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Zinc Stress Responses in Cajanus Cajan and Sorghum Bicolor Stomatal Behavior and Growth Dynamics

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ABSTRACT

In this study we investigated the Zn effect on stomatal behavior and plant growth of *Cajanus* and *Sorghum*. The anatomical changes of leaf was observed under Zn effect. Pollution negatively impacts on stomata structure by reducing the size of the stomata. Low concentrations of Zn initially increased the stomatal opening, followed by a closed stomata was found with high Zn concentrations. Tolerance index of *Cajanus* and *Sorghum* plants studied increase up to post flowering stage. *Sorghum* registered greater tolerance index values than *Cajanus*. The highest decrease in total plant length was noted at 100ppm Zn in both *Cajanus* and *Sorghum*. Zn toxicity in plants results in reduced growth of both root and shoot, however end of the research total plant length was decreased with increasing Zn concentration in *Cajanus* than *Sorghum.* The growth rate was exhibited slowly in pre flowering stage, followed by a rapid increase until post flowering stage, this growth rate showed a sharp decline in the end of the harvest period in all areas *Cajanus* and *Sorghum* plants studied. Flowering stage of *Cajanus* and *Sorghum* exhibited nearly one month delay in 50ppm and 100ppm Zn concentrations when compare to the controls. Some plants were not even produce flowers at 100ppm Zn.

Keywords: *Cajanus, Sorghum*, stomatal behavior, Zn toxicity, tolerance index.

INTRODUCTION

Zinc (Zn) is one of the eight trace element, the other trace elements are boron, chlorine, copper, iron, manganese, molybdenum, and nickel. These elements are referred to as essential trace elements or micronutrients. Zn phytotoxicity showed considerable attention because it is a part of the long term utilization of fertilizers, industries increased its content in the surface soil (Tsonev&Lidon, 2012). Plants absorb most of the minerals from the soil via their roots. Soil is important for plants because it holds roots that provide support for plants and store nutrients. Soil pollution takes place due to fertilizers, insecticides, and pesticides used in the fields. Soils may become contaminated by the accumulation of heavy metals and metalloids, mine tailings, disposal of high metal wastes, leaded gasoline and paints, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition (Khan *et al*., 2008). According to the Warne *et al.,* (2008) described that the increased concentration of Zn in the soil hinders metabolic functions of plants that causes senescence and delayed growth. At increased concentration of Zn create the cytotoxic effect on

plant growth and metabolism. It leads to major changes in the nucleolus of the root tips cells, cortical cells displayed disruption, and dilution of nuclear membrane at 7.5 mM dose of Zn (Rout and Das, 2003). Similar results in *Solanum nigrum* obtained by Liu *et al.,* (2016). Pollution negatively impacts on stomata structure by reducing the size of the stomata. Jitin Rahul and Manish Kumar Jain, (2016) studied to comparatively analyze the anatomical and morphological changes in roadside plants and heavy metal accumulation in selective roadside plants. The decrease of the happening in plants under exogenous use of Zn was potentially credited to a reduction in stomatal conductance.

MATERIALS AND METHODS

Site description

The experiment were conducted at Rampachodavaram and Rajahmundry rural areas (East Godavari dt). The district regional climate is Agro Ecological Sub Region on Eastern Coastal plain, hot sub-humid to semi arid eco region (12.1, 18.4). Agro-Climatic Region East Coast plain and hill region (XI). Geographic coordinates of district latitude 16o 58' 60"N longitude 18o 46' 60" E altitude 13m AMSL.

Field preparation and experimental design

The experimental field was ploughed with tractor and harrowed during the first week of July. Six fields (three field for *Cajanus* and three for *Sorghum*) were selected based on the Zn concentration. Six fields (three fields for *Cajanus* and three for *Sorghum*) were taken based on the Zn concentration. Out of the six fields, two fields had high Zn concentrations belong to Area-1 (10 km distance from the industries), having ~50 ppm(50ppm) of Zn, and two fields belong to Area-2 (5 km distance from the industries), having ~100ppm of Zn. Remaining two fields were normal area (30-50 km from industries.), having ~ 2ppm Zn which is located in Rampachodavaram rural areas, is considered as controlled field. The seeds are placed into the furrows ploughed in the field either continuously or at specific distance manually by a man working behind plough. The depth of sowing depends on the depth of plough. In present experimental fields depth of seed sowing is 25mm in *Sorghum* and 5cm in *Cajanus.*

Plant material

Two types of plants Pigeonpea (*Cajanus cajan* (L.) Millspaugh) and *Sorghum bicolour* L. were selected for the investigation. The seeds were obtained from agriculture research stations Rampachodavaram and Rajahmundry east Godavari district Andhra Pradesh.

