Journal of Stress Physiology & Biochemistry, Vol. 10 No. 3 2014, pp. 259-272 ISSN 1997-0838

ORIGINAL ARTICLE

Biochemical Response of *Glycine max* (L.) Merr. to Cobalt and Lead stress

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Received April 22, 2014

Background: Heavy metal pollution of soil has become a global concern, largely due to the fact that the heavy metals accumulated in plants may either directly or indirectly find their way into animals and human beings. Present study was carried out on the phytotoxicity of cobalt (Co) and lead (Pb) on biochemical constituent's *viz*. chlorophyll a, b, carotenoids, proline, protein and carbohydrate content of three different varieties of *Glycine max viz*. SL-688, PS-1347, DS-9712 treated with 50, 100 and 150 µM concentrations.

Results: The exposure of soybean varieties to Pb and Co resulted in the reduction of chlorophyll, carotenoids, carbohydrate and protein content and addition in proline content. Test plants were more sensitive to lead in comparison to cobalt. PS-1347 variety was found to be more susceptible to both the heavy metals. Maximum deleterious effect was observed at higher concentrations (100 & 150µM). However, an additional supply of nitrogen not only minimized the inhibitory effect of these two heavy metals but also decreased the proline content of plants.

Conclusions: The findings of the present study indicate that effect of heavy metals with different treatments on biochemical content was significantly different at 0.05 level of probability. Soils contaminated by heavy metals probably lead to substantial losses in seed yield of soybean plant.

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Pollution by heavy metals is one of the key environmental problems in the world today. Heavy metals make a significant contribution to environmental pollution as a result of human activities such as mining, smelting, power transmission and intensive agriculture (Nedelkoska and Doran, 2000). Heavy metals can affect plant's growth and production by inhibiting physiological (Garg and Singla, 2011) and morphological and biochemical (Ozdener and Kutbay, 2011) processes. They are shown to cause disturbance in plant ion concentration (Barcelo *et al*., 1986; Wallace *et al*., 1992) and water balance to interfere with protein metabolism by influencing nitrate and sulphate reduction (Nussbaum *et al*., 1988). A major environmental concern due to dispersal of urban and industrial waste generated by human activities is the contamination of soil with heavy metals. Polluted soil poses a severe problem to both health and land (Akhionbare *et al*., 2010). The discharge of heavy metals as a byproduct of various human activities has been accompanied by large scale soil pollution (Shivhare and Sharma, 2012).

Cobalt is usually described as "beneficial" to the plant, however, it can be a contaminant in soils due to agricultural additives or metal refineries (Nies, 1999; Simon 2001). Cobalt is known to cause irreversible damage to a number of vital metabolic constituents and plant cell and cell membrane. Toxic concentration of cobalt inhibits active ion transport and greening and reduces shoot weight in plants (Jayakumar and Vijayrengan, 2006).

Lead (Pb), a potentially toxic heavy metal has attracted considerable attention of scientists due to its widespread distribution and potential risk to the environment. Lead contamination in soils not only aroused the changes of soil microorganism and its activities and resulted in soil fertility deterioration but also directly affected the change of physiological indices and, furthermore, resulted in yield decline (Majer *et al*., 2002).

Soybean (*Glycine max*) is a species of legume which is native to East Asia, widely grown for its edible bean. The plant is classed as an oilseed rather than a pulse by the UN Food and Agricultural Organization (FAO). The main producers of soy are the United States (35%), Brazil (27%), Argentina (19%), China (6%) and India (4%). Soybean production constitutes around 55% of the total world production of oilseeds and figures around 170-185 million tons. It is favoured by wide variety of climates and soils and thus considered to be the most economical crop. It contains about 20 % oil and 40 % high quality protein (as against 7.0 % in rice, 12 % in wheat, 10 % in maize and 20-25 % in other pulses). Soybean protein is rich in valuable amino acids (5%) in which most of the cereals are deficient. In addition, it contains a good amount of minerals, unsaturated fats, salts and vitamins (thiamine and riboflavin) and its sprouting grains contain a considerable amount of Vitamin C. Besides, Vitamin A is present in the form of precursor carotene, which is converted into vitamin A in the intestine.

Using chemical products as nutrients, fertilizers and pesticides, it is believed that safety gets compromised. Therefore, it is necessary to study the effects of heavy metals from these compounds. In this work we tried to present the effects of the two heavy metals (Pb and Co) on biochemical processes of three different varieties (SL-688, PS-1347 and DS-9712) of soybean plant.

