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Effects of lead on *Lathyrus sativus* seeds

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ABSTRACT

This study was conducted to evaluate the effect of Lead on the seed germination and seedling growth of Lathyrus sativus. Seeds were subjected separately to five levels (0, 0.001%, 0.01%,0.1% and 1% w/v) of the metal salt. Results showed that the germination increased significantly with different metal concentrations. However, no germination occurred at 1% concentration's of lead salt. Shoot length was more affected by metal lead than root length. Our results exhibited that less concentrations of lead had more effect on seed germination and growth parameters of Lathyrus sativus are studied. Finally the investigation of interaction impacts of these element lead on Lathyrus sativus seeds is recommended.

Key words: *Lathyrus sativus* seeds, Metal:lead, Lead Effect on shoots, Lead Effect on Roots, Atomic Absorption spectrophotometer.

INTRODUCTION

Lathyrus sativus is a legume (family Fabaceae) commonly grown for human consumption and livestock feed in Asia and East Africa. Like other grain legumes, *L. sativus* produces a high-protein. The lead(Pb) is one of the most widespread heavy metal of anthropogenic origin(Sharma and Dubey,2005) . Lead is one of the most widely distributed trace metals. Higher Concentration of Pb or longer treatment inhibit cell metabolism and Hydrogen peroxide (H2O2) Production, resulting in a decrease in the activity of some antioxidant enzymes(CAT, Verma and Dubey,2003, Malecka et al,2009) Plant antioxidant defense system vary with the plant species.

Fig.1.*Lathyrus sativus* (L.S) Plant

Fig.2.*Lathyrus sativus* seeds

MATERIALS AND METHODS

MATERIALS REQUIRED: Petridishes, Cotton, Lathyrus sativus seeds, Flasks, Funnel, Pipette, Distilled water, Millipore water, Metal-Lead.

PROCEDURE: Take few good seeds of *Lathyrus sativus* and five petridishes are taken for this experiment , total 15 seeds of *Lathyrus sativus* germination are seen .Three seeds are placed in each petridish on the moist cotton .4ml of lead solution were applied separately for each petridish daily for 10 days at 0.001%,0.01%,0.1% and 1%(w/v) concentrations. For each metal treatment tested ,A control was set up, in which the filter paper was moistened with deionized water (3ml). Petridishes were then wrapped (Carlson et al., 1991) in order to avoid evaporation during experiment. The seeds were germinated under room temperature and daily *Lathyrus sativus* seeds germination were checked. The first day there was no growth of Lathyrus seeds. Slowly seeds starts germinating. Seeds start germinating from Day2. Seed germination was observed more on 6th day and Each *Lathyrus sativus* seeds shoot length and root length was measured in centimeters.

TREATMENTS: *Lathyrus sativus* plants are valued for green manure, Seeds were purchased from Medak district of Andhra Pradesh, India. Prior to germination, all the seeds were surface –sterilized in 10% Sodium hypochlorite solution for 10 minutes to protect them from fungal diseases and then rinsed three times with distilled water. Seed germination and shoots, roots elongation were tested in to the petridishes (9 Cm in diameter and 1.5 Cm in height) with a layer of filter paper (140 mm Whatman No.1 filter) on the bottom and moistened the cotton with water and this is placed on the moist Whatman filter paper and *Lathyrus sativus* seeds are placed for germination and lead metal solution's are added to the *Lathyrus sativus* seeds with appropriate concentrations for 10 days.

