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Retardation of Toxic Effect of Cadmium and Lead in *Vigna radiata* (L) R.Wilczek by using *Allium sativum* (L), *Eichhornia crassipes* (Mart) Solms and *Piper betle* (L)'

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Abstract: ***The seeds of Vigna radiata were treated with various concentrations of heavy metal solutions of cadmium chloride and lead acetate. Germination studies were conducted and observations were recorded. These entities were then subjected to remediation using Allium sativum, Eichhornia crassipes and Piper betle. A comparitive analysis was done between the heavy metals and phytoremediated entities. It was evident that the phytoremediation processes were effective in reducing the metal toxicity. With the reduction of toxic effect of heavy metals root and shoot growth of Vigna radiata was found to be enhanced.***

Keywords: Phytoremediation, phytotoxicity, Heavy metals

1. Introduction

One of the serious issues now facing by today world was environmental pollution. Non biodegradable pollutants cannot be easily degraded either by natural process or by artificial treatment method. They include heavy metals, plastics pesticides etc. When accumulated in excess ,these pollutants including toxic metals can function as stresses that causing physiological constraints and alterations in various vital growth processes. Hsu and Chang (1992) studied the inhibitory effects of heavy metals on seed germination and seedling growth of *Miscanthus* species and the results also supported that seed germination percentages and the rate of germination of each species decreased with increasing concentration. So many strategies are there for reducing pollution. Among them phytoremediation is a novel strategy which requires more concern due to its environmental friendliness.

Phytoremediation is the use of living green plants for insitu risk reduction or removal of contaminants from contaminated water, soil, sediments, air etc.There are so many phytoremediation procedures as Phytoextraction, Phytostabilization, Phytotransformation,Phytostimulation, Phytovolatilization, rhizofiltration,Hydraulic control etc. The main advantage of phytoremediation is its reduced cost and environmental friendliness.

The aim of the present study is to analyse the effect of toxic metals cadmium and lead on the germination of seeds of *Vigna radiata* belonging to family Fabaceae and also to determine the effects, after treating the seeds using heavy metal solution which was undergone phytoremediation using *Piper betle* belonging to family Piperaceae, *Allium sativum* belonging to family Amaryllidaceae and *Eichhornia crassipes* belonging to the family Pontederiaceae seperately. Germination can be taken as a mirror to reflect the toxicity study. A comparitive analysis was made to determine the extent to which phytoremediation was made by *Piper betle*, *Allium sativum* and *Eichhornia crassipes* to remove the toxic metals from the solution by morphological analysis of the sprouted seeds.

2. Materials and Methods

The seeds selected for the present study was *Vigna radiata*. The heavy metals selected were cadmium and lead in the form of their respective salts. The plants selected for phytoremediation were *Allium sativum*, *Eichhornia crassipes* and *Pipe r betle*. Cadmium chloride and lead acetate solutions are prepared in distilled water in ppm units. Five different concentrations of solutions are prepared , Sterilized petridishes were used for the treatment. Surface sterilized seeds (10 numbers) were taken in sterilized petriplates lined with WhatmanNo: 1 filter paper and moistened with respective heavy metal solutions . The control seeds were raised in distilled water. Triplicates were maintained for each varying concentration of the two heavy metal solutions. This is maintained for 4 days under room temperature. Estimation of number of seeds germinated, radicle length and hypocotyle length were also done during these days. On the fourth day fresh weight was estimated using an electronic balance. Then the seedlings were allowed to dry in an oven for 24 hrs at 50 degree Celsius and dry weight was estimated. Maximum adverse effect of heavy metal treatment was observed in the 20 ppm and 100 ppm concentrations of both heavy metals. These concentrations were considered for further study.

In similar manner as prescribed above , the effect of heavy metal solution was studied after treating the solutions with young water hyacinth , garlic and betel vine. Young water hyacinth plants (5 number of about 27cm) were selected for the study and betel leaf (5 number) was teared so as to facilitate effective absorption. Water hyacinth and betle leaf was immersed for 48 hrs and after that these metal solutions were used for the germination study. In case of garlic treatment , garlic solution was prepared by grinding 1gm of garlic in distilled water and making it upto 100 ml. The mung bean seeds were then treated for remediation studies.

