International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

EFFECT OF CADMIUM ON MORPHOMETRIC AND BIOCHEMICAL PLANT PARAMETERS OF *TRIGONELLA FOENUM - GRAECUM* L.

Dr Anjali Bhartiya, Lecturer
Department of Botany
Rameshwari Devi Girls College, Bharatpur (Raj.)

Introduction

In an agriculture-based country like India,livestock production is of utmost vitalityand plays a key rolein providing employment especially in Indian agriculture andrural economy. Livestock adds 9% to National GDP and25% to agricultural GDP respectively and contribution fromlivestock is about 15-20% to the farmer's household income(Hegde, 2006).Nutrition plays a major role by driving thefunctional efficacy, and efficiency of the livestock productionsystem. The fodder from common cereal crops are rich inenergy and the leguminous crops are rich in proteins. Thegreen fodder crops are known to be the best source ofnutrients as compared to concentrates and hence useful inbringing down the cost of feeding and reduce the need forthe purchase of feeds from the market(Roy et.al., 1993).

Heavy metal contamination of soil is a far more seriousproblem than air or water pollution because heavy metalsare usually tightly bound by the organic components in thesurface layers of the soil. Consequently, the soil is animportant geochemical sink which accumulates heavymetals quickly and usually depletes them very slowly byleaching into groundwater aquifers or bioaccumulating intoplants (Infotox, 2000). Heavy metals can also be very quicklytranslocated through the environment by the erosion of thesoil particles to which they may adsorb or bound andredeposited elsewhere. Industrial processing and intensiveagricultural practices, resulting in the contaminationof forage, feed, and water, are sources of Cd exposure forfarmed ruminants. From here, they migrate into the foodchain by direct or indirect usage of respective crops.Although some heavy metals like Cu, Fe, Mn, Zn arerequired for the growth of plants in

trace amounts but provefatal if present beyond their maximum permissible limits(Freitas et al., 2010). Various heavy metals along with thecd, viz., arsenic, copper, cobalt, lead, manganese, mercury,nickel, and zinc are reported to cause genotoxicity uponreaching the living systems (Chandra et al., 2005; Bertin etal., 2006).

Cd accumulates over time, primarily in the kidney andliver (Langlands et al., 1988). Cd can cross various biologicalmembranes and, once intracellular, to bind to ligands withexceptional affinity. Cd is a known human carcinogen (Filipicet al., 2006).Exposure to low Cd concentrations can also adversely affect bovine reproduction (Kreis et al., 1993).Cd contamination in food crops and their effects on humanhealth have not been extensively reported.An improvedunderstanding of the interaction of Cd with other elementsmay provide the key to understanding the effects of Cd onhealth. Therefore, in the present investigation, the effectsof heavy metal Cadmium (Cd) on fodder plant Trigonellafoenum-graecum from Rajasthan state was studied.

Material and methods-

Certified seeds of Trigonellafoenum-graecum were obtained from Durgapura AgricultureResearch Station, Jaipur. The seeds were stored in sterilizedglass stoppered bottles.

1. Morphometric evaluation of plant parameters underCd stress-

Uniform, healthy and viable seeds weretaken for the experiment. The seeds were surface sterilizedwith 0.01% mercuric chloride solution for three minutesand thoroughly washed with distilled water three timeseach for 5 minutes and then dried with a paper towel. Dryseeds were placed in 90-mm-diameter Petri dishes on alayer of Whatman No. 1 filter paper with a thin uniform padof sterilized cotton placed beneath, and then moistenedwith 6 different cadmium sulfate concentration (i.e.,10, 50,100, 200, 500 and 1000ppm),and seeds also grown in distilledwater as a control. Seeds were kept at room temperature(25°C ± 1°C) under normal light for germination.Each treatment includes 3 Petri dishes as replicates whichcontain 10 healthy and homogenous seeds (10 Seeds/ Petridish)

International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

arranged in a concentric ring and equidistant fromeach other on the filter paper. 30 seeds of Trigonella foenumgraecumwere used. The emergence of radical (not lessthan 2 mm) was considered an indication of successfulgermination. The number of germinated seeds was counteddaily until there was no further seed germination. The totalnumber of seeds germinated was recorded. The length ofplumule and radical was recorded on the 10th after germination.For radical and plumule length and fresh and driedweight average of 10 seedlings was taken. The data thusobtained were used to calculate germination percentage,the kinetics of plumule emergence, seedling length, freshand dry weight, growth index, and seed vigor. Followingformulae were used to calculate the parameters:

$$\text{(a) Seed germination} = \frac{No.\ of\ \ Seed\ \ ger\min ation}{Total\ \ no.\ of\ \ seed\ \ sowed}$$

(b) Shoot and Root Length (cm)-

The length of rootand shoot were separately measured of 10 days old seedlingswith the help of a ruler (in cm).

