Research Paper

Volume : 3 | Issue : 2 | February 2014 • ISSN No 2277 - 8179

Biochemical Changes in Seedling of Pisum Sativum Linn. Under The Lead And Nickel Stress

Botany

KEYWORDS : Chlorophyll, Lead, Nickel, Biochemical

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ABSTRACT

An investigation was conducted to study the different stress of heavy metals such as Lead and Nickel on biochemical parameters of Pisum sativum. These heavy metals at all concentration exhibited significant reduction in chlorophyll-a, chlorophyll-b, protein, sugar and ascorbic acid were performed at 10th and 20th day of growth. Primary leaves of Pisum sativum were significantly affected by Lead and Nickel, which resulted in a decline of chlorophyll content. Pisum sativum when treated with Lead and Nickel protein and sugar content was decreased in various organs. Maximum decrease in protein and sugar content was found in the root and minimum decrease was observed in the leaves. Nickel was reduced more protein and sugar content than Lead. The ascorbic acid content was significantly affected by the treatment of Lead and Nickel. The ascorbic acid was absent in root. Chlorophyll-a and Chlorophyll-b were significantly affected by Lead and Nickel. Nickel was found to be more effective inhibitor of Chlorophyll-a and Chlorophyll-b than Lead as its inhibitory effect begins at lower concentration.

INTRODUCTION

Lead (Pb) is one of the potentially toxic heavy metal pollutants of the environment with no known biological function and its concentrations are rapidly increased in agricultural soil (Hamid *et al*., 2010). The most significant factors which can distribute lead as a pollutant in the environment are burning of fossil fuels, agricultural manufacturing, mining, pesticides and fertilizers (Ross, 1994; Eick *et al*., 1999). In plants, it has been widely reported that accumulation of Pb may cause many physiological, biochemical and structural changes like decline in photosynthetic rate and essential elements absorption (Larbi *et al***.,** 2002).

Nickel is a metal found in the earth's crust. Its average world wide concentration is 8 parts per million (ppm). At the level of 100 ppm or higher nickel is considered to be phytotoxic or harmful to plants. Toxic effects of Ni on plant growth and photosynthesis have been reported in higher plants as well as in algae (Sheoran et al., 1990). Higher concentrations of Ni are responsible for reductions in Hill activity and oxygen evolution (Singh and Singh, 1987). The higher concentrations of nickel have been shown to bring the toxicity effects on growth (Gerendas et al., 1999) and metabolic disorders in plants (Baccouch et al., 1998; Gopal et al., 2002; Rahman et al., 2005).

MATERIALS AND METHODS

The seeds of *Pisum sativum* were obtained from Gandhi Agro, Surat and were surface sterilized wi^t^h H_2O_2 for the prevention of surface fungal/bacterial contamination. The germination was carried out in petri dishes. Ten seeds were placed on cotton in each petri dish and 40 ml solution of different concentration of $Pb(No_3)_2$ and $Nicl_2$ were supplied once for seed germination. Distilled water was applied every alternate day after this treatment. For each metal sub lethal (LC_{20}), lethal (LC_{50}) and super lethal (LC_{80}) values were determined for the experimental plant (Table 1).

Table 1: Lethal concentration of zinc and cadmium.

Metal	LC(20)	LC(50)	LC(80)
Lead	100ppm	200ppm	300ppm
Nickel	75ppm	100ppm	125ppm

For experiments solutions of these concentrations of Lead and Nickel were selected for the treatment. Plants grown in distilled water were control. The biochemical parameters viz., protein and sugar of seedlings were studied on 10^{th} and 20^{th} ^{d}ay after sowing of seeds in root, leaf and stem. Chlorophyll-a and chlorophyll-b were studies only in primary leaf of plants. Methods were used for the estimation of protein, sugar and chlorophylls are as under. The estimation of protein was carried out by following methods of Lowery (1951); sugars by following the techniques given by Nelson (1944) and Somagyi(1945). The chlorophyll pigments were estimated by employing the methods of Arnon (1949).

