International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Influence of Cadmium on Physiological and Biochemical Characteristics and Nutrient Uptake in Cowpea (*Vigna Unguiculata* (L.) Walp.)

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Abstract: ***Cadmium is extremely hazardous to life and has been involved in historic poisoning episodes of human, animal and plant populations. Cowpea (Vigna unguiculata (L.) Walp.) is a staple food crop in terms of its high protein value. The present investigation in realistic field conditions is to study the effect of five different concentration of Cadmium (100µM, 250µM, 500µM, 750µM and 1mM) on bioaccumulation, carbohydrate, protein, leaf area and stomatal characteristics of Vigna unguiculata (L.) Walp). Cd markedly hindered the uptake and transport of nutrients like Mg, Fe, Cu, Zn and Mn. Accumulation of Cd, was more in pod in lower concentration but it is more in root in higher of concentration Cd. Cadmium adversely affected total carbohydrate and the leaf area. However total content of protein in leaves was positively affected in all treatments, but it was not consistent in different treatments. It has been noticed that, number of stomata, stomatal index, length and width of stomata were not much influenced by Cd.***

Keywords: Bioaccumulation, stomatal index, carbohydrate, Protein, leaf area

1. Introduction

Plants are the target of a wide range of pollutants that vary in concentration, speciation and toxicity. Among common pollutants that affect plants, heavy metal Cadmium (Cd) is recognized as an extremely significant one due to its high toxicity (Pinto *et al*., 2004). Cadmium is thought to enter the environment mainly from anthropogenic activities, such as mining, electroplating, metallurgy, waste combustion and the abuse of Cd-containing pesticides and fertilizers (Cheng *et al*. 2014; Fagerberg *et al*. 2015); hence, it can be of high levels in agricultural soils. It is a major pollutant present in areas with heavy road traffic and near smelters and sewage sludge areas (Rascio *et al*., 1993).

Cadmium is of increasing scientific interest due to its relatively high mobility in soil and potential toxicity at low concentrations (Das *et al*., 1997); its toxicity is generally considered to be 2–20 times higher than that of other heavy metals (Jagodin *et al*., 1995); large solubility in water (Pinto *et al*., 2004) and subsequent movement through the food chain (Mc bride, 2003). It is a serious lethal, occupational and environmental toxin, known for its high toxicity, which may affect living systems in various ways. In addition, Cd is classified as a probable human carcinogen, which may cause cardiovascular disease, skeletal damage and lung, prostate and kidney cancer in human bodies (Hong and Yan, 2015; Brodziak-Dopierała *et al*., 2015).

There are ample references for the inhibitory effect of cadmium on plants such as lettuce (Maier *et al*., 2003); *Ceratopteris pteridoides* (Deng, *et al*., 2014); *Brassica juncea* (Ahmad *et al*., 2015); *Vicia faba* (Awatif and Wael, 2016); Mung Bean (Jayanta Kumar *et al*, 2016); *Cajanus cajan* (Garg and Singh, 2017).

Cadmium adversely affected a wide range of morphological, physiological and biochemical processes in plants. Many scientists have done extensive work on the morphological impact of Cd in plants and evaluated toxic effects on the processes such as, inhibition of root elongation (Heidari and Sarani, 2011*);* plant height (Pandey and Tripathi, 2011); root, shoot and leaf fresh biomass (Sun *et al*., 2011*)*; dry biomass (Singh *et al*., 2011*)*; leaf area (Domínguez *et al*., 2011*)*; stomata (Rehman *et al*., 2011); flowering (Rehman *et al*., 2011); yield (Rahmanian *et al*., 2011); relative growth rate (Domínguez *et al*., 2011*)*; tolerance index (Chen *et al*., 2011*)* etc. At high concentration Cd may eventually lead to death

