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RESEARCH ARTICLE

EFFECT OF HEAVY METALS ON BIOCHEMICAL PARAMETERS IN *VIGNA RADIATA* (GREEN GRAM)

***Damera. Vineeth, Chinna. Venkateshwar and Syeda.Azeem Unnisa**

Department of Botany, University College of Science, Osmania University, Hyderabad-500007, Telangana State, India

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ABSTRACT

To study the effect of heavy metals (Cd,Cr and Ni) on biochemical parameters in *Vigna radiata* (Green gram). *Vigna radiata* is an important legume species belongs to the family *Fabaceae*, commonly known as Green gram. Mung bean or green gram is an important cultivated pulses crop which has long been a food crop in Asia. Green gram is a protein rich staple food. It contains about 25 percent protein, which is almost three times that of cereals. It is also known as a useful green manure crop. The present research study was conducted to know the toxicity nature of heavy metals in *Vigna radiata* leaf and its remediation. Pot culture experiments were conducted with three treatments till productivity levels at Greenhouse of Botanical Garden, Department of Botany, Osmania University, Hyderabad. The three treatments consist of Treatment I control without any addition of heavy metals to the soil, Treatment II - heavy metals spiked into the soil and Treatment. III, 1 % of calcium hydroxide added along with heavy metals to the soil. The results showed when compared to treatment I and III the high concentrations of heavy metals (Ni, Cd and Cr) are found in leaf of *Vigna radiata* in (Treatment II). In addition, the plants grown in treatment III with 1% Calcium hydroxide treated soil, reversed the growth suppression and inhibited the heavy metal toxicity in plants as evidenced by reduced heavy metal concentration in leaf. The study concludes that food crop *Vigna radiata* affected with heavy metals can be treated by using calcium hydroxide.

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INTRODUCTION

Heavy metal stress is one of the major abiotic stresses that cause environmental pollution in recent decades (Castro *et al*., 2011; Gisbert *et al.,* 2003). Environmental deterioration has generated an increase of stress in all forms of life. Of these, stress on agricultural crops is of prime importance since agricultural is life time global society. The toxic heavy metal stress is an emerging and more dangerous stress for major crops. Environmental abiotic stress as heavy metals, drought, extreme temperature, cold or high salinity, severely impair plant growth and productivity (Anjum *et al.,* 2011). Therefore there is an increasing interest in effects of heavy metals on higher plants and their responses to excessive metal concentrations as stressors (Zhang *et al.,* 2010). Plant is an member of food chain create high risk to man and animals through contamination of food materials (Fargasova, 1994). The aim of present study to is assess the effect of heavy metals (Ni,Cd and Cr) on biochemical parameters of *Vigna radiata* (Green gram).

****Corresponding author: Damera. Vineeth,***
Department of Botany, University College of Science, Osmania University, Hyderabad-500007, Telangana State, India.

MATERIALS AND METHODS

Vigna radiata (Green gram)

Green gramWGG-2 var seeds was collected from Regional Agriculture Research Station, Warangal, Telangana State, India. 20Kgs of black soil was used for the pot experiments, the soil consists of 15.4% of clay and 3.5% of total carbon was maintained at pH 6.5.The heavy metal solution was prepared in the laboratory by following the (APHA.1992) guidelines .The different concentrations of heavy metals prepared are cadmium (10ppm), chromium (20ppm), nickel (16ppm). These heavy metals were dissolved in 150 litres of distilled water and sprayed on 600kg of black soil and dried in shade for 10 days for proper mixing of heavy metals in soil.1.5 kg of 1% Calcium hydroxide was added to the 300kgs of soil spiked with heavy metals.

Treatments

Treatment-I(T-I): 20 Kg of black soil was filled in 15 clay pots as control, Treatment-II(T-II): 20 Kg of black soil spiked with heavy metals was filled in 15clay pots, Treatment-III(T-III):

20 Kg of black soil spiked with heavy metals and 1% Ca $(OH)_2$ was filled in 15 clay pots. Seeds of *Vigna radiata* were sown in earthen pots at Greenhouse of Botanical Garden, Department of Botany, Osmania University Hyderabad. The plants were harvested till productivity level by following the standard protocol.

