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Effect of Heavy Metal Stress (Cadmium) on Morphological Physiological Activity and Anatomy of Cow Pea Plant (Vigna Uncuigulata)

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***Abstract:** This study was done to examine the effect of heavy metal on the growth, lipid peroxidation, antioxidant enzyme activity and some key physio-biochemical attributes in cowpea (Vigna unguiculata [L]. In this study 21 days old seedlings of Cow pea plant (Vigna unguiculata L) were subject to different heavy metal stress levels (0g, 0.1g and 0.2g Cd) at germination and early seedling growth stage of plant development. Data were analyzed for growth parameters such as plant height, fresh and dry weight, leaf water content (LWC), and length of radicle and plumule during germination period, and biochemical parameters such as proline content, membrane stability index (MSI), malondealdehyde (MDA) content, chlorophyll content, and antioxidant enzyme activity Catalase (CAT) and Peroxidase (POD). It was seen that the effect of heavy metal stress reduced plant height, fresh and dry weight, LWC, radical and plumule length. Heavy metal stress reduced the biochemical activities and also chlorophyll a, chlorophyll b and total pigment content. The decrease was 0.03 and 0.003 respectively. The result showed an increase in the activity of CAT enzyme in leaves and root with increasing salt concentration. An increase CAT activity were found with 0.1g, 0.2g Cd treatment which represented values of relative increasing of 15.88 and 18.5 g/L in leaves and 9.48 g/L and 12.44 g/L in root respectively. There was increase in the activity of POD enzyme in leaves and root with increasing heavy metal cadmium concentration. The level of POD activity was found with 0.1g,0.2g Cd treatment, which represented relative reduction80.55 g/L and 86.46g/L in leave and root 81.2 g/L and 85.3g/L.*
Unguiculata.
Keywords: Heavy metal, plant stress, Cadmium, Cow pea, Vigna.

I. INTRODUCTION

Environmental abiotic stresses, such as presence of heavy metals have been found to a stress factor for plants throughout the world. Many experiments described that cadmium (Cd) known as a non-essential toxic heavy metal produces physiological and morphological alterations in plants such as reduction in photosynthesis or growth as well as results in chlorosis in leaves. These indicators results in oxidative stress which is expressed as increase of lipid peroxidation (LP), formation of reactive oxygen species (ROS) and decrease in the activity of enzymatic and non-enzymatic antioxidants. Worldwide agricultural soils are slightly to considerably contaminated from heavy metals that limit the crop plants to achieve their full genetic potential and also reduce their productivity. Soil pollution by heavy metals has reasonably increased in last few decades due to discharge of wastewater and waste from anthropogenic sources. There are many illustrations which show that some aspects of Cd stress that make changes in morphological, physiological and biochemical changes of plants. Cd stress declines photosynthetic rate. This is due to limited access of CO_2 which results in a decrease in the gas exchange process and the results triggers reduction in the growth and productivity of the plant. It also reduce leaf size, stems extension and root proliferation and decrease water absorption and transportation by causing turgor loss through decreasing the cell wall elasticity. Cd stress increases reactive oxygen species (ROS) production. ROS is harmful for the cell components. Cadmium toxicity also results in alterations in the antioxidant systems. This research focuses on the ability and strategies of higher plants to respond adapt and overcome the Cd stress.

In this study the 21 days old seedings of Cow pea plant (Vigna unguiculata L) were subject to different Cadmium levels (0g, 0.1g and 0.2g NaCl) at germination and early seedling growth stage of plant development. Data were analyzed for growth parameters such as plant height, fresh and dry weight, leaf water content (LWC), and length of radicle and plumule during germination period, and biochemical parameters such as proline content, membrane stability index (MSI), malondealdehyde (MDA) content, chlorophyll content, and antioxidant enzyme activity Catalase (CAT) and Peroxidase (POD). In this study the effect of hevy metal

stress reduced plant height, fresh and dry weight, LWC, radical and plumule length. Salt stress reduced the biochemical activities and also chlorophyll a, chlorophyll b and total pigment content.

