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EFFECT OF CADMIUM CHLORIDE ($CdCl_2$) ON BIOCHEMICAL CONTENTS OF *ARACHIS HYPOGAEA* L.

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Abstract: *Environmental pollution is increasing due to the lack of development of a culture of pollution control; there has resulted a heavy backlog of gaseous, liquid and solid pollution. The aim of the present study was to estimate the effect of Cadmium Chloride on edible crop plants Arachis hypogaea L. (VRT. Narayani). Plants were grown in pots with different cadmium chloride concentrations viz., 0, 25µM/L, 50 µM/L 75 µM/L and 100 µM/L for a period of 30 days. Growth parameters and Biochemical contents of Arachis hypogaea L. was evaluated. The experiment was observed in the day's interval 0, 10^{th}, 20^{th} and 30^{th}. The result has been showed that at 25µM/L, 50 µM/L 75 µM/L and 100 µM/L cadmium chloride treatment root length was significantly decreased with increased concentration of cadmium chloride compare to control plants. Chlorophyll 'a', chlorophyll 'b' total chlorophyll, Total carotinoids and Total Carbohydate content in Arachis hypogaea. L. Chlorophyll content and Total carotinoids and Total Carbohydate content were increased up to 20^{th} day and then decreased from 21^{st} day onwards in control plants whereas in treated plants decreased with increasing concentration of cadmium treatment at all the day intervals.*

Key Words: *$Cdcl_2$, Arachis hypogeae L., Chlorophyll, Carbohydrate*

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INTRODUCTION

One of the greatest problems that the world is facing today is that of environmental pollution increasing with every passing year and causing grave and irreparable damage to the earth. In the beginning of 21st century our earth got stressed so much that it retaliated man's unsustainable consumption of its resources. Agriculture was the first man made modification of the ecosystem which occurred due to the compulsion of increasing human population and difficulty in procuring food. The presence of heavy metals in industrial effluents is a major concern of environmental pollution (Sagar T.; Sankpal and Pratap V. Naikwade, 2012). The air, soil and water pollution by industrial effluent are associated with various diseases and could be a reason for the current shorter life expectancy (WHO, 2002 & 2003). In India the environmental pollution through industrial effluents has become a cause of concern at various levels (Chauhan SS. and Chauhan DS, 2000). Various devastating ecological effects and human disasters in the last 40 years have arisen majorly from industrial wastes causing environmental degradation (Abdel-Shafy HI, and Abdel-Basir SE (1991); Sridhar MKC, 2000). Unmanaged human activities such as livestock grazing, logging, and intentional fire can result in environmental degradation. Burning of fossil fuel, flooding, mining and eutrophication further deteriorate the environment (Muhammad, 2008). Living organisms require varying amounts of heavy metals. Iron, cobalt, copper, manganese, molybdenum and zinc are required by humans (Lane TW, Morel FM, 2009). Heavy metals Fe, Cu and Zn are essential for plants and animals (Wintz, H. 2002) some of them pose a number of undesired properties that affect humans and the environment. In addition to these elements Mn and Mo also play significant role in biochemical and physiological functions of biological organisms. Cu, Zn, Fe, Mn, Mo, Ni and Co is referred to as micronutrients (Reeves, R.D and A.J.M. Baker, 2000). Cadmium enters into the environment through weathering of rocks, forest fires and volcanic eruptions. It may be naturally present in air, water, soil and foodstuffs. Rapid industrialization has increased the natural limit of cadmium to a toxic level (D. Thamayanthi, P.S. 2011). Cadmium is highly toxic, mobility and easy accumulation of heavy metals in the environment. In recent years, environmental pollution is being increased due to pesticides, fertilizers and other chemical products as well as long-term heavy use of inappropriate management practices such as irrigation, sludge and slag soil improvement, organic fertilizers containing cadmium and other metals, a lot of agricultural land have been affected to varying degrees cadmium pollution, in which land,

crop plants and orchard land vegetables more serious. Contamination of soil and water with organic or inorganic waste poses major environmental and human health problems (Raskin, I., 1997). Cadmium contained soil is taken up by roots and then transported across plant tissues, and finally accumulated in roots, shoots, fruits, and grain (Qian H, 2009). Of all the heavy metals, cadmium is well known as a highly toxic environmental element due to its great toxicity and high mobility from soil to plants and further down the food chain(Vig, K., 2003). The main course of cadmium is through discharge of effluents from industries, such as electroplating, paints, plastic, battery, zinc mining and refining. Because of its high toxicity, interest in cadmium contamination began after the outbreak of "itai-itai" diseases in Japan. Cadmium is extremely toxic to organisms because it inhibits a large number of metabolic enzyme system, forms complexes with aminoacids, peptides and proteins (Goutam C. Das et al., 2013).

