ISSN 1533-9211

DOI: 10.5281/zenodo.8431074

# EFFECT OF CADMIUM ON MORPHOPHYSIOLOGICAL CHARACTERISTICS AND BIOACTIVE COMPOUNDS OF *PHASEOLUS VULGARIS* L

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**Abstract**

*Phaseolus vulgaris* L. is the best known species of the genus Phaseolus in the family Fabaceae. It is an important food source for humans all around the world, with increasing consumption and economic significance. The purpose of this study was to see how cadmium (Cd) effects physico-chemical parameters like germination rate, plant height, root length, pod length, number of seeds per pod, number of pods per plant, 100 seed weight, chlorophyll, protein content, starch content, total soluble sugars, total flavonoid content, tannins, proline content, and total phenolic content in *Phaseolus vulgaris* variety (HUR-137). Seedlings were exposed to different concentrations of cadmium 25 mg/kg, 50 mg/kg, 75 mg/kg, and 100 mg/kg. Results indicated that cadmium significantly decrease germination rate, plant height, root length, pod length, number of seeds per pod, number of pods per plant, and 100 seed weight. Cadmium also induced a decrease in chlorophyll content, protein content and starch content at 25 mg/kg, 50 mg/kg, 75 mg/kg, and 100 mg/kg cadmium treatments; however total soluble sugars and proline content increased significantly with increasing cadmium concentration. Total phenolic content, and total flavonoid content also increases by increasing Cd concentration. But hydrolysable tannins and condensed tannins showed a significant decrease at higher cadmium concentration.

**Keywords**: Physico-Chemical Parameters, Cadmium, Phenolic Compounds, Chlorophyll, Germination, Flavonoid

## INTRODUCTION

The kidney bean (*Phaseolus vulgaris* L.), a self-pollinated, diploid (2n = 2x = 22) legume crop with a genomic size of 473 Mb, belongs to the Fabaceae (legume or bean family), (Priya and Manickavasagan, 2020). It is grown for both its green pods and its dry seeds all throughout the world (Adikshita and Kansak, 2017). The kidney bean (*Phaseolus vulgaris* L.), also known as "Rajmash" in India, is one of the world's most frequently planted grain legume crops.(Mavric, I. and Vozlic, S.J. 2004).The seed pods are slender, measuring 8-20 cm × 1-2 cm, and can have up to 12 seeds per pod, though most types only have 4-6 seeds. Depending on the cultivar, seeds come in a wide range of colors (Purseglove, 1968; Wortmann, 2006), and the size of the seeds varies greatly, ranging from 150 to 900 g per 1000 seeds (Brink and Belay 2006; Wortmann, 2006). Kidney bean is a high-quality commercial pulse crop grown in the northwestern Himalayan area of India. Small and marginal hill farmers primarily cultivate the crop in Himachal Pradesh, Jammu & Kashmir, and Uttrakhand. In terms of nutrition, kidney beans stand out among plant-based diets because they are an affordable source of high-quality

ISSN 1533-9211
DOI: 10.5281/zenodo.8431074

protein (between 21 and 25 percent), earning them the nickname "Poor Man's Meat" (USDA Dietary Guidelines, 2010). Kidney beans are more than just a food source because they are abundant in a variety of other substances, including vitamins, particularly folates from the B group of vitamins, antioxidants, polyunsaturated fatty acids, minerals like sulphur, potassium, phosphorus, calcium, magnesium, and magic wands, as well as micronutrients like iron, zinc, copper, iodine, and manganese (Sofora et al., 2021). There are several negative environmental factors that have an impact on plant production, with HM pollution ranking as one of the most significant ones (Jócsák, et al., 2022). Heavy metals (HMs) are naturally occurring, non-biodegradable components of the Earth's crust that, as a result of frequent anthropogenic activity, build up excessively and persist forever in the ecosystem (Bucker-Neto et al., 2017). Arable soil contamination with HMs is a serious environmental threat that is becoming a global issue. Cadmium (Cd), one of many HMs, is a non-essential element that causes concern because it is toxic to both plants and animals even at low concentrations and have a tendency to enrich the biosphere through the food chain (Chunhabundit, R. 2016). Poor growth and low biomass production are the results of Cd interactions with numerous critical processes at the level of the entire plant. Plant species, Cd concentration, and developmental stages are all factors that affect how sensitive a plant is to Cd toxicity (He et al., 2010). One of the naturally occurring heavy metals that are extremely toxic to both plants and people is cadmium. Farmland has recently been severely and widely contaminated by the rise in Cd content in soils. The buildup of Cd in plants has harmful effects on their ability to grow normally. For instance, Cd alters the activity of enzymes, the uptake and utilisation of vital nutrients, produces reactive oxygen species (ROS), and damages membrane systems, respiration, and photosynthesis (Li et al., 2023). In agricultural soil, 100 mg/kg of cadmium (Cd) is the recommended limit. High levels of Cd in the soil cause visible signs of injury in plants, such as chlorosis, growth inhibition, browning of the root tips, and ultimately death (Usman et al., 2020). The uptake, transport, and utilisation of several elements (Ca, Mg, P, and K) as well as water by plants have all been shown to be hampered by Cd. Through the inhibition of nitrate reductase activity in the shoots, Cd also decreased nitrate absorption and its transport from roots to shoots (Saadaoui et al., 2022). The aim of present study is to assess the effect of different concentrations of Cadmium on physicochemical and bioactive parameters of *Phaseolus vulgaris* L. variety (HUR-137).

