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Cadmium induced biochemical shift in maize

Shipa Rani Dey and Prasann Kumar

Abstract
Hardship of acknowledged assets is maybe one of the deadly slips mankind has ever arranged in its voyage of advance and civilization. All the normal assets are sullied with deadly slips. Among them the arrive and water assets are most noticeably awful influenced and beneath persistent stretch with both biotic and abiotic, due to anthropogenic mediations. In the event that we conversation around the soul of boundless life i.e., SOIL at that point it appears to seem the essential beneficiary by plan or mischance of a bunch of squander items and chemicals utilized in advanced society. Soil defilement can be characterized as the, expansion of any substance to soil that will apply antagonistic impacts on its working and capacity to abdicate a edit. Defilement of overwhelming metal is of uncommon stress due to well-known reports radiating both from India and overseas. Different infections and disarranges watched both in human and animals due to metal harmfulness. A researcher detailed that, the most prominent issues most likely to include mercury, cadmium, lead, chromium, arsenic, nickel etc. To a more prominent or lower degree all of these components are harmful to people and any others creatures. Cadmium is amazingly harmful, causing heart and kidney infection, bone embrittleness; Cr, Ni, and Pb are decently so which are capable for mutagenic, lung cancer, shaking and brain harm like a few deadlier infections.

Keywords: Agriculture, biotic, cadmium, density, effect, gap, high

Introduction
Anthropogenic annoyances of biosphere showed in a wide cluster of worldwide wonders, counting quickened rate of industrialization, seriously horticulture, and broad mining went with by burgeoning populace and quick urbanization have not as it were wreaked the destruction on the accessibility of normal assets but too caused far reaching and grave defilement of the basic components of life on the planet (Adeledo *et al*., 2002, Ackerson and Krig, 1977, Akdeniz *et al*., 2006) [1, 2, 3]. Of the suggestions of human-induced unsettling influence of characteristic biogeochemical cycles, emphasizd amassing of overwhelming metals (HMs) could be a issue of vital significance for biological, wholesome, and natural reasons. HMs have a place to a bunch of nonbiodegradable, determined inorganic chemical constituents with the nuclear mass over 20 and the thickness higher than 5−3 g/cm that have cytotoxic, genotoxic, and mutagenic impacts on people or creatures and plants through impacting and polluting nourishment chains, soil, water system or consumable water, aquifers, and encompassing air. Overwhelming metals (HM) are customary components with properties like ductility, conductivity, soundness as cations, ligand specificity, etc., and an nuclear number. HM such as Cu, Zn, Mn, Fe, Ni, and Co are fundamental micronutrients for plant digestion system but when show in abundance, these, as well as moo levels of non-essential HM such as Cd, Hg, and Pb, can gotten to be greatly harmful.

Materials and Method
The present investigation entitled **"**cadmium induced biochemical shift in maize**"** was carried out in the Laboratory of Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. A detailed account of the materials used and methods adopted during the course of investigation are presented in this paper. The experiment was a pot experiment which was conducted in the net-house of Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University. The University is located in the south-eastern part of Varanasi city which is at $25^0$18' N latitude, $83^0$03' E longitude and at an altitude of 75.7 m above mean sea level. Varanasi belongs to a sub tropical climate and is subjected to extremes of weather condition i.e., extremely hot summer and cold winter. Disease free, healthy seeds of maize variety (BIO-9544) were procured from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi. Bold and uniform seeds were selected where as small, discoloured, infected seeds were discarded.

Total Soluble Sugar

The total soluble sugar content in the plant sample was estimated by following the method proposed by Sadasuvam and Manickam (1992) [32].

Principle

The anthrone reaction is the basis of a rapid and convienient method for the determination oftotal soluble sugar in the plant sample. Carbohydrates are dehydrated by conc. H_2SO_4 to from furfural. Furfural condenses with anthorne to from blue-green coloured complex which is measured colorimetrically at 630 nm.

Reagents

1. Ethanol (80%)
2. Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice cold 95% sulphuric acid. Prepare fresh before use.
3. Standard glucose: Stcok-dissolve 100mg of glucose in 100ml water, working standard-10 ml of the stock diluted to 100ml with distilled water.

Procedure

100 mg of leaf sample was homogenized with 10 ml of ethanol till all the leaf tissues were fully digested. Then extract of the sample was centrifuged at 5000 rpm for 15 min. Volume of the extract was made 100 ml by adding distilled water. One ml of the extract was taken in test tube and 6 ml of the anthrone reagent were added to each test tube. The tube was then placed in a boiling water bath for 10 min, after which they were allowed to cool in running water. A blank was prepared in a similar way, but without a leaf sample. After some time blue colour developed in the test tubes and the intensity of the blue colour was measured at 620 nm by a spectrophotometer. The amount of the sugar in the leaf sample was calculated by standard curve.

Estimation of Total Phenol

The amount of total phenol was measured according to the protocol given by Mahadevan and Sridhar (1982) [28].