Due to detrimental effects of various abiotic stresses, food productivity is decreasing all-round the globe. Cadmium (Cd) is one of the most non-essential toxic heavy metal which produced physiological and morphological alterations in plants[1].In plant, cellular activities that are mostly affected by heavy metal pollution include mineral nutrition, photosynthesis, respiration, membrane structure, gene expression and other properties. Plant membrane structure is the first target of heavy metal toxicity. Cadmium toxicity causes harm to plants, especially in the arid and semi arid regions. Cadmium cause negative effects in plants such as decrease in leaf chlorophyll content, inhibition of photosynthesis etc (e.g. reduction in photosynthesis or growth as well as chloroplast in leaves). These indicators results in oxidative stress namely increase of lipid per oxidation (LP), formation of reactive oxygen species (ROS) and decrease in the activity of enzymatic and non-enzymatic antioxidants, (Soluble protein, CAT, POD).

II. MATERIALS AND METHODS

The present study is done to analyse the effect of the Heavy Metal (a biotic environmental stress) response on plants.

III. STUDY MATERIALS

The plant material selected for the study is Cow pea plant (Vigna unguilata L) considering the ease in the growth of the plant

Plate 1 (Control) Plate 2 (0.1g Cd) Plate 3(0.2g Cd)

Plate 1, 2 and 3 Effect of various concentration of heavy metal on germination

IV. SAMPLE COLLECTION

Cow pea plant (Vigna unguiculata L) seeds were collected from College of Agriculture, Kerala Agricultural College, Vellayani, Thiruvananthapuram, Kerala, India.

V. MORPHOLOGICAL PARAMETERS

A. Determination of plant height

The plant height (centimeters) was measured with the help of scale at the time of harvest. The length was measured from the point where the root and shoot joins to the end of root for root length and to the top of shoot for shoot length

B. Determination of fresh and dry weight of shoot and root:

After harvesting the seeding, the shoot was cut from root at the point where they joined together. The fresh weight was recorded for each part separately. And the sample was dried in an oven at 70°C up to constant dry weight.

C. Determination of length of radicle and plumule

The radicle and plumule length (centimeter) were measured with the help of a centimeter scale at time of germination. The length was measured from the point where the root tip and shoot joints to the end of root.

D. Determination of leaf water content

Leaf water content was estimated according to the method described by Smart and Bingham (1974). Leaf discs were punched from each treated plant and the fresh weight was determined. The same leaf discs were kept on water for 4 hrs and turgid weight recorded. The leaf sample was dried in oven at 85°C for dry weight.

E. Determination of pigment content

The chlorophyll content was determined according to[7]. Fresh leaf materials (1g) were ground properly in 50ml of 100% acetone then centrifuged for 10min at 2500g, absorbance was read spectrophotometrically at 662,645 and 470nm.Pigment content was estimated following formula;

Chl a = (0.0127xODx663) - (0.00269xODx645)
Chl b= (0.0229xODx645) - (0.00468xODx663)
Total Chl = (0.0202xODx645) + (0.00802xODx663)

F. Biochemical Parameters

The study materials were analyzed for the following biochemical parameters.

G. Enzymatic Antioxidants

1) Determination of Catalase (CAT): The enzyme extract of V. *unguiculata* was prepared in phosphate buffer. The homogenate was centrifuged and supernatant was used for enzyme assay. H_2O_2 - phosphate buffer was taken in an experimental cuvette, followed by the rapid addition of 0.1ml of enzyme extract and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units recorded at 240 nm in a spectrophotometer (UV-1800 Shimadzu). The enzyme solution containing H_2O_2 - free phosphate buffer was kept as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05units.

G. Determination of Peroxidase (POD)

Enzyme extract A (20% homogenate) was prepared in 0.1M Phosphate buffer (pH 6.5) from the various parts of the plant, clarified by centrifugation and supernatant was used for the assay. To 3.0ml of pyrogallol solution, 0.1ml of the enzyme extract was added and the spectrophotometer was adjusted to read zero at 430nm.To the test cuvette, 0.5ml of H_2O_2 was added and was mixed. The change in absorbance was recorded every 30 seconds up to 3minute minutes in a spectrophotometer (UV-1800 Shimadzu). One unit of peroxidase is defined as the change in absorbance/minute at 430nm.

H. Non enzymatic Antioxidant

1) Determination of proline contents: Dry weight (0.5g) was extracted by homogenization in 3% (w/v) aqueous sulphosalicylic acid. After the 20 minutes of centrifugation at 3000 x g, supernatant collected was mixed with acetic acid and ninhydrin. The mixture was boiled for 1 hour and then absorbance was read spectrophotometrically at 520 nm using toluene as blank.

