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Research Article

UPTAKE OF HEAVY METAL CADMIUM AFFECTING LEAF PHYSIOLOGY OF PEA CULTIVARS

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ABSTRACT
A comprehensive study on three pea cultivars namely *Pisum sativum* L. namely *Arvense, Arkel* and *RE-89* showed inhibited growth of root, shoot and leaves in the presence of heavy metal Cd. Vital activities such as transpiration and photosynthetic rate and chlorophyll content get affected. Interestingly, *Arvense* has better tolerance towards Cd induced toxicity than the other two cultivars. Radical elongation is most affected in *Arkel* followed by *RE-89* and *Arvense,* as the radical length has a direct bearing on the absorption capacity of seedlings. The dry biomass accumulations were least in *Arvense* confirming its better water absorption capacity. This morphological adaptation has an added advantage with high altitude cultivar. Cd enhanced surface diffusion resistance (SDR) was more in *RE-89* followed by *Arvense* and *Arkel.* Reduced rate of transpiration and increased stomatal resistance were noticed due to Cd. Reduced leaf surface humidity accompanied with lower transpiration rates lead to a significant rise of leaf surface temperature (3.1 ℃) which appears an adaptation to avoid unfavorable conditions.

Keywords: *Pisum sativum, Hydroponics, Transpiration, Surface Diffusion Resistance (SDR)*

INTRODUCTION
Pea (*Pisum sativum* L.) is one of the world's oldest domesticated legume crops for the humans, livestock and improving the soil fertility as well. Seeds are rich in many nutrient compounds including proteins (19.5-20.2%), starch, dietary fiber (6-10%), certain fatty acids and micronutrient (vitamins, trace minerals 3-4%). It is a leading vegetable crop in the tropics grown at higher altitudes in the NW Himalayas with a temperature range of 7-30°C particularly in the North Indian States like Himachal Pradesh, Jammu & Kashmir and Uttarakhand.

Heavy metals are causing widespread contamination of the soil and groundwater resources (Lombi *et al.*, 2001) and their continuous inflow into terrestrial food chains via agricultural crops is a serious concern (Krishnamurti *et al.,* 2006; Aulakh *et al.,* 2009). Amongst the heavy metals As, Hg, Ag, Cd, Pb have no role in a plant system as nutrients and seem to be more or less toxic. Cadmium exists in combined forms like CdO, $CdCl_2$ or $CdSO_4$. Cd is readily absorbed from the soil atmosphere and has a tendency to accumulate in plant tissues (Kabata-Pendias and Pendias, 1992). Cadmium as such has no known function in the biological organisms including vascular plants (Roosens *et al.,* 2003). It is highly mutagenic/ carcinogenic and WHO has set a maximum permissible limit of 0.3 ppm in case of medicinal plants. Cd enters primarily via the root system and at root surface Cd^{2+} bind to the carboxy group of mucilage uronic acids. About 30% inhibition of root growth with 1µM Cd (24h) exhibits a positive correlation with the viability of root cells (Siroka *et al.,* 2004).

It has been shown that pea (*Pisum sativum* L.) is less tolerant to Cd toxicity in comparison to other cereals (Inouhe *et al.,* 1994). Generally, legumes show a spectrum of responses to heavy metals like reduced growth and yield (Leita *et al.,* 1993; Demirevska-Kepova, 2006), photosynthetic activity (Kumar and Kumar, 1999), nodulation and nitrogen fixation (Chugh *et al.,* 1992) and uptake of nutrients in pea (Hernandez *et al.,* 96; Obata and Umebayershi, 1997). The morphology of plants gets affected, turn pale yellow, weak and unable to stand erect with the presence of Cd in rooting medium (Azmat *et al.,* 2005). Not much information is available regarding translocation behavior of the heavy metal Cd induced phytotoxicity affecting transpiration, leaf surface humidity, photosynthesis and leaf thermal variations in pea cultivars.

MATERIALS AND METHODS

Three cultivars of *P. sativum* L. (pea) namely *Arvense* (a wild cultivar from the temperate zone of Leh Ladakh); *Arkel* and *RE-89* (two locally grown cultivars from a sub-tropical zone in the plains of northern India) were selected for the seed germination studies. Seven day old healthy seedlings grown in the pots were shifted to hydroponics culture bottles (500ml, Tarson) filled with Hoagland's nutrient medium. Before shifting them to hydroponics, seedlings were acclimatized for 3 days in lab to a 12h day/night cycle (photon flux density 2046±40 lux; temperature 27±3.5°C; RH 55-61%). Nutrient solution was aerated continuously and replaced every 3rd day till completion of the trial. $CdSO_4$ (7, 14 and 21µM) treatment was supplemented to the nutrient medium itself.

