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# Element content, growth and metabolic changes in Cu- and Cd-stressed *Phaseolus vulgaris* plants

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### ABSTRACT

A large-scale pot experiment was accomplished for investigation of the varied effects of different concentrations of Cu and Cd on certain growth and metabolic attributes of roots and shoots of *Phaseolus vulgaris* plants, over a period of three weeks. Plants supplemented with Cu and Cd at the concentrations of  $10^{-6}$  and  $10^{-3}$  M, showed increased levels of Cu and Cd in both shoots and roots, above those levels in controls. However, Cu or Cd accumulation was lower in shoots than in roots. As compared with control levels, the low ( $10^{-6}$  M) concentration of Cu induced either a significant or an insignificant increase in growth parameters, photosynthetic pigments, PS II activity, glucose, proline and glycine contents in both roots and shoots. Otherwise, insignificant decreases in fructose, sucrose, polysaccharides, total saccharides, total soluble-N, protein -N, DNA and RNA contents, in the same test plant parts, were obtained. A reverse situation was however observed with the high concentration ( $10^{-3}$  M) of Cu as well as with the low and high concentrations ( $10^{-3}$  and  $10^{-6}$  M) of Cd. In general, the observed adverse effects were more pronounced with Cd at ( $10^{-6}$  M) as compared with those maintained with Cu at the same concentration. Furthermore, the most detrimental adverse effects were apparent upon administration of the high ( $10^{-3}$  M) concentration of Cd. The prominence of the above mentioned changes in growth and metabolism to stress tolerance in common bean is discussed.

**Keywords:** *Phaseolus vulgaris*, Cu, Cd, growth parameters, photosynthetic components, carbohydrate and nitrogenous constituents and nucleic acids.

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## INTRODUCTION

Heavy metals, being a heterogeneous group of elements, comprise essential, such as Cu, and non-essential ones, such as Cd. Broadly, the metal ions will be absorbed by plants and can be accumulated in both roots and shoots. The excess presence of these metals in plant parts may become toxic (Mediouni et al., 2006). No attempt at a review of literature needs be made here and only a brief account will be given. Thus, diverse studies have shown that Cd stimulates the inhibition of growth and causes chlorosis in plants whereas, extensive exposition to Cu causes synoptic disturbances of root and shoot growth, root morphology and leaf chlorosis (Wisniewski and Dickinson, 2003; Mediouni et al., 2006).

When legumes, e.g. black gram plants, are grown in heavy metal contaminated soils, the photosynthetic pigments and the photosynthetic reduction of carbon, have been found to be affected negatively (Bibi and Hussain, 2005). Yurekli and Porgali (2006) reported that chlorophyll a (Chl a) and chlorophyll b (Chl b) are highly sensitive to different concentrations of metals like Cu, in bean plants. Furthermore, the decrease in the Chl a/b ratio, subsequent to Cd treatment, has been observed by Hatata and Abdel-Aal (2008).

Sugars, being important metabolites in plant metabolism, have been shown to be subject to dramatic changes upon treatment with varied concentrations of heavy metals. Thus, Deef (2007) found an increase in the total carbohydrates in *Rosmarinus officinalis* seedlings at low concentration of Cu treatments and the reverse was true at high concentration. On the other hand, Cd toxicity extremely disturbed not only the degradation of soluble sugars but also the transportation of soluble sugars to the growing axis of the embryo (Kuriakose and Prasad, 2008). At the cellular level, nitrogen is the main component of amino acids, protein and other biomolecules. Thus, Abdel-Fattah et al. (2003) observed, a significant reduction in total nitrogen contents

and proteins of *Ambrosia maritima* but soluble nitrogen content was increased in consequence to treatment with Cd.

Accumulation of compatible organic osmolytes is confirmed by Schat et al. (1997) who determined an increase in the levels of proline contents, in response to Zn, Cu and Cd, in nontolerant and metal-tolerant *Silene vulgaris* (Moench); the proline level in leaves appeared 5 to 6 times higher in the metal-tolerant ecotype than in the nontolerant ecotype. Furthermore, Zengin and Munzuroglu (2005) reported a considerable increase in proline level of *Phaseolus vulgaris* seedlings after Cu and Cd treatment.

Hamid et al. (2010) found a decline in DNA and RNA contents under heavy metals stress. In plants, reduced adequacy of DNA synthesis, weaker DNA protection from damaged chromatin protein and increased DNase activity have been reported for Pb, Ni, Cd, Cu and Hg (Prasad and Strzalka, 2002). Furthermore, Zeid and Abou Elghate (2007) found decreased nucleic acids and total-N in leaves of *Phaseolus vulgaris* seedlings irrigated with a mixture of Pb, Cu, Zn, and Cd at concentration of sewage water.

The contamination of large areas of agricultural soils by heavy metals seems to enhance plant uptake causing their bioaccumulation tendency and toxicity leading to massive losses in plant productivity and hazardous health (Demirevska-Kepova et al., 2004). For better understanding towards the induced heavy metal stress, Cu and Cd elements were chosen for usage in the present study. As a test leguminous plant, common bean is the most important economic variety of the genus *Phaseolus* which is cultivated in large irrigated areas of Egypt for its beans. The main target of this study was thus to investigate the effects of low and high levels of the two elements on growth, photosynthetic parameters as well as on the changes in carbohydrate and nitrogen constituents, in *Phaseolus vulgaris*.

## MATERIALS AND METHODS

### Plant material and chemicals

Pure strain seeds of *Phaseolus vulgaris* L. var. Nibraska was used in this study. Analytical grade chemicals, obtained from different local suppliers, were used throughout this investigation.

### Estimation of element contents

As described by Motsara and Roy (2008), a known weight of dried and finely ground plant parts was mixed with conc. HNO<sub>3</sub> and heated at 80-90°C. Heating was continued until the production of red NO<sub>2</sub> fumes ceases and the volume was reduced to 3-4 cm<sup>3</sup> and became colourless. After cooling, the contents were made up to volume with distilled water and then filtered. The obtained solution was used for estimation of Cu and Cd contents, and the analyses were done by the atomic absorption spectrophotometer (Analyst 300; Perkin Elmer, Germany).

### Estimation of photosynthetic parameters

#### a- Estimation of pigments

Photosynthetic pigments were determined following the method of Metzner et al. (1965). By the use of spectrophotometer, the extract was measured, against a blank of acetone, at 3 wavelengths of 452.5, 644 and 663 nm. The concentrations of the pigment fractions were calculated as µg g<sup>-1</sup> fresh weight using the following equations:

$$\text{Chl a} = 10.3 E_{663} - 0.918 E_{644} = \mu\text{g g}^{-1} \text{ f. weight}$$

$$\text{Chl b} = 19.7 E_{644} - 3.87 E_{663} = \mu\text{g g}^{-1} \text{ f. weight}$$

$$\text{Cars} = 4.2 E_{452.5} - (0.0264 \text{ Chl a} + 0.426 \text{ Chl b}) = \mu\text{g g}^{-1} \text{ f. weight}$$

#### b- Estimation of photosynthetic activity

For preparation of chloroplast pellets the method of Trebst (1972) was essentially used. The reaction mixture for determination of photosystem II activity contained 0.5 mM 2,6-DCPIP, 2 mM MgCl<sub>2</sub> and 200 mM sodium phosphate buffer (pH 7.2). As adopted by Dean

and Miskiewicz (2003), a calibration curve, in terms of micromoles of dye reduced, was made using 2,6-DCPIP range between 10-50 µM in the reaction mixture (4 cm<sup>3</sup>).