## MATERIALS AND METHODS

The seeds of bean (*Phaseolus vulgaris*) variety HUR-135 were obtained from Indian Institute of Pulses Research, Kanpur (IIPR). Seeds with uniform size, colour and weight were chosen for the experimental purpose. The soil used in the experiment was red soil 40%+sandy loam 60% in nature and pH of the soils was 7.2. It contains major nutrients of 118 kg available N, 88 kg P and 106 kg k/ha and micronutrients of 21.89 mg available Cu, 219.11 mg Fe, 168 mg Mn and 28.13 mg Zn/kg, cadmium was not available in this experimental soil. The cadmium chloride ($Cd\,Cl_2$ ½ $H_2O$) was used as cadmium source.

ISSN 1533-9211
DOI: 10.5281/zenodo.8431074

The pot culture experiments were conducted in green house of Department of Botany, Glocal University. Bean plants (*Phaseolus vulgaris*) were grown in pots containing untreated soil (Control) and soil mixed with various levels of cadmium (viz., 0, 25, 50, 75 and 100 mg/kg). The inner surfaces of pots were lined with a polythene sheet. Each pot contained 3 kg of air dried soil. Six seeds were sown in each pot. All pots were watered to field capacity daily. Plants were thinned to a maximum of three per pots, after a week of germination. Each treatment including the control was replicated three times. Three plants from each replicates of pot were analyzed for the various physico chemical and bioactive parameters.

**Physical Parameters**

**Percent Germination:**

To calculate the percentage of germination, we counted seeds sprouted per pot every day until the seventh day, knowing that germination is positive when its radical reaches 5 mm in length. After the seventh day of seed germination, radical's length of germinated seeds was measured on cm.

To obtain percentage of germination, we used the formula:

$$G\ (\%) = (\text{number of germinated seeds} / \text{total number of seeds}) \times 100$$

**Plant height:** The Tape rule was used to determine plant height, which was measured from the plant's base above ground to the last expanded leaf at the growing tip and expressed in cm.

**Pod length:** The length of the pod was measured from the commencement of the stalk to the peak, as well as the breadth and thickness of the pod in a cutting plane perpendicular to the fruit's main axis, leading through its centre, and computed using an electronic slide caliper with 0.01 mm accuracy.

**Root length**: A ruler was used to measure the root length in cm.

**Number of pods/ plant:** We counted and recorded the total number of pods per plant.

**Number of seeds / pod:** The quantity of seeds in each plant's randomly picked pods was counted, and the average was calculated.

**100 seed weight:** Three randomly picked samples of sun dried seeds from the experimental field were weighed in grams and averaged at 100 seed weight.

**Bio-Chemical Parameters**

Total protein content: The protein content in leaf tissue was determined according to Bradford (1976), Fresh leaves were homogenized and extracted with 5 mM buffer Tris-HCl, pH 7.2, 0.25 M sucrose and 1 mM $MgCl_2$. The extract was incubated with Bradford reagent in darkness. Absorbance was measured at 595 nm and the amount of proteins was expressed in equivalents of BSA bovin serum albumin (mg eq. BSA g-1 FW), used as standard.