Phytotoxicity Index (Chou and Muller, 1972):

$$PI = \frac{\text{Root length in control - root length in treatment}}{\text{Root length in control}} \times 100$$

Physiological Studies: Steady State Porometer (Model Li-1600; Li Cor. Inc.) used for observations in hydroponics such as Stomatal Diffusive Resistance (s cm^{-1}), Transpiration Rate (µg/cm^2 /s), Foliar Relative Humidity (units) and Leaf Temperature (°C).

Statistical Analysis: One way analysis of variance (ANOVA) applied using SPSS for windows v. 16.0.1. Test of Significance for the differences among treatments considered at $P<0.05$ according to Tukey test.

RESULTS AND DISCUSSION

Firstly, pea seeds were allowed to germinate in earthenware pots and 10-12 day old seedlings were transferred to hydroponic culture bottles under room temperature conditions. The plants were allowed to acclimatize atleast for three days in the hydroponics before Cd treatment. Alternate periods of 12 hours lights (artificial) and dark were arranged in the culture racks for the trial. The observations were noted after 24, 48, 72, 96, 120 h of the treatment.

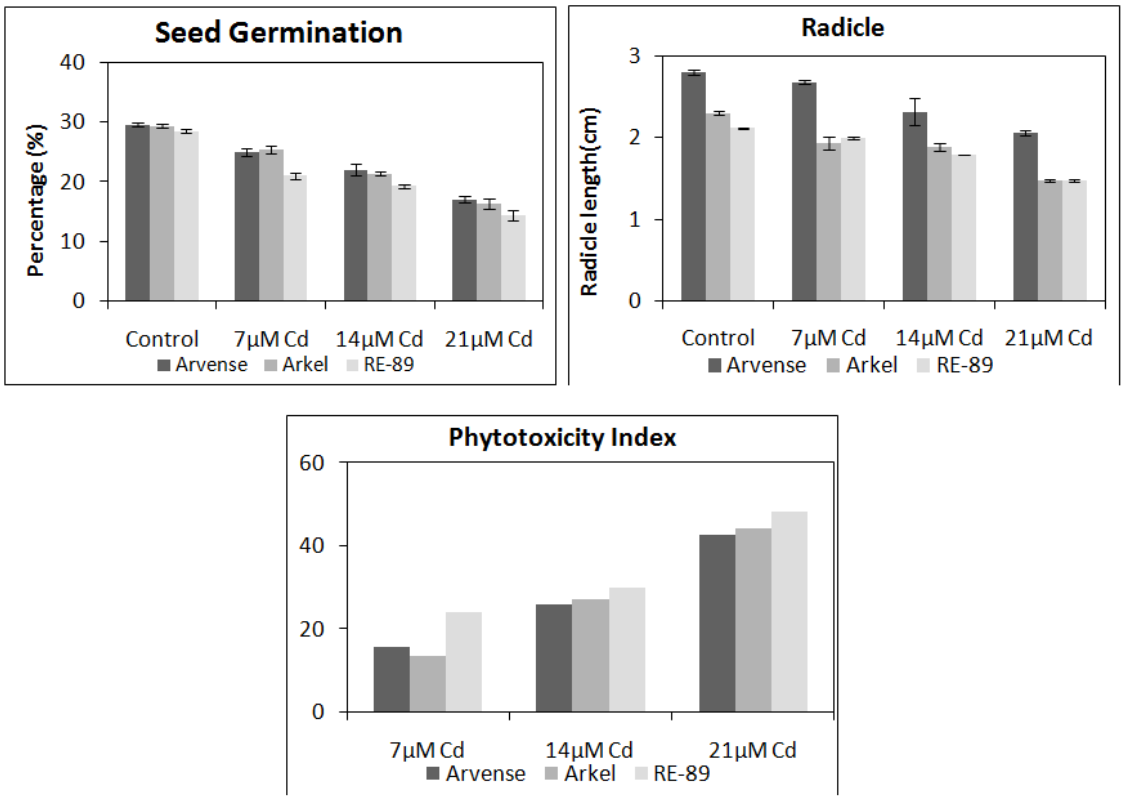

Figure 1: Effect of Cd treatments on Seed Germination percentage, Radicle Length and Phytotoxicity Index of three pea cultivars

Seed germination studies showed a significant reduction in percentage after 72h in comparison to control in all the three cultivars with Cd treatment. It was highest in *Arvense* (99%) followed by Arkel (98%) and RE-89 (95%) out of 30 seeds under control conditions. The effect of Cd treatment was visible affecting viability of seeds with reduced germination in three cultivars to 83%, 73%, 57% in *Arvense*; 84%, 71%, 54% in *Arkel* and 70%, 64%, 48% in *RE-89* cultivars with 7, 14, and 21 µM $CdSO_4$ levels, respectively. Interestingly, high altitude wild cultivar *Arvense* showed minimum effect of Cd toxicity whereas the locally grown cultivars *Arkel* and *RE-89* were more prone to Cd treatment levels (Figure 1).