(c) Fresh and dry weight (gm)-

After proper washing,excess water was removed and then the fresh weight ofseedling was measured, and for dry weight the seedlingwas dried in an oven at 80oC for 24 hours and then weighed.

2. Biochemical evaluation of plant parameters under Cd stress–

For biochemical evaluation, seeds were sownin experimental pots andhydroponic systems in the Departmentof Botany, Rameshwari Devi Girls College, Bharatpur (Raj.). The variousattributes considered for the present work were studiedat a different time interval after germination. The followingattributes were analyzed for fenugreek crop:

(a) Protein estimation-

The leaf proteins of control andtreated plants extracted by Lowry et al. 1951 method. Alkaline$CuSO_4$ catalyzes the oxidation of aromatic aminoacids with subsequentreduction of sodium-potassium molybdatetungstate of Folin's reagent giving a purple colorcomplex the intensity of the color is directly

International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

proposition tothe concentration of the aromatic amino acid in the givensample solution. For extraction, grind 500mg of 10 daysold plant sample with pestle and mortar in 5-10 ml of thephosphate buffer. Centrifuge the homogenate at 8000 rpmfor 20 min. Collect the supernatant and repeat the extraction4-5 times. Combine the supernatants and make thevolume to 50 ml with phosphate buffer. Take 0.1 ml of theabove extract and add 0.1 ml of 20% TCA. Keep it for halfan hour and centrifuged at 8000rpm for 20 min. Wash thepellet with acetone twice and again centrifuge it. Discardthe supernatant. Dissolve the pellet in 5 ml of 0.1 N NaOHand mix well till it gets dissolved. For estimation,a suitablealiquot (1 ml) of the above solution is taken and adds to it4.5 ml of freshly prepared alkaline copper sulfate reagent.Mix properly and after 10 min add 0.5 m of Folin's reagent.Mix the content instantaneously. Allow the color to developfor 30 min. Record the absorbance at 660 nm after settingthe instrument with reagent blank which contains 1 ml of0.1 N NaOH instead of the sample aliquot. In another setof tubes take suitable aliquots of BSA solution (in a rangeof 10-100µg) make the total volume to 1 ml with 0.1 N NaOHand develop the color as described above. Draw a standardcurve of absorbance at 660 nm versus µg of BSA.From the standard graph the amount of protein in the givenunknown solution is calculated and expressed as mg gm^{-1}fw of leaves.

(b) Total soluble sugar-

Total soluble sugar was estimatedby using anthrone reagent (Dubois et al., 1951).25mg ofthe sample was crushed in 10 ml of 80% ethanoland then centrifuged for 10 minutes at 4000rpm. Take thesupernatant as an extract. Add 0.1ml of the extract to 4mlof the anthrone solution and heated for 10 minutes in boilingwater and was allowed to cool at room temperature. The intensity of blue-green color was measured in UV-VISspectrophotometer at 625 nm against a reagent blank (anthronereagent was responsible for color development). Thesugar content was estimated from a standard curve preparedwith a known concentration of glucose.

(c) Estimation of Phenols –

The deproteinized test materials(200mg each) were macerated with 10 mL of 80%ethanol for 2 hours, and left overnight at room temperature.The mixtures were centrifuged, and the supernatantswere collected separately and

International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

maintained up to 40 mL byadding 80% ethanol.Total phenol content in each samplewas estimated by the spectrophotometer method of Brayand Thorpe, 1954. It includes the preparation of a regressioncurve of a standard phenol (Tannic acid). A stock solutionof tannic acid was prepared by mixing 40 mg of standardphenol in 1 mL of 80%ethanol. Eight concentrationsranging from 0.1 to 0.8 mL were prepared in the test tubeand volume was raised to 1mL by addition of 80% ethanol.To each test tube, 1mL of Folin-Ciocalteau reagent (commerciallyavailable reagent was diluted by distilled water in1:2 ratio just before use) and 2 mL of 20% sodium carbonatesolution was added and the mixture was shaken thoroughly.The samples were placed in boiling water for 1 minand cooled under running water. These reaction mixtureswere diluted to 25 mL by adding distilled water and opticaldensity was read at 750 nm against a blank. The opticaldensity of each sample was plotted against the respectiveconcentration of total phenols to compute the regressioncurve. The concentrations in the test samples were calculatedby referring the respective optical density of the testsample against a standard curve of tannic acid (or standardphenol).