Principle

Phenol reactss with an oxidizing agent phosphomolybdate in Folin-Ciocalteu reagent under alkaline conditions and result is the formation of blue coloured complex, the molybdenum blue which is measured at 650nm with the help of spectrophotometer.

Reagents

1. 80% Ethanol
2. Folin-Ciocalteu reagent (FCR)
3. 20% Na_2CO_3
4. Stock Standard (100mg catechol in 100ml of water). Working standard is prepared by dilution of Stock Standard 10 times.

Procedure

Five hundred mg leaves samples were crushed in 3 ml 80% ethanol and centrifuged at 10,000 rpm for 20 minutes. Residue and supernatant were separated out. Supernatant was saved. Residue was again washed with 2 ml of 80% ethanol and supernatant was saved. Finally both the supernatants were mixed and final volume was made to 5 ml by 80% ethanol. 1 ml of the above supernatant (extract) was taken and 1ml of Folinsciocalteau reagent and 2ml of sodium carbonate was added to it. The mixture was heated for 1 minute and absorbance was recorded at 650 nm. A calibration curve of known dilution of pyrocatechol was made following the same procedure as that of sample and the amount of phenols in the sample was expressed as mg g^{-1} fresh weight.

Estimation of Ortho-dihydruic Phenol

The amount Ortho-dihydruic Phenol was measured according to the protocol given by Mahadevan and Sridhar (1982) [28].

Principle

Arrow's reagent specifically reacts with ortho-dihydruic phenols and produces a pink coloured complex which is measured spectrophotometrically at 515nm.

Reagent

1. 80% Ethanol
2. 0.5N HCl
3. 1N NaOH
4. Arnow's reagent: Dissolve 10g of sodium nitrite and 10 g of sodium molybdate in 100ml of water.
5. Standard catechol: 100mg Catechol /100ml of water
6. Working standard: Dilute the standard catechol solution, 1:10 with water (100µg/ml)

Procedure

Five hundred mg leaves samples were crushed in 3 ml 80% ethanol and centrifuged at 10,000 rpm for 20 minutes. Residue and supernatant were separated out. Supernatant was saved. Residue was again washed with 2 ml of 80% ethanol and supernatant was saved. Finally, both the supernatants were mixed and final volume was made to 5 ml by 80% ethanol. 1 ml of the above supernatant (extract) was taken and 1 ml of 0.05 N HCl, 1 ml of Arnow's reagent, 10 ml of water and 2 ml of 1N NaOHmix thoroughly. Pink colour was developed. The blank was prepared similarly without extract. The absorbance was measured at 515nm. The amount of ortho- dihydruic phenols present in the sample was calculated with the help of standard curve prepared from working standard catechol solution.

Estimation of Bound Phenol

The amount Bound Phenol was measured according to the protocol given by Chattopadhyay and Samaddar (1980) [7].

Principle

The bound phenols may be liberated by treatment of plant tissues with NaOH at room temperature. The alkali extract contains the released phenols which are measured spectrophotometrically at 290nm.

Reagents

1. 80% Ethanol
2. Diethyl ether
3. 0.5M NaOH
4. 3% Sodium lauryl sulphate solution (SDS solution)

Procedure

100mg of plant sample was grind in mortar and pestle with 5 ml of SDS solution. This was centrifuged at 2000g for 5 minute. The supernatant was discarded. Residue was washed successively once with 5 ml of SDS solution, twice with 5ml of water, twice with 5 ml of ethanol and twice with 10 ml of diethyl ether. This reside was dried and suspended in 3 ml of 0.5M NaOH. This was kept at room temperature for overnight. The mixture was again centrifuged at 2000g for 15

minute and the supernatant was saved. 1 ml of the above supernatant (extract) was taken and 1 ml of Folinsciocalteau reagent was added to it. The mixture was heated for 1 minute and absorbance was recorded at 650 nm. A calibration curve of known dilution of pyrocatechol was made following the same procedure as that of sample and the amount of phenols in the sample was expressed as mg g^{-1} fresh weight.

Estimation of Total Soluble Protein

The method developed by Bradford, MM. (1976) [5] is sensitive enough to give a moderately constant value and hence largely followed.

Principle

The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Both hydrophobic and ionicinteraction stabilizes the anionic form of the dye causing a visible colour change.The assay is useful since the extinction coefficient of a dye-albumin complex solution is constant over a 10-fold concentration range.

Reagents

1. Sodium phosphate buffer (pH 7.4)

Solution A: To prepare the Sodium phosphate buffer, 13.9 g of 0.1 M sodium dihydrogen phosphate (NaH_2PO_4) was dissolved in distilled water and the volume was made up to 1000 ml.

Solution B: To prepare the Sodium phosphate buffer, 26.82 g of 0.1 M disodium hydrogen phosphate (Na_2HPO_4) was dissolved in distilled water and the volume was made up to 1000 ml.

The solution A and the solution B were mixed in the ratio of 19:81 and the final pH (7.4) was adjusted with the help of pH meter.