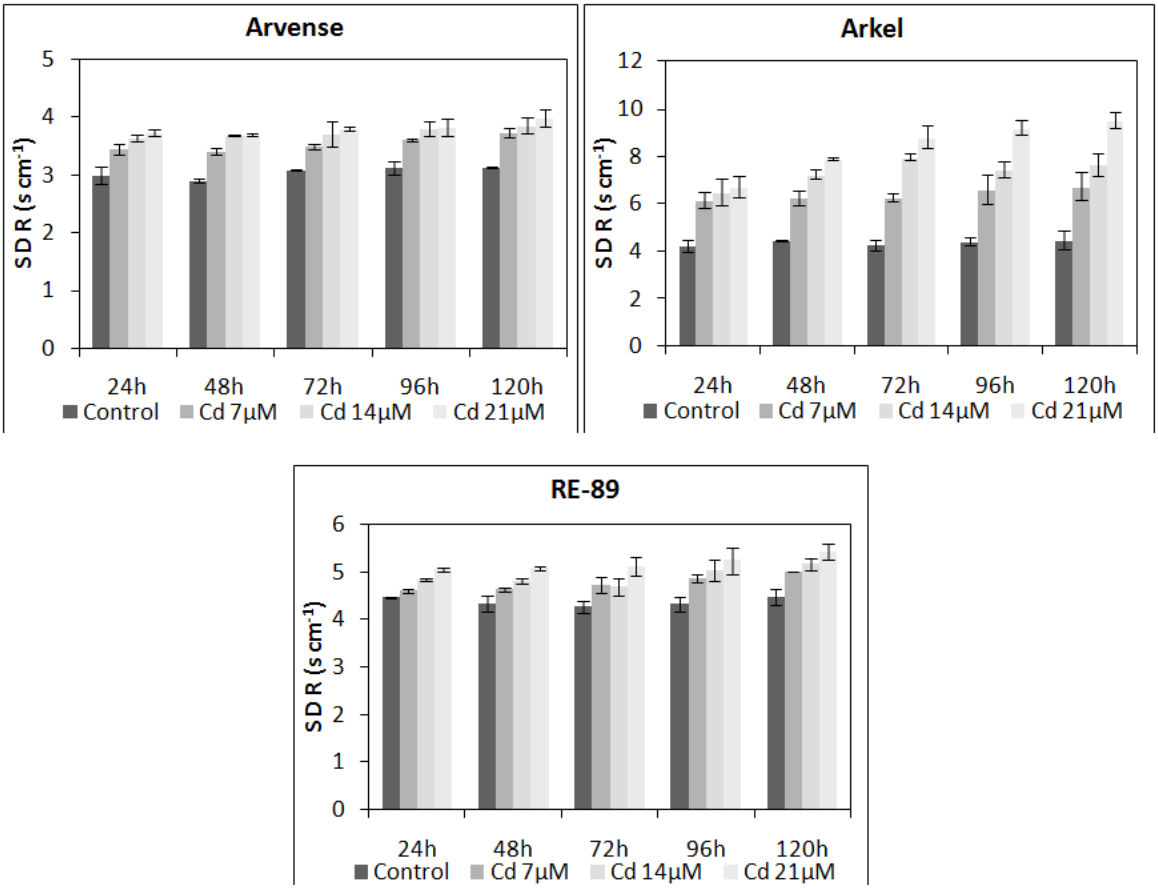

Figure 2: Effect of Cd treatments on Stomatal Diffusive Resistance (SDR) of three pea cultivars

The toxicity effect of Cd on seed germination in an increasing order was *RE-89>Arkel>Arvense*. The latter cultivar, although not native to this part of the country, had shown maximum tolerance towards Cd induced adverse conditions than the other cultivars *Arkel* and *RE-89*.