(d) Lipid Estimation-

The test sample was dried, powderedand 100mg was macerated with 10 mL distilled water,transferred to a conical flask containing 30 mL of chloroformand methanol (2/1: v/v; Jayaram, 1981). The mixturewas thoroughly mixed and left overnight at room temperaturein dark for complete extraction. Later, 20 mL ofchloroform mixed with 2 mL of water was added and centrifuged.Two layers were separated, the lower layer of chloroform,which contained all the lipids, was carefully collectedin the pre-weighed glass vials and the colored aqueouslayer of methanol which contained all the water-solublesubstances and thick interface layer were discarded in eachtest sample. The chloroform layers dried in vacuum andweighed. Each treatment was repeated thrice and theirmean values were calculated.

RESULTS AND DISCUSSION-

The accumulation of heavymetals in agricultural soils is of increasing concern due tothe food safety issues and potential health risks as well

International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

asits detrimental effects on soil ecosystems (Qishlaqi andMoore, 2007). The heavy metal concentration in the soilsolution plays an important role in controlling metalbioavailability to plants. Presently, due to constraints in theavailability of freshwater for irrigation, wastewater is beingused for irrigation of agricultural fields resulting in toxic metalcontamination. Heavy metals are either leach into groundor surface water and enter into the growing food crops(Janos et al., 2010). From here, they migrate into the foodchain by direct or indirect usage of respective crops.Although some heavy metals like Cu, Fe, Mn, Zn arerequired for the growth of plants in trace amounts but provefatal if present beyond their maximum permissible limits(Freitas et al., 2010). Various heavy metals along with thecd, viz., arsenic, copper, cobalt, lead, manganese, mercury,nickel, and zinc are reported to cause genotoxicity uponreaching the living systems (Chandra et al., 2005; Bertin etal., 2006).

Therefore, in the current investigation, the effect ofcadmium stress on morphometric and biochemical plantparameters of fodder plant Trigonella foenum-graecum wastested. For that, seeds of Trigonella foenum-graecum sown

in 3 treatment groups of pots of triplicates of threeexperiments each were observed for every undertakenexperiment.

1. Morphometric observation of plant parameters under Cadmium stress

(a) Seed germination percentage-

Seed germination isa vital developmental event in plants and considered animportant growth stage that is frequently subjected to highmortality rates. The percentage of germination has beenused as an indicator of heavy metal toxicity in plants. Theeffect of metals on the development and reproduction ofplants can be firstly quantified by determining the germinationcharacteristics of seeds (Munzuroglu and Geckil, 2002).The effect of heavy metals on seed germination dependson their ability to penetrate the seed coat and disturb variousphysiological processes involved in germination(Khattab, 2004; Faheed, 2005; Wang and Zhou, 2005).

The seeds of Trigonella foenum-graecum werescreened for cadmium salt tolerance deriving germinationpercentage. Results indicated that Cadmium stress givenin the form of Cadmium sulfate had a significant inhibitoryeffect on

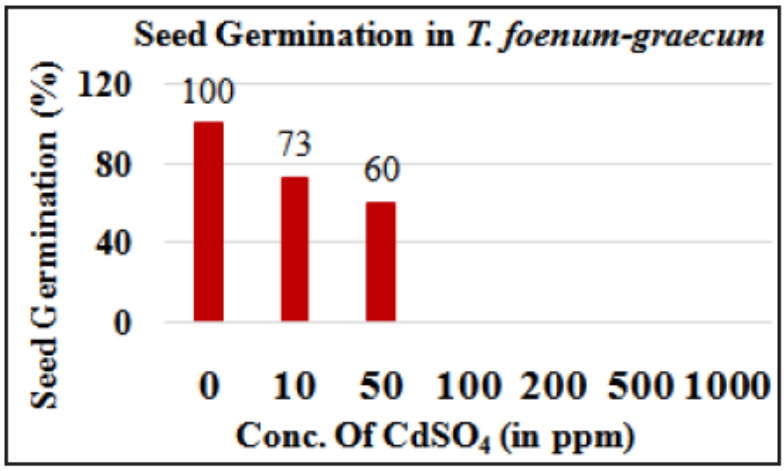
International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

germination percentage. By increasingconcentrations of cadmium sulfate, the germinationpercentage gradually decreased concerning the control inthe groups treated with increasing concentrations ofcadmium sulfate from 10, 50, 100, 200, 500, and 1000 ppm.Treatment on the seeds of Trigonella foenum-graecumexhibited only 73% germination at 10 ppm, 60% at 50 ppm,and null at 100 ppm or higher treatment. However, 100%germination under control condition when cadmium sulfatewas absent (0 ppm). The effect of cadmium stress on seedgermination of Trigonella foenum-graecum is shown inTable-1 and Figure-1(a), 1(b)

The inhibition percentage of germination in Trigonellawas high in heavy metals stress. In high-level treatments,germination percentages were detrimentally affected,implying that a higher concentration of cadmium was notconducive to seed germination. This may be attributed todepression of oxygen uptake and physiological disturbancein the mobilization of reserved food materials (Mondal etal., 2013). CdCl2 and HgCl2 (0.05-50 mM) have reducedseed germination and the shoot development with anincrease of metal concentrations and exposure time(Alhelal, 1995).

