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# Influence of Heavy Metal Toxicity on the Metabolism of *Vigna umbellata* T. Seeds

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## *ABSTRACT*

*The influence of three heavy metals Cd, Pb and Zn have been studied on two cultivars of Vigna umbellata T. viz. Haday and Thangray. Heavy metal stress severely affected the vigor of the seeds as observed by the reduction in TTC stainibility, dehydrogenase activity and the content of macromolecules. Of the three chemicals used the effect of Cd was found to be most deleterious and the cv. Thangray more susceptible to such effects.*

## INTRODUCTION

The legume crops have traditionally been used for rotation with cereal crops in order to increase the soil fertility. Rice bean *(Vigna umbellata* T.) is a less known legume and has recently been identified as a new addition to the presently existing list of pulses (Maggo *et al.*, 1999). This plant is used all over the temperate Himalayas as an important crop offering high quality of protein and increasing the soil fertility by combining $N_2$ into the soil.

Heavy metal plays a vital role in the growth and development of plants and may act as cofactors of some enzymes useful in the formation of intermediate metabolites (Bhattacharjee and Mukherjee, 1994.) Rise in the levels of heavy metals in the soil could be attributed to many factors like agricultural practices, soil properties, waste disposal, industrial sewage to agricultural land etc (Foy *et al.*, 1978). Heavy metal toxicity causes multiple direct and indirect effects on plant growth and alters many physiological functions (Woolhouse, 1983). In view of the present global scenario a complete eradication of heavy metal pollution has become an unrealistic goal. Understanding the responses of plants to their external environment is an attractive target for improving stress tolerance (Madhusudhan *et al.*, 2002). Heavy metals cause many deleterious effects to plants such as inhibition of seed germination (Mrozek, 1980), reduction in plant growth (Coughtrey and Martin, 1978) and metabolic disturbances by altering essential biochemical reactions (Krupa *et al.*, 1983).

Zinc in an essential plant nutrients and is involved in a multitude of functions. Zn catalyses different chemical reactions that influences metabolism (Marschner, 1995). Cadmium is a non essential toxic element that enters the environments through various industrial processes (Somashekaraiah *et al.*, 1992). Cadmium is a wide spread trace pollutant of high toxicity with a long biological half life (Hilmy *et al.*, 1985). Lead, with its increasing concentrations causes the reduction in the levels of RNA, DNA and protein with concomitant increase in amino acid content in rice embryo (Maitra and Mukherjee, 1979).

Studies on the physiological and biochemical responses on *Vigna umbellata* under the influence of heavy metal toxicity is lacking. An understanding of the

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physiological mechanisms and identification of specific characteristics conferring heavy metal tolerance could play a major role in development of new varieties suitable for such stress conditions.

## MATERIALS AND METHODS

Fresh seeds of *Vigna umbellata* T. cv *Haday* and cv. *Thangray* were obtained from Sikkim. The seeds were surface sterilized with 0.1% $HgCl_2$ for 2 minutes and washed for 10 minutes in distilled water (3 times). The washed seeds were allowed to germinate in Petri plates on filter papers soaked separately in the aqueous solution on $PbCl_2$ (Pb 10,100,500 and 1000 µM), $CdCl_2$ (Cd 10,100,500 and 1000 µM) and $ZnCl_2$ (Zn 10,100,500 and 1000 µM ). For each treatment 50 seeds were taken. A control set was prepared by soaking the filter paper in distilled water. Each seed lots were irrigated with the pretreating solution at an interval of 36 hrs till the experiment was over. During the experiment the environmental conditions were 88±2% relative humidity (RH), 18±2°C temperature and a photoperiod of 10 h at an altitude of 2168 m amsl. The physiological, biochemical and growth analysis were made after 3, 7 and 10 days.

To determine the TTC (2, 3, 5- Triphenyl tetrazolium chloride) stainibility, the dehusked seeds of each treatment (40 seeds each) were allowed to imbibe in 1% (w/v) TTC solution in petridishes and kept overnight in the dark. The percentage TTC stained seeds (deep-red) were calculated from the total number of seeds of each treatment (Chhetri *et al.*, 1993). The activity of total dehydrogenase of intact seeds was analysed by the reactions with TTC according to the method of Rudrapal and Basu (1979). The extraction for protease was done by homogenizing 200mg of seed tissue in 5ml of chilled 0.1 M Na-phosphate buffer (pH 6.5) and centrifugation of the homogenate for 10 minutes at 10,000 rpm. The supernatant was used as the enzyme source. The assay mixture contained 1 ml of the enzyme extract, 0.1 ml of $MgSO_4$, $7H_2O$ (0.1 M) and 1 ml of BSA (0.5 mg/ml) and the assay was done following the method of Snell and Snell (1971). The activity of these enzymes was calculated according to Fick and Qualset ( 1976).

