EFFECTS OF SOIL ACCUMULATED ZINC ON CHLOROPHYLL AND CARBOHYDRATE METABOLISM OF *CAJANUS CAJAN* (L.) AND *SORGHUM BICOLOUR* (L.)

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Abstract: *Zinc is one of the essential micronutrient for plant growth and development. In addition, zinc is a heavy metal and too much zinc can cause toxicity in most plants. Heavy metals due to industrial activities and frequently use of chemical fertilizers cause damage to the plants. In this study examined the effect of different concentrations of Zn control (2ppm), contaminated area-1 50ppm and contaminated area-2 100ppm on chlorophyll a, b, starch, total soluble sugars, reducing, non reducing sugars, starch and amylase content in Cajanus and sorghum. The results showed that that high zinc concentrations, decrease of photosynthetic pigments such as chlorophyll a, b, and soluble sugars, reducing, non reducing sugars, starch were decreased with increase in Zn concentration. Amylase activity also decreased with increasing Zn but slightly increase in leaf at the stage of post flowering in both the plants Cajanus and sorghum.*

Keywords: Zinc, toxicity, sugars, chlorophyll, amylase, *Cajanus and Sorghum.*

I. INTRODUCTION

Heavy metals are essential and important for plants growth, and play as key components of many vital compounds. However, when they increase in concentration, heavy metals show symptoms such as growth delay and inhibition of biochemical reactions (Aldoobie, N. F. and Beltagi, M.S. 2013). However elevated concentrations of both essential and non-essential heavy metals in the soil can lead to toxicity symptoms and growth inhibition in most plants (Hall, 2002; Li et al., 2010). Heavy metal stress is one of the major abiotic stresses that cause environmental pollution in recent decades (Gisbert et al., 2003; Castro et al., 2011). High zinc concentrations, like other heavy metals, are toxic for plants (Zhao et al., 2003).

Zn is one of the essential micro nutrient in plants. It plays an important role in plant growth and development. Although excessive amount of Zn leads to toxicity in plants. And high absorption of Zn suppresses copper and iron absorption and cause severe growth reduction and damage to the roots, yellowing wilting of leaves and reduced yields and stunted growth. Fe deficiency-induced chlorosis through reductions in chlorophyll synthesis and chloroplast degradation, and interference with (P and Mg and Mn) uptake (Foy et al., 1978; Chaney, 1993), And disturbance in the intensity of basic physiological processes, i.e., photosynthesis, respiration, and transpiration, and decrease of reproductive performance, too (Ali et al., 2000; Khudsar et al., 2004; Kholodova et al., 2005).Too much of it in most plants are poisonous. Zn2+ ions in high concentrations due to the overproduction of reactive oxygen caused oxidative damage to the plants (Lin and Mark, 2012; Khan *et al*, 2014).

Zinc toxicity in agricultural soils irrigated with sewage and indiscriminate use of fertilizers to occur (Yadav, 2010; Muntean*et al*, 2012) and toxicity occurs in soils contaminated by mining and smelting activities, in agricultural soils treated with sewage sludge, and in urban and peri-urban soils enriched by anthropogenic inputs of Zn, especially in low-pH soils (Chaney, 1993).In soluble zinc in soils can contaminate groundwater and excessive applications of zinc contain fertilizers or pesticides and use of zinc contaminated sewage sludges (Giuffre et al., 2012). Detailed studies indicate that heavy metals have effects on chlorophyll content in plants. Heavy metals are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient (van Assche and Clíjsters, 1990; Meers et al., 2010). Distinct concentrations of metals induce different biochemical responses in plants. In sensitive plants, high concentration of these metals inhibits enzymes involved in photosynthetic reaction (Smirnoval et al., 2006). *Brassica juncea*(Indian mustard), a high biomass producing plant can accumulate lead, chromium, cadmium, copper, nickel, zinc, boron and selenium (Palmer et al., 2001; Akbaş et al., 2009).

The purpose of this study was to investigate the effects of Zn concentration in three different areas and three plant stages. From preliminary experiments were conduct on percentage of germination under Zn contaminated areas and control areas and inhibition of plant growth. We examined the chlorophyll, total carbohydrates and soluble, non reducing, reducing sugars and starch in both the plants *Cajanus* and *Sorghum.*

II. MATERIALS AND METHODS

Site description

The experiment were conducted at Katheru, Gandepalli, Rajahmundry, Rampachodavaram (East Godavari dt) areas. 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 were selected based on the Zn concentration. Where the four sampling fields of soil is contaminated with heavy metal. Area-1 (10 km distance from the industries), 50 ppm of Zinc, Area-2 (5 km distance from the industries), having a concentration of 100 ppm of Zinc. The normal area (30-50km from industries.), which is located in Rampachodavaram rural areas, considered as Controlled fields (2ppm). 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 and depth of soil type. In our experimental fields depth of sowing 25mm in *Sorghum* and 5cm in *Cajanus.*

Plant material

Two types of plants Pigeonpea (*Cajanu 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.

Germination percentage: Seeds of pigeon pea and sorghum were selected and cultivated separately in fields containing 2, 50, 100 ppm Zinc contamination soils. Germinate at natural climatic conditions.

Length: The length of the root stem and leaf samples were measured using a mm scale. The length of the plant parts were expressed in cm.

Fresh Weight: The plant parts were separated into root, stem and leaf and their fresh weights were determined immediately.

Dry Weight: The root, stem and leaf kept for drying in a hot air oven maintained at 80^0C for 48 h, by which time constant dry weights were obtained.

Chlorophyll

Chlorophyll contents (Total chlorophyll, chl a and chl b) were estimated using the method of Arnon (1949).The absorbance of the 80% acetone extract was read at two wave lengths, 645 and 663 nm using Shimadzu (UV-240) spectrophotometer. The amount of total chlorophyll, chlorophyll and chlorophyll b were calculated as milligrams of chlorophyll content per gram of plant tissue.

Total Carbohydrate estimation

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630nm.

Starch and Total soluble sugars

The starch and total soluble sugars were estimated according to the method of McCready et al. (1950) as modified by Clegg (1956). Soluble sugars were separated by alcohol extraction and the residue containing starch was brought into solution with perchloric acid.

Reducing sugars: Total reducing sugars were estimated according to the phenol-sulphuric acid method of Dubois et al., (1956) as followed by Smyth and Dugger (1980).

Non reducing sugars

The reducing sugar content subtracted from the total soluble sugar content was considered as non reducing sugars.

Amylase

Amylase activity was estimated by the method of Filner and Varner (1967) as followed by Kapoor and Sachar (1979). One gram of plant material was homogenized in 15 ml of 50 mM phosphate buffer pH 6.5. The homogenase was centrifugate at 10,000 g for 20 min. The supernatant containing crude enzyme extract was collected and used for measuring the starch hydrolyzing activity.

III. RESULTS

Percent seed germination of both the *Cajanus* and *sorghum* decreased with increasing concentrations of zinc. The seed germination of controls (2ppm) exhibited 98 per cent in *Cajanus* and 96 per cent in *Sorghum*(Fig no.1) The area 2 (100 ppm) recorded lowest per cent germination. The seeds of *Cajanus* recorded 85 and 63 per cent seed germination in area-1, area-2 zinc concentrations respectively. The *Sorghum* showed 70 and 55 per cent germination in area-2, area-3 zinc concentrations respectively. *Sorghum* exhibited greater reduction in per cent seed germination than *Cajanus* . Area-3 effected per cent germination more intensely than both control and area-2.

