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Research article

Influence of Chromium and Lead toxicity on biochemical parameters of Soybean (*Glycine max L*)

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ABSTRACT

Heavy metals like lead and chromium that are present in the environment in large amounts are a serious environmental concern. An investigation was carried out to determine the effect of different concentrations (50ml, 100ml, 150ml and 200ml) of two kinds of heavy metals chromium (Cr), and lead (Pb)on biochemical parameters of three varieties (JS:335, JS:80-21, JS:75-46) of soybean. The result of the present study showed that the exposure of *Glycine max* to Pb and Cr in Normal concentrations of heavy metal would not be shown any visible toxic symptoms in the increase of heavy metal concentration, resulting in a reduction of total chlorophyll content total carbohydrate content and total protein content. Among the three varieties of (*JS:335, JS:80-21, JS:75-46*) of *Glycine max*, JS:80-21 showed a maximum reduction of chlorophyll content and protein content while in carbohydrate content, the maximum decrease was found in JS:75:46 at 200ml concentration. The results clearly indicated that Lead (Pb) treatment proved more toxic than chromium (Cr) treatment for all three different varieties (*JS: 335, JS: 80-21, JS: 75-46*) of *Glycine max.*

Keywords: Heavy metal, Chromium, Lead, Glycine max Biochemical parameters

Introduction

Heavy metals are the most important element of the environment that frequently accumulates in the soil due to unauthorized municipal waste disposal, mining, the use of numerous pesticides and chemical fertilizers (M. Paksoy *et al* 2009, Nagajyoti *et al.*, 2010). High concentrations of ROS are produced by heavy metal stress, which later causes the oxidation of key metabolic biomolecules (Choudhury et al., 2013). Heavy metal contamination of agricultural soils is a global issue that endangers the environment, human, and animals (Khanet al., 2021). A common effect of heavy metals in plants is a decrease in photosynthetic pigments and the appearance of chlorosis effects. This can be caused by decreased chlorophyll synthesis (caused by the inhibition of the respective enzymes), increased chlorophyll destruction, or altered element uptake (either by inhibition of the uptake or by competition with other heavy metals). Inhibition of the Calvin cycle is one of the additional causes of disrupted photosynthesis (Ali et al 2015). According to numerous studies, heavy metals are toxic to photosynthesis and the antioxidant system because they alter the amount of chlorophyll and increase reactive oxygen species (ROS) (Ahmad, 2020, Kovacevic, 2020, Mansoor, 2022). The seventh most abundant environmental pollutant in the earth's crust is chromium (Chandra et al 1997). Chromium (Cr) is a metal that is widely used in a variety of industries, including glass, textile, and film production, metallurgy, leather tanning, and electroplating (Fu et al., 2023). Chromium toxicity affects the amount of chlorophyll and photosynthesis in the *Pisum Sativam* plant (Bishnoiet al 1993). Cr toxicity affects physiological and biochemical processes like photosynthesis, transpiration, pigment biosynthesis, root growth, and nutrient uptake, which disturbs redox homeostasis and signaling, damages membrane lipids, DNA, proteins, and enzymes, and also affects enzymatic activities linked to starch synthesis and metabolism, which reduces crop yield and lowers the quality of food (Stambulskaet al., 2018; Christou et al., 2021). Lead contamination in soils also affects the physiological indices, which further reduced crop yield (Majer etal., 2002). Agricultural soils that have been contaminated with heavy metals may become undesirable and decrease crop productivity (Cai et al., 2012). Soybean a generally consumed food crop is commonly grown in stressful environments, such as soils with high arsenic levels (Bundschuhet al., 2012). Throughout the world, soybeans (*Glycine max*) are well known for their low-cost protein foods, edible oils, fertilizers, sprouts, milk, and other produce. More than 7000 years have passed since people first began to cultivate and consume soybeans. Due to its ease of cultivation, soy has become one of the most widely consumed foods in the world (Graham and Vance, 2003). Heavy metals can cause a variety of morphological, physiological, and biochemical disorders inside of plants, which in turn lower crop productivity.

The current experiment was undertaken to investigate a change in the level of biochemical aspects *Glycine max* treated with Cr and Pb were examined for total carbohydrate, total protein, and total chlorophyll content to better understand how soybeans adapt to their environment.