For the analysis of insoluble carbohydrates 100mg of seed kernels from each treatment at the end of each analysis cycle were homogenized with 5 ml of boiling 80% ethanol and centrifuged at 6,000 rpm for 10 min. The pellet was taken and hydrolyzed with 5 ml of 25% $H_2SO_4$ (v/v) at 80°C in a water bath for 30 minutes (Raj *et al.*, 1992). The extracted materials were taken as sources of insoluble carbohydrates and the estimation was done by following the method of Mc Cready *et al.*, (1950). In the above study, the pooled supernatant (volume adjusted to 10 ml with distilled water) obtained after the centrifugation was taken as the source of soluble carbohydrates and the estimation was done as per Mc Cready *et al.*, (1950).

For the extraction of amino acid from the seeds 100 mg. of dehusked seeds were taken and homogenized with 5 ml of 80% ethanol and centrifuged. The volume of the supernatant was made up to 10 ml with 80% ethanol which served as the source of free amino acids (Sadasivam and Manickam, 1996). The estimation was done with ninhydrin reagent as per Moore and Stein (1948).

## RESULTS AND DISCUSSION

TTC stainibility may be considered as an index of seed viability. The percentage of TTC stained seed decreases as the seed experienced prolonged treatment with heavy metals. However, the decreasing trend was not that significant in the treatment with Zn, particularly at the initial stages. Of the three heavy metals used Cd showed the most deleterious effect and the damage was proportionally increased with the increase in the concentration from 10 to 1000 µM. Of the two cultivars cv. *Thangray* was more susceptible to the damage caused by the heavy metals (Fig.1)

The level of dehydrogenase steeply declined with the advancing time period of heavy metal treatment and with advancing concentration of the same (Fig.2). Dehydrogenase activity is generally used as a reliable index for the evaluation of seed viability (Abdul-Baki and Anderson, 1972). Therefore, it is pertinent to conclude here that with increasing duration and increasing concentration of heavy metal treatment the vigor of *Vigna umbellata* seed is correspondingly reduced. The activity of protease steadily decreased with the concentration and duration of heavy metal treatment. Though it may be expected that the activity of protease will be higher for degrading the protein content, however, at an increasing magnitude of heavy metal stress most possibly the disruption of the enzyme nature of protease takes place causing the decline in its activity. The rate of protease decline was found to be slower in cv, *Haday* than the cv. *Thangray*. (Fig.3)

The carbohydrate content showed a general trend of gradual increase in the level of soluble carbohydrates (Table 2) and decrease in the level of insoluble carbohydrates (Table 1) with the advancement of the duration of heavy metal treatment. The same trend is seen with the increase in the concentration of the heavy metals. Amino acid content showed a progressive increase with advancing period of heavy metal treatment and with increasing concentration of the same. The magnitude of increase in the amino acid level is negligible at the initial stage but rapid with advancing days of treatment (Table 3). In case of cv. *Haday* the heavy metal Zn causes more damage while in the cv. *Thangray* all the chemicals had equally deleterious effects.

**Table 1. Effect of heavy metal treatment with $CdCl_2$ (Cd 10, 100, 500 and 1000 µM), $PbCl_2$ (Pb 10, 100, 500 and 1000 µM) and $ZnCl_2$ (Zn 10, 100, 500 and 1000 µM) on the insoluble carbohydrate content (mg./g) of *Vigna umbellata* cv. *Haday* and cv. *Thangray* seeds under laboratory conditions.**