2) Determination of malonaldehyde content: Fresh leaves were ground in 1% (10 ml/g fresh weight) trichloro acetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 5 minutes. Reaction mixture containing 1.0 ml of supernatant and 4.0 ml of 0.5% (w/v) thiobarbituric acid (TBA) was heated at 95°C for 30 min, cooled on ice bath and centrifuged at 5000 rpm for 5 min for clarification. Absorbance of the supernatant was taken at 532 and 600 nm.

3) Determination of membrane stability index: Fresh leaf samples (0.1 g) were taken in test tubes in two sets containing 10 ml of double distilled water. One set was kept in water bath for half an hour at 40°C and the electric conductivity was recorded (C1). Another set was kept in water bath at boiling temperature (100°C) and EC was recorded (C2). MSI was calculated as per the formula:

$$(MSI) = [1 - (C1/C2)] \times 100$$

I. Anatomy of stem

Two to three centimeter long pieces of the material were taken. Thin sections were taken using a razor. The thinnest section of the material was taken with the help of delicate brush. For staining, the sections were left for 3 – 5 minutes in a watch glass with stain. Leave 3-5 minutes. The stained sections were mounded on a watch galss and were viewed under a compound microscope with a camera attached.

VI. RESULT AND DISCUSSION

Exposure of plants to toxic metals can lead to numerous physiological and biochemical disorders. The inhibition of plant seedling growth can be regarded as general responses associated with heavy metal toxicity.

A. Morphological parameters

The effect of salt stress on plant growth parameters is shown in Table1. Exposure of cow pea plant to various salinity conditions reduced fresh and dry weight of plant, plant height, leaf water content, length of plumule and radical during germination period with increasing Cadmium Concentration treatment. Cadmium induced reduction in weight was reported to be 1.6g (0.1g Cd), 0.95g (0.2g Cd l), 0.93g (0.1g Cd) and 0.66g (0.2g Cd) in fresh and dry weight of plant against 2.12 g of fresh weight of control plant and 1.88g of dry weight of control plant. The length of the plumule was measured to be 2.8cm (0.1g Cd), 1.52.46 cm (0.2g Cd) against 5.63 cm (control) and the length of the radicle were 1.9cm (0.1g Cd) and 0.98 cm (0.2g Cd), against 8.5cm in control plant, respectively at various treatments. Leaf water content (LWC) is 62.2.55(0.1g Cd), 54.1(0.2g Cd) against 92.11 in the control plant.

The present study shows that cadmium markedly reduced root elongation and shoot length. Occurrence of these symptoms was associated with reductions in dry matter production. Cadmium affected root growth more than shoot growth, especially at elevated Cadmium levels, confirming the results found in pea plant,[2] radish and barley[15]. Greater sensitivity of roots to cadmium than shoots might be related to the fact that roots are the first organs to be in contact with cadmium, accumulating it at much higher amounts than shoots.The water content in pea plants decreased gradually and significantly with the increase of Cd concentration (Table 1). To examine the osmotic effect of abiotic stress treated plant tissues, the water content was frequently measured[4]and it was observed that plant water status was highly affected by heavy metal stress These results indicate that an excess level of cadmium has a toxic and an osmotic effect on pea plants.

Table1. Influence of heavy metal stress on growth parameters of Cowpea plant

Sl No	Treatment	Plant height(cm)	Fresh weight (g)	Dry weight(g)	Leaf water content (%)	Length of plumule (cm)	Length of radicle (cm)
01	Control	30±1.00	2.12±0.03	1.88±0.036	92.11±2.43	5.63±0.057	8.5±0.141
02	Cd (0.1g)	18.2 ±0.25	1.6±0.26	0.93±0.45	62.2±0.03	2.8±0.04	1.9±0.04
03	Cd (0.2g)	15.5±0.12	0.95±0.033	0.66±0.002	54.1±0.052	1.52±0.03	0.98±0.01

The change in the growth parameters considered in the control treatment can be better represented as graph (Figure 1).