### Estimation of carbohydrate components

The procedures of extraction of carbohydrate fractions were patterned after those adopted by Van Handel (1968). The estimation of glucose was carried out in the ethanolic extract by the anthrone method of Van Hndel (1968). Fructose content was estimated in the ethanolic extract using the resorcinol method described by Devi (2002). Sucrose was determined by first degrading reactive sucrose present in 0.1 cm<sup>3</sup> extract with 0.1 cm<sup>3</sup> 5.4 N KOH at 97°C for 10 min then, three cm<sup>3</sup> of freshly prepared anthrone reagent were added to the reaction products after cooling. The mixture was heated at 97 °C for 5 min, cooled and the developed colour was read at 620 nm (Van Handel, 1968). Polysaccharides (being considered mainly as starch) were estimated by the method of Thayumanavan and Sadasivam (1984).

### Estimation of nitrogenous constituents

For extraction of soluble nitrogen constituents, the method of Yemm and Willis (1956) was essentially followed. The total soluble N (TSN in plant extracts) and total-N (TN in the dried powdered tissue) were determined by the conventional semi-micromodification of Kjeldahl method, using H<sub>2</sub>SO<sub>4</sub> and SeO<sub>2</sub>, 0.3g; K<sub>2</sub>SO<sub>4</sub>, 80 g; CuSO<sub>4</sub>.5H<sub>2</sub>O, 20g as catalyst. Subtracting the TSN from TN gave the value for protein-N. The method adopted by Muting and Kaiser (1963) for estimation of glycine was herein used. The extract was deproteinized with ethanol/acetone mixture and the glycine content was determined colourimetrically with ninhydrin. The method adopted for extraction and estimation of proline was essentially that described by Bates et al. (1973).

### Estimation of nucleic acids

According to Sadasivam and Manicham (1996) and Devi (2002), one g of fresh shoots or roots of the tested bean plants was ground with

ethanol. The homogenate was filtered through cheesecloth and centrifuged at 3000 rpm for 10 min. The clear supernatant was collected and made up to 10 cm<sup>3</sup> with ethanol. For RNA determination, the orcinol reagent was used and the optical density of the developed green colour was read at 660 nm with a blank. For DNA, the diphenylamine was used and the optical density of the blue colour developed was measured at 600 nm against a blank.

### Time course experiment

A homogenous lot of seeds of *Phaseolus vulgaris* L. var. Nibraska was chosen and surface sterilized by immersion in 10<sup>-3</sup> M HgCl<sub>2</sub> for 3 min, thoroughly washed several times with distilled water and then sown in plastic pots (25 cm in diameter); 10 to 15 seeds per pot, containing 8 kg of acid washed sand. The pots were maintained in a glass house under semi-controlled normal day / night conditions. Each pot was irrigated with 0.5 l of distilled water every other day until appropriate germination occurred. Thinning was carried out after 14 days of sowing the seeds, so as to leave 5 uniform young plants per pot for experimentation. On this day, using young uniform plants from few pots, initial sampling was carried out and this day is presented as 0 time in figures. Simultaneously, thirty pots were divided into five groups; one of them was kept without treatment to serve as control and the other four groups were separately treated with 10<sup>-6</sup> M Cu, 10<sup>-3</sup> M Cu (as CuSO<sub>4</sub>.5H<sub>2</sub>O), 10<sup>-6</sup> M Cd and 10<sup>-3</sup> M Cd (as 3CdSO<sub>4</sub>.8H<sub>2</sub>O) respectively. The appropriate amounts of Cu or Cd salts were calculated and dissolved in half-strength Hoagland nutrient solution to maintain the appropriate adopted levels as above mentioned. Thus, on the thinning day, 0 time, the pots allotted for untreated control group were watered with 0.5 l of half-strength Hoagland's solution every other day till the end of the experimental period. The pots of the other four groups were watered every other day with 0.5 l of the appropriate level of Cu or Cd maintained in Hoagland's nutrient solution.

After 1, 2 and 3 weeks of treatment, ten plants from each treatment were taken at random for measuring the different growth and metabolic parameters in the separated shoots and roots. The samples were taken in a way so as to include all plants from those allotted for each treatment in the 5 pots.

### Statistical analysis

The results obtained from duplicate determinations and triplicate samples were remarkably close, thus the data presented in the corresponding figures are the means of triplicate samples. The full data were statistically analyzed using one way analysis of variance (ANOVA) and comparison among means was carried out by calculating the Post Hoc L.S.D. at 5% level. All the analyses were made using the SPSS 13.0 for windows software package (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Copper and cadmium contents

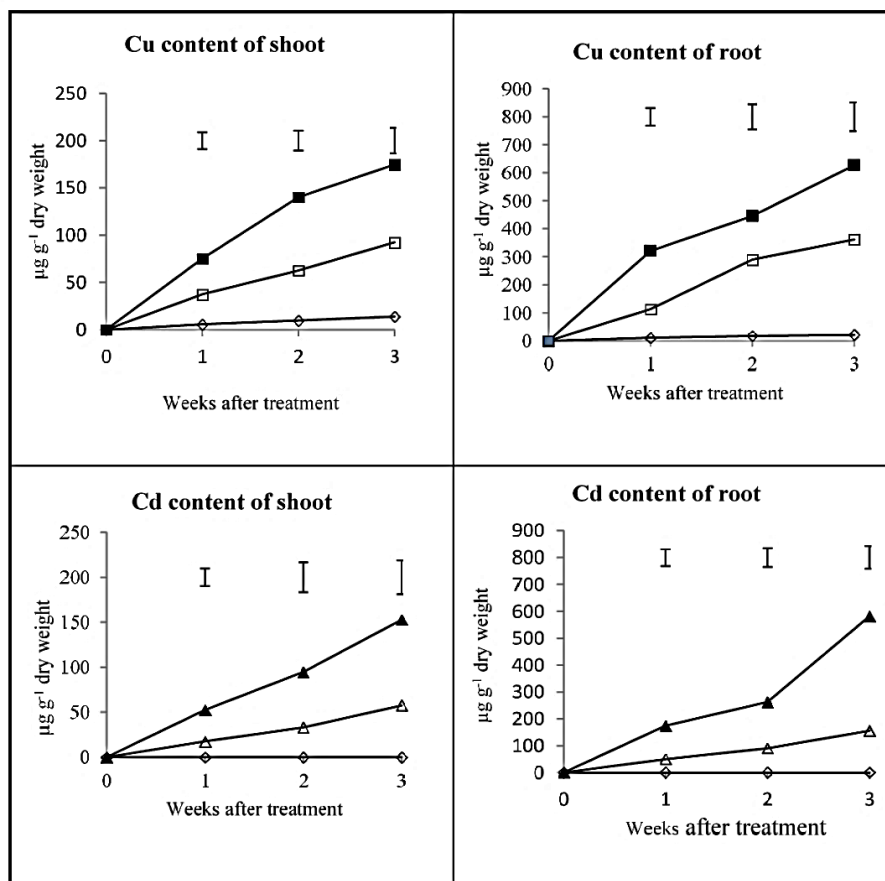
As being evident from figure 1, administration of all Cu and Cd concentrations to common bean plants were characterized by significant increased levels of Cu or Cd contents, above those of control levels, in both shoots and roots. However, Cu or Cd accumulation was high in roots as compared with that in shoots. Furthermore, accumulation of Cu, in shoots and roots of the treated plants, appeared higher than that of Cd. Throughout the experimental period, the following sequence of treatments (10<sup>-3</sup> M Cu > 10<sup>-3</sup> M Cd > 10<sup>-6</sup> M Cu > 10<sup>-6</sup> M Cd) was, in general, displayed with respect to the magnitude of increase in Cu and Cd contents above the control values in both shoots and roots of common bean plants.