Cadmium disturbs physiological processes in plants like transpiration, photosynthesis, respiration, nitrogen assimilation, etc. It is attributable to chloroplast damage (Baszyński *et al*., 1988), affects chloroplast function or CO_2 fixation (Krupa and Baszynski, 1995; Seidlecka *et al*., 1997), disrupt action of metals on photo system efficiency (Chugh *et al*., 1997) and chlorophyll content (Larsson *et al*., 1998). Additionally, Cd excess in the environment inhibited stomatal opening (Perfus-Barbeoch *et al*., 2002), plant water balance (Zhou and Qiu, 2005), photo activation of photo system II by competitive binding to the essential Ca_{2+} site (Faller *et al*., 2005), the activity of photo systems I and II (Küpper *et al*., 2007), the activity of photosynthetic enzymes (Mobin and Khan, 2007), carotenoid content (Thapar *et al*., 2008) etc.

Cadmium influences the overall distribution of nutritional elements within the different organs of the plant. Results from multiple studies demonstrate that nutrient uptake and overall distribution of nutritional elements within the different organs of the plant by plants are significantly affected by the presence of cadmium (korshunoval *et al*., 1999). Most of the observed actions of cd appear to be indirect as a result of mineral imbalance within the tissues.

Cadmium accumulation of different parts of plants was studied in *Lupinus albus* (Zhao *et al*., 2002); *Oryza sativa*

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(Liu *et al*., 2004); *Silene dioica* (Martinka and Lux, 2006); L*obelia chinensis* (Peng *et al*., 2009).

The metal can affect the overall metabolism through alterations in both the behavior of the key enzymes of important pathways (Shah et al., 2001), membrane composition and function (Fodor *et al*., 1995; Madejón *et al*., 2006), including lowering the control of the cell redox state which ultimately causes oxidative stress (Gratao *et al*., 2005). Cadmium inhibits the photoactivation of photosystem 2 (ps2) by inhibiting electron transfer (Kudo *et al*., 2011). Hence, Cd can lead to the generation of reactive oxygen species (ROS) indirectly by production of disturbances in the chloroplasts. As a non-redox metal, cd is unable to perform single electron transfer reactions and hence does not directly produce ROS such as the superoxide anion (o2•–), singlet oxygen (1o2), hydrogen peroxide (h2o2) and hydroxyl radical (oh), but thought to generate oxidative stress by interfering with the antioxidant defense system (Tran and Popova, 2013). In addition, some other reports suggested that Cd may stimulate the production of ROS in the mitochondrial electron transfer chain (Hassan and Aarts, 2011). Acid phosphatase and alkaline phosphatase activity can be used as marker enzymes for Cd toxicity in plant as the enzymes have been shown to be sensitive to Cd. Also the toxicity of Cd has been related with the increase of lipid peroxidation and alterations in antioxidant systems in plants (Fornazier *et al*., 2002; Tran and Popova, 2013).

Comparatively very little research has been conducted on the impact of Cd in cow pea, a staple food crop of tropics. The aim of this study is to evaluate the effect of Cd on (1) the accumulation and distribution of Cd and micro and macro nutrients in roots, shoots leaves and fruits. (2) protein and carbohydrate content of leaves (3) leaf area (4) stomatal characteristics.

2. Materials and Methods

The experiment was carried out in natural conditions. Soil, sand and farm yard manure were mixed in the ratio of 4:1:1.5., as per the recommendation by Kerala Agriculture University. Twenty kg of this potting mixture was filled in gunny bag. Different concentrations namely 100µM, 250µM, 500µM, 750µM, 1mM of cadmium as $CdCl_2.2_{1/2}H_2O$ were applied to the respective gunny bags as basal dose. Sufficient surface sterilized healthy seeds were sown in each gunny bags. Plants were studied from seed to seed and analyzed for bioaccumulation of elements and various biochemical, physiological and Foliar epidermal studies at definite intervals.

Bioaccumulation of Cd, Mg, Fe, Cu and Zn in root, stem, leaves and pod were analyzed by Atomic Absorption Spectrophotometer (Varian Spectra 220) on 60th day. Total protein was determined on 60th day by standard micro–kjeldhal method and estimation of carbohydrate through the method adopted by Shirlaw and Giltchrist (1967).