Phytotoxicity index calculated for the three cultivars of Pea showed toxicity levels of Cd in an increasing order of *Arvense>Arkel>RE-89*. It was 16, 26, 43 in *Arvense*; 14, 27, 44 in *Arkel* and 24, 30, 48 in *RE-89* when range of Cd exposure was enhanced from 7-14-21 µM, respectively. As expected, other two locally grown cultivars have showed lesser tolerance to Cd toxicity in comparison to *Arvense*, a wild high altitude cultivar of temperate zone Leh-Ladakh of Northern India (Figure 1).

Stomatal Diffusive Resistance (SDR) of leaves in *Arvense* increased by 4-6% in all the plants by keeping them in hydroponics cultures upto 120 h irrespective of the treatment. Further, SDR increase got stimulated with the application of Cd (7, 14, 21µM) treatments. In general, SDR increase in comparison to control was in the range of 15 to 25% with enhancing Cd exposure from 7 to 21 µM. In Arkel, SDR increase due to hydroponic (24-120 h) conditions irrespective of the treatments was 6%, 9%, 18% and 42% in Control, 7, 14 and 21 µM Cd, respectively. The effect of Cd application on SDR values was 46,

53, 59% (24h) and 50, 71, 113% (120 h) in 7, 14 and 21µM Cd levels, respectively in comparison to their control. Similarly, SDR values of *RE-89* cultivar after 24, 48, 72, 96 and 120 h in hydroponics showed an increase of 0.45% (Control), 9% (7µM), 7% (14µM) 8% (21 µM). SDR increased from 3, 9, 13% (24hr); 12, 15, 21% (120 h) in 7, 14 and 21µM levels of Cd, respectively (Figure 2).

It was noticed that Cd application has increased SDR of the leaves and the effect in an increasing order was *RE-89* (21%)>*Arvense* (25%)>*Arkel* (113%).

Rate of leaf transpiration stabilized within 24-48 h in hydroponics culture and there was hardly any change upto 120 h in control *Arvense* cultivar. It was noticed in Cd treated plants that transpiration rate lowered by 17, 19, 34% (48 h); 43, 48, 52% (72 h); 23; 30, 42% (96 h) and 42, 47, 53% (120 h) in comparison to controls with 7, 14, 21µM levels of Cd, respectively. In the pea cultivar *Arkel*, transpiration rate was also stable under control conditions in hydroponics culture (24-120 h). The effect of Cd was noticeable within 24 h as transpiration rate decreased with an increase of Cd concentration. Total percentage decrease 10, 19, 25 (24 h); 9, 18, 23% (72 h) and 16, 22, 28% (120 h) in comparison to control with Cd treatment levels 7, 14 and 21µM, respectively. Rate of transpiration in *RE-89* decreased slightly (6%) in the control plants in hydroponics culture from 34.87 units (24 h) to 32.70 units (120 h). Cd treatment levels of 7, 14 and 21µM reduced transpiration rate by 15, 28, 30% after 24h; 12, 30, 30% after 48h and 27, 42, 43% after 120h, in comparison to control respectively (Figure 3).

The effect of Cd toxicity in reducing transpiration rate in hydroponics culture was highest in *Arvense* (53%) > *RE-89* (43%) > *Arkel* (28%).

