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# PERTURBATIONS IN THE GROWTH AND **BIOCHEMICAL CONTENTS OF CAJANUS** CAJAN L. (PIGEON-PEA) AGAINST CADMIUM CHLORIDE (CdCl<sub>2</sub>), A HEAVY **METAL COMPOUND**

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Abstract: In this current period of time, environmental heavy metal contamination is an increasing concern that affects all life forms. Among the issues concerning the heavy metals, cadmium is a toxic, silver-white metal element that can be readily absorbed by plants, enters the food chain, and poses a major health risk to humans. When crops are grown in regions where heavy metal levels are above threshold levels they become an integral part of the food chain. Pigeon pea is one among the major cultivated edible legume crops of India with incredible medicinal and nutritional values. Thus, it is very imperative to determine the effect cadmium on plant growth and physiochemical of pigeon pea. Hence, a pot experiment was carried out to study the effect of 25 µM/L, 50 µM/L, 75 µM/L and 100 µM/L CdCl<sub>2</sub> concentration on growth and physiology of pigeon pea and it showed that the  $CdCl_2$  induced remarkable decrease in root length, shoot length, chlorophyll and carotenoids levels, carbohydrate and proteins contents of the test plant. This can be attributed to the nutritional deficiency, reduced photosynthetic activities and enhanced reactive oxygen species production in response to cadmium stress. Finally, this manuscript may help to determine the essential remediation strategies to overcome the contamination of edible legume crops with Cd in agricultural soils in order to reduce the it's entry into human body through the food chain, that causes chronic poisoning and endangering human health by their consumption.

# IndexTerms - Cadmium Chloride, Heavy Metal Compound, Growth, Physiology, Food Chain.

# I. INTRODUCTION

The decline of the environment and ecosystem devastation has dramatically increased in recent years as a result of the industrialization process at accelerating pace. Both qualitatively and quantitatively, food security remains a primary issue for sustainable development worldwide. Thus human health and food security are at risk due to pollutants unanticipated negative impacts on the nutritional value of crops. The metabolomics of the organisms that live can be disrupted by metallic substance (such as Hg, As, Pb, Cd, and Cr), that can result in a rise in sickness as well as death. Cadmium (Cd), one of the metals, is known to be extremely poisonous and has physiological effects in both land and aquatic animals (Chellaiah, 2018). The use of phosphatic fertilisers that include Cd in crop production and other industry segments has resulted in a greater amount of Cd in the soils of agriculture (Bojorquez et al., 2016; Madhu and Sadagopan, 2020). According to Abbas et al., (2014), cd penetrates environments through a variety of human-induced processes and environmental pollutants. A poisonous, silvery-white metal substance called

cadmium (Cd) causes substantial risks for the well-being of people and animals when it accumulates in plant and land, due to its harmful effects in polluted soils, mobility, and ability to dissolve in water (Chen et al., 2016). Cd is readily consumed through plants, where it ultimately enters into the food chain thus poses a serious threat to people. According to research done by Satarug et al., (2010), human beings can absorb eighty percent of the metallic Cd which has been contaminated in vegetables and cereals through food consumption. Malignancy, urinary tract problem, fragility of the bones, cardiovascular disease, nephritis, as well as renal issues are among the most serious ailments caused by Cd toxicity (Nishijo et al., 2006; Nordberg et al., 2002; Yanggui et al., 2021). Since Cd could stay throughout the atmosphere for a period of time exceeding twenty years, it represents a potentially hazardous factor for humans (Ruiz et al., 2009).

