Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231– 6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2015 Vol. 5 (4) October-December, pp. 152-158/Monalisa et al.
Research Article

EFFECTS OF CERTAIN HEAVY METALS ON BIOCHEMICAL CONSTITUENTS OF *PHASEOLUS ACONITIFOLIUS* JACQ. CV RMO-40

***Monalisa[1], Mohil P[2] and Jain U.[2]**
[1]S. S. Jain Subodh P. G. College, Jaipur
[2]Department of Botany, University of Rajasthan, Jaipur
**Author for Correspondence*

ABSTRACT
Biochemical constituents of moth (*Phaseolus aconitifolius*) Jacq. CV. RMO-40, seedling growths were studied under heavy metal concentrations of 25ppm to1000ppm. These concentrations significantly affected total soluble sugars and protein content. Total soluble sugar content increased at lower concentration (10-50ppm concentrations) almost in all the treatments except Cd where sugars reduce gradually. However, with increase in concentration (500-10000 ppm) in presence of all pollutants total soluble sugar content markedly reduced. Protein content also increased at lower concentration in Cu, Zn and Pb. Soluble protein content remains unaffected at lower concentration in Ni only. In all the treatments of heavy metals, protein content decreased at higher concentration. Cd was found to be most effective heavy metal in all respect.

***Keywords**: Phaseolus Aconitifolius, Seeds, Sugars, Protein*

INTRODUCTION
Heavy metals have significant biological roles as metallo-enzyme and are required as micronutrients by all organs. Some heavy metals at low doses are essential micronutrients for plants but in higher doses they cause metabolic disorders and growth inhibition for most of the plant species with the ongoing technological advancement in industrialization and urbanization process, release of toxic contaminants like heavy metals in natural resources has become a serious problem worldwide (Sethy *et al.,* 2013). The toxicity of heavy metals is a problem for ecological, evolutionary and environmental reasons (Nagajyoti *et al.,* 2008).
Excessive levels of metals naturally present in the environment due to normal geological phenomenon like mining of ore, erosion of surface deposit of minerals due to weathering of rocks or leaching, forest fires and volcanic activity contribute to environmental pollution. They can be carried to places many far off from the source by winds depending on their physical state, namely, whether, they are in the gaseous form or as particulate. Such pollutants are ultimately washed out of the air by rain on to land or in the water bodies and absorbed by plants. Most of the heavy metal pollution of river and consequently of soil is caused by agriculture, industrial and domestic water. Therefore, there is an increasing interest in effects of heavy metals on higher plants and their responses to excessive metal concentrations as stressors (Zhang *et al*., 2010). The aim of present study to is assess the effect of heavy metals on biochemical constituents of *phaseolus aconitifolius.*

MATERIALS AND METHODS
Certified veritiety RMO 40 of *Phaseolus aconitifolius* Jacq. was obtained from agriculture research Station, Beechwal, Agriculture University, Bikaner. Seeds were stored in glass stoppered bottles. After a preliminary selection for uniformity criteria (size and colour of seeds), the seeds were surface sterilized with 0.1% $HgCl_2$ for two minutes (Misra, 1968), then washed with distilled water three times and then soaked for two hours in respective solutions of different concentrations (10, 50, 100, 200, 500 and 1000 ppm) of copper sulphate, cadmium sulphate, lead sulphate, nickel sulphate and zinc sulphate. Seeds soaked in distilled water for two hours constituted the control. After the above treatments, seeds were removed and allowed to germinate in petri plates on filter paper soaked in each of the above metallic solution. Three replicates each of 10 seeds were kept for each concentration of every heavy metal. The

filter paper was moistened with metallic solutions. The experiments were carried out for ten days under laboratory conditions of temperature (25±2°C) and diffuse light.

On the day of termination of experiment, (10^{th} day) germinated, total soluble sugar and protein contents were analysed.

(a) Total Soluble Sugar

The phenol sulphuric acid reagent method of Dubois *et al*., (1956) was followed for estimating the amount of total soluble sugars. 500.0 mg of fresh tissue was homogenized with 10.0 ml of 80.0 percent ethanol and centrifuged at 2000 rpm for 20 minutes. The supernatant was collected.

