3	0.247 ± 0.03	110.5
50	29.1 ± 11.3	0.205 ± 0.02	141.26
100	21.9 ± 9.6	0.192 ± 0.21	114.06
150	16.4 ± 5.0	0.1921 ± 0.03	85.37
200	14.14 ± 6.3	0.234 ± 0.03	60.34
250	12.30 ± 4.9	0.0787 ± 0.01	156.22
Lens culinaris			
0	17.60 ± 3.9	0.084 ± 0.03	208.53
50	18.40 ± 3.8	0.057 ± 0.05	323.15
100	11.80 ± 2.9	0.668 ± 0.06	176.55
150	11.38 ± 4.1	0.0578 ± 0.03	196.65
200	4.66 ± 4.6	0.0577 ± 0.01	80.46
250	-	-	-

Stomata and Trichomes as Biomonitor of Environmental Contamination: Results showed that seed germination remained unaffected at low concentrations in both species but was completely inhibited at 250 mg dm^{-3} of Pb in case of *Lens culinaris* (Azmat *et al.,* 2006). Leaf stomatal density and leaf trichome density and water relations reflects drought resistance in relation with Pb toxicity.

Increase in number of stomata with trichomes on the surface of leaves of *Phaseolus mungo* and *Lens culinaris* were observed in the 15 d old seedling of plant (Figs. 1-4). An increase in the number of stomata and trichomes per unit area with simultaneous reduction in the size of the guard cells appeared due to self defense system; develop in plants under stress condition which provided the support to the plant for their survival in contaminated environment. Pb toxicity commonly reduced the absorption of CO_2 due to reduced leaf surface area whereas an increase in number of stomata may be helpful to absorb the CO_2 in stress condition for the production of glucose molecule. A comparison of number of stomata and leaf hairs with the leaves of control plant ($p<0.001$) represented that the size of stomata was large but in Pb treated plant the number of stomata and leaf hairs were more with reduced size of stomata (Figs. 1-4), suggest that increase in number of stomata and leaf hairs may be compassionate to the plant to release the excesses of Pb in the environment to protect the plants by the toxicity of nonessential element Pb.

Foliar morphological variability in *Phaseolus mungo* and *Lens culinaris* may be considered an adaptive advantage that enables leaves to develop and function in habitats marked by strong variations of Pb toxicity with solar radiation, air temperature and humidity. This decrease in the relative plant water content (Azmat *et al*., 2009) with reduced leaf area and transpiration intensity suggests that the presence of excess lead in the nutrient medium reduces water uptake and transport (Azmat *et al.*, 2006; Novica *et al*., 1998) which may effect the photosynthesis system of plant (Haider *et al.,* 2006; Romanowska *et al*., 1980).