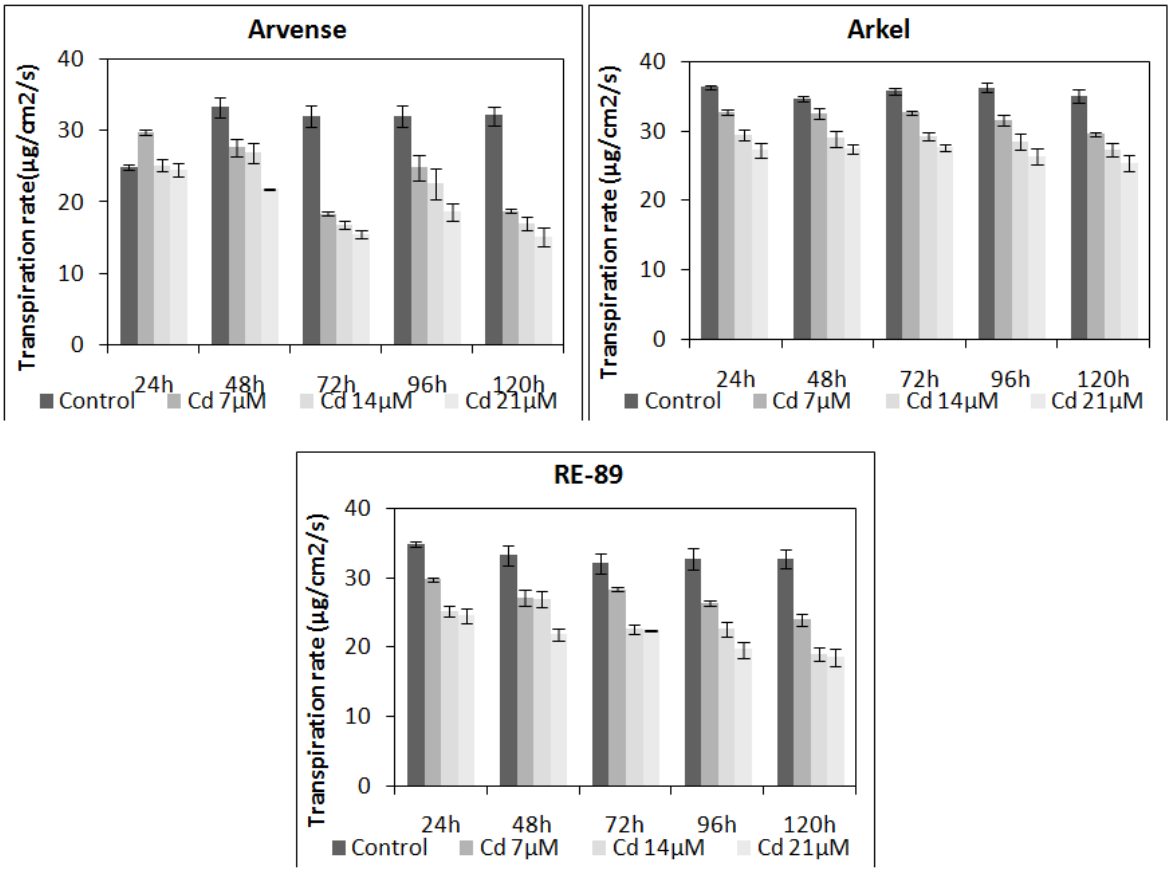

Figure 3: Effect of Cd treatments on Leaf Transpiration Rate of three pea cultivars

Leaf surface relative humidity in *Arvense* reduced upto 2.8%, 6.2%, 5.6% and 7.4% in hydroponics culture from 24 to 120 h in Control, 7, 14 and 21 µM, respectively. The percentage reduction in surface humidity was 1.2, 2.7, 5.3% (24h); 2.8, 5.0, 10.1% (72h); 4.6, 5.5, 9.7% (120h) in comparison to control

with Cd levels 7, 14 and 21µM, respectively. The percentage decline in leaf surface humidity in cultivar *Arkel* was 0.6, 2.3, 2.4% (24h); 5.6, 6.0, 6.2% (72h) and 12.0, 19.3, 21.0% (120h) indicating Cd severity levels at 7, 14 and 21µM, respectively. Also, the effect hydroponics culture conditions (24 to 120 h) in reducing leaf surface humidity was 1.4% (Control), 12.7% (7 µM); 18.5% (14 µM) and 20.1% (21 µM). Leaf surface humidity in cultivar *RE-89* was almost stable in control even after 120h where as it reduced by 8.0% (7 µM), 7.7 (14 µM) and 4.9% (21 µM) by 120h of treatment. Similarly, the percentage reduction in leaf surface humidity noticed was 4.5, 7.4, 13.0% (24h); 12.3, 11.8, 15.3% (72h) and 12.1, 14.5, 17.3% (120h) with Cd levels of 7, 14 and 21µM, respectively (Figure 4).
Surface humidity loss in an increasing order was *Arvense* (10%)>*RE-89* (17%)>*Arkel* (21%).