2. Dye concentration

Dissolve 100mg ofCoomassie brilliant blue G 250 in 50 ml of 95% ethanol. Add 100 ml of concentrated ortho-phosphoric acid. Add distilled water to final volume of 200 ml. Store in amber bottle in refrigerator, the solution is stable atleast six months. Mixed concentrated dye solution with distilled water at the ratio of 1:4. Filter with Whatman No. 1 paper if any precipitate occurs.

Procedure

100mg of plant sample was taken and it was transferred in to a mortar. The 10 ml of cold extraction was added. The mortar was kept into the ice bucket and it was cursed with the help of pestile, till fine slurry was made. The homogenates was centrifuged at 15,000 rpm for 15 minutes. Supernatant was collected and used as crude protein extract. Took 5ml diluted dye, 0.2 ml of leaf crude protein extract an d0.8 ml of distilled water; mix well and allow the colour to develop for at least five minute but not longer than 30 minute. The red dye turn blue when it binds to proteins, Read the absorbanceat 595 nm in spectrophotometer.

Estimation of Membrane Stability Index and Injury Index

The MSI was calculatedusing the formula described by Premchandra *et al*. (1990).

Principle

Membrane damage can be evaluated indirectly by measuring solute leakage (electrolyte leakage) from cells and the MSI. The stimulation effect of stress on Electro Leyte leakage might be attributed to injury of the plasma membrane.

Reagent

1. Double Distilled Water

Procedure

The leaves were taken from the youngest fully grown leaf. The membrane stability index (MSI) and Membrane Injury Index was estimated by placing 200 mg of leaves in 10 ml double distilled water in two sets. One set was heated at 40℃ for 30min in a water bath and the electrical conductivity (C1) was measured. The second set was boiled at100℃ in a boiling water bath for 10 min and the conductivity (C2) was measured; both conductivities were measured using a conductivity meter (ME977-C,Max Electronics, India). The MSI and MII were calculated using the formula described below

$$MSI = 100\left(1 - \frac{C1}{C2}\right)$$

$$MII = 100\left(C1/C2\right)$$

Results and discussion

The present research work entitled "Cadmium Induced Biochemical Shift in Maize**"** was carried out during the summer season in the Department of Plant Physiology as the pot culture experiment in the polyhouse, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The results obtained are presented and discussed in this chapter.

Total phenol (µg/ml)

Effect of cadmium nitrate on total phenol (µg/ml) content was studied in maize. Data were recorded at the two leaf stage after germination (Fig. 1). During 2016-17, it is evident that the average total phenol content was significantly increased by 0.011743, 3.41828, 7.985547, 8.434508,9.9195122, 11.47787, 17.68022, 17.71635, 25.33243, 26.41915, 31.98735 and 32.55285 µg/ml, respectively when exposed to heavy metal stress as compared to control (T0) at germination. Essentially, when plants were uncovered to higher dosages of overwhelming metal than its add up to phenol substance was altogether expanded as compared to control (T0) on the dates of proposed interim. The antioxidant action of phenolic compounds is due to their tall slant to chelate metals. Phenolics have hydroxyl and carboxyl bunches, able to tie particularly iron and copper. The roots of various plants revealed to heavy metals overflow tall levels of phenolics. From the figure the decreased regard of phenolics with extended concentration of overpower ing metal copper and cadmium shows up that phenol outline chelation with metal and in this way diminish the hurtfulness of the plant in the midst of the conglomeration of the metal (Bhardwaj *et al*., 2009, Cao *et al*., 2007, Devitt *et al*., 1984) [4, 6, 8].