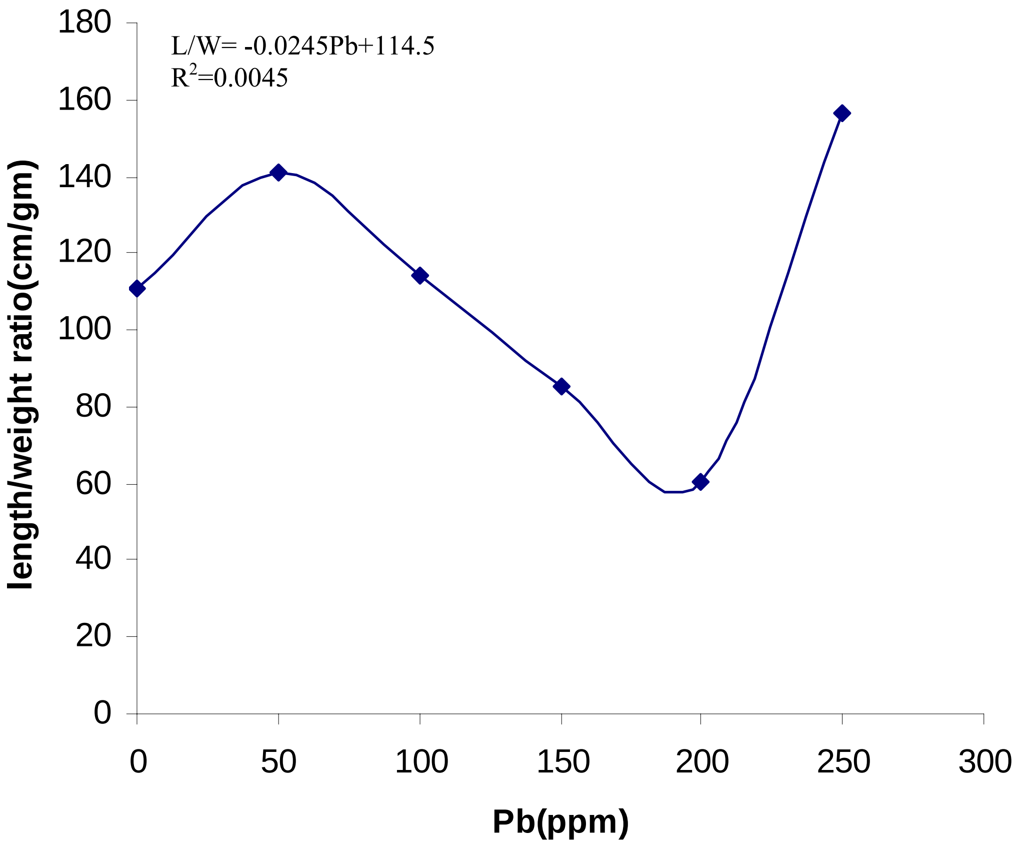

Fig. 1. Length –weight ratio of *Phaseolus mungo* under Pb stress

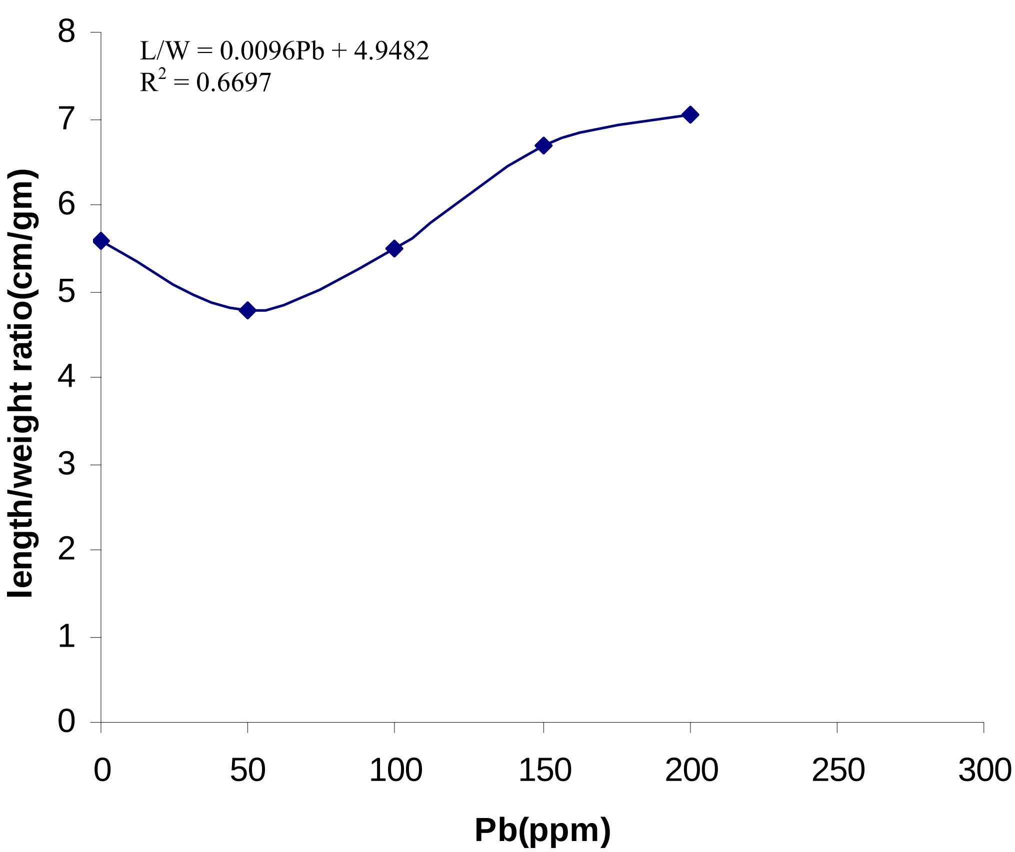

Fig. 2. Length –weight ratio of *Lens culinaris* under Pb stress.