ISSN 1533-9211

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**Total chlorophyll content**. For photosynthetic pigments (chlorophyll *a* and *b*, carotenoids) analysis, fully developed trifoliate leaves were extracted with 80% acetone. Pigments contents were determined spectrophotometrically (spectrophotometer Shimadzu UV 1800) at following wavelengths: 663, 646 and 470 nm and calculated according to Lichtenthaler and Wellburn (1983).

Starch content: Starch was analyzed by the enzymic/colorimetric method of Holm et al. (1986). This method comprises the following main steps: incubation with Termamyl for 15 min at boiling temperature, digestion with amyloglucosidase at 60 "C (30 min), and free glucose measurement using the combined glucose oxidase/peroxidase colorimetric assay.

**Total soluble sugars:** Leaves were used for soluble sugar determination; extraction was done with 80% (v/v) ethanol. Sugar content of resulting solution was determined following to Dische (1962) using anthrone reagent. Absorbance was measured at 620 nm. Standards of glucose were prepared and analyzed in the same way to obtain a calibration curve. Soluble sugars content was calculated as mg glucose equivalent g-1 FW.

**Total phenolic content:** The Folin-ciocalter method was used to determine the total phenolic content (Heimler *et al*. 2005). Phenolic compounds were extracted from shoot and roots dried samples of *Phaseolus vulgaris* L. The amount of total phenolics in the extract was determined using Folin Ciocalteu reagent as described by Meyers *et al*. (2003). Absorbance was measured at 750 nm and the results expressed in equivalents of gallic acid (mg eq. GA g-1 DW), used as standard.

**Total Flavonoid content:** Colorimetric aluminum chloride method was used for flavonoids and flavonols determination, using optimized protocols established by Bahorun *et al*. (1996); Kumaranand Karunakaran (2007a, b), respectively. Absorbance was measured at 430 and 440 nm respectively, and the amount of flavonoids and flavonol were expressed in equivalents of Quercetin (mg eq. Q g-1 DW), used as standard.

**Tannins:** Hydrolysable tannins content was determined as described by Mole and Waterman (1987) and Swere quantified according to standard curve prepared for tannic acid. Results were expressed in equivalent of tannic acid (mg eq. TA g-1 DW). Condensed tannins contents were determined following the method described by Swain and Hillis (1959). The absorbance was measured at 500 nm. The amount of condensed tannins was expressed in equivalents of catéchin (mg eq. CAg-1 DW), used as standard

Proline content: Proline content was determined following to Bates et al. (1973), using ninhydrin reagent. The absorbance was measured at 520 nm. Reference standards of proline were prepared and analyzed in the same way to obtain a calibration curve. Results were expressed on µg.g-1 DW.

**Statistical analyses**

All data presented are the mean values of three replicates ± standard deviation (SD). Statistical analysis was carried out by student analysis at 5%, 1% and 0.1% significance level, using statistical software package STATISTICA version 8.0.

The Seybold REPORT
ISSN 1533-9211
DOI: 10.5281/zenodo.8431074

## RESULT

In the present study, *Phaseolus vulgaris* L. plants were exposed to different concentrations of cadmium. Physico-chemical parameters including changes in plant growth, Chlorophyll content, oxidative stress, antioxidant enzymes and metabolites activities etc were deduced.

### Physical parameters

Effect of various concentrations of Cd on Seed germination, Plant height, root length, pod length, number of seeds/pod, number of pods/plant and 100 seed weight

Smaller number of seeds germinated in comparison with control variant was identified when different concentrations of Cd were added. Seed germination was affected by different cadmium concentrations; it was lowered in *Phaseolus vulgaris* variety HUR-137 when Exposure to Cd. Cadmium caused a significant reduction in the germination percentage. In the Cd 100 mg/kg treatment, seed germination of HUR-137 variety was significantly affected compared to the control; germination was reduced by 68.32%. At 25 mg/kg Cd treatment, there was no significant difference between the control and HUR-137 variety. At 50 mg/kg and 75 mg/kg Cd treatment, the germination rate showed a significant decrease of 76.32%, 70.44% in HUR-137, compared to the control. (Table. 1).