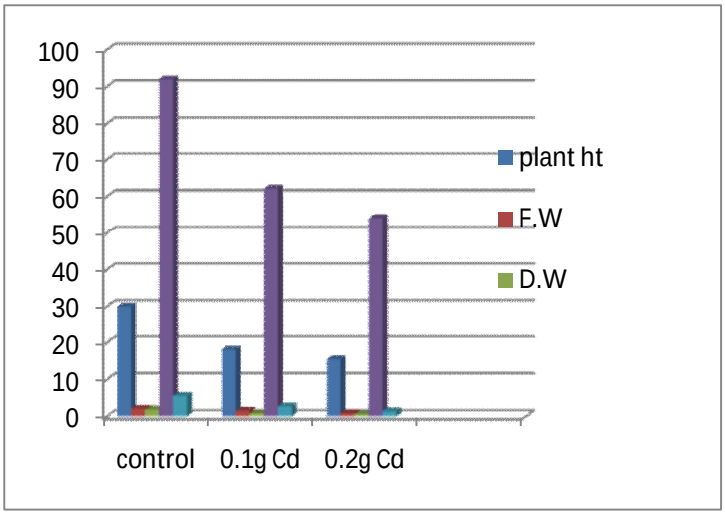

Figure.1 Influence of heavy metal (Cd) morphological parameters of cow pea palnt

B. Chlorophyll content

The effect of heavy metal stress on the production of plant pigment is presented in Table 2. It is seen that Cadmium reduced chlorophyll a, chlorophyll b and total pigment content in the experimental plant. The values obtained with respect to the hevy metal stress given were as follows. Chlorophyll a showed values such as 0.03(0.1g Cd) and 0.002 (0.2g Cd), where the corresponding values for the control plant was 2.14. The content of chlorophyll b was 0.01 (0.1g Cd) and 0.001(0.2g Cd) as compared to 0.84 in

control and total pigment content showed values0.03(0.1g Cd)and 0.003(0.2g Cd), when the control plant showed a total chlorophyll content of 2.98 mg/g of fresh weight

Table 2 Influence of heavy metal stress on pigment system of *Vigna unguiculata*

SL No:	Treatment	Chlorophyll a (mg/g fresh weight)	Chlorophyll b (mg/g fresh weight)	Total chlorophyll content (mg/g fresh weight)
01	Control	2.14±0.16	0.84±0.06	2.98±0.11
	Cd (0.1g)	0.03±0.01	0.01±0.02	0.04±0.01
03	Cd (0.2g)	0.002±0.02	0.001±0.01	0.003±0.03

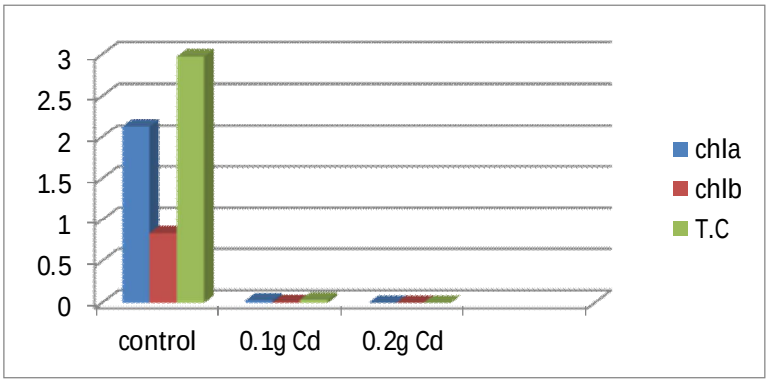

Figure 2 Influence of heavy metal stress on chlorophyll content of cow pea plant

C. Biochemical Parameters

1) Enzymatic antioxidant activity Catalase (CAT): Heavy metal (Cd) cause a wide range of responses in plants such as decreased growth, increased osmotic potential and most of important production of ROS due to oxidative stress in the cell. ROS are highly reactive species which readily oxidize protein, lipids, nucleic acid. As reported in many plants both enzymatic and non-enzymatic antioxidant plays an important role in scavenging the ROS. Result of antioxidant content in this study showed that CAT and POD activities increased with heavy metal stress. Increase in CAT and POD of Cadmium enhancement was 15.88 (0.1g Cd) 18.5and (0.2g Cd) as compared to 12. 58 in control plant in leaf samples. The activity was recorded as 9.48 (0.1 g Cd) and12.44 (0.2 g Cd) as compared to a 7.16 in control in root samples. From this study, it can be concluded that enzymatic antioxidants CAT and POD do play an important role in cowpea plant under heavy metal stress. Catalase (CAT) is an important enzyme in the protection against oxidative stress in all aerobic organisms. It catalyze rapid decomposition of hydrogen peroxide into oxygen and water, thereby protecting cells from oxidizing effects caused of excessive H_2O_2,[16]. Earlier data in the literature concerning the catalase response in plants leaves exposed to cadmium stress are contradictory since both enzyme activation,[20]and inhibition,[19]. In our investigations exposure of wheat plants to cadmium markedly induced an increase of CAT activity in leaves. In response to the in ROS accumulation, the antioxidant defence system comprising SOD and CAT plays important roles in their scavenging,[17],[3]. SOD could eliminate superoxide, a harmful substance to cell membranes, produced in the aero-metabolism process. H_2O_2 is also toxic to plant cells, could be eliminated by CAT[9].