SEEDS GERMINATION OF *LATHYRUS SATIVUS:*

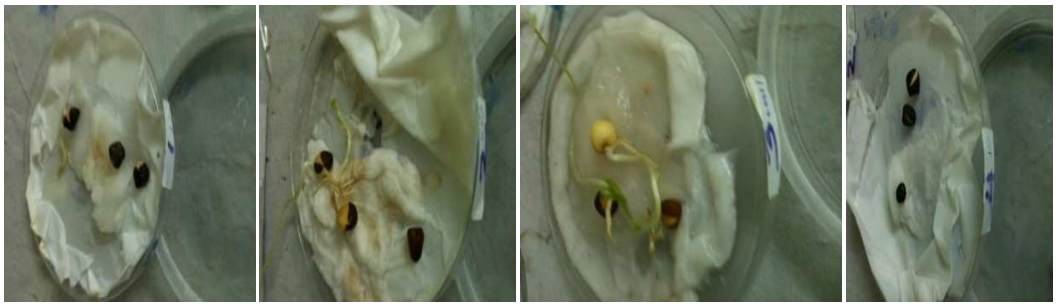
1st Day

2nd Day

3th Day

Day4

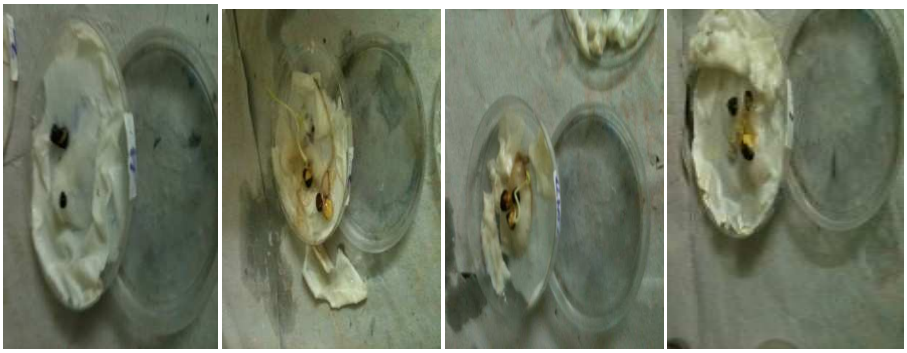
5th Day



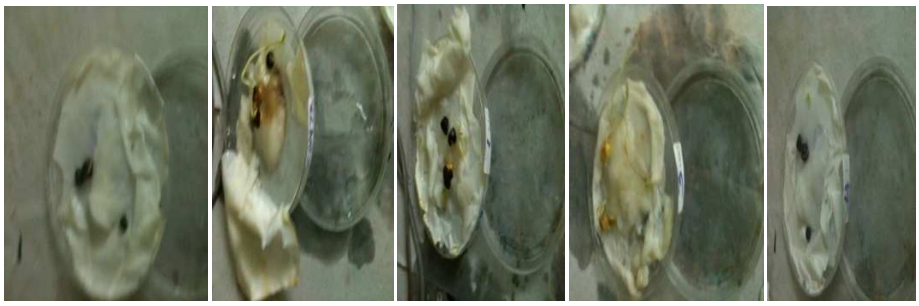
6th Day

7th Day

8th Day



9th Day



10th Day

PREPARATION OF LEAD STOCK SOLUTION'S: Weigh 1 gm of Lead and dilute with 100ml of distilled water to make 1% of solution .

STOCK SOLUTIONS PREPARATION'S: Take Control as Water, Four concentrations of Lead are taken:

STOCK SOLUTION'S:
1)Solution 1:0.1 %=Take10ml of stock solution dissolved in 90ml distilled water.
2)Solution 2: 0.01%=Take 1ml of stock solution dissolved in 99ml distilled water.
3)Solution 3:0.001%=Take 0.1ml stock solution dissolved in 99.9 ml distilled water.
4)Solution 4: Take 1 gm in 100ml (1%)

The Control weight of *Lathyrus sativus* shoot material was 6000mg, shoots material of 0.1%=22mg ,0.01%=40mg, 0.001%=17 mg, 1%=Nil, Root material of weight =3 mg are taken and Acid digestion of plant materials are done.

EFFECT OF ROOT LENGTH BY METAL LEAD: Roots rapidly respond to the presence of Lead by forming mechanical barrier .After exposure to Lead, Cell mechanisms that minimize the potential for toxicity are rapidly activated. The cell walls, the first barrier against Lead stress, can immobilize and accumulate some or even most Lead ions. The important role of the cell wall in the defense response of plants to trace metals was recently reviewed by Krzeslowska (2011).The capacity of cell walls to bind divalent metal cations mainly depends on the amount of polysaccharides with many carboxyl groups.(Inove etal 2013). Brunet etal.(2008) showed that the root of *Lathyrus sativus* L. exposed to lead contained much less calcium than control plants, and explained the reduction in calcium content by the replacement of calcium ions by Lead ions, which have a high affinity for pectin in cell walls. The cell wall is one of the preferred and essential compartments for Lead accumulation, deposition and sequestration. Therefore ,these results shed a new light on the functioning of the cell walls in plant cell defense strategy against lead. Heavy metals including lead are likely to enter plant cells via essential cations transporters. At CNGC(Cyclic Nucleotide gated channel) homologous to a non –selective cation channel ,was suggested to enable lead entry since over expression of the truncated gene resulted in tolerance to Lead (Pb^{2+})(Sunkar et al.,2000).Lead enters the root cells via Ca^{2+}/Mg^{2+} gated channel(Kim etal.,2002)