MATERIALS AND METHODS

Pot culture studies were conducted in the department of environmental sciences, Sri Venkteswara University, Tirupati. Soil sample was collected from Tirumala Forest nearby I-Block, Sri Venkateswara University, Tirupati. Seeds of *Arachis hypogeae* L. and *Cicer arietinum* L. were obtained from N. G. Ranga Agricultural Regional Research Station Tirupati, Chittoor District, Andhra Pradesh, India and treated with 0.2 N Mercuric Chloride for 2 min and washed with running tap water to remove contamination of seed coat, prior to germination studies. Experimental studies were carried out with *Arachis hypogeae* L. and *Cicer arietinum* L. using different concentrations of cadmium chloride concentrations viz, 25 µM/L, 50 µM/L, 75 µM/L and 100 µM/L, control was maintained with tap water which served as control. Each experiment including control was performed in five replicates and in every pot 10 seeds were used. A measured volume (50 ml) of different cadmium chloride concentrations was applied to pots every day. Seedlings were maintained under natural day light and night temperature of 30^0 C ± 2^0 C. Seedlings were removed at regular intervals (10, 20 and 30^{th} day) and used for experimental studies. The plants were uprooted carefully, leaves and roots were washed with distilled water and then grinded using mortar and pestle for physiological and biochemical studies. After thorough washing of the plant tissues like leaf with double distilled water various physiological and biochemical compositions were determined in experimental plants. Fresh leaves were used for estimation of chlorophyll a, chlorophyll b, total chlorophyll content and total carbohydrates. The chlorophyll content

was estimated according to the method of Arnon (1949). Total carotenoids were determined as per the method of Jensen and Jensen (1971). Total carbohydrates were measured by the Anthrone method (Hedge *et al*., 1962). Total protein content was estimated by Lowry et al., 1951.

Fig: 1. *Arachis hypogeae L.* seeds

Fig: 2. **Laboratory experiments showing the growth of *Arachis hypogeae* L. in the soils containing different concentrations (25, 50, 75 and 100** µM/L**) *of cadmium chloride and control without cadmium chloride***

Fig: 3. 10th day *Arachis hypogeae* L. plants grown in different cadmium chloride concentrations

Fig:4. 20th day *Arachis hypogeae* L. plants grown in different cadmium chloride concentrations

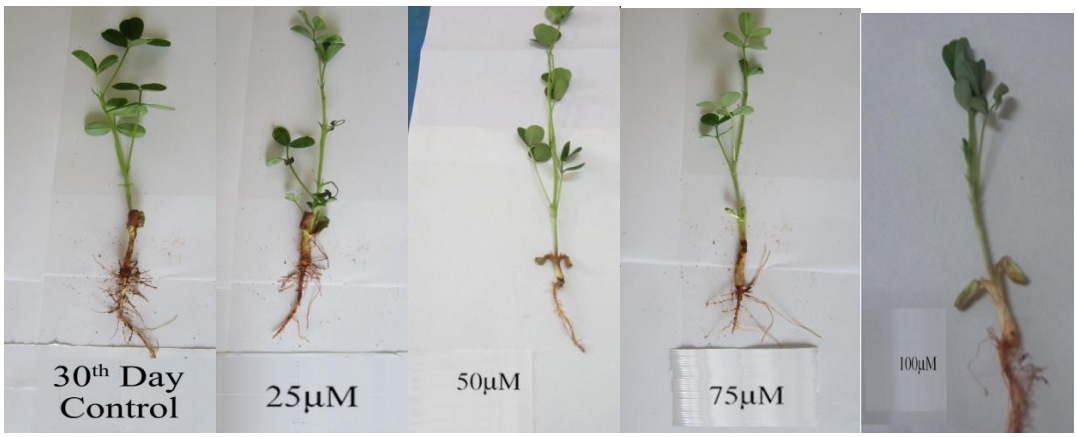


Fig: 5. 30th day *Arachis hypogeae* L. plants grown in different cadmium chloride concentrations

RESULTS

The present work comprises of nurturing *Arachis hypogeae* L. and *Cicer arietinum* L. crop plants grown under laboratory conditions. The seeds were germinated in earthen pots and

allowed to grow for the 10 days. After 10th day the plants were treated with $CdCl_2$ solution at different concentrations (25 µM/L, 50 µM/L, 75 µM/L and 100 µM/L) and further phytotoxicity of cadmium on *Arachis hypogeae* L. and *Cicer arietinum* L. were observed after 10, 20 and 30th days interval respectively.

The objective of this work was to see the effects of cadmium chloride on the chosen 10 day old plant species in circumstances of photosynthetic activity, pigmental changes, amino acid (total protein content, total free amino acids and proline), starch and enzymatic activities (nitrate and nitrite activities). The changes in the plant physiology at different concentrations 0, 25 µM/L, 50 µM/L, 75 µM/L and 100 µM/L at different periods 10, 20 and 30th day respectively. The results were obtained from the experimental observations and have been described below.

Morphological Studies

The growth parameters were observed in crop plant species *Arachis hypogeae* L.. Root and shoot length was measured at different concentrations of cadmium chloride on 10, 20 and 30th day intervals are indicated and presented in table: 1, 2 and fig: 1, 2. It has been observed that length in shoot and root of *Arachis hypogeae* L. was decreased with increased concentration of cadmium chloride treatment compare to control plants at all experimental days.