SEM (Scanning Electron Microscopy):

Scanning Electron Microscopy works on the principle of scattering of electrons on the surfaces of the sample. The electrons reflected from the sample were detected in the form of secondary electrons with SEM technology. In this technology electrons beam is scanned in Raster Scan Pattern i.e. object is scanned side by side so 3-D images found Goldstein, *et al., (*1981).

For scanning electron microscopic studies both *Cajanus* and *Sorghum* 1-month-old fresh leaves were used from every three concentrations i.e. 2ppm, 50ppm, and 100ppm. The fresh leaves after cutting immediately transferred into 2.5% glutaraldehyde in 0.01 M phosphate buffer (pH 7.2) and fixed overnight. Post fixed in Osmium tetroxide in the same buffer for 4 hours. After the post-fixation, samples were dehydrated in a series of 30%, 50%, 70%, 90%, 100% alcohol every 20 minutes continuing the last step for another time. After dehydration samples were allowed for drying under critical point drying with the Electron Microscopy Science CPD unit. Then dried samples were mounted over the stubs with double-sided carbon tape. Finally, a thin layer of gold and palladium plating over the

sample using an automated sputter coater (Model: JEOL JFC- 1600) for about 10 min. Then scanned the samples under SEM ((Model: JEOL-JSM 6610LV) at various magnifications (500X). This experiment was conducted at Advanced Analytical Lab, Andhra University, and Visakhapatnam. This method was previously used by Priyadarshini and Sujatha, (2011) in *Cajanus cajan for* Cadmium-induced plant stress.

Tolerance Index (%)

The tolerance index of each concentration against each of the Zn concentration at the end of the research both of the plants *Cajanus* and *Sorghum* plants height was calculated as follows:

Mean plant length of plants in contaminated areas

$$\text{Toleranceindex} = \frac{\text{Mean plant length of plants in contrareas}}{\text{Mean plant length of plants in controlareas}} \times 100$$

RESULTS

Stomatal Behavior

The leaves (control, and Zn contaminated area) were taken for SEM studies. The stomatal aperture size of *Cajanus* showed a certain initial increase at 2ppm Zn, followed by a decline with increasing concentration of Zn at 50 and 100ppm Zn concentrations. 50 and 100ppm Zn concentrations resulted in a continuous decrease of stomatal size and closed stomata. In *Cajanus*, well-developed stomata and clear aperture had been seen in control plant leaves. And we are noticed conspicuous stomata with completely developed guard cells (Plate-1). The dumbbell shape of the guard cells had been seen clearly. In the case of 50ppm, the stomata were partially opened with wrinkled guard cells (Plate-2), 100ppm Zn decreased the stomatal aperture size with increasing Zn concentration, defective stomata with collapsed subsidiary cells. The 100 ppm Zn concentration affected the stomatal aperture size to a maximum extent compared to the two concentrations 2ppm and 50ppm. Whereas leaves of *Sorghum*, the stomatal aperture size of 2ppm Zn increased and also we observed well-developed ledges . On the other hand, 50ppm of the stomata were closed and wrinkled guard cells (Plate-2). Completely closed and undeveloped stomata were we have seen at the 100ppm Zn (plate-3)

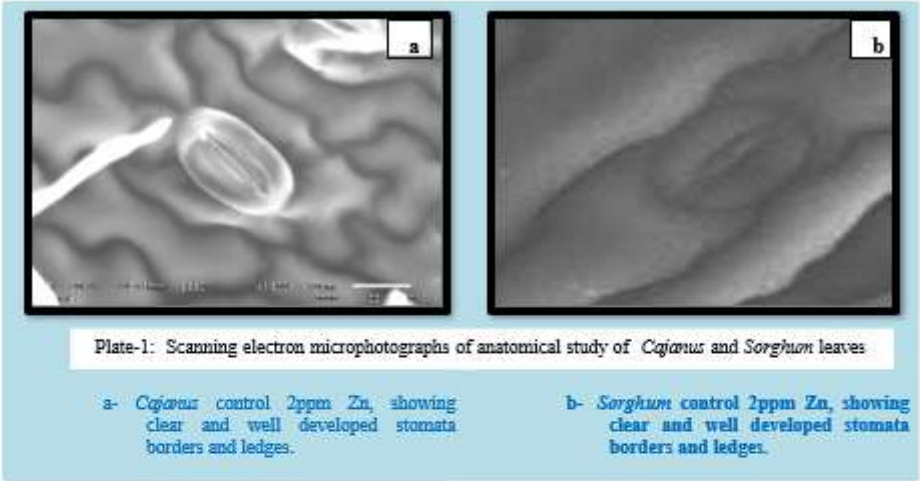


Plate-1: Scanning electron microphotographs of anatomical study of *Cajanus* and *Sorghum* leaves

a- *Cajanus* control 2ppm Zn, showing clear and well developed stomata borders and ledges.

b- *Sorghum* control 2ppm Zn, showing clear and well developed stomata borders and ledges.

