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MATERIALS & METHODS

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Chapter-2

MATERIALS & METHODS

The impact of lead in the form of its Acetate ((CH₃COO)₂Pb) was studied on the following systems :

1. Seed germination.
2. Seedling growth of test plant.
3. Effect of lead polluted water irrigation on seed germination and seedling growth.

The seed germination studies were done on *Vigna radiata* cv., *Oryza sativa* cv. *Zeamays* cv. and *Phaseolus mungo*. Seedling growth studies were done on *Zea mays* cvs. and *Vigna* cvs. growth and yield studies were carried out on *Vigna radiata* cvs.

Impact of lead amended soil on growth was carried out on *Zea mays* cvs. for the investigation on the impact of pre-phasic treatment of lead on the seedling growth of *Zea mays* cvs. were selected.

Seeds of the above cultivars were procured from K. Chandra, B. Ram seed store Jaunpur,. The procured seeds were selected for uniformity based on the parameter size, shape and colour of the seed. Selected seeds were sterilized with 0.01% HgCl₂ and washed with distilled water.

STUDIES ON SEED GERMINATION :

To investigate the impact of lead on seed germination the solution of lead acetate of different concentrations were prepared and seeds were soaked in different concentrations of lead acetate for their full imbibition period. Seeds imbibed in distilled water for their full imbibition period constituted the control set, thereafter imbibed, seeds were washed with distilled water and transferred to moistened filter paper in petridishes for seed germination studies. The percentage of germination was recorded after 48 hours of incubation.

SEEDLING GROWTH OF TEST PLANT :

To investigate seedling growth studies two types of lead acetate treatments were given to the seeds of test plant.

1. Pretreatment :

Seeds of test plant were given pretreatment of different concentrations of lead acetate.

2. Post radicle emergence treatment :

Treatment of various concentrations of lead acetate were given after the emergence of radicle. For the study

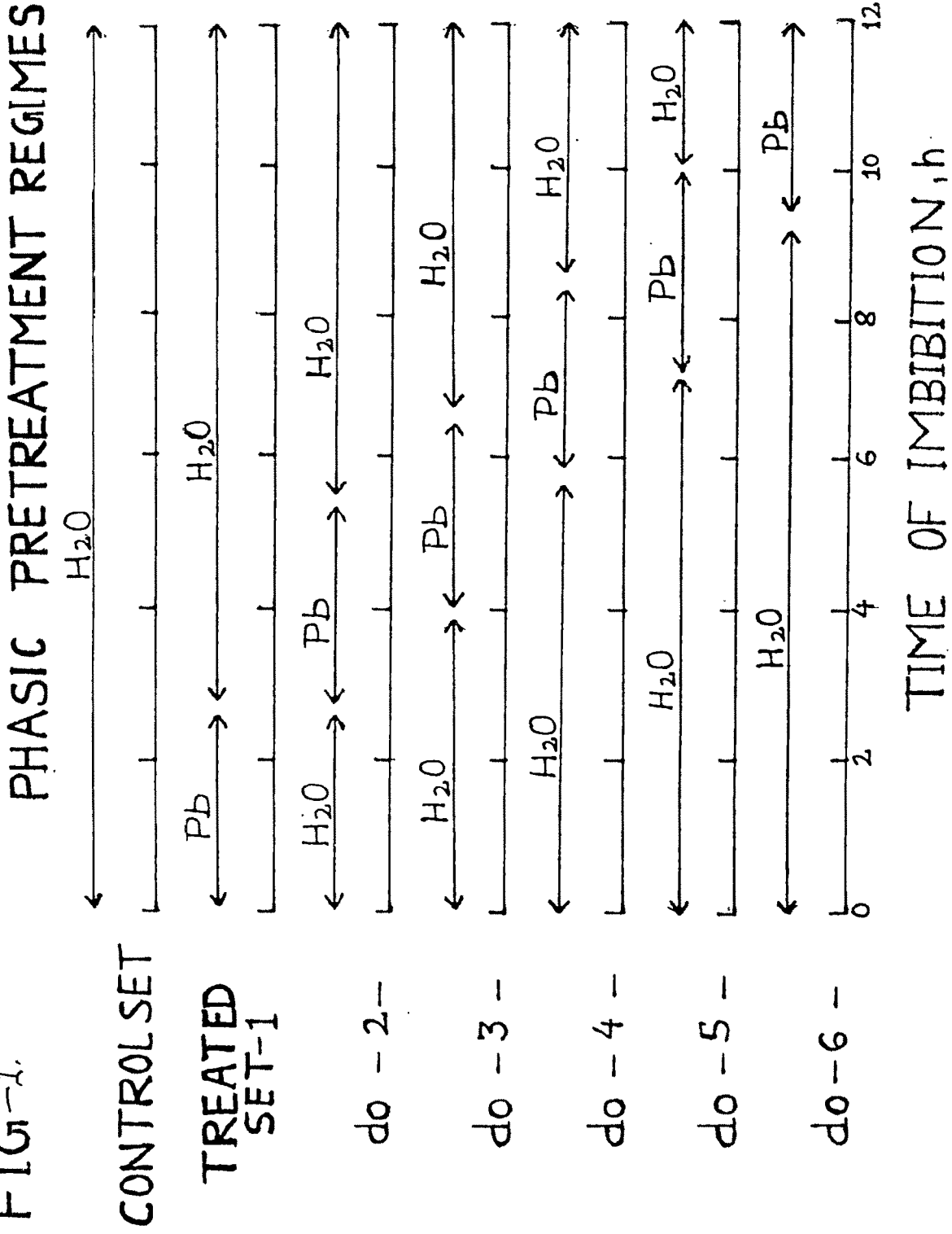
seeds were soaked in distilled water, allowed to germinate on moistened filter paper in petriplates. After 48 hours of incubation the germinated seeds were given treatment of various concentrations of lead acetate separately. To assess the impact of lead acetate on seedling growth of test plant, the seedling growth was studied in terms of length measurement on suitable day (i.e. 5th days after the emergence of radicle). The selected concentrations of lead acetate used for above investigation were 10 mg/l, 20mg/l, 0.50 mg/l. Seedling growth studies were done on 7th, 9th, 11th days after the emergence of radicle.