Pigments (Chlorophyll a, Chlorophyll b, Total Chlorophyll)

The pigments (chlorophyll a, chlorophyll b, total chlorophyll) have been assessed in two crop plant species *Arachis hypogeae* L. It has been observed that there is a decreasing trend in both the plant species. The trend was observed for *Arachis hypogeae* L. has been visualized in tables 3, 4, 5 and fig: 3, 4, 5 for three consecutive experimental days i.e. 10, 20 and 30th days respectively. It reflects that there has been a decrease in the total chlorophyll, chlorophyll a and chlorophyll b contents at 0, 25, 50, 75 and 100 µM/L $CdCl_2$ concentrations with progress in experimental days. As expected there was an increase in the pigmental compositions of both species in control under similar experimental conditions. In *Arachis hypogeae* L. changes were observed for chlorophyll 'a' after 10th day at 25, 50, 75 and 100 µM/L cadmium concentrations were 1.190, 1.083, 1.036, 0.813 respectively, 20th day 1.163, 1.064, 0.927, 0.720 and 30th day 1.120, 1.028, 0.838, 0.612. The changes were observed for chlorophyll 'b' in 10th day at 25, 50, 75 and 100 µM/L cadmium concentrations were 1.189,

1.181, 1.079, 1.028, 0.776 respectively, 20th day 1.154, 1.059, 0.907, 0.681 and 30th day 1.187, 1.018, 0.719, 0.580. It was observed for total chlorophyll in 10th day 2.147, 1.717, 1. 421, 0.936 respectively, 20th day 2.116, 1.603, 1.345, 0.818 and 30th day 2.083, 1.510, 1.217, 0.802.

Total Carotenoids

Total carotenoids content was estimated in both control and treated plants and results are shown in table: 6 and fig: 6. Carotenoids content in *Arachis hypogeae* L. leaves shown a decreasing trend with increasing concentrations of cadmium chloride (25, 50, 75 and 100 µM/L) at the end of all the experimental days on 10th , 20th and 30th day compared to control plants. In control and treated plants, total carotenoids content values on 10th day in experimental plants at 25, 50, 75 and 100 µM/ L were 1.750, 1.625, 1.201, 0.881, 0.520. On 20th day at 25, 50, 75 and 100 µM/ L concentrations of cadmium chloride were 1. 902, 1.520, 1.082, 0.701, where as on 30th day at 25, 50, 75 and 100 µM/ L concentrations of cadmium chloride were 1.655, 1.440, 0.954, 0.620, 0.320 respectively.

Total Carbohydrate Content

Total carbohydrate content was estimated in both control and treated plants and results are shown in Table: 7 and Fig: 7. Carbohydrate content in *Arachis hypogeae* L. of plant leaves have been showed decreasing trend with increasing concentrations of cadmium chloride (25, 50, 75 and 100 µM/L) at all the experimental days on 10th, 20th and 30th day compared to control plants. In control and treated plants, total carbohydrate content values on 10th day in both experimental plants at 25, 50, 75 and 100 µM/ L were 19.031, 18.134, 17.016, 17.001, 16. 819 respectively. On 20th day at 25, 50, 75 and 100 µM/ L concentrations of cadmium chloride were 20.368, 17.181, 16.708, 15.613, 14.718 where as on 30th day at 25, 50, 75 and 100 µM/ L concentrations of cadmium chloride were 18.613, 14.087, 13.914, 12.814, 10.217 respectively.

Total Protein Content

Total protein content was estimated in both control and treated plants and results are shown in Table 8 and Fig 8. Total protein content in *Arachis hypogeae* L. of plant leaves have shown a decreasing trend with increasing concentrations of cadmium chloride (25, 50, 75 and 100 µM/L) at the end of all the experimental days on 10th, 20th and 30th day compare to control plants. In control and treated plants, total protein content values on 10th day in both

experimental plants at 25, 50, 75 and 100 µM/ L were 13.829, 12.428, 10.189, 9.802, 8.901 respectively. On 20th day at 25, 50, 75 and 100 µM/ L concentrations of cadmium chloride were 15.872, 11.279, 9.172, 8.641, 7.133 where as on 30th day at 25, 50, 75 and 100 µM/ L concentrations of cadmium chloride were 14.097, 9.268, 8.181, 7.163, 6.093 respectively.