RESULTS

In *Pisum sativum* leaves modified into tendril and stipules modified into leaves. Primary leaves of *Pisum sativum* were significantly affected by Lead and Nickel, which resulted in a decline of chlorophyll content. Presence of 100 ppm of Lead and 75 ppm of Nickel resulted in a significant decrease of chlorophyll. Beyond that concentration decrease of chlorophyll content was observed with elevated concentration level of Lead and Nickel. When *Pisum sativum* were exposed to 300 ppm to Lead and 125 ppm of Nickel, the amount of chlorophyll reached to a minimum value.

Chlorophyll-a content was 0.9395 mg/gm under control condition on 10th day of growth after sowing. The treatment of 75 ppm, 100 ppm and 125 ppm of Nickel, resulted more reduction in chlorophyll – a content compared to Lead treated plants (Table 2). Thus Nickel affected at lower concentration, compared to Lead. Chlorophyll – b content was more affected than chlorophyll-a content in Lead and Nickel treated plants. Both chlorophyll-a and chlorophyll–b were significantly affected by Lead and Nickel. Nickel was found to be more effective inhibitor of chlorophyll–a and chlorophyll–b than Lead as its inhibitory effect begins at lower concentration.

At lower concentration Lead increased protein content in plants. But at higher concentration it reduced the protein content (Harmens *et al.,*1993). Maximum concentration of protein was observed in the leaves and it was lower in stem and root. Protein content was significantly affected by the treatment of Lead and Nickel. *Pisum sativum* when treated with Lead and Nickel protein content was decreased in various organs. Maximum decrease in protein content was found in the root and minimum decrease was observed in the leaves. Nickel has reduced more protein content than Lead. The reducing effect of Nickel on protein content was found at lower concentration while Lead was effective at relatively higher concentration. *Pisum sativum* was also affected by Nickel like Lead but this plant was affected at lower concentration of Nickel. The sugar content on 10th day was reduced when the plants were treated with 75 ppm, 100 ppm and 125 ppm Nickel solution as shown in Table 3. Similarly the reduction in sugar content was observed in the plants of 20th day when treated with same doses of Nickel (Table 3)

The maximum reduction in sugar content was observed in the roots of *Pisum sativum* and minimum reduction was found in the leaves of plant. Nickel was more adversely affected than Lead. The sugar content was significantly affected by the treatment of Lead and Nickel. The sugar content was 9.83 mg/gm in leaf, 3.79 mg/gm in stem and 3.32 mg/gm in root of control plants on 10th day of growth.

Table. 2 Effects of Lead on Chlorophyll-a, Chlorophyll-b, Protein and Sugar mg/gm fresh weight) content of *Pisum sativum.*

Content	Organs	10th day					20th day		
		Control	100 ppm	200 ppm	300 ppm	Control	100 ppm	200 ppm	300 ppm
Chlorophyll-a	Leaf	0.9395± 0.0073	0.8512± 0.0013 (9.39)	0.8315± 0.0182 (11.49)	0.8057 ±0.236 (14.24)	0.9810± 0.0065	0.8190± 0.0155 (16.51)	0.7954±0.218 (18.91)	0.7615±0.0050 (22.37)
Chlorophyll-b	Leaf	0.6315± 0.0285	0.5819± 0.0475 (7.80)	0.5675± 0.0309 (10.13)	0.5178 ±0.0158 (18.32)	0.6710± 0.0042	0.5419± 0.0988 (19.23)	0.5216±0.0653 (22.26)	0.4519±0.0321 (32.65)
Protein	Leaf	19.73± 0.045	14.90± 0.045 (24.48)	12.97± 0.252 (34.26)	11.03 ± 0.0153 (44.09)	17.80± 0.055	12.97± 0.0451 (27.13)	10.07±0.075 (43.42)	8.17±0.083 (54.26)
	Stem	10.07± 0.030	8.14± 0.043 (19.16)	7.17± 0.0400 (28.79)	6.11± 0.030 (39.32)	8.14± 0.086	6.21± 0.085 (23.71)	5.05±0.056 (37.96)	3.79±0.0350 (53.43)
	Root	8.14± 0.0100	7.17± 0.040 (11.91)	5.92± 0.030 (27.27)	4.95± 0.0368 (39.18)	6.21± 0.030	5.24± 0.0674 (15.61)	4.18±0.041 (32.38)	3.12±0.0252 (49.75)
Sugar	Leaf	9.83± 0.050	7.04± 0.040 (28.38)	6.11± 0.020 (37.84)	4.72± 0.005 (51.98)	8.43± 0.045	5.65± 0.010 (32.97)	4.72±0.005 (44.32)	3.32±0.030 (60.61)
	Stem	3.79± 0.015	2.86± 0.020 (24.53)	2.39± 0.023 (36.93)	1.93± 0.035 (49.07)	3.32± 0.035	2.39± 0.0153 (28.01)	1.93±0.005 (41.86)	1.47±0.012 (55.72)
	Root	3.32± 0.020	2.63± 0.003 (20.78)	2.12± 0.012 (36.14)	1.74± 0.0015 (47.59)	2.86± 0.018	2.30±0.007 (19.5)	1.70±0.0180 (40.55)	1.42±0.0128 (50.32)