Table-1: Effect of Heavy Metal Cd on Seed Germination(%) in *Trigonella foenum-graecum* (Values are meansof three replicates each)

Concentration of $CdSO_4$ (in ppm)	0	10	50	100	200	500	1000
Seed Germination (%)	100	73	60	-	-	-	-



International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

Figure-1 (a): Effect of Heavy Metal Cdon Seed Germination (%) in Trigonella foenum-graecum

Figure-1 (b): Trigonella foenum-graecumseeds under Cd stress of varied concentration

(b) Shoot Length

In the present study, the results recordedsignificant reductions in shoot length with all thetreated groups from 10, 50, 100, 200, 500, and 1000 ppmconcentrations of cadmium sulfate salt as compared withthe control in *Trigonella foenum-graecum*. Shoot length wasdecreased from 4 cm in control to 2.4 cm and 1.1 cm at 10and 50 ppm respectively. No growth was observed at higherconcentrations (100, 200, 500,and 1000 ppm) of cadmiumsulfate. The effect of cadmium stress on shoot length ofTrigonella foenum-graecum has been shown in Table-2 andFigure-2.

The reasons for the inhibition effect of heavy metal to plantgrowth were probably due to a series of physical andchemical reactions between excess heavy metal and soilcomponents which changes soil properties, thus affectingsoil fertility levels (Cieslinski et al., 1996; Chang and Wu,2005). However, the level of significance varied in differentvarieties, cadmium sulfate levels, and at different

International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

durations.Mukherjee and Dalal, 2014 demonstrated that root andshoot length was drastically changed under heavy metalstress. After the accumulation of heavy metals, the lengthof root and shoot were decreased. There was a decreasein root and shoot length in Trigonella foenum graecum underlead and cadmium stress. Pb and Cd treated *Trigonella foenum-graecum* showed a decreased level of toleranceindices which also dose-dependent and Cd shows themaximum effect for the decreased root length. Perveen etal., 2011 also showed that Cd treated Trigonella foenumgraecumwas exhibit blackening of the root system(Godbold and Kettner, 1991). Ahmad et al., 2005 reportedthat there was a significant decrease in root and shoot lengthin lead and cadmium treated *Trigonella*.

Table-2: Effect of Heavy Metal Cdon Shoot Length (cm) of Seedlings in Trigonella foenum-graecum(Values are means of three replicates each)

Concentration of $CdSO_4$ (in ppm)	0	10	50	100	200	500	1000
Shoot Length (cm)	4	2.4	1.1	-	-	-	-

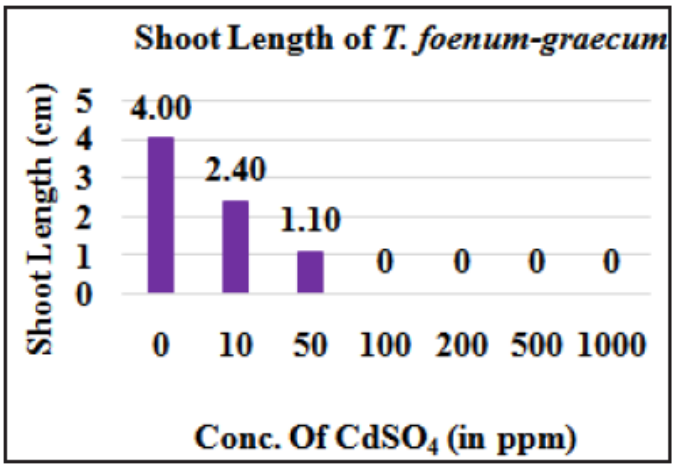


Figure-2: Effect of Heavy Metal Cdon Shoot Length (cm) of Seedlings in Trigonella foenum-graecum

(c) Root Length

International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

In the present study, similar to shootlength, root length has also been adversely affected undertreatedoncentrations of cadmium sulfate. Under nonstressedconditions, when cadmium sulfate was absent (0ppm), root length in T. foenum-graecum was 4.2 cm. Intreated groups, it was noticed that the root length wasdecreased at 10 ppm and 50 ppm to 1.5 cm and 0.4 cm,and further, there was no growth was observed. The effectsof cadmium stress on root length of Trigonella foenumgraecumhave been shown in Table-3 and Figure-3.