Table: 1. Effect of cadmium chloride on root length of *Arachis hypogeae* L. (cm)

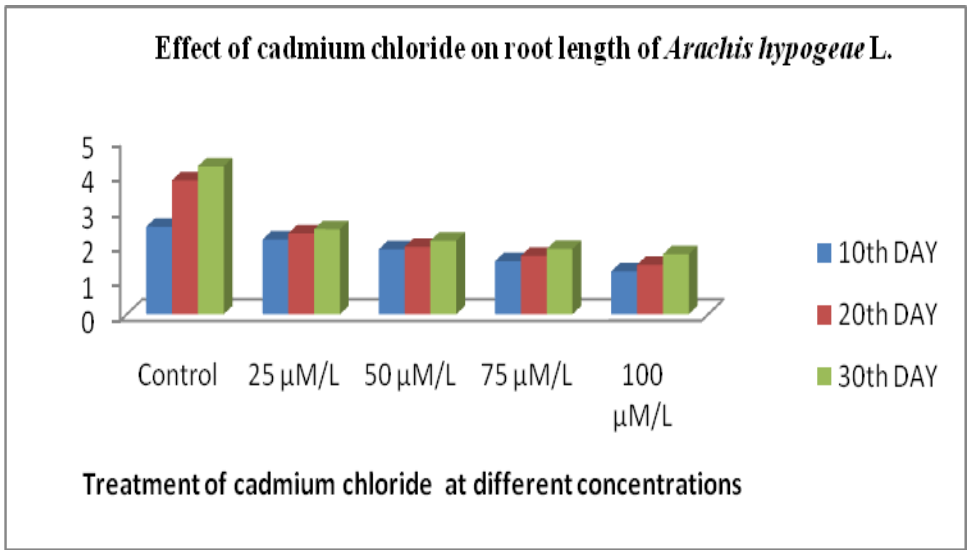
Concentration µM/L	10th DAY	20th DAY	30th DAY
Control	2.51 ± 0.122	3.84 ± 0.112	4.24 ± 0.220
25 µM/L	2.14 ± 0.011	2.32 ± 0.020	2.44 ± 0.100
50 µM/L	1. 86 ± 0. 120	1.93 ± 0. 110	2.11 ± 0. 122
75 µM/L	1.52 ± 0.082	1.67 ± 0.011	1.87 ± 0.121
100 µM/L	1.23 ± 0.003	1.42 ± 0.031	1.72 ± 0.017

SE ± : Values are mean of 5 replications

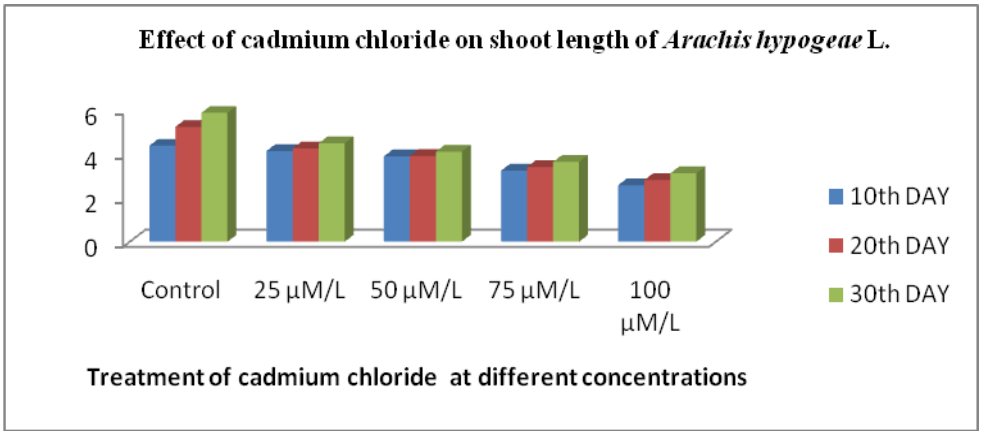
Table: 2. Effect of cadmium chloride on shoot length of *Arachis hypogeae* L. (cm)

Concentration µM/L	10th DAY	20th DAY	30th DAY
Control	4.38 ± 0.102	5.24 ± 0.115	5.88 ± 0.104
25 µM/L	4.14 ± 0.118	4.26 ± 0.290	4.49 ± 0. 212
50 µM/L	3.89 ± 0.077	3.91 ± 0.092	4.11 ± 0.115
75 µM/L	3.24 ± 0.110	3.41 ± 0.071	3.64 ± 0.051
100 µM/L	2.58 ± 0.013	2.81 ± 0.125	3.12 ± 0.032

SE ± : Values are mean of 5 replications



Graph: 1. Effect of cadmium chloride on root length of *Arachis hypogeae* L. (cm)



Graph: 2. Effect of cadmium chloride on shoot length of *Arachis hypogeae* L. (cm)

Table: 3. Effect of cadmium chloride on chlorophyll 'a' content of *Arachis hypogeae* L. and *Cicer arietinum* L. (mg g^{-1} fwt)

Concentration µM/L	10th DAY	20th DAY	30th DAY
Control	1.201± 0.120	1.381±0.110	1.193±10.201
25 µM/L	1.190 ± 0.032	1.163 ± 0.051	1.120 ± 0.512
50 µM/L	1.083 ± 0.264	1.064 ± 0.032	1.028 ± 0.030
75 µM/L	1.036 ± 0.039	0.927 ± 0.085	0.838 ± 0.361
100 µM/L	0.813 ± 0.038	0.720 ± 0.023	0.612 ± 0.061