Table 2. Some biogeochemical indicator of *Phaseolus mungo* and *Lens culinaris* under Pb stress.

Pb (ppm)	Reducing sugars g/dl	Glucose g/dl	Creatinine mg/dl	CK.10^{3-}activity (nm/min)
Phaseolus mungo				
0	1.161 ± 0.05	80.630 ± 15.0	1.6 ± 0.04	3.739 ± 0.0
50	1.207 ± 0.05	17.764 ± 5.0	2.00 ± 0.5	3.756 ± 0.02
100	1.438 ± 0.07	20.626 ± 3.0	7.60 ± 0.09	3.950 ± 0.03
150	1.685 ± 0.08	21.063 ± 4.0	22.0 ± 3.4	3.982 ± 0.4
200	1.406 ± 0.02	21.806 ± 3.0	32.4 ± 8.2	4.144 ± 0.3
250	1.159 ± 0.02	14.486 ± 2.4	38.0 ± 12.0	4.630 ± 0.3
Lens culinaris				
0	1.636±0.09	5.588±1.2	5.588±0.5	3.278±0.89
50	1.656±0.6	4.763±1.3	4.763±0.6	3.464±0.34
100	1.331±0.7	5.487±1.1	5.487±0.4	3.488±0.5
150	0.88±0.3	6.678±1.7	6.678±0.5	4.606±0.6
200	0.74±0.08	7.036±1.5	7.036±0.3	5.739±0.3
250	-	-	-	-

Energy providing compounds as a biogeochemical indicator: The level of energy providing compounds like reducing sugars, glucose, creatine and cretinen kinase (CK) enzyme activity of both species were significantly higher ($p<0.001$) in the Pb treated plants as compared to the control ones (Table 2). Which indicated that glucose formed may not be utilized by the plant due to physical presence of Pb in the tissues due to which CK activity of plants were found to increase which may be attributed with function of cretinine. Creatinine is an energy producing substance, found in much lesser amount in plants. Creatine helps in those conditions where a large burst of energy for a short period of time is required (Anon., 1999). It also supplies energy for the first few minutes, and effective if one has developed good nutrition. Increased in creatinine contents under Pb stress may be due to damaging in cell structure (Azmat *et al.,* 2006) or have an energy generating action to overcome the stress condition for survival of plants in presence of toxic metal. It provides the energy buffering system to the plants with enhanced tolerance level. This shows the alternative system of plant adapted in the stress conditions for support of the plants biochemically. The results related to the soluble sugar content (Table 2) revealed that lower concentrations of Pb increased the soluble sugar content however, higher concentrations of 250 mg/l of Pd showed a decrease of 50% in soluble sugar content after 15 days which may be attributed with reduced leaf size and thickening of guard cell which may be responsible of abridged exchange of gases (Xing *et al*., 2008).

Our results corroborate with the findings of Ahmad *et al*., (2006) who found an increase in soluble sugars at low concentrations of salt stress and decrease at higher concentrations in *Pisum sativum*. The decrease in total sugar content of stressed leaves probably corresponded with the photosynthetic inhibition or stimulation of respiration rate. Tzvetkova & Kolaro (1996) reported higher starch accumulation in damaged leaves of *Tilia argentea* and *Quercus cerris* may result both in the higher resistance of their photosynthetic apparatus and low starch export from the mesophyll. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive centre of ribulosebisphosphate zarboxylase (Stiborova *et al*., 1987). Verma

& Dubey (2001) observed an increase in the content of total soluble sugars and reducing sugars and decrease in the content of non-reducing sugars with toxic metal Cd on rice. Cd-induced increase in the sugar content was greater in shoots than in roots. The nitrogen content, soluble sugars, starch and lipid content were lower in Cu-grown plants. Lower carbohydrate levels appear to result from low photosynthetic activity. Stress caused by high Cu concentration in the soil appears to affect the light reactions of photosynthesis leading to growth inhibition (Lanaras *et al.,* 2006) and increasing trend of sucrose was observed in shoots of cadmium-stressed seedlings of pea (Devi *et al*., 2007).