A continuous elongation of the plant length was observed in control areas in both the plants of *Cajanus* and *Sorghum* At the end of the month the length of the plants were showed 3.0 folds decrease in *Cajanus,* 3.75 folds decrease in *Sorghum* in area-2. They always registered lower values when compared to their respective controls (Plate.1), length of plants in both *Cajanus* and *Sorghum* decreased with increasing concentrations of Zn. The fresh weight (fig.no.2) and dry weight (fig.no.3) recorded highest values in control lowest values in contaminated areas. Always lowest values recorded in area 2 both *Cajanus* and *Sorghum.*

The chlorophyll a content of *Cajanus* and *Sorghum* plants were recorded in three plant stages (pre flowering, flowering and post flowering) and three different contaminated areas (control, area-1 and area-2). The chlorophyll a content was gradually decreased from control to area- 2 fields. Among the three areas plants both *Cajanus* and *Sorghum* those exposed to high Zn contaminated area-2 (100 ppm) recorded lower levels of chlorophyll a and b content. This trend was followed in three plant stages.(fig: no 4,5). Total carbohydrates (fig.no.6) and starch (fig.no.7,8) were gradually decreased from control to area- 2 field both the plants *Cajanus* and *Sorghum* . Total soluble sugars were also recorded lowest values compare to their controls respectively (fig.no.9,10). And the decreased was observed in reducing sugars (fig.no.11,12) and non reducing sugars (fig.no.14,15) also in three plant stages.

Amylase activity of *Cajanus* and *Sorghum* plants were recorded in three plant stages and three different contaminated areas. Amylase activity was gradually decreased from control to area- 2 fields (fig no: 15,16). But in our study post flowering stage *Cajanus* and *Sorghum* amylase activity was slightly increase in leaf. 1.17 folds increase in *Cajanus* leaf and 1.21 folds in *Sorghum* leaf from control to area-2.

