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Full Length Research Paper

Effect of lead and nickel toxicity on chlorophyll and proline content of Urd (*Vigna mungo* L.) seedlings

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The effect of Pb and Ni on chlorophyll a, b, carotenoids and proline content of Black gram (*Vigna mungo* L.) seedlings were evaluated under 10, 50 and 100 µM concentration. These concentrations significantly affected chlorophyll, carotenoid and proline content of Black gram as compared to control. Pb and Ni at 10 µM concentration resulted in less significant effect on chlorophyll, a, b and carotenoids. Carotenoids were less affected compared to Chl a and b, while higher concentrations (50 and 100 µM) significantly reduced chlorophyll and carotenoid contents of the seedlings. However, the addition of nitrogen (5 mM) somehow minimized the effect of these heavy metals. Nitrogen increased the chlorophyll content of metal treated plants. The proline content of plants was increased under Pb and Ni treatments (10, 50 and 100 µM). In this case, the additional supply of nitrogen in the form of ammonium nitrate decreased proline content of plants treated with Pb and Ni.

Key words: Carotenoid, chlorophyll, lead, nickel, proline, *Vigna mungo*.

INTRODUCTION

Black gram (*Vigna mungo* L.) is a member of family Fabaceae. It is an annual and important short duration pulse crop growing in many parts of India and cultivated both in kharif and rabi season. It is native to Central and South East Asia. The optimum temperature for better growth of black gram ranges between 25 to 35°C, but it can tolerate upto 42°C which permit to cultivate during summer and winter seasons. Black gram is cultivated both in hilly and plain regions and commonly grown upto 1800 m amsl with short duration (90-120) and high nutritive value (El-Karamany, 2006).

It is quite drought resistant but intolerant of frost and prolonged cloudiness. Black gram is sown in most of the soils but it can grow better on heavier soils (pH 5.5 – 7.5) with an annual rainfall of 600 – 1000 mm (Chauhan et al., 2010). Amongst many abiotic stresses, heavy metal toxicity is very important, especially of crop species that are grown in the vicinity of sites of heavy industry, particularly in developing countries (Bi et al., 2006; Ona et al., 2006).

The toxicity of heavy metals is a problem for ecological, evolutionary and environmental reasons (Nagajyoti et al., 2008). Pb tends to accumulate in the ground surface layer and its concentration decreases with soil depth (De Abreu et al., 1998). Pb is considered as a general protoplasmic poison which is cumulative slow acting and subtle. Soil contaminated with Pb cause sharp decrease in crop productivity thereby posing a serious problem for agriculture (Johnson and Eaton, 1980). Maximum Pb content is found in senescing leaves and least in young leaves (Godzik, 1993). Pb moves predominantly into the root apoplast and thereby in a redial manner across the cortex and accumulates near the endodermis. The endodermis acts as a partial barrier to the movement of Pb between the root and the shoot (Jones et al., 1973; Verma and Dubey, 2003). Pb decreases germination percentage of plant, root/shoot length, tolerance index and dry mass of roots and shoots (Mishra and Chaudhary, 1998). The key enzyme for chlorophyll biosynthesis that is, α-amino laevulinate dehydrogenase is strongly inhibited by Pb ions (Prasad and Prasad, 1987).

Nickel, one of the important heavy metal pollutants is of considerable concern because its concentration is rapidly increasing in soils of different parts of the world (Echevarria et al., 1998; Faryal et al., 2007; Atiq-ur-Rehman and Iqbal, 2008). The increasing concentration of Ni^{2+} has been shown to inhibit seed germination and

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Table 1. Effect of Lead and Nickel on chlorophyll, carotenoids and proline content in Black gram (*V. mungo* L.) grown with or without nitrogen (N).