EFFECTS OF PHASIC PRETREATMENT OF Pb ON SEEDLING GROWTH :

It was of interest to see whether the whole imbibition period is important for inhibition of seedling growth or a particular phase. For this, entire imbibition period was divided into six equal phases of two hours each in Vigna radiata cvs.-PS-7 and PS-16 having the imbibition period of 12 hours. For the phasic pretreatment, chosen concentrations were 1×10^{-5} M Pb, a low less effective concentration and 1×10^{-3} M Pb, a high inhibitory concentration.

These six phasic pretreatment regimes (regimes - 1, 2, 3, 4, 5, 6) and control set of Vignaradiata are shown in fig...3 In the control set the seeds were imbibed in distilled water for their whole imbibition period of 12 hours while on the other hand in phasic pretreatment seeds were

FIG 1-1.



treated with 1×10^{-5} M Pb, 1×10^{-3} M Pb separately. In the treated set 1 (regime 1), seeds were imbibed in Pb solution during the first phase, i.e. 0-2 hours followed by imbibition in distilled water for remaining period i.e. 2-12 hours, in the set 2 (regime-2) seeds were imbibed in Pb solution during the second phase i.e. 2-4 hours, in the preceding and following periods while in distilled water i.e. from 0-2 hours and 4-12 hours, in the set-3 (regime-3), seeds were imbibed in lead solution for the third phase, i.e. between 4-6 hours, while for the rest of the phases i.e. 0-4 hours and 6-12 hours in distilled water, in set -4 (regime - 4) seeds were imbibed in Pb solution during the fourth phase i.e. 6-8 hours, while during the rest of the phases in water i.e. 0-6 hours and 8-12 hours in set-5 (regime-5), imbibition in Pb solution was done during the fifth phase i.e. 8-10 hours while during rest of the phases in water, i.e. 0-8 hours and 10-12 hours, similarly, in the set-6 (regime-6) lead treatment was given during 6th i.e. 10-12 hours, which was preceded by imbibition in distilled water i.e. from 0-10 hours (fig. 1) -Prephasic treatment was also given to two cultivars *Oryza sativa*.

SEEDLING GROWTH UNDER THE INFLUENCE OF VARIOUS CONCENTRATIONS OF LEAD AMENDED SOIL :

For these studies cultivar of *Vigna radiata* cv. were grown in lead amended and control (non-amended) soil. For this purpose, soil was taken from lilak Dhari College exper-

imental plot, which was sandy, clayey loam type, rich in organic nutrients and had acidic pH. This soil was amended with lead acetate so as to make the final concentrations of lead 10mg/kg soil and 50mg/kg soil, lead treated soil (experimental) as well as blank soil (control) were filled in polythene bags and placed in the open field.