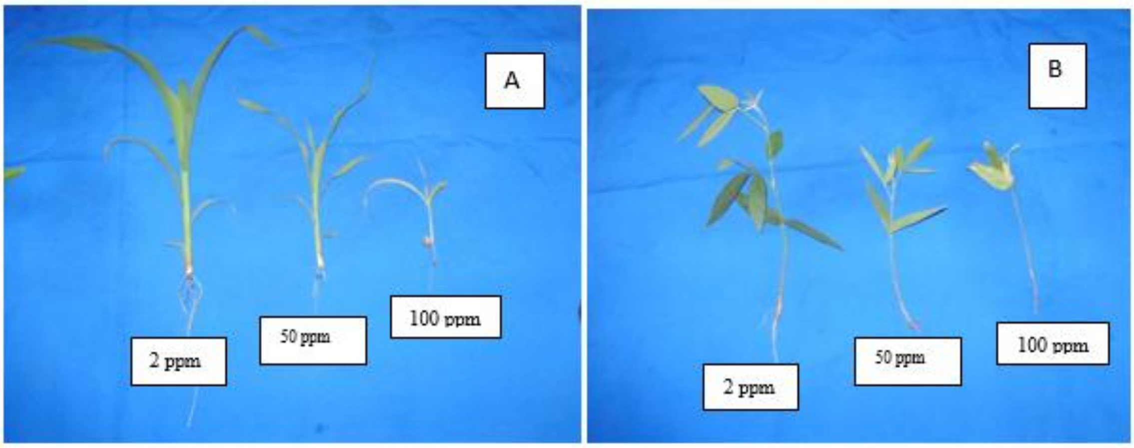


Plate no.1: One month old plants a).*Sorghum*, b).*Cajanus*

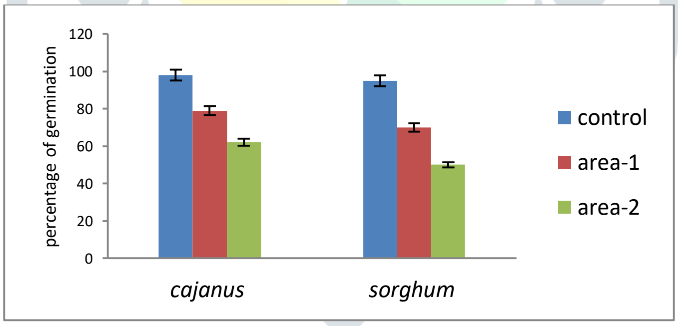


Fig no: 1The effect of Zn on seed germination of *Cajanus* and *Sorghum*

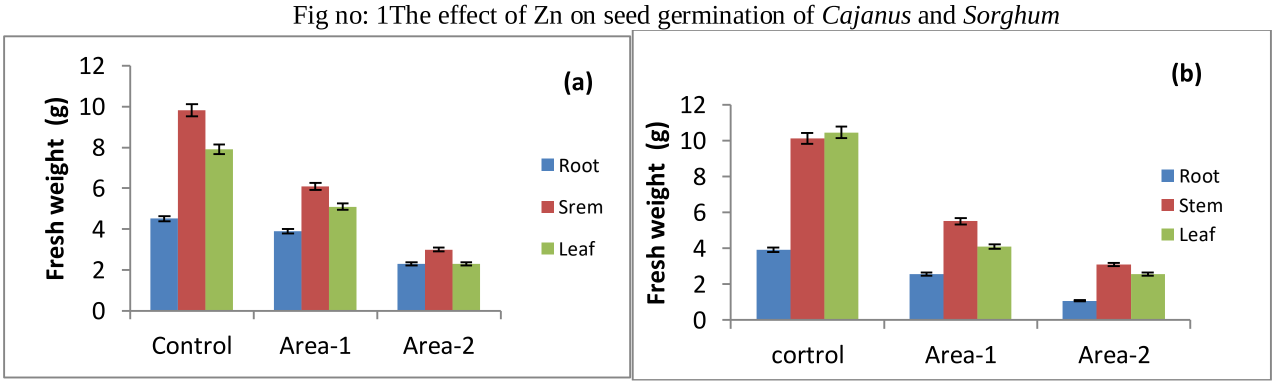


Fig no: 2 The effect of Zn on Fresh Weight a). *Cajanus* and b). *Sorghum*

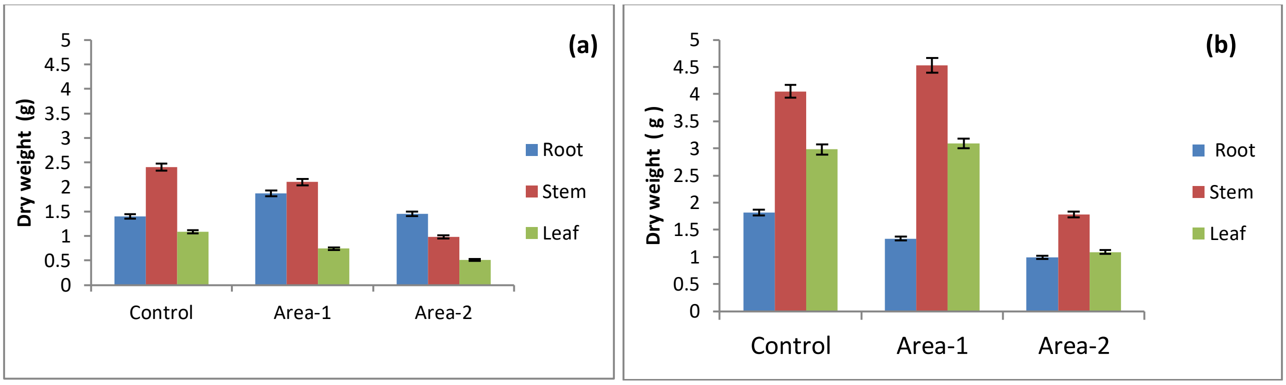


Fig no: 3 The effect of Zn on Dry Weight a).*Cajanus* and b). *Sorghum*

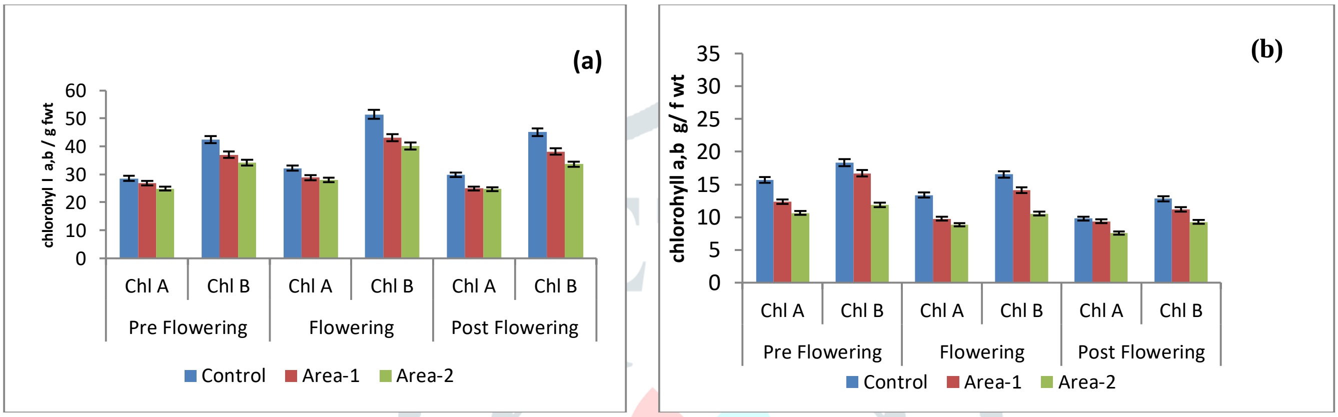


Fig no: 4 The effect of Zn on Chlorophyll in *Cajanus* a). Leaf andb). Stem

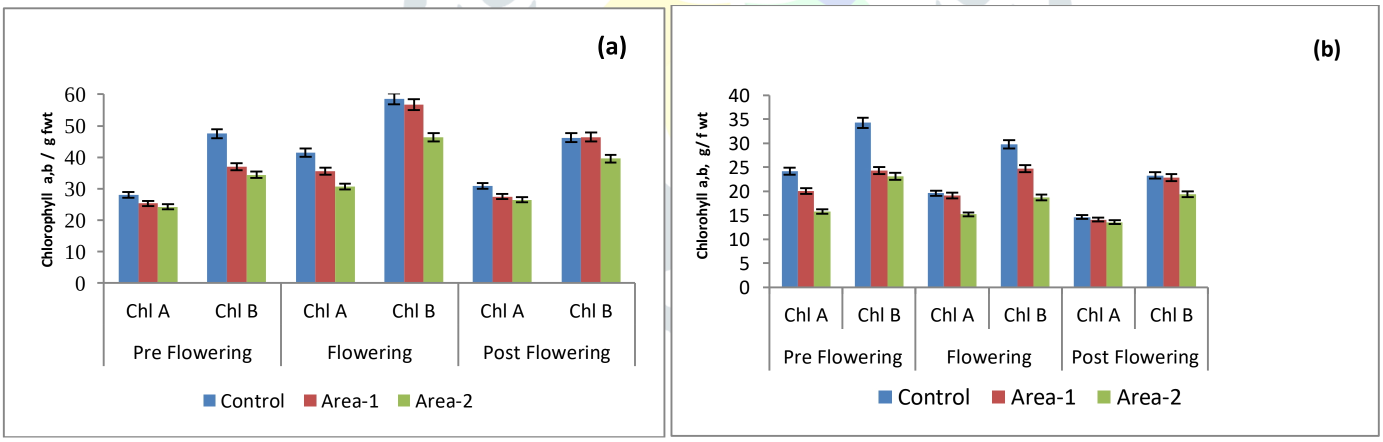
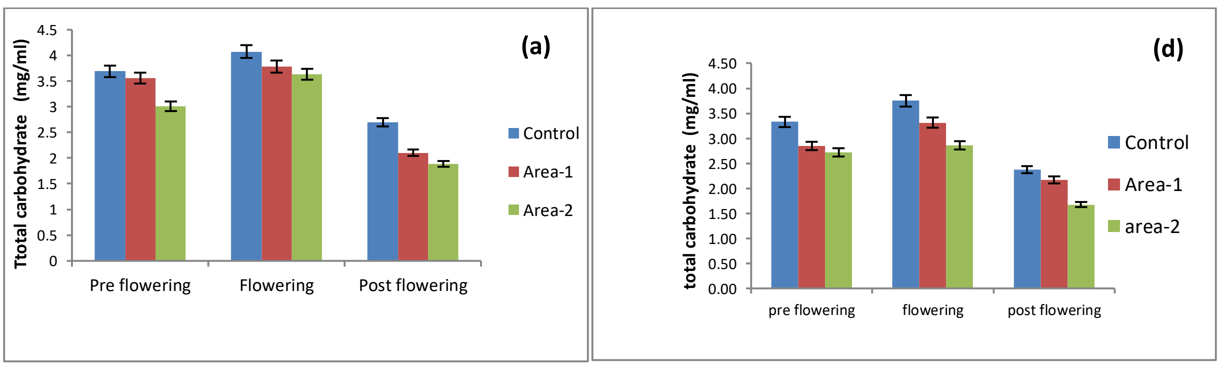


Fig no: 5 The effect of Zn on Chlorophyll in *Sorghum* a).Leaf and b).Stem



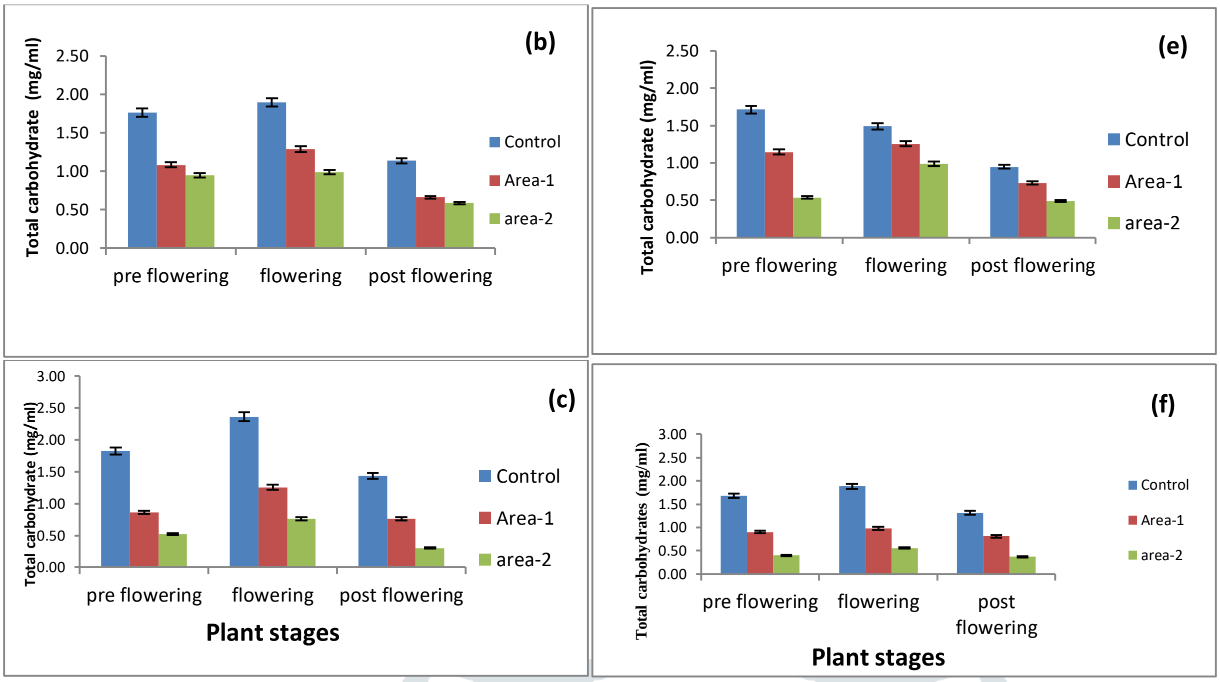


Fig no: 6. The effect of Zn on the total carbohydrates of *Cajanus* a-leaf, b-stem, c-root, and *Sorghum* d-leaf, e-stem, f-root.