In the present study, similar to shoot length, root lengthhas also been adversely affected under variousconcentrations of cadmium sulfate. Zayneb et al., 2015 reported that cadmium affected various plant growthparameters. Its accumulation was markedly lower in shootsas compared to roots, reducing root biomass by almost 50%. Chugh and Sawhney, 1995 also investigated thedeleterious effect of cadmium on fenugreek á- and âamylase,which were quite sensitive to metal: both wereaffected equally by35%and46% inthepresenceof0.25mMand0.50 mM cadmium, respectively.

Table-3: Effect of Heavy Metal Cd on Root Length (cm)of Seedlings in Trigonella foenum-graecum(Values aremeans of three replicates each)

Concentration of $CdSO_4$ (in ppm)	0	10	50	100	200	500	1000
Root Length (cm)	4.2	1.5	0.4	-	-	-	-

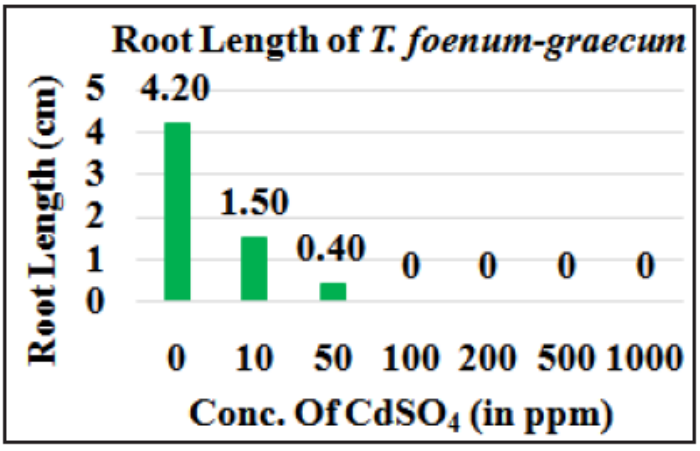


Figure-3: Effect of Heavy Metal Cd on Root Length (cm) of Seedlings in *Trigonella foenum-graecum*

International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

(d) Fresh weight of seedlings-

The fresh weight ofuntreated seedlings in Trigonella foenum-graecumwas0.06g. A decrease in weight was observed with 0.04 and0.02 g at 10 and 50 ppm and further no growth was observedat higher concentrations. The effects of cadmium stresson the fresh weight of Trigonella foenum-graecum areshown in Table-4 and Figure-4.

The fresh weight of seedlings in Trigonella foenumgraecumwas significantly affected by increasing Cddosage. Pirselova et al., 2016 reported that each of thetested doses of Cd resulted in a decrease of root freshweight by 31.70 and 28.68%. Biyani et al., 2019 alsodescribed that Cd stress caused a significant decrease inshoot fresh and dry weight. The fresh and drybiomassaccumulation of Cd-treated plants was significantly lowerthan in plants that were adequately supplemented with Fe(+Fe/+Cd). The greatest reduction was observed in "Fe/+Cd plants after 10 days of treatment, whereas this effectwas reversed after the addition of Fe to the plants (+Fe/+Cd), even in the presence of Cd treatment.

Table-4: Effect of Heavy Metal Cd on Fresh Weight (g) of Seedlings in *Trigonella foenum-graecum* (Values are means of three replicates each)

Concentration of $CdSO_4$ (in ppm)	0	10	50	100	200	500	1000
Fresh White (g)	0.06	0.04	0.02	-	-	-	-

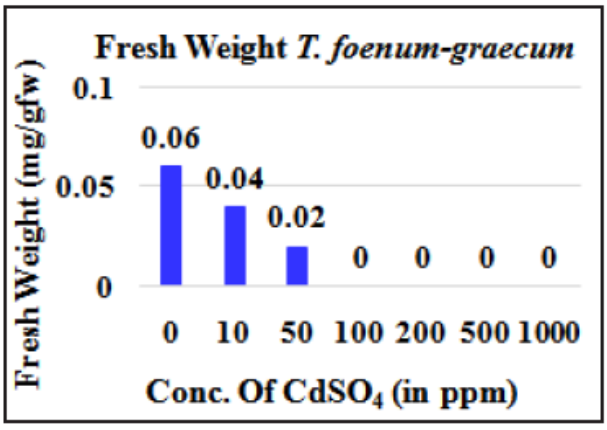


Figure-4: Effect of Heavy Metal Cd on Fresh Weight (g) of Seedlings in *Trigonella foenum-graecum*