Materials and methods

Certified seeds of three varieties of soybean (*Glycine max*) (*JS:335, JS:80-21, JS:75-46*) were procured from Vindhya herbal testing laboratory and nursery Bhopal, M.P India. Seeds were surface sterilized with 0.1% $HgCl_2$ (mercuric chloride) for two minutes. The seeds were washed with distilled water twice and air dried. The soil was divided into 27 parts of 4kg each. 3 parts each were treated with four different concentrations i.e., 50ml, 100ml, 150ml and 200ml of two heavy metals Lead (II) nitrate and Potassium dichromate. Seeds were sown at 2-3cm depth in pot and watering was done once in two days till transfer of plantlets for experiments all experimental pots were placed outdoor for growing after reaching the maturity stage the plants were removed and were used for biochemical analysis.

Methods of extraction and estimation

Estimation of Total chlorophyll: the total chlorophyll was estimated by the method of Arnon (1949) and Lichtenthaler, (1983). One gram of fresh leaves and stems was ground in pre-chilled mortar and pestle with 40ml of 80% acetone and centrifuged at 10000rpm for 5mins. Absorbance of the supernatant was measured at 645nm and 663nm against the 80% acetone as blank using spectrophotometer. Concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated according to the following equations:

Total Chlorophyll: 20.2 (A645) + 8.02 (A663)
Chlorophyll a: 12.7(A663) – 2.69(A645)
Chlorophyll b: 22.9(A645) – 4.68(A663)

Estimation of Total carbohydrate content: total carbohydrate content was estimated in the leaf of *Glycine max* by the method of Mc Gready (1950) and Nelson (1941) with some modification this was done by using Somogy's Alkaline Copper Tartrate reagent and Arsenomolybdate reagent. 1gm of leaves and stem samples was ground in a mortar and pestle with 10ml of 80% ethanol by adding acid free sand and was then hydrolyzed with 2ml of conc Hclfor 30min. Neutralized it with sodium carbonate (Na_2CO_3) until the effervescence ceases. Supernatant was taken in a conical flask. Distilled water was added and filtered. Residue was discarded and filtrate was considered as reducing sugar sample. 20ml of reducing sugar sample was taken in 150 ml conical flask. it was then hydrolyzed with 2mlof conc. HCl for 30 minutes. Neutralized it with sodium carbonate Filtered extract was considered as total sugar sample. 0.5ml of aliquot sample was taken in test tube and 1 ml of Somogy's reagent was added. Test tubes were placed in boiling water bath for 30 minutes. The test tubes were then cooled at room temperature. 1ml of Arsenomolyb date reagent was added in each tube and mixed vigorously. The volume of each tube was made up to 10ml with distilled water. Absorbance was measured at 560 nm in Spectrophotometer and carbohydrate was calculated as:

Absorbance correspondence to 0.1ml of test = x mg of glucose

10ml contains = x / 0.1 x 10mg of glucose

= % of reducing sugar.

Estimation Total protein content: protein content of the plant was estimated by the method of Lowry et al (1951).

Sample preparation: 1gm of sample was crushed in mortar pestle and with approximately 20ml of freshly prepared phosphate buffer. Solutions were transferred in a centrifuge tube and centrifuged at 10000rpm for 10 min. Supernatants were collected for quantification of samples.

Quantification of Total Protein Content

Bovine Serum Albumin (BSA) was used to prepare the standard curve against unknown concentration of protein. 4.5 ml of reagent I was added to extracted sample and incubated for 15min. 0.5ml of freshly prepared reagent II was mixed and left for 30 min in a dark place for incubation. Absorbance was measured at 660nm. Standard curve was made first and amount of protein in unknown samples were expressed as mg/ g of fresh weight.

Results

The effect of different concentrations of Heavy metals (Lead and Chromium) on biochemical parameters of *Glycine max* as shown in table 1

Variety	Treatment	Doses	Total Carbohydrate Content (mg/g)	Total Protein Content (mg/g)	Total Chlorophyll Content (mg/g)
JS:335	**Control**	**ACtrl**	26.00	4.49	1.20
	Lead (Pb)	**AL1**	21.23	3.86	1.03
		AL2	18.96	3.12	0.91
		AL3	16.12	2.79	0.83
		AL4	14.32	2.23	0.74
	Chromium (Cr)	**AC1**	23.61	4.12	1.12
		AC2	21.36	3.89	1.02
		AC3	20.01	3.46	0.96
		AC4	18.96	3.21	0.91
JS:80-21	**Control**	**BCtrl**	24.17	4.16	1.11
	Lead (Pb)	**BL1**	19.62	3.46	0.97
		BL2	18.96	2.93	0.87
		BL3	14.92	2.57	0.78
		BL4	13.61	2.03	0.68
	Chromium (Cr)	**BC1**	22.01	3.84	1.01
		BC2	19.87	3.64	0.92
		BC3	18.52	3.21	0.87
		BC4	17.46	2.84	0.81
JS:75-46	**Control**	**CCtrl**	24.95	4.29	1.13
	Lead (Pb)	**CL1**	20.12	3.67	0.98
		CL2	18.05	2.99	0.86
		CL3	15.23	2.54	0.82
		CL4	13.62	2.11	0.70
	Chromium (Cr)	**CC1**	22.53	3.81	1.06
		CC2	20.46	3.64	0.97
		CC3	19.23	3.35	0.94
		CC4	18.24	2.98	0.88