Selected seeds of vigna radiata cv. were sown in the month of July in these polythene bags. After germination of seeds only one seedling was left in each bag and rest were removed. Control and experimental sets were irrigated with tubewell water at definite intervals when ever necessary. Seedling emergence (Ca. 5th day after sowing) was considered as the first day of emergence. For vegetative growth analysis, samples were taken at 40th day and 60th day of seedling emergence. The above two days selected for vegetative growth analysis were at nodulation time and flowering time. For yield analysis, plants were harvested at 90th day.

SEEDLING GROWTH UNDER THE INFLUENCE OF IRRIGATION BY POLLUTED WATER :

Effect of irrigation by polluted (treated) and tubewell water (control) on growth and yield were studied on seeds of Oryza sativa cv. seeds were sown in normal (control) soil irrigated by tubewll water) as well as polluted soil (i.e. soil irrigated by polluted water) and were irrigated by normal and polluted water respectively. Growth measurements

were done after 60 days of sprouting. The yield analysis were done after 120 days of sprouting. The data have statistically analysed.

Besides the above studies effect of irrigation by polluted water on branching (tillering) of Oryza sativa cv. was also studied. Number of tillers/plants was counted after 60th day of sprouting.

LEAD PHASIC PRETREATMENT Vs. SEEDLING GROWTH

The control as well as treated seeds were washed with water and then transferred to distilled water moistened filter papers kept in petriplates in dark for seedling growth. At a suitable day (e.g. 5 days after radicle emergence) seedling were dissected into different parts and their lengths measured.

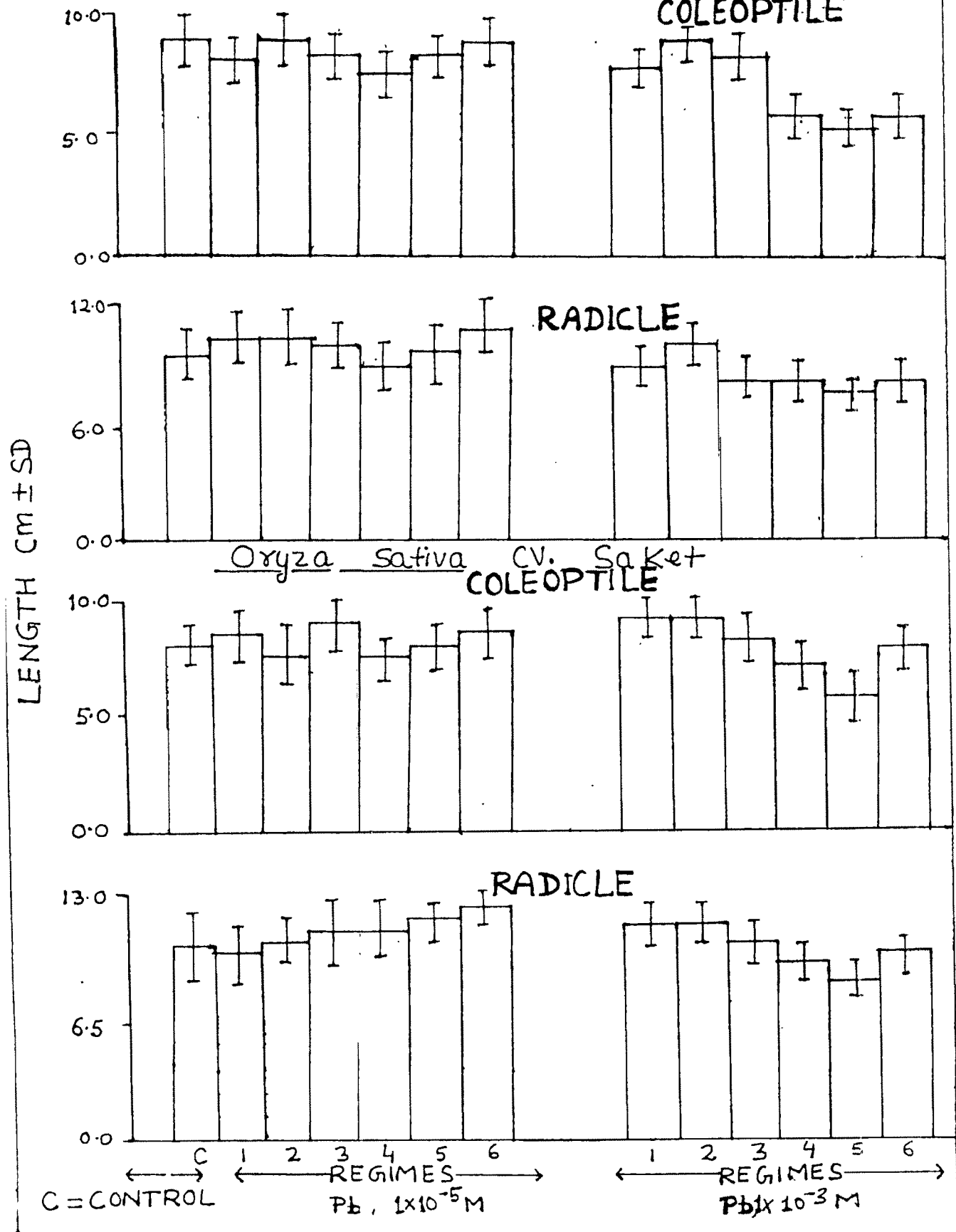
The studies were carried out on Oryza sativa cv. Saket and Sarju 52 and Vigna radata cv. PS-7 and PS-16.

