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EFFECTS OF COBALT INDUCED STRESS ON *TRITICUM AESTIVUM* L. CROP

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ABSTRACT

This experiment was conducted with different concentrations of cobalt (100, 200, 300, 400 and 500 ppm) on wheat (*Triticum aestivum* L.) plants for quantification of its effects on growth performance in a sand culture medium using Hoagland solution. Germination was tested in petri dishes using same treatments. Inhibitory effect of Co on germination percent was observed from 200ppm of Co while vigor index decreased with increasing Co concentration. No negative effect of Co on germination index was found up to 300 ppm while beyond that higher percent inhibition of wheat seed germination was recorded. An enhancing effect on plant height, leaf number, leaf area and dry matter production was observed up to 200ppm of Co treatment while higher concentrations showed detrimental effect of the same. Chlorophyll a/b increased and chlorophyll stability index decreased with increasing Co concentration from 300 ppm onwards. Results from analysis of above ground plant biomass showed higher accumulation of Co with increasing concentration in the medium. However, at 100 ppm of Co treatment, significantly higher grain accumulation of Co was recorded compared to 500ppm of Co treatment. From these findings, it can be summarised that at lower concentrations (up to 200ppm) Co has an enhancing effect on growth of wheat crop and it has a good phyto-extracting ability for cobalt

Keywords: Cobalt, Wheat, Morphological parameters, Chlorophyll pigment, Plant accumulation

INTRODUCTION

Cobalt is a heavy metal that occurs naturally in many different chemical forms in our environment. Though, it is an essential element for both plant and animal but at higher concentration Co is reported to be toxic (Jayakumar and Vijayarengan, 2006). The toxicity of Co is quite low compared to many other metals in soil, but its accumulation in agricultural crops is especially very important because it contributes toxicity into the human food chain. The levels of Co toxicity depend on plant species, soil type and soil chemistry (Bakkus *et al.,* 2005). More acidic the soil, the greater is the potential for Co toxicity at any concentration (Hasan *et al.,* 2011).

Co has both beneficial as well as harmful effects to plants. At lower concentration, Co has been reported to have a positive effect on plants. Being a component of cobalamin, Co at lower concentrations has positive effect on legume plants in nitrogen fixation (Palit *et al.*, 1994). Rathsooriya and Nagarajah (2003) reported that Co has beneficial effect on growth of salinized pea plants to increase leaf water potential relative to those untreated plants. At higher Co concentration detrimental effects on plant growth along with chlorosis and necrosis was reported by Caselles *et al.* (1997). It also inhibits root growth by retarding cell division hindering the uptake and translocation of nutrient and water (Jayakumar *et al.,* 2008). Accumulation of free proline in response to heavy metal exposure helps to maintain the water balance in higher plants (Costa and Morel, 1994). Co inhibits the synthesis of chlorophyll pigments by obstructing its biosynthesis pathway (Mysliva-Kurdziel *et al.,* 2004).

Wheat *(Triticum aestivum* L.*)*, one of the most important strategy crops is grown worldwide in a variety of climates. The present experiment was conducted in a sand culture medium to evaluate the effects of various levels of Co on morphological and biochemical parameters of wheat. The accumulation of Co in different above ground parts of the plant was also estimated at harvest.

MATERIALS AND METHODS

Wheat (*Triticum aestivum* L.) seeds cv. DBW-39 was used for this experiment. The seeds were collected from Regional Agricultural Research Station, Shilongoni, Nagaon, Assam, India. Hoagland solution as developed by Hoagland and Arnon (1950) was prepared and used as the nutrient solution for this experiment. For preparation of Hoagland

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solution $CaNO_3$, KNO_3, $MgSO_4$, KH_2PO_4, Fe-EDTA were used as sources of macronutrient and H_3BO_3, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $MnSO_4$and $NaMoO_4$ were used as micronutrients in proportion as described by Hoagland and Arnon (1950). Cobalt in the form of $CoNO_3$ was applied in different concentrations to the nutrient solution. The treatments include 100ppm (T_1), 200ppm (T_2), 300ppm (T_3), 400ppm (T_4), 500ppm (T_5) of Co and control (C) with only Hoagland solution. Treatments were applied from 30 days after sowing (DAS).