To one ml of the alcoholic extract, one ml of 5.0% phenol reagent was added. To this 5 ml of concentrated (96%) H_2SO_4 was added rapidly.

It was gently agitated during addition of suphuric acid and then allowed to stand in water bath at 26-30°C for 20 minutes.

Optical density (O.D.) of the characteristic yellow orange colour thus developed was measured at 490 nm in a spectrophotometer (Electronic Corporation of India Limited, (GS 570IV) after setting for 100% transmission against the blank (by substituting distilled water for test solution). The standard curve was prepared by using known concentration of glucose. The quantity of sugar was expressed as mg/g fresh weight of tissue.

(b) Protein Estimation

Protein content was estimated using the method of Lowry *et al*., (1951). 200 mg (fresh weight) plant tissue samples were extracted with 5.0 ml of 5% TCA (Trichloroacetic acid). The homogenate was centrifuged at 2000 rpm.

For 20 minutes and the supernatant discarded. The residue was dissolved in 10.0 ml of 0.1 N NaOH. 0.1 ml of this solution was made up to 1.0 ml by adding distilled water.

The following reagents were prepared:-

(a) Alkaline sodium carbonate solution (50 ml of 2% Na_2CO_3 in 0.1 N NaOH).

(b) Copper sulphate sodium tartarate solution

(0.5 ml of 0.5% $CuSO_4.5H_2O$ in 0.5 ml of 1.0% sodium potassium tartarate, prepared fresh).

(c) 50 ml of reagent 'a' was mixed with 1.0 ml of reagent 'b'. This was mixed just before use.

(d) Folin-ciocalteau reagent (diluted with equal volume of distilled water i.e. in the ratio of 1:1.

(e) Stock standard solution – 100 mg of bovine serum albumin dissolved in 100 ml of 1N NaOH (1 mg/ml).

3.5 ml of alkaline copper reagent or Lowery reagent was added, to the dissolved residue and allow standing for 10 minutes. 0.5 ml of folin-ciocalteau reagent was added in last, and O.D. was measured at 720 nm in spe3.5 ml of alkaline copper reagent or Lowery reagent was added, to the dissolved residue and allows standing for 10 minutes. 0.5 ml of folin-ciocalteau reagent was added in last, and O.D. was measured at 720 nm in spectrophotometer. The reference curve was prepared by using known concentration of BSA (bovine serum albumin) in 0.1 N NaOH. The quantity of protein was expressed as mg/g fresh weight of tissue.

RESULTS AND DISCUSSION

Results

In the present study an attempt was made to study influence of heavy metals on different metabolic events in *Phaseolus aconitifolius*. In RMO 40 of *Phaseolus*, pollutants namely Cu, Cd, Pb, Zn and Ni, affect total soluble sugar content and protein. Growth stimulation or inhibition by different heavy metals depends on concentration and nature of heavy metals.

Significant change in the concentrations of total sugar in seedling treated with heavy metal was observed (Table 1and Figure 1). Treatment with heavy metals like Cu, Zn (10 ppm to 50 ppm), Pb and Ni (10 ppm to 200 ppm) was marked by an increase in soluble sugars. However, at high concentration (500 ppm, 1000 ppm) a decline in sugar level was observed. Cd led to sharp decline in sugar content with increasing concentration Table-1; Figure 1.

Table 1: Showing the Effect of Heavy Metals on the Carbohydrate Contents in Terms of Soluble Sugars (mg/g fresh weight) in the Seedling of *Phaseolus Aconitifolius* CV. RMO 40

Heavy Metals	Concentrations (ppm)						
	Control ± SD	**10 ± SD**	**50 ± SD**	**100 ± SD**	**200 ± SD**	**500 ± SD**	**1000 ± SD**
Cu	0.063± 0.002	0.064± 0.001	0.066± 0.002	0.061± 0.001	0.053± 0.001	0.053± 0.003	0.044± 0.002
Cd	0.063± 0.002	0.063± 0.002	0.054± 0.001	0.045± 0.003	0.033± 0.002	0.028± 0.001	0.018± 0.002
Zn	0.063± 0.002	0.081± 0.001	0.076± 0.001	0.061± 0.002	0.053± 0.002	0.041± 0.001	0.032± 0.001
Pb	0.063± 0.002	0.086± 0.002	0.123± 0.002	0.133± 0.002	0.098± 0.002	0.073± 0.002	0.050± 0.001
Ni	0.063± 0.002	0.085± 0.001	0.121± 0.001	0.103± 0.002	0.081± 0.001	0.064± 0.001	0.044± 0.002