Mechanism of survival: Results showed that Pb physically block the uptake of water and water stress led to substantial losses in dry weight, leaf area, root dry weight and length (Azmat *et al.,* 2006). Figures 1-11 of leaves suggest that the existence of trichomes in *Phaseolus mungo* and *Lens culinaris* leaves may constitute a protective light-filtering mechanism for the photosynthetic machinery. There is also evidence that *Arabidopsis* trichomes play additional or alternative roles in response to abiotic stress conditions such as the detoxification of heavy metals (Ager *et al*., 2003). In cadmium-contaminated soil, leaf trichomes accumulate the toxic metal at a concentration of up to 10-fold. Many specific functions have been ascribed to trichomes, including protection against herbivores and UV light, storage of toxic metal ions, and increased freezing tolerance. In addition to the chemical defense, trichomes have been implicated in protecting the plant mechanically against herbivorous insects. Trichomes exhibit a very rigid cell wall, exemplified by the high mechanical and biochemical resistance against protoplasting (Zhang & Oppenheimer, 2004).

Generally deficit water uptake produced greater leaf trichome densities and number of stomata in leaves (Bañon *et al*., 2004) and accumulation depends on the plant species as well as on the organ of the plant whereas the accumulation of Pb in roots inhibit the translocation of water. Our experiments also suggest that the roots of the both plants are a barrier for Pb absorption by the stalk with leaves and grains. The study clearly shows that increase in heavy metal concentration in foliage of plants grown in heavy metals caused unfavorable changes in physiological and biochemical characteristics of plants leading to reductions in morphological characteristics, biomass accumulation and yield. These hairs may constitute a shield against Pb pollution and act as a physical barrier for the protection of plants and can act as a biomonitor of environmental contamination and biogeochemical indicator of Pb. Enhancement in the hairs on leaves at high dose of Pb may be related with reducing evaporation of water from the surface of leaves in stress condition The protection provided by the trichomes could afford advantages under stress conditions, especially during leaf development while high dose of Pb rupture the leaf structure which results in the failure of all adaptive mechanism consequently the plants experience the death.

Conclusion

Plants undertake many adaptive mechanism for their survival under abiotic stress which includes morphological as well biogeochemical ones in which leaf pubescence has adaptive value to both species because the hairs allow the leaf to gain a higher rate of carbon under arid condition than the leaf acquire without hairs to avoid potentially lethal condition of soil and loss of water which allows the plant to extend its growth for a longer period into the drought. The sugar, glucose, creatinine and CK enzyme activity showed the powerful tool for the survival of plants to provide energy when damaging in photosynthesis apparatus occurred on accumulation of Pb.

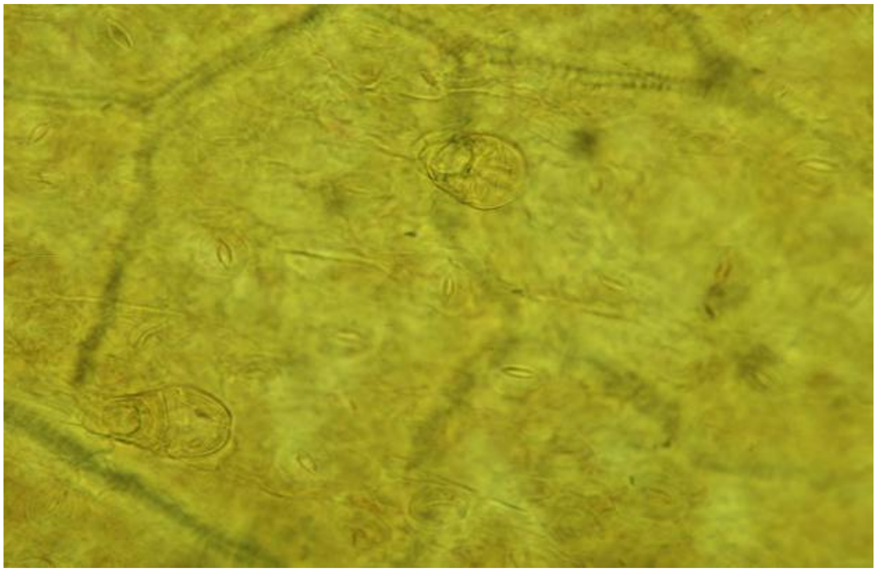

Fig. 3. Stomata and trichomes of *Phaseolus mungo* of control plant.