MATERIALS AND METHODS

Chemicals

In the present investigation the chemicals used were obtained from Qualigen and Merck companies. Distilled water was used for preparation of all the solutions.

Plant materials

The seeds of different varieties of experimental plant, *Glycine max* (SL-688, PS-1347 and DS-9712) were procured from Indian Agricultural Research Institute, Pusa, New Delhi, India

Pot and sand culture experiment

The seeds were surface sterilized with dilute solution of sodium hypochlorite to prevent any fungal contamination and then rinsed three times with distilled water. The sand was thoroughly washed with water and then treated with 2% sodium hypochlorite and dried. The seeds were sown is earthen pots containing equal quantities (2kg) of washed and acid treated loamy sand soil. The soil was treated with Evans and Nason nutrient solution. Co and Pb (control, 50, 100 and 150µM) were provided along with nutrient solution twice a week. Nitrogen was also provided along with nutrient solution. Three identical sets were maintained during the whole experiment and experiments were conducted in green house to provide controlled conditions.

Treatments and Sampling

Metal treatments of Co and Pb was prepared using cobalt chloride and lead chloride solutions with concentrations of 50, 100 and 150 µM/L. Nitrogen was prepared using calcium nitrate at 5mM/L concentration. The samples were taken from three week old seedlings for biochemical analysis.

Biochemical analysis

Determination of chlorophyll and carotenoid content

Chlorophyll and carotenoids were extracted from leaves and estimated by the method of Arnon (1949). In this method 1g sample of green tissues was weighed and grinded in pestle and mortar with mixture of acetone and ethyl alcohol (3:1) by repeated homogenization. Supernatant was filtered and was made up to 100 ml. The amount of chlorophyll ‘a', ‘b' and carotenoid was determined by measuring the optical density on (UV-VIS spectrophotometer) at 663, 645 and 470nm.The chlorophyll and carotenoids were calculated by using standard formula.

Total carotenoids (mg g^{-1} fw) =A/(196xLxW), where:

A = OD of acetone extract at 440 nm

L = diameter of calorimeter tube (quartz)

W = gm sample per total volume

Proline determination

Proline content was measured according to the method of Bates *et al.* (1973). Fresh leaves were ground in 1.5ml of 3 % (w/v) and proline was estimated by ninhydrin reagent. The ninhydrin reaction mixture was partitioned against toluene and absorbance of the toluene phase was read at 520nm .Proline concentration was determined by plotting a standard curve and as expressed in mg/g proline.

Protein estimation

Protein was quantified according to Lowry (1951) method. Samples were homogenized in 0.1M phosphate buffer (pH=6.8). After adding reagent absorbance was recorded at 750nm and protein was calculated using the standard curve prepared with different concentration of Bovine Serum Albumin (BSA, Sigma: 20 – 200 µg).

Estimation of carbohydrates

Dried powder (50 mg) was extracted with 80% ethanol at room temperature for 1 hour and centrifuged at 1000 rpm at 25^{0}C for 60 minutes. The pellet was re-extracted with 80% ethanol for 10 minutes and centrifuged at 10,000 rpm at 25^{0}C. The two supernatants were cooled and the volume was reduced to 25 ml, which was used for the analysis of total soluble sugars, reducing sugar and non-reducing sugars.

The pellet was used to estimate starch. It was washed with 80% ethanol and then with water. To this 2 ml of water and 2 ml of 52% Perchloric acid (v/v) were added and the solution was left to hydrolyze at room temperature for 20 minutes. The mixture was then centrifuged at 10,000 rpm at 25ºC for 30 min and the supernatant was made up to 40 ml with distilled water. This was used for estimation of starch.

Total soluble sugars

Total soluble sugars were estimated by following Dubois *et al.* (1956). To 2 ml of extract, 0.5 ml phenol reagent was added. The colour was developed by rapidly adding 5 ml conc. H_2SO_4 and the solution was allowed to stand at room temperature for 30 minutes. The absorbance was recorded at 485 nm. The sugar concentration was calculated using a standard curve prepared by different concentrations (20-200 mM/L) of D-glucose.

Reducing sugars

For the estimation of reducing sugars Sumner (1935) method was followed. To 0.5 ml of ethanolic extract, 0.5 ml of water and 1 ml of Dinitrosalicylic acid (DNSA) reagent were added. The reaction mixture was boiled for 10 min and allowed to cool at room temperature. The absorbance was recorded at 560 nm. The concentration of reducing was calculated using standard curve prepared by taking different concentrations (20-200 mM/L) of D-glucose.