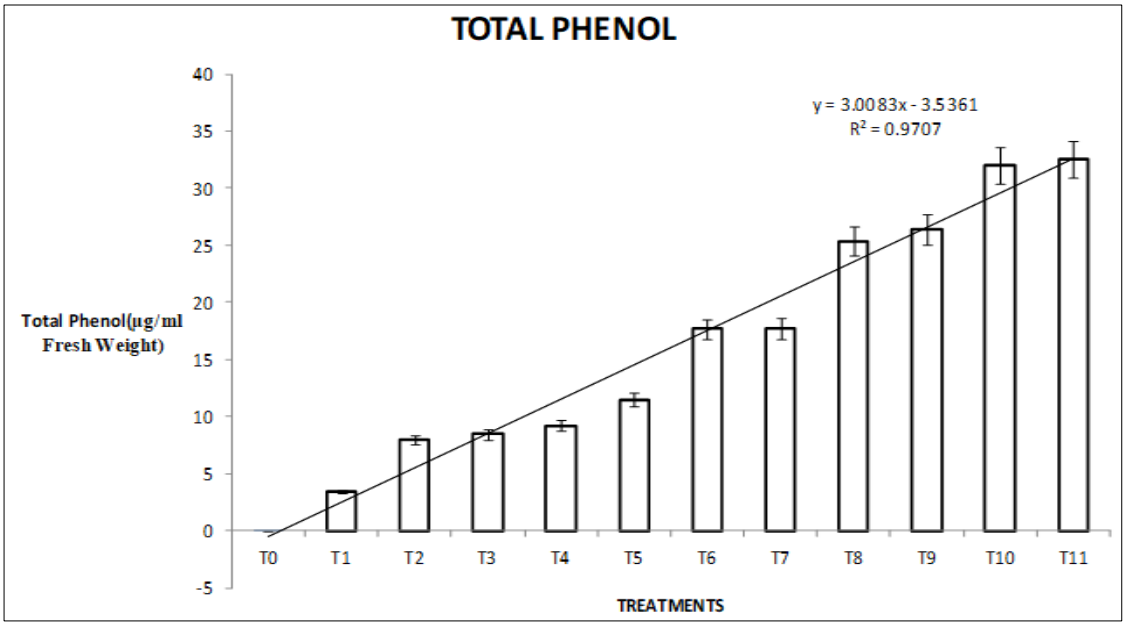

Fig 2: Total Phenol of Maize during Rabi

where, T0= Control, T1= 110ppm $Cd(NO_3)_2$, T2= 120 ppm $Cd(NO_3)_2$, T3= 130 ppm $Cd(NO_3)_2$, T4= 140 ppm $Cd(NO_3)_2$, T5= 150ppm $Cd(NO_3)_2$, T6= 160ppm $Cd(NO_3)_2$, T7= 170ppm$Cd(NO_3)_2$, T8= 180ppm $Cd(NO_3)_2$, T9= 190ppm $Cd(NO_3)_2$, T10= 200ppm Cd $(NO_3)_2$, T11= 210ppm Cd $(NO_3)_2$, Data is in the form of Mean ± SEM, LSD at P≤0.05 and P≤0.01

Bound Phenol (µg/ml)

Effect of cadmium nitrate on bound phenol (µg/ml) content was studied in maize. Data were recorded at the two leaf stage after germination (Fig. 2). During 2016, it is evident that the average bound phenol content was significantly increased by 0.00271, 12.39837, 14.10659, 14.29539, 14.62782, 15.88799, 16.52575, 17.42096, 18.46341, 19.1382, 24.66305 and 27.51671 µg/ml, respectively when exposed to heavy metal stress as compared to control (T0) at germination. Similarly, when plants were exposed to higher doses of heavy metal than its bound phenol content was significantly increased as compared to control (T0) on the dates of proposed interval. The antioxidant action of phenolic compounds is due to their high slant to chelate metals. Phenolics have hydroxyl and carboxyl bunches, able to tie particularly press and copper. The roots of various plants revealed to overwhelming metals emanate tall levels of phenolics. The diminished esteem of phenolics with expanded concentration of overwhelming metal, copper and cadmium appears that phenol shape chelation with metal and subsequently diminish the poisonous quality of the plant amid the amassing of the metal (Gisbert *et al*., 2003) [9]. Increase in solvent phenolics such as intermediates in lignin biosynthesis can reflect the commonplace anatomical modify started by stretch increase in cell divider continuation and the creation of physical boundaries maintaining a strategic distance from calls against the harmful movement of overpowering metals (Jalil *et al*., 1994) [10]. An increment of phenolics related to the increment in action of chemicals included in phenolic compound digestion system was detailed, recommending de novo amalgamation of phenolics beneath overwhelming metal stress. In differentiate, a few prove shows that the increment in flavonoid concentration is primarily the result of conjugate hydrolysis and not due to de novo biosynthesis (Krantev *et al*., 2008) [11].