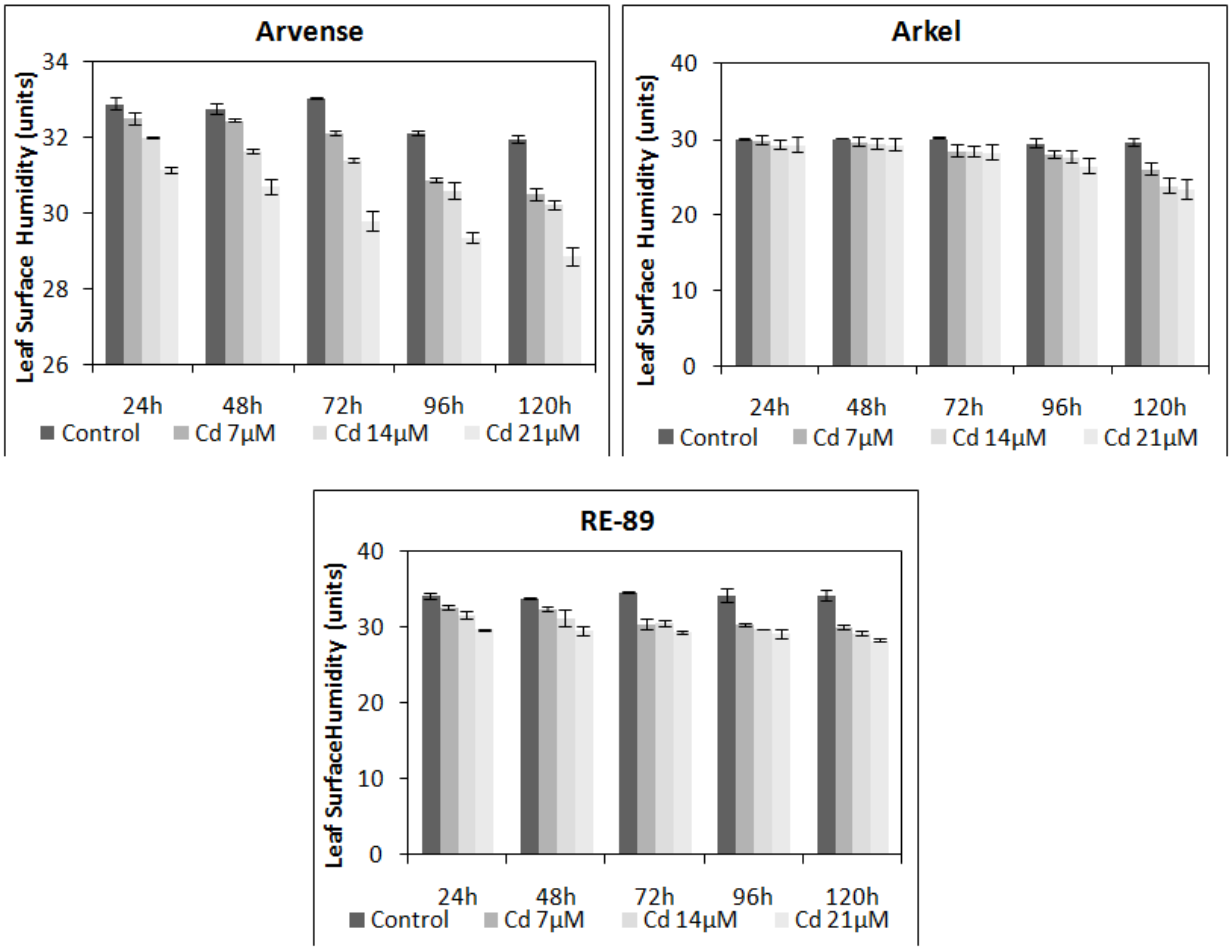

Figure 4: Effect of Cd treatments on Leaf Surface Humidity of three pea cultivars.

Leaf surface temperature increase was noticed within 24h to 120h of the trial in pea cultivars *Arvense* with cadmium treatment. A gradual rise in leaf temperature noticed was 0.16, 0.33, 0.50 °C (24 hr); 0.50, 0.84, 0.90 °C (48hr); 0.39, 0.65, 1.41 °C (72hr); 0.45, 0.91, 2.31 °C (96hr) and 1.84, 1.90, 3.01 °C (120hr) with Cd levels (7, 14, 21µM) in comparison to control, respectively. All the values were significant with respect to control. Also, a corresponding rise in leaf surface temperature due to hydroponics culture (24-120 h) was 0.33 (Control), 2.01 (7 µM), 1.90 (14 (µM) and 2.84 (21 µM). Cd severity was significant in raising leaf surface temperature from 2-3 °C. A similar trend was noticed in cultivar *Arkel* showing a linear and gradual increase of 0.03 to 0.24 °C (24hr); 0.18 to 0.88 °C (48hr); 0.26 to 0.99°C (72hr); 0.93

to 1.47 °C (96h); 1.18 to 1.66 °C (120hr) in comparison to control on raising Cd level 7 to 21µM, respectively. The plants in hydroponics cultures also experience increased temperature 0.11 (Control), 1.26 (7 µM), 1.29 (14 µM) and 1.30 (21 µM) during treatment (24 to 120h). Similarly, an appreciable increase in leaf surface temperature of cultivar *RE-89* was 0.23, 0.25 and 0.38 °C (24h), 0.70, 0.82, 1.08 °C (72h) and 0.78, 1.10, 1.24 °C (120h) in comparison to control plants with Cd (7 to 21µM), respectively (Figure 5). The corresponding increase due hydroponics culture (24-120h) was Nil (Control), 0.52 °C (7 µM), 0.82 °C (14 µM) and 0.83 °C (21 µM).

