

## Effect of heavy metals on the Photosynthetic pigments of *Leptadenia reticulata* (Wight & Arn)

<sup>1</sup>Hafiz Ul Islam, <sup>2</sup>Irfan Rashid Thokar, <sup>3</sup>Shafat Ahmad Mir & <sup>4</sup>S.D. Singh

Department of Botany Govt MVM Bhopal (MP) (India)

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### ABSTRACT

In plants, heavy metals have been seen to deploy their toxic action mostly by damaging chloroplast and interrupting photosynthesis. The hindrance of photosynthesis is the outcome of interference of metal ions with photosynthetic enzymes and chloroplast membranes. The present investigation was undertaken to evaluate the effect of heavy metals (Cr, Pb and Cd) on the content of chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids of the *Leptadenia reticulata*. The plants were exposed to different concentrations (10mg/kg, 20mg/kg, 30mg/kg, 40mg/kg and 50mg/kg) of heavy metals (Cr, Pb and Cd). The study revealed that as the heavy metal concentration gets increased the content of these photosynthetic pigments viz. chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids got reduced as compared to control. However the maximum reduction was seen in case of cadmium.

### 1. Introduction

*Leptadenia reticulata* is a very important medicinal plant commonly known as jiwanti belongs to family Asclepiadaceae. The true origin of *Leptadenia reticulata* is not identified until now, the description in the oldest scripture of Hinduism (Atharveda) indicates that it originated in India and is mainly found in Rajasthan, Gujarat, Punjab, The Himalayan ranges, Sikkim, Kerala, Karnataka up to an altitude of 2000m. Apart from India it is also revealed to be distributed in tropical and subtropical parts of Africa, srilanka, Burma, Nepal, Cambodia, Philippines and Madagascar. It is specifically known for its stimulant and restorative properties in Ayurveda and it also an important ingredient of many well reputed ayurvedic formulations like Chywanprash, speman etc. In ayurveda, it is recognized for its rejuvenating, revitalizing and lactogenic properties. Its principal constituents are leptadenol, leptidin,  $\beta$ -sitosterol,  $\alpha$ -amyrin,  $\beta$ -amyrin acetate. *Leptadenia reticulata* grows from April to September and flowering is seen from May to July. During November to February fruits are formed and seed germination can be seen from June to September.

Recently, increase in heavy metal content of the environment has turn out to be one of the stern ecological problems, which threatens ecosystems and human health (Lasat, 2002 and Kachout et al., 2009). Metals with a specific gravity  $> 4\text{g/cm}^3$  are heavy metals (Anonymous, 1964; Nieboer and Richardson, 1980; Nagajyoti et al., 2010), or  $5\text{g/cm}^3$  or even higher (Lapedes, 1974; Nieboer and Richardson, 1980). Heavy metals are mainly obtained from naturally occurring geochemical materials. The heavy metal pollution may well occur naturally (surface mineralization's, forest fires, volcanic outgassings, spontaneous combustions, etc.) or all the way through human activities such as agriculture and mining (Shaw et al., 2004). However human interference has been seen to have much higher contributions towards environmental pollution than the natural occurrence (Shaw et al., 2004).

One of the main biochemical make over occurring in plants that are subjected to heavy metal stress such as cadmium toxicity is the production of reactive oxygen species (Cho and Park, 2000). Cadmium is taken up by minerals, gets into the plants, and accumulates at different levels. However after that it influences the food chain and causes human carcinogen (Hoolev et al., 2016). Cadmium in leaf results in leaf chlorosis, decline in photosynthetic pigment content, chlorophyll a, and phycobiliproteins (Simek et al., 2016, Simonetti et al., 2016) and then ultimately leads to the reduction of plant biomass.

Lead is well-known to be one of the most harmful materials in the soil and in the air, as a result of which a trace of lead can cause problems for the environment and human life (Krzyszowska et al., 2016 and Li et al., 2016). Lead is considered as an immobilized property in the soil so that plants can easily access it; though, it should be noticed how lead enters the plant body, as the roots do not have any sites for Pb uptake at all, and lead would be taken up through the root surface by carboxylic structures of mucilage uranic acids (Peralta-Videa et al., 2009). One of the main affect of lead toxicity is the production of reactive oxygen species in plant cells, which could substitute the essential ions in the cell and damage other processes such as cell adhesion and cell signalling (Lyer et al., 2015).

Chromium (Cr) being used as test metal as chromium compounds have a range of industrial uses and broadly employed in leather processing, manufacture of refractory steel, and specialty chemicals. These activities have led to the extensive contamination of Cr and increased bioavailability and bio mobility of Cr. Chromium is found in the trivalent ( $\text{Cr}^{3+}$ ) and hexavalent ( $\text{Cr}^{6+}$ ) forms, where the former has lower toxicity than the latter. Chromium induces the oxidative stress which leads to the deprivation of photosynthetic pigments resulting in declining of growth. Phytotoxicity due to Cr leads to reduction in seed germination or of early seedling development,

decrease in root growth, leaf chlorosis and reduced biomass (Sharma *et al.*, 1995).