At the opposite conjunction, the full process for Cd to harm crops is still not completely known. The primary methods that Cd absorbed to initiate the negative consequences in crops that hamper physiological and/or metabolic processes, which decrease the development of crop qualities, are now being investigated by researchers (Ashfaq et al., 2022). The metabolic processes of plants is hampered as a result of inhibition of metabolic processes such as primarily respiration, transpiration, flow of water, and exchange of gas by Cd either directly or through indirect means. Furthermore, Cd can disrupt the defences of antioxidants through raising the generation of oxygen species that are reactive, which in turn affects the metabolic processes and formation of chlorophyll of plants (Zhao et al., 2021). Since more Cd was absorbed and moved through plants as a consequence of chlorosis, the uptake of iron as well as lowering were dramatically increased (Genchi et al., 2020; Larbi et al., 2002). Furthermore, it disrupts plants ability to absorb metals including calcium, magnesium, and potassium at their optimum levels, leading to nutritional shortages (Dong et al., 2005; Greger et al., 1991; Larbi et al., 2002). Overall insufficient uptake of iron, zinc, and manganese (Lasat, 2002), that has been noted as a significant contributor to poorer production (Dong et al., 2005), additionally obstructs the proper function of a cell in a plant. Plants that have been exposed to Cd within the permissible limit experience oxidative damage and reduced electron transport chain performance, therefore affects the nucleic acid-related activities that control plant growth (Cuypers et al., 2010). Therefore, being one of the more significant culinary crops of legumes in India, it is crucial to understand the impact of cadmium poisoning on the biochemical aspects of pigeon peas. In an effort to establish a conceptual basis over subsequent studies upon the requirement to explore mitigation and approaches for remediation within crops regarding Cd exposure, which has a direct relationship with safety, quality of food, as well as well-being of humans, the current research was carried out to explore the impact of  $CdCl_2$  upon growth, as well as biological components of Cajanus cajan L.(Pigeon pea).

#### **II. MATERIALS AND METHODS:**

#### 2.1. Plant material and growth conditions:

Pigeon pea (*Cajanus cajan* L.) seeds that had been authenticated had been bought from the Sri Venkateswara Agriculture College (Acharya N G Ranga Agricultural University), Tirupati, India. For the experimentation, the chosen seeds having a consistent size along with no infections were employed. The seeds of *C. cajan* are meticulously cleaned using tap water before being sterilised using 80% ethanol over thirty seconds, after which they were changed to a 5% solution consisting of sodium hypochlorite for fifteen minutes and finally washed using de-ionized water. Following sterilisation, seeds were planted in pots (10 cm in height, 8.5 cm in diameter) that contained a compost-based mix of vermiculite & peat (1:4, w/w) combined along with sand (3:1, w/w) for propagation. In the greenhouse situated at Sri Venkateswara University in Tirupati, the pot experiment carried out in pursuant to the following standard growth conditions: temperatures of 28°C/20°C (Day/Light), a 14-hour photoperiod, 410–570 m<sup>-2</sup> s<sup>-1</sup> of average per day photosynthetic active radiation, and 75–85% of relative humidity.

#### 2.2. Soil characteristics and Experimental design:

The soil was obtained within the botanical conservatory (1-30 cm depth) at Sri Venkateswara University in Tirupati. The finely crushed soil then sterilised by autoclave allowed to dry for seven days, then pulverised using a mortar and pestle before being put over 2 mm tubes of sieving. Considering the research of Ramesh and Damodharam, (2017) the fundamental characteristics about the soil was determined. Clay (54.2%), silt (10.6%), soil (33.9%), pH (6.8), electrical conductivity (0.39 µMhos/cm), organic

matter (6.2 g/kg), available phosphorus (59.26 mg/kg), available potassium (97.45 mg/kg), total nitrogen (55.13 mg/kg), total copper (11 mg/kg), and soil Cd (0.16 mg/kg) had been it's physiochemical attributes. The subsequent full randomised block design was used with five replications, and every single pot received with a comparable amount of soil. For the experiment of the control, water has been provided everyday to keep the soil moistened at a level between 75 - 85%. In contrast to the treatment condition, soil had been experimentally supplied with various CdCl<sub>2</sub> concentrations (25, 50, 75, and 100  $\mu$ M/L).

#### 2.3. Determination of growth and biochemical parameters:

In order to compare the growth rate and physiological responses of being treated and untreated seedlings subjected to varying amounts of CdCl<sub>2</sub> stress, offsets of pigeon pea that were 10, 20, and 30 days old were collected.

**2.3.1.** *Root length:* The plants were cautiously removed so as not to harm their root system. To get rid of the sandy and clay granules stuck on its root system, everything was washed underneath running water. With a scale to measure, the root length was assessed from the top of the root area to its tip, and the results have been recorded as (cm) centimetres.

**2.3.2.** *Shoot length:* The young plants were gently removed so as not to harm the shoot structure. Using a normal scale, each plant's length of shoot was determined by measuring starting at the collar area to the apex of the plant, then the results was recorded as cm.