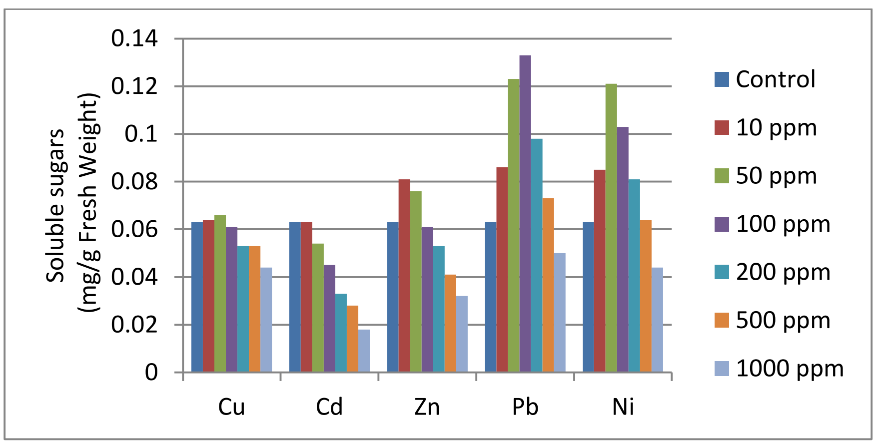

Figure 1: Showing the Effect of Heavy Metals on the Carbohydrate Contents in Terms of Soluble Sugars (mg/g fresh weight) in the Seedling of *Phaseolus Aconitifolius* Cv. RMO 40

Effect of Cu, Cd, Zn, Pb and Ni on accumulation of protein has been presented in Table 2 and Figure 2. Significant changes in the concentration of protein were recorded in the treated seedlings. Higher concentration of protein was the features of seedling raised in Cu (10-200 ppm), Zn and Pb (10-100 ppm) and Ni (50-100 ppm).

Protein content decreased at higher concentration. However, in this respect Cd was found to be the most effective heavy metals. Remarkable reduction in protein content was observed in the presence of Cd. Table 2; Figure 2.

Discussion

Effect of Cd, Cr, and Ni on biochemical parameter was studied by Vineeth *et al.*, (2015) in *Vigna radiata* (green gram), reducing sugar content were significantly decreased in plants treated with Treatment II (heavy metals spiked in soil) 1.268+-0.015mg/g when compare with Treatment I (without heavy metal) 0.281+-0.002mg/g and Treatment III (1% of calcium hydroxide added along with heavy metals. Similarly non reducing sugar and protein content were also significantly decreased in plant treated with Treatment –II as compared to Treatment I and III.

Table 2: Showing the Effect of Heavy Metals on Protein Contents (mg/g Fresh Weight) in the Seedling of *Phaseolus Aconitifolius* Cv. RMO 40

Heavy Metals	Concentrations (ppm)_						
	Control± SD	**10± SD**	**50± SD**	**100± SD**	**200± SD**	**500± SD**	**1000± SD**
Cu	0.233± 0.003	0.251± 0.001	0.264± 0.001	0.280± 0.001	0.268± 0.003	0.228± 0.001	0.212± 0.003
Cd	0.233± 0.003	0.221± 0.001	0.212± 0.002	0.207± 0.002	0.137± 0.015	0.083± 0.006	0.000± 0.000
Zn	0.233± 0.003	0.242± 0.002	0.263± 0.002	0.244± 0.003	0.231± 0.001	0.156± 0.115	0.205± 0.002
Pb	0.233± 0.003	0.236± 0.001	0.250± 0.001	0.275± 0.001	0.186± 0.001	0.166± 0.002	0.148± 0.003
Ni	0.233± 0.003	0.229± 0.001	0.233± 0.002	0.241± 0.001	0.223± 0.002	0.211± 0.001	0.193± 0.003