Starch

Starch was extracted from leaves by following Mc Cready *et al.* (1950). To 0.1 ml of the extract 0.9 ml of distilled water was added followed by the addition of chilled anthrone reagent (5 ml). The mixture was heated in a boiling water bath for 10 min and allowed to cool at room temperature. The absorbance was recorded at 630 nm against anthrone as blank. The concentration of starch was calculated using a standard curve prepared by taking different concentration (20-200 mM/L) of D-glucose.

Statistical analysis

The data was analyzed by one-way analysis of variance (ANOVA) to determine the statistical significance of the differences between means of treatments.

RESULTS

Pigment composition (chlorophyll and carotenoids)

The deleterious effect of Co and Pb on pigment composition in all the three varieties of soybean is presented in Table 1-3. Both cobalt and lead reduced the chlorophyll ‘a', ‘b' and carotenoids content in all the three varieties. Plants were more susceptible to lead than cobalt. At 150µM concentration of both heavy metals chlorophyll a showed reduction of 0.640 and 0.320 (SL-688), 0.653 and 0.213 (PS-1347) and 0.511 and 0.226 (DS-9712) mg g^{-1} f.w, respectively. Maximum reduction in chlorophyll 'b' (0.692 and 0.350 f.w.) was found in DS-9712 variety. The carotenoids of PS-1347 plants were found to be most susceptible to both the heavy metals with maximum reduction up to 0.981 and 0.587 f.w, when compared to control (1.262 f.w) Application of nitrogen ($Ca_2 (No_3)_2$) at 05 mM in the nutrient medium somehow minimized the effect of heavy metals. Increase in chlorophyll 'b' was more than the chlorophyll 'a' (Table 1-3 and Fig. 1-3). Carotenoids appear to be more tolerant to heavy metals as compared to other pigments thus showed an increase in the presence of the nitrogen (Table1-3 and Fig. 4).

Proline content

Plants treated with the different concentrations (50, 100 and 150 µM) of Pb and Co showed high accumulation of proline and again the effect was more due to lead then cobalt. At 150 µM concentration of Co and Pb, enhancement was found to be 1.395 and 1.790, 0.441 and 1.080 and 1.052 and 1.575 mg g^{-1} in variety SL-688, PS-1347 and DS-9712, respectively. However with the application of nitrogen the proline content was reduced to some extent. Plants showed1.328 and 1.756 (SL-688), 0.441 and 1.080 (PS-1347) and 1.040 and 1.555 mg g^{-1} f.w. (DS-9712) decline in proline content when treated with the media supplemented with additional source of nitrogen (Table 4).

Protein content

The results pertaining to the effect of Co and Pb on protein content in all the three varieties is presented in Table 5. Plants showed reduction in protein content when exposed to Pb and Co at different concentrations. This detrimental effect was more by Pb as compared to Co at all the three concentrations. The protein content of DS-9712 plants was found to be more sensitive to both the heavy metals with maximum reduction up to 58 and 90% when compared to control (1.065 mg/g) at 150 µM concentration of Co and Pb, respectively. Addition of nitrogen in nutrient medium increased protein content and proved to be beneficial for plants (Table 5 and Fig. 1-4)

Carbohydrate content

Carbohydrate (soluble sugars, reducing sugars and starch) content was significantly decreased (at 5% level of probability) in all the test varieties treated with different concentrations (50, 100 and 150µm) of both heavy metals (Table 6). Decrease in carbohydrate content was more at higher concentrations (100 & 150 µM). Comparably Pb had more inhibitory effect on carbohydrate content than Co at all the three concentrations. Maximum reduction was observed in PS-1347 variety and it was found to be 0.59 and 0.40 (soluble sugars), 0.64 and 0.44 (reducing sugars) and 0.09 and 0.03 mg g-1 (starch) at 150 µM concentration of Co and Pb, respectively. Application of nitrogen was found to be effective in minimizing the deleterious effect of both the heavy metals (Table 6 and Fig. 1-4).