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For each experimental set up 10 seeds were used in each petriplate and triplicates were maintained . The set up was maintained for 4 days under room temperature. The germination parameters selected for the present study were:-

1) Germination Percentage:-

Germination percentage is calculated by the formula

$$\text{Germination percentage} = \frac{\text{number of seeds germinated}}{\text{Total number of seed}} \times 100$$

2) VigourIndex :-

The method described by Sharma and Saran (1992) was adopted for determining vigour index. It was calculated by using the formula

Vigour Index = germination percentage x axis length

3) Phytotoxicity:-

The percentage of phytotoxicity was calculated by following method adopted by Chou and Muller (1972)

$$\text{Phytotoxicity} = \frac{\text{radicle length of control-radicle length of testx100}}{\text{radicle length of control}}$$

4) Growth Index:-

Growth index was calculated by the following formula:

$$\text{Growth index} = \frac{\text{growth in cm/day in presence of heavy metal}}{\text{Growth in cm/day in control}}$$

3. Results and Discussion

Impact of pollutants on ecosystem and human health is an urgent and international issue, since there is an ever increasing number of examples of environmental disturbance , likely to affect the biota and humans by both natural and anthropogenic stress. The principal objective of this study is to know the effect of the heavy metals cadmium and lead on the germination of *Vigna radiata*(L.)R. Wilczek and the phytoremediation of these heavy metals using *Eichhornia crassipes , Allium sativum and Piper betle*.

Cadmium and lead are heavy metals that are present in low levels in the soil. At minimum concentration they are not much harmful. But the emerging industries contribute a lot of effluents which contain high concentration of these heavy metals.*Vigna radiata* is a common vegetable crop used by us.There are several supporting studies that reveal the toxic effect of these heavy metals. Studies by Maitra and Mukharji (1979) revealed that heavy metals affect physiological and biochemical functions of plants. Hsu and Chang (1992) studied inhibitory effects of heavy metals on seed germination and seedling growth in *Miscanthus* sps and it revealed that seed germination and seedling growth decreased with increasing concentrations. Heavy metals mainly affect the root growth and root hair initiation. Compared to the control there was reduced root hairs in the heavy metal treated test seedlings. In the present study the decrease in the root/shoot ratios clearly shows the impact of heavy metals. When heavy metal ions are present at elevated levels in water and soil, they are readily taken up by plants and accumulated in different parts , there by inducing impaired metabolism and reduced growth , (Ernst , 1980). M.Kabir*et al.,* conducted the studies on effect of cadmium and lead on seed germination, root, shoot and seedling growth, seedling dry weight and seedling vigour index of *Thespesia populnea* and found that seedling vigour index decreased with increasing concentrations of lead and cadmium. He also noticed that the inhibitory effect of cadmium treatment were more prominent than lead at its higher concentrations, which was found to be a parallel evidence with the selected work. Like this numerous citations were available in the concerned topic.

Phytoremediation is a novel strategy brought up with a view to eliminate the adverse effects of various environmental pollutants. Phytoremediation process is normally termed as **'green clean'** , as green plants are used for the purpose of remediation. Many plants such as sunflower , Indian mustard, poplar, and various aquatic and semi aquatic plants are used in a sense of phytoremediation and was found to be effective.*Eichhorniacrassipes* is normally considered as a noxious weed spreading very quickly in the aquatic ecosystems rich in nutrient contents. Some studies have revaeled that it's roots has the ability to absorb some amount of toxic metals like cadmium, copper, mercury, etc. Studies conducted by Dr. Kelley (1999) *et al.,* found that water hyacinth have the ability to absorb toxic metals. Studies conducted by W.S.Jiang *et al.,*(2009) on effect of cadmium in the root meristematic cells of Allium sativum revealed that the cell walls, plasma membrane and main organelles actively participated in cadmium detoxification and tolerance at low cadmium concentrations. The remediation study revealed that *Eichhorniacrassipes* and *Allium sativum* were found to be more effective in remediation of the toxic effect of heavy metals.

In the present study in the first part of the experiments the seeds were treated with heavy metal solutions. Heavy metal solution treated seeds showed maximum germination in the low concentration 0.1ppm than in the higher concentration of 100ppm. The radicle and hypocotyle length also showed a reduction with increasing concentration of heavy metals. The maximum radicle length was also found in 0.1ppm and minimum in 100ppm. Maximum hypocotyle length was found in 0.1ppm and minimum in 100ppm. Lower concentrations of heavy metals showed higher vigour index and lower vigour index was observed for higher heavy metal concentrations. The growth index was higher for 0.1ppm and lower for 100ppm of heavy metal. Phytotoxicity was observed higher for 100ppm and in lower 0.1ppm. 20ppm and 100ppm entities were used for the second part of the experiment.