Metals	Concentration (µM)	Chlorophyll a		Chlorophyll b		Total Chlorophyll		Carotenoids		Proline	
Control		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
Control	0	0.176 ± 0.003	0.298± 0.002	0.309± 0.001	0.419± 0.015	0.485± 0.004	0.717± 0.001	0.524± 0.001	0.639± 0.015	0.029± 0.002	0.015± 0.001
	10	0.166± 0.001	0.224± 0.002	0.252± 0.015	0.380± 0.015	0.418± 0.003	0.604± 0.002	0.466± 0.015	0.516± 0.003	0.032± 0.003	0.020± 0.001
Pb	50	0.150± 0.002	0.209± 0.004	0.230± 0.015	0.362± 0.015	0.380± 0.003	0.571± 0.002	0.441± 0.001	0.481± 0.002	0.034± 0.001	0.022± 0.002
	100	0.147± 0.002	0.197± 0.002	0.204± 0.001	0.324± 0.015	0.351± 0.001	0.521± 0.001	0.420± 0.015	0.460± 0.003	0.038± 0.002	0.023± 0.015
	10	0.140± 0.003	0.180± 0.003	0.202± 0.002	0.310± 0.015	0.342± 0.002	0.490± 0.005	0.409± 0.001	0.469± 0.002	0.040± 0.001	0.025± 0.001
Ni	50	0.064± 0.002	0.123± 0.002	0.085± 0.001	0.315± 0.002	0.149± 0.004	0.258± 0.003	0.272± 0.015	0.312± 0.001	0.042± 0.015	0.027± 0.015
	100	0.057± 0.002	0.117± 0.002	0.068± 0.015	0.123± 0.015	0.125± 0.002	0.240± 0.003	0.245± 0.001	0.280± 0.002	0.049± 0.015	0.029± 0.001

N – Nitrogen, Mean ± - Standard Error.

seedling growth of different plant species (Espen et al., 1997; Farooqi et al., 2009). Nickel stimulates many enzymatic activities at very low concentration and it found a part of active metallo center of hexamer enzyme urease (Gerendas et al., 1999). Nickel is rapidly taken up by the plant root system and research with different plant species have shown that Ni is able to inhibit a large number of plant enzymes such as those of Calvin cycle and chlorophyll biosynthesis (Van Assche and Clijsters, 1990).

Proline is an amino acid which could play a therapeutic role in plants (Singh et al., 1973; Flower et al., 1977). Proline, sugar, glycine, betaine and other organic solutes are believed to improve metal tolerance by contributing to osmosis and preserving enzyme activity in presence of toxic ions (Greenway and Munns, 1980).

It has been shown that nitrogen has significant effect on crop plants under stress conditions. Nitrogen induced the pigment composition and leaf area in various crops when provided exogenously. The availability of nitrogen fixation sensitivity to drought has been analyzed with several grain legumes including *V. mungo* L. (Sinclain and Seeraj, 1995). Keeping all these effects in mind the present investigation was executed with an objective to study the role of lead and nickel on cholrophyll, carotenoid and proline content of *Vigna mungo* at different concentrations.

MATERIALS AND METHODS

The seeds of *V. mungo* L. Hepper var. Shekhar 3 (KU – 309) were obtained from Agricultural Seed Company, Belanganj, Agra. The seeds were surface sterilized with 0.1% of sodium hypochlorite to prevent any fungal contamination. Then after washing with distilled water and were then soaked in 5% bavistin (a systemic fungicide) for 15 min. The sand was thoroughly washed with sodium hypochlorite and dried. The seeds were sown in earthen pots containing 4:1 soil and farm yard manure. The soil was treated with Long Ashton nutrient solution (Hewitt, 1966). Metal treatments of Pb and Ni were prepared using lead nitrate and nickel chloride solutions (10, 50 and 100 µmol/L). Nitrogen was prepared using ammonium nitrate at 5 mM/L concentration. Pb and Ni (control, 10, 50 and 100 µM) 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 the experiments were conducted in green house to provide control conditions. The samples were taken from three week old seedlings for biochemical analysis. The chlorophylls and carotenoids were estimated by Arnon's (1949) technique using double beam UV-visible spectrophotometer (systronics). The proline was estimated by using Bates et al. (1973) method.