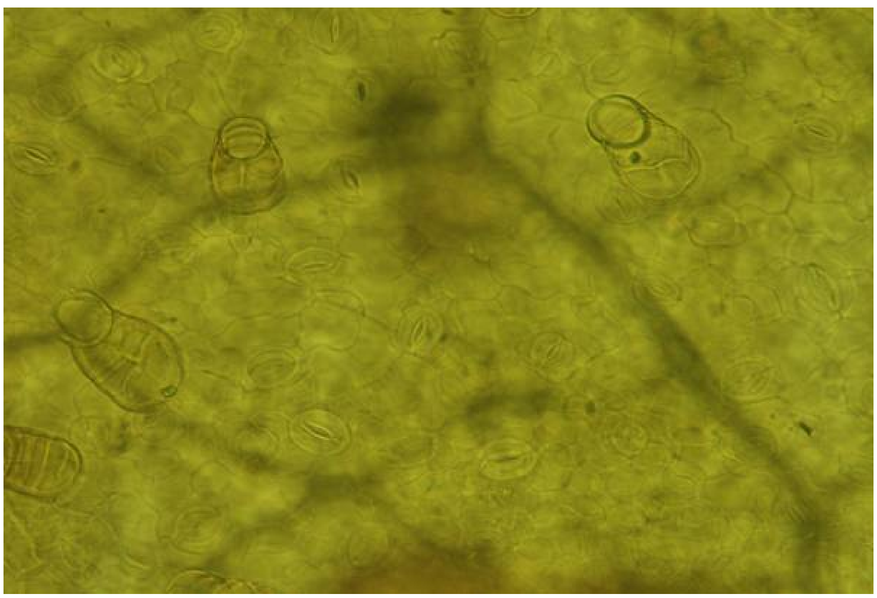

Fig. 4. Stomata and trichomes of Pb treated plant *Phaseolus mungo*.

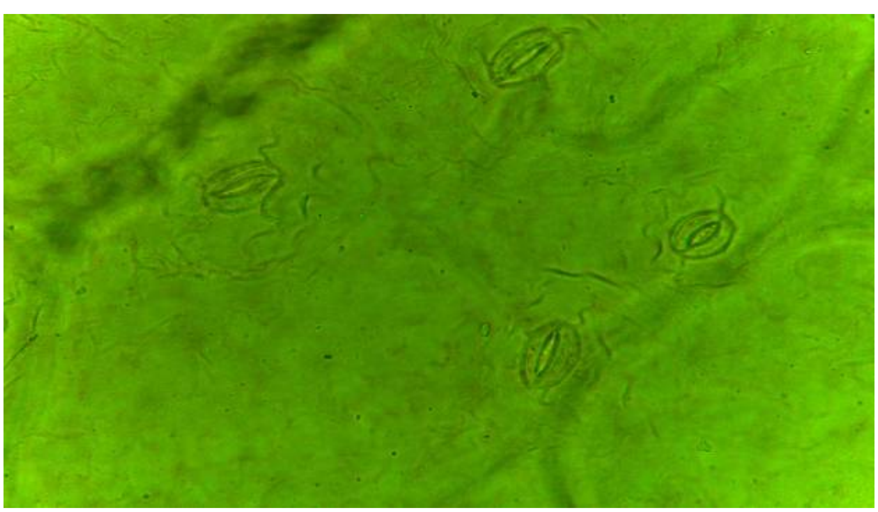

Fig. 5. Stomata and trichomes of *Lens culinaris* of control plant.