SE ± : Values are mean of 5 replications

Table: 4. Effect of cadmium chloride on chlorophyll 'b' content of *Arachis hypogeae* L. and *Cicer arietinum* L. (mg g^{-1} fwt)

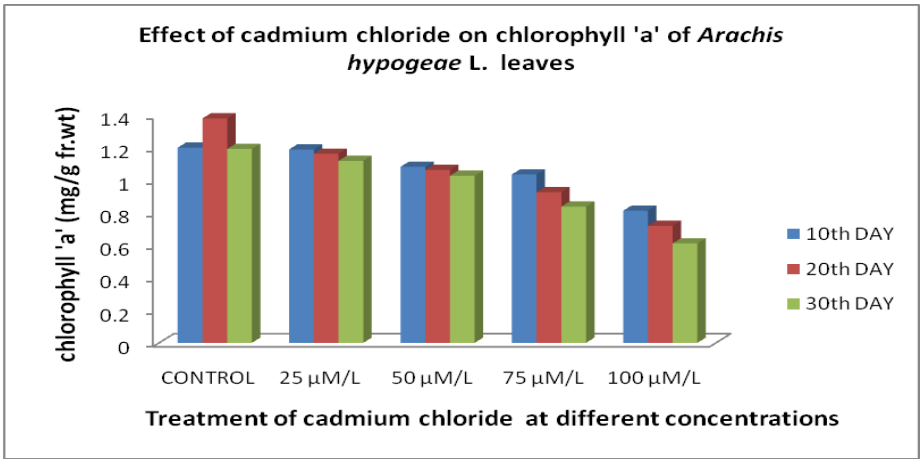
Concentration µM/L	10th DAY	20th DAY	30th DAY
Control	1.189 ± 0.008	1.199 ± 0.039	1.186 ± 0.015
25 µM/L	1.181 ± 0.053	1.154 ± 0.035	1.187 ± 0.039
50 µM/L	1.079 ± 0.091	1.059 ± 0.113	1.018 ± 0.068
75 µM/L	1.028 ± 0.035	0.907 ± 0.108	0.719 ± 0.091
100 µM/L	0.776 ± 0.079	0.681 ± 0.031	0.580 ± 0.091

SE ± : Values are mean of 5 replications

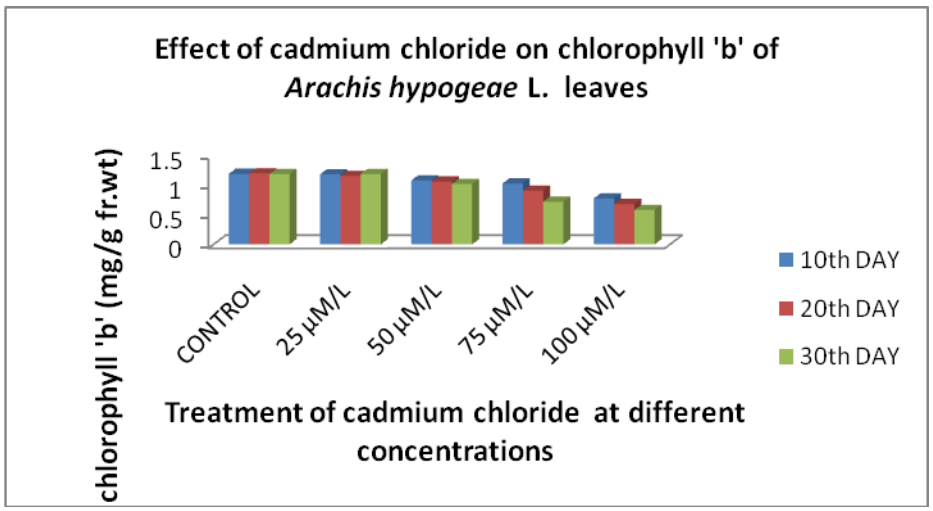
Table: 5. Effect of cadmium chloride on total chlorophyll content of *Arachis hypogeae* L. and *Cicer arietinum* L. (mg g^{-1} fwt)

Concentration µM/L	10th DAY	20th DAY	30th DAY
Control	2.330 ± 0.077	2.580 ± 0.071	2.108 ± 0.051
25 µM/L	2.147 ± 0.027	2.116 ± 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. 421 ± 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