Leaf area was measured by using graph paper on 20th Day. Stomatal distribution on the abaxial (lower) and adaxial (upper) leaf surface was determined using nail polish imprints. Number of stomata was examined through high power (10 x 40) on 20th day. The number of stomata was counted from different fields and different leaves of same age. Length and width of stomata were recorded by using calibrated ocular micrometer. Stomatal index was calculated by a methodology used by Meinder and Mansfield (1968).

$$\text{Stomatal index} = \frac{S}{S+E} \times 100$$

S= Number of stomata / unit area.
E= number of epidermal cell / unit area.

The data was analyzed statistically using proper statistical tools like Mean, Standard Deviation and ANOVA to make a significant conclusion.

3. Results

3.1Bioaccumulation

a) Cadmium uptake and accumulation

In control, Cd was not at all detected in root, stem, leaves and pods. Applications of Cd caused an increase in Cd concentration in root, stem, leaves and pod (Fig-.1). Accumulation of Cd, was more in pod in lower concentration but it is more in root in higher of concentration Cd. In pod its presence was detected even in the low concentration. At 1mM, 111.270 % amplification of Cd was recorded in pod. In root, the presence of Cd was not detected in 100, 250 and 500µM. The addition of Cd in the soil when amplified to 500, 750 µM and 1mM, the Cd level in the root raised to 2.064, 4.052 and 7.450ppm respectively. Similarly in leaves Cd was not seen in 100, 250 and 500µM treatment, but in 750 µM and 1mM, level of Cd, mounted to 0.054 and 0.317 ppm, respectively. Presence of Cd was not at all detected in stem.

b) Nutrient uptake and accumulation

Cadmium was found to interfere with the uptake and transport of several micro and macro nutrients like Mg, Fe, Cu, Zn and Mn. The higher doses of Cd clearly blocked and the lower doses accelerated the uptake and translocation of all these elements in the present studies.

Cd lessened the quantity of Mg in root and stem, but it was found to be augmented in leaves (Fig- 2). As the concentration of Cd intensified the absorption of Mg was suppressed in root. Thereby 33.09, 58.05 and 66.56% suppression were identified in its accumulation in 500 and 750 µM, 1mM respectively. In shoot 8.95, 20.05, 26.76 28.80 and 64.39% reduction were noted in its accumulation in 100, 250 500, 750 µM and 1mM respectively. However leaves showed boosting of Mg as much as 25.60% for 100µM, 61.27% for 250 µM, 102.23% for 500 µM, 77.19% for 1mM.The accumulation of Mg in different plant parts were in the order stem > leaf > root.

The Fe content in root was curtailed in all Cd treatments. In stem and leaves, it was negatively affected only in higher concentration (1mM), but in all other treatments, it was found to be enhanced (Fig- 3). In root, an inhibition of 5.42 % in 100 µM, 9.50% in 250 µM, 39.14% in 500 µM, 58.05% in 750 µM, 65.57% in 1mM, were identified. In

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stem amount of Fe were gradually escalated up to 500µM, then it descended, thereby 9.30% and 24.62% decrease were recorded in 750µM and 1mM respectively. In leaves it gradually magnified up to 750µM then diminished and hence 25.34% deduction was registered in 1mM. The order of distribution of Fe was root>stem> leaf.

The impact of Cd in the absorption and translocation of Cu differ in different plant parts. In root and leaves, it was negatively affected only in higher treatments, but in all other treatments it was found to be boosting (Fig-4). In stem, content of Cu was reduced in all Cd treatments. In root 20% increase was detected up to 250 µM, then diminishing gradually to 9.65% in 500µM, 29.48% in 750µM and 47.08% in 1mM. However in leaves it intensified progressively up to 750 µM and declined in 1mM over control. Thereby 5.63, 6.91, 13.26 and 16.57 amplification in 100, 250, 500, 750 µM and 18.30% inhibition in 1mM were calculated. In stem linear reduction was observed. Accordingly 1.52, 5.75, 7.12, 17.30 and 35.90% deduction was ascertained in 100, 250, 500, 750µM and 1mM respectively. The distribution of Cu was root>stem> leaf.