Table 3 Catalase content in stressed Cow pea plant (*Vigna unguiculata* L)

SL No:	Treatment	Catalase (CAT) (g)	
		Leaf	Root
01	Control	12.58±0.106	7.16±0.029
02	Cd (0.1g)	15.88±0.051	9.48±0,04

03	Cd (0.2g)	18.5±0.141	12.44±0.503

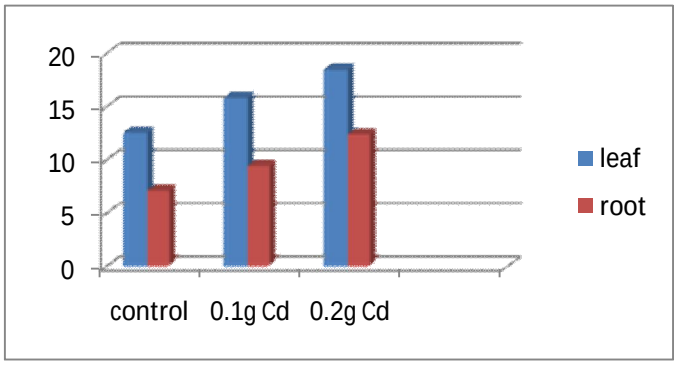

Figure 3 Influence of heavy metal (Cd) catalase activity of cow pea palnt

D. Peroxidase (POD)

Table 4 shows the result of peroxiadase enzyme activity in the study plants. High levels of peroxidase enzyme (POD) activity in leaves were found with 0.1g and 0.2g Cadmium treatment (80.55mg and 86.46 mg respectively) with respect to 71.14 mg in leaf tissues of control plant. The values of peroxidase in root tissue in control plant was found to be 1549 mg and the stressed plant showed values 1749.43 mg and 1931.1 mg for 0.1 g Cd and 0.2 g Cd concentrations respectivelyThese results showed that, Cd treatment significantly increased POD activities in leaves of pea plants (Figure 4)[21]. showed that cadmium could increase POD activities in pea plant leaves. The role of POD is to eliminate the excess of H_2O_2. POD catalyzes H_2O_2 dependent oxidation of substrate, while CAT H_2O_2 by breaking it down directly to form water and oxygen [22].

Table 4. Peroxidase content in stressed Cowpea plant (*Vigna unguiculata* L)

SL No:	Treatment	Peroxidase	
		Leaf	Root
01	Control	71.14±0.056	74.2±0.33
02	Cd (0.1g)	80.55±0.04	81.2±0.166
0.3	Cd(0.2g)	86.46±0.036	85.3±0.54