Biochemical parameters analysis

The Biochemical parameters were analyzed in fresh leaves of *Vigna radiata.* The biochemical components like Chlorophyll (Arnon. 1949), Protein (Lowry *et al.,* 1957), Carbohydrates (Yoshida *et al*., 1976), Reducing sugars (Nelson, 1944), Non–reducing sugars (Loomis, 1980), Starch (McCready *et al*., 1950), Phenols (Swain *et al.,* 1959), Proline (Bates *et al*., 1973), DNA (Burton, 1968), RNA (Schmeider, 1957) and enzymes Catalse (Barber, 1976), Peroxidase (Kar *et al*., 1976) and Polyphenoloxidase (Kar *et al*.,1976).

RESULTS AND DISCUSSION

The results of biochemical parameters are presented in Table 1 and Fig. 1-5

Chlorophyll content

Chlorophyll is vital for photosynthesis, which allows plants to absorb energy from light (Carter J. Stein. 1996) .Chlorophyll absorbs sun light and converts it to chemical energy (Yakar and Bilge 1987). The importance of chlorophyll for photosynthesis is that it captures light energy from the sun to produce glucose via a chemical reaction. Data presented in Fig.1 show that chlorophyll content in leaf was significantly decreased in Treatment II which is 0.480±0.004 mg/gm when compared with Treatment –I 0.831±0.005 mg/gm and Treatment –III 0.817±0.005 mg/gm.

Protein content

They are complex combinations of smaller chemical compounds called *amino acids*. Proteins are involved in processes such as catalyzing chemical reactions (enzymes), facilitating membrane transport, intracellular structure and energy generating reactions involving electron transport. A challenge lies in the fact that proteins have a finite life span and must be constantly translated from m-RNA in order for plant growth and development to continue.

Table 1. Treatment comparative study of *Vigna radiate* (Greengram) on biochemical parameters

S.N0	BIOCHEMICAL PARAMETERS	T-I	T-II	T-III
1.	CHLOROPHYLL mg/gm	0.831±0.005	0.418±0.004	0.817±0.005
2.	PROTEINS mg/gm	0.664±0.005	0.325±0.003	0.670±0.005
3.	CARBOHYDRATES mg/gm	2.588±0.024	1.549±0.015	2.546±0.023
4.	REDUCING SUGARS mg/gm	2.186±0.023	1.268±0.015	2.067±0.021
5.	NON -REDUCING SUGARS mg/gm	0.452±0.003	0.281±0.002	0.449±0.003
6	STARCH mg/gm	1.708±0.009	0.736±0.006	1.652±0.009
7.	PHENOLS mg/gm	0.496±0.006	0.908±0.008	0.478±0.006
8.	PROLINE mg/gm	0.468±0.006	0.815±0.009	0.421±0.006
9.	DNA mg/gm	3.376±0.045	1.926±0.021	3.464±0.041
10.	RNA mg/gm	0.386±0.006	0.160±0.004	0.382±0.006
11.	CATALASE ENZYME mg/gm	0.696±0.008	0.352±0.006	0.674±0.008
12.	PEROXIDASE ENZYME mg/gm	0.976±0.009	0.478±0.007	0.982±0.009
13.	POLY PHENOLOXIDASEENZYME mg/g	1.540±0.010	0.682±0.009	1.544±0.010