Cd shared a retarding effect in the accumulation of Zn in root and leaves but it was found to be enhanced in stem (Fig-5). By the addition of Cd ,the level of Zn subsided in root there by 9.69, 16.42, 39.77, 51.68 and 58.50% depreciation were noticed in its accumulation in 100, 250, 500, 750µM and 1mM respectively. Zn concentration in leaves was affected the least and lessened for about 1.73% in 100 µM, 9.76% in 250 µM, 13.54% in 500 µM, 17.5 % in 750 µM and 17.08% in 1mM. However in stem 6.45, 46.07, 38.08 and 0.22% increase in 100, 250, 500 and 750 µM and 17.13% decrease in 1mM could be seen. The order of distribution of Zn was root>stem> leaf.

Mn was the most severely affected element due to Cd toxicity. In root presence of Mn was not at all noticed in any of the treatments other than control *i.e.*, 100% inhibition was noted in all treatments of Cd (Fig-6). However, in stem successive inhibition was noticed i.e., 21.90, 29.25, 62.39, 75.41 and 80.65% deduction in 100, 250, 500, 750 µM and 1mM respectively. But in leaves more Mn was found in 100 µM and 250 µM, thereafter it declined over control. Accordingly 8.14, & 21.36% increase in 100 and 250 M and 4.42, 19.34 and 30.04% reduction were sighted in 500, 750 µM and 1mM respectively. The accumulation of Mn in different plant parts was in the sequence leaf > stem >root.

3.2 Biochemical Studies

a) Protein

Cadmium taken up by plants induces stress reactions, which is manifested as enhancements of the levels of mRNA with subsequent changes in protein profile. Total content of protein increased in leaves of all treatments, but it is not consistent in different treatments. The total protein of leaves in control was 12.77 mg/gm. It was elevated to 14.63 mg/gm in 250µM, 13.83 mg/gm in 500µM, 23.738 mg/gm in 750µM and 18.06 mg/gm in 1mM (Fig-7).

b) Carbohydrate

The V*igna unguiculata* plants when supplied with Cd, (100 µM, 250µM, 500µM, 750 µM and 1mM) suppression were observed in the total carbohydrate of leaves in all concentrations. Maximum decrease was exhibited by 100µM (33.99%) followed by 250 µM (33.82%), 500µM (30.58%), 750 µM (25.23%) and 1mM (10.27%) (Fig-7).

3.3 Physiological Studies

An overall shrinkage in the leaf area with increasing concentration of Cd was measured. It was recognized that a prominent compaction by about 44.33, 41.37, 64.69, 56.06 and 77.82% in 100, 250, 500, 750µM and 1mM of Cd subsequently. Leaf area was 649.88 mm ^{2} in control and 144.16 mm^2 in 1mM on 20th day (Fig-8).

3.4 Foliar Epidermal Studies

It has been suggested that, stomatal number, stomatal index, length and width of stomata were not much influenced by Cadmium.

a) Number of Stomata

Number of stomata was examined on the abaxial and adaxial leaf surfaces. Largest number of stomata per microscopic field was on the abaxial surfaces. After the treatment with Cd, the leaves exhibited variations in the number of stomata per field. Positive and negative correlations were noticed among the Cd concentrations and the number of stomata on the adaxial and abaxial surfaces of the leaves. In control it was 26/ field in the abaxial surface and 22/field in the adaxial surface of leaves. In the abaxial surface the number of stomata per field was not consistent in different treatments (Table- 1). Yet in the adaxial surface it decreased in different treatments except 1mM. It was 15, 14, 10 and 13 in 100, 250, 500 and 750 µM respectively.