**2.3.3.** *Photosynthetic pigments (total chlorophyll and carotenoids):* The technique outlined by Arnon, (1949) has been applied for estimating the concentrations of both chlorophyll plus carotenoids. With cold acetone at a concentration of 80%, photosynthetic pigments have been extracted using a frozen leaf powder sample. Employing a UV-visible spectrophotometer, the supernatant absorption was measured at 480, 645, and 663 nm after centrifuged at 10,000 g for ten minutes.

**2.3.4.** *Total carbohydrates:* In accordance with the procedure outlined by DuBois et al., (1956), the total levels of carbohydrates were determined utilising a phenol-sulfuric acid test with glucose as a basis for comparison.

**2.3.5.** *Total protein:* This was calculated by multiplying a sample's organic nitrogen content with the protein conversion factor, whose value is typically 6.25 (Lang, 1958). The total amount of protein was colorimetrically quantified at 540 nm against a blank using a spectrophotometer in accordance with Gopal and Rizvi, (2008).

#### 2.4. Statistical Analysis:

The results were expressed as the mean  $\pm$  standard error of the five determinations.

#### **III. RESULTS:**

The findings of this research appraisal of the root lengths of pigeon pea plants collected across three offset ages (10, 20, and 30) under the presence a variety of  $CdCl_2$  levels are shown in Table 1 & Fig 1.

Concentration	10 <sup>th</sup> Day	20 <sup>th</sup> Day	30 <sup>th</sup> Day
Control	2.41±0.189	3.81±0.112	4.14±0.220
25 µM/L	2.12±0.011	2.34±0.020	2.41±0.100
50 µM/L	1.84±0.120	1.89±0.110	2.11±0.122
75 µM/L	1.52±0.082	1.62±0.011	1.87±0.121
100 µM/L	1.27±0.003	1.45±0.031	1.72±0.017

Table 1. Effect of cadmium chloride on root length (cms) of Cajanus cajan L

Values are mean  $\pm$  standard error of five replications

Within all, the length of the roots proved greater (( $2.41\pm0.10$ ,  $2.11\pm0.12$ ;  $1.87\pm0.12$ ,  $1.72\pm0.01$  and  $2.34\pm0.02$ ,  $1.89\pm0.110$ ,  $1.62\pm0.011$ ,  $1.45\pm0.031$  cms) in the 30<sup>th</sup> and 20<sup>th</sup> days of offsets upon being provided alongside 25, 50, 75, and 100  $\mu$ M/L of CdCl<sub>2</sub>, respectively, instead of 10<sup>th</sup> day offsets ( $2.12\pm0.011$ ,  $1.84\pm0.120$ ,  $1.52\pm0.082$ ,  $1.27\pm0.003$  cms), following being made augmented with 25, 50, and 75  $\mu$ M/L of CdCl<sub>2</sub>.

Fig 1. Effect of cadmium chloride on root length (cms) of Cajanus cajan L.



For offsets that were 10, 20, and 30 days old, the instillation of  $CdCl_2$  under levels ranging from 25, 50, 75, and 100  $\mu$ M/L prescribed to a shorter root length than untreated controls. Likewise, after intake alongside varying levels about 25, 50, 75, and 100  $\mu$ M/L during the 10<sup>th</sup>, 20<sup>th</sup>, and 30<sup>th</sup> day of the pigeon pea offsets, their shoot length reduced to 4.12±0.118, 3.86±0.077, 3.24±0.110 and 2.59±0.013; 4.26±0.290, 3.93±0.092, 3.41±0.071 and 2.81±0.125; 4.46±0.212, 4.11±0.115, 3.64±0.051, and 3.14±0.032 cms, correspondingly (Table 2 & Fig 2).

Table 2. Effect of cadmium chloride on shoot length (cms) of Cajanus cajan L

Concentration	10 <sup>th</sup> Day	20 <sup>th</sup> Day	30 <sup>th</sup> Day
Control	4.28±0.102	5.20±0.115	5.84±0.105
25 µM/L	4.12±0.118	4.26±0.290	4.46±0.212
50 µM/L	3.86±0.077	3.93±0.092	4.11±0.115
75 μM/L	3.24 <mark>±0.110</mark>	3.41±0.071	3.64±0.051
100 µM/L	2.59±0.013	2.81±0.125	3.14±0.032

Values are mean ± standard error of five replications

Fig 2. Effect of cadmium chloride on shoot length (cms) of Cajanus cajan L.