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In general the lower 1×10^{-5} M Pb concentration has no significant effect on seedling growth while the higher 1×10^{-3} M Pb concentration is inhibitory to seedling growth. Interestingly the extent of inhibition varies in different phases, being maximum in mid phase (regime-4), minimum in the first phase (regime-1) and in the rest of the phases the effect is in between.

FIG-2 Pb PHASIC PRETREATMENT vs LENGTH

Oryza sativa CV. Sakju 52



Seeds of the Oryza sativa cv. Saket and Sarju 52 were given phasic pretreatment with two concentrations i.e. low 1×10^{-5} M Pb and High 1×10^{-3} M Pb, as shown in the fig. From the figure it is quite evident that the lower concentration has no significant effect on seedling growth in both the cultivars, while the higher concentration is inhibitory to the seedling growth, with varying extent of inhibition in different phases of treatment. For instance in cv. Sarju 52 at different pretreatment regimes i.e. 1, 2, 3, 4, 5, and 6 and Coleoptile length is ca. 102%, 103%, 88%, 66%, 73%, and 85% of control, while radicle length is ca. 89%, 89%, 78%, 60%, 50%, and 65% of control respectively. So, in all the regimes there is a decrease in coleoptile as well as radicle length which is maximum in mid phase treated seeds (regime-) while minimum in the intitial regime i.e. regime-1 extent of inhibition is inbetween the initial and mid phase. The same is the pattern with cv. Saket.

Seeds of Vigna radiata cvs. PS-7 and PS-16 were given phasic pretreatment with high 1×10^{-5} M Pb concentration only. Like in the case of Oryza this higher 1×10^{-3} M Pb concentration is inhibitory to seedling growth of Vigna radiata cv. also, as shown in fig. with varying extents of inhibition in different regimes 1,2,3,4,5 and 6 the epicotyl length is Ca. 100%, 104%, 90%, 60%, 68% and 93% of control, hypocotyl length is Ca. 98% 106%, 69%, 67%, 65% and 102% of control and radicle length is Ca. 103%, 112%, 67%, 60%, 77% and 96%

FIG-3 Pb PHASIC PRETREATMENT VS LENGTH

Vigna radiata CVS.