In the present study, the toxic effects of different Cd concentrations on physical parameters, including plant height, root length, pod length, number of seeds/pod, number of pods/plant and 100 seed weight in *Phaseolus vulgaris* variety HUR-137 was recorded. In HUR-137 these physical parameters were less affected at lower Cd concentrations than at higher Cd concentrations as compared to the control. But at higher cadmium concentrations the root length, plant height, number of seeds/pod, number of pods/plant and 100 seed weight were highly affected. Treatment with 25 mg/kg and 50 mg/kg Cd concentrations, plant height was decreased 37.01cm and 32.14 cm compared to control. At 75 mg/kg and 100 mg/kg plant height was highly affected 27.08 cm and 22.33 cm. Root length at 25 mg/kg and 50 mg/kg was decreased 3.18 cm and 3.1 cm also at 75 mg/kg and 100 mg/kg root length was decreased 2.5 cm and 1.5 cm compared to control. At different concentrations of Cd viz; 25 mg/kg, 50 mg/kg, 75 mg/kg and 100 mg/kg pod length was decreased to 4.6 cm, 3.1 cm, 2.5 cm and 1 cm respectively. Number of pods per plant was decreased at different concentrations of Cadmium viz; 25 mg/kg, 50 mg/kg, 75 mg/kg and 100 mg/kg to 30, 25, 20 and 15 respectively. Number of seeds per pod was also decreased at different concentrations of Cd viz; 25 mg/kg, 50 mg/kg, 75 mg/kg and 100 mg/kg to 7, 6, 5 and 4 respectively. In *Phaseolus vulgaris* L. HUR-137 variety, 100 seed weight at different Cd concentrations 25 mg/kg, 50 mg/kg, 75 mg/kg and 100 mg/kg was reduced to 32.35 g, 28.21g, 22.08g and 18.33g respectively. (Table.1)

### Bio-Chemical parameters

Cd treatment results in decline in chlorophyll content with increasing Cd concentrations in *Phaseolus vulgaris* HUR-137 variety. Different levels of cadmium stress significantly reduced both chlorophyll a and b contents. Total chlorophyll content at different concentrations of Cd viz; 25 mg/kg, 50 mg/kg, 75 mg/kg and 100 mg/kg decreased to 20.5 mg $g^{-1}$ FW, 20 mg $g^{-1}$

ISSN 1533-9211
DOI: 10.5281/zenodo.8431074

FW, 19.5 mg $g^{-1}$ FW and 19 mg $g^{-1}$ FW respectively compared to control. *Phaseolus vulgaris* L. HUR-137 variety exhibited a significant decrease in protein content at different concentrations of Cd viz; 25 mg/kg, 50 mg/kg, 75 mg/kg and 100 mg/kg to 22.47 mg/g fr wt, 21.84 mg/g fr wt, 20.32 mg/g fr wt and 16.56 mg/g fr wt respectively. Starch content at different concentrations of Cd viz; 25 mg/kg, 50 mg/kg, 75 mg/kg and 100 mg/kg was decreased to 16.4 mg/g fr wt, 14.3 mg/g fr wt, 10.2 mg/g fr wt and 8.5 mg/g fr wt respectively. Total soluble sugars increases at different concentrations of Cd viz; 25 mg/kg, 50 mg/kg, 75 mg/kg and 100 mg/kg to 320 $g^{-1}$FW, 420 $g^{-1}$FW, 520 $g^{-1}$FW and 620 $g^{-1}$FW respectively. **(Table 2)**

**Changes in total phenolic content and total flavonoid content**

Total Phenolic content and flavonoid content in *Phaseolus vulgaris* L. HUR-137 variety increases by Increasing concentrations of Cd markedly increased synthesis of these metabolites in all treatments in both above ground part and roots in contrast to control. At different concentrations of Cd viz; 25 mg/kg, 50 mg/kg, 75 mg/kg and 100 mg/kg total phenolic content increases to 16.01 mg eq. GA g-1 DW, 21.06 mg eq. GA g-1 DW, 28.21 mg eq. GA g-1 DW and 32.83 mg eq. GA g-1 DW respectively. Total flavonoid content also increases at different concentrations of Cd viz; 25 mg/kg, 50 mg/kg, 75 mg/kg and 100 mg/kg to20 mg eq. Q g-1 DW, 26.01 mg eq. Q g-1 DW, 32.05 mg eq. Q g-1 DW and 37mg eq. Q g-1 DW respectively. (Table.3)