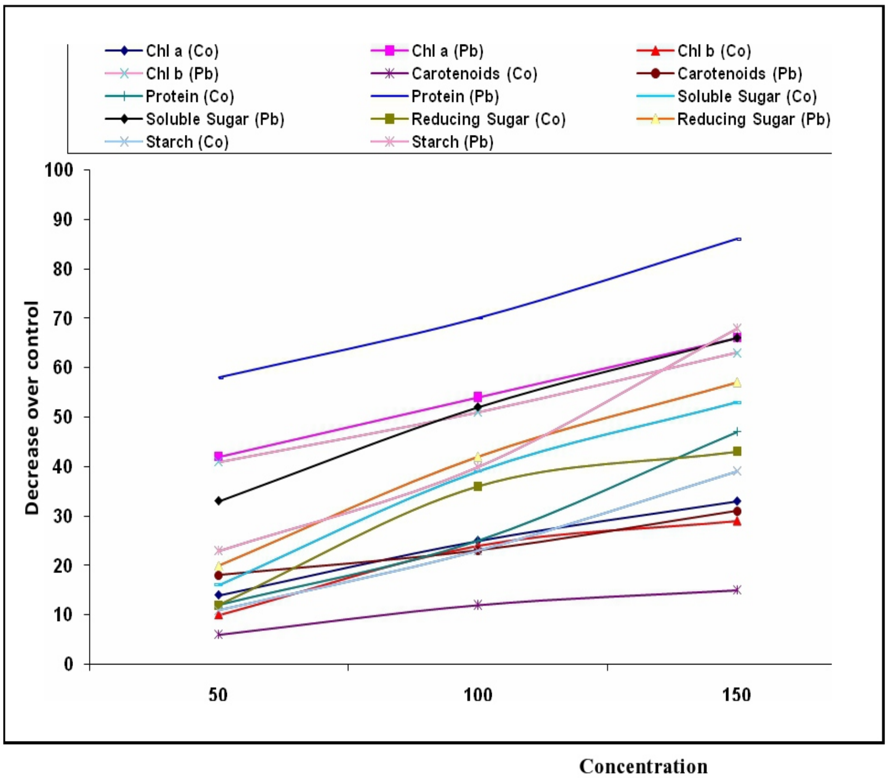

Figure 1. Co and Pb induced heavy metal stress on some biochemical parameters in SL-688 variety of *Glycine max*

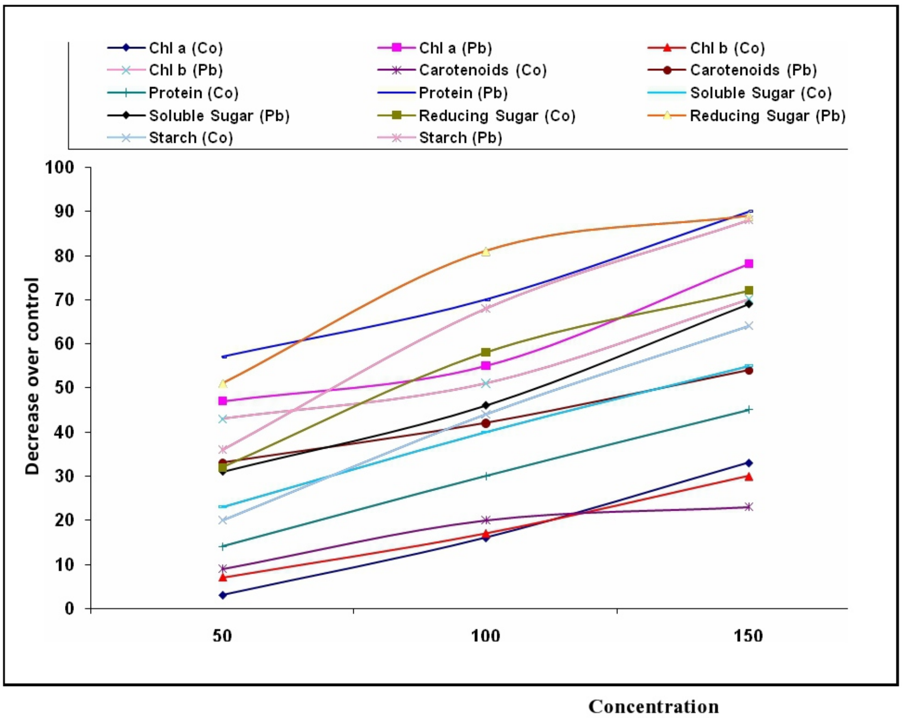

Figure 2. Effect of heavy metals on some biochemical parameters in PS-1347 variety of *Glycine max*