The observations clearly showed the rise of leaf surface temperature amongst the three pea cultivars in an increasing order was *RE-89* (0.86 °C)>*Arkel* (1.66 °C) >*Arvense* (3.1°C).

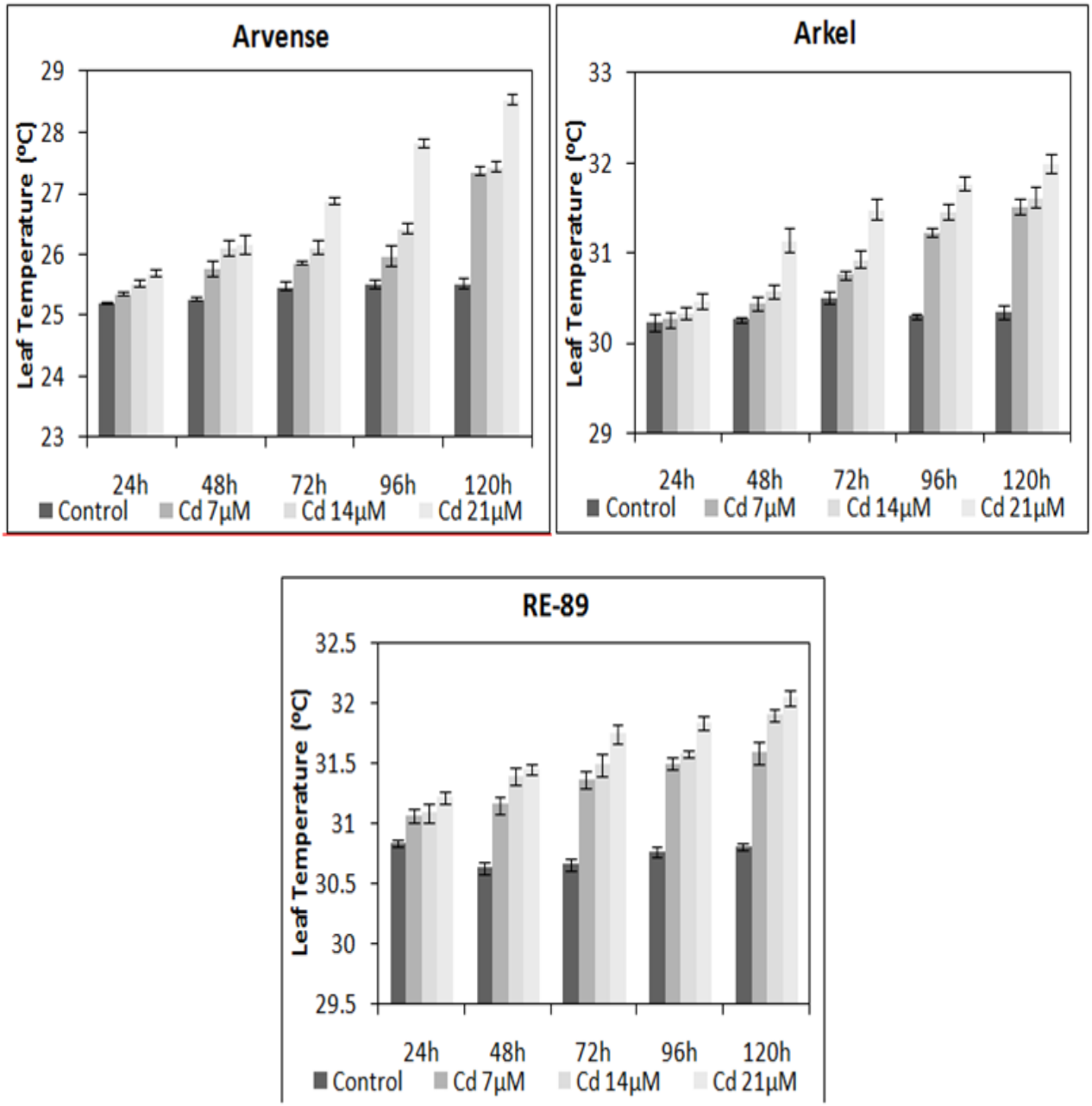

Figure 5: Effect of Cd treatments on Leaf Surface Temperature of three pea cultivars