Table 1: Effect of different concentrations of Heavy metals

Total chlorophyll content

The effect of four different doses (50ml,100ml,150ml,200ml) of heavy metals (lead and chromium) on the total chlorophyll content in fresh leaves of *Glycine max* in comparison to control were evaluated after harvesting . The maximum reduction in total chlorophyll content of *Glycine max* was observed at

200ml treatment in JS: 80-21 variety and minimum reduction was found at 200ml concentration in JS: 75-46.After chromium treatment the maximum reduction in total chlorophyll content was observed at 200ml concentration in JS: 80-21 variety and minimum reduction was found at 200ml concentration in JS: 75-46 as shown in figure 1

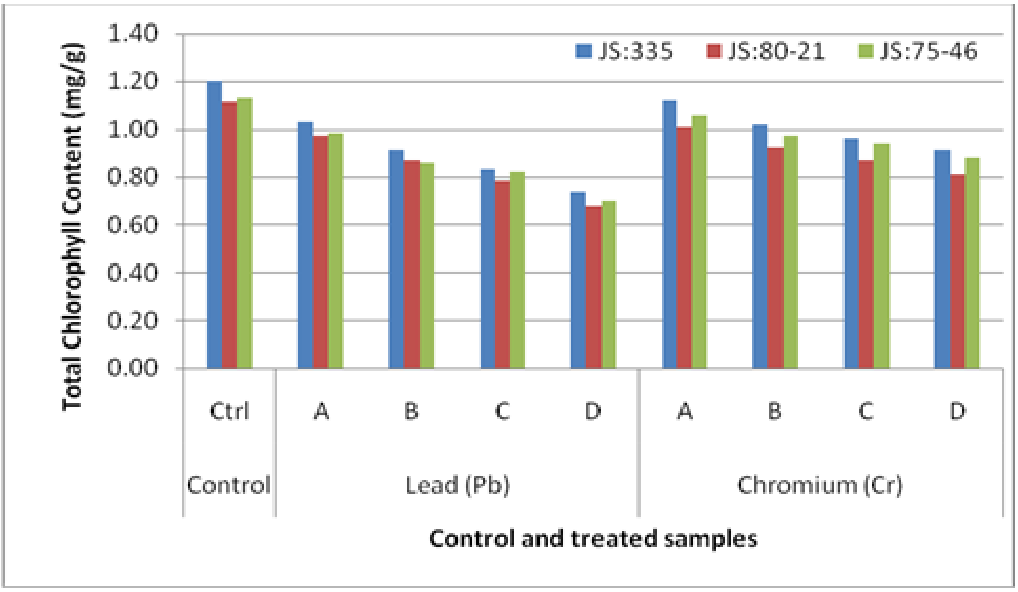

Figure 1: The figure demonstrates the Comparative effect of lead and chromium on chlorophyll content of different varieties of soybean plant

Total carbohydrate content

The effect of four different doses (50ml,100ml,150ml,200ml) of heavy metals (lead and chromium) on the total carbohydrate content in fresh leaves of Glycine max in comparison to control were evaluated after harvesting . The maximum reduction in total carbohydrate content of Glycine max was observed at higher dose (200ml) in JS:75-46 variety and minimum reduction was found at 200ml concentration inJS:80-21.After chromium treatment the maximum reduction in total carbohydrate content was observed at 200ml concentration in JS:80-21 variety and minimum reduction was found at 200ml concentration inJS:75-46 as shown in figure 2