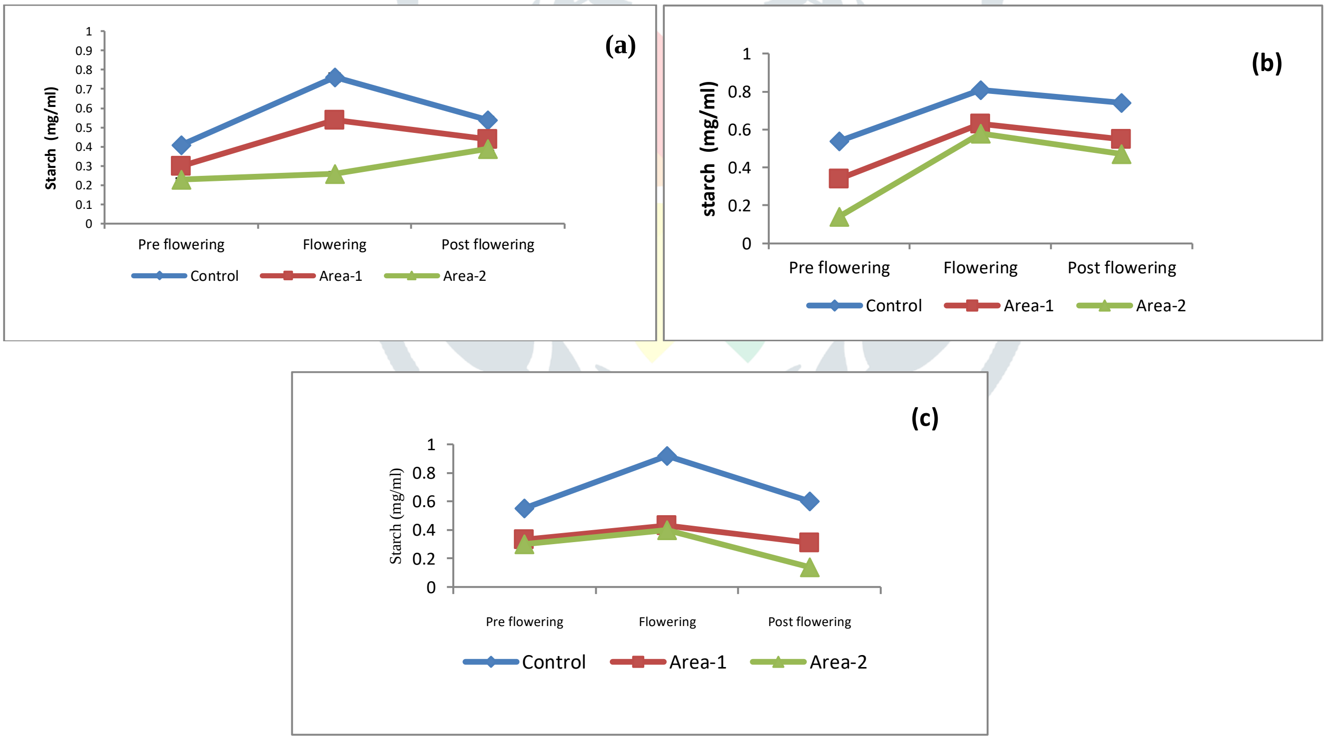


Fig no: 7 The effect of Zn on the Starch of *Cajanus* a). Leaf, b).Stem and c). Root.

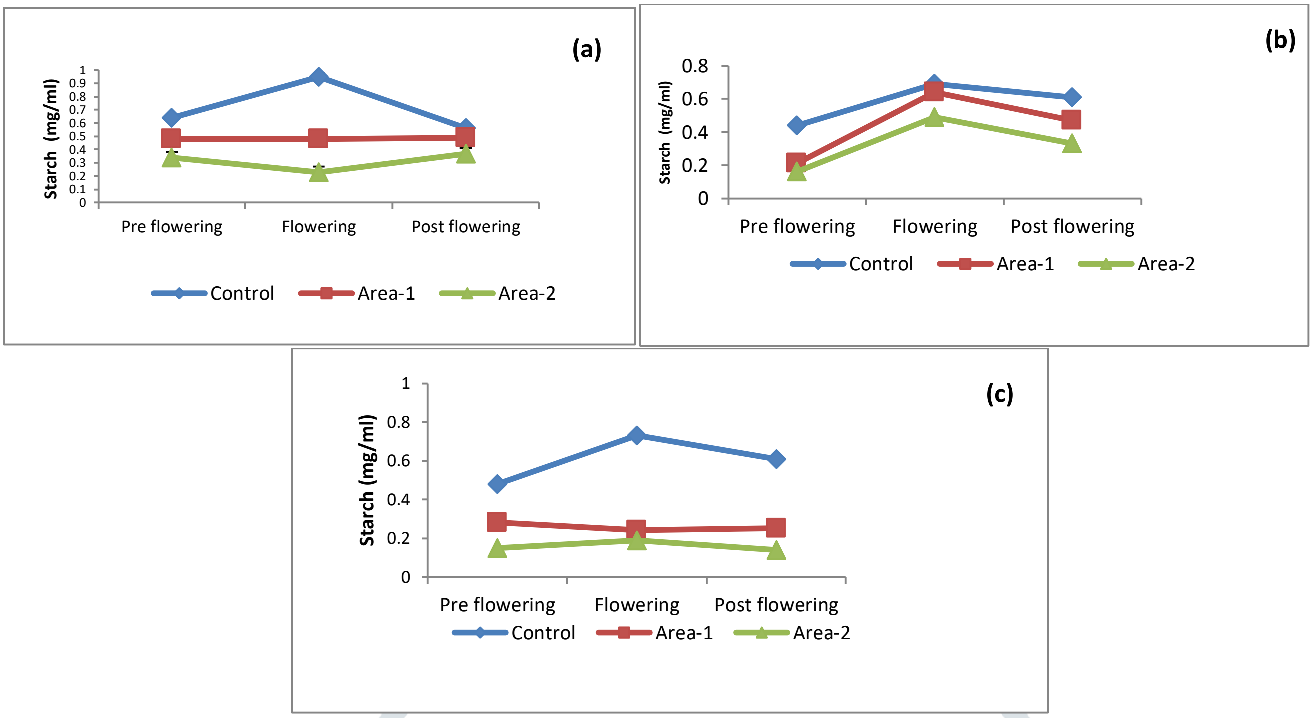


Fig no: 8 The effect of Zn on the Starch content of *Sorghum* a). Leaf, b).Stem and c) Root.