b) Stomatal index

It appeared that excess Cd had little influence on the distribution of stomata in adaxial and abaxial surface (Table-1). The stomatal index of abaxial surface was higher than the other surface. Cd did not affect a consistent variation on both surfaces in this regard.

c) Stomatal length

The stomata length remains same on both surfaces. Cd influences a marginal variation in its length on both surfaces but more variations in abaxial surface (Table-1). Length of stomata ranges between 6.6 mµ to 7.76 mµ in the abaxial surface and 7.36mµ to 8.48mµ in the adaxial surface. In the both surfaces a decreasing trend was noticed but it was not linear. In 1Mm, 10 % & 3.36 % reduction were registered on abaxial and adaxial surface respectively.

d) Stomatal width

The stomatal width in both surfaces also had no consistent variation in various Cd level. The mean values ranges between 4.64 - 5.62 mµ in lower surface and 5.28-6 mµ in upper surface (Table-1).

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4. Discussion

In the present studies, accumulation of Cd was more in pod in lower concentration but it is more in root in higher of concentration Cd. The accumulation of Cd^{2+} in pods has been reported in the plants grown in hydroponic, pot culture and under field conditions in; *Sesamum indicum* (Bharti and Singh, 1994); *Vigna radiate* (Chaudhary and Singh, 2000); *Brassica juncea* (Sharma *et al*., 2010). However, Zhu *et al*. (1999) observed higher accumulation of Cd in the roots of *Brassica juncea* as compared to parts above the ground part. Normally, Cd ions are mainly retained in the roots (Ramos *et al*., 2002) and only small amounts are transported to the shoots (Sheng and Xia, 2006*)*. In general, the content of Cd in plants decreases in the order: roots > stems > leaves > fruits > seeds (Blum, 1997). But, Gupta and Goldsbrough (1991) reported maximum Cd accumulation in stem of tomato. Cd accumulation in stem and inactivation in root cells are probably related to its binding in cell walls, compartmentalization in vacuoles and complexation with metal binding proteins and peptides, especially phytochelatin and metallothioneins (Gupta and Goldsbrough, 1991).

The higher doses of Cd markedly hampered the uptake and translocation of Mg, Fe, Cu, Zn and Mn in the present studies (Fig-2- 6). These results are in accordance with the views of several authors.

In general, Cd has been shown to interfere with the uptake, transport and use of several elements like Fe, Mn, Cu, Zn and Ni in plants (Clarkson and Luttge,1989); Ca, Mg, P, K and water in plants (Das *et al*., 1997): Mn, Ca, Mg, Cu, Zn in Maize (Maksimovic *et al*., 2007); Mn and Zn in Spinach (Rahimi and Ronaghi, 2012)

The uptake of Cd ions seems to be in competition for the same transmembrane carrier with nutrients, such as K, Ca, Mg, Fe, Mn, Cu, Zn and Ni (Clarkson and Luttge, 1989; Rivetta *et al*., 1997). Domínguez *et al*. (2011) reported that, the root of plants were more effected as compared to the shoot and therefore mineral uptake of the root was markedly suppressed and regular decline in concentration of mineral ion with the increase in concentration of Cd.

Cd exposure of *Helianthus annuus* led to substantial reduction in Fe in leaves. Iron deficiency was reported in several plants species treated by Cd (Siedlecka *et al*., 1993; Gussarsson *et al*., 1996). Decrease of this nutrient could be the major origin of chlorosis and necrosis. Mg was also reduced by Cd treatment. Cd inhibits Mg transport to the shoots of sugar beet and could be the cause of leaf area decrease and loss of chlorophyll (Greger, *et al*., 1987).Growth reduction due to Cd may also result from decreased photosynthesis and impaired mineral nutrition (Sumanasinghe *et al*., 1983*)*.

