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Changes in the Levels of Photosynthetic Pigments in *Phaseolus aureus* Roxb. Exposed to Hg and Cd at Two Stages of Development: A Comparative Study

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Since the recognition of the toxic potential of heavy metals, their concentration levels in the environmentally sensitive places are being monitored rigorously. In addition, their biological effects are being studied to better assess their impact on the environment, and to identify suitable test systems for biomonitoring low level contaminations (Dash et al. 1988).

Photosynthetic processes are widely studied endpoints in metal toxicity testing involving plants (Rai et al. 1991; Ferretti et al. 1993; Krupa et al. 1993a, b; Shaw et al. 1988). Considering the importance of photosynthetic pigments (chlorophylls and carotenoids) in energy transduction, variations in their levels, especially that of chlorophylls, in response to heavy metals have also been studied by some workers (Shaw et al. 1989; Ferretti et al. 1993; Foy et al. 1978; Krupa et al. 1993a). However, a detailed and comparative study of such variations giving importance not only to chlorophylls but also to carotenoids is lacking. The present study was designed to study the same. Keeping in view the report of Patro (1993) that the cultivated lands in and around the Angul-Talcher industrial belt, adjacent to Bhubaneswar, are under severe threats of pollution by heavy metals released from a thermal power plant and various industrial and mining activities in the region, a widely cultivated legume, Phaseolus aureus Roxb., was selected as the test species. The metals used were Hg and Cd which are well recognized land pollutants from secondary sources (Fergusson 1990). This study also reports how the changes in photosynthetic pigments and the toxicity of the two metals are dependent on the stage of development at which the test species is exposed to the metals, information on which is scant.

MATERIALS AND METHODS

The seeds of Phaseolus aureus Roxb., presoaked overnight in distilled water, were germinated on nylon nets placed over 100 mL of Hoagland's solution (Hoagland and Arnon 1950). The seedlings were grown at 25 ± 2 °C and a light intensity of 300 μmol quanta m^{-2} s^{-1} in 12:12 hr light:dark cycles. Mercury and cadmium were

added in the medium in the form of $HgCl_2$ and $Cd(NO_3)_2$, respectively. Two types of treatments were followed: 1) Germination-stage treatment (GST) respresenting treatment on the 1st day, i.e., while placing the seeds for germination, in concentrations of 0.5, 5, 10, 20 and 50 µM, selected on the basis of the growth of the roots. 2) Seedling-stage treatment (SST) representing treatment on the 6th day of germination in concentrations of 0.5, 5 and 20 µM.

Failure of seeds to germinate and the failure of seedlings to grow in the GST were considered as unsuccessful establishment of seedlings. For the determination of pigments, the primary leaves were selected randomly from both the treatments on the 8th day of germination, cut into pieces after removing the mid ribs and weighed quickly and accurately (around 50 mg) on a digital analytical balance (reproducibility $\pm$ 0.1 mg). The pigments, chlorophylls (chl) a and b and carotenoids (car), in the weighed samples were quantified spectrophotometrically (Lichtenthaler and Wellburn 1983). Each estimation was done 6 times, 3 each in two independent sets of experiments. The results were expressed as means $\pm$ standard deviations (SD). F-test and t-test were performed following Gomez and Gomez (1983). LSD (least significant difference) values were calculated only when the F-test revealed significant variations in a parameter in response to treatment concentrations.

RESULTS AND DISCUSSION

Both Hg and Cd significantly inhibited the establishment of seedlings and the elongation of roots in GST in a concentration-dependent manner (Fig. 1). However, their effects were significantly different only on root elongation, Hg being much more toxic than Cd in 2, 5 and 10 µM concentrations. Greater toxicity of Hg than Cd has also been reported by other workers (Rai et al. 1981; Nordberg 1976). Notwithstanding, the seedlings, when treated on the 6th or later days of germination (SST), were more susceptible to Cd than Hg, contrary to the results of GST; all of them died within 48 hr of receiving 30 µM or higher Cd treatment, but survived even 100-µM Hg treatment (beyond this the result was not tested). Because of this difference in their effects, the highest concentration of both the metals for SST was reduced to 20 µM. The study clearly revealed that the toxicity of a metal to a test species may depend upon its stage of development. The factor underlying such toxicity expression is not known and no report supporting this observation is available.