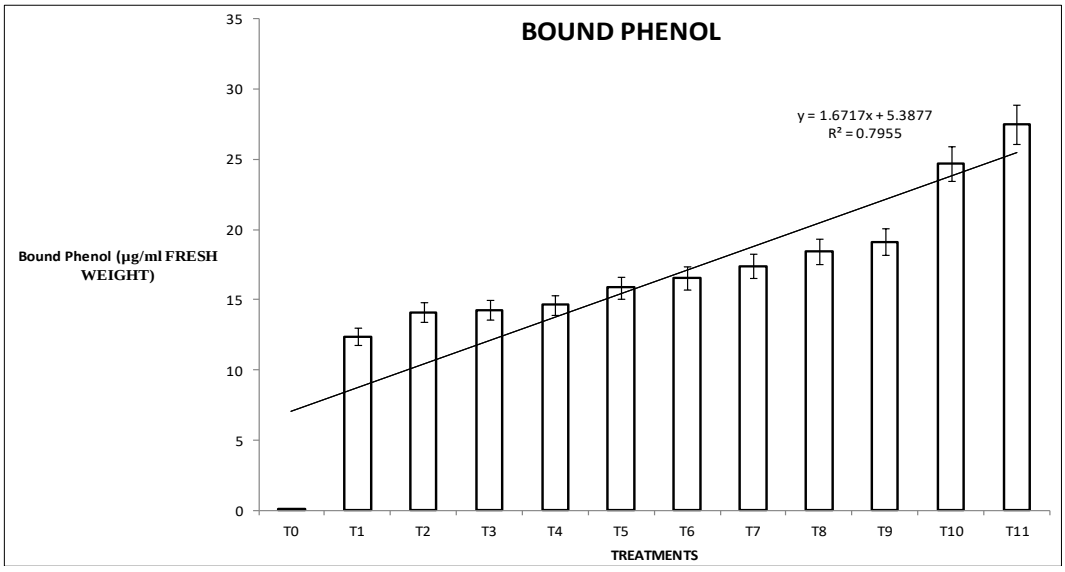

Fig 3: Bound Phenol of Maize during Rabi

where, T0= Control, T1= 110ppm $Cd(NO_3)_2$, T2= 120ppm $Cd(NO_3)_2$, T3= 130ppm $Cd(NO_3)_2$, T4=140ppm $Cd(NO_3)_2$, T5= 150ppm $Cd(NO_3)_2$, T6= 160ppm $Cd(NO_3)_2$, T7= 170ppm$Cd(NO_3)_2$, T8= 180ppm $Cd(NO_3)_2$, T9= 190ppm $Cd(NO_3)_2$, T10= 200ppm Cd $(NO_3)_2$, T11= 210ppm Cd $(NO_3)_2$, Data is in the form of Mean ± SEM, LSD at P≤0.05 and P≤0.01

Ortho dihydruic phenol (µg/ml)

Effect of cadmium nitrate on Ortho dihydruic phenol (µg/ml) content was studied in maize during the years 2016-17. Data were recorded at the two leaf stage after germination (Fig. 4). It is evident that the average Ortho dihydruic phenol content was significantly increased by 9.938573, 1.133695, 1.139115, 1.323397, 1.355917, 1.388437, 1.516712, 1.919603, 2.440831, 2.69196, 3.4182 and 3.5583 µg/ml, respectively when exposed to heavy metal stress as compared to control (T0) at germination. Essentially, when plants were uncovered to higher dosages of overwhelming metal than its Ortho dihydruic phenol substance was essentially expanded as compared to control (T0) on the dates of proposed interim. Arora *et al*. 2003, appear that phenolics (particularly flavonoids) are able to modify peroxidation energy by adjusting the lipid pressing arrange. They stabilize films by diminishing layer smoothness (in a concentration-dependent way) and prevent the dissemination of free radicals and confine peroxidative response (Kumar and Dwivedi, 2014, Kumar and Dwivedi, 2011) [12, 27].