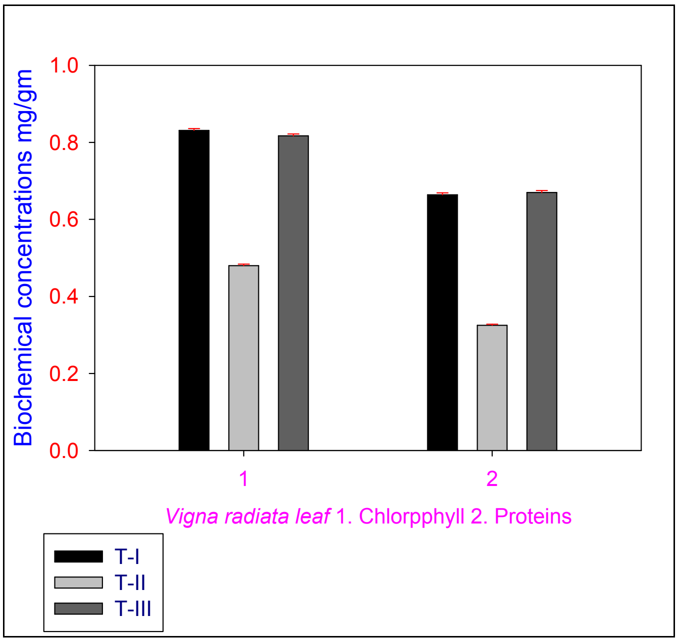

Figure 1. ***Vigna radiata*** **Leaf chlorophyll and Proteins**

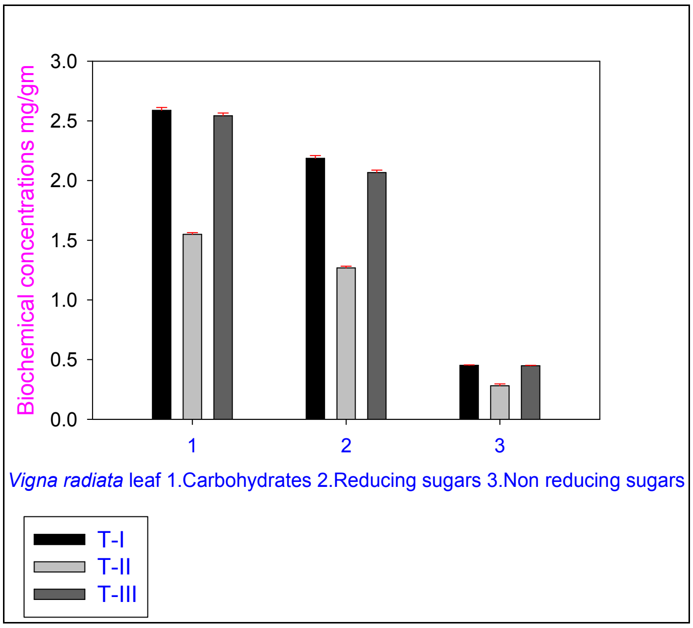

Figure 2. ***Vigna radiata*** **Leaf biochemical contents**

Data presented in Fig.2 show that protein content were significantly decreased in plant treated with Treatment-II 0.325±0.003 mg/gm when compared with Treatment –I 0.664±0.005 mg/gm in plant material and Treatment –III 0.670±0.005 mg/gm.

Carbohydrates content

Carbohydrates are formed by green plants from carbon dioxide and water during the process of photosynthesis. A source of energy for the body e.g. glucose and a store of energy, e.g. starch in plants. Components of other molecules eg. DNA, RNA, glycolipids, glycoproteins and ATP. Data presented in Fig.2 show that carbohydrates content were significantly decreased in plant treated with Treatment-II 1.549±0.015 is mg/gm when compared with Treatment-I 2.588±0.024 mg/gm and Treatment –III 2.543±0.023 mg/gm.

Reducing sugars content

Glucose is a carbohydrate, and is the most important simple sugar in human metabolism. Glucose is made during photosynthesis from water and carbon dioxide, using energy from sunlight, is a very important source of power for cellular respiration. Glucose may be stored in plants as the polymers starch and cellulose. Sugars such as glucose, fructose, and sucrose are recognized as signaling molecules in plants (Rolland *et al*., 2006; Bolouri-Moghaddam *et al*., 2010). in addition to their typical roles as carbon and energy sources (Koch, 2004). Invertases play crucial roles in the regulation of sucrose levels, sink strength, and sucrose:hexose ratios linked to sugar signaling. Vacuolar, cell wall, and neutral/alkaline invertases can be discerned (Koch, 2004; Xiang *et al*., 2011). Sugar signaling might also be of great importance in plant (defence) responses under biotic and abiotic stresses. Data presented in Fig.2 show that Reducing sugars content were significantly decreased in plant treated with Treatment-II is 1.268±0.015 mg/gm when compared with Treatment –I 2.186±0.023 mg/gm and Treatment –III 2.067±0.021 mg/gm.

Non reducing sugars content

Sucrose is an important carbohydrate in most plants. It has multiple functions such as regulating photosynthesis and respiration, serving as storage compound and helping to maintain the osmotic pressure in the cytosol. It provides protection to plants from stress by contributing to cellular osmotic adjustment, ROS detoxification, protection of membrane integrity and enzymes/protein stabilization (Ashraf *et al.,* 2007; Bohnert *et al.,* 1996; Yancey, 1994). Data presented in Fig.2 show that Non-reducing sugars content were significantly decreased in plant treated with Treatment-II is 0.281±0.002 mg/gm when compared with Treatment –I 0.452±0.003 mg/gm and Treatment –III 0.449±0.003 mg/gm.