Germination test

Different concentrations (100ppm, 200ppm, 300ppm, 400ppm, 500ppm) of $CoNO_3$ solution in distilled water were prepared. Distilled water was taken as control. Germination was investigated in sterilized petri plates with whatmann No. 1 filter paper. Ten seeds were placed in each petri plates and the treatments were replicated 5 times. The plates were then kept at 25 ± 2^oC for 7 days. Proper care was taken to keep the plates wet throughout the experiment with addition of respective solutions periodically. The radicle and plumule lengths were recorded at the end of the experiment (7 days). The germination performances under different treatments were calculated as follows:

Percent germination:

(No. of seeds germinated/ Total no of seeds taken) × 100

Germination index (GI):

$$2(7x +6x+ 5x+ 4x+3x+2x+x)$$

Where, x is the number of seeds germinated in 24 hours.

Percent inhibition of germination:

100 – (GI of treatment /GI of control ×100)

Vigour index (VI):

radicle length + plumule length × percent germination.

Preparation of sand culture medium and experimental layout

Sand was collected and repeatedly washed under running tap water and then with distilled water till the soil, mud or dust portion washed out. Then it was treated with 0.1% of HCL and kept for 2 days followed by repeated washing with distilled water to make it free from acid. To ascertain the presence of no acid, the sand was tested with litmus paper. Then the pots were filled with 1.5kg of the nutrient free sand. Total thirty six pots were taken for the experiment (6 for each treatment). 3 replications were kept intact and were used to measure the morpho-physiological and yield attributes. The remaining three replicates were used for destructive sampling for analyzing various biochemical parameters at regular interval. Wheat seeds (@5 seeds per pot) were sown in sand medium on 5th November, 2012. After germination of the wheat seeds, nutrient solution was added to the pots at 3-4 days interval till harvest of the crop.

Morphological parameters

Changes in various plant morphological parameters like plant height, leaf number, leaf area and dry biomass were recorded at 15 days interval from 30 DAS till harvest. Leaf area was recorded by using laser leaf area meter (CI-203, USA). Dry biomass of the plant was taken after oven drying the plant at 70^oC for 24 hours.

Biochemical parameter

Total chlorophyll, chlorophyll-a and chlorophyll-b was estimated by using standard method described by Anderson and Boardman (1964) and chlorophyll a/b was calculated. The pigment composition was measured by a double-beam spectrophotometer (UV 1700 Pharma Spec, Japan). Chlorophyll stability index (CSI) was calculated using the following formula:

CSI= (Total chlorophyll of the stressed / Total chlorophyll of the control) × 100.

The leaf proline content was estimated using the method of Bates *et al.* (1973).

Estimation of cobalt in plant, sand medium and grain

For estimation of Co in sand, the sand was digested by following the method of Shapiro and Brannock (1962). 0.5g of sand was digested with 10 ml concentrated HF and 5ml concentrated HNO_3 with 1ml $HClO_4$ in sealed Teflon crucible at 85^oC to 90^oC on a hot plate for 4 hours. After 4 hours, the crucible was opened and heated until almost dry. In the second phase, 5ml concentrated HF and 10ml concentrated HNO_3 were added and heated till dryness at 90^oC. In the third phase 10ml concentrated HNO_3 was added and heated at 90^oC till dryness. Finally 20ml of 2N HCl was

added to dry crucible and transferred to 100 ml volumetric flask and cooled and volume make up was done with ultra pure water.

At harvest, the grains and above ground plant biomass were collected, dried and ground properly and subsequently digested to analyse for Co concentration. 1gm of grind material was taken for di-acid digestion using HNO_3 and $HClO_4$ (9:4).

The digested samples (sand, plant and grains) were then analysed for Co concentration in Atomic absorption spectrophotometer (AAS).

Statistical analysis

Standard errors of means (of three replicates) were calculated for all the parameters. Data obtained were subjected to one-way analysis of variance (ANOVA) in SPSS for windows 16.0.20. Critical Differences (CD) among the means and Duncan's Multiple Range Test (DMRT) at 5% level of probability were used to test the significance of differences between treatment means.