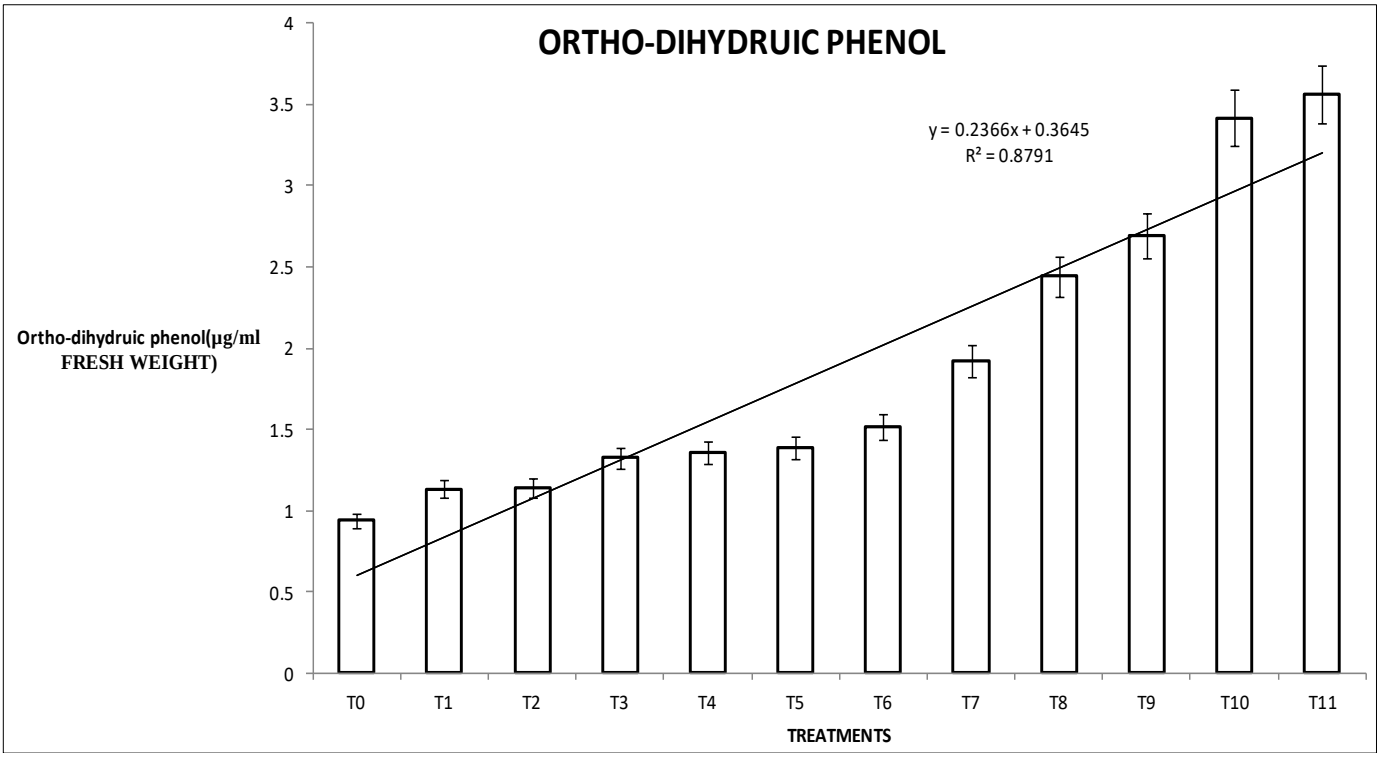

Fig 4: Ortho dihydruic phenol of Maize during Rabi 2016-17

where, T0= Control, T1= 110ppm $Cd(NO_3)_2$, T2= 120ppm $Cd(NO_3)_2$, T3= 130ppm $Cd(NO_3)_2$, T4=140ppm $Cd(NO_3)_2$, T5= 150ppm $Cd(NO_3)_2$, T6= 160ppm $Cd(NO_3)_2$, T7= 170ppm$Cd(NO_3)_2$, T8= 180ppm $Cd(NO_3)_2$, T9= 190ppm $Cd(NO_3)_2$, T10= 200ppm Cd $(NO_3)_2$, T11= 210ppm Cd $(NO_3)_2$, Data is in the form of Mean ± SEM, LSD at P≤0.05 and P≤0.01

Electrical Conductivity [dSm^{-1}]

Effect of cadmium nitrate on Electrical Conductivity [dSm^{-1}] content was and data were recorded at the two leaf stage after germination (Fig. 5). The average Electrical Conductivity [dSm^{-1}]was significantly increased by 33.21, 147.03, 149.27, 163.19, 173.21, 173.67, 185.03, 193.13, 198.17, 200.23, 210.09 & 240.0 respectively when exposed to heavy metal stress as compared to control (T0) at germination. Similarly, when plants were exposed to higher doses of heavy metal than its Electrical Conductivity [dSm^{-1}] was significantly increased as compared to control (T0) on the dates of proposed interval. It was obvious from the results that the physiological characters like electrical character under different treatment were affected by the presence of Cu and Cd but combined effect of both Cu and Cd was less synergistic on the traits like EC under study and less reduction in the average of these traits was shown compared to Cd as single (Kumar and Dwivedi, 2014) [12]. This result was supported by Kumar and Dwivedi, 2014 [12], who found that under the same stressed environment such as heavy metals the adaptation and yield stability of sorghum was more enhanced than that of poaceae plants.