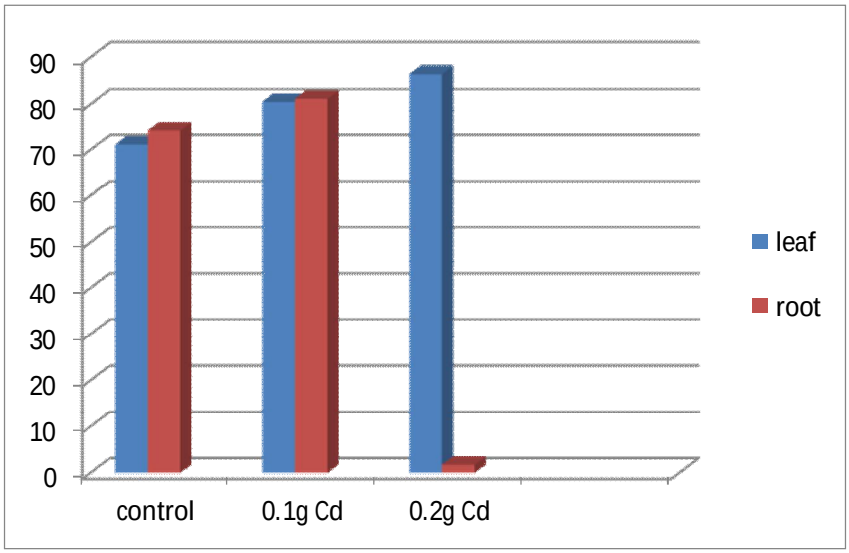

Figure 4 Influence of Heavy Metal on peroxidase content

E. Non Enzymatic Activity

1) Proline content : As seen in Table 5, increase in proline content was conspicuous in heavy metal stressed plant. Heavy metal stress 0.1g, 0.2g induced treatment showed a proline content of 9.92g and 13.66g respectively, where in the control plant showed 1.93 g only. The effect of cadmium stress on the proline content of pea leaves. Exposure of pea plants to cadmium significantly increased proline content. The table 5 shows that proline content was highly affected with the $CdCl_2$ concentration. In higher plants, proline is accumulated under stress, both due to an increase in production by reducing its degradation[21],[6] .The accumulation of proline occurs after the development of resistance is a consequence rather than a cause of hardening[14]. In the present study proline increased significantly in the cadmium treated pea plants. Enhanced proline accumulation in reponse to Cd toxicity has been earlier demonstrated in *Triticum aestivum, Vigna radiate, Helianthus annus and Phaseolus vulgaris* [10],[6],[13]Thus, proline accumulation is a potential indicator of stress tolerance[12]. Proline also acts directly as an antioxidant to protect the cell from free radical damage and maintains a more reducing environment that is favorable for phytochelation synthesis and cadmium sequestration[8],[5] .

Table 5 Influence of heavy mtal stress on proline content of Vigna *unguiculata*

SL No	Treatment	Proline content(g)
01	Control	1.93 ±0.25
02	Cd (0.1g)	9.2±0.026
03	Cd (0.2g)	13.66±0.15

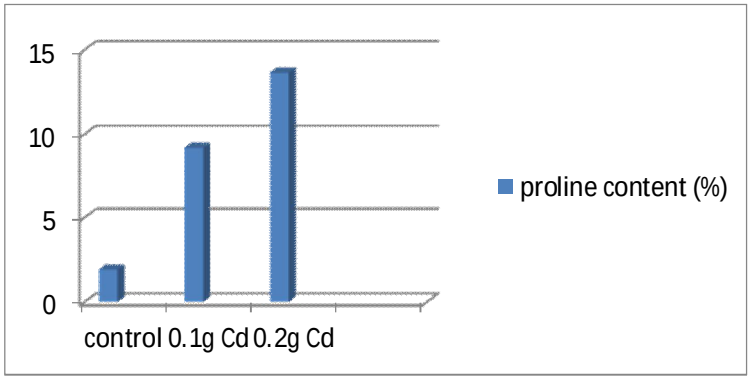

Figure 5 Influence of heavy metal stress on cowpea plant

F. Malondialdehyde (MDA)

In given table 6, Lipid peroxidation measured in terms of MDA content was reported to increase by 4.12(0.1g Cd) and 9.2 (0.2g Cd) treated cow pea plant. The control plant showed only 4.12 g of MDA. The lipid peroxidation level, as indicated by MDA accumulation, increased significantly under the heavy metal stress.

Table 6 Influence of salt stress Malondealdehyde

SL No:	Treatment	Malondialdehyde (MDA) (g)
01	Control	4.12±0.42
02	Cd (0.1g)	9.2±0.026
03	Cd (0.2g)	12.44±0.503

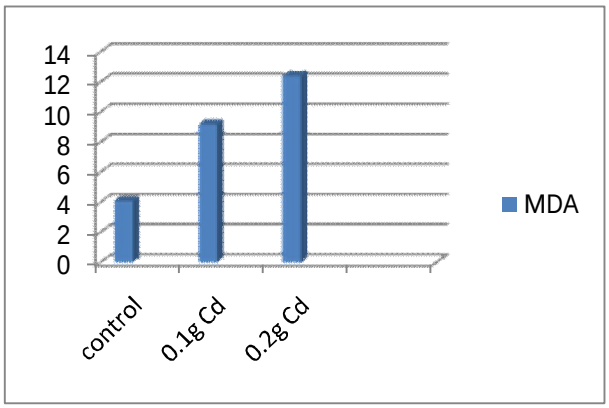

Figure 6 Influence of heavy metal stress on MDA content of cowpea plant

G. Membrane stability (MSI)

The results of MSI are given in table 7. It can be seen that both level of heavy metal stress induced reduced MSI in comparison to control. Cadmium induced reduction is reported to be 71.06% (0.1g NaCl) and 52.4% (0.2gNaCl).