**2. Materials and Methods:**

**Plant material:** The certified seeds of *Leptadenia reticulata* plant were purchased from the Bhoj, Gujarat India in the Month of April. These Certified seeds were sown in the month of June under controlled conditions in pots in the mixture of sand, soil and vermicompost material. The experiment was carried up to 100 days. After one month of establishment and normal growth of the plants raised from the seeds. The plants were grouped and treated with different dosage of heavy metals ( Pb, Cd and Cr) and a separate untreated group was also raised for comparison. The stock solution of these heavy metals were prepared and were given in the relative amount of 10mg, 20mg, 30mg, 40mg and 50mg/kg of soil to the different groups respectively at different time intervals. The first dose was given after 30 days of normal growth. The estimation was done in two intervals to check the heavy metals affect on chl-a, chl-b, total chlorophyll and carotenoid content of the plant.

**3. Estimation of Chlorophyll and Carotenoid content:**

The estimation of chlorophyll and carotenoid content was done by the procedure of Arnon (1949). 100 mg of fresh leaf tissues was weighed and grinded in pre-chilled mortar and pestle with 10 ml 80% acetone, and centrifuge at 10000xg for 10 min with 80% acetone serves as blank. After that the absorbance of supernatant was measured at 480, 510, 645 and 663nm by using UV-Vis spectrophotometer. The concentration of these pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid) was estimated according to the following formula and expressed as µg /ml fresh weight of leaves.

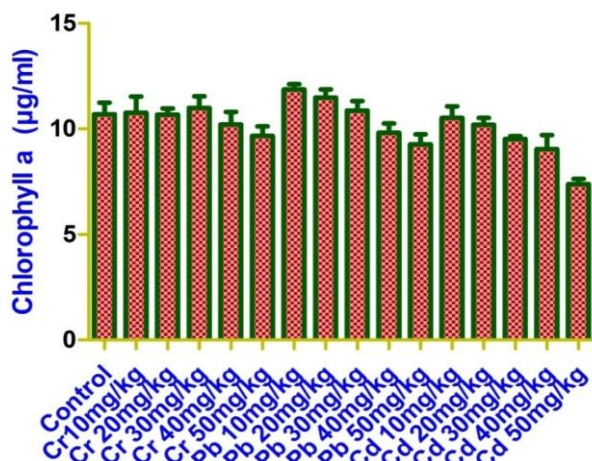
- a. Chlorophyll a (µg/ml) = (12.7× A<sub>663</sub> – 2.63 × A<sub>645</sub>)
- b. Chlorophyll b (µg/ml) = (22.9× A<sub>645</sub> – 4.48 × A<sub>663</sub>)
- c. Total Chlorophyll (µg/ml) = (chl a + chl b)
- d. Total Carotenoid (µg/ml) = (7.60× A<sub>480</sub> – 1.49× A<sub>510</sub>)

**4. Results:**

**Effect of heavy metals on Chlorophyll**

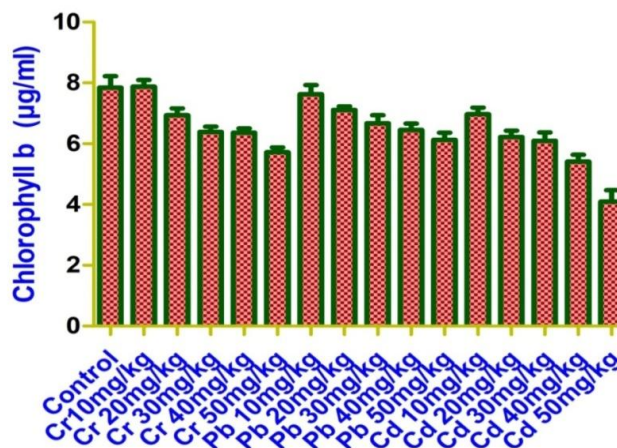
The effect of heavy metals on chlorophyll a and Chlorophyll b after 50<sup>th</sup> day of dosing:

**P=0.0001**



**Figure (1):** The figure demonstrates the heavy metal effect on chlorophyll a after 50<sup>th</sup> day of dosing.

**P=0.0001**

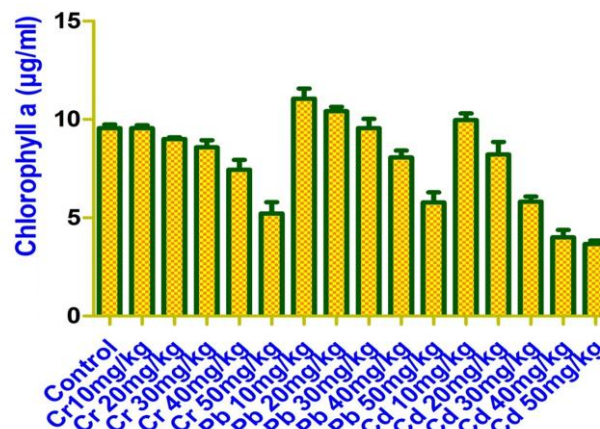


**Figure (2):** The figure demonstrates the heavy metal effect on chlorophyll b after 50<sup>th</sup> day of dosing .

The plants were treated with different doses (10mg/Kg, 20 mg/Kg, 30 mg/Kg, 40 mg/Kg and 50 mg/Kg) of lead (Pb), cadmium (Cd) and chromium (Cr) along with a controlled study. After 50<sup>th</sup> days of dosing significant reduction was seen in chlorophyll a in case of lead (Pb) and cadmium (Cd) (Figure 1), while in case of chlorophyll b significant reduction was seen in all the heavy metals as shown by the ANOVA (figure 2). Analysis of variance of each heavy metal shows significant variation (P= 0.0001).

**The effect of heavy metals on chlorophyll a and Chlorophyll b after 70<sup>th</sup> day of dosing:**

**P=0.0001**



**Figure (3):** The figure demonstrates the effect of heavy metals on chlorophyll a after 70<sup>th</sup> day of dosing.

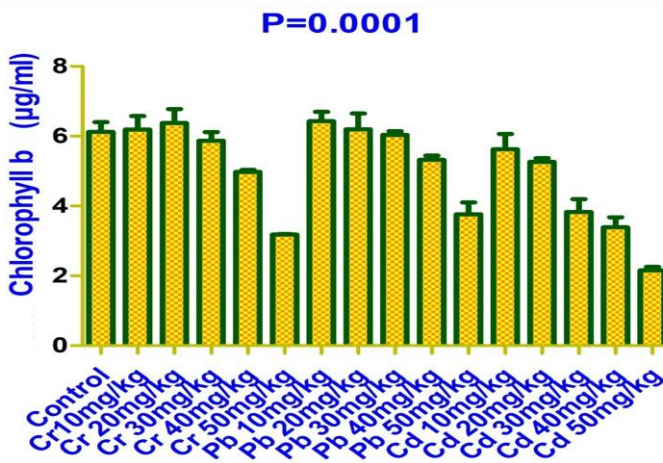


Figure (4): The figure demonstrates the effect of heavy metals on chlorophyll b after 70<sup>th</sup> day of dosing.

The plants were exposed with different doses (10mg/Kg, 20 mg/Kg, 30 mg/Kg, 40 mg/Kg and 50 mg/Kg) of lead (Pb), cadmium (Cd) and chromium (Cr) along with a controlled study. After 70<sup>th</sup> days of dosing significant reduction was seen in photosynthetic pigments i.e., chlorophyll a and b in case of all heavy metals (lead, cadmium and chromium) as depicted by ANOVA (figure 3 and 4).

The effect of heavy metals on Total chlorophyll after 50<sup>th</sup> and 70<sup>th</sup> day of dosing:

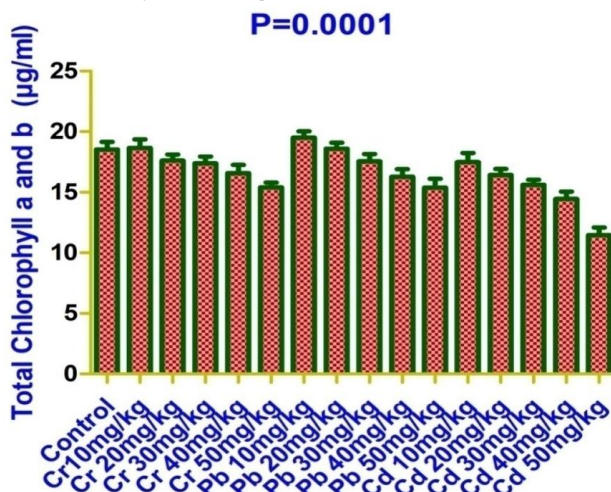


Figure (5): The figure demonstrates the effect of heavy metals on total chlorophyll after 50<sup>th</sup> day of dosing.