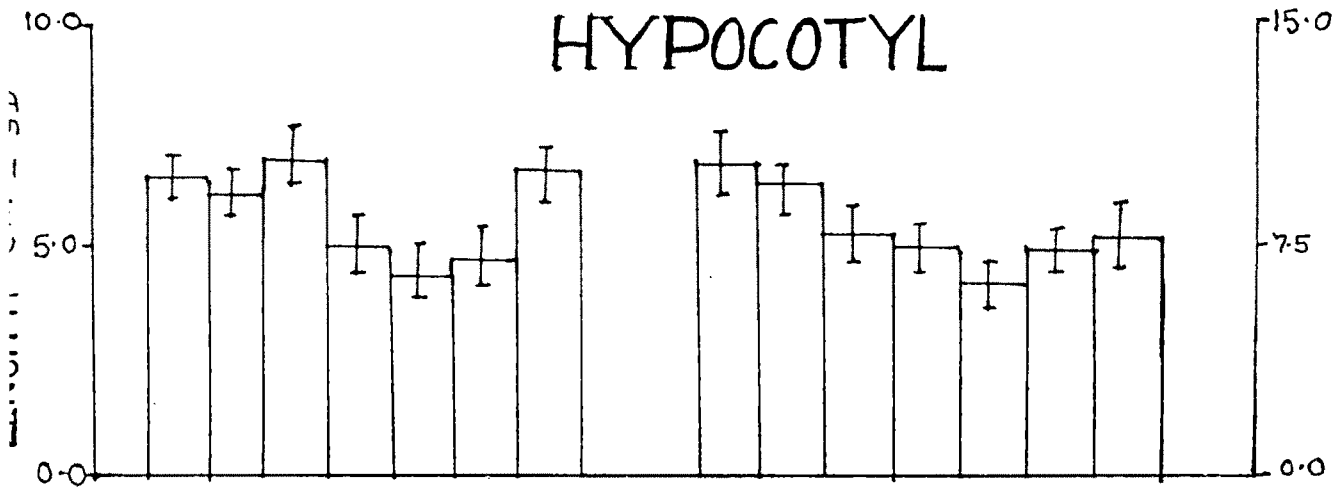
CV. PS.7

CV. PS. 16

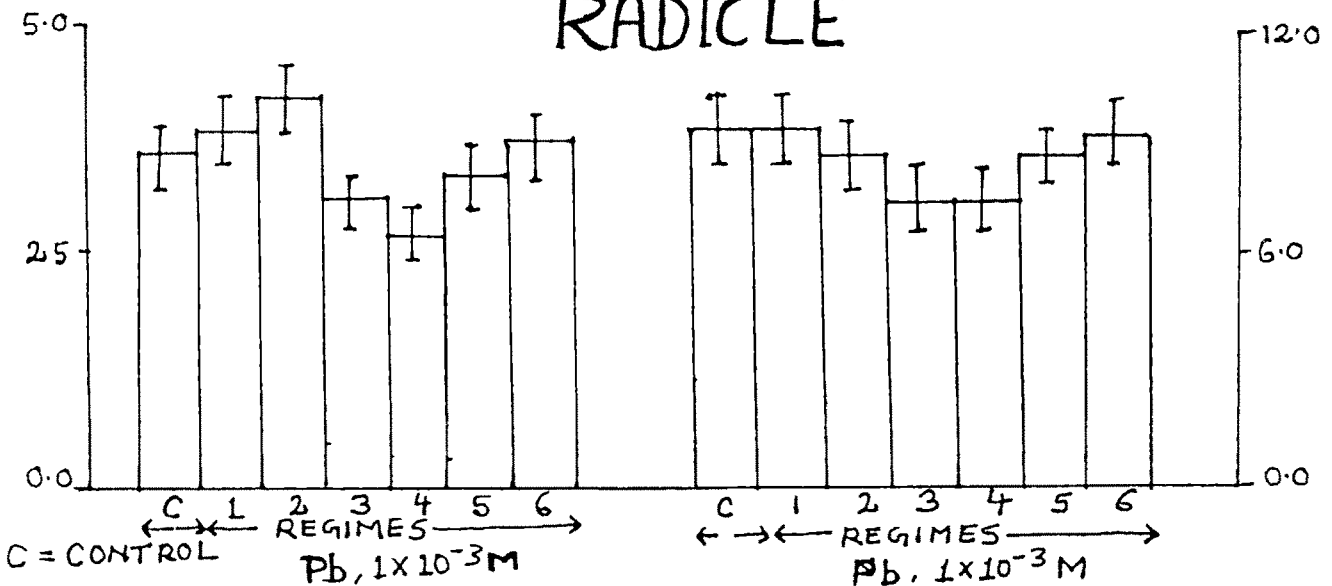
EPICOTYL



HYPOCOTYL



RADICLE



C = CONTROL
 ← → REGIMES
 Pb, $1 \times 10^{-3} M$

← → REGIMES
 Pb, $1 \times 10^{-3} M$

of control respectively. Thus as in *Oryza*, *Vigna radiata* cv. also shown maximum inhibition with mid phase treatment (regime -1) and minimum at first phase treatment i.e. regime-1.

The above results indicate that all the above species and cultivars exhibit maximum inhibition in mid phase (regime-4) treated sets and minimum in initial or first phase (regime-1) treated sets. The last phase treatment (regime-6) induces more inhibition than the first and less than the mid phase treatment.

SUMMARY OF THE OBSERVATIONS

1. In all the phases there is inhibition of seedling growth.
2. Maximum inhibition of seedling growth is in mid phase and minimum inhibition of seedling growth is in first phase while in the rest of the phases it is inbetween.