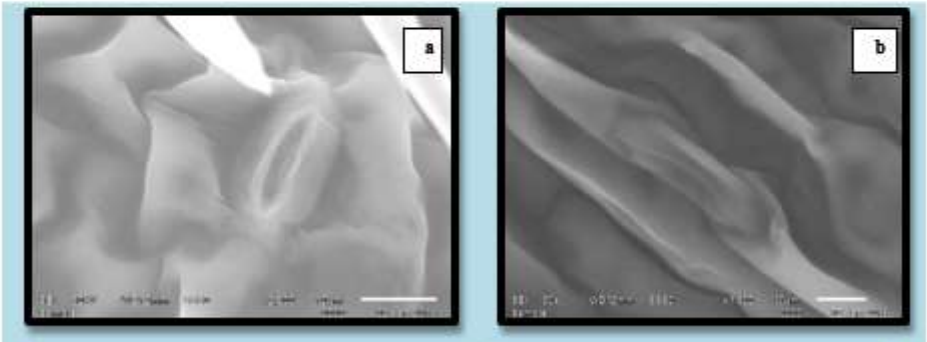


Plate-2: Scanning electron microphotographs of anatomical study of *Cajanus* and *Sorghum* leaves

a- *Cajanus* 50ppm Zn, showing disrupted trichome and stomata

b- *Sorghum* 50ppm Zn, showing disrupted trichome and closed stomata,

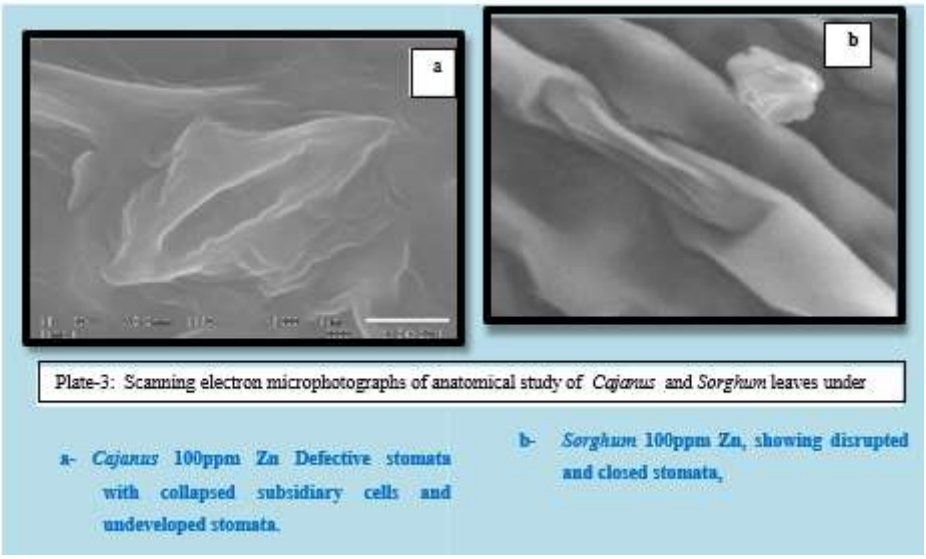


Plate-3: Scanning electron microphotographs of anatomical study of *Cajanus* and *Sorghum* leaves under

a- *Cajanus* 100ppm Zn Defective stomata with collapsed subsidiary cells and undeveloped stomata.

b- *Sorghum* 100ppm Zn, showing disrupted and closed stomata,

Growth stages

Plant growth analysis of *Cajanus* and *Sorghum* is carried out. Data were collected and analyzed after seed sowing at three stages pre-flowering, flowering, and post-flowering in three areas 2ppm(control) area- 1(50ppm), and area-2(100ppm) Zn concentrations (plate 4 and plate 5)