Table 1: Effect of different Co concentrations on germination parameters of wheat seed

	% Germination	Germination Index	% Inhibition	Vigor Index
C	100 ± 0^{c}	69.67 ± 2.37^{b}	0	1032.08 ± 17.11^{c}
T_1	100 ± 0^{c}	82 ± 2.31^{d}	-18.30 ± 7.68^{a}	672.29 ± 20.21^{b}
T_2	91.67 ± 4.17^{c}	79 ± 1.0^{cd}	-13.84 ± 5.68^{ab}	658.33 ± 50.88^{b}
T_3	70.83 ± 4.17^{b}	71.67 ± 3.84^{bc}	-3.09 ± 6.27^{ab}	101.52 ± 7.23^{a}
T_4	60.32 ± 2.04^{a}	50.67 ± 1.33^{a}	27.10 ± 2.96^{b}	96.82 ± 14.64^{a}
T_5	54.17 ± 2.08^{a}	43.67 ± 2.73^{a}	36.89 ± 6.07^{c}	61.57 ± 8.97^{a}
CD	8.27	7.73	16.70	75.87
P	0.00	0.00	0.00	0.00

Different letters within each column for each parameter indicate significant differences between treatments at 5% level of significance according to DMRT.

Table 2: Effect of different Co concentrations on plant height of wheat

	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	110 DAS
C	13.467 ± 0.33^{a}	20.33 ± 0.40^{ab}	28.50 ± 0.98^{a}	34.30 ± 0.57^{b}	36.50 ± 2.75^{b}	37.30 ± 3.65^{ab}
T_1	12.70 ± 0.56^{a}	18.80 ± 0.26^{ab}	28.93 ± 2.16^{a}	36.50 ± 0.29^{b}	37.70 ± 2.55^{b}	39.00 ± 3.18^{b}
T_2	13.11 ± 0.72^{a}	19.93 ± 0.76^{ab}	30.97 ± 2.30^{a}	37.03 ± 1.47^{b}	38.97 ± 3.15^{b}	41.17 ± 1.59^{b}
T_3	13.27 ± 0.27^{a}	20.78 ± 1.97^{ab}	28.47 ± 1.30^{a}	36.67 ± 2.24^{b}	37.00 ± 2.18^{b}	37.07 ± 2.75^{ab}
T_4	13.10 ± 0.23^{a}	15.26 ± 1.75^{a}	26.47 ± 1.67^{a}	31.17 ± 2.33^{ab}	32.20 ± 2.25^{ab}	33.30 ± 3.67^{ab}
T_5	13.73 ± 0.27^{a}	20.37 ± 2.78^{b}	24.17 ± 2.95^{a}	27.77 ± 2.63^{a}	28.13 ± 1.48^{a}	28.40 ± 2.39^{a}
CD	1.34	4.95	6.18	5.62	7.56	9.13
P	0.66	0.22	0.31	0.02	0.07	0.10

All values are Mean± SE (cm). Different letters within each column indicate significant differences between treatments at 5% level of significance according to DMRT.

Table 3: Effect of different Co concentrations on dry matter content of wheat

	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	110 DAS
C	0.232±0.016^{a}	0.493±0.009^{a}	0.782±0.004^{bc}	2.981±0.374^{a}	3.459±0.037^{b}	4.593±0.064^{ab}
T_1	0.270±0.011^{ab}	0.511±0.008^{ab}	0.952±0.078^{d}	3.018±0.118^{a}	4.392±0.015^{d}	5.340±0.279^{b}
T_2	0.317±0.027^{b}	0.495±0.014^{a}	0.899±0.029^{cd}	4.128±0.007^{b}	4.693±0.041^{e}	5.541±0.110^{b}
T_3	0.219±0.017^{a}	0.503±0.004^{a}	0.551±0.036^{a}	3.163±0.198^{a}	3.549±0.023^{c}	4.447±0.107^{ab}
T_4	0.248±0.013^{a}	0.535±0.005^{b}	0.712±0.011^{b}	3.229±0.039^{a}	3.599±0.012^{c}	4.064±0.172^{a}
T_5	0.242±0.020^{a}	0.509±0.009^{ab}	0.784±0.011^{bc}	2.650±0.017^{a}	3.219±0.025^{a}	3.841±0.372^{a}
CD	0.056	0.018	0.113	0.557	0.080	1.043
P	0.02	0.05	0.00	0.00	0.00	0.02

All values are Mean± SE (g/plant). Different letters within each column indicate significant differences between treatments at 5% level of significance according to DMRT.