**Proline content and Tannins**

The estimation of proline content showed that as Cd concentration increased, proline content also increased in the roots and shoots of HUR-137 variety compared to the control. At different concentrations of Cd viz; 25 mg/kg, 50 mg/kg, 75 mg/kg and 100 mg/kg proline content increases to 30.11 µg.g-1 DW, 35.21 µg.g-1 DW, 40.12 µg.g-1 DW and 45.32 µg.g-1 DW respectively. But tannins decreases by increasing concentrations of cadmium as compared to control. Tannins at different concentrations of Cd viz; 25 mg/kg, 50 mg/kg, 75 mg/kg and 100 mg/kg decreases to 3.26 mg/100g, 2.91mg/100g, 2.65mg/100g and 1.08 mg/100g respectively. (Table.3)

## DISCUSSION

Cadmium (Cd) is one of the most well-known heavy metals due to its high mobility and plant toxicity. In the present study results indicated that cadmium significantly decreases germination rate, plant height, root length, pod length, number of pods/plant, number of seeds/pod, and 100 seed weight in *Phaselous vulgaris* variety (HUR-137). Cadmium also induced a decrease in chlorophyll content, Starch content, and protein content with increase in cadmium treatment. But total soluble sugars increases with increasing cadmium concentration in *Phaseolus vulgaris* variety HUR-137. However, total flavonoid content, proline and total phenolic content increases significantly with the increase in cadmium. But with increasing concentration of cadmium in *Phaseolus vulgaris* variety HUR-137 tannins decrease. The germination-percentage was affected with different concentrations of Cd as compared with controls one. Several hydrolyzing enzymes become active and synthesized during seed germination (Bose

ISSN 1533-9211
DOI: 10.5281/zenodo.8431074

et al., 1982). A number of hazardous salts, such as Pb, Hg, Cu, Ni, Co, and others, have been shown to inhibit seed germination, hydrolyzing enzymes (amylase and proteases), and seedling growth in many plants (Sharma et al., 1995; Jain et al., 1998). The assessment of heavy metal phytotoxicity frequently involves seed germination and root elongation tests (Wang and Keturi, 1990; Munzuroglu and Geckil, 2002). Our results support earlier findings by Benhabiles et al. (2020) on Phaseolus vulgaris and showed a significant inhibition of seed germination and root elongation under Cd stress. Reduced water absorption and transport caused by Cd is another potential factor in decreased seed germination. Evidence suggests that Cd enters the cytosol through calcium channels found in the plasma lemma, changing the interactions between cells and water (Kaur et al., 2023). Kranner and Colville (2011) Claims those metals have an impact on seed germination through two different mechanisms: (a) toxicity and (b) altering water uptake during the imbibition process. According to Hsu and Chang-Hung Chou (1992), Cd was the heavy metal that was most toxic to Miscanthus seed germination. Fattahi et al. (2019) found that as when Cd concentrations are increased, germination time is increased and germination percentage is decreased. Munzurolu et al., 2008 show that the inhibition of germination was related to the accumulation of abscisic acid in seeds. Kranner and Colville 2011 claim that Cd hinders water uptake inhibiting seed germination and prolongs their dormancy at room temperature. Additionally, after being broken down by enzymes like amylase, starch is the most abundant stored material in seeds used in germination, so heavy metals that inhibit specific enzymatic reactions have a negative impact on germination (Ko et al., 2012). Cadmium stress is a prominent environmental element that causes stunted growth; root browning, chlorosis, necrosis, and cell apoptosis, among other phytotoxicity symptoms (Chang et al., 2013; Zemanová et al., 2015; Younis et al., 2018a). Cadmium stress hindered bean plant growth in our experiments; Yazdi et al. (2019) discovered that seedlings of lettuce fed with low levels of Cd (100, 200, and 300 g.g-1 perlite) had phytotoxic effects. Furthermore, Younis et al. (2018a) revealed a substantial decrease in growth indices in (Phaseolus vulgaris) treated with (10-6, 10-3M Cd). Three Miscanthus species were studied, and the same outcome was found (Guo et al., 2016). Cd could be preventing growth by limiting photosynthesis, respiration, water, and nutrient intake (Dias et al., 2013; Xue et al., 2013). Plant growth is slowed as a result of the generation of numerous forms of reactive oxygen species as a result of inductive oxidative stress caused by heavy metals, which could finally lead to plant death (Loi et al., 2018). The inhibition of root and shoot growth in the presence of heavy metals is thought to be due to aberrant cell division as well as interference of heavy metals with cofactors involved in photosynthesis, respiration, and protein synthesis in the root and shoot of plants (Volland et al., 2014). According to research by Molnarova et al. (2012) on Sinapis alba shoots, cadmium treatments result in a reduction in the pigmentation of common bean shoots. *P. vulgaris* L. study by Younis et al. (2018a) and Lactuca sativa Linn study by Yazdi et al. (2019) and Chen et al. (2019) on Kandelia obovata. According to Stobart et al. (1985; Van Assche and Clijsters, 1990), reduction in chlorophyll content is caused by inhibition of chlorophyll synthesis, either directly by interfering with the production of vital enzymes like d-aminolevulinic acid dehydratase (ALA-dehydratase) and proto-chlorophyllide reductase, or indirectly by causing Fe deficiency by blocking Fe (III), (Alkantara et al., 1994; Lang et al., 1995; He et al., 2017). Cd is also known to accelerate the enzymatic degradation of chlorophyll (Somashekaraiah et