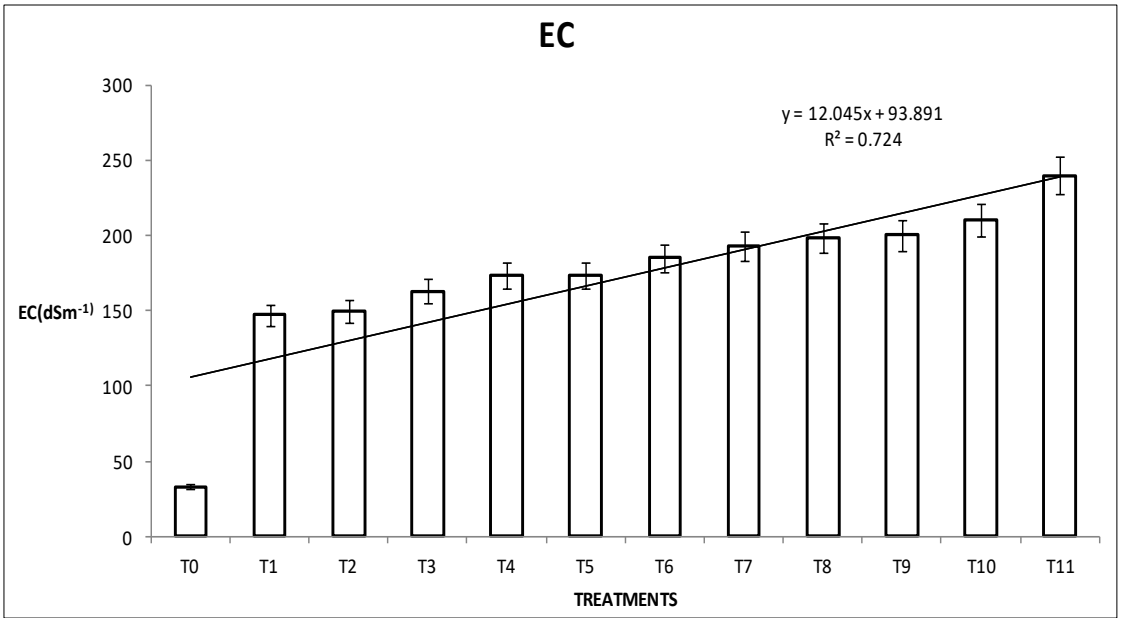

Fig 5: Electrical Conductivity [dSm^{-1}] of Maize during Rabi

where, T0= Control, T1= 110ppm $Cd(NO_3)_2$, T2= 120ppm $Cd(NO_3)_2$, T3= 130ppm $Cd(NO_3)_2$, T4=140ppm $Cd(NO_3)_2$, T5= 150ppm $Cd(NO_3)_2$, T6= 160ppm $Cd(NO_3)_2$, T7= 170ppm$Cd(NO_3)_2$, T8= 180ppm $Cd(NO_3)_2$, T9= 190ppm $Cd(NO_3)_2$, T10= 200ppm Cd $(NO_3)_2$, T11= 210ppm Cd $(NO_3)_2$, Data is in the form of Mean ± SEM, LSD at P≤0.05 and P≤0.01

Total soluble protein (mg/g Fresh Weight)

Effect of cadmium nitrate on total protein (mg/g fresh weight) content was studied in maize. Data were recorded at the two leaf stage after germination (Fig. 6). It is evident that the average total protein (mg/g fresh weight) was significantly decreased by 0.638476, 0.23513, 0.216543, 0.202602, 0.195167, 0.181227, 0.151487, 0.141264, 0.077138, 0.054833, 0.048327 and 0.021375 respectively when exposed to heavy metal stress as compared to control (T0) at germination. Similarly, when plants were exposed to higher doses of heavy metal than its total protein (mg/g fresh weight) was significantly decreased as compared to control (T0) on the dates of proposed interval. One of the components influenced by overwhelming metals in plants is protein amalgamation. It is known that solvent protein substance is an imperative pointer of physiological status of plants. In numerous ponders, researcher taken note a noteworthy diminish in protein substance from the foremost contaminated site (Kumar and Dwivedi, 2014) [12].

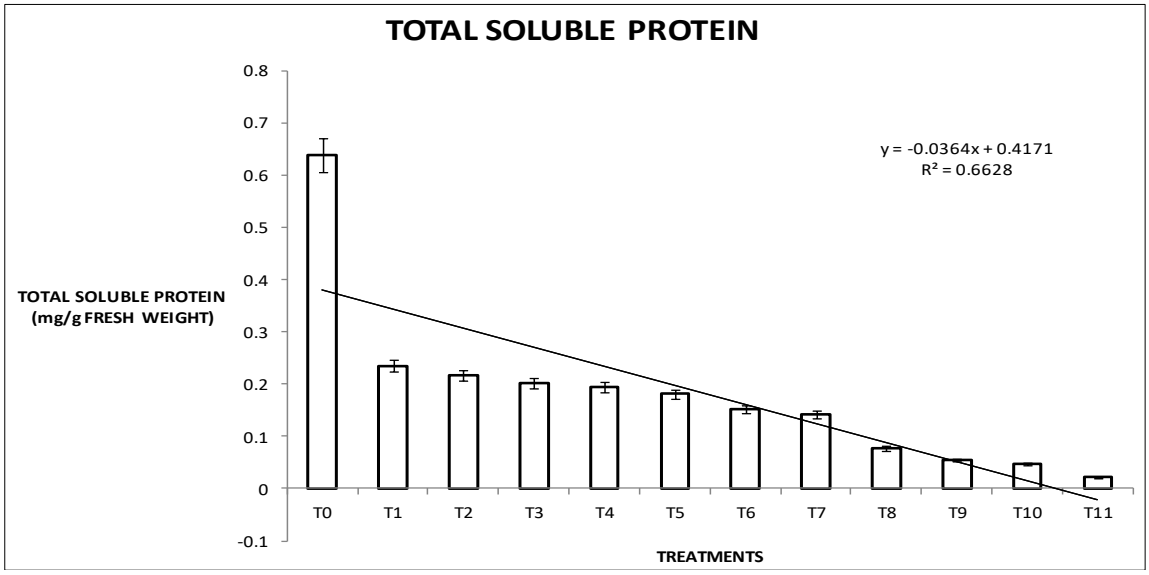

Fig 6: Total Protein of Maize during Rabi

where, T0= Control, T1= 110ppm $Cd(NO_3)_2$, T2= 120ppm $Cd(NO_3)_2$, T3= 130ppm $Cd(NO_3)_2$, T4=140ppm $Cd(NO_3)_2$, T5= 150ppm $Cd(NO_3)_2$, T6= 160ppm $Cd(NO_3)_2$, T7= 170ppm$Cd(NO_3)_2$, T8= 180ppm $Cd(NO_3)_2$, T9= 190ppm $Cd(NO_3)_2$, T10= 200ppm Cd $(NO_3)_2$, T11= 210ppm Cd $(NO_3)_2$, Data is in the form of Mean ± SEM, LSD at P≤0.05 and P≤0.01

Total soluble sugar (mg/g Fresh Weight)

Effect of cadmium nitrate on total soluble sugar (mg/g fresh weight) content was studied in maize. Data were recorded at the two leaf stage after germination (Fig. 6). It is evident that the average total soluble sugar (mg/g fresh weight) was significantly decreased by, 0.789655, 0.290805, 0.267816, 0.250575, 0.241379, 0.224138, 0.187356, 0.174713,