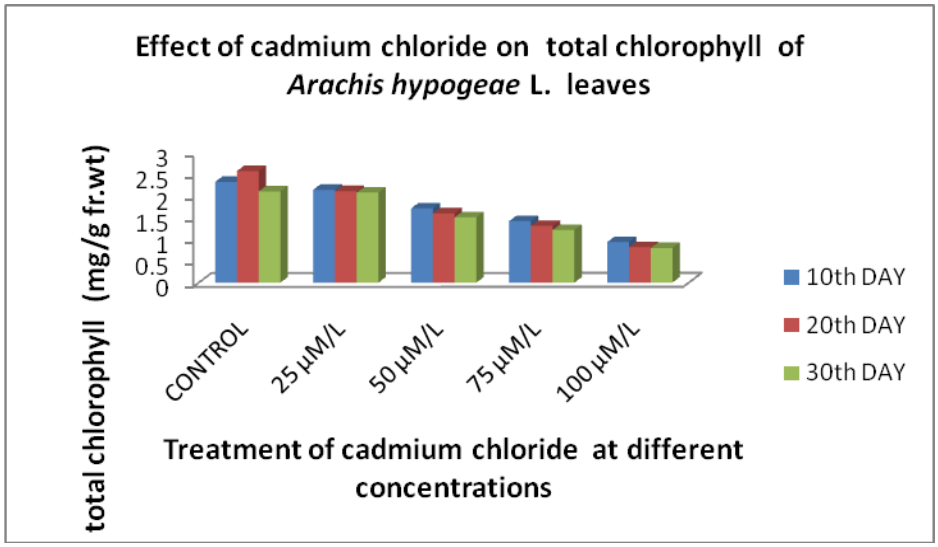
SE ± : Values are mean of 5 replications



Graph: 3. Effect of cadmium chloride on chlorophyll 'a' of *Arachis hypogeae* L.



Graph: 4. Effect of cadmium chloride on chlorophyll 'b' of *Arachis hypogeae* L.

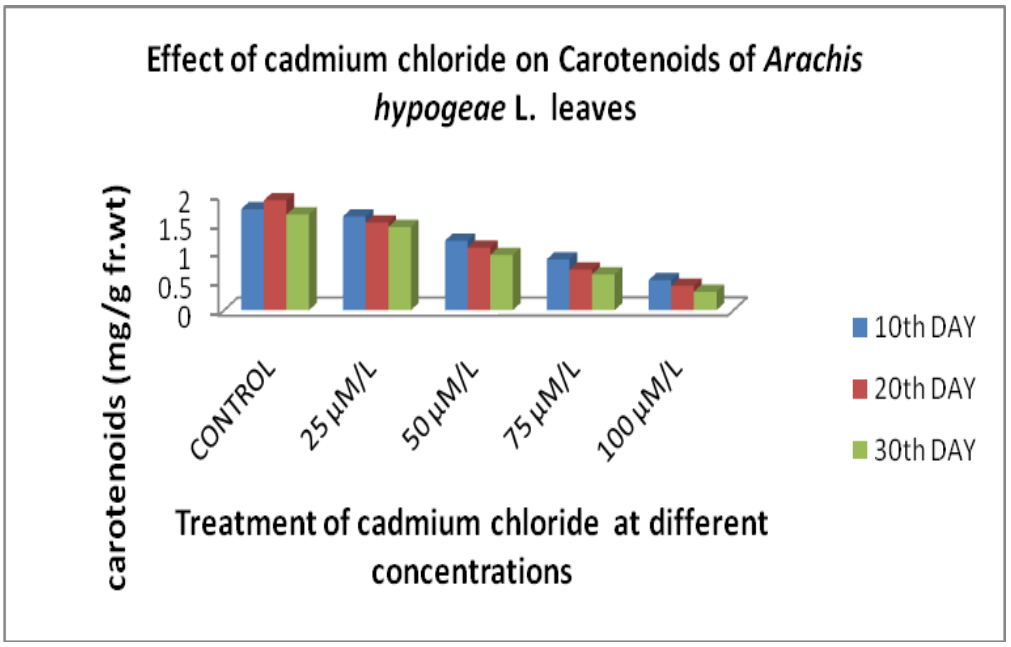


Graph: 5. Effect of cadmium chloride on total chlorophyll of *Arachis hypogeae* L.

Table: 6. Effect of cadmium chloride on total carotenoids content of *Arachis hypogeae* L. and *Cicer arietinum* L. (mg g^{-1} fwt)

Concentration µM/L	10th DAY	20th DAY	30th DAY
Control	1.750 ± 0.171	1.902 ± 0.201	1.655 ± 0.271
25 µM/L	1.625 ± 0.119	1.520 ± 0.011	1.440 ± 0.016
50 µM/L	1.201 ± 0.020	1.082 ± 0.014	0.954 ± 0.023
75 µM/L	0.881 ± 0.026	0.701 ± 0.117	0.620 ± 0.105
100 µM/L	0.520 ± 0.014	0.421 ± 0.020	0.320 ± 0.108