The Seybold REPORT
ISSN 1533-9211
DOI: 10.5281/zenodo.8431074

al., 1992). Sugars have an important function in biotic and abiotic stress responses; for example, they can act as osmoprotectants by stabilising cellular membranes and preserving turgor (Peshev and Ende, 2013) or as antioxidant molecules (Keunen et al., 2013). Our results demonstrate that Cd treatment increased the amount of total soluble sugars in *Phaseolus vulgaris*. Li et al. (2013) on wheat plants and Vezza et al. (2018) on soybean plants under Zn and As stress, respectively, reported similar outcomes. In contrast to these findings, *Phaseolus vulgaris* L. under Pb and Cd stress showed a decreasing sugar content (Bhardwaj et al., 2009; Younis et al., 2018a). In fact, cadmium perturbs the metabolism of carbohydrates by altering the activities of their enzymes, which leads to the buildup of sugar (Devi et al., 2007; Mishra and Dubey, 2013). Proline has long been thought of as a biomarker of salt stress or drought. Actually, a number of studies linked its buildup to heavy metal stress (Alia and Pardha, 1991; Choudhary et al., 2007; Vezza et al., 2018; Younis et al., 2018a). Proline ensures osmotic adjustment at the cellular level (Kasai et al., 1998) and encourages photosynthetic activity, water status, and antioxidative enzyme activities (Zouari et al., 2016). According to Alia and Pardha (1991), an increase in proline levels may be caused by a decrease in its utilisation in stressed plants or by a fresh synthesis of the amino acid. According to Li et al. (2013), proline accumulation is caused by the ornithine pathway, which stimulates the biosynthesis enzyme ornithine d-aminotransferase. On the other hand, proline catabolism is caused by a decrease in the activities of the enzymes glutamate kinase and proline dehydrogenase. Protein content is inversely related to Cd concentrations, and as Cd increases, protein content decreases. This could be attributed to increased protein hydrolysis caused by increased protease activity (Palma et al., 2002; Melnichuk et al., 1982) or fragmentation caused by reactive oxygen species' harmful effects. In fact, reactive oxygen species (ROS) generation in stressed cells is known to cause denaturation of functional and structural proteins (Davies et al., 1987; Bowler et al., 1992). Furthermore, a fall in protein content can be linked to their manufacturing being slowed due to a decrease in nitrate reductase activity (Fresneau et al., 2007). Several other plant species under Cd stress (Tamas et al., 1997; John et al., 2008; Aslam et al., 2014) or *Phaseolus vulgaris* L. have shown similar effects (Bhardwaj et al., 2009; Younis et al., 2018a). Cadmium toxicity is caused by oxidative stress, which is a key mechanism (Smeets et al., 2005). To prevent oxidative damage and keep reactive oxygen species concentrations within a restricted functional range, plants employ a complicated antioxidant defence system (Ozgur et al., 2013). Non-enzymatic radical scavengers such as carotenoids, flavonoides, and phenolic chemicals are key components of this system. By scavenging ROS, these components protect plant cells from oxidative injury (Schafer et al., 2002). To create methods for polluted crop soil, a greater knowledge of the underlying antioxidant mechanisms mediated by phenolic chemicals is required. In both portions of the plant, Cd-treated *Phaseolus vulgaris* L. demonstrated a considerable increase in phenolic compounds and total flavonoids. Under heavy metal stress, phenolic compounds can serve as transition metal ions chelators, ROS scavengers, (Vollmannova et al., 2014; Manquian-Cerda et al., 2016) and inhibit lipid peroxidation by breaking radical chain reactions during lipid peroxidation (Arora et al., 2000; Sakihama and Yamasaki, 2002). Abiotic stress, particularly heavy metals, typically induces flavonoids, which have a role in plant defence (Grace and Logan, 2000; Dai et al., 2006). Flavonols, such as quercetin, have been shown to have biological and antioxidant properties (Tsai et al., 2002).