Table 4: Correlation between the different parameters at harvest

	Plant Concentration	Proline	Chlorophyll a:b	CSI	Dry Matter	Leaf Area	Plant Height	Leaf Number
Plant Concentration	1							
Proline	0.982**	1						
Chlorophyll a:b	0.724	0.820*	1					
CSI	-0.885*	-0.912*	-0.802	1				
Dry Matter	-0.615	-0.714	-0.894*	0.635	1			
Leaf Area	-0.881*	-0.934**	-0.870*	0.925**	0.852*	1		
Plant Height	-0.791	-0.859*	-0.808	0.707	0.912*	0.915*	1	
Leaf Number	-0.861*	-0.926**	-0.914*	0.915*	0.884*	0.995**	0.916*	1

**. Correlation is significant at the 0.01 level (2-tailed)
*. Correlation is significant at the 0.05 level (2-tailed)

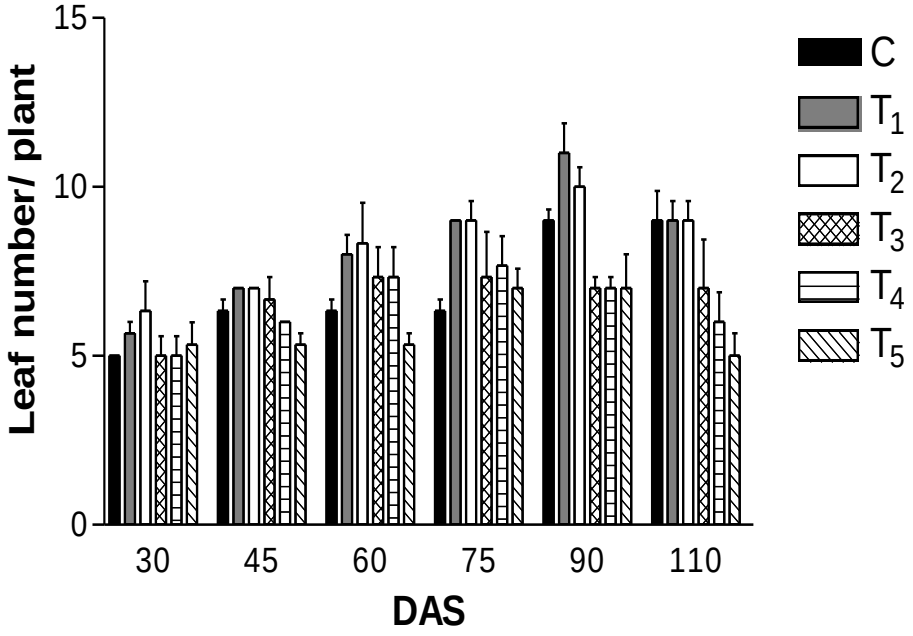

Figure 1. Effect of different Co concentrations on leaf number of wheat

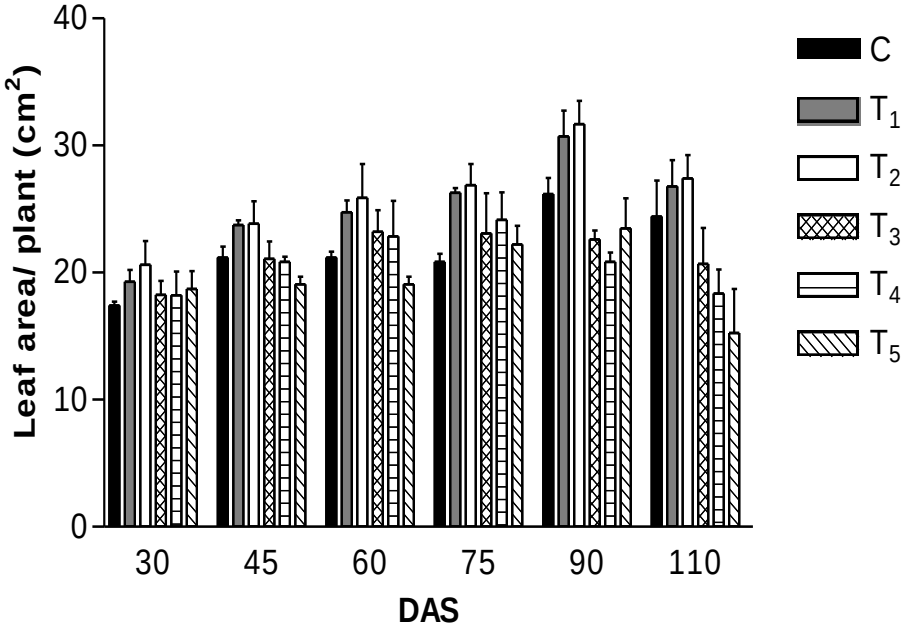