SD = Standard Deviation

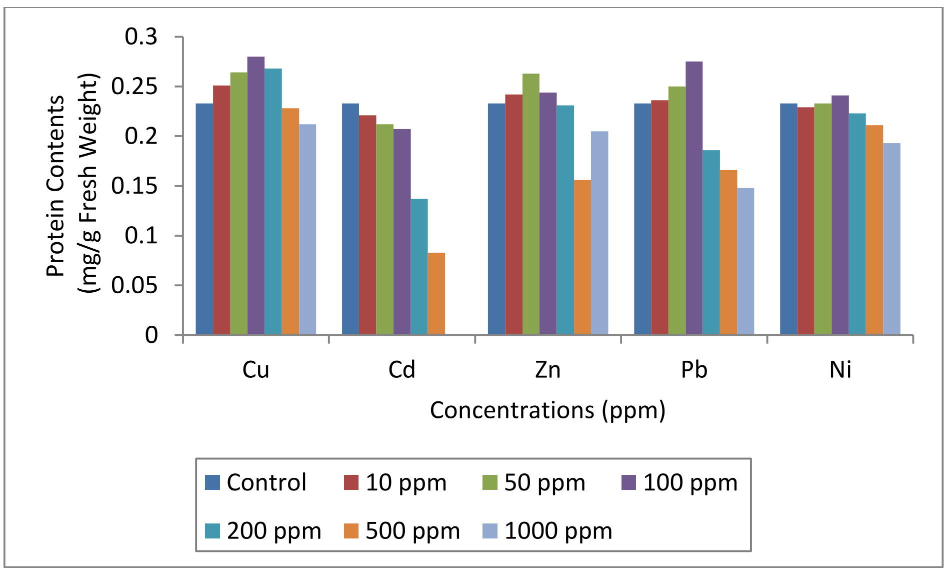

Figure 2: Showing the Effect of Heavy Metals on Protein Contents (mg/g Fresh Weight) in the Seedling of *Phaseolus Aconitifolius* CV. RMO 40

Effect of mercuric chloride and cadmium chloride on the biochemical parameters of the seedling of pigeon pea (*Cajanus cajan*) (L) Millsp.) were studied and found that sugar and Protein content decreased with increase in concentration of heavy metals (Patanik and Mohanty, 2013). The total sugar content of green gram seedlings were found to be more in control (0.0912mg/g/Fr.wt.) and a gradual decrease in sugar content (0.0690 mg/g/fr.wt.) found as the concentration of lead and copper increases (Sajid, 2008). The results are in conformity with results of Dhankar (2010), in black gram under Zinc and copper treatment.

Verma and Dubey (2004) studied effect of cadmium on soluble sugar and enzymes of metabolism of rice using two cultivars namely Ratna and Jaya and found similar results with the present study. During 5 to 20 days exposure at 100 µ M or 500 µ M Cd $(NO_3)_2$ in growth medium increase total soluble sugars and reducing sugars and decrease in the content of non reducing sugars. Deef (2007) observed application of Cu at rates of 0, 50 and 200 ppm were gradually increased the dry matters accumulation and sugar fraction. However, high rate up to 3200 ppm, the dry matter gradually diminished as well as sugar fraction in *Rosmarinus officinalis*.

Heavy metals have been shown to increase soluble proteins in leaves of soyabean treated with lead and cadmium (Lee *et al*., 1976) and rice treated with lead (Mukherjee and Maitra, 1976). Effect of copper and cadmium on duckweed (*Lemna minor*) was studied by Hou *et al*., (2007). Their results demonstrated that exposure to high concentration heavy metals (Cu>10 mg l^{-1}, Cd>0.5 mg l^{-1}) could result the disintegration of antioxidant system in duckweed and the significant decrease of content of soluble protein and photosynthetic pigment was observed to high level metal stress. Additionally cadmium was found to be more toxic than copper on plants as observed in present studies. Inhibition of soyabean metabolism by cadmium and lead was studied by Huang *et al*., (1974). They concluded that Pb and Cd inhibited plant metabolism generally, as shown by their reduction of shoot and root growth and pod's fresh weight. The reduction of pod fresh weight was correlated with the effect on shoot, root, leaf, nodule dry weight, carbohydrate and protein content. They also concluded that lead was less effective than Cd. The chloride salts of Cd or Ni were also examined in relation to biomass, seed production and metabolic pattern in soyabean (*Glycine max*) by Malan and Farrant (1978). Both metals markedly reduced plant biomass and seed production. Ni was found to be more mobile than Cd, reaching higher levels in all plant parts, especially in seeds. Cadmium reduced mature seed mass. This effect was mostly due to decreased yield of lipids, protein and carbohydrates. Copper tolerance in *Chlorella vulgaris* has been studied by comparing physiological properties (Fathi *et al*., 2005), and copper uptake in a wild type strain and a copper tolerant one. A concentration dependent reduction in growth rate, dry mass and content of chlorophyll, protein, sugar and amino acids was noticed in both strains at 1.0 and 400 mgl^{-1} copper. The reduction in all parameters was higher in wild type strain than in the tolerant one. Beside copper, chromium accumulation also reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L. (Vajpayee *et al.*, 1999).