PROCEDURE FOR ACID DIGESTION: Take five conical flask of 100ml and label them as Control, 0.1%, 0.01%, 0.001% stock solutions of Lead and 0.01% Sample of Root of *Lathyrus sativus*, To the solutions add 1 ml of Perchloric acid and 5 ml of Nitric acid (HNO_3),Keep on sand bath. After that add HCL 1:1 and Nitric acid (HNO_3) 2.5ml: 2.5 ml .After that add 5ml Conc. HCl. Keep it on sandbath. Immediately remove if fumes are coming out, Keep till dry if fumes are not coming and at last add 3ml of (M.Q)Millipore water. After Acid digestion of the samples, Evaluate the amount of Lead present in the samples of Shoots, Roots of *Lathyrus sativus* seeds by the help of Atomic Absorption spectrophotometer. Record the results.

Measurements of Roots, Shoots of *Lathyrus sativus* seeds in Centimeters

DAYS	METAL TREATMENT—LEAD USED.	ROOT LENGTH OF L.S SEED(R1)	SHOO T LENGTH OF L.S SEED (S1)	SHOOT LENGTH OF L.S SEED (S2)	SHOOT LENGTH OF L.SSEED (S3)
1st DAY	CONTROL	---	-----	----	----
	0.001%	---	0.2 cm	-----	-----
	0.01%	---	------	------	------
	0.1%	----	-----	------	-----
	1%	----	-----	------	-----
2nd DAY	CONTROL	----	-----	-----	-----
	0.001%	---	0. 4cm	------	0.4 cm
	0.01%	----	------	0.4 cm	-------
	0.1%	---	0.4 cm	-----	-----
	1%	---	------	-----	-----
3rd DAY	CONTROL	----	-----	------	------
	0.001%	---	1.4 cm	0.9 cm	0.9 cm
	0.01%	---	1 cm	0.9 cm	------
	0.1%	---	0.4 cm	1cm	------
	1%	---	------	------	------
4th DAY	CONTROL	---	1 cm	------	-----
	0.001%	---	3cm	1.3 cm	3cm
	0.01%	---	1cm	0.9 cm	----
	0.1%	---	2.9 cm	4 cm	----
	1%	---	------	------	------
5th DAY	CONTROL	---	1.9 cm	------	----
	0.001%	---	4.5 cm		5.1 cm
	0.01%	---	4.4 cm	4.2 cm	
	0.1%	---	2.5 cm		
	1%	---	------	-----	-----
6th DAY	CONTROL	---	1.9cm	-----	-----
	0.001%	---	6cm	1.3 cm	6cm
	0.01%	---	6 cm	4.5cm	------
	0.1%	---	2.6cm		
	1%	---	-----	-----	------
DAYS	**METAL TREATMENT--- LEAD USED.**	**ROOT LENGTH OF L.S SEED(R1)**	**SHOOT LENGTH OF L.S SEED (S1)**	**SHOOT LENGTH OF L.S SEED (S2)**	**SHOOT LENGTH OF L.S SEED (S3)**
7th DAY	CONTROL	---	1.9 cm		
	0.001%	--	6 cm	1.3 cm	6 cm
	0.01%	---	6 cm	4.5 cm	--
	0.1%	---	2.6 cm	---	----
	1%	---	-----	-----	----
8th DAY	CONTROL	---	2.5 cm	1.2 cm	----
	0.001%	---	10.5 cm	3.5 cm	6.5 cm
	0.01%	---	----	5.3 cm	4.3 cm
	0.1%	---	2.8 cm	-----	------
	1%	---	----	----	----
9th DAY	CONTROL	---		1.2 cm	
	0.001%	---	13.9 cm	3.5 cm	6.5 cm
	0.01%	---	6 cm	6 cm	2.5 cm
	0.1%	---	3 cm	1 cm	
	1%	---	-----	----	-----
10th DAY	CONTROL	1.9 cm	2.5 cm	1.2 cm	-------
	0.001%	2.2 cm	14.2 cm	3.5 cm	6.5 cm
	0.01%	4 cm	6 cm	6 cm	2.5 cm
	0.1%	1 cm	2.8 cm		
	1%	----	----	-----	-----

