RESEARCH ARTICLE

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Evaluation of Biochemical Parameters in Heavy Metals Stressed Crop *Phaseolus aconitifolius* Cv. RMO 225

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ABSTRACT

Background: Metabolic processes involve set of chemical reactions that occur in the cells of living organisms to sustain life and allow living organisms to maintain their structure and response to the surrounding environment. With increase in industrilisation and urbanisation heavy metal pollution is becoming a global problem. Plant growth and metabolism naturally affected by heavy metals, although they are required in various metabolic reactions and that is why there is need to study their role and impact on biochemical parameters in heavy metal stressed crop.

Method: The experiments were carried out during 2019-2020 under laboratory condition. In the present study biochemical constituents of moth (*Phaseolus aconitifolius*) Jacq. Cv. RMO-225, seedlings were studied under certain heavy metals (Cu, Zn, Pb, Ni and Cd) concentrations 25 ppm-1000 ppm. These concentrations significantly affected biochemical constituents e.g. soluble sugar, starch, phenol and protein content.

Result: In the present investigation total sugar and starch content increased at lower concentrations (10-50 ppm concentration) almost in all the treatments except cadmium. However with increase in the concentration (500-100 ppm) total content of both markedly reduced, In cadmium, starch content reduce gradually from 10-1000 ppm concentration. Phenol content also increased at 10-100 ppm concentrations of the heavy metals Cu, Zn, Pb and Ni and decreased at higher concentrations. Protein content sharply decline in all the treatments of cadmium, however in lower concentration of all heavy metals treatments protein content increased and decreased at higher concentrations (200-500 ppm). Cadmium was found to be most toxic in all respect in present study.

Key words: Metabolism, *Phaseolus aconitifolius*, Phenol, Protein, Seedlings, Starch, Sugar.

INTRODUCTION

Pollution is becoming a major issue on a global scale as industrialization and urbanisation develop. Plant development and metabolism are impacted by pollution, which ultimately results in a decrease in yield. Metals are now utilised in many facets of human endeavour. Metals are necessary for a variety of purposes in modern society. All organisms need heavy metals as micronutrients because of their important biological roles as metallo-enzymes (Wood *et al.*, 1975). Yet, they become dangerous when their concentration is slightly more than what is required for physiological interaction and nutritional requirements in traces. Environmental pollution is also a result of high levels of metals that are naturally present in the environment as a result of common geological phenomena including mining for ore, erosion of mineral deposits on the surface owing to weathering of rocks or leaching, forest fires and volcanic activity. The dispersion of metals in the biosphere is caused by improved technologies used in the extraction of metals from ore. Depending on their physical state, namely whether they are in the gaseous form or as particulates, they can be transported by winds to locations that are many miles away from the source. Eventually, these toxins from agriculture industry and domestic water consumption are the main contributors to the heavy metal pollution of rivers and soil. River water, the earth and soil are all contaminated by waste water (Pande *et al.*, 1999).

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Moreover, it has a detrimental impact on plant physiology, productivity, nutritional value, seed germination and seedling growth.

Certain heavy metals are required micronutrients for plants at low concentrations, but at greater concentrations, they can cause metabolic problems and stunt most plant species growth (Claire *et al.*, 1991). Thus, there is growing interest in how heavy metals affect higher plants (Zhang *et al.*, 2010). The aim of the present study is to assess the effect of heavy metals on Biochemical parameters of *Phaseolus aconitifolius* cv RMO-225.

MATERIALS AND METHODS

Certified seeds of variety RMO 225 of *Phaseolus aconitifolius*J acq. were obtained from Research Station Beechwal, Rajasthan 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^{o}C) and diffuse light.

On the day of termination of experiment, (10 day) germinated seeds were counted and total soluble sugar, starch, phenol and protein contents were estimated.

Estimation of carbohydrates

(a) Total soluble sugars

The phenol sulphuric acid reagent method of Dubois *et al.* (1956) was followed for estimating the amount of total soluble sugars.

(b) Starch

Starch estimation was done according to the anthrone reaction method (McCready *et al.*, 1950).

Estimation of phenolic contents

The estimation of total phenolic was made by the following method given by Mahadevan (1975).

Protein estimation

Protein content were estimated using the method of Lowry *et al.*, (1951).