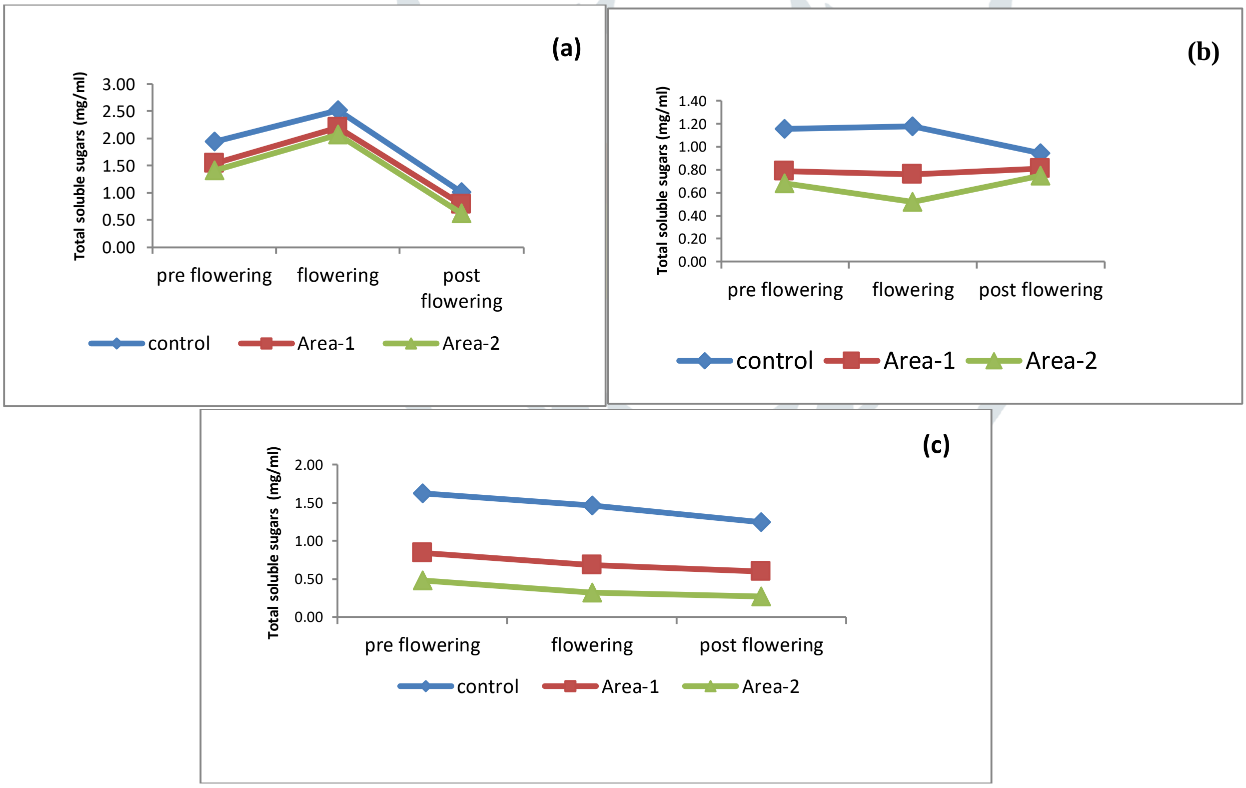


Fig no: 9. The effect of Zn on the total soluble sugars of *Cajanus* a). Leaf, b).Stem and c). Root

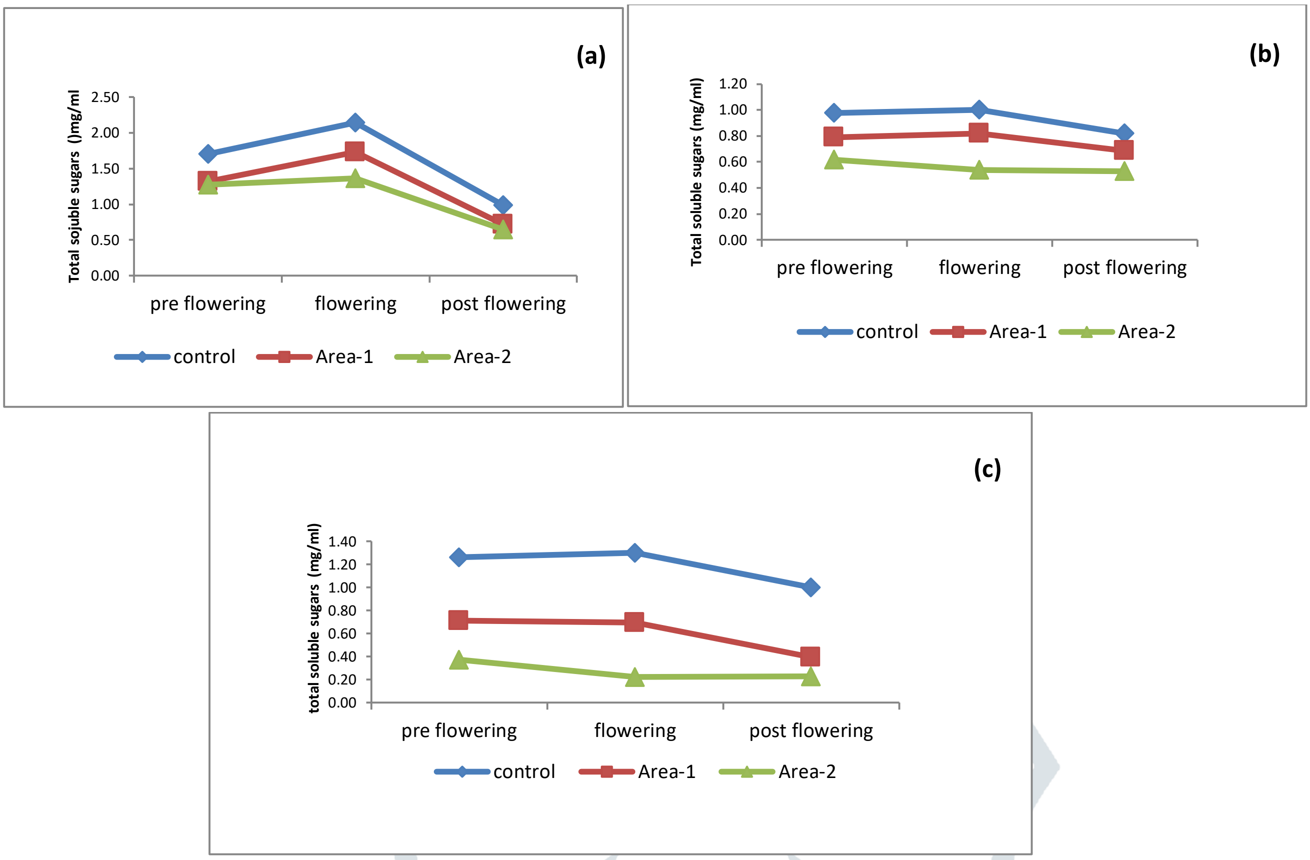


Fig no: 10. The effect of Zn on Total soluble sugars of *Sorghum* a). Leaf, b).Stem and c). Root