Cajanus and *Sorghum* showed a characteristic growth pattern with an initial slow growth up to 30 days after sowing followed by a rapid increase till last stages in controls. A continuous elongation of the plant length was observed in control area plants up to the time of harvest. The root and stem length was recorded high in controls both of the plants *Cajanus* and *Sorghum* (plate-4 and 5). At the end of the experimental period, the result revealed the length of plants showed 5.26 folds increase in *Cajanus* and 6.42 folds increase in *Sorghum*. Though the elongation of the plants of the contaminated areas showed a trend similar to that of controls, they always registered lower values when compared to their respective controls (Plate-4and 5). The length of plants in both *Cajanus* and *Sorghum* decreased with increasing concentrations of Zn (Fig. 1.3-1.4). Among the two contaminated areas plants exposed to Zn showed suppression of root growth in both of the plants. However, in both the areas of plants growth of roots was affected much greater than that of the stem.

The plant growth was gradually decreased in 100ppm Zn when compared to the control in both *Cajanus* and *Sorghum* plants. In *Cajanus* plant height was recorded as 1.5, 3.0 folds in the pre-flowering stage, 1.30, 1.93 folds in the flowering stage, and 1.37, 1.75 folds in the post-flowering stage decreased in 50 to 100ppm Zn concentrations respectively. On the other hand in *Sorghum* also followed the same pattern for plant growth, the decrease was recorded as 1.75, 3.5 folds in the pre-flowering stage, 1.29, 2.4 folds in the flowering stage, and 1.5, 1.83 folds in post-flowering stage 50 and 100ppm Zn concentrations respectively.

The end of the research result revealed plants of *Cajanus* recorded 27.21, 43.0 percent reduction in plant height 50ppm and 100ppm Zn concentrations respectively when compared to their controls. Similarly, *Sorghum* also resulted in 33.3, and 45.5 percent reduction of in-plant length at 50ppm and 100ppm concentrations respectively when compared to their controls. However, a rapid increase was shown in controls of both of the plants at the flowering stage. *Sorghum* exhibited a greater reduction in plant length than the *Cajanus*. Among the two areas, the highest growth reduction was noted in 100ppm Zn.

The days of flowering were broadly varied within the plants. The *Sorghum* flowering appeared early when compared to the *Cajanus*. In the case of *Cajanus* process of flowering continuously in each plant

almost up to the time of harvest. Control plants were flowering appeared nearly one month before when compared to the 50ppm and 100ppm Zn concentrations. The particular age of each stage differs according to the duration of the crop under Zn stress as indicated in the tables below. Some differences were exhibited under Zn stress when compared to the controls.

Table-1. Growth stages of *Cajanus* under Zn stress.

Zn Concentration	Days after Sowing			
	Pre flowering	Flowering	Post flowering	Days of harvest
2ppm	90	120	170	180
50ppm	110	140	180	200
100ppm	130	160	190	230

Table-2. Growth stages of *Sorghum* under Zn stress.

Zn Concentration	Days after Sowing			
	Pre flowering	Flowering	Post flowering	Days of harvest
2ppm	40	70	100	120
50ppm	60	90	120	150
100ppm	90	110	150	180

Plate 4 : Effect of Zn stress on *Cajanus* plants at the flowering stage.

2ppm Zn contration was treated as control area

50ppm Zn concentration was treated as contaminated area-1

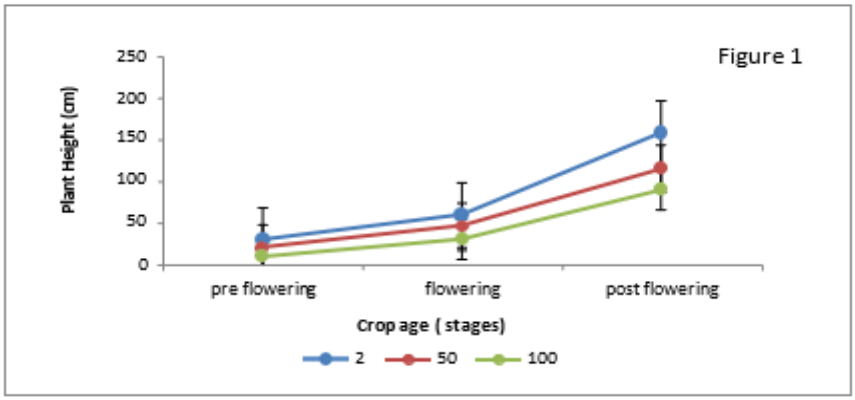
100ppm Zn concentration was treated as contaminated area-2

Plate 5 : Effect of Zn stress on *Sorghum* plants at the flowering stage.