Figure 2. Effect of different Co concentrations on leaf area of wheat

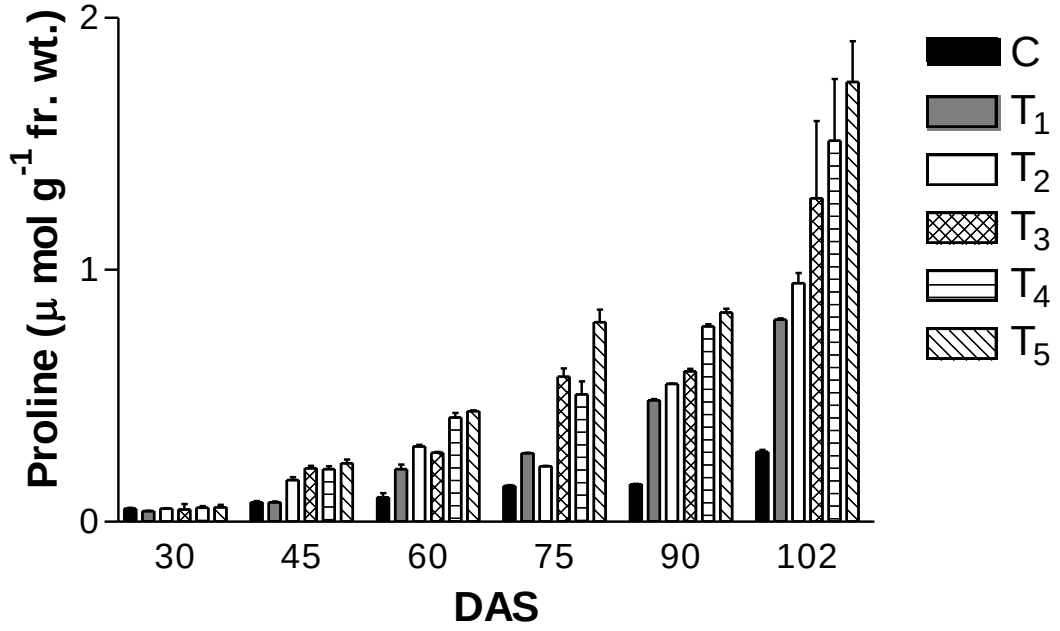

Figure 3. Effect of different Co concentrations on leaf proline content of wheat

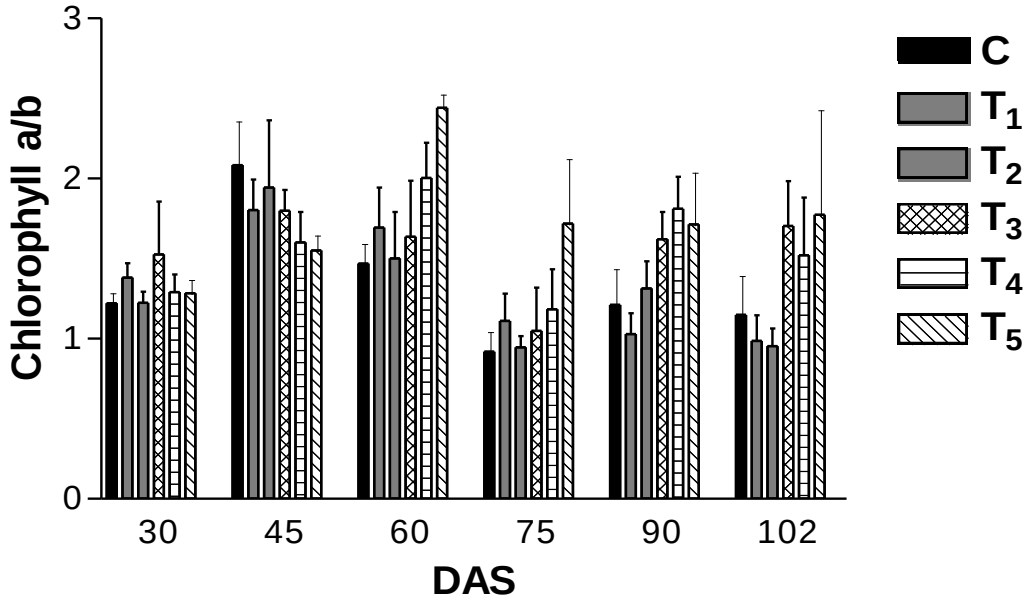

Figure 4. Effect of different Co concentrations on chlorophyll a/b of wheat

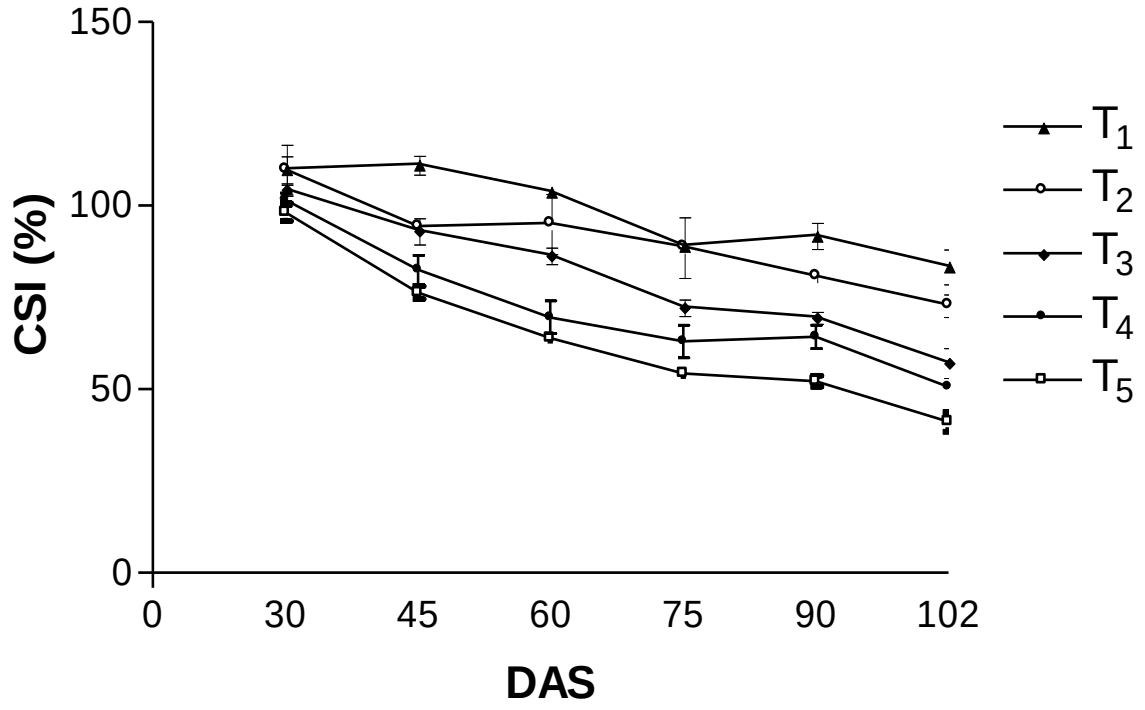

Figure 5. Effect of different Co concentrations on chlorophyll stability index of wheat