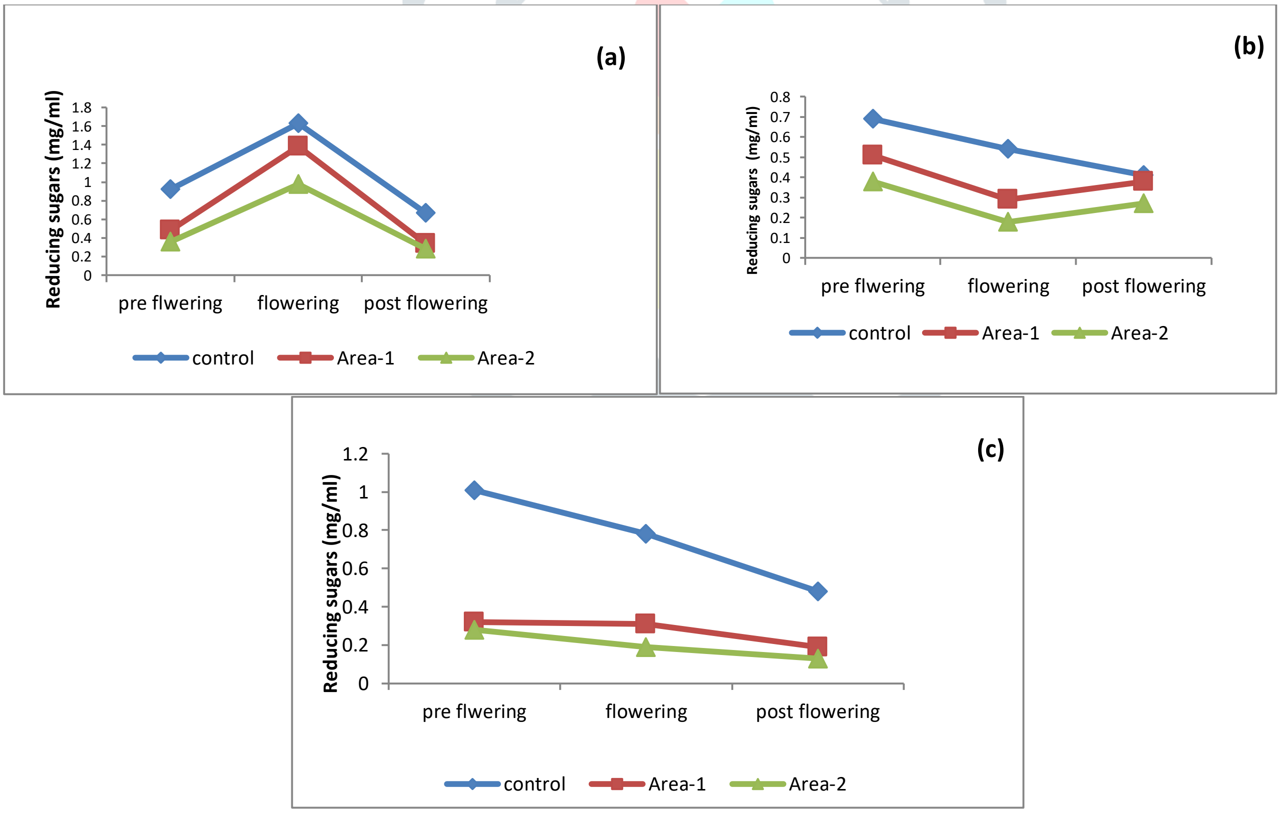


Fig.no: 11 The effect of Zn on the reducing sugars of*Cajanus* a). Leaf, b).Stem and c). Root

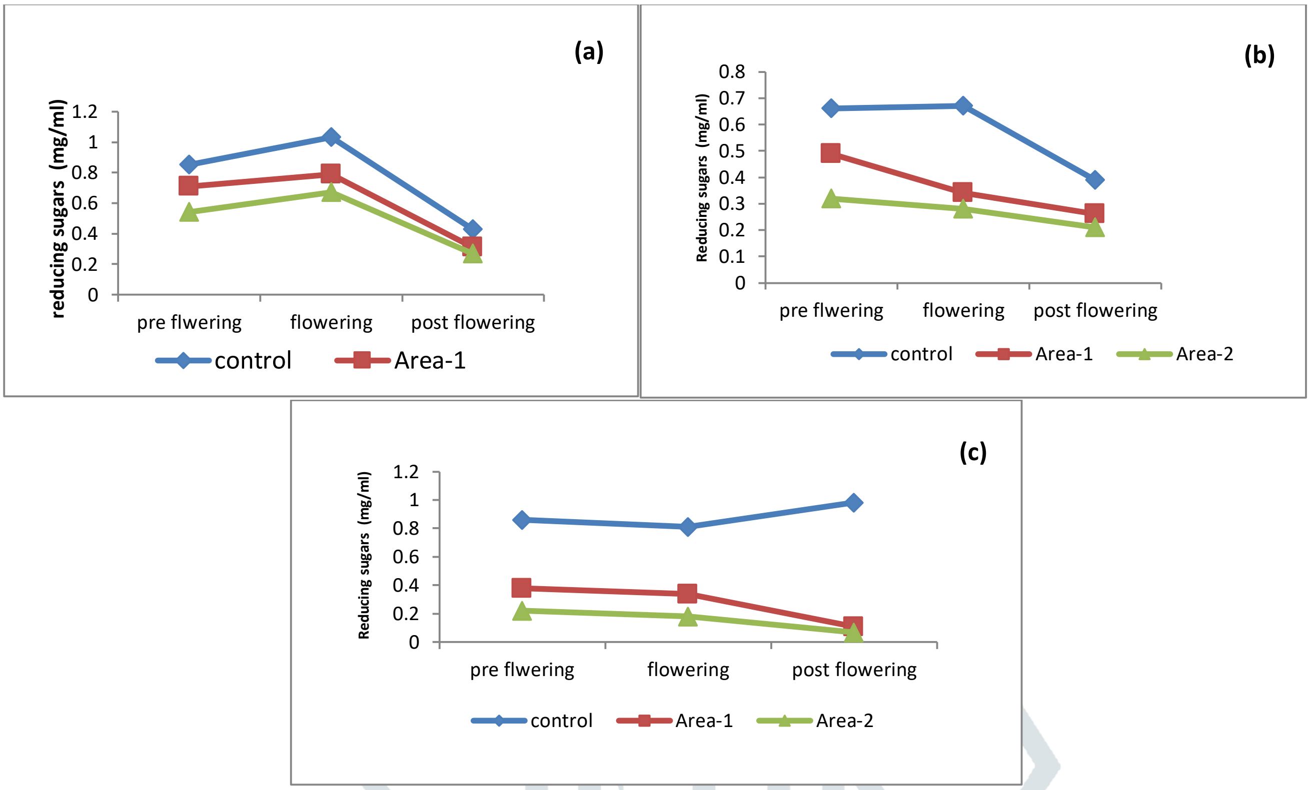


Fig no: 12. The effect of Zn on reducing sugars of *Sorghum* a). Leaf, b).Stem and c). Root

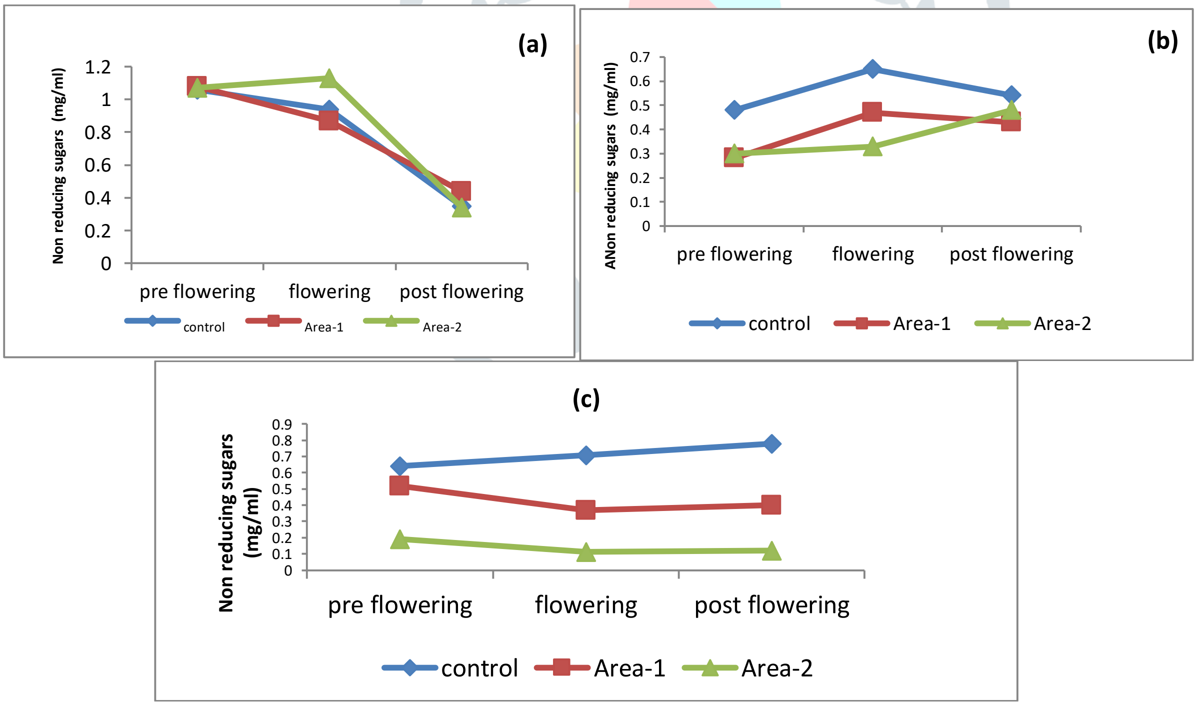


Fig no: 13. The effect of Zn on the non reducing sugars of *Cajanus* a). Leaf, b).Stem and c). Root