2ppm Zn contration was treated as control area

50ppm Zn concentration was treated as contaminated area-1

100ppm Zn concentration was treated as contaminated area-2



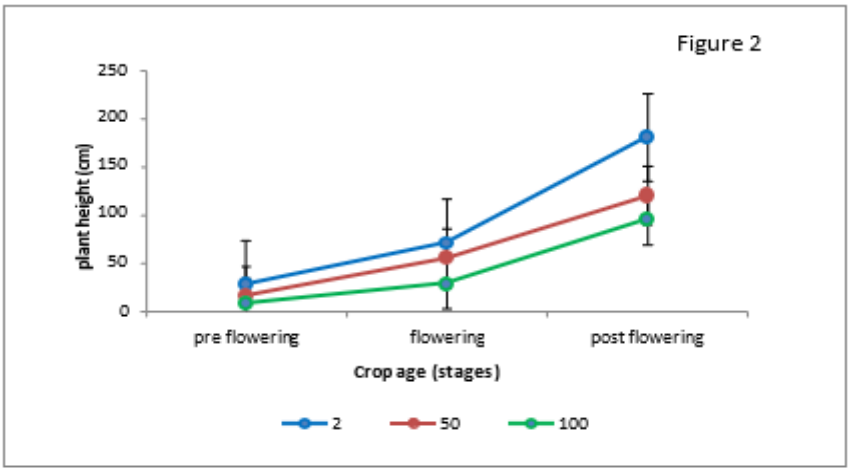


Figure 1: The effect of Zn stress on Plant length during three growth seasons of *Cajanus* (vertical lines represent S.E)

Figure 2: The effect of Zn stress on Plant length during three growth seasons of *Sorghum* (vertical lines represent S.E)

Plant Stages: Pre flowering
Flowering Post flowering
2 : 2ppm Zn contratio was treated as control area
50 : 50ppm Zn concentration was treated as contaminated area-1
100 : 100ppm Zn concentration was treaed as contaminated area-2

Tolerance index

The tolerance index of the plants of *Cajanus* and *Sorghum* at the end of the season decreased with increasing Zn concentration (Fig. 3 and 4). Among the two Zn concentrations, plants exposed to 100ppm Zn recorded the lowest index values. The *Cajanus* recorded 72.9, 56.9 percent tolerance index in 50ppm and 100ppm Zn concentrations respectively. *Sorghum* resulted in 66.6, 54.4 percent tolerance index at 50ppm and 100ppm Zn concentrations respectively. Greater tolerance index values were recorded in *Sorghum* than *Cajanus*.

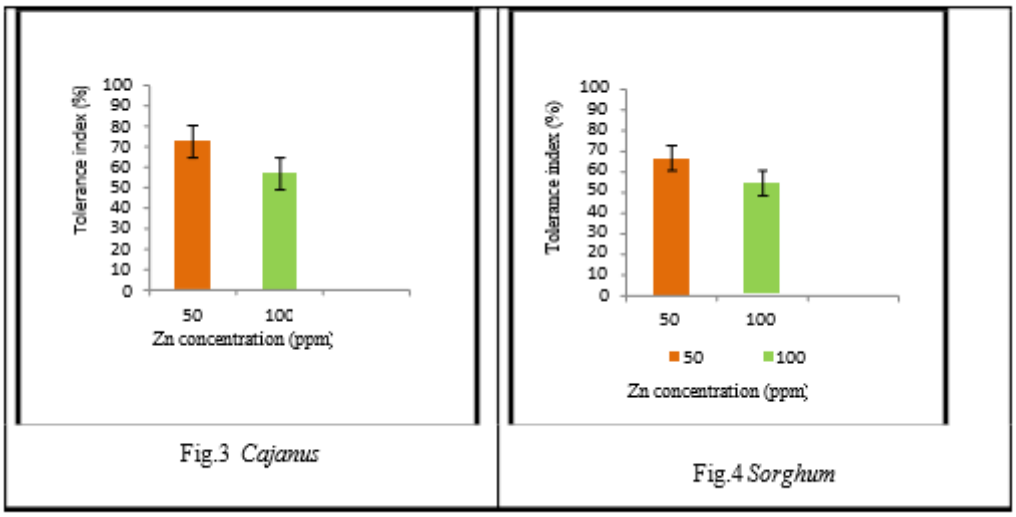


Fig.3 *Cajanus*

Fig.4 *Sorghum*

Figure 3 Tolerance index (per cent of control) of *Cajanus* at the end of the growth season under Zn stress. (Vertical lines represent S.E).