| Treatments | *Vigna umbellata* cv. *Haday* | | | *Vigna umhellata* cv. *Thangray* | | |
|---|---|---|---|---|---|---|
| | Days after treatment | | | Days after treatment | | |
| | 3 | 7 | 10 | 3 | 7 | 10 |
| Control | 47.2±0.80 | 28.35±0.25 | 18.6±0.3 | 47.5±0.15 | 29.9±0.2 | 17.7±0.05 |
| Cd 10 | 45.0±0.10 | 26.5±0.5 | 18.0± 0.60 | 46.65± 0.05 | 27.9± 0.35 | 17.4± 1.65 |
| Cd 100 | 36.3±0.10 | 23.4±0.22 | 14.3±0.55 | 38.6±0.10 | 23.9±0.40 | 12.7±0.40 |
| Cd 500 | 35.8±0.90 | 20.6±0.30 | 9.6±0.10 | 31.3±0.35 | 19.1±1.2 | 8.9±0.20 |
| Cd 1000 | 27.7±0.10 | 16.9±0.05 | 6.2±0.40 | 19.6±0.15 | 13.2±0.20 | 4.05±0.25 |
| Pb 10 | 47.6±0.34 | 27.8±0.08 | 19.9±1.50 | 46.3±0.15 | 26.9±0.20 | 17.7±0.70 |
| Pb 100 | 38.05±0.05 | 25.75±0.45 | 18.35±0.05 | 42.55±0.05 | 21.7±0.10 | 14.3±0.10 |
| Pb 500 | 35.6±0.30 | 22.1±0.10 | 14.6±0.90 | 38.45±0.05 | 20.1±0.45 | 12.3±0.20 |
| Pb 1000 | 32.9±1.30 | 18.5±0.50 | 10.5±0.90 | 24.65±0.05 | 17.4±0.10 | 8.65±0.15 |
| Zn 10 | 46.4±0.55 | 26.2±0.20 | 18.55±0.75 | 46.8±0.10 | 29.3±0.20 | 16.0±0.70 |
| Zn 100 | 40.6±0.40 | 23.2±0.20 | 17.95±1.35 | 41.7±0.25 | 27.45±0.85 | 14.25±0.25 |
| Zn 500 | 37.2±0.22 | 20.9±0.10 | 15.65±0.55 | 32.7±0.10 | 26.1±0.10 | 12.7±0.70 |
| Zn 1000 | 28.1±0.11 | 19.1±0.20 | 12.25±0.35 | 28.65±0.05 | 18.2±0.20 | 10.9±0.20 |

Values are mean ±SE of 3 replicates

**Table 2. Effect of heavy metal treatment with $CdCl_2$ (Cd 10, 100, 500 and 1000 µM), $PbCl_2$ (Pb 10, 100, 500 and 1000 µM) and $ZnCl_2$ (Zn 10, 100, 500 and 1000 µM) on the content of soluble carbohydrate (mg/g) of *Vigna umbellata* cv. *Haday* and cv. *Thangray* seeds under laboratory conditions.**

| Treatments | *Vigna umbellata* cv. *Haday* | | *Vigna umbellata* cv. *Thangray* | |
|---|---|---|---|---|
| | Days after treatment | | Days after treatment | |
| | 7 | 10 | 7 | 10 |
| Control | 14.3±0.20 | 20.85±0.45 | 13.25±0.45 | 21.95±0.15 |
| Cd 10 | 15.625±0.37 | 22.95±0.35 | 14.6±0.40 | 24.55±0.35 |
| Cd 100 | 16.6±0.70 | 24.8±0.11 | 15.00±0.20 | 27.75±0.75 |
| Cd 500 | 17.75±1.45 | 24.6±0.70 | 15.45±0.25 | 29.0±0.90 |
| Cd 1000 | 20.15±0.95 | 29.8±0.30 | 17.65±0.35 | 30.2±0.80 |
| Pb 10 | 15.25±0.45 | 24.95±0.55 | 17.15±0.15 | 24.4±0.30 |
| Pb 100 | 16.2±0.70 | 25.55±0.45 | 19.7±0.20 | 26.35±0.85 |
| Pb 500 | 14.95±1.05 | 25.35±0.95 | 18.15±0.55 | 27.6±0.70 |
| Pb 1000 | 17.00±0.80 | 27.7±0.2 | 20.2±0.10 | 29.4±0.50 |
| Zn 10 | 14.85±0.65 | 25.85±0.15 | 15.3±0.70 | 23.5±0.20 |
| Zn 100 | 18.1±0.70 | 26.4±0.60 | 16.2±0.40 | 25.5±0.35 |
| Zn 500 | 20.65±0.15 | 27.0±0.60 | 18.05±0.15 | 25.35±1.05 |
| Zn 1000 | 22.15±0.65 | 28.65±1.85 | 21.3±1.00 | 26.45±0.55 |