The Seybold REPORT
ISSN 1533-9211
DOI: 10.5281/zenodo.8431074

Anthocyanins concentration increased in response to 0.5 mM $Cu^{2+}$ stress, according to Posmyk et al. (2009), and anthocyanin production was an effective method against ROS generated by $Cu^{2+}$ stress. Flavonoids are known lipoxygenase inhibitors, which convert polyunsaturated fatty acids to oxygen-containing derivatives (Nijveldt et al., 2001). Posmyk et al. (2009), Mongkhonsin et al. (2016), and Younis et al. (2018b) all looked at common bean plants that had been exposed to metals like Cu and Cd and found an increase in flavonoids concentration in both the shoots and roots. Tannins, related to their phenol nuclei, have a high anti-oxidant capacity. Hydrolysable tannins, according to Bruneton (1999), are ROS and superoxide anion scavengers and excellent proton donors to ROS formed during peroxidation, resulting in more persistent tannic radical production. Tannins are proven to be effective at removing heavy metals from solutions when used in conjunction with green technologies (Sun et al., 2019). Metal ions could interact with hydroxyl groups of mangrove tannins through an ion exchange and/or complexation process, according to Oo et al., (2009).

**Table 1: Physical Parameters of *Phaseolus vulgaris* L. variety HUR – 137.**

| Treatment (Cd mg/kg) | Germination Rate (%) | Plant height (cm) | Root Length (cm) | Pod length (cm) | No. of pods/plant | No. of seeds/pod | 100Seed weight (g) |
|---|---|---|---|---|---|---|---|
| 0 | 96.21 | 41.13 | 5.42 | 5.8 | 35 | 8 | 37.64 |
| 25 | 90.44 | 37.01 | 3.18 | 4.6 | 30 | 7 | 32.35 |
| 50 | 76.32 | 32.14 | 3.1 | 3.5 | 25 | 6 | 28.21 |
| 75 | 70.44 | 27.08 | 2.5 | 2.8 | 20 | 5 | 22.08 |
| 100 | 68.32 | 22.33 | 1.5 | 1 | 15 | 4 | 18.33 |

**Table 2: Chemical Parameters of *Phaseolus vulgaris* variety HUR – 137**.

| Treatment (Cd mg/kg) | Chlorophyll content (mg g-1 FW) | Protein content (mg/g fr wt) | Starch content (mg/g fr wt) | Total soluble sugars (g-1 FW) |
|---|---|---|---|---|
| 0 | 21 | 23.56 | 18.44 | 220 |
| 25 | 20.5 | 22.47 | 16.4 | 320 |
| 50 | 20 | 21.84 | 14.3 | 420 |
| 75 | 19.5 | 20.32 | 10.2 | 520 |
| 100 | 19 | 16.56 | 8.5 | 620 |

**Table 3: Bioactive Parameters of *Phaseolus vulgaris* variety HUR – 137**.

| Treatment (Cd mg/kg) | Total flavonoid content (mg eq.Q g-1 DW) | Tannins (mg/100g) | Proline content (µg.g-1 DW) | Total phenolic content (mg eq.GA g-1 DW) |
|---|---|---|---|---|
| 0 | 15.12 | 5.19 | 25.14 | 11.15 |
| 25 | 20 | 3.26 | 30.11 | 16.01 |
| 50 | 26.01 | 2.91 | 35.21 | 21.06 |
| 75 | 32.05 | 2.65 | 40.12 | 28.21 |
| 100 | 37 | 1.08 | 45.32 | 32.83 |

ISSN 1533-9211

DOI: 10.5281/zenodo.8431074

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