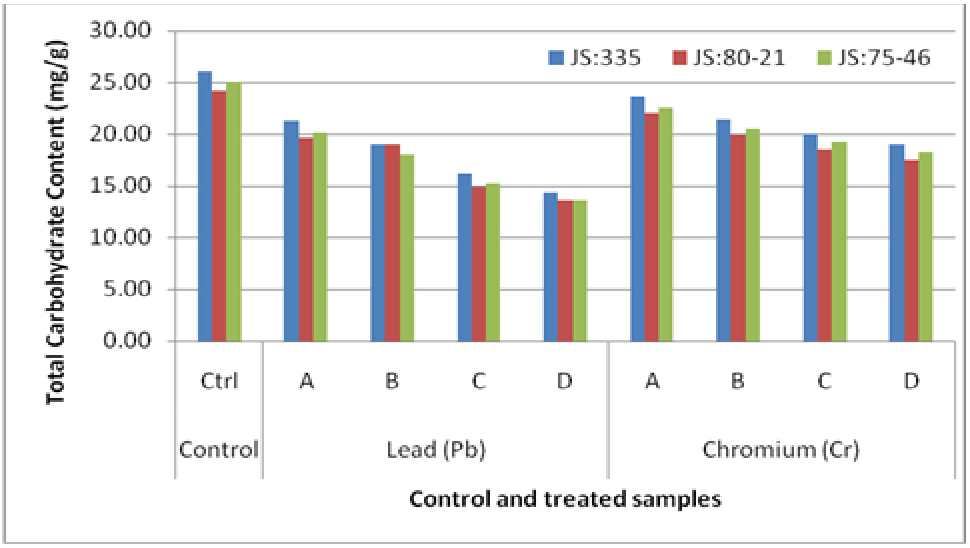

Figure 2: The figure demonstrates the Comparative effect of lead and chromium on total carbohydrate content of three different varieties of soybean plant

Total protein content

The effect of four different doses (50ml,100ml,150ml,200ml)of heavy metals (lead and chromium) on the total protein content in fresh leaves of Glycine *max* in comparison to control were evaluated after harvesting . The maximum reduction in total protein content of *Glycine max* was observed at higher dose (200ml) in JS: 80-21 variety and minimum reduction was found at 200ml concentration inJS: 335.In chromium treatment the maximum reduction in total protein content was observed at 200ml concentration in JS: 80-21 variety and minimum reduction was found at 200ml concentration in JS:335 variety as shown in figure 3

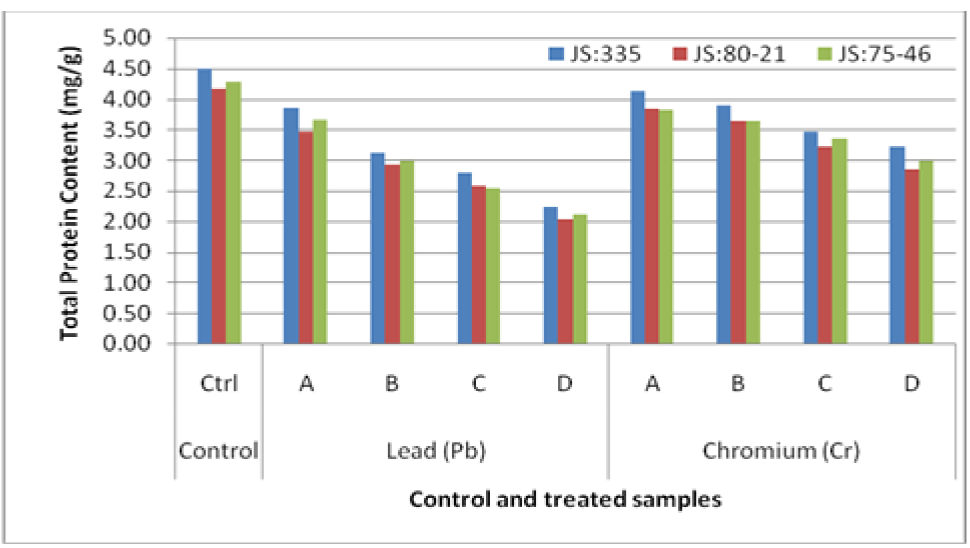

Figure 3: The figure depicts the Comparative effect of lead and chromium on total protein content of three different varieties of Glycine max (soybean)

Discussion

In the present study biochemical parameters of the three varieties (*JS:335, JS:80-21, JS:75-46*) of *Glycine max* treated four different concentrations (50ml, 100ml, 150ml and 200ml) of lead (Pb) and chromium(Cr). After the heavy metal treatment biochemical characteristics in terms of total carbohydrate content, total protein content and total chlorophyll content were observed.