Starch content

Starch is the main form by which plants store carbohydrate and is a major photosynthetic product in many species. Starch is the major energy reserve for plants; it is located mainly in the seeds, roots or tubers, stem pith, and fruit. The functional properties of native starch are determined by the granule structure. This is important for normal growth in a diurnal cycle and is finely controlled to suit the growth conditions (Gibon *et al.,* 2009). Data presented in Fig.3 show that starch content were significantly decreased plant treated with Treatment-II is 0.736±0.006 mg/gm when compared with Treatment –I1.708±0.009 mg/gm and Treatment –III 1.652±0.009 mg/gm.

Phenols content

Phenolic compounds are crucial for plants growth and reproduction, and are produced as a response to environmental factors (light, chilling, pollution etc) and to defend injured plants (Valentine *et al*., 2003) Phenolics acids are the most important groups of secondary metabolites and bioactive compounds in plants (Kim *et al.*, 2003). Data presented in Fig.3 show that phenols content were significantly increased in Treatment-II is 0.908±0.006 mg/gm when compared with Treatment –I 0.496±0.008 mg/gm and Treatment –III 0.478±0.008 mg/gm.

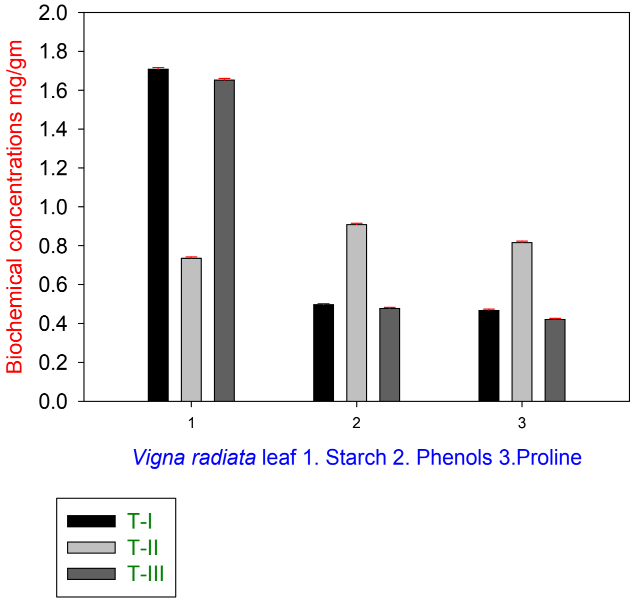

Figure 3. ***Vigna radiata*** **Leaf starch, phenols and proline**

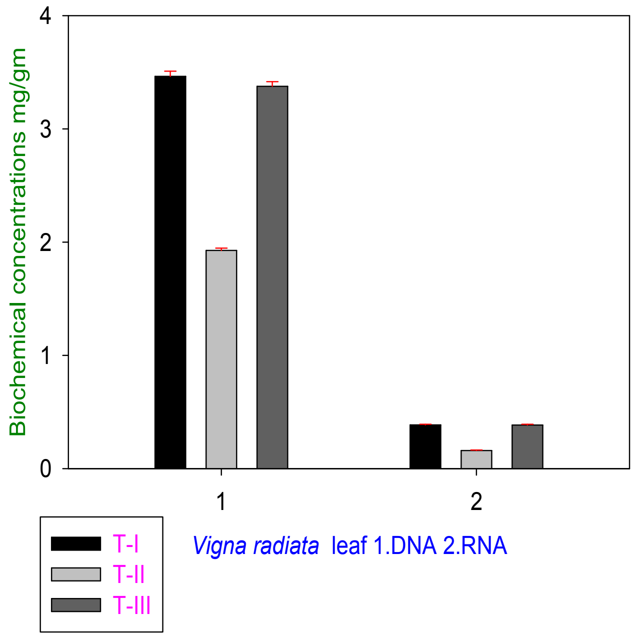

Figure 4. ***Vigna radiata*** **Leaf DNA and RNA contents**

Proline content

Proline provide protection to plants from stress by contributing to cellular osmotic adjustment, ROS detoxification, protection of membrane integrity and enzymes/protein stabilization (Ashraf *et al.,* 2007; Bohnert *et al*., 1996; Yancey, 1994). Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress. Data presented in Fig.3 show that Proline content were significantly increased in Treatment-II is 0.815±0.009 mg/gm when compared with Treatment –I 0.468±0.006 mg/gm and Treatment –III 0.421±0.006 mg/gm.