RESULTS AND DISCUSSION

In the current study attempt was to investigate how heavy metals affect several biochemical parameters in *Phaseolus aconitifolius* in cv RMO 225. Pollutants, specifically Cu, Cd, Pd, Zn and Ni, have an impact on total soluble sugars, starch, phenol and protein. The nature and concentration of specific metals have an impact on their effects. Total soluble sugar concentration is affected by contaminants such as Cu, Cd, Pb, Zn and Ni in the *Phaseolus* cultivar RMO 225.

Table 1 illustrate how heavy metals affect total sugar.The seedlings grown in Cu, Zn and Ni treatments, higher total sugar contents observed. In Cu, Zn and Ni concentrations sugar content increased by up to 50 ppm and 100 ppm concentrations. Higher concentrations of heavy metals adversely affected sugar concentration. In Table 2 the effect of heavy metals on the accumulation of starch is depicted. All heavy metals were discovered to significantly alter the amounts of starch at various concentrations, much as total soluble carbohydrates. Starch concentrations increased up to 200 ppm when Zn, Cu, Pb and Ni treatment given. However, in the presence of Cd, no effect was seen at 10 ppm, but it rapidly decreased as concentration increased. The starch content was decreased as all the heavy metal concentrations increased. Table 2 depicted how heavy metals affect the amount of starch (mg/g fresh weight) in the *Phaseolus aconitifolius* cv. RMO 225 seedlings.

Table 3 illustrate how heavy metals affect phenol content. Higher concentration of phenols than control plants observed in the presence of various heavy metals-10 ppm to 100 ppm of Cu, Zn, Pb and Ni. On the other hand, accumulation of phenols was noticeably diminished at larger quantities of these heavy metals. Of all the heavy metals, Cd has the biggest impact on the phenol content of the seedling. In case of Protein estimation, Cb had a larger negative impact on proteins than Cu, Ni, Zn and Pb. At Cu, Zn, Pb and Ni concentrations of 10 ppm and 50 ppm, respectively protein levels were higher than the control (Table 4). In various Cd concentrations, protein content gradually reduced. A decrease in protein content was caused by higher levels of all heavy metals (Cu, Zn, Pb and Ni). At

Table 1: 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 225.

Heavy metals	Concentrations (ppm)						
	Control±SD	10±SD	50±SD	100±SD	200±SD	500±SD	1000±SD
Cu	0.095±0.002	0.120±0.001	0.141±0.001	0.147±0.001	0.162±0.206	0.140±0.182	0.023±0.002
Cd	0.095±0.002	0.111±0.001	0.082±0.002	0.075±0.002	0.063±0.002	0.058±0.001	0.023±0.002
Zn	0.095±0.002	0.101±0.001	0.122±0.002	0.178±0.002	0.092±0.002	0.081±0.001	0.071±0.001
Pb	0.095±0.002	0.112±0.002	0.130±0.001	0.081±0.001	0.061±0.001	0.052±0.001	0.041±0.001
Ni	0.095±0.002	0.104±0.002	0.113±0.001	0.114±0.002	0.083±0.002	0.067±0.002	0.053±0.001

SD = Standard deviation.

Table 2: Effect of heavy metals on the carbohydrate contents in terms of starch (mg/g fresh weight) in the seedling of *phaseolus aconitifolius* cv. RMO 225.

Heavy metals	Concentrations (ppm)						
	Control±SD	10±SD	50±SD	100±SD	200±SD	500±SD	1000±SD
Cu	0.033±0.001	0.045±0.00 1	0.057±0.001	0.042±0.002	0.032±0.002	0.024±0.002	0.020±0.002
Cd	0.033±0.001	0.025±0.00 2	0.016±0.003	0.013±0.001	0.009±0.001	0.002±0.002	0.00±0.00
Zn	0.033±0.001	0.042±0.00 2	0.045±0.001	0.048±0.001	0.035±0.001	0.028±0.001	0.023±0.002
Pb	0.033±0.001	0.033±0.00 1	0.037±0.001	0.039±0.001	0.031±0.001	0.028±0.001	0.013±0.003
Ni	0.033±0.001	0.034±0.00 2	0.044±0.001	0.037±0.001	0.032±0.002	0.025±0.002	0.022±0.002

SD = Standard deviation

Table 3: Effect of heavy metals on phenol contents (mg/g Fresh Weight) in the seedling of *phaseolus aconitifolius* cv.RMO 225.