Figure 4 Tolerance index (per cent of control) of *Sorghum* at the end of the growth season under Zn stress. (Vertical lines represent S.E).

DISCUSSION

Great morphological variation exists in both of the plants at end of the research. In case of root and stem length, the result reveled that significant increase in controls both of the plants *Cajanus and Sorghum*. The anatomical changes of leaf was observed under Zn effect. Pollution negatively impacts on stomata structure by reducing the size of the stomata. We observed, in control plant stomata were fully grown with the well developed guard cells, borders and ledges (plate.1). Low concentrations of Zn initially increased the stomatal opening, followed by a closed stomata was found with high Zn concentration. In excess Mn reducing stomatal conductance (Li *et al.,* 2010). The decrease in cellular size and intercellular spaces with an increase in the metal concentration effected the thickenes of the leaf in Zn treated plants (Balaji *et al.,* 2007).

In the 50 ppm Zn contaminated plant leaves the defective stomata with collapsed subsidiary cells (plate.2 a, b). The stomata of the 100 ppm Zn exhibited undeveloped stomata both the plants *Cajanus and Sorghum*, and at 100ppm Zn showed completely collapsed, irregular thickness in guard cells in *Cajanus* (plate. 3 a) and their stomatal pore always remained closed in *Sorghum* (plate. 3 b). This indicates that stomata were severely affected by Zn ions. Priyadarshini *et al.,* (2015) described the stomata of pigeonpea and amaranth collapsed and lost their capacity of stomatal movement in response to SO_2. Similar results were found by Sandalio *et al.,* (2001) in *P. sativum* plants exposed to high concentrations of cadmium.

With the addition of heavy metal concentration of Fe, Zn and Cu, there was significant decrease in root/shoot length Nidhi mittal *et al.,* (2017). The reduced seedling length during metal stress could be due to low water potential, hampered nutrient uptake and secondary stress Jhon RP *et al.,* (2009). Due to excess amount of Zn reduction in cell division (Powell *et al.,* 1986). Moreover, we monitored the influence of Zn concentrations on length of root and stem. The length of root and stem significantly increased at 2ppm Zn compared with the high 50 and 100ppm Zn concentrations. The root growth of the plants sensitive to Zn was severely inhibited when subjected to high Zn concentration (Souza *et al.,* 2005). The root and stem length gradually decreased with increasing Zn in 50ppm and 100ppm compare to the control. Prabhu Inbaraj Michael *et al.,* (2014). Observed Zn toxicity symptoms appeared on all plants grown at excess Zn concentrations.. on the other hand leaf number also significantly increases at 2ppm compared with the high 50 and 100ppm Zn concentrations. Zn at 50 and 100ppm shown lowest number of leaves and leaf length was decreased. Cadmium caused visual toxicity symptoms in plants such as leaf roll and chlorosis (Gallego *et al.,* 2012). Cadmium reduced the root and shoot length, biomass, and photosynthetic pigments in numerous crop species (Rizwan *et al.,* 2016a; Younis *et al.,* 2016). Although very thick and green color leaves were observed in controls when compare to the 50 and 100ppm Zn concentrations. Gradually leaf color was decreased, they exhibited light green in color some of the plants shown yellowish green color because of excess amount of Zn at 50 and 100ppm. The growth rate was exhibited slowly in pre flowering stage, followed by a rapid increase until post flowering stage, this growth rate showed a sharp decline in the end of the harvest period in all areas *Cajanus* and *Sorghum* plants studied. Flowering stage of *Cajanus* and *Sorghum* exhibited nearly one month delay in 50ppm and 100ppm Zn concentrations when compare to the controls. Some plants were not even produce flowers at 100ppm Zn (Plate 4 and Plate 5).

However where as in 50 and 100ppm Zn, decline in plant length at pre flowering stage in *Cajanus* and flowering stage in *Sorghum* compare to the controls. R. John *et al.,* (2009) reported the effect of Cd and Pb on *B.juncea*. decline in root length in pre flowering stage and stem height declined from 4% (with 300 µM Cd) to 51% (with 900 µM Cd) at the post flowering stage.

Tolerance index of *Cajanus* and *Sorghum* plants studied increase up to post flowering stage. *Sorghum* registered greater tolerance index values than *Cajanus* (Fig.3 and 4). Rengel (2000) reported that the response of the plants to high Zn concentration was related to their tolerance capacity of Zn. The decrease of the relative yield at higher concentrations could be due to the toxic effect of Zn that damages plant growth (Atici *et al.,* 2005). TI decreases progressively with the increase of Zn concentration in solution tolerance index values lower than 50 % were recorded at concentrations between 200 mg/l and 600mg/l (Anişoara Stratu and Naela Costica 2015).