International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

2. Biochemical observation of plant parameters under Cadmium stress

(a) Protein content-

There was a slight decrease in theprotein content of *Trigonella foenum-graecum* from control42.62mg/g at 10 ppm level (37.62 mg/g) and the slight increaseat 50 ppm level (38.96 mg/g) was obtained comparedto control. The effects of cadmium stress on the proteincontent of Trigonella foenum-graecum is shown inTable-5 and Figure-5

Abiotic stress may inhibit the synthesis of someproteins and promote others (Ericson and Alfinito, 1984).In different crop plant heavy metal toxicity also reduced thelevel of proteins (Tamas et al., 1997). Various types of grainsdeveloped on the cadmium treated soils also show a lowerlevel of protein content (Salgare and Achareke, 1992). Cdstress could trigger the production of free ROS and resultin oxidative damage in plants.

Table-5: Effect of Heavy Metal Cd on Protein content (mg/g) in the Seedlings of Trigonella foenum-graecum (Values are means of three replicates each)

Concentration of $CdSO_4$ (in ppm)	0	10	50	100	200	500	1000
Fresh White (g)	42.62	37.62	38.96	-	-	-	-

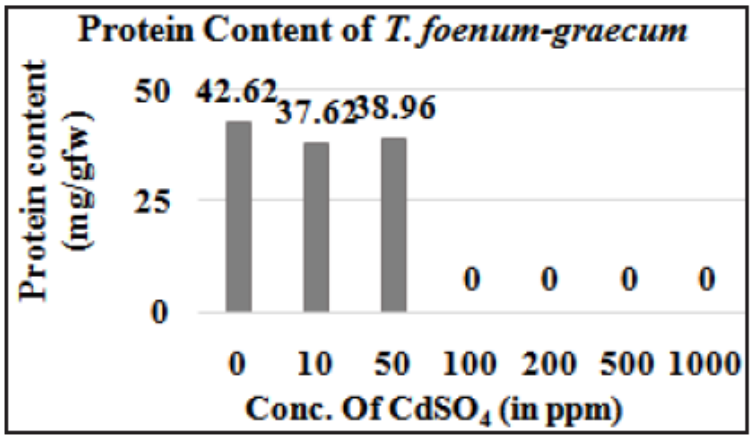


Figure-5: Effect of Heavy Metal Cd on Protein content (mg/g) in the Seedlings of Trigonella foenum-graecum

International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

(b) Total Soluble Sugar content

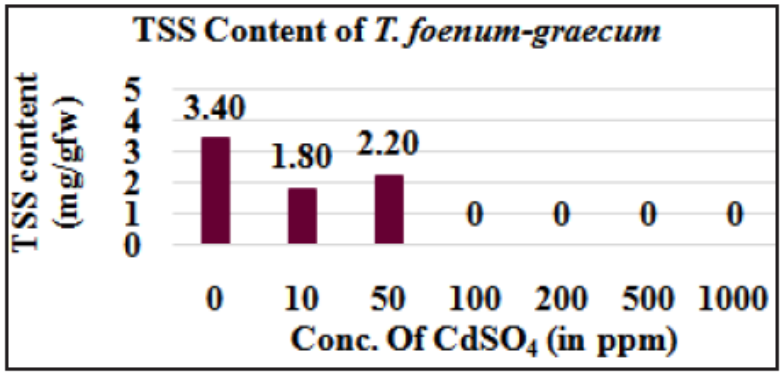
The soluble carbohydratecontent in Trigonella foenum-graecum was higher atnon treated conditions with 3.4 mg/g. Initially the decreasein the TSS content was obtained in Trigonella foenumgraecumwith 1.8 mg/g and 2.2 mg/g at 10 and 50 ppmlevel and further no content was exhibited at higher concentrations

(100, 200, 500 and 1000 ppm concentrations)of Cd stress. The effects of Cd stress on the total solublesugar content of Trigonella foenum-graecum is shown inTable-6 and Figure-6.

Sugar metabolism has adversely affected the plantsgrown under stressed conditions (Jha and Dubey, 2004).Saleh and Garni, 2006, reported that more than 5mg/kgconcentration of Cd inhibited the total carbohydrate content.Ci et al., 2009, also demonstrated that the total soluble sugarconcentration decreased in four wheat (Triticum aestivumL.) lines differing in cadmium (Cd) tolerance were subjectedto 50 µM CdCl2 from the three-leaf stage for 24 days.