RESULTS AND DISCUSSION

Effect of heavy metals on chlorophyll and carotenoids

Data presented in Table 1 shows that the effect of Pb and Ni (10, 50 and 100 µM/L) and their interaction with nitrogen (5 mM/L) on chlorophyll and carotenoids content of black gram plants. Pb and Ni at 10 µM reduced chlorophyll a to 0.1666 and 0.140 mg g^{-1} Fw respectively in comparison to control. Chlorophyll b at 10 µM reduced upto 0.252 and 0.202 mg g^{-1} Fw compared to control (0.309 mg g^{-1} Fw), while chlorophyll a at 50 µM/L concentration reduced upto 15 and 64% but chlorophyll b reduced upto 26 and 72% as compared to control at 50 µM/L concentration (Figure 1a). The chlorophyll a reduced 16 and

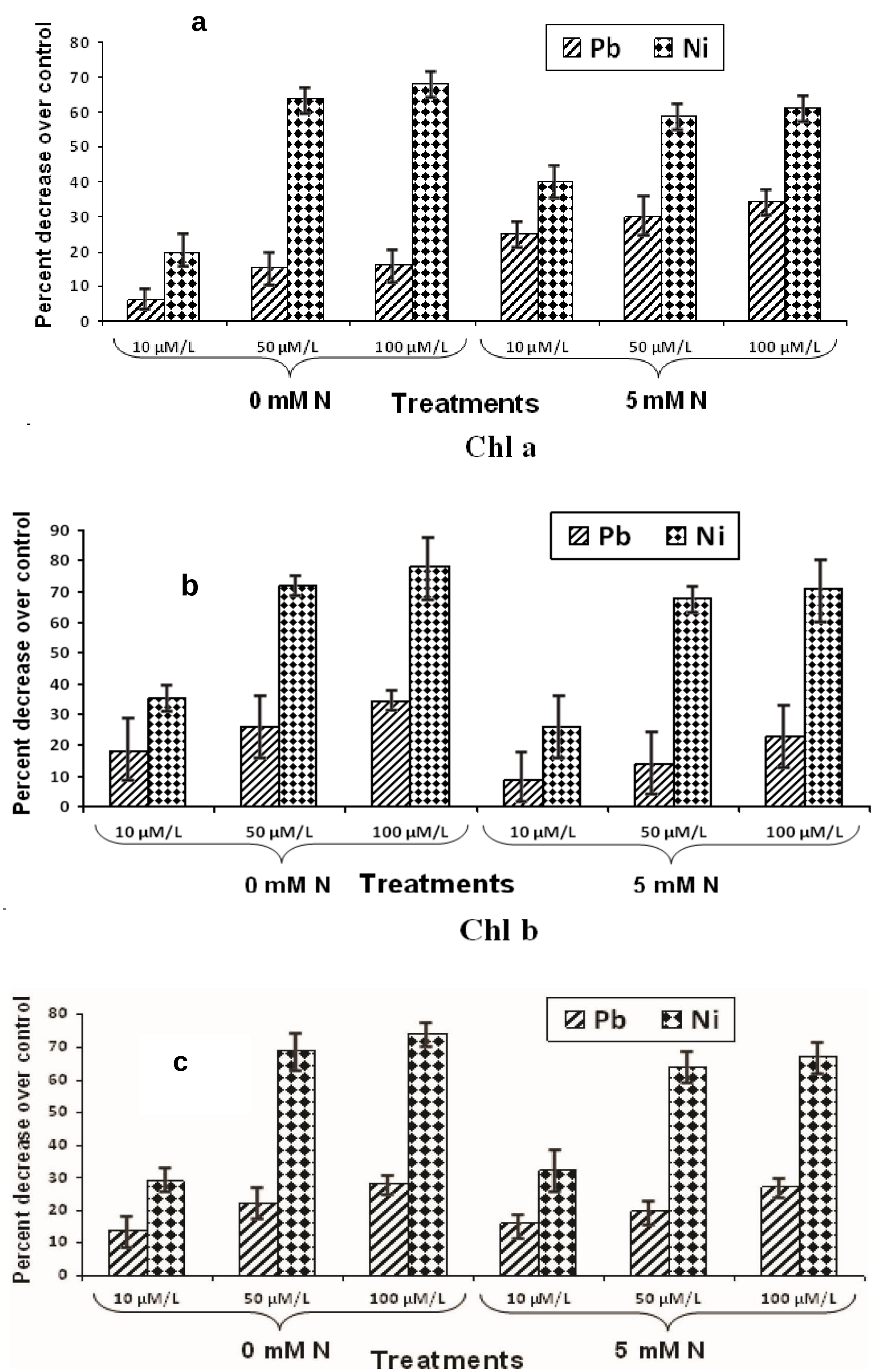

Figure 1. Total chlorophyll.

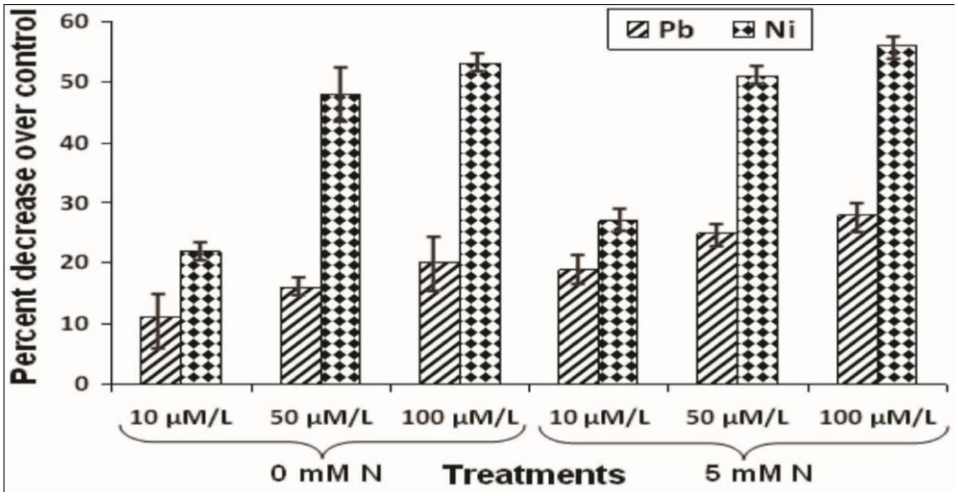

Figure 2. Carotenoids.

68% by Pb and Ni at 100 µM concentration compared to control. A corresponding result in chlorophyll b were 34 and 78% (Figure 1b). Total chlorophyll was reduced upto 16 and 32% at 10 µM/L concentration. At 50 µM/L concentration total chlorophyll was reduced upto 20 and 64%. But at higher concentration (100 µM/L), the reduction was 27 and 67% as compared to control (Figure 1c). On the contrary, additional supply of nitrogen (Ammonium nitrate) 5 mM/L in nutrient medium somehow minimized the effect of heavy metals and proved beneficial for all pigments studied (Figure 1a, b, c).

An examination of Figure 2 reveals that plants under control without nitrogen exhibited 0.524 mg g^{-1} Fw and with nitrogen 0.639 mg g^{-1} Fw of carotenoid content. However, when the Pb and Ni at 10 µM without nitrogen were supplied the decrease in carotenoid content was 11 and 22%, respectively. When the nitrogen was applied the increase was found to be 19 and 27% over control. Similarly at higher concentration of both these metals the decrease in carotenoid content was found to be 20 and 53%, however, when nitrogen was applied the increase in carotenoid content was found to be 28 and 56%. This is in accordance with the results reported by Panda and Khan (2003) who found carotenoid content decreased in rice under heavy metals.