The effect of Zn, Cd, Cu and Hg on the soluble protein bands during germination of lentil seeds was investigated by Ayaz and Kadioglu (1997) and found contradictory results with the presence study. Manios *et al*., (2002) the effect of the heavy metals (Cd, Cu, Ni, Pb and Zn) on the total protein concentration of *Typha latifolia* plants growing in a substrate containing sewage sludge compost and watered with metaliferous waste water and found that higher concentrations of soluble protein was observed at lower concentration of heavy metals and inhibition occurred in case of stronger solutions. Protein synthesis were also significantly reduced with the treatment of Cu^{+2} in pea plants (Angelov *et al*., 1993), treatment of Cd in wheat seedling (Lesko *et al*., 2002), treatment of Pb in maize (Jana and Choudhary, 1984). Changes in some important protein involved in CO_2 fixation (Rubisoo, Rubisico activase, Rubisco binding protein, NH^{4+} assimilation and glutamate synthase, as a result of excess in barley leaves (*Hordeum vulgare* L. cv. Obzer) was determined by Kepova *et al*., (2004). Excess of Cu affected mainly the non protein SH groups, while Mn influenced the ascorbic acid content. Oxidative stress under Cu or Mn toxicity was most probably the consequence of depletion of low molecular antioxidant as a result of their involvement in detoxification processes and disbalanced in antioxidation enzymes. A comparative study was carried out with Mo, Ni, Cd and Pb using concentration of 1×10^{-7}, 1×10^{-5} and 1×10^{-3} mol dm^{-3}, on the metabolism of nitrogen and proteins in young pea plants (*Pisum sativum* L.) (Kevresen *et al*., 2001). Nitrate and protein metabolism decreased according to the order of Cd>Pb>Ni>Mo.

Conclusion

In present study effect of heavy metals on total soluble sugar, protein was studied in *Phaseolus aconitifolius* cv. RMO 40. Among heavy metals, cadmium was found to be the most toxic in all the concentrations. All the concentrations of all the heavy metals were lowered the total soluble sugars and protein contents in *Phaseolus aconitifolius* except certain lower concentrations of Cu, Zn, Pb and Ni.

REFERENCES

Angelov M, Tsonev T, Uzunova A and Gaidardjieva K (1993). Cu^{+2} effect upon photosynthesis, chloroplasts structure, RNA and protein synthesis of pea plants. *Photosynthetica* **28** 341-350.

Ayaz AF and Kadioglu A (1997). Effect of heavy metals (Zn, Cd, Cu and Hg) on the soluble protein bands of germinating *Lens esculentum* L. seeds. *Turkey Journal of Botany* **21** 85-88.

Deef HES (2007). Copper treatment and their effects on growth, carbohydrates, minerals and essential oils contents of *Rosmarinus officinalis* L. *World Journal of Agriculture Science* **3** 322-328.

Dhankar R (2011). Zinc and copper induced change s in physiological characterstics of *Vigna mungo* (L.) *Journal of Environmental Biology* **32** 747-751.

Dubios M, Gilles KA, Hamilton JK, Rebers PA and Smith F (1956). Colorimetric method for determination of sugar and related substances. *Analytical Chemistry* **28**(3) 350-356.