Heavy metals	Concentrations						
	Control±SD	10±SD	50±SD	100±SD	200±SD	500±SD	1000±SD
Cu	0.013±0.003	0.016±0.001	0.017±0.001	0.020±0.001	0.016±0.001	0.009±0.001	0.004±0.001
Cd	0.013±0.003	0.012±0.003	0.010±0.002	0.012±0.003	0.010±0.001	0.005±0.001	0.000±0.000
Zn	0.013±0.003	0.019±0.00	0.022±0.00	0.023±0.00	0.017±0.00	0.009±0.00	0.011±0.00
Pb	0.013±0.003	0.013±0.002	0.016±0.001	0.020±0.001	0.024±0.002	0.020±0.005	0.015±0.001
Ni	0.013±0.003	0.013±0.003	0.022±0.001	0.024±0.004	0.015±0.001	0.010±0.001	0.009±0.001

SD = Standard deviation

Table 4: Effect of heavy metals on protein contents (mg/g fresh weight) in the seedling of *phaseolus aconitifolius* cv. RMO 225.

Heavy metals	Concentrations (ppm)						
	Control±S D	10± SD	50± SD	100± SD	200± SD	500± SD	1000± SD
Cu	0.242±0.003	0.256±0.002	0.245±0.001	0.235±0.001	0.242±0.002	0.221±0.001	0.213±0.003
Cd	0.242±0.003	0.224±0.001	0.195±0.002	0.171±0.001	0.116±0.021	0.015±0.003	0.000±0.000
Zn	0.242±0.003	0.262±0.002	0.254±0.002	0.256±0.002	0.235±0.003	0.224±0.002	0.208±0.003
Pb	0.242±0.003	0.264±0.002	0.278±0.00	0.294±0.001	0.281±0.001	0.271±0.001	0.241±0.001
Ni	0.242±0.003	0.255±0.001	0.262±0.002	0.242±0.002	0.233±0.002	0.211±0.001	0.202±0.001

SD = Standard deviation

a concentration of 1000 ppm, the protein contents of Cu, Zn, Pb and Ni fresh weights all reduced from the control's 0.242 mg/g to 0.213 mg/g, 0.208 mg/g, 0.241 mg/g and 0.202 mg/g, respectively. At 1000 ppm Cd exposure, seedlings did not survive.

With the exception of Cd, where sugar content steadily decreased, practically all treatments in RMO 225, slight rise observed in total sugar content at lower concentrations (10 ppm). However, with a rise in concentration (between 500 and 1000 ppm) of all the contaminants soluble sugar content significantly reduced. In the current study, starch content increased at lower concentrations (10 ppm to 100 ppm) and reduced at high concentrations (500 ppm to 1000 ppm) in the presence of Cu, Zn, Pb and Ni, similar to the effect of heavy metals on total soluble sugars. The most harmful of the five heavy metals appears to be Cd.

Verma and Dubey (2004) used the Ratna and Jaya cultivars to examine the impact of cadmium on rice's soluble sugar and metabolic enzymes and discovered findings that were consistent with those of the current study. Growth medium contains more total soluble sugars and reducing sugars and less non-reducing sugars after exposure to 100 M or 500 M Cd (NOM or 500 M or 500 M Cd (NO3) 2 for 5 to 20 days. Vineeth *et al.* (2015) studied, how Cd, Cr and Ni affected biochemical parameters in the plant *Vigna radiata*. Comparing heavy metal treatment to control and treatment with calcium hydroxide and heavy metals together, reducing, non-reducing sugars and protein levels were considerably reduced.

Patnaik and Mohanty (2014) investigated the impact of mercury chloride and cadmium chloride on the biochemical parameters of seedlings of the pigeon pea [*Cajanus cajan* (L.) Millsp], including photosynthetic pigments, sugars, protein, amino acids, DNA and RNA content. The seedlings' metabolic components and photosynthetic pigments gradually decreased as metal concentrations increased.

The impact of heavy metals on the biochemical profile of *Azolla filiculoides* was examined by Anand *et al.* in 2017. It was shown that the heavy metals Pb, Cd, Hg and Zn had a significant impact on the amount of total sugar and protein in *Azolla filiculoides*. However, the kind and quantity of heavy metals had an impact on the metabolic profile of *A. filiculoides*. Insoluble sugar, reducing sugar, amylolytic activity, amylase activity, total nitrogen content, protein content and free amino acids were all significantly decreased by all four heavy metals.