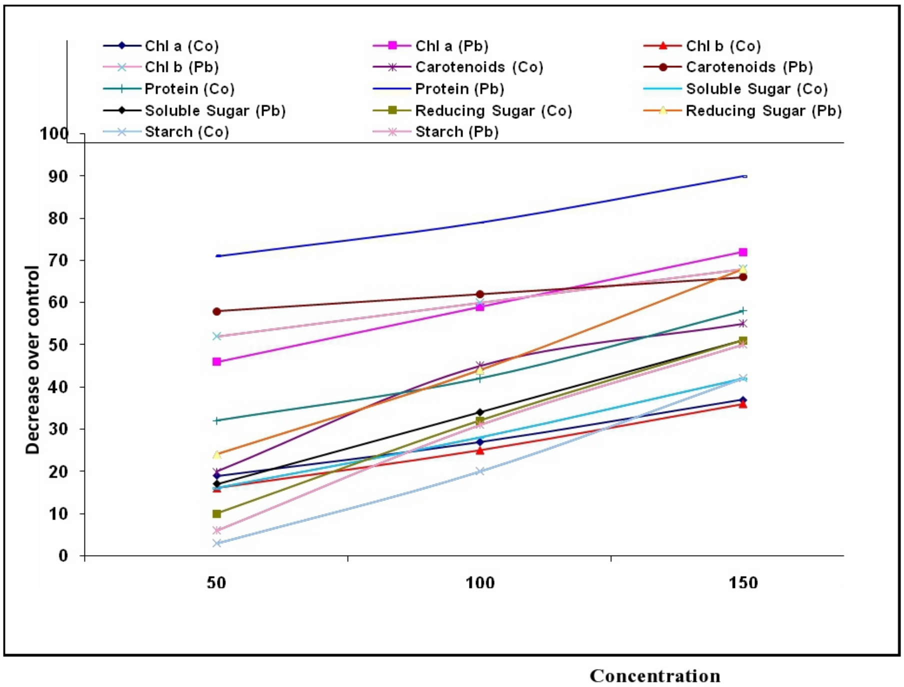

Figure 3. Effect of cobalt and lead on some biochemical parameters in DS-9712 variety.

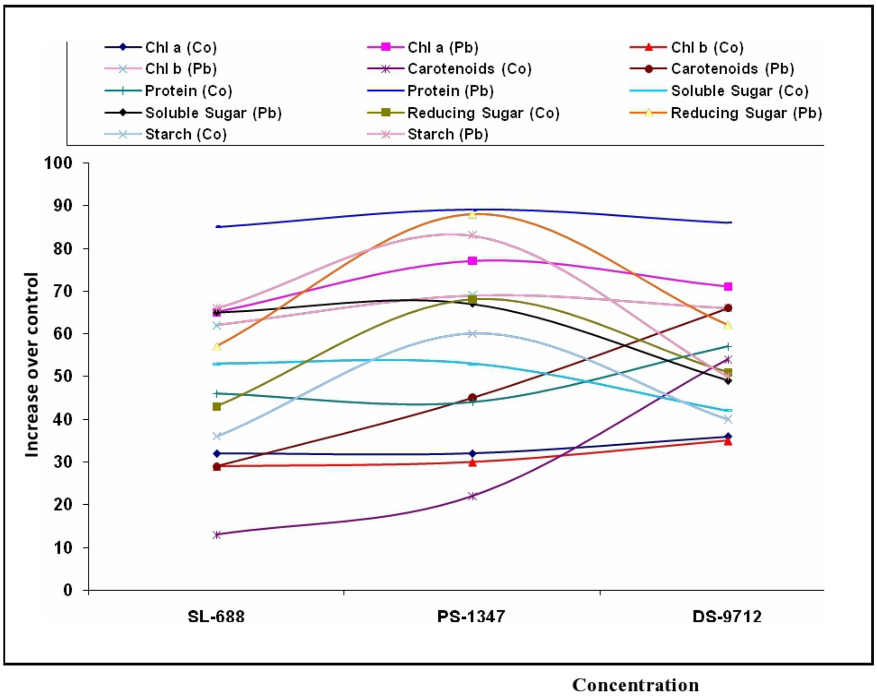

Figure 4. Percentage increase over control in biochemical parameters in different varieties of *Glycine max* on application of nitrogen.