Table 7.Influence of heavy metal stress on Membrane Stability Index (MSI %)

SL No:	Treatment	Membrane stability Index (MSI) (%)
01	Control	87.12±2.11
02	Cd (0.1g)	50.83±0.08
03	Cd(0.2g)	40±0.05

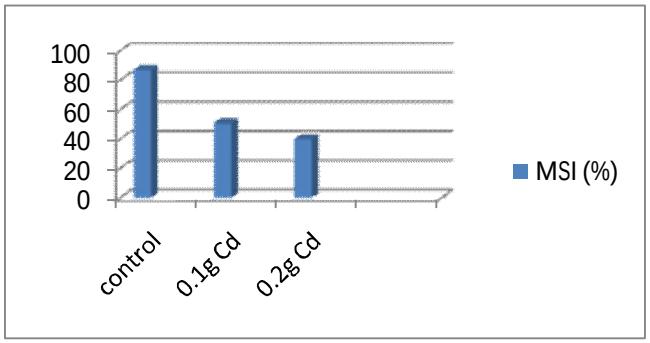

Figure 7 Influence of heavy metal (Cd) MSI of cow peaplant

H. Anatomy of stem

The cross section (anatomy) of stem of *Vigna unguiculata* was analyzed to assess the effect of various Cadmium concentrations and the anatomical adaptation of this plant to be acclimatized under drought stress. There was significant alteration in anatomical feature of stem of cow pea seedings imposed to various level of drought . Transverse section of stem *V. unguiculata* showed decrease in thickness of upper epidermal layer at 0.1g Cd and 0.2g Cd compared to control. The thickness of cortex layers of stem was reduced by different concentration level. The thickness of hypodermal layer and pith area of stem were decreased by 0.1g Cd, 0.2g Cd

treated plants. The pith cell diameter increased significantly in stem of Cadmium treated seedling as compared to control. There was significant change observed in the xylem vessels diameter of the stem of cow pea plant.

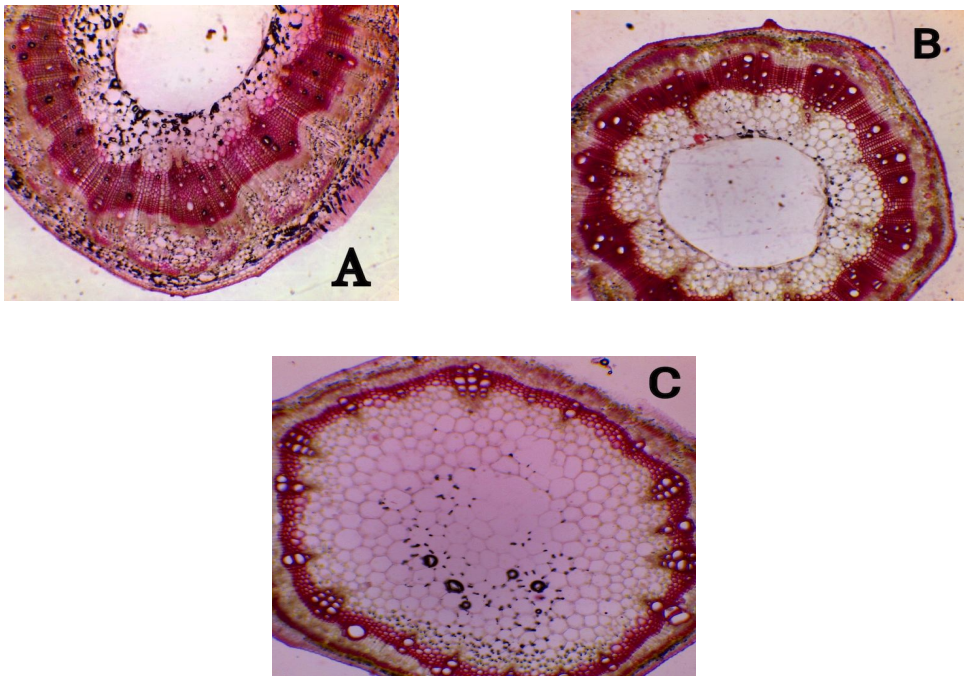

Plate 4: Figures show effects of Cd on stem anatomy (10 x magnifications) of *Vigna unguiculata* seedling treated with various concentration of drought
Figure (A) Control (0g Cd), Figure (B) 0.1g/L Cd, Figure(C) 0.2g/L Cd

VII. CONCLUSION

From the present study, it is concluded that plant morphological and biochemical parameters was decreased as the level of cadmium increased. Heavy metal cadmium resulted in altered morphological growth such as root and shoot length and increasing biochemical parameters antioxidant activity such as CAT and POD. The study concludes that environmental stress such as cadmium greatly influences the activity of both Catalase and Peroxidase. Heavy metal cause oxidative stress which affect thebiochemical and enzymatic component in plant cell heavy metal stress.