### Growth parameters

In relation to initial levels, a progressive increase in all determined growth parameters was observed throughout the duration of the experimental period (Fig. 2). The low concentration of Cu slightly increased the levels of the growth parameters, while the low

concentration of Cd was characterized by decreased levels of growth parameters of both shoots and roots of *Phaseolus vulgaris* plants below those of control. The high concentrations of Cu and Cd appeared to motivate a significant decrease in all growth parameters throughout

the experimental period. The magnitude of decrease appeared, in general, to increase with an increase in duration of the experimental period. Cd stress appeared to induce a higher decline in the levels of growth parameters as compared to those of Cu stress.



**Fig. 1:** Effects of different concentrations of copper or cadmium on element content of shoots and roots of *Phaseolus vulgaris* plants (—◇— Control; —□— 10<sup>-6</sup> M Cu; —■— 10<sup>-3</sup> M Cu; —△— 10<sup>-6</sup> M Cd; —▲— 10<sup>-3</sup> M Cd). Vertical bars represent the L.S.D at 5 % level.

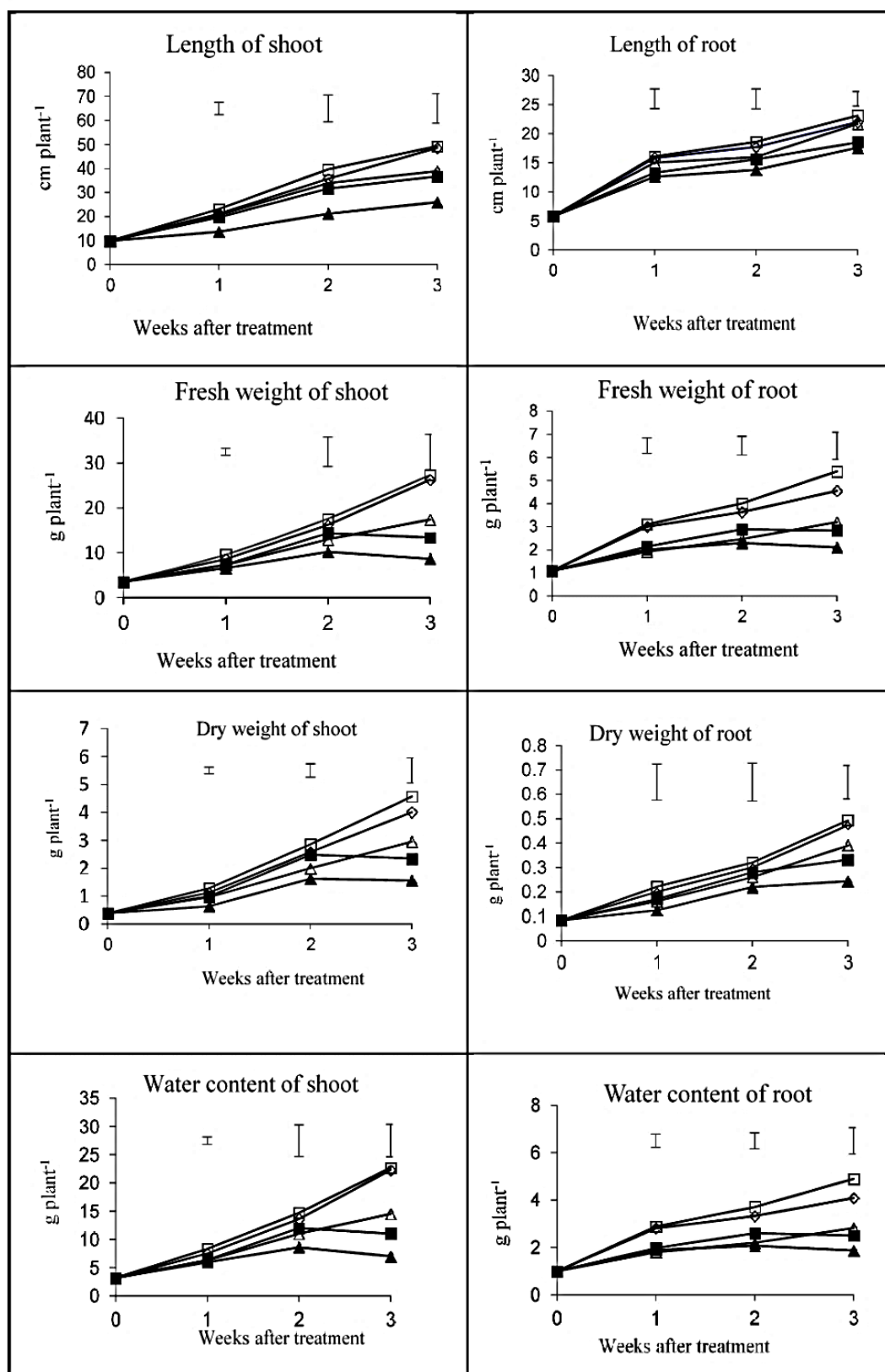
### Photosynthetic parameters

Chl a, Chl b, Cars and total pigment contents of common bean plants treated with low Cu showed a significant increase above the control levels throughout the experimental period. In all the other stressed plants, on the contrary, all the pigment fractions and consequently the total pigments appeared to decrease significantly below the control levels. As far as Chl a/b ratio is concerned, a progressive decrease was maintained throughout the experimental period. The following sequence of

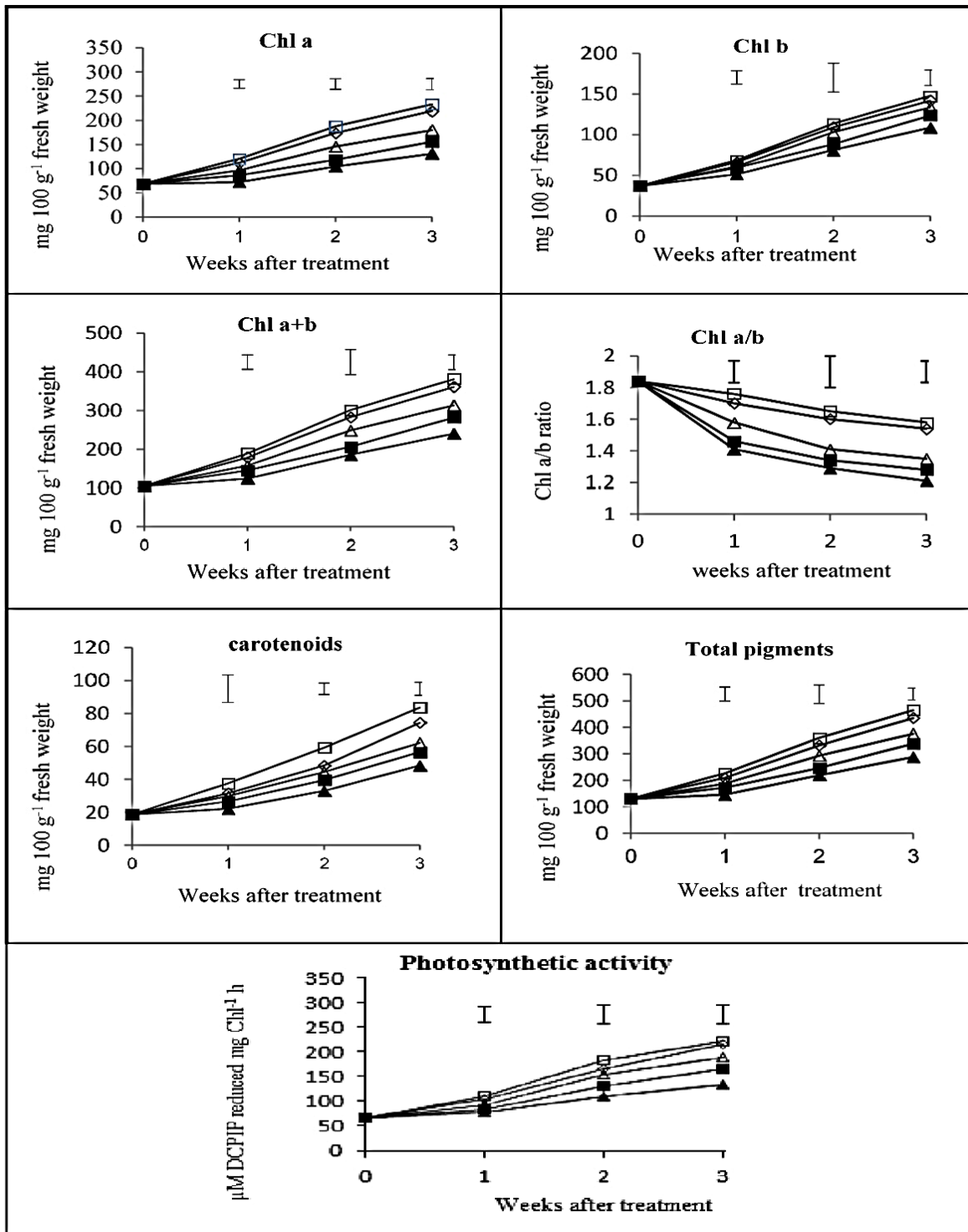
treatments: 10<sup>-3</sup> M Cd > 10<sup>-3</sup> M Cu > 10<sup>-6</sup> M Cd > control > 10<sup>-6</sup> M Cu, was displayed with respect to the maintained decrease in Chl a/b ratios (Fig. 3).