Table 1. Effect of cobalt and lead on pigment composition (mg g^{-1} f.w) in SL-688 variety of soybean

Metals	Concentration (µM)	Chlorophyll a		Chlorophyll b		Total Chlorophyll		Carotenoids	
		0 mM N	5 mM N	0 mM N	5 mM N	0 mM N	5 mM N	0 mM N	5 mM N
Control	0	0.955	0.965	1.069	1.070	2.035	2.035	2.205	2.190
	50	0.818^{ns}	0.830	0.956^{ns}	0.970	1.774^{ns}	1.800	2.079*	2.097
Co	100	0.718^{ns}	0.729	0.805^{ns}	0.822	1.523^{ns}	1.551	1.946*	1.960
	150	0.640^{ns}	0.658	0.745^{ns}	0.759	1.385^{ns}	1.417	1.870*	1.899
	50	0.551^{ns}	0.567	0.633^{ns}	0.647	1.184^{ns}	1.214	1.795*	1.812
Pb	100	0.440^{ns}	0.556	0.520^{ns}	0.635	0.960^{ns}	1.191	1.680*	1.696
	150	0.320^{ns}	0.336	0.390^{ns}	0.408	0.710^{ns}	0.744	1.520*	1.541

Data are average of 3 replicates, N – Nitrogen, ns = Non significant *= Significant at 5% level of probability.

Table 2. Effect of heavy metals on pigment composition (mg g^{-1} f.w) in PS-1347 variety of soybean

Metals	Concentration (µM)	Chlorophyll a		Chlorophyll b		Total Chlorophyll		Carotenoids	Carotenoids
		0 mM N	5 mM N	0 mM N	5 mM N	0 mM N	5 5mM N	0 mM N	5 mM N
Control	0	0.970	0.976	1.180	1.190	2.150	2.166	1.262	1.275
	50	0.944^{ns}	0.960	1.097^{ns}	1.110	2.041^{ns}	2.070	1.150*	1.165
Co	100	0.815^{ns}	0.828	0.980^{ns}	0.995	1.795^{ns}	1.823	1.010*	1.045
	150	0.653^{ns}	0.660	0.822^{ns}	0.830	1.475^{ns}	1.490	0.981*	0.997
	50	0.526^{ns}	0.535	0.676^{ns}	0.690	1.202^{ns}	1.225	0.851*	0.864
Pb	100	0.435^{ns}	0.442	0.580^{ns}	0.590	1.015^{ns}	1.032	0.731*	0.742
	150	0.213^{ns}	0.220	0.357^{ns}	0.370	0.570^{ns}	0.590	0.587*	0.695

Data are average of 3 replicates, N – Nitrogen, ns = Non significant *= Significant at 5% level of probability.

Table 3. Effect of Cobalt and lead induced heavy metal stress on pigment composition (mg g^{-1} f.w) in DS-9712 variety of soybean.

		Chlorophyll a		Chlorophyll b		Total Chlorophyll		Carotenoids	
		0 mM N	5 mM N	0 mM N	5 mM N	0 mM N	5 mM N	0 mM N	5 mM N
Control	0	0.815	0.828	1.080	1.095	1.895	1.923	2.098	2.110
	50	0.661^{ns}	0.684	0.905^{ns}	0.933	1.556^{ns}	1.581	1.675*	1.692
Co	100	0.595^{ns}	0.623	0.810^{ns}	0.846	1.405^{ns}	1.469	1.146*	1.660
	150	0.511^{ns}	0.530	0.692^{ns}	0.715	1.203^{ns}	1.245	0.953*	0.970
	50	0.439^{ns}	0.460	0.514^{ns}	0.538	0.953^{ns}	0.998	0.890*	0.915
Pb	100	0.331^{ns}	0.355	0.433^{ns}	0.455	0.764^{ns}	0.810	0.801*	0.817
	150	0.226^{ns}	0.240	0.350^{ns}	0.373	0.576^{ns}	0.613	0.709*	0.725

Data are average of 3 replicates, N – Nitrogen, ns = Non significant *= Significant at 5% level of probability.

Table 4: Effect of cobalt and lead on proline content in different varieties of *Glycine max*.