The 25  $\mu$ M/L solution had the longest shoot length between these days worth of the pigeon pea offsets as contrasted with the remaining regimens (50, 75, and 100  $\mu$ M/L). For 100  $\mu$ M/L level, the shortest shoot length of 2.59±0.013, 2.81±0.125 and 3.14±0.032 cms were observed when contrasted with untreated pigeon pea plants. Following treatment upon 100  $\mu$ M/L over the

Total chlorophyll (mg.g<sup>-1</sup> fwt)

1.6

1.2

0.8

0.4

0

Control

25 µM/L

30<sup>th</sup>, 20<sup>th</sup>, and 10<sup>th</sup> day of pigeon pea offsets, the total amount of chlorophyll level of the freshly developed plants was lowered to 0.802±0.010, 0.818±0.081 and 0.936±0.054105 mg.g<sup>-1</sup> fwt (fresh weight), equated with that of the untreated control group (2.106±0.051, 2.580±0.071 and 2.331±0.077 mg.g<sup>-1</sup> fwt), correspondingly (Table 3 & Fig 3).

Concentration	10 <sup>th</sup> Day	20 <sup>th</sup> Day	30 <sup>th</sup> Day
Control	2.331±0.077	2.580±0.071	2.106±0.051
25 µM/L	2.147±0.027	2.114±0.110	2.083±0.092
50 µM/L	1.717±0.011	1.603±0.115	1.510±0.021
75 µM/L	1.424±0.062	1.345±0.023	1.217±0.112
100 µM/L	0.936±0.054	0.818±0.081	0.802±0.010

Table 3. Effect of cadmium chloride on total chlorophyll (mg.g<sup>-1</sup> fwt) of *Cajanus cajan* L



Fig 3. Effect of cadmium chloride on total chlorophyll (mg.g<sup>-1</sup> fwt) of Cajanus cajan L.

However, compared with the remaining CdCl<sub>2</sub> regimens (50 µM/L, 75 µM/L, and 100 µM/L) used in this investigation, the greatest levels of chlorophyll was determined on days 10<sup>th</sup>, 20<sup>th</sup>, and 30<sup>th</sup> day plants under a level of 25 µM/L (2.147±0.027, 02.114±0.110 and 2.083±0.092 mg.g<sup>-1</sup> fwt, correspondingly. The overall levels of carotenoids appeared to be least in the 100  $\mu$ M/L of CdCl<sub>2</sub> regimens used in the present research on the 10<sup>th</sup>, 20<sup>th</sup>, and 30<sup>th</sup> day young pigeon pea offsetting (Table 4 & Fig 4). The least carotenoids in total amount of 1.623±0.119, 1.520±0.011 and 1.440±0.016; 1.201±0.020, 1.084±0.014 and  $0.952\pm0.023$ ;  $0.881\pm0.026$ ,  $0.701\pm0.117$  and  $0.620\pm0.105$  mg.g-1 fwt were determined following the application of 25  $\mu$ M/L, 50  $\mu$ M/L, and 75  $\mu$ M/L of CdCl<sub>2</sub> over 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day aged pigeon pea offsets as opposed to the untreated control offsets.

50 µM/L

Concentrations of cadmium chloride (CdCl<sub>2</sub>)

75 µM/L

100 µM/L

Table 4. Effect of cadmium chloride on to	al carotenoids (mg.g	<sup>1</sup> fwt) of <i>Cajanus</i>	cajan L.
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Concentration	10 <sup>th</sup> Day	20 <sup>th</sup> Day	30 <sup>th</sup> Day
Control	1.751±0.171	1.902±0.201	1.655±0.271
25 µM/L	1.623±0.119	1.520±0.011	1.440±0.016
50 µM/L	1.201±0.020	1.084±0.014	0.952±0.023
75 µM/L	0.881±0.026	0.701±0.117	0.620±0.105
100 µM/L	0.520±0.014	0.423±0.020	0.320±0.108

Values are mean ± standard error of five replications

Fig 4. Effect of cadmium chloride on total carotenoids (mg.g<sup>-1</sup> fwt) of Cajanus cajan L.