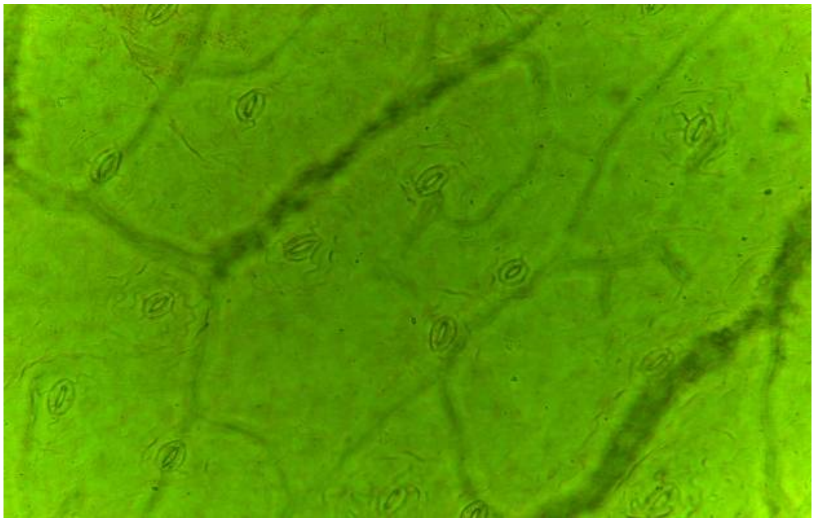

Fig. 6. Stomata and trichomes of *Lens culinaris* of control plant.

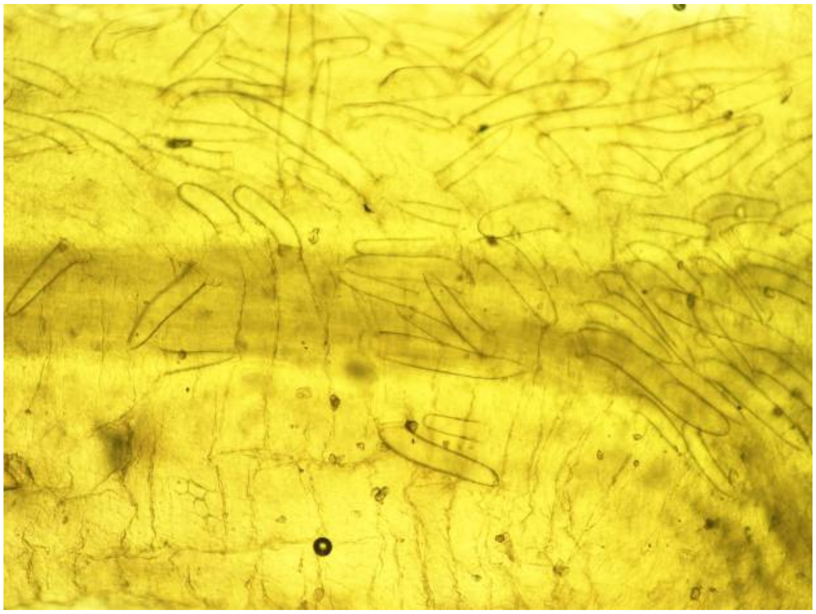

Fig. 7. Trichomes at mid rib of Pb treated plant of *Phaseolus mungo*.