It is also clear from figure 3, that the photosynthetic activity in leaves of the low (10<sup>-6</sup> M) Cu-treated common bean plants showed a significant increase above that activity of the control plants. On the other hand, leaves sampled from the high (10<sup>-3</sup> M) Cu-stressed and from the low (10<sup>-6</sup> M) and the high (10<sup>-3</sup> M) Cd-stressed plants showed PS II activities

significantly lower than those of the controls throughout the experimental period (Fig. 3).



**Fig. 2:** Effects of different concentrations of copper or cadmium on growth parameters of shoots and roots of *Phaseolus vulgaris* plants (—◇— Control; —□— 10<sup>-6</sup> M Cu; —■— 10<sup>-3</sup> M Cu; —△— 10<sup>-6</sup> M Cd; —▲— 10<sup>-3</sup> M Cd). Vertical bars represent the L.S.D at 5 % level.



**Fig. 3:** Effects of different concentrations of copper or cadmium on photosynthetic pigment fractions and photosynthetic activity (PS II activity) of *Phaseolus vulgaris* leaves (—◇— Control; —□— 10<sup>-6</sup> M Cu; —■— 10<sup>-3</sup> M Cu; —△— 10<sup>-6</sup> M Cd; —▲— 10<sup>-3</sup> M Cd). Vertical bars represent the L.S.D at 5 % level.

### Carbohydrate components

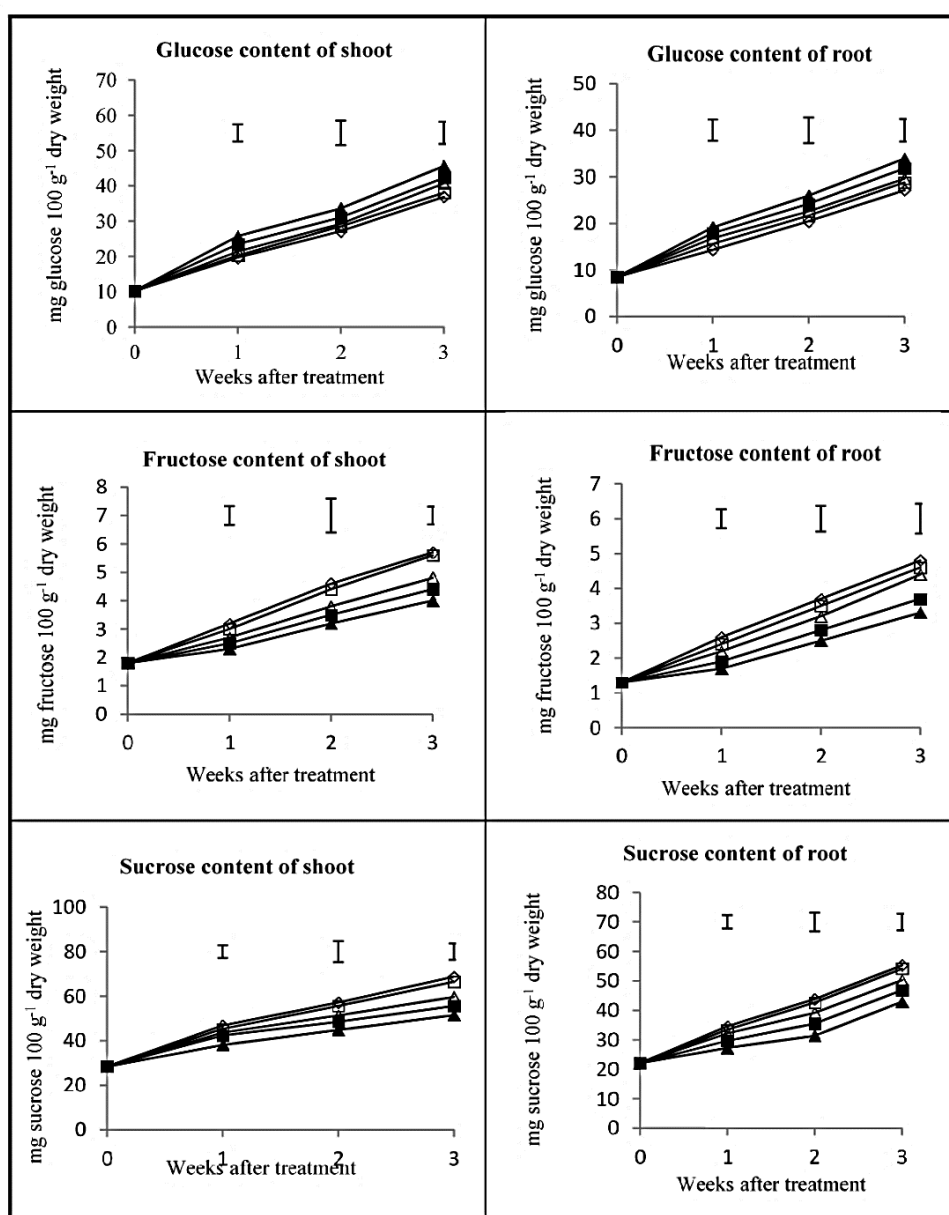
As being apparent from the data given in figures 4a and 4b, it is evident that carbohydrate contents in shoots and roots of common bean plants, variously treated with Cu

or Cd, increase progressively with an increase in duration of the experimental period. Treatment with 10<sup>-6</sup> M Cu induced an insignificant increase in glucose contents of both shoots and roots as compared with control

levels. On the other hand, treatments with the high concentration ( $10^{-3}\text{M}$ ) of Cu as well as with the low ( $10^{-6}\text{M}$ ) and the high ( $10^{-3}\text{M}$ ) concentrations of Cd induced marked significant increases in relation to control values throughout the three weeks. The higher the concentrations of the heavy metal used, the higher was the magnitude of increase in the glucose content, in both shoots and roots (Fig. 4a).