The overall amount of carbohydrates within pigeon pea offsets were observed to fall significantly with ageing ( $10^{th}$ ,  $20^{th}$ , and  $30^{th}$  day) along with dosage dependently with varied doses of CdCl<sub>2</sub> (Table 5 & Fig 5). In contrast with the remaining levels (25  $\mu$ M/L, 50  $\mu$ M/L, and 75  $\mu$ M/L) of CdCl<sub>2</sub>, the overall quantity of carbohydrates found in the 100  $\mu$ M/L treated pigeon pea seeds reduced to 16.819±0.039, 14.718±0.032 and 10.217±0.091 mg.g<sup>-1</sup> fwt, respectively.

Table 5. Effect of cadmium chloride on total carbonydrates (ing.g Twt) of Cajanus caj
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Concentration	10 <sup>th</sup> Day	20 <sup>th</sup> Day	30 <sup>th</sup> Day
Control	19.031±0.085	20.461±0.039	18.612±0.061
25 µM/L	18. <mark>124±0.052</mark>	17.184±0.080	14.087±0.035
50 µM/L	17.014±0.039	16.706±0.050	13.914±0.031
75 μM/L	17.003± <mark>0.036</mark>	15.211±0.038	12.818±0.079
100 µM/L	16.819±0. <mark>03</mark> 9	14.718±0.032	10.217±0.091

Values are mean ± standard error of five replications

Fig 5. Effect of cadmium chloride on total carbohydrates (mg.g<sup>-1</sup>fwt) of Cajanus cajan L.



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However, when compared against all levels of CdCl<sub>2</sub> treatment options, overall carbohydrates were greater in untreated controls, such as  $18.612\pm0.061$ ,  $19.031\pm0.085$  and  $20.461\pm0.039$  mg.g<sup>-1</sup> fwt. The results presented within Table 6 & Fig 6 showed how the varied treatments of CdCl<sub>2</sub> (25  $\mu$ M/L, 50  $\mu$ M/L, 75  $\mu$ M/L, and 100  $\mu$ M/L concentration) had an impact on the overall amount of protein and appeared to be lower in  $10^{\text{th}}$ ,  $20^{\text{th}}$ , and  $30^{\text{th}}$  day aged pigeon pea offsets. As juxtaposed with varied amounts of CdCl<sub>2</sub>, the pigeon pea seedlings those affected without any treatment of CdCl<sub>2</sub> on 10, 20, and 30 day offsets exhibited greater protein levels, with the corresponding values of  $13.629\pm0.321$ ,  $14.091\pm0.015$  and  $15.874\pm0.128$  mg.g<sup>-1</sup> fwt.

Concentration	10 <sup>th</sup> Day	20 <sup>th</sup> Day	30 <sup>th</sup> Day
Control	13.629±0.321	15.874±0.128	14.091±0.015
25 µM/L	12.426±0.013	11.275±0.032	9.268±0.081
50 µM/L	10.189±0.091	9.172±0.017	8.181±0.096
75 µM/L	9.802±0.016	8.641±0.030	7.163±0.021
100 µM/L	8.901±0.011	7.133±0.081	6.093±0.061

Table 6. Effect of cadmium chloride on total proteins (mg.g<sup>-1</sup> fwt) of Cajanus cajan L.

Values are mean ± standard error of five replications





#### **IV. DISCUSSION:**

Food quality and safety, atmospheric heavy metal pollutants, and the well-being of humans are all interconnected. According to El-Kady and Abdel-Wahhab (2018), the quantity about heavy metals within the natural environment has dramatically grown in recent times. Within developing as well as advanced nations, there are different ways to accumulate heavy metals found in food crops, such as atmospheric release, manure from livestock, the cultivation of crops through waste water and/or water that has been contaminated with metallopesticides or herbicides in particular phosphate-based fertilisers, as well as sewage sludge based supplements (Prabhat et al., 2019; Fasih et al., 2021). Because of their confined nature and reliance upon soil, crops are often subject to the adverse consequences of these contaminants (Ali et al., 2020). As a result, a variety of contaminants containing heavy metals which differ in quantity, variation, and severity frequently attack crops.