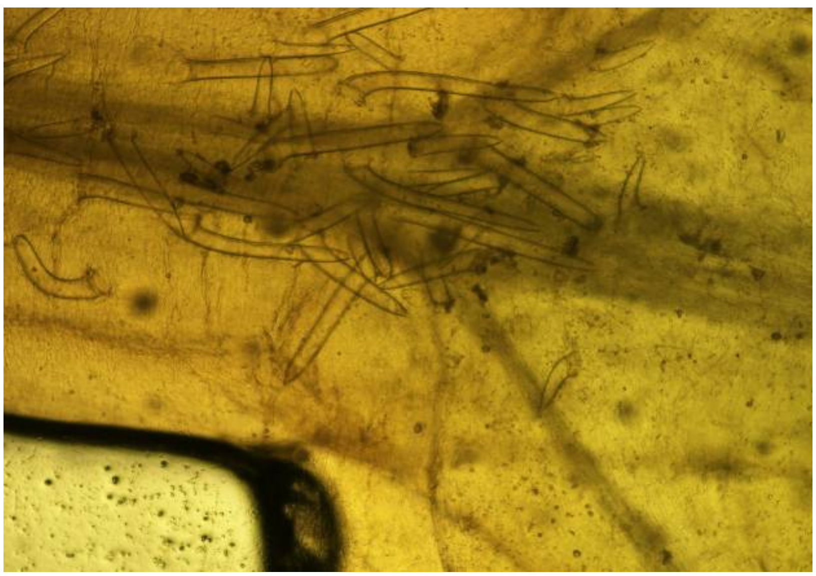

Fig. 8. Trichomes at mid rib of Pb treated plant of *Lens culinaris*.

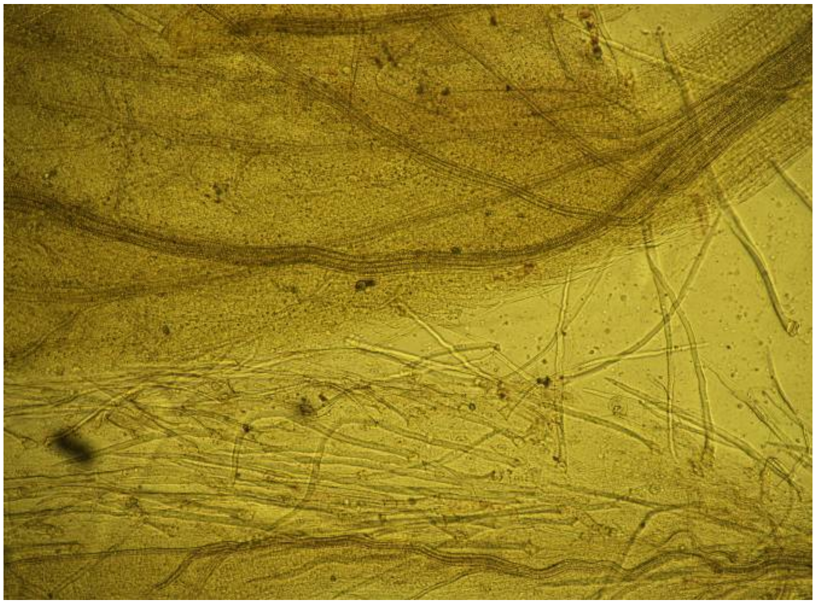

Fig. 9. Trichomes of Pb treated plant of *Phaseolus mungo*.

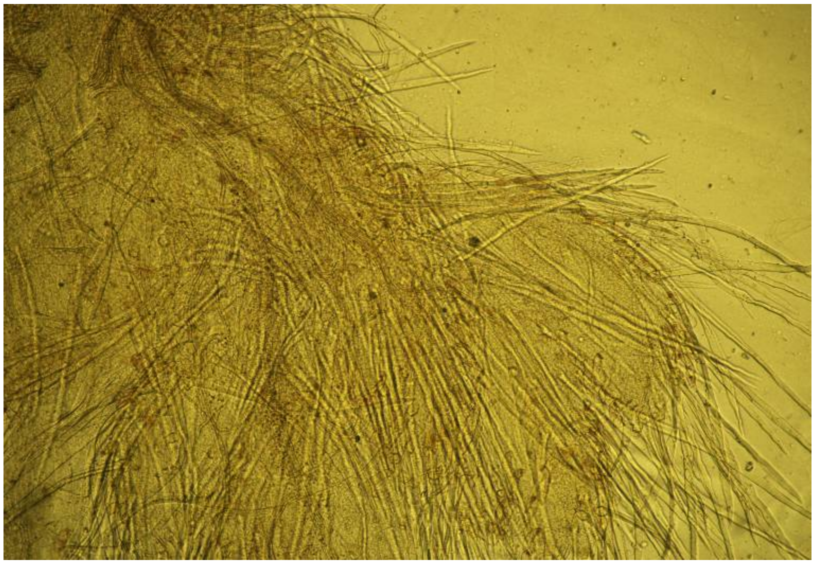

Fig. 10. Trichomes of Pb treated plant of *Lens culinaris*.

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