Ganeva and Zozikova (2007) discovered a disparity between the effects of rising Cu^{+2} concentration on growth and content of free phenols in two lines of wheat (*Triticum aestium*) with different tolerance levels. With increasing Cu concentration in the medium, it was discovered that the content of free phenols increased in both lines; this finding is comparable to the one made in the current investigation.

In the current investigation, it was discovered that the effects of heavy metals on proteins were more severe in Pb and Cd than in Cu, Ni and Zn. The pollutants copper sulphate, cadmium sulphate, zinc sulphate, lead sulphate and nickel sulphate also affected metabolism in terms of soluble protein in cv. RMO-225. At 10 ppm and 50 ppm concentrations of Cu, Zn, Pb and Ni, protein levels were higher than the control (Table 4). The protein content gradually declined in Cd ranging from 10 ppm to 1000 ppm. At a Cd dosage of 1000 ppm, no seedlings survived. The protein content of the cultivar under study was noticeably decreased at higher concentrations (500 ppm and 1000 ppm) of all the contaminants.

In *Noccaea caerulescens* and *Arabidopsis halleri,* Zemanova *et al.* (2018) investigated the change in concentration of specific free amino acids (glutamic acid, glutamine, aspartic acid, asparagines, proline and hydroxyproline) under cadmium (Cd1= 30, Cd2= 60 and Cd3= 90 mg/kg soil). The pot experiment's findings supported the higher stress tolerance of *A. halleri* and the differing effects of Cd on *N. caerulescens* compared to that of *A. halleri*. In both plant species, total free amino acid levels were considerably altered by cadmium exposure. Under Cd stress, the amount of aspartic acid was increased in *N. caerulescens*, while no changes were seen in *A. halleri*. It is inferred from this investigation that cadmium affects protein concentration in the same way as the present study, although few plant species can tolerate this.

In other investigations, Houet *et al.* (2007) examined the impact of copper and cadmium on duckweed (*Lemna minor*). Their findings showed that exposure to high concentrations of heavy metals (Cu>10 mg l^{-1}, Cd>0.5 mg l^{-1}) could cause reduction on antioxidant systems in duckweed and high levels of metal stress were also associated with a significant decrease in the content of soluble protein and photosynthetic pigment. Additionally, it was shown that cadmium is more hazardous to plants than copper. Huang *et al.* (1974) investigated how Pb and Cd generally hindered plant metabolism in soyabean. Additionally, they came to the conclusion that, similar to the current investigation of *Phaseolus aconotifolius* cv. RMO 225, lead was less effective than Cd. Copper tolerance in *Chlorella vulgaris* has been examined by Fathi *et al.* (2005). By contrasting physiological traits and copper uptake in a wild type strain and a copper tolerant one, Both strains showed a concentration-dependent decrease in growth rate, dry mass and chlorophyll, protein, sugar and amino acid content at 1.0 and 400 mgl^{-1} copper. In the wild type strain compared to the tolerant one, the fall in all parameters was greater.

Ayaz and Kadioglu (1997) studied the impact of Zn, Cd, Cu and Hg on the soluble protein bands during the germination of lentil seeds and discovered results that differ with the presence investigation. Kevrese *et al.* (2001) studied protein and nitrogen metabolism in young pea seedlings (*Pisum sativum* L.) at different concentration of Mo, Ni, Cd and Pb and their result conclude decrease in order Cd>Pb>Ni>Mo. The findings of this study are similar to those of the current investigation of the metals Cd, Pb and Ni.

CONCLUSION

In the current study, the impact of heavy metals Zn, Cu, Ni, Pb and Cd on total soluble sugar, starch, phenol and protein content was evaluated *Phaseolus aconitifolius* cv. 225. Cadmium was discovered to be the most harmful of all heavy metal treatments in all concentrations. Except for some lower concentrations (10-50 ppm) of Cu, Zn, Pb and Ni, where examined biochemical constituents enhanced in accordance with the nature of heavy metals, all the concentrations of all the heavy metals reduced the total soluble sugars, starch, phenols and protein contents in *Phaseolus aconitifolius.*

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Conflict of interest

The authors have no conflict of interest to declare.

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