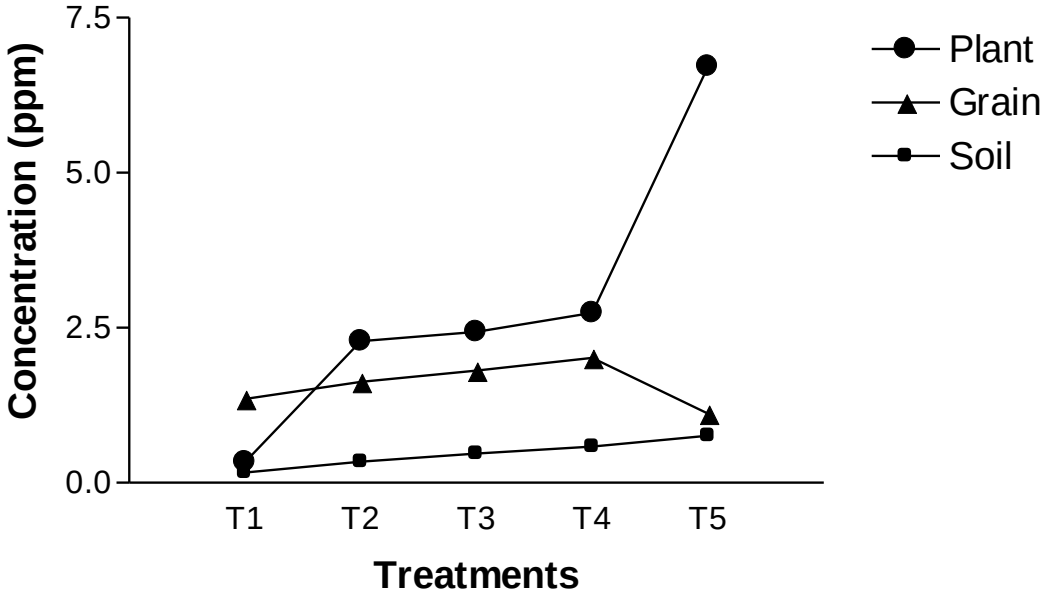

Figure 6. Co concentrations in plant, grain and sand after harvest of wheat

RESULT AND DISCUSSION

Germination responses of wheat to different Co concentration

Cobalt exposure significantly reduced the germination of wheat seed with increasing Co concentration (table 1). No negative effect on percent germination was recorded up to 100ppm of Co. The inhibitory effect of Co on germination of wheat seed was noted from 200ppm (T_2) onwards. At the highest concentration of Co (T_5), the reduction in percent germination was 50% of the control. Ionic toxicity induced by higher concentrations of Co might be the cause of reduced seed germination or it could be due to osmotic effect (Shaukat *et al.*, 1999). Similar results of reduced germination under Co exposure had been reported in various crops such as ragi (Jayakumar *et al.*, 2008), paddy (Jayakumar and Vijayarengan, 2006) and *Vigna mungo* (Munzuroglu and Geckil, 2002). Highest germination index was obtained in T_1 with increasing values up to 300ppm of Co (table 1). This may be attributed to the triggering effect of Co on germination of wheat seed as Co enhances the activity of enzyme α-amylase, one of the important enzymes involved in the process of seed germination (Zeid, 2001). The percent inhibition of germination was recorded from 400 ppm of Co onwards with highest value at 500 ppm (table 1). Cobalt had a stimulatory effect up to 300ppm. The recorded vigor index was concentration dependent and the control showed the highest vigor index of 1032.08. These results of vigor index indicate that addition of Co even at lowest concentration did not have any positive impact on elongation of plumule and radicle. This might be due to the effect of Co in the process of cell division of these growing parts.

Morphological responses of wheat to different Co concentration

Plant height was recorded throughout the experimental period and enhancement in the linear growth of shoot was observed up to 200ppm of Co at all the stages of growth when compared with control plants (table 2). While significant reduction of shoot growth rate was observed after 60 DAS with increasing (from 300ppm onwards) concentration of Co. T_3 maintained the equal plant height with that of control plants. However, at lower concentrations (up to 200 ppm) Co has an enhancing effect on all these physiological parameters as been previously reported by Jaleel *et al.* (2009). Enhancing effect of lower doses of Co on plant height and leaf area has previously been reported by Kadhim (2011).

Throughout the growth period more leaf number and leaf area were recorded in treatment T_1 and T_2 (100 and 200 ppm of Co) compared to control and other treatments. Leaf number and area increased till 90DAS and then started to decrease in all the treatments (figure 1 and 2). Highest leaf senescence was observed in T_4 and T_5 treatments. The decreasing growth rate of these morphological parameters under higher concentration of Co (300ppm and above) is the direct inhibitory effect of Co in cell division or cell elongation or combination of both (Jayakumar *et al.*, 2007). Plant accumulation of Co at harvest had showed a significant negative correlation with leaf area (r=0.881) and leaf number (r=0.861) (table 4).

Plant dry matter increased in all the treatments with the growth of the crop, but the rate of increase was lesser in T_3, T_4 and T_5. Enhanced production of plant biomass was seen up to 200ppm of Co concentration while same was retarded from 300ppm onwards (table 3). Due to panicle initiation, higher dry plant biomass was recorded at 75 DAS compared to 45 and 60 DAS irrespective of treatments. These results showed an enhancing effect of Co on wheat plant biomass production at lower concentrations (up to 200ppm). Similar results of higher dry matter production at lower Co concentration have been reported by Aery and Jagetiya (2000). At higher doses, Co ion might inhibit cell division resulting in stunted growth and lower dry matter production of wheat plants.