On treatment with *Eichhornia crassipes* the values for germination percentage, radicle length, hypocotyle length, vigour index and growth index increased and phytotoxicity reduced. Maximum effect was found in 20ppm. On treatment with betle leaf also the effect was almost similar to that in *Eichhornia crassipes* treatment. On treatment with various concentrations of garlic solutions parameters like germination percentage, radicle length, hypocotyle length, vigour index and growth index increased , but phytotoxicity reduced. Maximum effect was found in the combination of 20ppm+25% garlic solution treatment in case of both the heavy metal solutions. Correlation studies were made for germination percentage and phytotoxicity values. Correlation coefficient for germination percentage for cadmium and lead were -0.8369 and -0.9691 respectively.

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Correlation coefficient for phytotoxicity for cadmium and lead were 0.5145 and 0.7224 respectively.

From the various results of the present study it assures that cadmium and lead were highly toxic to *Vigna radiata* at higher concentrations which is reflected in the first part of the experiment. In the second part of the experiment it was shown that betle leaf, water hyacinth and garlic were effective in the remediation process. With the reduction of toxic effect of heavy metals the root and shoot growth of *Vigna radiata* was enhanced along with reduction in dry weight.

Table 1: Effect of 'Cadmium' on the germination studies in Vigna radiata (L.) R. Wilczek

Sl. No	Concentration (ppm)	Germination percentage (%)	Radicle length(cm)	Hypocotyle length(cm)	Vigour index(cm)	Growth index(cm)	Phytotoxicity	Fresh weight(g)	Dry weight(g)
1	Control	100	3.3	6.71	1001	_____	_____	0.36	0.03
2	0.1ppm	90	1.53	5.46	629.1	0.69	53.64	0.26	0.03
3	1 ppm	83.33	0.42	1.63	170.83	0.21	87.27	0.18	0.03
4	10 ppm	50	0.23	0.03	13	0.03	93.03	0.12	0.04
5	20 ppm	26.67	0.096	0.01	2.83	0.011	97.09	0.12	0.04
6	100 ppm	3.33	0.02	0.006	0.09	0.002	99.4	0.10	0.05

Table 2: Effect of 'Lead' on the germination studies in Vigna radiata (L.) R. Wilczek

Sl. No	Concentration (ppm)	Germination percentage(%)	Radicle length(cm)	Hypocotyle length(cm)	Vigour index(cm)	Growth index(cm)	Phytotoxicity	Fresh weight(g)	Dry weight (g)
1	Control	100	3.3	6.71	1001	_____	_____	0.3	0.03
2	0.1 ppm	90	2.76	6.63	845.1	0.94	16.36	0.3	0.03
3	1 ppm	83.33	2.69	6.43	759.97	0.91	18.48	0.3	0.03
4	10 ppm	80	1.10	4.86	476.8	0.59	66.67	0.27	0.03
5	20 ppm	76.67	0.39	1.59	151.81	0.19	88.2	0.25	0.03
6	100 ppm	56.67	0.10	0.07	9.86	0.02	96.8	0.13	0.05

Table 3: Effect of 'Cadmium' on germination of Vigna radiata (L.)R.Wilczek after treatment with Eichhornia crassipes (Mart) Solms

Sl. No	Concentration (ppm)	Germination percentage (%)	Radicle length(cm)	Hypocotyle length(cm)	Vigour index(cm)	Growth index(cm)	Phytotoxicity	Fresh weight(g)	Dry weight(g)
1	Control	100	4.62	7.68	1230	_____	_____	0.32	0.08
2	20 ppm	40	0.83	0.01	33.6	0.07	82.03	0.12	0.05
3	100 ppm	10	0.03	0.006	0.36	0.003	99.39	0.11	0.05

Table 4: Effect of 'Lead ' on germination of Vigna radiata (L.) R.Wilczek after treatment with Eichhornia crassipes (Mart) Solms

Sl.No	Concentration (ppm)	Germination percentage(%)	Radicle length(cm)	Hypocotyle length(cm)	Vigour index(cm)	Growth index(cm)	Phytotoxicity	Fresh weight(g)	Dry weight(g)
1	Control	100	4.62	7.68	1230	_____	_____	0.32	0.08
2	20 ppm	86.67	0.70	3.33	349.28	0.33	84.85	0.21	0.08
3	100 ppm	63.33	0.28	0.39	42.43	0.05	93.93	0.12	0.05

Table 5: Effect of 'Cadmium' on germination of Vigna radiata (L.)R.Wilczek after treatment with Piper betle (L)