Notably lowest tolerance index values were noticed at 100ppm in both of the plants *Cajanus and Sorghum* when compare to their respective controls (fig.3 and 4). The increase in copper and Zn

concentrations decreased vigor and tolerance indexes of tomato seedlings compared to control, treatment (Ashagre *et al.,* 2013).

CONCLUSION

Pollution negatively impacted on stomata structure by reducing the size of the stomata. However, the Zn concentrations that was responsible for the stomatal opening differe in plants. The scanning electron microscopic studies of stomatal structure was observed in both types of plants under Zn stress. It was observed stomata of primary leaves of control ones were fully grown with well-developed guard cells, borders and ledges. Zn exerted a negative effect on leaf anatomy. The data showed that lower Zn concentrations initially increased the stomatal opening, followed by a closure with increasing Zn concentration (Plate-1,2 and 3). Zn toxicity has produced a negative interaction promoting stomatal closing, and consequently, reduction in stomatal conductance. This effect can be attributed to excess Zn, in which it will induce a minor density and size of these structures, with structural differences in minor stomatal aperture sizes. The stomata were the most effected as observed in 100ppm Zn in *Cajanus* and *Sorghum*. In *Sorghum* it was appeared to be closed stomata on the other hand, collapsed stomata was observed in *Cajanus*. Total height significantly differed because of excess amountof zinc.

Zn essentiality as a micronutrient up to certain concentration level. Maximum plant height was recorded in both types of plants *Cajanus* and *Sorghum* at the post flowering stage. The height was decreased in total plant length, noted at 100ppm Zn in both *Cajanus* and *Sorghum*. Flowering stage of *Cajanus* and *Sorghum* was exhibited nearly with one-month delay in 50ppm and 100ppm Zn concentrations when compared to the controls.

Stomatal closing, and consequently, reduction in stomatal conductance. This effect can be attributed to excess Zn, in which it will induce a minor density and size of these structures, with structural differences in minor stomatal aperture sizes. The stomata were the most effected as observed in 100ppm Zn in *Cajanus* and *Sorghum*. In *Sorghum*it was appeared to be closed stomata on the other hand, collapsed stomata was observed in *Cajanus*. (plate 4 and 5)

Maximum plant height was recorded in both types of plants *Cajanus* and *Sorghum*at the post flowering stage. The height was decreased in total plant length, noted at 100ppm Zn in both *Cajanus* and *Sorghum*. Flowering stage of *Cajanus* and *Sorghum* was exhibited nearly with one-month delay in 50ppm and 100ppm Zn concentrations when compared to the controls (Table 1 and 2).

REFERENCES

1. Anişoara Stratu , Naela Costica., (2015).The Influence Of Zinc On Seed Germination And Growth In The First Ontogenetic Stages In The Species *Cucumis Melo* L. *Pesd, Vol.* 9, No. 2, 2015. https://colab.ws/articles/10.1515%2Fpesd-2015-0038
2. Gallego SM, Pena LB, Barcia RA, Azpilicueta CE, Iannone MF, Rosales EP, Benavides MP., (2012) Unravelling cadmium toxicity and tolerance in plants: insight into regulatory mechanisms. *Environ Exp Bot* 83:33–46. https://www.researchgate.net/publication/249314301
3. Goldstein, G.I., Newbury, D.E., Echlin, P., Joy, D.C., Fiori, C. andLifshin, E., (1981). Scanning electron microscopy and x-ray microanalysis. New York: *Plenum Press*. https://rd.springer.com/content/pd f/bf m:978-1-4 939-6 676-9 /1.pdf
4. Jitin Rahul and Manish Kumar Jain., (2016) Effect of Heavy Metals on Some Selected Roadside

Plants and its Morphological Study. Nature Environment and Pollution Technology An International Quarterly Scientific Journal, Vol. 15, No. 4, 2016. https://neptjournal.com/upload-images/NL-58-5-(3)B-3291.pdf.