DNA content

The DNA segments that carry genetic information are called genes, but other DNA sequences have structural purposes, or are involved in regulating the expression of genetic information The DNA segments that carry genetic information are called genes in eukaryotes such plants and animals. Data presented in Fig.4 show that DNA content were significantly decreased in Treatment-II is 1.926±0.021 mg/gm when compared with Treatment –I 3.464±0.045 mg/gm and Treatment –III 3.376±0.041mg/gm.

RNA content

Ribonucleic acid or RNA is a nucleic acid polymer consisting of nucleotide monomers that plays several important roles in the processes that translate genetic information from deoxyribonucleic acid (DNA) into protein products.. Some RNA molecules play an active role within cells by catalyzing biological reactions, controlling gene expression, or sensing and communicating responses to cellular signals. One of these active processes is protein synthesis, a universal function whereby mRNA molecules direct the assembly of proteins on ribosomes. This process uses transfer RNA (tRNA) molecules to deliver amino acids to the ribosome, where ribosomal RNA (rRNA) links amino acids together to form proteins. Data presented in Fig.4 show that RNA content were significantly decreased in Treatment-II which is 0.160±0.004 mg/gm in plant material as compared with Treatment –I 0.386±0.006 mg/gm in plant material and Treatment –III 0.382±0.006 mg/gm.

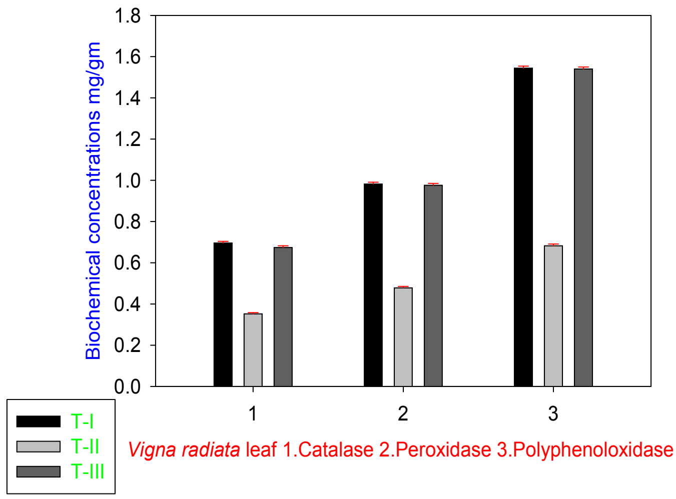

Figure 5. ***Vigna radiata*** **Leaf enzymes**

Catalase content

In green leaves a majority of CAT activity is found in peroxisomes (Foyer and Noctor 2000; Scandalios *et al*., 1997). Catalase (CAT, H_2O_2:H_2O_2 oxidoreductase; EC 1.11.1.6) by scavenging hydrogen peroxide to water and oxygen is an important enzyme of cell defense mechanisms against oxidative stress in plants (Dat *et al.,* 2003; Foyer and Noctor. 2000). Catalase is the most efficient enzyme as an antioxidative enzyme which lowers, hydrogen peroxide or superoxide to accumulate to toxic levels in plant growth (Bowler *et al.,* 1992; Brenan and Frenkel, 1977). Data presented in Fig.5 show that catalase enzyme content were significantly decreased Treatment-II is 0.352±0.006 mg/gm as compared with Treatment –I 0.696±0.008 mg/gm and Treatment -III 0.674±0.008 mg/gm.