Values are mean ±SE of 3 replicates

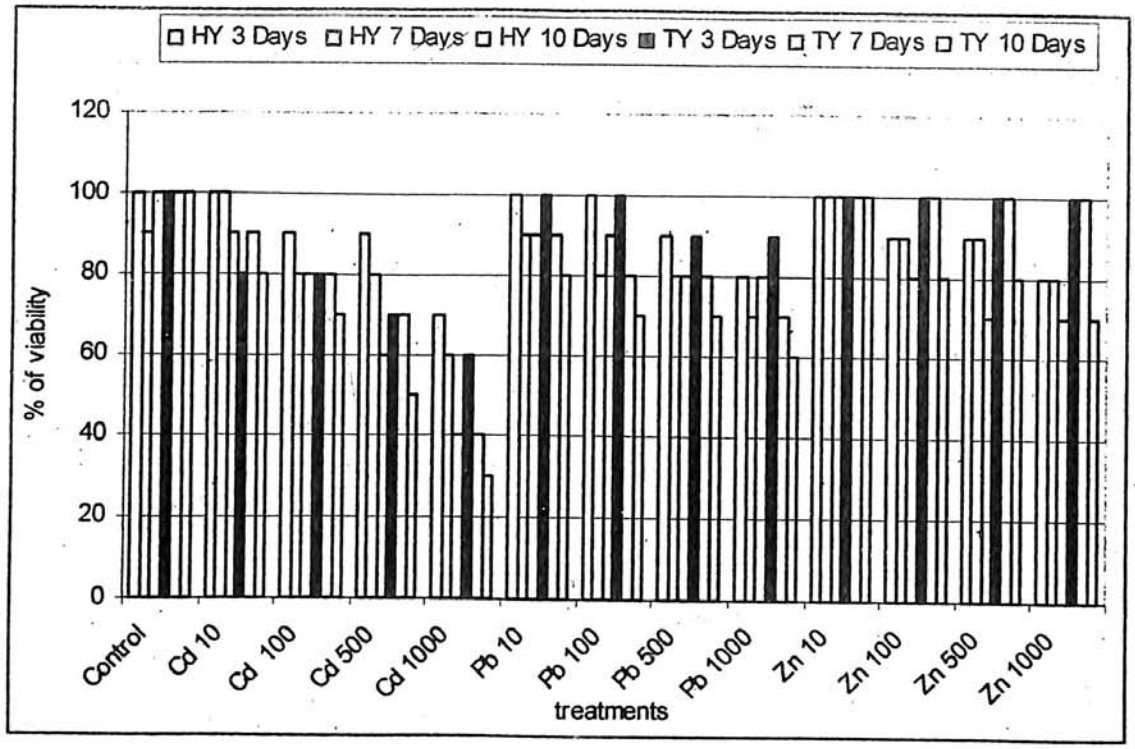


**Fig. 1. Effect of heavy metal treatment with $CdCl_2$ (Cd 10, 100, 500 and 1000 µM), $PbCl_2$ (Pb 10, 100, 500 and 1000 µM) and $ZnCl_2$ (Zn 10, 100, 500 and 1000 µM) on the percentage of TTC stained seeds (deep red) of *Vigna umbellata* cv. *Haday* HY and cv. *Thangray* TY seeds under laboratory conditions.**