Metals	Concentration (µM)	Proline content (mg g^{-1} f.w.)					
		SL-688		PS-1347		DS-9712	
		0 Mmn	5 mMN	0 mMN	5 mMN	0 mMN	5 mMN
Control	0	0.226	0.201	0.097	0.080	0.273	0.260
Co	50 µM	0.780*	0.750	0.235*	0.205	0.446*	0.439
	100 µM	1.295*	1.270	0.327*	0.316	0.669*	0.656
	150 µM	1.359*	1.328	0.459*	0.441	1.052*	1.010
Pb	50 µM	1.593**	1.565	0.565**	0.551	1.262**	1.250
	100 µM	1.650**	1.627	0.783**	0.769	1.386**	1.365
	150 µM	1.780**	1.756	1.101**	1.080	1.575**	1.555

Data are average of 3 replicates, N – Nitrogen, ns = Non significant *= Significant, **= Highly Significant at 5% level of probability.

Table 5: Effect of cobalt and lead on protein content in different varieties of soybean.

Metals	Concentration (µM)	Protein content (mg/g)					
		SL-688		PS-1347		DS-9712	DS-9712
		0 mMN	5 Mmn	0 mMN	5 mMN	0 mMN	5 Mmn
Control	0	0.971	0.985	0.933	0.940	1.065	1.092
	50	0.850^{ns}	0.865	0.801^{ns}	0.810	0.725^{ns}	0.748
Co	100	0.725^{ns}	0.737	0.657^{ns}	0.670	0.620^{ns}	0.650
	150	0.517^{ns}	0.530	0.516^{ns}	0.528	0.443^{ns}	0.465
	50	0.405^{ns}	0.424	0.401^{ns}	0.418	0.310^{ns}	0.335
Pb	100	0.287^{ns}	0.305	0.280^{ns}	0.301	0.225^{ns}	0.252
	150	0.133^{ns}	0.151	0.090^{ns}	0.099	0.105^{ns}	0.125

Data are average of 3 replicates, N – Nitrogen, ns = Non significant at 5% level of probability.

Table 6: Effect of heavy metals (Co and Pb) on carbohydrate composition in different varieties of soybean.

	Concentration (µM)	SL-688						PS-1347						DS-9712					
		Soluble Sugars		Reducing Sugars		Starch		Soluble Sugars		Reducing Sugars		Starch		Soluble Sugars		Reducing Sugars		Starch	
		0 mM N	5 mM N	0 mM N	5 mM N	0 mM N	5 mM N	0 mM N	5 mM N	0 mM N	5 mM N	0 mM N	5 mM N	0mM N	5 mM N	0 mM N	5 mM N	0 mM N	5 mM N
Control	0	1.31	1.36	1.06	1.12	0.79	0.83	1.31	1.35	0.57	0.60	0.25	0.38	1.35	1.40	0.41	0.45	1.24	1.37
	50	1.10^{ns}	1.18	0.92^{**}	0.97	0.70^{**}	0.74	1.01^{ns}	1.06	0.39^{**}	0.41	0.20^{**}	0.24	1.13^{ns}	1.19	0.37^{**}	0.41	1.20^{**}	1.23
Co	100	0.80^{ns}	0.85	0.73^{**}	0.77	0.61^{**}	0.66	0.78^{ns}	0.85	0.24^{**}	0.28	0.14^{**}	0.17	0.97^{ns}	1.04	0.28^{**}	0.33	0.99^{**}	1.04
	150	0.61^{ns}	0.64	0.59^{**}	0.63	0.48^{**}	0.52	0.59^{ns}	0.64	0.16^{**}	0.19	0.09^{**}	0.12	0.78^{ns}	0.81	0.20^{**}	0.22	0.72^{**}	0.76
	50	0.87^{ns}	0.93	0.82^{*}	0.88	0.64^{**}	0.69	0.91^{ns}	0.95	0.28^{*}	0.34	0.11^{**}	0.20	1.09^{ns}	1.17	0.31^{*}	0.35	1.16^{**}	1.19
Pb	100	0.63^{ns}	0.67	0.58^{*}	0.64	0.47^{**}	0.51	0.71^{ns}	0.77	0.11^{*}	0.13	0.08^{**}	0.11	0.89^{ns}	0.95	0.23^{*}	0.28	0.82^{**}	0.82
	150	0.44^{ns}	0.48	0.43^{*}	0.47	0.25^{**}	0.28	0.40^{ns}	0.44	0.06^{*}	0.07	0.03^{**}	0.05	0.65^{ns}	0.71	0.13^{*}	0.17	0.61^{**}	0.63

Data are average of 3 replicates, N – Nitrogen, ns = Non significant, *= Significant, **= Highly Significant at 5% level of probability