The total protein content increased in all treatments of Cd (Fig- 7). The increase in total soluble protein content under heavy metal stress may be related to; induced synthesis of stress proteins such as enzymes involved in Krebs cycle, glutathione and phytochelatin biosynthesis and some heat shock proteins (Verma and Dubey, 2003); accumulation of proline, a factor counteracting oxidative stress (Seregin and Ivanov, 2001) intensified metabolic activity within plant defense response (Mihailović, 2010).

However, Rastgoo (2011), evaluated that there was a negative relationship between Cd concentration (50µM and 100µM) and protein content in *Aeluropus littoralis*. Costa and Spitz (1997), also reported a decrease in soluble protein content under heavy metal stress in *Lupinus albus*.A decrease in carbohydrate accumulation in the roots and shoots of *Vigna unguiculata* seedlings was reported by Al-Rumaih (2001). It is supported by Saharan *et al*., 2000 in wheat; Podar *et al*., 2000 in maize (*Zea maize*); Rafia-Azmat *et al*., 2005 in bean (*Vigna radiata*), Pandey and Tripathi, 2011, in *Albizia procera;* where as Cd increased soluble carbohydrate concentration and decreased starch concentration in the seedlings of *Pinus sylvestris* (Kim *et al*., 2003).

A general reduction in sugar and starch in particular may presumably be due to increased respiration rates of its organ and reduced the photosynthetic rates as reported in lettuce and oats (Ahmed 1978); due to the inhibition of heavy metals on photosynthesis as reported in maize (Sandmann and Bogger 1983); due to carbon metabolism as a result of their possible interaction with the reactive centre of ribulose bisphosphate carboxylase as in barley and maize (Stiborova *et al*. 1987).

Increasing of Cd soil addition decreased nitrate uptake, nitrate concentration, nitrate reductase activity as well as nitrate translocation to the shoot plants (Korshunoval *et al*., 1999; Hernandez *et al*., 1997),Which may negatively affect net photosynthesis, chlorophyll and protein synthesis as well as essential nutrients contents.

An overall shrinkage in the leaf area with increasing concentration of Cd was measured.(Fig). The adverse effects of Cd on plant growth in general and leaf area in particular have been reported by: Sterckeman *et al*., 2004 in *Zea mays* and *Thlaspi caerulescens*; Wahid *et al*. 2007 in mungbean (*Vigna radiata*). Barcelo *et al*. (1988a) suggested that reduced cell turgour potential and cell-wall elasticity led to formation of small cells and intercellular spaces and also a decrease in the production of leaves in Cd-treated plants, there by the decrease in the leaf area.

Stomatal parameters can be used as signs of stressful condition. Stomatal number, stomatal index, length and width of stomata were not much influenced by Cadmium (Table- 1). Rehman *et al*., (2011) noticed *a* consistent decrease in the stomatal frequency on both the leaf surfaces of *Lycopersicon esculentum* with the increase in the dose of Cd.

5. Conclusion

Accumulation of Cd, was more in pod in lower concentration but it is more in root in higher of concentration Cd. Cadmium negatively affected the uptake and translocation of Mg, Fe, Cu, Zn and Mn: total carbohydrate in leaves and the leaf area: However total content of protein in leaves was positively affected in all

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treatments number of stomata, stomatal index, length and width of stomata were not much influenced by Cd**.**

6. Acknowledgements

The author thank to University Grant Commission, Bangalore, for granting financial assistance and also to Central Institute of Fisheries Technology, Kochi for providing laboratory facilities.