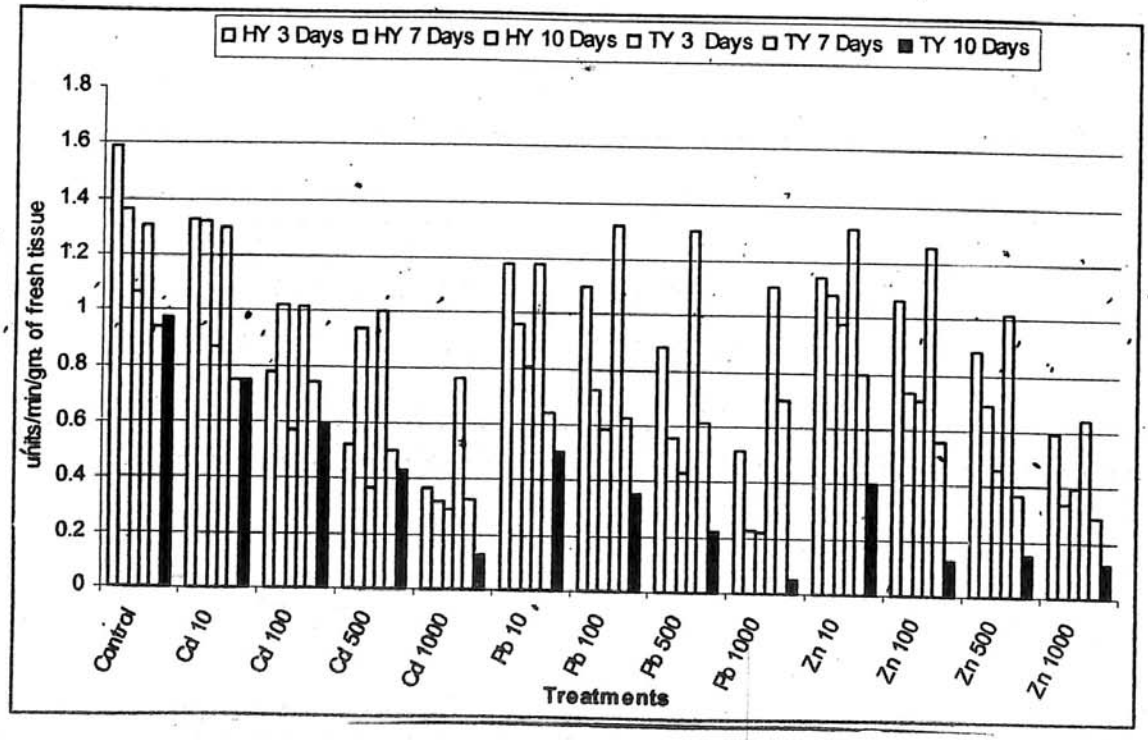


**Fig. 2. Effect of heavy metal treatment with $CdCl_2$ (Cd 10, 100, 500 and 1000 µM), $PbCl_2$ (Pb 10, 100, 500 and 1000 µM) and $ZnCl_2$ (Zn 10, 100, 500 and 1000 µM) on the dehydrogenase activity (units/min./gm. of fresh tissue) of *Vigna umbellata* cv. *Haday* HY and cv. *Thangray* TY seeds under laboratory conditions.**

**Table 3. Effect of heavy metal treatment with $CdCl_2$ (Cd 10, 100, 500 and 1000 µM), $PbCl_2$ (Pb 10, 100, 500 and 1000 µM) and $ZnCl_2$ (Zn 10, 100, 500 and 1000 µM) on the content of free amino acids (mg/g) of *Vigna umbellata* cv. *Haday* and cv. *Thangray* seeds under laboratory conditions.**

| Treatments | *Vigna umbellata* cv. *Haday* | | *Vigna umbellata* cv. *Thangray* | |
|---|---|---|---|---|
| | Days after treatment | | Days after treatment | |
| | 7 | 10 | 7 | 10 |
| Control | 5.9±0.65 | 8.5±1.45 | 7.3±0.50 | 9.7±1.10 |
| Cd 10 | 8.4±1.00 | 8.5±0.95 | 9.3±1.00 | 14.6±0.50 |
| Cd 100 | 9.35±0.75 | 11.6±0.80 | 10.5±1.00 | 17.2±0.80 |
| Cd 500 | 9.9±0.10 | 10.9±0.45 | 10.8±0.70 | 18.0±1.25 |
| Cd 1000 | 11.32±0.42 | 15.6±0.70 | 11.5±0.50 | 21.5±0.95 |
| Pb 10 | 7.8±2.00 | 10.2±1.70 | 8.2±0.30 | 10.4±0.25 |
| Pb 100 | 11.4±0.40 | 9.95±0.65 | 9.2±0.30 | 14.2±0.75 |
| Pb 500 | 10.2±2.60 | 10.45±0.35 | 10.7±0.30 | 17.0±0.90 |
| Pb 1000 | 11.8±0.10 | 12.7±1.90 | 11.5±1.00 | 18.3±1.35 |
| Zn 10 | 7.8±0.40 | 15.4±0.35 | 8.4±0.80 | 10.2±0.85 |
| Zn 100 | 11.85±0.95 | 16.2±0.80 | 10.45±0.55 | 14.1±0.25 |
| Zn 500 | 12.7±0.90 | 14.7±1.20 | 12.8±1.30 | 14.9±1.20 |
| Zn 1000 | 13.0±0.60 | 15.1±1.40 | 12.8±0.70 | 16.1±0.30 |