The data incorporated in Table 1 displays that the results obtained from the present study showed that after Lead (Pb) and chromium (Cr) treatments (50, 100 and 150 and 200 ml), Glycine max plant exhibits the reduction in total carbohydrate content, total protein content and total chlorophyll content at all concentration over control.

For photosynthesis, Chlorophyll is extremely important and is also highly sensitive to environmental stress like heavy metals (Ekmekciet al., 2008). Plants exposed to Pb ions showed a noticeably decrease in dry weight as well as a decline in total chlorophyll and, consequently, photosynthetic efficiency. (Kambhampatiet al., 2005). In our results, A gradual decrease in total chlorophyll content under Lead stress showed maximum reduction at 200ml concentration in *JS:80-21*variety(38.7%) followed by *JS:335*(38.3%)and minimum reduction was found at 200ml concentration in *JS:75-46*(38%).After chromium treatment the maximum reduction in total chlorophyll content was observed at 200ml concentration in *JS:80-21* variety (27%) followed by *JS:335*(24%) and minimum reduction was found at 200ml concentration in *JS:75-46*(22%).our results are parallel with the study of (Dey et al.,2009), which showed that as the concentration of Cr increased, the total chlorophyll content in *T. aestivum* shoots decreased.

The majority of a plant's energy comes from total carbohydrates, which are also directly related to the growth and development under stress conditions. Crop yield and productivity are increased by the synthesis, translocation, storage, and utilization of carbohydrates (Slewinskiet al., 2012). The carbohydrate and sugar metabolism is changed when maize plants are exposed to Cr contamination (Mahajan et al., 2013). Previous research has also documented the decrease in carbohydrate content in crop plants, including cereals like maize, exposed to different heavy metals (Mahajan et al., 2013). In our results, at Lead (Pb) treatment maximum decrease of 45.4% in total carbohydrate content was observed at 200ml concentration in *JS:75-46* variety followed by *JS:335*(44.9%) and minimum reduction was observed in *JS:80-21* variety (43.6%). Similarly in case of chromium(Cr) treatment maximum reduction of 27.7% in total carbohydrate content was found at 200ml concentration in *JS:80-21*, followed by 27% in *JS:335* and minimum reduction was found in *JS:75-46 (*26.8%).

Proteins are Essential cellular components that are easily damage under stressful environmental conditions (Wuet al., 2010). The induced synthesis of stress proteins, such as those involved in the Krebs cycle, glutathione and phytochelatin biosynthesis, as well as some heat shock proteins, may be the cause of the increase in total soluble protein content under heavy metal stress (Mishra et al., 2006). Protein content in *Brasiccajuncea* has decreased, according to reports of Mobin and khan, 2007). In our results, maximum reduction of protein was found at 200ml concentration in *JS: 80-21*(51.2%), followed by *JS: 75-46*(50.8%) and minimum reduction was found in *JS: 335* (50.3%) Under Lead treatment. After chromium treatment the maximum reduction in total protein content was observed at 200ml concentration in *JS: 80-21* variety (31.7%) followed by *JS: 75-46* (30.5 %) and minimum reduction was found at 200ml concentration in *JS: 335* (28.5%). Results indicate that heavy metal affect the biochemical parameters of soybean leaves with increasing concentrations of heavy metals. The investigation revealed that significant reduction in total carbohydrate content, protein and chlorophyll content of *Glycine max* in Pb treatment than Cr treated plants. Lead (Pb) proved more toxic than Chromium (Cr).

Conclusion

The current study revealed that the application of Lead (Pb) and Chromium (Cr) to soybean (*Glycine max*) plants had several effects that cannot be neglected. Our results indicated that the exposure of Glycine max to Lead (Pb) and Chromium(Cr) resulted in a decrease in total carbohydrate content, protein content and total chlorophyll content at higher concentrations of both heavy metals. Among the three varieties of (*JS:335*, *JS:80-21*, *JS:75-46*) of Glycine max, JS*:80-21* shown maximum reduction of chlorophyll content and protein content while in carbohydrate content, maximum decrease was found in *JS:75:46* at 200ml concentration. Pb treatment proved more toxic than Cr treatment. Since the problem of Lead (Pb) and Chromium (Cr) accumulation in agricultural soils is increasing rapidly, It is urgent to find the strategies and mechanisms to lower its toxicity in crops. Further molecular mechanism is needed in order to investigate the process of proper toxic signal pathways of heavy metals exhibited by treated plants.

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