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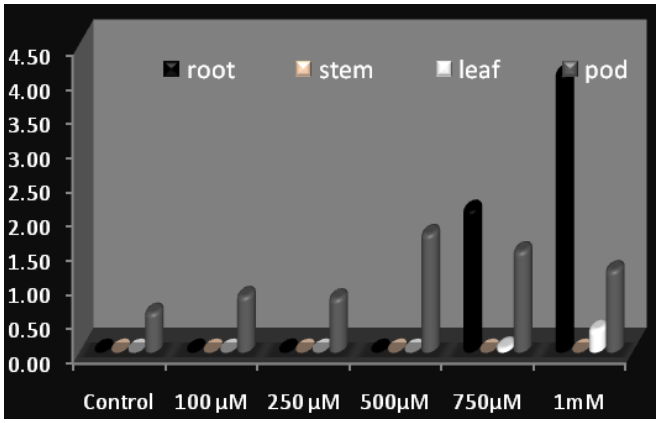

Figure 1: Bioaccumulation of Cadmium

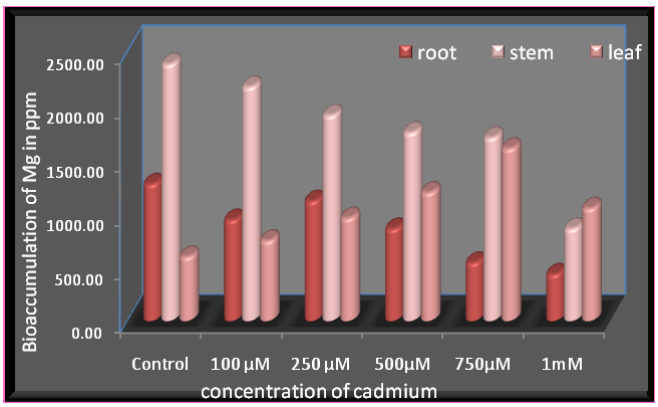

Figure 2: Impact of Cadmium on the Bioaccumulation of Mg

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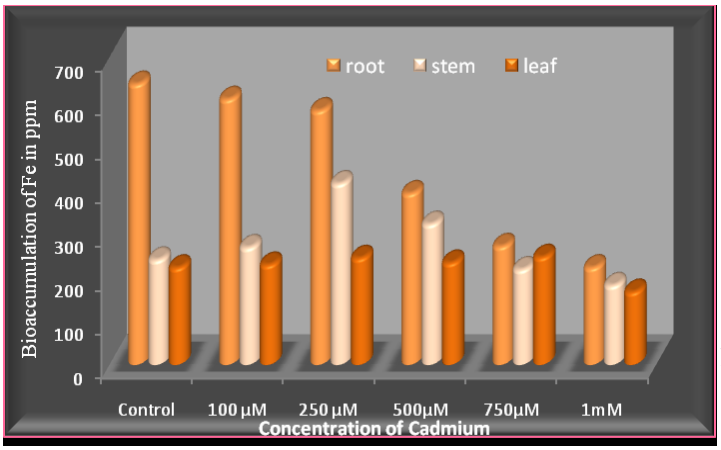