OBSERVATIONS :
CONCENTRATION'S OF LEAD PRESENT IN *LATHYRUS SATIVUS* SEEDS EVALUATED BY ATOMIC ABSORPTION SPECTROPHOTOMETER

LEAD STANDARDS SOLUTIONS(%)`	PART TAKEN FOR STUDY	WEIGHT OF THE PLANT MATERIAL.	CORR. ABSORBANCE.	CONC.OFLEAD PRESENT IN *LATHYRUS SATIVUS* SEEDS
CONTROL	SHOOT	6000mg	0.0297	2.369 mg/l
0.1%	SHOOT	22 mg	0.2289	19.97mg/l
0.01%	SHOOT	40 mg	0.0274	2.184 mg/l
0.001%	SHOOT	17 mg	0.0257	2.046 mg/l
0.01%	ROOT	3 mg	0.0123	0.971 mg/l

RESULTS

The growth of germinated seeds at each concentration of single metal has shown in figure. In contrast to control, only at highly used concentrations, the metals were observed to have negative effect on seeds. At 0.001% concentrations of lead was significantly affected when compared to control, but shoots of sample 0.001% markedly increased .The lead concentration's is highest in 0.1% sample i.e 19.97 mg/l ,and control value is 2.369 mg/l, The 0.01% concentration of lead is 2.184 mg/l, The 0.001% ,the concentration of lead is 2.046 mg/l. The 0.01% root sample the concentration of lead is 0.971 mg/l.

DISCUSSION

In this investigation study is carried out with Effects of Lead on seed germination of *Lathyrus sativus*.

WORKING WITH ATOMIC ABSORPTION SPECTROPHOTOMETER USING LEAD SAMPLES: First calibrate the system using lead standards, Aspirate the sample through nebulizer, Result will be saved in the system using Winlab software.

Uptake of metal Lead by seeds is measured by Atomic Absorption spectrophotometer. The lead is the only heavy metal which has an impact on mineral homeostasis, founded that the roots of *Lathyrus sativus* exposed to lead

showed an increase in lead content along with an increase in sodium levels, which is absorbed to compensate the loss in potassium. Finally lead induces genotoxicity in plants(Rucinska etal ,2004).The highest germination was observed in the 0.001%.so less concentration of lead leads to the high growth of shoot (Seed S1) i.e 14.2 cm observed in 0.001% on 10^{th} day and no germination was observed with the treatment at 1%.

CONCLUSION

The present study was concluded that heavy metal on seeds is very detrimental to seed germination. The reason for high percentage in germination of *Lathyrus sativus* seeds might be due to physiological mechanism .Further work is recommended to increase the enhancement of heavy metal lead in low concentrations in the *Lathyrus sativus* plant for growth.

Overall it was revealed from our results that less amount of lead had more growth effect on seed germination and growth parameters of *Lathyrus sativus*. High concentration of Lead is inhibiting the growth of the *Lathyrus sativus* seeds.

Thus less concentrations of lead 0.1ml of stock solution dissolved in 99.9ml distilled water i.e 0.001% shows 14.2cm of shoot length of seed (S1) on 10 th day..No growth seen in 1%.The more amount of lead present in the 0.1% sample i.e 19.97 mg/l.

0.001% Concentration of sample of lead is 2.046mg/l
0.01% The concentration of sample of lead is 2.184 mg/l
0.01% Root sample, The concentration of sample of lead is 0.971 mg/l
0.1% The concentration of lead is 19.97 ,as the control is 2.369 mg/l

Thus 0.1% had more amount of lead i.e 19.97 mg/l .Thus it is concluded that greater the amount of lead present in the stock solution ,greater will be the amount of lead present in the Lathyrus sativus seeds .Here 0.1% has more concentration of lead.

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