Whenever agricultural crops are grown in locations where levels of heavy metals stand over regulatory boundaries, such heavy metals eventually enter into the food chain (Ashfaq et al., 2022). For common culinary legume plant grown throughout the tropics

and semi-arid regions within India and across the rest of the world is the pigeon pea (*Cajanus cajan* L.). It is a fast-growing shrub that is a member of the Fabaceae family. Its seeds provide a lot of protein. The kernels and foliage can be utilised as animal feed, while the green pods have been eaten as a vegetable. Having a wealth of compounds that are bioactive, it has been used as a plant for medical purposes (Zavinon and Sagbadja, 2019). Their tender leaves can be cooked to eliminate infections, stop the flow of blood, and strengthen immunity across traditional Chinese medicine. It also serves as an analgesic to ease agony (Saxena et al., 2010; Hayat et al., 2021). Such agricultural crop food often include high concentrations of naturally occurring bioactive chemicals with antihypertensive, anti-allergic, and anti-oxidative effects. As a result, these advantages coming from food become unavailable or diminished in crops as long as heavy metal exposure alter their metabolic activity (Oladele et al., 2019). Because of the aforementioned, the present investigation was undertaken to assess the impact of CdCl<sub>2</sub> on the plant *Cajanus cajan* L. (pigeon pea) development and physiological components.

When viewed alongside the control group without treatment in the present investigation,  $CdCl_2$  treatments (25 µM/L, 50 µM/L, 75 µM/L, and 100 µM/L) negatively impacted both the root and shoot length of 10, 20, and 30 day old pigeon pea offsets (Table 3 & Fig 3). Previous investigation has shown that Cd stress results in comparable decreased growth metrics (shoot length and root length) within various plant species (Swapna, 2016; Aprile et al., 2019; Hassan et al., 2020; Ullah et al., 2020; Hayat et al., 2021). The connection of cadmium with the walls of cells as well as middle lamellae, fosters the cross-linking of pectin and minimises the development of cells, and this could possibly be responsible for the development-inhibiting consequences caused by Cd against pigeon pea (*Cicer arietinum* L.), lentil (*Lens culinaris* L.), and various other legumes (Yruela, 2005; Llases et al., 2019; Fasih et al., 2021). Furthermore, the pigeon pea plant shoot as well as root length may be inhibited by lower activity of photosynthesis, nutritional shortage, and increased generation of reactive oxygen species (ROS) when exposed to cadmium (Hayat et al., 2021).

As contrasted to controls without treatment, the level of chlorophyll present in the plants that were treated with increasing  $CdCl_2$ concentration (25  $\mu$ M/L, 50  $\mu$ M/L, and 75  $\mu$ M/L) dramatically decreased, notably for 100  $\mu$ M/L CdCl<sub>2</sub> concentration across all the plants that were treated. The amount of total chlorophyll reduced within the plants that were impacted as the Cd content ascended. Among different biosynthetic enzymes which cd had been demonstrated to inhibit, resulting in the degradation of chlorophyll, include protochlorophyllide reductase as well as  $\delta$ -aminolaevulinic acid dehydratase (Prasad et al., 1999). The finding we made was in line with earlier studies wherein different kinds of plants showed a comparable restriction in photosynthetic properties. Researchers also mentioned that a reduction in chlorophyll following exposure to heavy metals could have been brought on by the inhibition of enzymes involved in the production of chlorophyll or its breakdown (Chettri et al., 1998; Bachaieb et al., 2016). An additional potential explanation is that the affinity for binding of cadmium may replace the  $Mg^{2+}$ ion in the chlorophyll reducing the former's ability to absorb light. Furthermore, the abundance of all these metals near the apparatus for photosynthesis and a drop in the partial pressure of  $CO_2$  throughout the stroma cause the apertures in the stomata to close down, which lowers the pace of transpiration, stomatal conductivity, and intracellular levels of CO<sub>2</sub> (Hayat et al., 2021). A significant decrease in the photosynthesis rate was also seen for many varieties of plants undergoing stress from Cd, according to many research efforts (Sawhney et al., 1990; Sheoran et al., 1990a; 1990b). According to research by Dong et al., (2005), Padmaja et al., (1990), and Satyakala, (1997) the plants grown in high Cd soil revealed negative effects on the production of chlorophyll along with additional pigments involved in photosynthesis as well as other physiological activities. The amount regarding chlorophyll appears inversely proportional to the rate of photosynthesis while can serve as an indicator on how well leaves are capable of absorbing and utilising light energy. Chlorophyll a, which is the primary component in photosynthesis, transforms sunlight into chemical activity at the light reaction centre, whereas Chlorophyll b is in charge of absorbing and transferring sunlight (Sun et al., 2010). Surprisingly, the Zhao et al., (2021) research findings, where minimal cadmium level encouraged an upsurge in the amount of chlorophyll whereas an elevated level reduced the production of chlorophyll, seem to be at odds regarding our findings. In their research, the amount of chlorophyll initially raised after which it decreased at escalating levels of Cd in sassafras plants. This could have been caused by a small quantity of Cd ions stimulating the development in