Table-6: Effect of Heavy Metal Cd on TSS content (mg/g fresh weight) in the Seedlings of Trigonella foenumgraecum (Values are means of three replicates each)

Concentration of $CdSO_4$ (in ppm)	0	10	50	100	200	500	1000
TSS Content	3.4	1.8	2.2	-	-	-	-



International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

Figure-6: Effect of Heavy Metal Cd on TSS content (mg/ g fresh weight) in the Seedlings of Trigonella foenumgraecum

(c) Phenol content-

The phenol content at the controlconditions of Trigonella foenum-graecum was 2.8 mg/g, itreduces to 2.2 mg/g at 10 ppm level, and then it was observedto increase at 50 ppm upto 4.3 mg/g which is greaterthan control. However, no phenol content was observed athigher Cd stress concentrations. The effects of Cd stresson phenol content of Trigonella foenum-graecum is shownin Table-7 and Figure-7.

Patel et al., 2013 results agree with the findings of Hamid et al., 2010 who found that the phenolic content ofplants was decreasing with increasing levels of heavy metal.These reports justify the initial decline in the phenol content.Korat et al., 2019 also studied the total phenol content fromseedlings (10, 20, and 30 days after germination) of differentfenugreek genotypes that were found statistically significantfor a different stage. Among the genotypes, the mean totalphenol content varied from 0.424 to 0.570 mg g-1. In thecase of 10 to 30 days after germination, there was anincrease in the total phenol content in a leaf of all fenugreekgenotypes. At 30 days, among the 10 genotypes the totalphenol was remained significantly higher in JFG-80 (0.759mg g-1) and the lowest was found in GM-2 (0.527 mg g-1).Mukherjee and Dalal, 2014, reported that lead and cadmiumstressed Trigonella shows a marked increase level of totalphenol in a dose-dependent manner and a higherconcentration of cadmium shows maximum level. It wasalso revealed gradual accumulation of proline and totalphenols in seedling leaves. Phenols in plants may performuseful effects through free radicals scavenging (Chun etal., 2003). This also reflects an increase in antioxidantactivity and the reason why phenol content was increasedat higher Cd levels.

Table-7: Effect of Heavy Metal Cd on Phenol content (mg/g fresh weight) in the Seedlings of Trigonella foenum-graecum (Values are means of three replicateseach)

Concentration of $CdSO_4$	0	10	50	100	200	500	1000

International Journal of Engineering, Science and Mathematics
Vol. 5 Issue 3, September 2016,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

(in ppm)							
Phenol content (mg/gfw)	**2.8**	**2.2**	**4.3**	**-**	**-**	**-**	**-**

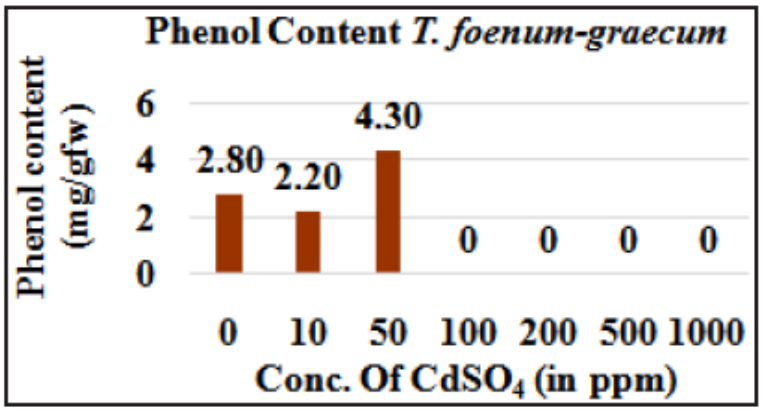


Figure-7: Effect of Heavy Metal Cd on Phenol content (mg/g fresh weight) in the Seedlings of Trigonella foenum-graecum

(d) Lipid content-

Lipid peroxidation (MDA) is a biochemicalmarker for free radical-mediated injury. Under the oxidativestress MDA is the final product of lipid peroxidationand the most prominent indicator of oxidative stress in variousstressed plants (Sima et al., 2012). As per the resultsobtained in the present study, a decrease in lipid contentwas observed in Trigonella foenum-graecum with increasingCd concentration. Lipid content of the control group was10 mg/g which was reduced to 3.33 mg/g at 10 ppm but at50 ppm it was found to be 6.66 mg/g and further no lipidcontent was determined at higher concentrations of Cdstress. The effects of Cd stress on the lipid content ofTrigonella foenum-graecum have been shown in Table-8 and Figure-8.

In the study conducted by Zhan et al., 2017, the levelsof lipid peroxidation and cell death were higher in Cd-treatedseedlings compared with those of the controls. Similarly, inanother study as well, Trigonella foenum-graecum inresponse to cadmium stress showed significantly higherdoses of MDA than the control and it was greatly affectedby the highest doses of Cd. All the concentrations of Cdtreatment effect MDA production.