DISCUSSION

The present investigation showed that both heavy metals (Co and Pb) caused marked reduction in pigment composition, protein content and carbohydrate composition in all the test varieties of soybean. Decrease in pigment composition under heavy metal stress has been reported by several workers on various crops (El-Sheekh *et al.,* 2003; Jayakumar *et al.,* 2007; Jayakumar et *al.,* 2009; Jaleel *et al.,* 2009). Dubey and Pandey (2011) also observed decrease in pigment composition, plant growth and yield of chick pea when exposed to nickel and cobalt stress. Similar results were found by Shakir *et al.* (2010) in *Triticum aestivum.* Heavy metal severely inhibits plant growth and even causes plant death by disturbing the uptake of nutrients and inhibiting photosynthesis through degradation of chlorophyll (Zhang *et al.*, 2007). Nagajyoti *et al.* (2008) reported the reduction in chlorophyll 'a', chlorophyll 'b' and total chlorophyll in groundnut by industrial effluents containing heavy metals.

Results indicated that increasing levels of cobalt and lead treatment markedly increased the proline content of all the test varieties of soybean. The findings of our study are in agreement with other workers (John *et al.,* 2009; Singh *et al.,* 2012). Under stress proline is a source of nitrogen and energy in metabolism for the plants. Associated with the stress, the accumulation of proline or some other organic solute may be serving as a compatible solute for maintaining the osmotic balance between the cytoplasm and vacuoles (John *et al.*, 2009; Dar, 2009; Tantary, 2009; Shakir *et al.,* 2010; Asgharipou *et al.*, 2011) In response to the heavy metal stress from different sources, proline has also been observed to accumulate in (Fatima *et al.*, 2007).

In the present study, it was observed that the protein content get reduced in the test plants when exposed to both lead and cobalt. Our results are in agreement with the findings of other workers (Balestrasse *et al*., 2003; Bavi *et al*., 2011) in different crop plants. The reduction in the amount of protein could be due to decrease in protein synthesis or an increase in protein degradation (Balestrasse *et al*., 2003). Heavy metals are known to promote protein denaturation (Gadd and Griffth, 1978) and increase the activities of proteases, RNAase and DNAase enzymes (Lee *et al*., 1976).

In our study metal toxicity reduced the carbohydrate content like soluble sugars, reducing sugar, non reducing sugar and starch of three tested varieties of soybean. These results are in concern with the findings of several other workers. Salch and Al-Garni (2006) reported that the total carbohydrates may get inhibited if cadmium concentration in soil is more than 5mg/kg. Considerable reduction in the carbohydrate content of *Lemna polyrrhiza* L. was observed when treated with different concentrations of cadmium and lead (John *et al*., 2009). Similar results were also reported by (Gaweda, 2007) in vegetables and Hamid *et al*. (2010) in *Phaseolus vulgaris* when treated with different concentrations of lead. Carbohydrates are the polyhydroxy aldehydes or polyhydroxy ketones which are widely distributed in plants as changes in the carbohydrate fractions during growth and development are consistent with the changes in photosynthetic pigments. Pb toxicity caused a marked reduction in carbohydrate content. Hemaltha *et al*. (1997) observed that Pb^{2+} exposed old seedlings of rice (*Oryza sativa*) showed a marked decrease in carbohydrate content.

CONCLUSION

Findings of the present study revealed that imposition of both metals viz. cobalt and lead induced considerable changes in all the biochemical parameters studied in all the three varieties of *Glycine max*. Lead proved more toxic than cobalt which is more consistent in its effect on various plant parameters studied. The effect of cobalt and lead was comparably more at higher concentrations (100 and 150 mM). Carotenoids were less affected as compared to total chlorophyll. Carbohydrate content was also reduced under the exposure of cobalt and lead in the tested plants. Starch was more reduced as compared to other carbohydrates. Additional supply of nitrogen minimized the inhibitory effect of lead and cobalt with varying degree. Exceptionally, proline content in plant tissues increased under cobalt and lead induced stress. In contrast to other parameters, additional supply of nitrogen repressed the proline content. The deadly effects of heavy metals may be reduced in plants if provided with appropriate concentration and form of nitrogen. However, the extent of minimization or reduction depends on the type and concentration of metals and crop used. Thus cultivation of soybean in metal polluted soils should be avoided or appropriate control measures should be adopted to maintain the heavy metal content of soil below the damage threshold level.

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