SE ± : Values are mean of 5 replications

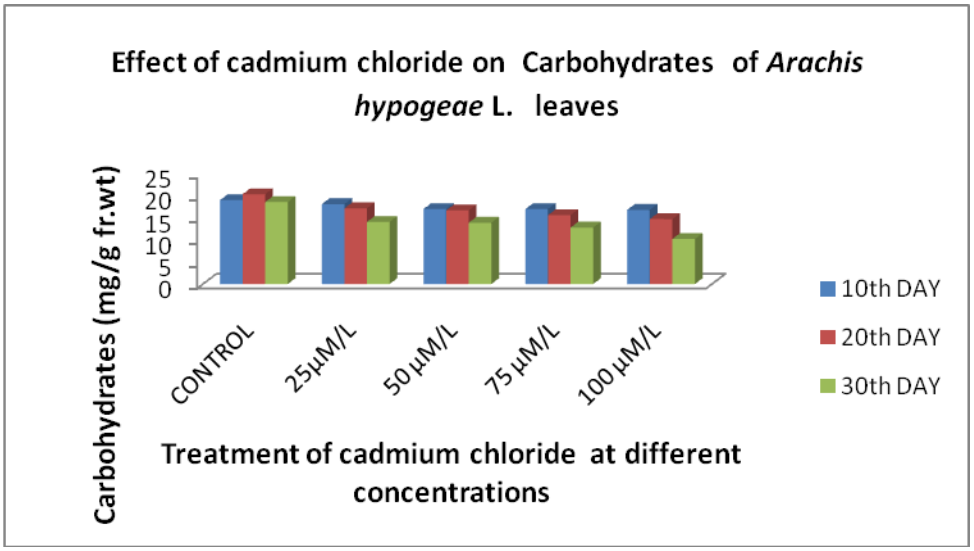


Graph: 6. Effect of cadmium chloride on total carotenoids content of *Arachis hypogeae* L.

Table: 7. Effect of cadmium chloride on total carbohydarte content of *Arachis hypogeae* L. (mg g^{-1} fwt).

Concentration µM/L	10th DAY	20th DAY	30th DAY
Control	19.031 ± 0.085	20.368 ± 0.039	18.613 ± 0.061
25 µM/L	18.134 ± 0.052	17.181 ± 0.080	14.087 ± 0.035
50 µM/L	17.016 ± 0.039	16.708 ± 0.050	13.914 ± 0.031
75 µM/L	17.001 ± 0.036	15.613 ± 0.038	12.814 ± 0.079
100 µM/L	16.819 ± 0.039	14.718 ± 0.032	10.217 ± 0.091

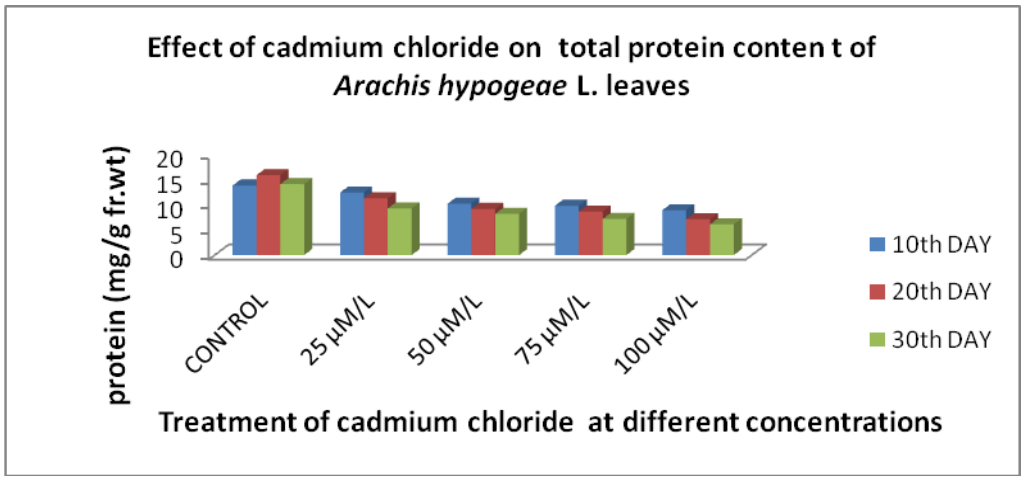
SE ± : Values are mean of 5 replications



Graph: 7. Effect of cadmium chloride on total carbohydarte content of *Arachis hypogeae* L.

Table: 8. Effect of cadmium chloride on total protein content of *Arachis hypogeae* L. (mg g^{-1} fwt)

Concentration µM/L	10th DAY	20th DAY	30th DAY
Control	13.829 ± 0.321	15.872 ± 0.128	14.097 ± 0.015
25 µM/L	12.428 ± 0.013	11.279 ± ` 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



Graph: 8. Effect of cadmium chloride on total protein content of *Arachis hypogeae* L.