Table-8: Effect of Heavy Metal Cd on Lipid content (mg/g fresh weight) in the Seedlings of Trigonella foenumgraecum (values are means of three replicates each)

Concentration of $CdSO_4$ (in ppm)	0	10	50	100	200	500	1000
Lipid (mg/gfw)	10	3.33	6.66	-	-	-	-

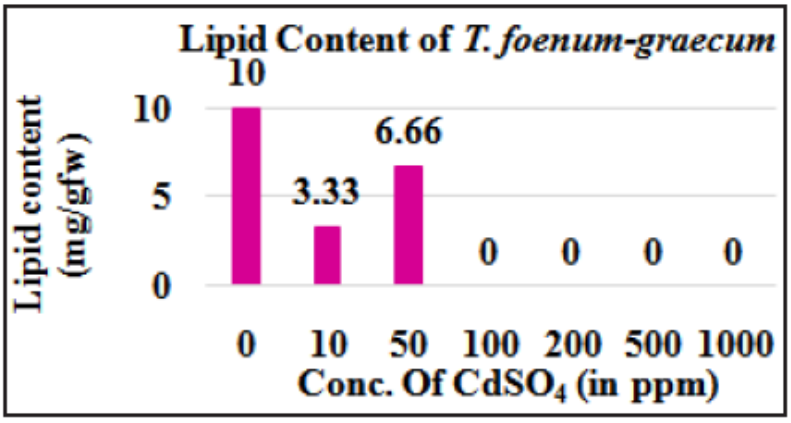


Figure-8: Effect of Heavy Metal Cd on Lipid content (mg/g fresh weight) in the Seedlings of Trigonella foenum-graecum

REFERENCES

1. Ahmad, S.H., Reshi, Z., Ahmad, J. and Iqbal, M. (2005):Morpho-anatomical responses of Trigonella foenumgraecumLinn. To induced cadmium and lead stress.J. of plant biology. Vol. 48(1), pp. 64-84.
2. Alhelal, A. A. (1995): Effect of cadmium and mercuryon seed germination and early seedling growth of Riceand Alfalfa. J. Univ. Kuwait. Sci. Vol. 22, pp. 76-83.
3. Arnon DI. (1949). Copper enzymes in isolatedchloroplasts, polyphenoxidase in Beta vulgaris. PlantPhysiol; 24:1-15.

4. Bertin G and Averbeck D. 2006. Cadmium: cellulareffects, modifications of biomolecules, modulation ofDNA repair and genotoxic consequences Biochimie,88, 1549-1559.

5. Biyani, K. Tripathi, D. K. Lee, J. H. Muneer S. 2019.Dynamic role of iron supply in amelioration of cadmiumstress by modulating antioxidative pathways and peroxidaseenzymes in mungbean. AoB Plants,Vol 11(2).

8. Chun OK, Kim DO, Lee CY. Superoxide radical scavengingactivity of the major polyphenols in fresh plums.Journal Agriculture and Food Chemistry. 2003;51:8067-8072.

9. Freitas M, Gomes A, Porto G and Fernandes E. 2010.Nickel induces oxidative burst, NF-kappa B activationand interleukin-8 production in human neutrophils,Journal Biology Inorganic Chemistry. 15, 1275-1283.

10. Hegde NG. "Positive Attitude for Good Health and Happiness".Nature Cure Ashram, Urulikanchan, Pune(2006).

11. Jha, A. B. and Dubey, R. S. (2004): Carbohydratemetabolism in growing ice seedlings under arsenictoxicity. J. Plant Physiol. Vol. 161, pp. 867-872.

12. Khattab, H. (2004) Metabolic and oxidative responsesassociated with exposure of Eruca sativa (Rocket)plants to different levels of selenium. International Journalof Agriculture & Biology 6: 1101-1106.

13. Kreis,I.A. , M. deDoes, J.A. Hoekstra, C. de LezenneCoulander, P.W. Peters, G.H. Wentink. Effects of cadmiumon reproduction, an epizootological study.Teratology, 48 (1993), pp. 189-196

14. Mondal, N. K. Das, C. Roy, S. Datta J. K. and BanerjeeA. (2013). Effect Of Varying Cadmium Stress OnChickpea (Cicer Arietinum L) Seedlings: An UltrastructuralStudy. Annals of Environmental Science, Vol 7,59-70.

15. National Research Council Mineral Tolerance of DomesticAnimals. National Academy of SciencesPress, Washington DC, USA (1980)