Sl. No	Concentration (ppm)	Germination percentage(%)	Radicle length(cm)	Hypocotyle length(cm)	Vigour index(cm)	Growth index(cm)	Phytoto xicity	Fresh weight(g)	Dry weight(g)
1	Control	100	3.02	7.40	1042	_____	_____	0.29	0.03
2	20 ppm	40	0.12	0.03	6	0.014	96.02	0.11	0.04
3	100 ppm	6.67	0.03	0.02	0.33	0.005	99.01	0.09	0.05

Table 6: Effect of 'Lead' on germination of Vigna radiata(L.)R.Wilczek after treatment with Piper betle (L):

Sl.No	Concentration (ppm)	Germination percentage(%)	Radicle length(cm)	Hypocotyle length(cm)	Vigour index(cm)	Growth index(cm)	Phytotoxicity	Fresh weight(g)	Dry weight(g)
1	Control	100	3.02	7.40	1042	_____	_____	0.29	0.03
2	20 ppm	83.33	0.42	1.62	169.99	0.196	86.09	0.15	0.03
3	100 ppm	57	0.12	0.08	11.4	0.019	96.02	0.11	0.04

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Table 7: Effect of 'Cd' on germination of Vigna radiata(L.)R.Wilczek under various concentrations of garlic solution (Allium sativum)

Sl.No	Concentration (ppm)	Germination percentage(%)	Radicle length(cm)	Hypocotyle length(cm)	Vigour index(cm)	Growth index(cm)	Phytotoxicity	Fresh weight(g)	Dry weight(g)
1	Control	100	3.77	6.29	1006	_____	_____	0.29	0.05
2	20 ppm								
a)	100 %	50	0.19	0.02	10.6	0.02	94.96	0.13	0.07
b)	75 %	70	0.39	0.05	30.8	0.04	89.65	0.12	0.03
c)	50 %	80	0.4	0.05	36	0.045	89.38	0.12	0.04
d)	25 %	80	0.41	0.06	37.6	0.047	89.12	0.12	0.04
3	100 ppm								
a)	100 %	30	0.04	0.02	1.8	0.006	98.93	0.12	0.07
b)	75 %	30	0.06	0.02	2.4	0.008	98.40	0.11	0.04
c)	50 %	30	0.14	0.02	4.8	0.016	96.29	0.11	0.04
d)	25 %	40	0.27	0.04	12.4	0.031	92.84	0.11	0.04

Table 8: Effect of 'Lead' on germination of Vigna radiata(L.)R.Wilczek under various concentrations of garlic solution (Allium sativum)

S. No	Concentration (ppm)	Germination percentage(%)	Radicle length(cm)	Hypocotyle length(cm)	Vigour index(cm)	Growth index(cm)	Phytotoxicity	Fresh weight(g)	Dry weight(g)
1	Control	100	3.77	6.29	1006	_____	_____	0.29	0.05
2	20 ppm								
a)	100%	90	0.43	0.72	103.68	0.115	88.5	0.18	0.08
b)	75%	90	0.57	1.08	149.4	0.165	84.88	0.22	0.04
c)	50%	100	0.65	1.09	173	0.172	82.76	0.17	0.04
d)	25%	100	0.83	2.4	323	0.32	77.98	0.19	0.04
3	100 ppm								
a)	100%	70	0.2	0.07	16.24	0.023	94.69	0.11	0.07
b)	75%	70	0.3	0.09	27.3	0.039	92.04	0.12	0.04
c)	50%	80	0.45	0.67	89.6	0.11	88.06	0.14	0.04
d)	25%	80	0.47	1.03	120	0.15	87.53	0.13	0.05

Table 9: Correlation coefficient for germination percentage and phytotoxicity in Vigna radiata after treatment with cadmium and lead

Sl No	Concentration (ppm)	Effect of Cd on GP	Effect of Pb on GP	'r' for GP on Cd treatment	'r' for GP on Pb treatment	Phytotoxic effect of Cd	Phytotoxic effect of Pb	'r' for phytotoxic effect of Cd treatment	'r' for phytotoxic effect of Pb treatment
1	0.1ppm	90	90	-0.8369	-0.9691	53.64	16.36	0.5145	0.7224
2	1ppm	83.33	83.33			87.27	18.48		
3	10ppm	50	80			93.03	66.67		
4	20ppm	26.67	76.67			97.09	88.2		
5	100ppm	3.33	56.67			99.4	96.8		

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