The variations in concentration levels of the photosynthetic pigments in leaves in response to both the metals were also found to be dependent on the stage of development at which the test species was exposed. In the case of GST, the levels of chlorophylls, whether chl a, chl b or total, increased significantly with increase in the treatment concentrations of both Hg and Cd (Table 1). Notwithstanding, the levels of chlorophylls in the case of SST exhibited only an insignificant

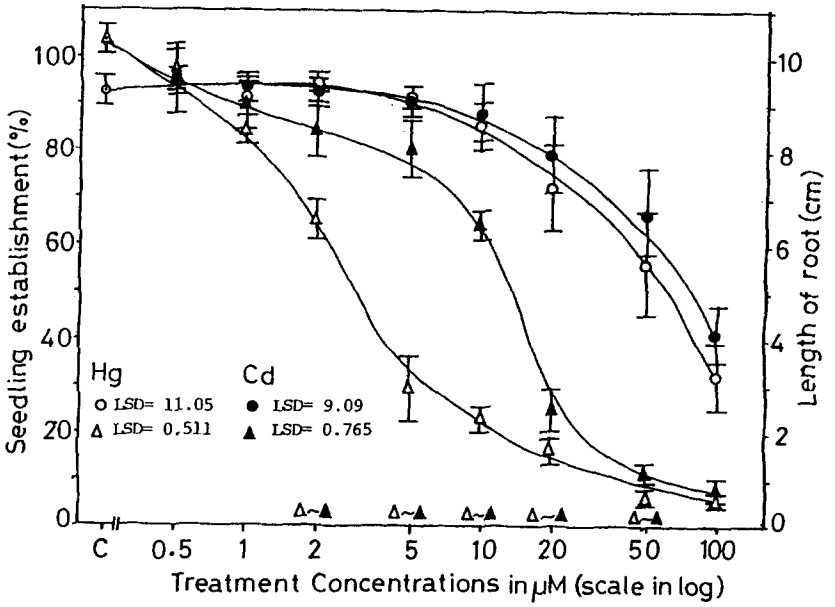

Figure 1 : The percentage of seedlings showing successful establishment (data means ± SD, n = 3) in response to Hg (o) and Cd (●) in germination-stage treatment (observation on the 8th day of germination), and the effect of the treatments (Hg-Δ, Cd -▲) on the growth of roots of the seedlings (data means ± SD, n = 4; observation on the 5th day of germination). The symbols in empty and bold pairs at the bottom of the graph against a concentration indicate significant difference between the results of the two metal treatments in that concentration.

increase in response to increasing concentrations of the metals (Table 1). Contrary to the present results, many workers have reported a decrease in the levels of chlorophylls in terrestrial plants when exposed to metals or industrial effluents containing metals, whether at germination stage (Behera and Misra 1983; Ramasubramanian and Ravichandran 1993) or seedling stage (Ferretti et al. 1993). Recently, however, Krupa et al. (1993a) have reported an increase in the levels of chlorophylls in Phaseolus vulgaris when exposed to Ni at seedling stage. The increase might have been due either to an increase in the number of chloroplasts per cell or a decrease in the cells' volume leading to increase in the number of cells per unit weight, or both.

Both metals affected the relative levels of chl a and chl b in the GST, resulting in concentration-dependent significant variations in the chl a to chl b ratio (Table 1). Their effects, however, were quite different. Hg caused a significant increase in the ratio, when compared to the control, at 0.5, 5 and 10 μM concentrations. Cd on the other hand caused a significant decrease in the ratio at 10 μM concentration and above. Thus, while both the metals increased the levels of chlorophylls, most probably by decreasing the cells' volume, only Hg had a stimulatory effect on

Table 1. Changes in the levels of the photosynthetic pigments (mg g^{-1} Fr. wt) in Phaseolus aureus Roxb. in response to Hg and Cd in germination-stage and seedling-stage treatments. Data means ± SD, n = 6.