5. L M Sandalio, H C Dalurzo,Gomez,M C Romero-Puertas, L.A del .Rio., (2001). Cadmium-induced changes in the growth and oxidative metabolism of pea plants. https://pubmed.ncbi.nlm.nih.gov/11604450/
6. Liu, X., Chen, J., Wang, G. H., Wang, W. H., Shen, Z. J., Luo, M. R., (2016). Hydrogen sulfide alleviates zinc toxicity by reducing zinc uptake and regulating genes expression of antioxidative enzymes and metallothioneins in roots of the cadmium/zinc hyperaccumulator *Solanum nigrum* L. *Plant Soil* 400, 177–192. https://link.springer.com/article/10.10 07 /s11104-015-2719-7
7. M. K. Zhang, Z. Y. Liu, and H. Wang., (2010). "Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice," *Communications in Soil Science and Plant Analysis*, vol. 41, no. 7, pp. 820–831. https://www.tandfo nline.com /doi/abs/ 10 .1080 /0010362100 3592341
8. Nidhi Mittal, Kirti Sharma, Oshin, Avneet Kaur., (2017). Toxicity of metals such as iron, Nickel and cobalt on seedlings of broad beans (*Viciafabal*). *International Education & Research Journal* [IERJ]. Volume : 3, Issue : 5. https://www.researchgate.net/publicat ion/327135402
9. Prabhu Inbaraj Michael and Muthuchelian Krishnaswamy., (2014). Membrane Damage and Activity of Antioxidant Enzymes in Response to Zinc and High Irradiance Stress in Cowpea Plant. *International journal of current research and academic review* 2014; 2(10):112-128. https://www.sem antic scholar.org/pap er/Membrane-Damage-and-Activity-of1Antioxidant-Enzymes- Michael-Krishnaswam y/c47 3ac8191f0f245 6976292441 12170b0 4747437
10. Priyadarshini, B. and Sujatha, B., (2011). Cadmium-induced plant stress investigated by scanning electron microscopy in *Cajanus cajan*. *Journal of Pharmacy Research*, 4(3):938-941. https://scholar.google.co.in/scholar?q =cadmium+induced+plant+stress+inve stigated+by+scanning+electron+m icro scopy+in+cajanus+cajan&hl=en&as_sd t=0 &as_vis=1&oi=scho lart
11. Rengel Z., (2000). Ecotypes of Holcus lanatus tolerant to zinc toxicity als tolerate zinc deficiency. *Ann Bot* 86:1119-1126. https://www.sciencedirect.com/science/article/abs/pii/S0305736400912824
12. Rizwan M, Ali S, Abbas T, Rehman MZ, Hannan F, Keller C, Al-Wabel MI, OK YS., (2016a). Cadmium minimization in wheat: a critical review. *Ecotoxicol Environ Saf*: 130:43–53. https://pubmed.ncbi.nlm.nih.gov/27062345/
13. R. Johna, P. Ahmad, K. Gadgila, S. Sharmab., (2009). Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. *International Journal of Plant Production* 3 (3). https://ijpp.gau.ac.ir/artic le_653 .html
14. S. Khan, Q. Cao, Y. M. Zheng, Y. Z. Huang, and Y. G. Zhu., (2008). "Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China," *Environmental Pollution*, vol. 152, no. 3, pp. 686–692. https://pubmed.ncbi.nlm.nih.gov/1772 0286/
15. Rout, G.R. and Das, P., (2003). Effect of metal toxicity on plant growth and metabolism. I. Zinc.

Agronomic
23: 3-11. https://link.springer.com/chapter/10.1007/978-90-481-2666-8_53

16. Souza JF, Dolder H. Cortelazzo AL., (2005). Effect of excess cadmium and zinc ions on roots and shoots of maize seedlings. *J Plant Nutr* 28:1923-1931. https://www.tandfo nline.com /doi/full/ 10 .1080 /0190416050 0310435
17. Tsonevt, lidon F.J.C., (2012). Zinc plants-An overview. *Emir. J. food agr.* 24:322-333. https://www.researchgate.net/publicat ion/267030914_Zinc_in_plants_-_An_o verview
18. Warne, M. S. J., Heemsbergen, D., Stevens, D., McLaughlin, M., Cozens, G., Whatmuff, M., (2008). Modeling the toxicity of copper and zinc salts to wheat in 14 soils. *Envirol Toxicol. Chem*. 27, 786–792. https://pubmed.ncbi.nlm .nih.gov/1833 36 81/
19. Younis U, Malik SA, Rizwan M, Qayyum MF, Ok YS, Shah MHR, Rehman RA, Ahmad N., (2016) Biochar enhances the cadmium tolerance in spinach (*Spinacia oleracea*) through modification of Cd uptake and physiological and biochemical attributes. *Environ Sci Pollut Res* 23:21385–21394. https://pubm ed.ncbi.nlm .nih.gov/2750 25 64