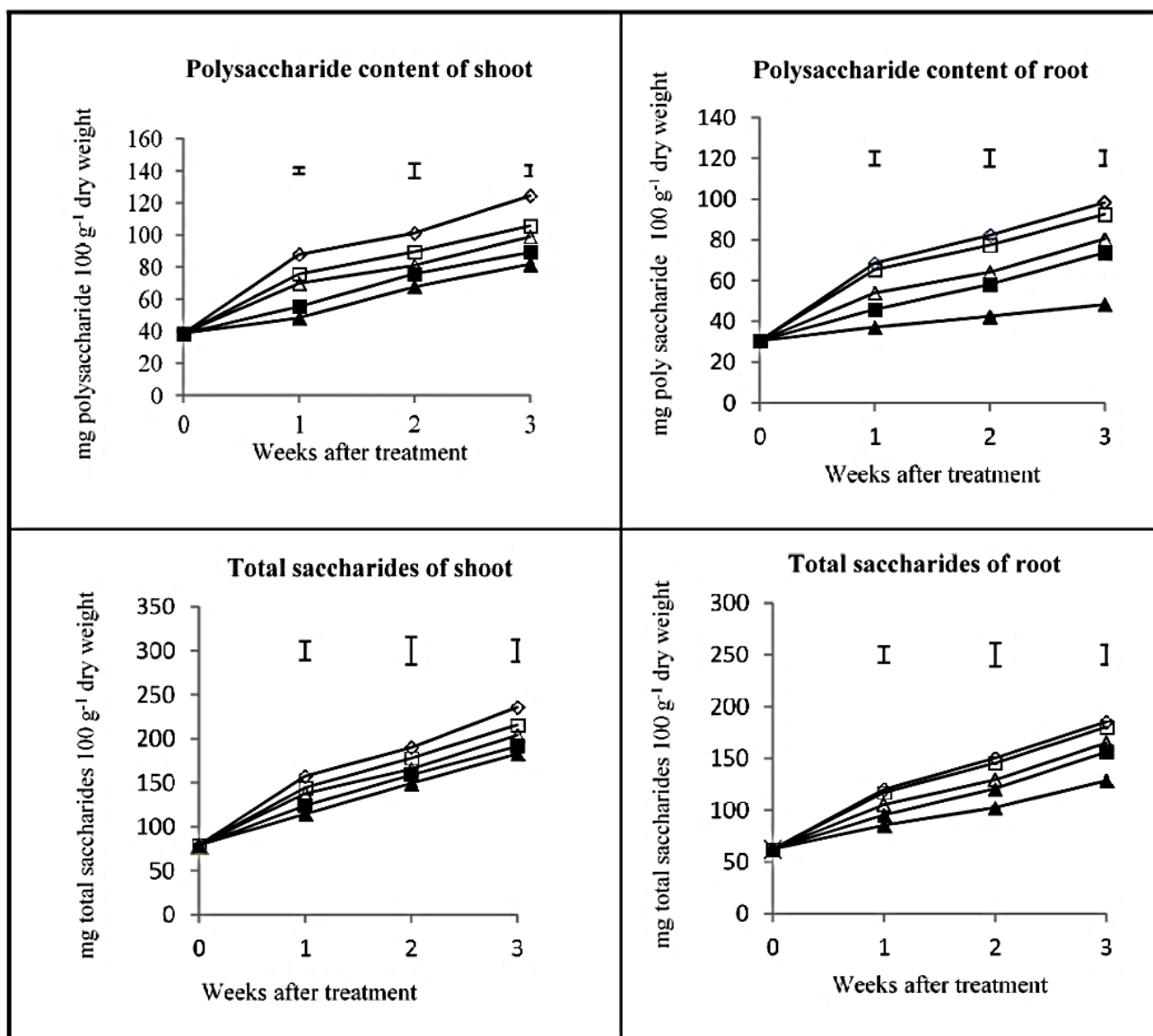
Nevertheless, all the concentrations of Cu or Cd caused decreases in fructose, sucrose, polysaccharide and total saccharide contents,

throughout the experimental period. Only with the low concentration ( $10^{-6}\text{M}$ ) of Cu, the maintained decrease in fructose, sucrose, polysaccharide and total saccharide contents were insignificant in relation to the control values. Thus, the following sequence of treatments: ( $10^{-3}\text{M Cd} > 10^{-3}\text{M Cu} > 10^{-6}\text{M Cd} > 10^{-6}\text{M Cu} > \text{control}$ ), was displayed with respect to the magnitude of decrease in fructose, sucrose, polysaccharides and total saccharide contents in both shoots and roots of common bean plants (Figs. 4a and 4b).



**Fig. 4a:** Effects of different concentrations of copper or cadmium on carbohydrate fractions of shoots and roots of *Phaseolus vulgaris* plants (—◇— Control; —□—  $10^{-6}\text{M Cu}$ ; —■—  $10^{-3}\text{M Cu}$ ; —△—  $10^{-6}\text{M Cd}$ ; —▲—  $10^{-3}\text{M Cd}$ ). Vertical bars represent the L.S.D at 5 % level.





**Fig. 4b:** Effects of different concentrations of copper or cadmium on carbohydrate fractions of shoots and roots of *Phaseolus vulgaris* plants (—◇— Control; —□— 10<sup>-6</sup> M Cu; —■— 10<sup>-3</sup> M Cu; —△— 10<sup>-6</sup> M Cd; —▲— 10<sup>-3</sup> M Cd). Vertical bars represent the L.S.D at 5 % level.