Figure 3: Impact of Cadmium on the Bioaccumulation of Fe

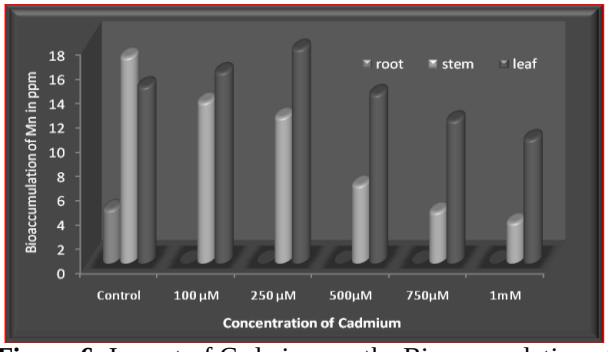

Figure 6: Impact of Cadmium on the Bioaccumulation of Mn

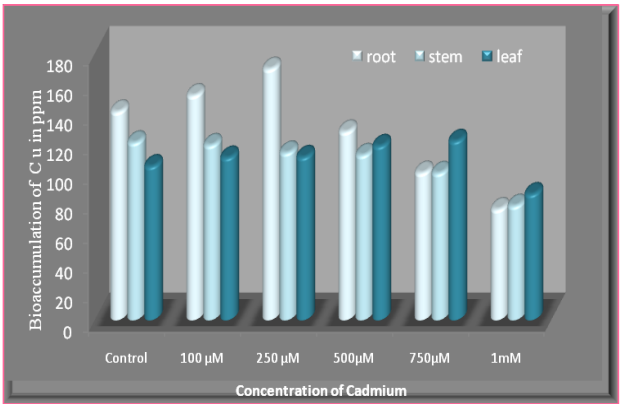

Figure 4: Impact of Cadmium on the Bioaccumulation of Cu

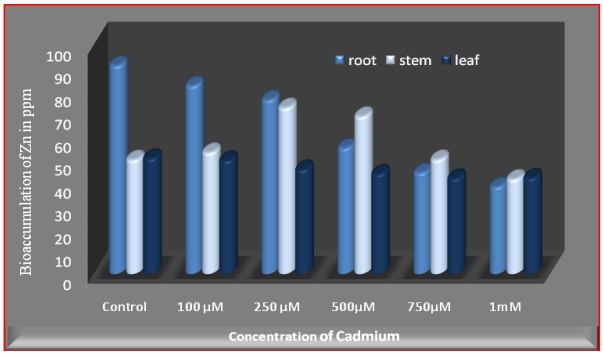

Figure 5: Impact of Cadmium on the Bioaccumulation of Zn

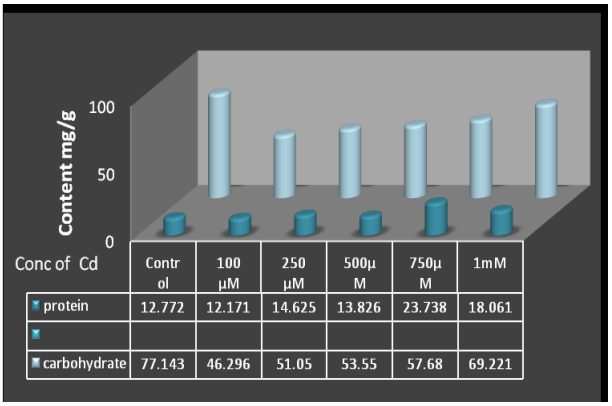

Figure 7: Impact of Cadmium on the protein and carbohydrate

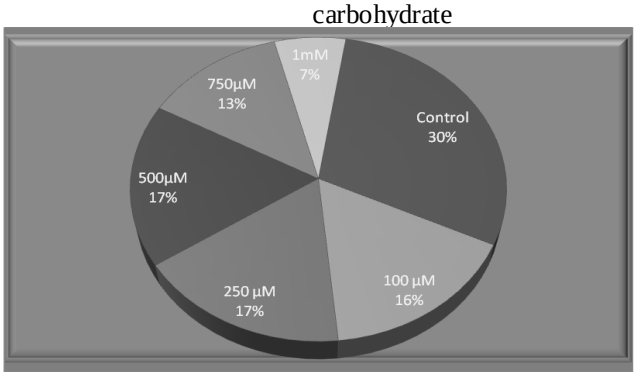

Figure 8: Impact of Cadmium on Leaf Area

Table 1: Impact of Cadmium on Stomatal Variations in cowpea on 20th day

	Abaxial (lower) side					Adaxial (upper) side				
Treatment	No. of stomata	No. of epidermal cells	Stomatal index	Length (µm)	Width (µm)	No. of stomata	No. of epidermal cells	Stomatal index	Length (µm)	Width (µm)
Control	26	45	0.365	7.7	5.06	22	50	0.322	7.76	5.54
100µM	23	42	0.356	7.76	5.62	15	39	0.272	8.48	6
250µM	26	41	0.39	6.64	4.64	14	34	0.288	7.6	5.48
500µM	33	55	0.324	7.24	5.32	10	49	0.166	7.68	5.28
750µM	35	62	0.352	7.08	5.4	13	46	0.212	7.36	5.64
1mM	28	43	0.445	6.93	4.9	27	55	0.352	7.5	5.63

Author Profile

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