Discussion

Intra-specific variation in response to heavy metals was reported in legumes (Sanita di toppi and Gabbrielli, 1999). Heavy metals impair stomata conductance, nutrient relations, photosynthesis and protein synthesis (Yang *et al.*, 2004). Altered water relations during abiotic stress increases ABA titer thus, interfering with stomata functioning (Poschenrieder *et al.*, 1989). Cd^{2+} replace Ca^{2+} in the guard cells inducing stomata closure and prevent them from responding to any stimuli (Perfus-Barbeoch *et al.*, 2002). Transpiration regulates leaf surface humidity and temperature and helps in the ambient temperature adjustment (Salisbury and Ross, 1992).

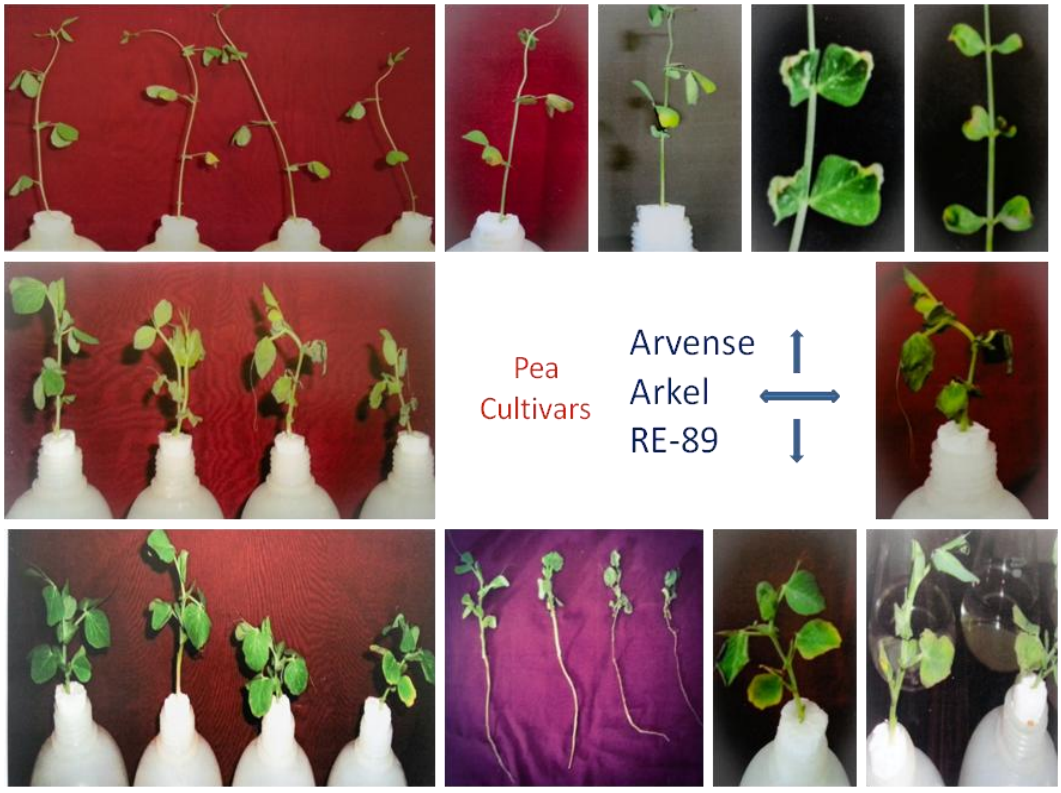

Plate I: Effect of cadmium treatments (Control 7, 14, 21 µM) on growth of pea seedlings in hydroponics culture showing chlorosis, necrotic spots, leaf curling and leaf burns

Inhibited transpiration and increased SDR leads dehydrated leaf surface and higher leaf temperatures (Thakur and Singh, 2012). The minimum dry biomass accumulations in *Arvense* showed its higher water absorption capacity. Highest radical elongation severity in *Arkel* was followed by *RE-89* and *Arvense* cultivars. Radical length has a direct bearing on the absorption capacity of seedlings. Increased SDR was highest in cultivar *Arkel* followed by *Arvense* and *RE-89*. This increase in SDR accompanied reduced rate of leaf transpiration. Leaf surface humidity reduces because of lower transpiration rate thus, affecting its leaf temperature which was highest in pea cultivar *Arvense* followed by *Arkel* and *RE-89*. The study can go a long way in remote thermal sensing and geo-spatial monitoring of the heavy metal polluted soil sites as bio-indicators.

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