porphyrin rings of leaf, speeding the assimilation of Mg, Fe, K, and P micronutrients through the soil, consequently boosting the amount of chlorophyll (Chen et al., 2020).

From the current study, overall carotenoids content of 10, 20, and 30 day aged pigeon pea offsets had decreased when each the crops underwent exposure to higher CdCl<sub>2</sub> concentrations (25  $\mu$ M/L, 50  $\mu$ M/L, and 75  $\mu$ M/L). Similar findings to those in the current study were made by Shu et al., (2012) and Saadaoui et al., (2022), who found that cuttings and juveniles of the plants Jatropha curcas L. and Vicia faba L. displayed less amount of carotenoid when exposed to elevated concentrations of the heavy metals being worked. The apparatus for photosynthesis and associated pigments, the metabolism regarding carotenoids as well as chlorophyll were main sites where Cd acts (Rafiq et al., 2014). Additionally, carotenoids have been demonstrated to shield the pigments found in chlorophyll from stress caused by heavy metals (Boamponsem et al., 2012), therefore if the process of making them is disrupted, this would have an immediate impact on the concentration of chlorophyll within plant leaves (Wang et al., 2020). The results of our investigation (Table 3, 4 and Fig 3, 4) show a substantial association among the overall chlorophyll concentration and carotenoids, which supports the argument. The impacts of various CdCl<sub>2</sub> levels upon overall protein as well as overall carbohydrate levels are displayed in Table 5, 6 and Fig 5, 6. In 10th, 20th, and 30th day age pigeon pea offsets, CdCl<sub>2</sub> levels of 25, 50, 75, and 100  $\mu$ M/L dramatically reduced the total amount of proteins and total carbohydrates content found in the plants that were treated. Chhetri et al., (2004) found that elevated levels of stress from heavy metals exerted a negative impact upon the content of Vigna umbellata's proteins and Phaseolus vulgaris's carbohydrates (Hamid et al., 2010). The similar pattern versus the impact of heavy metal impact on the average of total carbohydrate and protein content was seen with test crops such as Vigna subterranean L. and Zea mays L. (Oladele et al., 2019). This was presumably due to the harmful effects of cadmium at high concentrations in crops, resulting in changes in the regulation of carbohydrate as well as protein synthesis (Moya et al., 1993; Dong et al., 2005).

The results of this research generally demonstrated that the cultivation of pigeon-pea plant seeds with 25  $\mu$ M/L, 50  $\mu$ M/L, 75  $\mu$ M/L, and 100  $\mu$ M/L levels alleviated the growth along with physiological variables, likely as a result of 1) deficiencies in nutrients, 2) the hindered the production of chlorophyll, pigments needed for photosynthesis and their photosynthetic activities, 3) perturbed protein and carbohydrate metabolisms, and 4) pectin's increased cross-linking enhanced cell damage caused by the abundant reactive oxygen species that rose through the aftermath of cadmium stress. In order to comprehend the underlying causes of cadmium toxicity's on *Cajanus cajan* L. (pigeon-pea) growth along with physiological traits, additional research on proper remediation techniques that primarily focus on providing optimal plant nutrition ought to be conducted with the goal to reduce the adverse impacts of cadmium on crops while avoiding it from entering the food chain.

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