Biochemical responses of wheat to different Co concentration

Figure 3 clearly indicate that leaf proline concentration increased with increasing Co concentration as well as with growth stages of wheat. From 45DAS significant (P=0.00) increase in proline content was recorded with increasing levels of Co. Highly significant increase in proline content was obtained at 102 DAS in all the Co treatments. Significant positive correlation of proline (r = 0.982) was observed with increased accumulation of Co in plants at harvest (table 4). Treatment T_5 maintained the highest proline concentration throughout the experiment while control plants

were found to accumulate the least amount of proline. Increased proline content under Co stress might be due to Co induced physiological drought. As osmo-protectant, proline accumulation up to 200 ppm of Co, can induce resistance in wheat plants and nullify the Co induced stress on wheat crop (Zengin and Munzuroglu, 2005). Proline accumulation in response to Co stress is reported to increase tolerance by osmoregulation, resisting enzyme denaturation and stabilization of protein synthesis (Kuznetsov and Shevyakova, 1997).

At 45 DAS, increase in chlorophyll a/b was found in all the treatments. However, at 60 DAS significant increase in chlorophyll a/b ratio was obtained in T_4 and T_5 (figure 4). However, from 90 DAS a/b ratio were found to increase with increasing Co concentration except for T_1. At 102 DAS up to 200 ppm of Co chlorophyll a/b ratio was at par to that of control. Also, addition of 100ppm of Co (T_1) maintained highest CSI value throughout the growing period whereas, significantly higher reduction in CSI value were observed from 300ppm of Co (T_3) treatments from 45 DAS till end of the crop growth period (figure 5). Co up to 200 ppm had anabolic effect on chlorophyll content of wheat crop due to its positive role in hormonal synthesis and metabolic activities. Anabolic effect of lower Co concentration (up to 200 ppm) on chlorophyll biosynthesis resulted higher a/b ratio throughout the experiment through enhanced uptake of Mg (Zeid, 2001). Heavy metals were found to affect the chlorophyll biosynthesis/degradation by enzymatic modulation. Co enhances the activity of chlorophyll degrading enzyme chlorophyllase (Reddy and Vora, 1986) and inhibits the activity of chlorophyll synthesizing enzyme 5-aminolevulinic acid and protoporphyrin (Mysliva-Kurdziel *et al.*, 2004). At higher concentration Co showed prominent catabolic effect on chlorophyll synthesis. However, the negative effect of Co on chlorophyll b was lesser than on chlorophyll a as has previously been described by Zengin and Munzuroglu (2005).

Plant, grain and sand accumulation of Co

From figure 6, it was observed that when wheat plants were exposed to higher Co concentration, the plant accumulation was relatively higher compared to grain. But under lower (100ppm) soil Co exposure, wheat plants accumulate more amount of Co in grain compared to other plant parts. This is due to the tendency to accumulate in the sink compared to other plant parts under low Co exposure. In control (plants, grain and sand) Co concentration was beyond the detectable limit. Plant accumulation of Co was found to be lowest in 100ppm (T_1) of Co exposure while with increasing concentration, Co content in plant parts was found to be higher. 500 ppm (T_5) exposure of Co recorded the highest Co accumulation in above ground plant biomass. Accumulation of Co is always higher in the above ground plant parts of wheat than that of grains. This is attributed to the fact that Co has a lesser mobility in the leaf tissues compared to the vascular system of wheat (Collins *et al.*, 2010). Significant increase in wheat grain Co concentration was found up to 400 ppm (T_4) of Co exposure. T_4 recorded the highest (2.013ppm) grain accumulation while interestingly T_5 showed the lowest (1.117ppm) grain Co concentration. This might be due to stimulatory effect of Co in phloem translocation pathway which might be disrupted under higher Co concentration. Lesser accumulation of Co in grains of wheat has already been confirmed by Zeller and Feller, 1998.

Co content in the sand medium also showed an increasing trend with increasing dose of Co application. T_1 (0.167ppm) recorded the lowest while T_5 (0.757ppm) recorded the highest concentration of Co in sand medium.

SUMMARY

Results of germination index and percent inhibition of germination showed that Co up to 300 ppm has an enhancing effect on germination of wheat seed while it has a negative impact on enlargement of plumule and radicle. Exposure of wheat crop to lower concentration of Co (up to 200 ppm) is useful for growth and development of morpho-physiological attributes but higher had detrimental effects of the same. Results on various biochemical (CSI, chlorophyll a/b, proline) analyses also support this. Wheat is found to be a good phyto accumulator for cobalt.

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