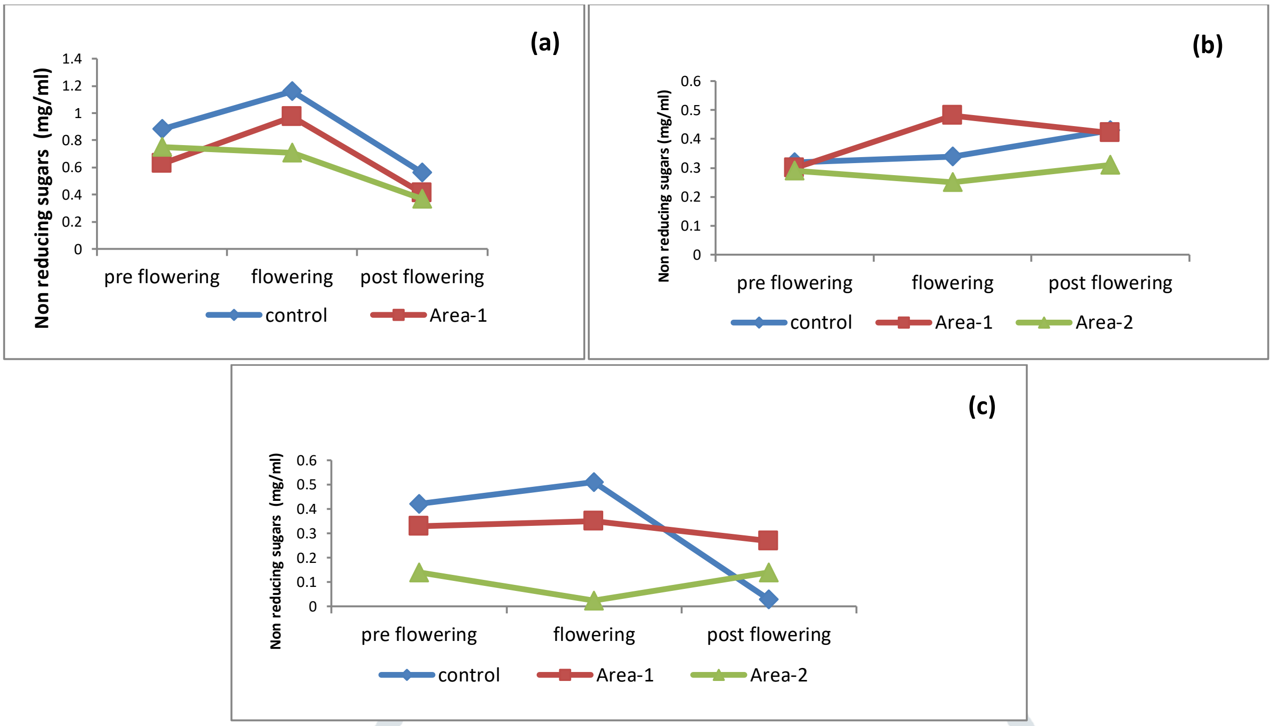


Fig no: 14. The effect of Zn non reducing sugars of *Sorghum* a). Leaf, b).Stem and c). Root

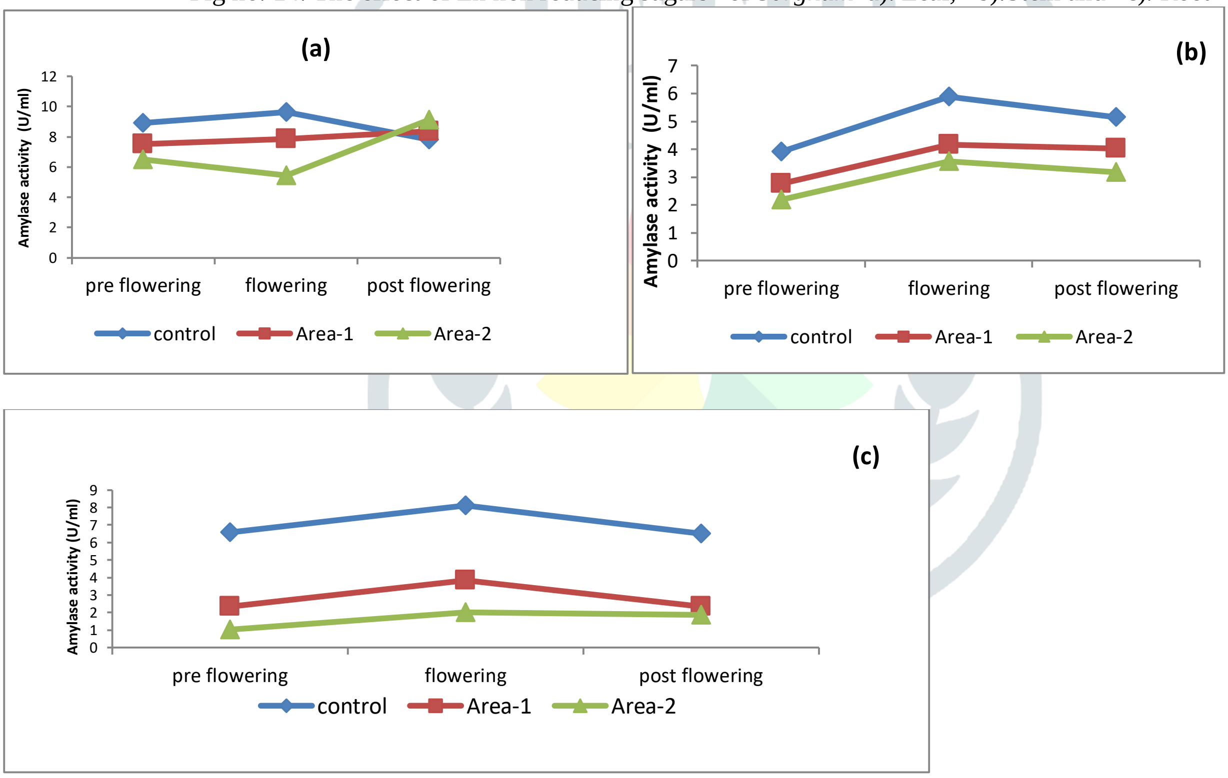
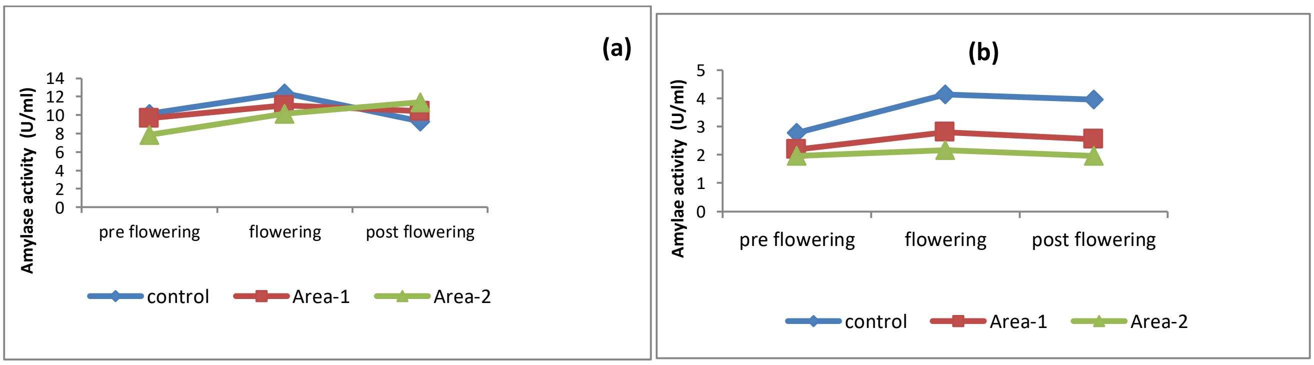