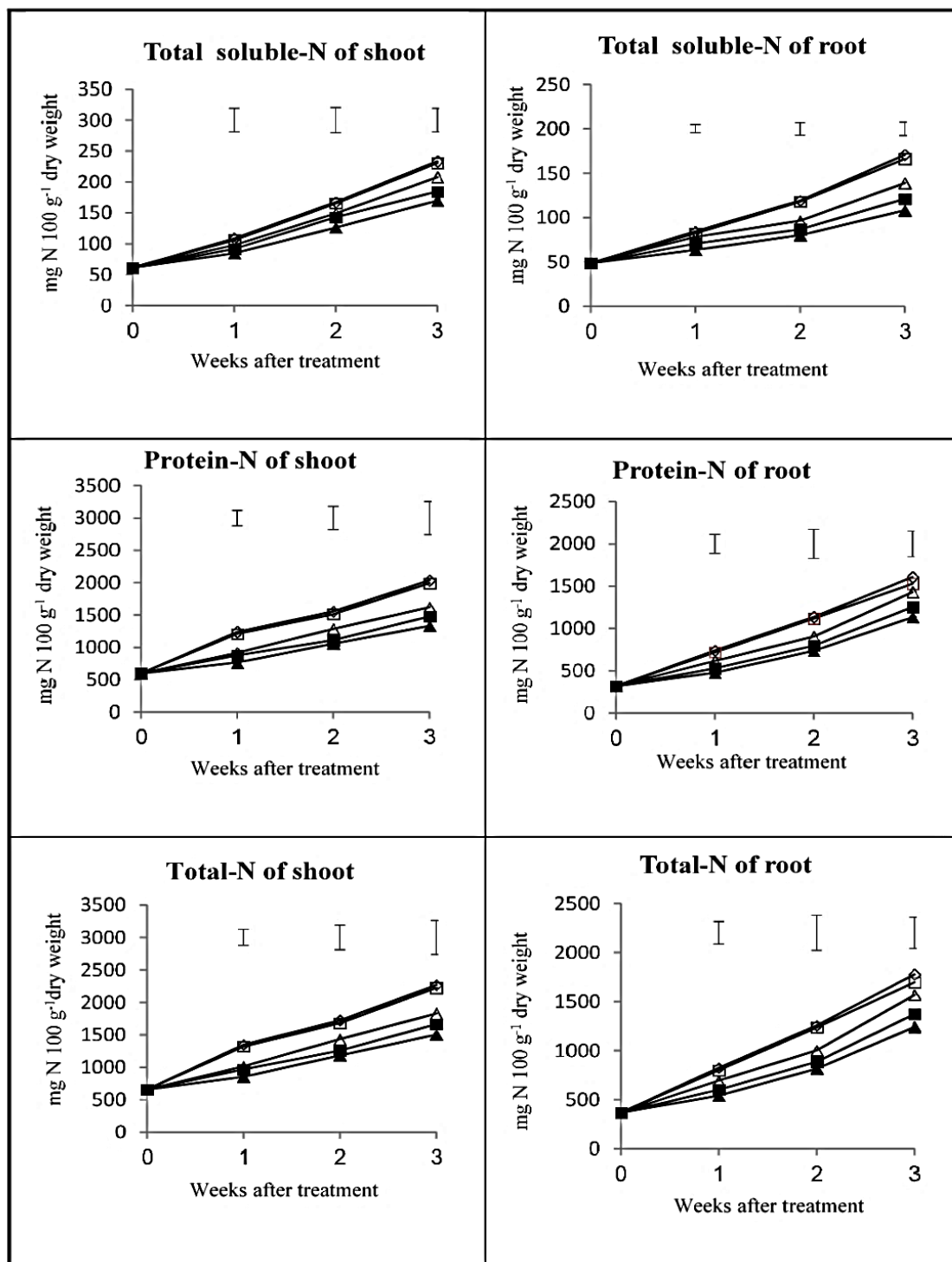
## Nitrogen constituents

### a- Changes in total soluble-N and protein-N

All the main fractions determined (total soluble-N, protein-N and total-N) appeared, in general, to increase progressively, in both shoots and roots, with an increase in duration of the experimental period. It is also evident that protein -N is the predominant component of the nitrogen pool in both shoots and roots of *Phaseolus vulgaris* (Fig. 5).

Treatment with the low concentration (10<sup>-6</sup> M) of Cu appeared to induce a slight, but insignificant, decrease in total soluble -N,

protein -N and total -N fractions in both shoots and roots of the treated plants, below the comparable control values. On the other hand, treatments with the high concentration (10<sup>-3</sup> M) of Cu as well as with the low (10<sup>-6</sup> M) and the high (10<sup>-3</sup> M) concentrations of Cd induced marked and varied significant decreases in the main nitrogen fractions, in relation to the appropriate control values. Nevertheless, the magnitude of decline in response to the high concentration (10<sup>-3</sup> M) of Cu appeared, in general, to be more or less comparable with that of the low concentration (10<sup>-6</sup> M) of Cd (Fig. 5).



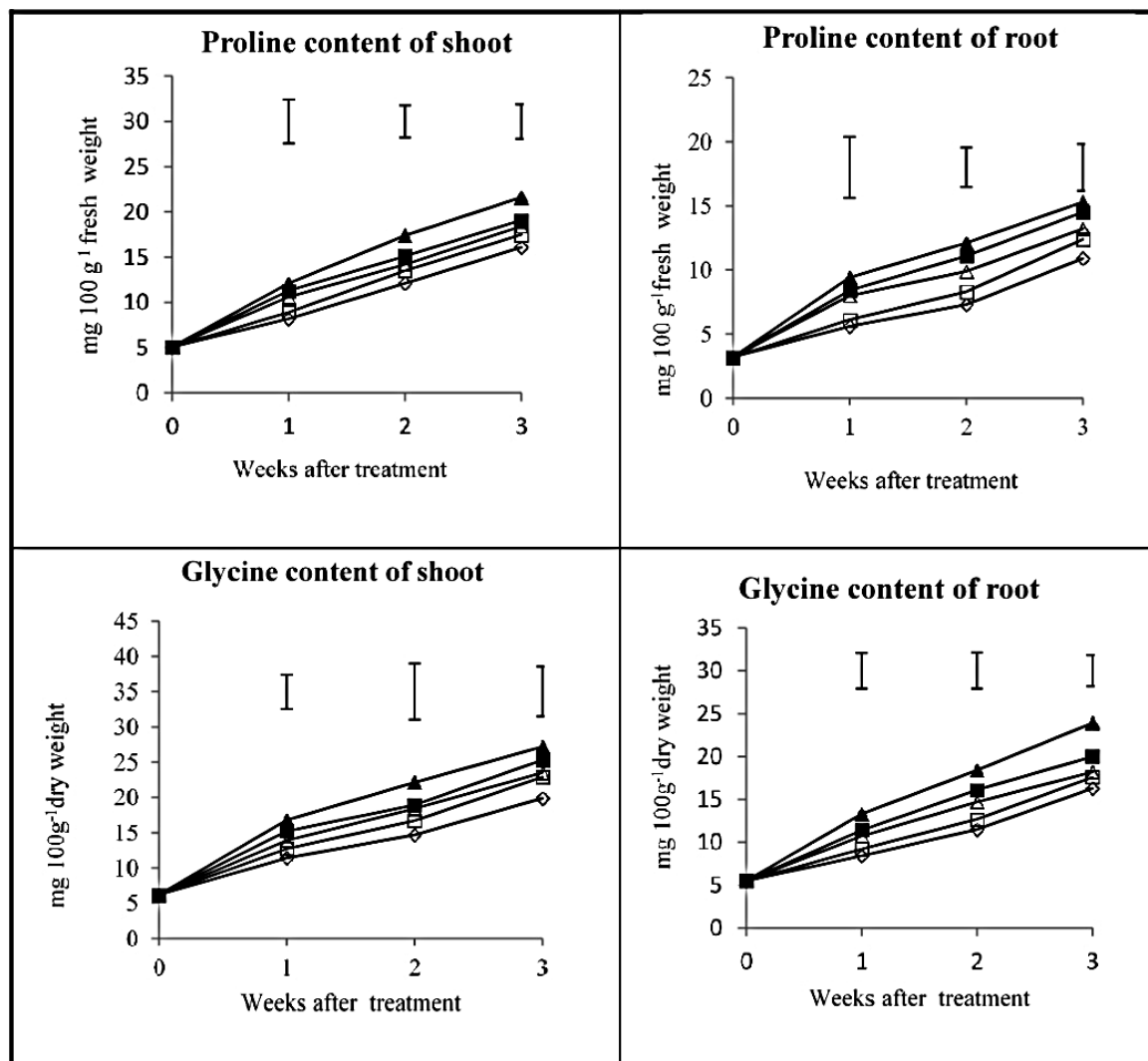
**Fig. 5:** Effects of different concentrations of copper or cadmium on nitrogen fractions of shoots and roots of *Phaseolus vulgaris* plants (—◇— Control; —□— 10<sup>-6</sup> M Cu; —■— 10<sup>-3</sup> M Cu; —△— 10<sup>-6</sup> M Cd; —▲— 10<sup>-3</sup> M Cd). Vertical bars represent the L.S.D at 5 % level.

**b- Changes in proline and glycine contents**

Proline and glycine contents showed a progressive increase throughout the period of the experiment (Fig. 6). Treatment with low (10<sup>-6</sup> M) Cu induced insignificant increases in proline and glycine contents in shoots and roots above the respective control values. On the

other hand, upon treatment with high (10<sup>-3</sup> M) Cu concentration or with the low (10<sup>-6</sup> M) and high (10<sup>-3</sup> M) Cd concentrations, marked significant increases in both proline and glycine contents were obtained above the appropriate control values. The following sequence of treatments (10<sup>-3</sup> M Cd > 10<sup>-3</sup> M Cu > 10<sup>-6</sup> M Cd

>  $10^{-6}$  M Cu) appeared, in general, to be displayed with respect to the magnitude of increase in proline and glycine contents in both shoots and roots above the appropriate controls (Fig. 6).