Fathi AA, Zaki FT and Ibrahim HA (2005). Response of tolerant and wild type strains of *Chlorella vulgaris* to copper with special reference to copper uptake system. *Protistology* **4** 73-78.

Hou W, Chen X, Song G, Wang Q and Chang CC (2007). Effect of copper and cadmium on heavy metal polluted water body restoration by duck weed (*Lemina minor*). *Plant Physiology & Biochemistry* **45** 62-69.

Huang CY, Bazzaz FA and Vanderhoef LN (1974). The inhibition of soyabean metabolism by cadmium and lead. *Plant Physiology* **54** 122-124

Jana S and Choudhary MA (1984). Synergistic effect of heavy metal pollutants on senescence in submerged aquatic plants. *Water Air Soil Pollution* **21** 351-357.

Kepova KD, Stoilova LS, Stoyanova Z, Holzer R and Feller U (2004). Biochemical changes in barley plants after excessive supply of copper and manganese. *Environment Experimental Botany* **52** 253-266.

Kevresan S, Petrovic N, Popovic M and Kandrac J (2001). Nitrogen and protein metabolism in young pea plants as affected by different concentrations of nickel, cadmium, lead and molybdenum. *Journal of Plant Nutrition* **24** 1633-1644.

Lesko K, Banyai ES, Pais I and Sarkadi LS (2002). Effect of cadmium and titanium – ascorbate stress on biological active compounds in wheat seedlings. *Journal of Plant Nutrition* **25** 2571-2581.

Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ (1951). Protein measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry* **193** 265-275.

Lee KC, Cunningham BA, Poulsen GM, Liang JM and Moore RB (1976). Effects of cadmium on respiration rate and activities of several enzymes in soyabean seedlings. *Plant Physiology* **36** 4-7.

Malan HL and Farrant JM (1998). Effect of the metal pollutants cadmium and nickel on soyabean seed development. Seed Science Research 8 445-453.

Dubios M, Gilles KA, Hamilton JK, Rebers PA and Smith F (1956). Colorimetric method for determination of sugar and related substances. *Analytical Chemistry* **28**(3) 350-356.

Manios T, Stentiford I and Millner P (2002). The effect of heavy metals on the total protein concentration of *Typha latifolia* plants growing in a substrate containing sewage sludge compost and water with metaliferous wastewater. *Journal of Environment Science and Health* **37** 1441-1451.

Misra R (1968). *Ecology Work Book*, (Oxford and IBH Publishing Co. New Delhi, Bombay, Calcutta, India).

Mukerji S and Maitra P (1976). Toxic effects of lead on growth and metabolism of germinating rice (*Oryza sativa* L.) seeds and on mitosis of onion (*Allium cepa* L.) root tip cell. *Indian Journal of Experimental Biology* **14** 519-521.

Nagajyoti PC, Lee KD and Sreekanth TVM (2010). Heavy Metals, Occurance and toxicity for Plants, *Environmental Chemistry Literature* **8** 199-216.

Patnaik A and Mohanty BK (2014). Effect of the stress induced by mercury and cadmium on the biochemical parameters of the seedlings of pigeon pea (cajanus cajan (L.) Millsp.) *International Journal of Research in Bioscience* **3** 19-24.

Sajid Md (2008). Photosynthetic performance of two mung bean cultivars under lead and copper stress. *International Journal of Agriculture and Biology* 18.

Sethy SK and Ghosh S (2013). Effect of heavy metals on germination of seeds. *Journal of Natural Science, Biology and Medicine* **4**(2) 272-275.

Vajpayee P, Tripathi RD, Rai UN, Ali MB and Singh SN (1999). Chromium (VI) accumulation reduce chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L. *Chemosphere* **41** 1075-1082.

Verma S and Dubey RS (2004). Effect of cadmium on soluble sugars and enzymes of their metabolism in rice. *Biological Plantarum* **44** 117-123.

Vineeth D, Venkateshwar C and Unnisa A (2015). Effect of heavy metals on Biochemical parameters in Vigna radiate green gram *International Journal of Current Research* **7**(8) 18936-18942.

Zhang W, Huang H, Tan F. Wang Hand, Qui R (2010). Influence of EDTA: Washing on the species and mobility of heavy metals residual in soils. *Journal of Hazard Mater* **173**(1-3) 369-376.