Peroxidase content

Peroxidases, a class of enzymes in animal, plant and microorganism tissues, catalyze oxidoreduction between H_2O_2 and various reductants. Peroxidase is involved in a large number of biochemical and physiological processes and may change quantitatively and qualitatively during growth and development (Shannon, 1969). Enzymes peroxidase and catalaseare high-molecular, which are capable of eliminating the hydrogen peroxide formed during non enzymatic or enzymatic dismutation (Merzlyak, 1999). When plants are subjected to stress this tends to result in the release of reactive oxygen species (ROS).It is thought that peroxidase remove ROS, helping prevent damage. Data presented in Table 1 & Fig.5 show that Peroxidase enzyme content were significantly decreased is Treatment-II is 0.478±0.007 mg/gm when compared with Treatment –I 0.982±0.009 mg/gm and Treatment –III 0.976±0.009 mg/gm.

Polyphenoloxidase content

Physiological function of polyphenol oxidase in higher plants has been unequivocally determined; there is evidence, which suggests that the enzyme plays an important role in plant defense also (Mayer. 1987). The plant polyphenol oxidase is induced in response to mechanical wounding and signaling molecules, which suggests that polyphenol oxidase may play a role in plant defense (Boss *et al.,* 1995; Thipyapong and Steffens, 1997). These enzymes Data presented in Table 1 & Fig.5 show that Polyphenoloxidase enzyme content were significantly decreased in Treatment-II is 0.682±0.010 mg/gm when compared with Treatment –I 1.544±0.009 mg/gm and Treatment -III1.540±0.009 mg/gm.

The results revealed that exposure of *Vigna radiata* (Green gram) to heavy metals decrease in chlorophyll, detail studies indicates that heavy metals have effects on chlorophyll content in plants. Heavy metal like lead also decreased the carbohydrate and protein content in Wheat (Tiwari *et al.,* 2013). Toxicity may result from binding of metals to sulphydryl groups in proteins, leading to inhibition of activity or disruption of structure, or form displacement of an essential element, resulting in deficiency effects (Van Assche and Clijsters, 1990; Capuana, 2011). In plant tissue the accumulation of heavy metal cause alterations in various physiological process (Jayakumar and Jallel, 2009). Heavy metals are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient (Van Assche and Clijsters. 1990; Meers *et al.*, 2010) It has been determined that as a proline has increased with stress of heavy metals (Zengin and Kirbag, 2007). Among different environmental stress, heavy metal is strongest inducer for proline accumulation (Sharma and Dietz. 2006). Accumulation of the phenol compounds under heavy stress has been reported by (Ustun *et al.,* 2000; Diaz *et al*., 2001) Decrease of chlorophyll content with heavy metal stress in *Zea mays* and *Acer rubrum* (Siedelka and Krupa, 1996). Similar results protein content of leaves show that there was decrease of effect of three heavy metals Ni, Cd and Cr. Proline accumulation, accepted as an indicator of environmental stress, is also considered to have important protective roles Heavy metal stress to proline accumulation (Alia and Saradhi, 1991). The phenomenon of proline accumulation is known to occur under heavy metal (Schat *et al.*, 1997). It is well described that under stress conditions many plant species accumulate proline as an adaptive response to adverse conditions. A large body of data suggests a a positive correlation between proline accumulation and plant stress. Therefore proposed technique may solve the disposal problem of various heavy metals. This kind of study is beneficial for saving the environment from heavy metals. The interaction between nutrients, heavy metal and soil may occur at the level of plant and/or in the soil. In soil, nutrients and metal interact at the level of precipitation, surface absorption and formation of complexes with organic compounds (Pantazis *et al.,* 2007). The present study recommends that limestone be used in some heavy metals polluted agricultural soils to maintain crop productivity.

Conclusion

Study reveals that there is a decrease in biochemical contents in Treatment II when compared with Treatment I and Treatment III of *Vigna radiata*. It was also observe that the phenol and proline parameters are increased in Treatment II and the heavy accumulation in the leaf parts, where these are indicators of heavy metal where as in Treatment I and Treatment III phenol and proline contents decreased. Remaining biochemical contents such as Chlorophyll, Protein, Total sugar/Carbohydrates, Reducing sugars, Non -reducing sugars, Starch, DNA and RNA and Enzymes Catalase, Peroxidase and Polyphenoloxidase content is decrease in Treatment –II compared with Treatment I and Treatment III. It may conclude that direct relationship between heavy metals and biochemical in plant *Vigna radiata*. It may concluded that there is relationship between heavy metal and biochemical parameters. Heavy metal content in the plants, growing in polluted areas can be reduced when the soils are treated with of 1% Ca $(OH)_2$.

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