Values are mean ± SE of 3 replicates

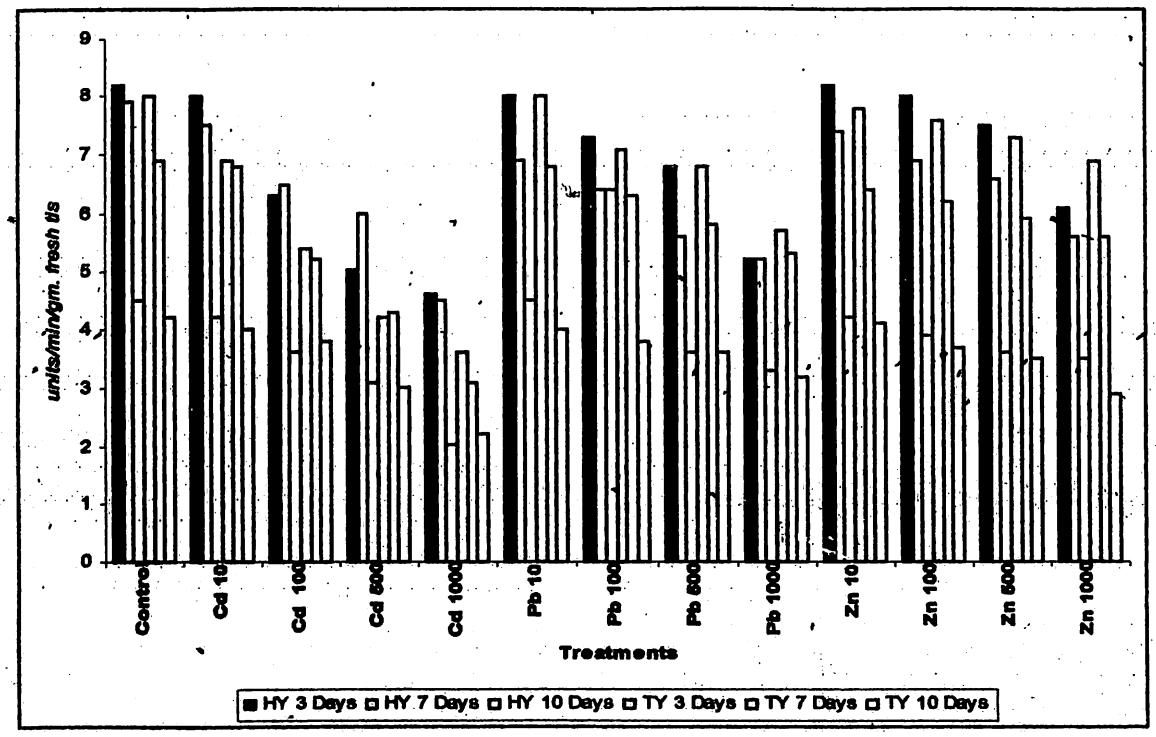


**Fig. 3. Effect of heavy metal treatment with $CdCl_2$ (Cd 10, 100, 500 and 1000 µM), $PbCl_2$ (Pb 10, 100, 500 and 1000 µM) and $ZnCl_2$ (Zn 10, 100, 500 and 1000 µM) on the on the protcase activity (units/min./gm. of fresh tissue) of *Vigna umbellata* cv. *Haday* HY and cv. *Thangray* TY seeds under laboratory conditions.**

The results enumerated in the present study revealed that the heavy metal toxicity caused by the treatment with Cd, Zn and Pb for some length of time cause severe impairment of metabolic activities of the *Vigna umbellata* cv. *Haday* and cv. *Thangray*. The disrupted cellular activities may be due to retardation of biosynthetic activities caused mainly by jeopardizing the action of specific enzymes. The breakdown of complex organic molecules like Proteins and Carbohydrates may be the cause of the increase in the levels of amino acids and sugars. The results demonstrated that the two cultivars of the rice bean *Haday* and *Thangray* slightly differed in their sensitivity to heavy metal stress. The cultivar *Thangray* was more susceptible to damage caused by heavy metals as compared to the cultivar *Haday* and Cd at a concentration of 100 μM and above showed the most damaging effect. The loss of seed vigor as exemplified by the dehydrogenase activity and TTC stainibility may be attributed to the disruption of biochemical activity at the enzymatic level and probably at the genetic level.

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