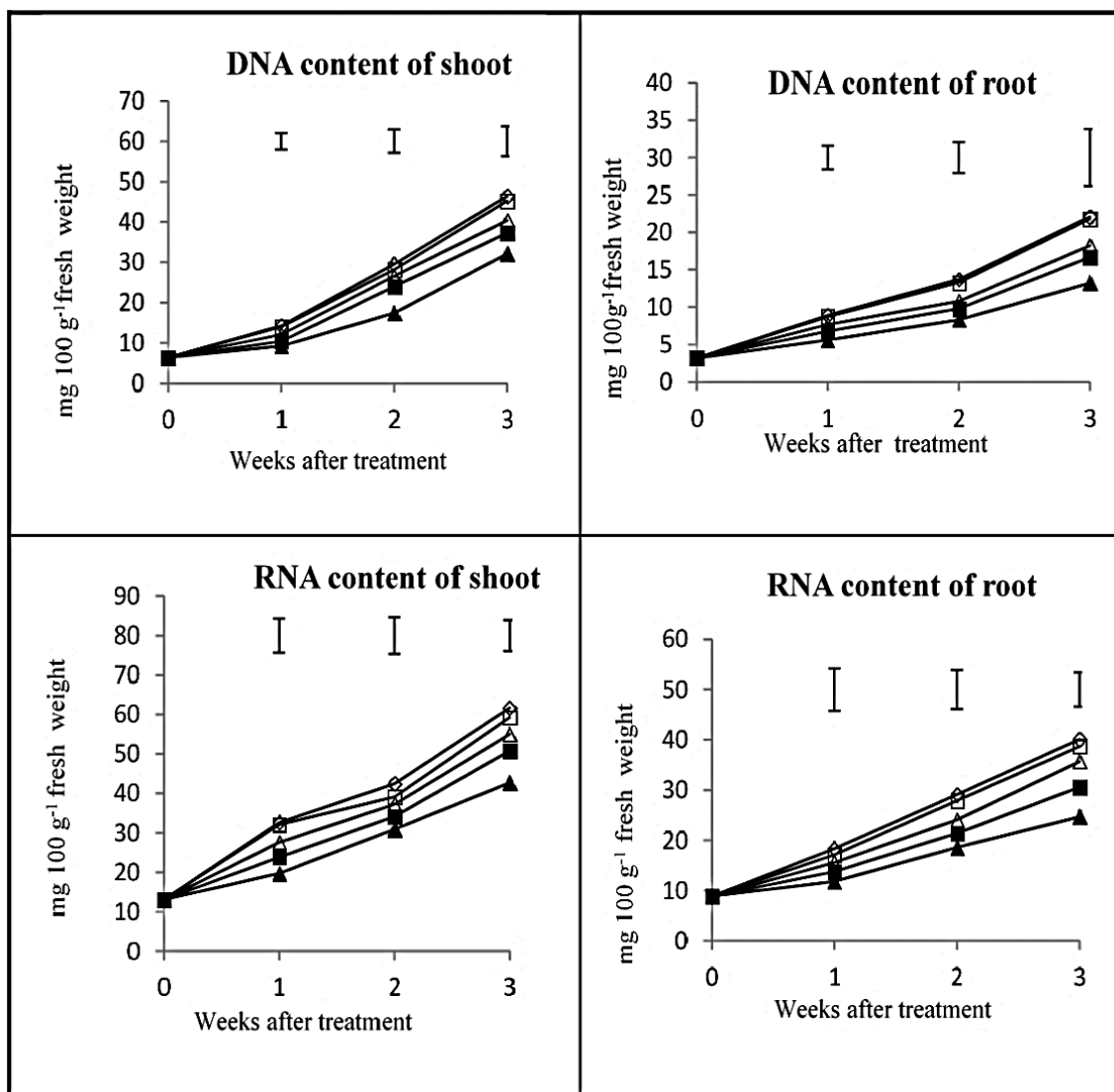


**Fig. 6:** Effects of different concentrations of copper or cadmium on amino acids content of shoots and roots of *Phaseolus vulgaris* plants (—◇— Control; —□—  $10^{-6}$  M Cu; —■—  $10^{-3}$  M Cu; —△—  $10^{-6}$  M Cd; —▲—  $10^{-3}$  M Cd). Vertical bars represent the L.S.D at 5 % level.

### Changes in nucleic acid contents

The nucleotide levels detected in both shoots and roots appeared to increase with an increase in the duration of the experimental period. Furthermore, in both shoots and roots of the common bean plants, RNA appears to be the dominant component of the nucleotide pool. As compared with controls, DNA and RNA contents in both shoots and roots of the common bean-treated plants showed a slight insignificant decrease (with the low ( $10^{-6}$  M)

concentration of Cu) and marked significant decreases with the high ( $10^{-3}$  M) concentration of Cu as well as with the low ( $10^{-6}$  M) and the high ( $10^{-3}$  M) concentrations of Cd. Again, it is evident from figure 7 that the magnitude of decline induced by both Cu ( $10^{-3}$  M) and Cd ( $10^{-6}$  M) appears more or less comparable; Cd ( $10^{-3}$  M) appeared to be the most detrimental concentration used within the present set of experimental conditions (Fig. 7).



**Fig. 7:** Effects of different concentrations of copper or cadmium on nucleic acids contents of shoots and roots of *Phaseolus vulgaris* plants (—◇— Control; —□—  $10^{-6}$  M Cu; —■—  $10^{-3}$  M Cu; —△—  $10^{-6}$  M Cd; —▲—  $10^{-3}$  M Cd). Vertical bars represent the L.S.D at 5 % level.

## DISCUSSION

Growth is the best index for evaluating plant response to environmental stress. The present general manner of plant response to heavy metals stress is a growth suppression which seemed to be a function of the concentration of the heavy metal used. Thus, as shown in figure 2, varied insignificant changes; increase and decrease were obtained with the low concentrations of Cu and Cd, respectively. Further, significant varied reductions in all the determined growth parameters were obtained throughout. Furthermore, careful examination of figures 1 and 2 indicates that a positive

correlation appears to exist between the progressive degree of uptake and accumulation of both heavy metals and the progressive increase in the resulting adverse effects observed throughout the experimental period.

The Cu and Cd contents in roots and in shoots appeared to increase with increasing the available Cu and Cd concentrations in soil. As a micronutrient for plants, the role played by Cu in assimilation of  $\text{CO}_2$  and generation of ATP is of utmost importance. Cu is also considered as a basic component for several proteins like cytochrome oxidase of respiratory transport chain and plastocyanin of photosynthetic

system (Demirevska-Kepova et al., 2004). But, augmentation of human activities and industrial and mining activities have participated in the increasing existence of Cu in ecosystem. Excess Cu performs a cytotoxic role, stimulates stress and leads to great damage of plants (Wisniewski and Dickinson, 2003). Plants growing in soils saturated with elevated levels of Cd exhibit apparent symptoms of damage, reflected in expressions of chlorosis, growth suppression, browning of root tips and finally death (Mohanpuria et al., 2007). Exposure to over doses of Cu and Cd generates oxidative stress with consequent accumulation of ROS that leads to perturbation of metabolic pathways and harm macromolecules (Hegedus et al., 2001).

The application of Cu at low ( $10^{-6}$  M) concentration appeared to increase shoots and roots length, water content and plant's dry weight. The promotion effect of Cu, at this low concentration, on the biomass production may indicate the valuable position of Cu as a fertilizer in plant growth. However, the distinctive reduction obtained in all growth parameters, in consequence to application of excess Cu as well as to application of the low and the high concentrations of Cd, may indicate the observed toxic effects on the growth of the common bean plants, due to chromosomal aberrations and suppression of both cell division and elongation (Souguir et al., 2008).

In corroboration of the current results, Manivasagaperumal et al., (2011) working on greengram (*Vigna radiate*), reported that treatment of plants with low level of Cu induced significant increases in shoot length, root length, leaf area and dry matter production in relation to control values; a reverse situation being observed with the high concentrations. The dry matter yield of greengram also showed similar reduction at the high concentrations due to Cd (Madhavi and Rao 1999). The reduction in biomass in excess Cu treated greengram as well as in excess Cd treated plants might be due to low protein formation, resulting in

suppression of photosynthesis, as well as disturbed carbohydrate translocation (Wani et al., 2007). This conclusion is well supported by our results showing inhibition of chlorophyll formation and photosynthetic activity (Fig. 3), inhibition of carbohydrate formation (Figs. 4a and 4b) as well as protein breakdown (Fig. 5).