0.095402, 0.067816, 0.05977 & 0.026437, respectively when exposed to heavy metal stress as compared to control (T0) at germination. Similarly, when plants were exposed to higher doses of heavy metal than its total soluble sugar (mg/g fresh weight) was significantly decreased as compared to control (T0) on the dates of proposed interval. The watched decrease in total sugar substance with regard to the high levels of zinc may be due to its part within the enzymatic responses related to the cycles of carbohydrate catabolism (Kumar and Dwivedi, 2014) [12] or likely compared with the photosynthetic hindrance or incitement of respiration rate (Kumar and Dwivedi, 2014) [12]. Pandey and Tripathi (2011) detailed a diminish in add up to dissolvable sugars in Albizia sprocera beneath overwhelming metal stretch. All overwhelming metals have diminished the substance withincreasing concentration in agrarian crops as appeared by Kumar and Dwivedi, 2014 [12].

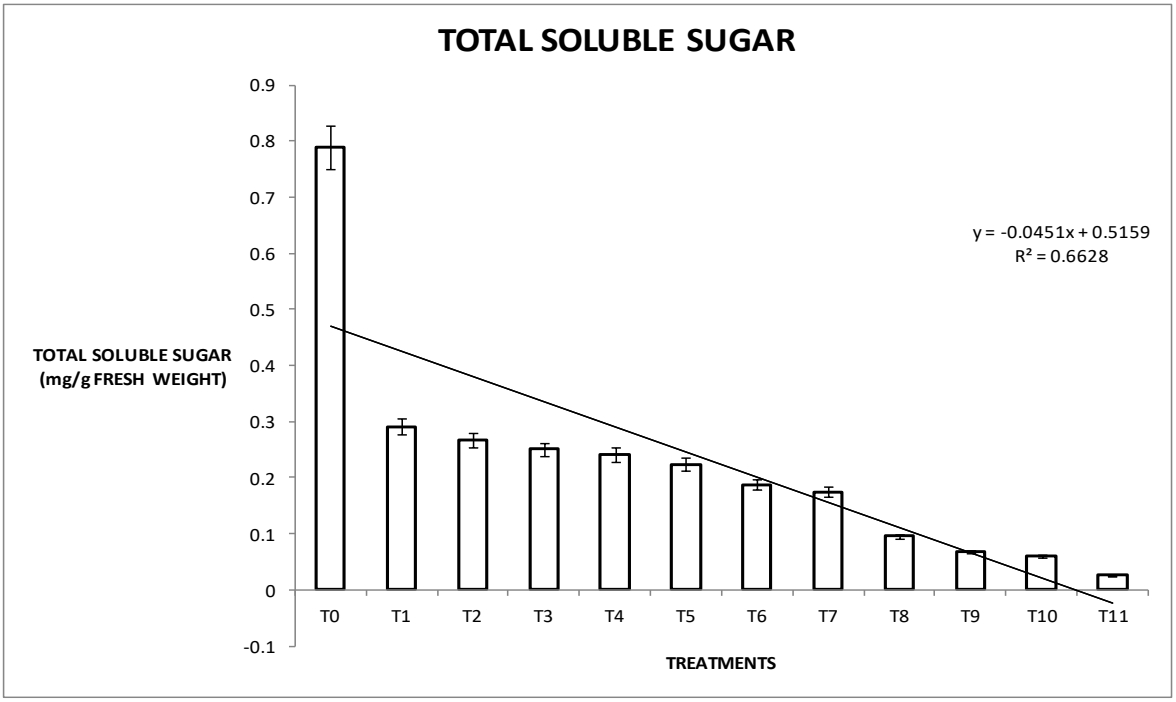

Fig 7: Total Soluble Sugar of Maize during Rabi

where, T0= Control, T1= 110ppm $Cd(NO_3)_2$, T2= 120ppm $Cd(NO_3)_2$, T3= 130ppm $Cd(NO_3)_2$, T4=140ppm $Cd(NO_3)_2$, T5= 150ppm $Cd(NO_3)_2$, T6= 160ppm $Cd(NO_3)_2$, T7= 170ppm$Cd(NO_3)_2$, T8= 180ppm $Cd(NO_3)_2$, T9= 190ppm $Cd(NO_3)_2$, T10= 200ppm Cd $(NO_3)_2$, T11= 210ppm Cd $(NO_3)_2$, Data is in the form of Mean ± SEM, LSD at $P \leq 0.05$ and $P \leq 0.01$

Conclusion

Plants that have efficient detoxification methods are generally distinguished as being the shift in the biochemical changes in maize. Better understanding of ecological interaction has been seen in the experiment. The increased level of phenol and reduced level of sugar in the sample indicates the appreciation of the effect of the remediation process on ecological interactions and that's why the knowledge of the entry and movement of the cadmium in the ecosystem.

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Conflict of interest statement

Authors have no conflict of interest.

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