All the values are means ± S.D. (mg/gm fresh weight).

Values in bracket are percentage decreased, compared to control.

Table. 3 Effects of Nickel on Chlorophyll-a, Chlorophyll-b, Protein and Sugar mg/gm fresh weight) content of *Pisum sativum.*

Content	Organs	10th day					20th day		
		Control	75 ppm	100 ppm	125 ppm	Control	75 ppm	100 ppm	125 ppm
Chlorophyll-a	Leaf	0.9395± 0.0080	0.8019± 0.0092 (14.64)	0.7815± 0.0157 (16.81)	0.7519± 0.0254 (19.96)	0.9810± 0.0162	0.7419± 0.0127 (24.37)	0.7213± 0.0131 (26.47)	0.6915± 0.0512 (29.51)
Chlorophyll-b	Leaf	0.6315± 0.0285	0.5416± 0.0277 (14.23)	0.5219± 0.0362 (17.35)	0.4985± 0.0578 (21.06)	0.6710± 0.0042	0.4956± 0.0281 (26.14)	0.4681± 0.0251 (30.23)	0.4285 ± 0.0236 (36.14)
Protein	Leaf	12.97 ± 0.451	11.03 ± 0.020 (14.95)	9.10 ± 0.0.030 (29.83)	7.17 ± 0.028 (44.71)	11.03 ± 0.088	9.10 ± 0.008 (17.49)	7.17 ± 0.033 (34.99)	5.24 ± 0.039 (52.49)
	Stem	8.14 ± 0.040	7.17 ± 0.032 (11.91)	5.72 ± 0.029 (29.62)	4.70 ± 0.046 (42.26)	6.21 ± 0.113	5.24 ± 0.038 (15.61)	4.27 ± 0.049 (31.23)	3.21 ± 0.0456 (48.30)
	Root	6.21 ± 0.107	5.53 ± 0.031 (10.95)	4.37 ± 0.047 (29.62)	3.60 ± 0.051 (42.02)	5.24 ± 0.038	4.47 ± 0.049 (14.69)	3.62 ± 0.0.050 (30.91)	2.73 ± 0.036 (47.90)
Sugar	Leaf	8.43 ± 0.020	6.11 ± 0.030 (27.52)	5.18 ± 0.015 (38.55)	4.25 ± 0.0351 (49.58)	7.50 ± 0.050	5.18 ± 0.0451 (30.93)	4.25 ± 0.020 (43.33)	3.32 ± 0.011 (55.73)
	Stem	4.25 ± 0.030	3.32 ± 0.017 (21.88)	2.86 ± 0.005 (32.70)	2.35 ± 0.016 (44.70)	3.32 ± 0.010	2.39 ± 0.020 (28.01)	1.93 ± 0.031 (41.86)	1.70 ± 0.022 (48.79)
	Root	2.86 ± 0.006	2.39 ± 0.005 (16.43)	1.98 ± 0.030 (30.76)	1.70 ± 0.027 (40.55)	1.93 ± 0.014	1.47 ± 0.019 (23.83)	1.23 ± 0.016 (36.26)	1.05 ± 0.018 (47.50)

All the values are means ± S.D. (mg/gm fresh weight of organs).
Values in bracket are percentage decreased, compared to control.