DISCUSSION

The present study has been carried out to show the effect of cadmium chloride on crop plant species of *Arachis hypogeae* L.. The metal cadmium is a highly toxic, metallic soil

contaminant, having no metabolic use which adversely affects the plant growth especially at early stages and results in the loss of crop productivity (Faizan S, Kausar S, et al., 2011). Cadmium is one of the highly toxic metal pollutants present in the environment (Wagner, 1993). Root and shoot length was drastically decreased in experimental plants species with increasing concentration of cadmium chloride at all the treatments on 10th, 20th and 30th day when compared to control. These results were corroborating with the findings of Saravanamoorthy and Ranjita Kumari (2005) in peanut and green gram Srivastava et al., 2012, in Solanum melongena, Bahmani R, et al., 2012 in *Phaseolus vulgaris* L., Mamta Hirve and Angoorbala Bafna, 2013, in *Vigna radiata* L. The pigmental contents showed an increasing trend in control plants of *Arachis hypogea*e L. up to 20th day afterwards decreasing trend was observed from 21st day onwards. The results have been revealed according to the findings of Laspina NV et al, 2005, in sunflower leaves. In control plants chlorophyll a, b and total chlorophyll content was increased up to 20th day and decreased on 21st day onwards where as at 25, 50, 75 and 100 µM/L concentrations of cadmium chloride chlorophyll content was drastically decreased at all the experimental days i.e. 10th, 20th and 30th day. Smeets et al., 2005; Mishra et al., 2006 reported, it might have been caused due to inhibition of leaf rolls chlorosis. It has been reported according to the findings of Khadijeh Bavi et al., 2011, in peanuts, Faizan S, 2011, in chickpea plant. The reduction of biomass by cadmium toxicity could be the direct consequence of the inhibition of chlorophyll synthesis and photosynthesis inhibition of important enzymes, such as ð aminolevulinic acid dehydratase (Padmaja et al., 1990). According to Cheng SF., and Huang CY., 2006, higher levels of cadmium chloride inhibition of enzymes activities, reduction of cell metabolism, reduction of photosynthesis, Mamta Hirve and Angoorbala Bafna, 2013, in *Vigna radiate* L., Udita Gubrelay et al., 2013, in Barley. Total carotenoids content was increased in control plants upto 20th day afterwards it was decreased from 21st day onwards. It has been observed that carotenoids content was decreasing with increasing concentration of cadmium treatment on 10th, 20th and 30th day at 25 µM/L, 50 µM/L, 75 µM/L and 100 µM/L concentrations in experimental plants *Arachis hypogeae* L. days i.e. 1oth , 20th and 30th day. The reduction of carotene content has been attributed by the interference of cadmium chloride with desideration steps of carotenoid biosynthesis and thus prevented the accumulation of carotenoid (Bartels and McCullough, 1972; Vaisberg chiff, 1976). Hence, it

can be presumed that decrease in carotinoid content in the present study may be due to the action of cadmium chloride on their synthesis and accumulation. The results have been agreed with the findings of Udita Gubrelay, 2013, in Barly seeds. Total carbohydrates in *Arachis hypogeae* L. plant leaves showed the carbohydrate content decreased with the increased of cadmium chloride concentrations when compared to control plants. In control plants carbohydrates increased upto 20th day whereas decreased from 21st day onwards in *Arachis hypogeae* L. plants. The decrease in the total carbohydrates may be due to inhibition of RUBP carboxylase activity, thereby resulting in reduced levels of carbohydrate. (Abdul Razak., 1985). It has been reported by Udita Gubrelay et al., 2013, in Barly seeds. The decrease in total carbohydrates content in sugar industry effluent treated plants may be due to reduced rates of photochemical activities and the pigment composition, and these are required for Co_2 assimilation and carbohydrate formation.

Protein content of *Arachis hypogeae* L. plant leaves have shown an increasing trend upto 20th day and decreasing trend from 21st day onwards in control plants where as decreasing trend was observed at all concentrations of cadmium treatment i.e., 25 µM/L, 50 µM/L, 75 µM/L and 100 µM/L on 10th, 20th and 30th day. Several authors contributed various reasons for the reduced amounts of amino acid and protein contents due to cadmium toxicity in plant species. The reduction in the amount of protein could be due to decrease in protein synthesis or an increase in the rate of protein degradation (Blaestrasse et al., 2003). The reduction in protein content in plants exposed to Cd2+ stress is believed due to cadmium bound with three families of peptides forming high molecular weight Cd+2 binding complexes such as (g- glutamic acid-cysteine)n- glycin [(g-glu-cys)n-Gly] (g-glu-cys)n-Glu; so the free peptides decreased and consequently protein synthesis inhibited (Winfried,1995) inhibit protein penetration by Arun rtal, 2005, protein synthesis was greatly affected it was reported by the findings of Khadijeh Bavi et al., 2011. The protein content in the studied plant samples varied between *Arachis hypogeae* L.. It is very interesting to notice that the plant shows the quantity of the total proteins was the plant samples that combined highest weight of number of plant samples and heavy vice versa. This variation was attributed to environmental factors such as geographical area, season of collection, elevation and annual temperature precipitation, soil fertility and geno type's variation (Vollmann et al., 2000).

CONCLUSION

The results of the present study have been shown that on the effect different cadmium chloride concentrations in *Arachis hypogaea* L., it can be concluded that certain concentrations of cadmium chloride inhibit plant growth, affect photosynthetic activities carbohydrate content, total protein content of *Arachis hypogaea* L.. Therefore, further investigation on a cellular level is necessary to understand the mechanism of cadmium chloride reaction in plants.

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