Fig no: 15. The effect of Zn on the amylase of *Cajanus* a). Leaf, b).Stem and c). Root



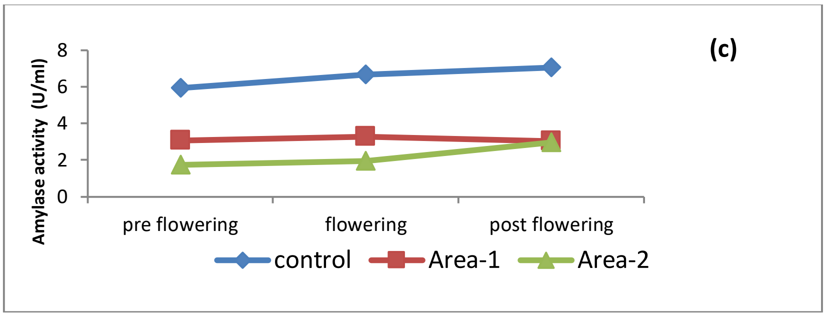


Fig no: 16. The effect of Zn on the amylase of *Cajanus* a). Leaf, b).Stem and c). Root

IV. DISCUSSION

Per cent seed germination of both the *Cajanus* and *sorghum* decreased with increasing concentrations of zinc. The seed germination of controls exhibited 98 per cent in pigeonpea and 96 per cent in sorghum. Heavy metal plays important role in governing germination and growth of plants. Their effects on plants are dependent on the amount of heavy metals take up from the given environment. Germination and seedling growth are valuable stages in the life of plants (Vange et al., 2004). The decrease in seed germination of cluster bean can be attribute to the accelerated breakdown of stored food materials in seed by the application of Zn. Reduction in seed germination can also be attributed to alterations of selection permeability properties of cell membrane (Manivasagaperumal et al., 2011). The decrease in seed germination of *Cajanus* and *Sorghum* due to zinc treatment is in conformity with the previous findings (Mahalakshmi. G et al., 2003, Mahmoodet al., 2005).

Hediji et al. (2010) reported fresh weight reduction of *Solanum lycopersicum* under high Cd levels. Similarly a sharp reduction in leaf number was observed by Jing et al. (2005) and Rehman et al. (2011). Zn at lower concentrations enhanced *Cicerarietinum* seed germination. This is because Zn is a micronutrient and indispensable for plant growth (Wierzbicka and Obidzinska, 1998). But at higher quantity it abridged the germination percentage (Sudarshana Sharma et al., 2010).

With the addition of heavy metal concentration of Fe, Zn and Cu, there was significant decrease in root/shoot length Nidhimittal et al., (2015). The reduced seedling length during metal stress could be due to low water potential, hampered nutrient uptake and secondary stress Jhon et al., (2009). Fe toxicity causes reduction in root/shoot length due to toxic effect on photosynthesis, respiration and protein synthesis Sonmez et al., (2006).

In our study the chlorophyll a and b content was gradually decreased in high contaminated areas respectively when compared their control areas in both *cajanus and sorghum* plants. The excess zinc treatment brought about a marked depression in photosynthetic pigment in plants. It might be due to excess supply of zinc resulting in interference with the synthesis of chlorophyll. The formation of chlorophyll pigment depends on the adequate supply of iron (Manivasagaperumal et al., 2011). The low Chl a/b ratio recorded in P. australis in the first part of the season, together with the low level of total chlorophyll, could be the effects of possible stress conditions, as reported in other studies (Manios et al., 2003; Pagter et al., 2005). All heavy metals (lead, chromium, nickel, cadmium, zinc) significantly lowered the leaf con-tents of the photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids). Chlorophyll a showed high sensitivity to lead, nickel, zinc and then cadmium, but low sensitivity to chromium. Chlorophyll b was less sensitive to heavy metal stress, but more sensitive to nickel (Aldoobie and Beltagi 2013).

Generally, metalstress caused changes in carbohydrate concentrations in leaves of plants. The Zn stress induced increases of glucose, fructose and sucrose in leaves of Salix (Klaudia et al., 2015). In regular plant metabolismassimilates are produced in leaves, then transferred to different tissues and stored in response to sink–source relations regulated by partitioning of starch and sucrose biosynthesis (Taiz and Zeiger2006).

Soluble sugar, is an important constituent manufactured during photosynthesis and breakdown during respiration by plants. In our study total soluble sugars, reducing sugars, non reducing sugars and starch content was gradually decreased from control to area- 2 fields both *cajanus and sorghum* plants. This trend was followed in three plant stages. Total soluble sugar content at lower level but total sugar content showed a decreasing trend with progressive increase in Zn concentration in treatment similar to wheat leaves (Lanaras et al 1993). Low concentration of copper treatments exhibits an increase in the total carbohydrates values in seedlings and reverse was true at high concentration (Deef 2007). All metals have decreased the content with increasing concentration as reported in agricultural crops (Hemalatha et al., 1997; Rascio and Navari-Izzo, 2011). Such inhibition of photosynthesis in higher plants by heavy metals has been reported (Bazzaz et al., 1975). The obtained results confirmed our earlier studies concerning the influence of metals (Ni and Cu) on accumulation of soluble sugars in leaves (Drzewiecka et al. 2012; Gą̧secka et al. 2012).

Amylase and its important role during seed germination through hydrolysis of reserve starch and release in the energy has been worked out by Thevenot et al. (1992). Inhibitory effect of excess chromium on germination, growth, cell division and metabolic parameters at early stages of growth was also studied by Jain et al. (1999). Amylase activity showed lower level in plants with increase in concentration of heavy metals (Devi et al. 2007; Gopal et al. 2008). Adityaverma et al., (2009) reported in old cucumber (*Cucumissativus*) 0.20 and 0.50 Mm Zn the amylase activity (total amylase, α-amylase and β amylase) was found to be significantly inhibited in higher doses of the treatments. The poor germination rate and seedling growth in treatments seem due to the poor break down of starch by amylase activity.

V. CONCLUSION

In this study it was found that high zinc concentrations decrease of seed germination both the plants *Cajanus* and *Sorghum*. *Sorghum* exhibited greater reduction in per cent seed germination than *Cajanus*. The length of plants in both *Cajanus* and *Sorghum* decreased with increasing concentrations of Zn. The chlorophyll a, b content was gradually decreased from control to area- 2 fields. Reduction in starch content and an accumulation of total soluble sugars, reducing sugars and non reducing sugars increased with increasing Zn concentration in three plant stages both the plant species. However, amylase increased in leaf both the plants *Cajanus* and *sorghum* at the end of the study.

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