Discussion

In the present investigation lead and nickel treatment result in decline in total sugar content of *Pisum sativum.* Our studies coincide with Costa and Spitz (1997) who also reported a decrease in soluble protein content under heavy metal stress in *Lupinus albus.* 100 ppm of lead treatment showed much more toxic effect on total protein content *Leucaena leucocephala* as compared to 25 and 50 ppm concentration (Shafiq *et al*., 2008). Similarly in the present study also it was found that the 100 ppm of lead treatment showed much more toxic effect on total protein content of *Pisum sativum.* The decrease in protein content was observed at higher concentrations of Cd and Pb in/ *Brassica juncea* may be because of enhanced protein degradation process as a result of increased protease activity (Palma *et al.,* 2002).

Volume : 3 | Issue : 2 | February 2014 • ISSN No 2277 - 8179

In the present study the reduction in protein content was observe by the different doses of lead and nickel in *Pisum* sativum. Such reduction in protein contents due to the treatment of lead and nickel was reported by other workers also. Protein content increased in *Lycopersicon esculentum* with increase in Nickel concentration up to 0.5 ppm and reduced with further increase in nickel concentration at 5 and 50 ppm (Sanghpariya, 2008). The increase in protein content was due to exposure of various heavy metals, enhanced accumulation of total soluble protein (Mishra and Dubey, 2006). The higher concentration of nickel in leaf tissue of *Pisum sativum* significantly declined the total protein contents but increased sugar level. The level of protein was increased in recovery treatments (Verma and Sharma, 2009).

In this investigation it has been found that sugar contents were reduced in *Pisum sativum* by the different doses of lead and nickel. The other workers also observed similar decline in sugar contents due to the effects of lead, nickel and other heavy metals. At 20, 50 and 100 ppm lead treatment in *Phaseolus vulgaris*, carbohydrate content decrease with compare to the control (Hamid, 2010).

It was observed that increasing treatment of lead resulted in decrease in total chlorophyll content of *Pisum sativum* leaf total chlorophyll significantly decreased by increase in lead and nickel amount which coincides with Hamid *et al.*(2010).

Our results of decrease in chlorophyll content corroborated with the finding of Siedlecka and Krupa (1996). Who also found a decrease in chlorophyll content with heavy metal stress in *Zea mays* and *Acer rubrum.* The loss in chlorophyll content can consequently lead to disruption of photosynthetic machinery. An enhancement of chlorophyll degradation occurs in lead treated plants due to increased chlorophyllase activity (Drazkiewicz, 1994). Chlorophyll b is reported to be more affected than chlorophyll a by lead treatment (Vodnik *et al.,* 1999). Decrease in total chlorophyll content was observed in lead treated samples as compared to control. Under the metal stress, the levels of photosynthetic pigments, namely Chl. 'a' and Chl 'b' and Carotenoid decreases as the concentrations of Pb in soil increases. Chl.'a' was more affected in comparison to carotenoids.in *Phaseolus vulgaris* (Bhardwaj *et al.,* 2009).

CONCLUSION

Lead (Pb) is one of the potentially toxic heavy metal pollutants of the environment with no known biological function and its concentrations are rapidly increased in agricultural soil. In plants, it has been widely reported that accumulation of Pb may cause many physiological, biochemical and structural changes like decline in photosynthetic rate and essential elements absorption, the roots and shoots growth inhibition, chlorosis and decrease in water potential and plant hormones. Nickel (Ni) is an essential micronutrient for plant growth. Trace elements are necessary for normal metabolic functions in plants, but higher concentrations of these metals are toxic and may severely interfere with many physiological and biochemical processes of plants.

In biochemical study, it was observed that increasing treatment of lead and nickel resulted in decrease in total chlorophyll content, chlorophyll a and chlorophyll b of plant. Plant when treated with lead and nickel protein content was decreased in various organs. Maximum decrease in protein content was found in the root and minimum decrease was observed in the leaves. In this investigation it has been found that sugar contents were reduced in *Pisum sativum* by the different doses of lead and nickel.

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