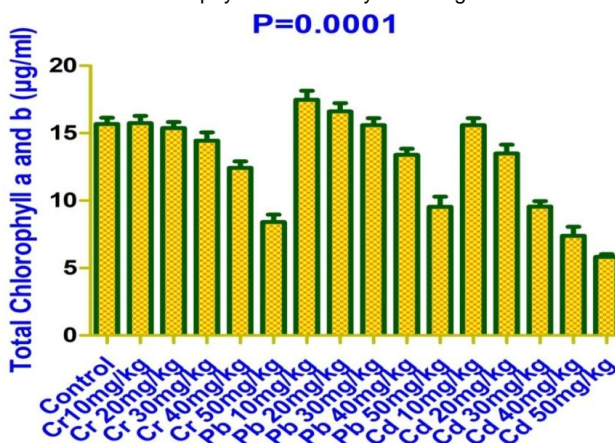


Figure (6): The figure demonstrates the effect of heavy metals on total chlorophyll after 70<sup>th</sup> day of dosing.

Total chlorophyll was analyzed after 50 days of dosing and was shown to be suppressed by heavy metal dosing at higher concentration. Analysis of variance of each heavy metal shows significant variation (P= 0.0001) as depicted in figure (5). Total chlorophyll was also analyzed after 70 days and it follows the similar pattern. It was suppressed by heavy metal dosing. One way ANOVA have been performed between the quantitative values of untreated and treated plant (Pb, Cd and Cr ) and was recorded significant (p= 0.0001) as revealed in figure (6).

Effect of Heavy metals on Carotenoid content after 50<sup>th</sup> and 70<sup>th</sup> day of dosing:

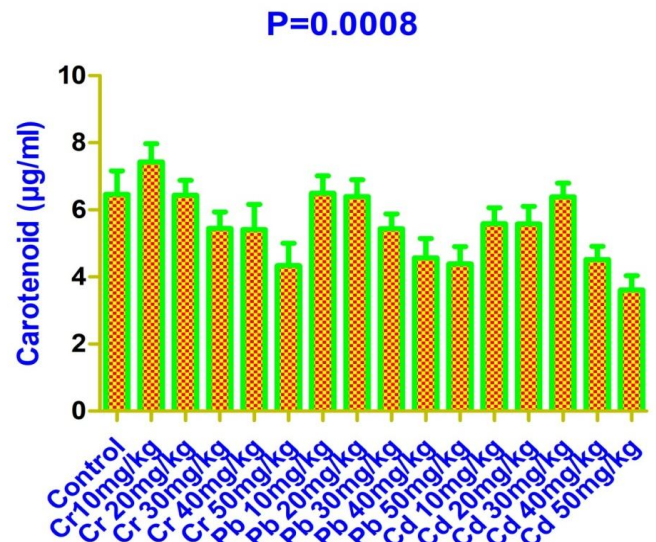


Figure (7): The figure demonstrates the heavy metal effect on Carotenoid content after 50<sup>th</sup> day of dosing.

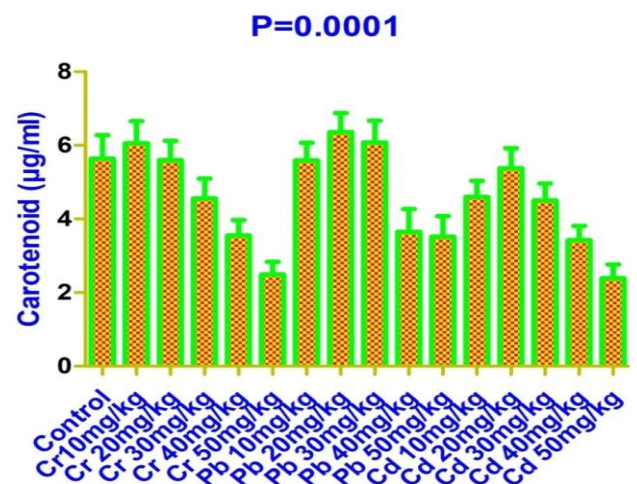


Figure (8): The figure demonstrates the effect of heavy metals on Carotenoid content after 70<sup>th</sup> day of dosing.

Carotenoid content was analyzed after 50th day of dosing with heavy metals (Cr, Pb and Cd) and was seen to be down regulated in heavy doses at higher concentrations for all studied heavy metals. Analysis of variance of each heavy metal shows significant variation (P= 0.0008) as depicted in figure (7). Carotenoid content was also evaluated after 70 days and shows the similar variations. Analysis of variance of each heavy metal shows significant variation (P= 0.0001) as shown in figure (8).

## 5. Discussion:

The present study entitled Effect of heavy metals on the photosynthetic pigments of *Leptadenia reticulata*, which showed deleterious effects on the content of photosynthetic pigments viz, chl-a, chl-b, total chlorophyll and carotenoids. The reduction in photosynthetic pigments was found to be dose dependent and decreased with the increase in the heavy metal concentration. However among the three heavy metals

(Cr, Pb and Cd) studied the maximum reduction was seen in case of cadmium (Cd). Our results corroborate with the findings of Bharadwaj *et al* (2009) who reported that under heavy metal stress the level of photosynthetic pigments decreases as the concentration of heavy metals increases. These results were also found to be consistent with the findings of Das (2017).

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