Similar results have been obtained by several workers working on various crops. The study on *V. mungo* leaves suggested that decline in Chl. content in shoots of metal treated plants resulted mostly from its enhanced degradation or reduced synthesis (Stobart et al., 1985; Somashekaraiah et al., 1992). Nagajyoti et al. (2008) reported the reduction in Chlorophyll a, Chl. b and total chlorophyll in groundnut by industrial effluents containing heavy metals. Chlorophyll content decreased in leaves exposed to Cd stress were also reported in *V. mungo* (Singh et al., 2008). Reduced chlorophyll content due to nickel toxicity in different plant species has been well documented (Pandey and Pathak, 2006).

Effect of heavy metals on proline

The effect of Pb and Ni on proline content of Black gram and their interaction with nitrogen is shown in Figure 3. Plants grown with Pb and Ni showed rapid accumulation of proline. As compared to control (0.029) mg g^{-1} proline, plants showed an increase upto 0.032 and 0.040 mg g^{-1} proline under Pb and Ni treatments at 10 µmol/L concentrations. At (50 µmol/L) concentration, proline content was enhanced upto 15 and 31% under Pb and Ni treatments over control. However at higher concentration (100 µmol/L) proline content was further enhanced up to 31 and 69% over control under Pb and Ni treatments. In this case, nitrogen application reduced the proline content in *V. mungo* L. plants.

At higher concentration (100 µmol/L), proline content was reduced upto 55 and 93% over control under Pb and Ni treatment. Our results are in agreement with several workers worked on several crops (Singh et al., 1973; Flower et al., 1977; Chris et al., 2006; John et al., 2009). Proline, an osmoprotectant, accumulate under heavy metal stress, and give rise to a series of reactions, which generate numerous free radicals by altered levels of major anions and accumulation (Alia and Saradhi, 1991). Proline is supposed to participate in the reconstruction of chlorophyll, activate the Krebs cycle and also in the energy source (Saxe, 1991).

Conclusion

Based on the results it can be concluded that chlorophyll, carotenoid, and proline contents were seriously affected by heavy metals (both these). Chlorophyll a was affected

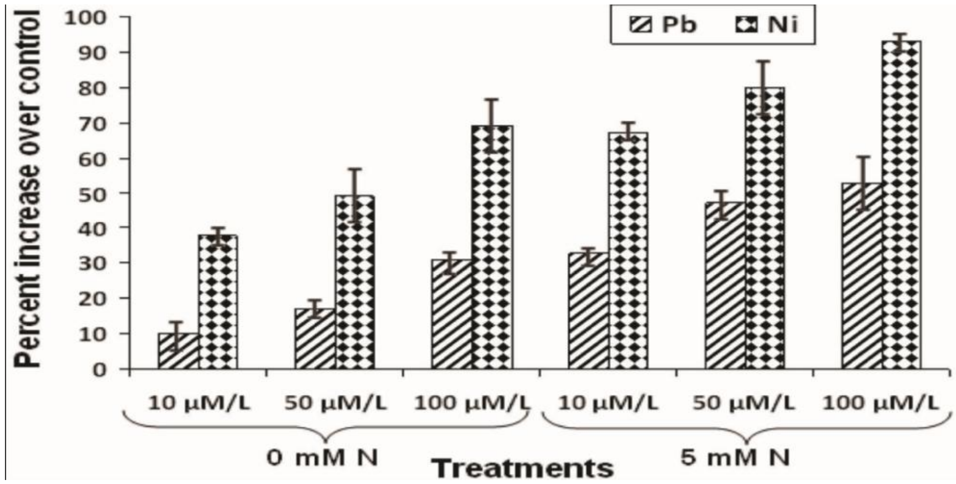

Figure 3. Proline content.

more as compared to chlorophyll b whereas carotenoids were less affected then Chl a and b. The amount of proline increased in plants under stress caused by these heavy metals. Nickel proved to be more toxic than lead at all the three concentrations (10, 50 and 100 µM). The deleterious effects of heavy metals may be alleviated in plants if provided with appropriate concentration and form of nitrogen in nutrient medium. Further more research is needed in order to evaluate the effect of different heavy metals on various crops especially in the Braj (Agra and Mathura) region.

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