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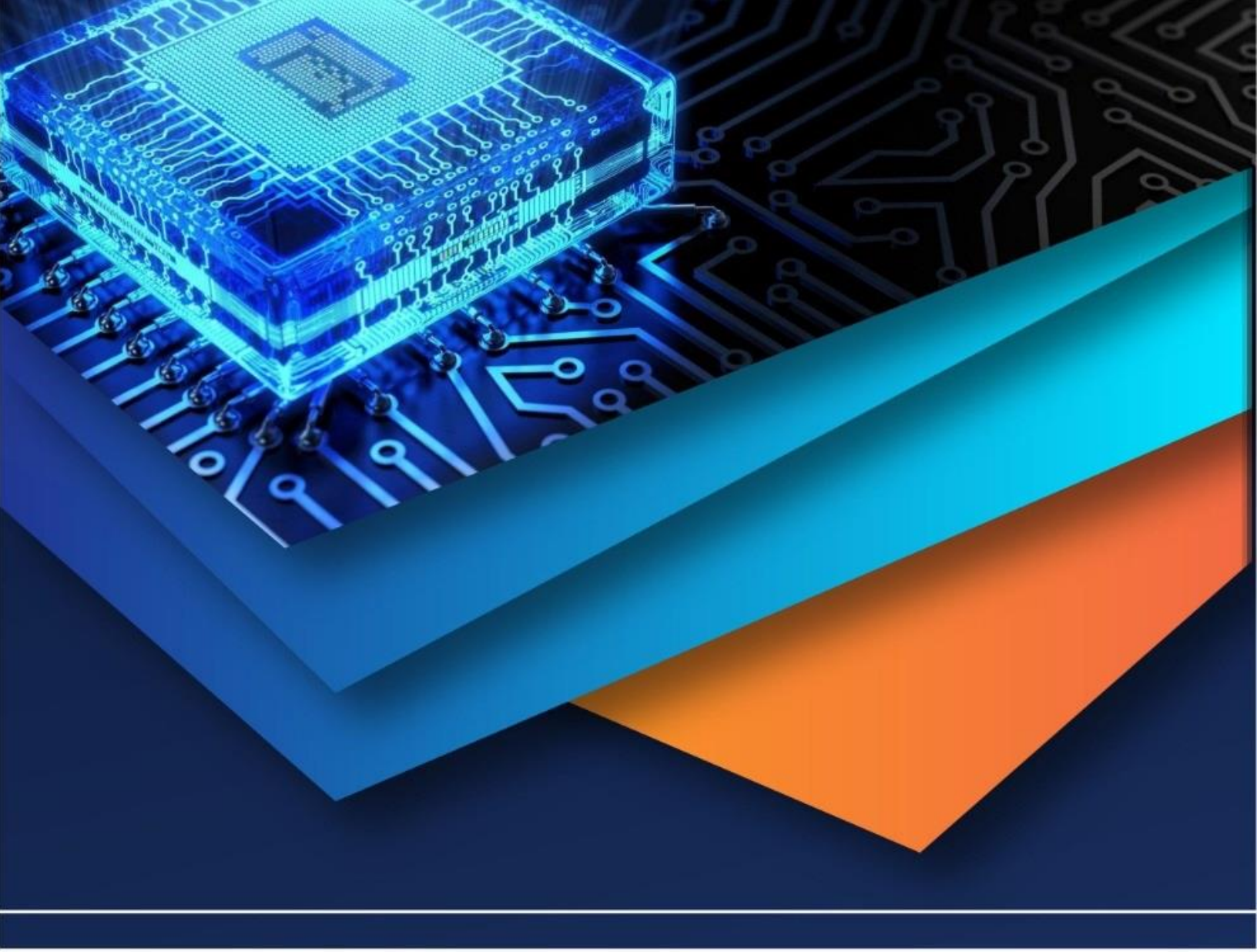

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