The present results concerning the effects of both heavy metals on the photosynthetic machinery appeared to coincide with those effects maintained in growth parameters, including dry mass accumulation, in common bean plants. The positive significant values maintained in growth parameters and photosynthetic efficiency, in response to application of the low ( $10^{-6}$  M) Cu concentration, led us to suggest that one of the factors leading to high rates of growth, including dry matter accumulation, in treated plants was the increased capacity for assimilation of  $\text{CO}_2$ . Again, the role of this Cu concentration as a micronutrient was more evident than its toxicity. The decreasing trend of photosynthetic machinery can be tentatively imputed to the fact that, both Cu (at  $10^{-3}$  M) concentration and Cd (at the low ( $10^{-6}$  M) and the high ( $10^{-3}$  M) concentrations), increase the activity of chlorophyllase depending enzyme, leading to instability of pigment protein complex and demolition of chloroplast structure.

The chlorophylls are the most important active pigments in photosynthetic process. The interrelationships between Chl a and Chl b fractions can be better evaluated when the values of Chl a / b ratio are taken into consideration. As compared with control values, the trend of changes in the contents of Chl a and b were comparable with respect to the type and the concentrations of the heavy metals used. Thus, throughout the experimental period, the progressive increase in Chl a and b contents is correlated with a gradual decrease in the values of Chl a/b maintained, throughout.

In support of the present results, the photosynthetic machinery have been established to be negatively affected when

legumes as well as other plants are grown in heavy-metal contaminated soils (Abdel-Fattah et al., 2003; Bibi and Hussain, 2005; Yurekli and Porgali, 2006; Hatata and Abdel-Aal., 2008). Furthermore, it was found, in isolated protoplasts reacted with Cd, the reactions of the Calvin cycle were the fundamental target of this metal action (Sheoran et al., 1990). Thus, considering our results (Fig. 3) and, in the light of current literature, we can tentatively deduce that excess Cu and Cd metals destroy firstly carbon reduction cycle and thereafter affect the photosynthetic electron transport.

Sugars are now well documented to be important metabolites in plant metabolism, not only because they are the first complex organic compounds formed in the plant as a result of photosynthesis, but they are also considered as the main origin of respiratory energy. The present results, in consequence to supplemental addition of different concentrations of Cu and Cd, show varied appreciable increases in glucose contents, whereas fructose, sucrose, polysaccharides and consequently total carbohydrate contents were variably decreased. The varied increased levels of glucose in the heavy metal-treated plants can be explained on the basis of contribution to energy requirements for maintenance of varied rates of growth. Nevertheless, it seems that carbohydrate metabolism, under stress conditions, can be considered as a dynamic process; including concomitantly occurring process of polysaccharide degradation and induction of soluble sugar accumulation. Glucose is always considered as the ultimate respiratory substrate which has to be activated giving the immediate respiratory substrate, which enters the sequence in respiratory metabolism.

In support of the present results, Deef (2007) reported that treatment with Cu at low concentration shows an increase in total carbohydrate in seedlings and the opposite was true at elevated concentration. The massive decrease of carbohydrate in Cd-treated plants

is stated to be a consequence of the reduction in chlorophyll biosynthesis, leading to a decrease in carbohydrate contents (Kupper et al., 1998). In further support of our results, Subbaiah and Sachs (2003) found that elevated doses of Cu and Cd decreased the total carbohydrates and increased the sugar contents in maize seedlings. These results seem to correspond with the photosynthesis reduction or the stimulation of respiration rate (Kumar and Rajam, 2002).

The present inhibited growth has previously been attributed to metabolic disorders. In this context, in the Cu- and Cd-treated common bean plants, a steady decline in TSN, PN and consequently total-N, in relation to control levels, were observed in shoots and roots (Fig. 5). In plant cells, nitrogen manipulates a vital role as a main component of amino acids, protein and other biomolecules. In support of the current results, Abdel-Fatah et al. (2003) observed a significant reduction in protein and total nitrogen contents of *Ambrosia maritima* but soluble-N was found to increase as a result of high concentration of Cd in the nutrient solution.

In different plant species, it has been observed that heavy metal-stress is accompanied with the capacity to accumulate free amino acids, especially proline, which is now well known to act as a suitable solute participating in stress control (Stewart and Lee, 1974; Younis et al., 2009). Although earlier, most interest has been concerned with the role of proline in stress resistance, yet, the role of glycine has suffered far less attention. Nevertheless, as stated by Waditee et al. (2005), glycine appears to meet the requirements of a compound that can have a role in stress resistance as (1) it is the master amino acid that can be synthesized *in vivo*, (2) ubiquitous amounts of glycine may be used for betaine synthesis under salt stress conditions, (3) the manifestation of the importance of glycine for the improvement of abiotic stress resistance in crop plants and (4) Waditee et al. (2005) obtained results referring that glycine

is limiting for maximal salt stress tolerance in higher plants. Furthermore, Hasaneen et al. (2008) observed a remarkable increase in glycine and proline contents in *Lactuca sativa* plants treated with various levels of NaCl. The higher was the salt concentration used, the higher was the accumulation of proline and glycine contents.

All the aforementioned parameters besides the present comparable fashions of changes in both proline and glycine contents, point to the fact that the function of glycine in heavy metal-stress tolerance cannot be eliminated. Thus, the accumulation of particular amino acids as proline and glycine in plants seems to be a common response to some abiotic stress (Jain et al., 2001; Hassaneen et al., 2008; Younis et al., 2009). In fact, the present proline as well as glycine accumulation (Fig. 6) are in accord with the present reduction in water content (Fig. 2).

In addition, exposition of common bean plants to varied concentrations of the heavy metals, herein used, induced a significant decrease in the total amount and in the relative composition of the nucleic acids contents (RNA and DNA) throughout the experimental period. Thus, one of the major results of heavy metals stressful action is enhanced production and accumulation of ROS, which usually harm the cellular components such as nucleic acids, membranes and chloroplasts (Bayer et al., 2006). In support of the present results, a decline in DNA and RNA contents in consequence either to weak protection or reduced efficiency of synthesis of nucleic acid is reported under heavy metal stress (Prasad and Strzalka, 2002; Begum et al., 2007; Hamid et al., 2010).

## CONCLUSIONS

Cu is metabolized by the plant and plays a vital role at low concentrations, but higher concentrations are toxic. In contrast, Cd is not metabolized by the plant and exhibits high toxicity even at low concentrations. In view of the above-mentioned changes in growth and metabolism, induced by uptake and

accumulation of Cu and Cd, we can finally conclude that a close parallelism appeared to exist among the growth pattern, photosynthetic machinery and dry matter accumulation. This appeared to be a consequence of the maintained disruption of the functional intensity of the photosynthetic apparatus leading, in general, to decreased levels in the various carbohydrate fractions as well as in the total carbohydrate content. The increased levels of glucose, proline and glycine contents can be explained on the basis of contribution to the maintenance of osmotic pressure in the expanding cells in shoots and roots of the common bean plant. However, the role of these compounds in acting as a reserve of carbon and nitrogen for repaid recovery from stress as well as in serving as stabilizer of macromolecules and free radical scavenger cannot be ignored (Jain et al., 2001). The results of this study on common bean plants well support the likelihood that heavy metal ion excess produces ROS leading to development of oxidative stress which appeared to be a function of the concentration of the element used. This conclusion is further substantiated by our second communication (Younis et al., 2018).

## CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

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