Treatment Conc.(μM)	Total Chloro-phylls	Chl a	Chl b	Chl a/ Chl b	Caro-tenoids	Chlorophylls/ Carotenoids
Germination-stage treatment						
			Hg			
Cont.	2.39 ± 0.14	1.77 ± 0.10	0.62 ± 0.03	2.03 ± 0.04	0.29 ± 0.02	8.24 ± 0.11
0.5	2.30 ± 0.13	1.71 ± 0.09	0.59 ± 0.03	2.91 ± 0.04	0.29 ± 0.02	8.07 ± 0.09
5	2.80 ± 0.15	2.09 ± 0.11	0.71 ± 0.04	2.97 ± 0.06	0.34 ± 0.02	8.13 ± 0.13
10	3.00 ± 0.17	2.24 ± 0.12	0.76 ± 0.05	2.95 ± 0.06	0.38 ± 0.02	7.96 ± 0.10
20	3.21 ± 0.15	2.37 ± 0.11	0.83 ± 0.05	2.85 ± 0.07	0.41 ± 0.02	7.85 ± 0.14
50	3.20 ± 0.11	2.36 ± 0.09	0.84 ± 0.04	2.80 ± 0.05	0.41 ± 0.02	7.81 ± 0.16
LSD=	0.179	0.112	0.059	0.049	0.014	0.146
			Cd			
Cont.	2.39 ± 0.14	1.77 ± 0.10	0.62 ± 0.03	2.83 ± 0.04	0.29 ± 0.02	8.24 ± 0.11
0.5	2.42 ± 0.15	1.79 ± 0.11	0.63 ± 0.04	2.87 ± 0.05	0.29 ± 0.02	8.19 ± 0.10
5	2.75 ± 0.10	2.03 ± 0.07	0.71 ± 0.04	2.86* ± 0.05	0.32 ± 0.02	8.26 ± 0.13
10	2.90 ± 0.17	2.11 ± 0.12	0.78 ± 0.05	2.70* ± 0.06	0.34* ± 0.02	8.60* ± 0.17
20	3.17 ± 0.17	2.30 ± 0.13	0.88 ± 0.05	2.62* ± 0.04	0.36* ± 0.03	8.76* ± 0.16
50	3.18 ± 0.15	2.30 ± 0.12	0.88 ± 0.04	2.60* ± 0.06	0.37* ± 0.02	8.72* ± 0.14
LSD=	0.171	0.123	0.045	0.059	0.029	0.159
Seedling-stage treatment						
			Hg			
Cont.	2.39 ± 0.14	1.77 ± 0.10	0.62 ± 0.03	2.83 ± 0.04	0.29 ± 0.02	8.24 ± 0.11
0.5	2.41 ± 0.11	1.79 ± 0.06	0.63 ± 0.03	2.85 ± 0.05	0.29 ± 0.02	8.33 ± 0.13
5	2.53 ± 0.13	1.88 ± 0.08	0.66 ± 0.04	2.86 ± 0.06	0.31 ± 0.02	8.09 ± 0.09
20	2.56 ± 0.14	1.89 ± 0.08	0.67 ± 0.03	2.85 ± 0.05	0.32 ± 0.02	8.01 ± 0.14
LSD=	NS	NS	NS	NS	0.019	0.148
			Cd			
Cont.	2.39 ± 0.14	1.77 ± 0.10	0.62 ± 0.03	2.83 ± 0.04	0.29 ± 0.02	8.24 ± 0.11
0.5	2.43 ± 0.13	1.80 ± 0.08	0.63 ± 0.03	2.85 ± 0.06	0.29 ± 0.02	8.27 ± 0.08
5	2.49 ± 0.13	1.84 ± 0.09	0.65 ± 0.03	2.84 ± 0.07	0.30 ± 0.01	8.40* ± 0.15
20	2.40 ± 0.12	1.75* ± 0.09	0.66 ± 0.03	2.65* ± 0.07	0.25* ± 0.02	9.57* ± 0.16
LSD=	NS	NS	NS	0.071	0.017	0.163

*The values are significantly different (t-test) from that obtained under Hg treatment at the same concentration at least at 0.05p. LSD = Least significant difference at 0.05p, NS = Variations not significant.

chl a synthesis. In the SST, however, no such stimulatory effect of Hg was observed, and Cd, as in GST, resulted in a significant decrease in the ratio in this case also. The decrease in chl a to chl b ratio might have been due to destruction of chlorophylls,

preferably of chl a, as a result of their reaction with singlet oxygen which is produced when the acceptor side of PS II is inhibited (Barber and Anderson 1992), and metals have been reported to do so (Rai et al. 1991; Krupa et al. 1993a, b). The decrease as a result of inhibition of chl a synthesis is ruled out since the levels of chl b increased, and the accepted view is that chl b is formed from chl a molecules (Castelfranco and Beale 1983).

The levels of carotenoids, similar to chlorophylls, increased significantly in response to both Hg and Cd in GST. However, the increase was significantly less in response to Cd than to Hg as was evident from the t-tests (Table 1). Thus, while the chl to carotenoid ratio decreased significantly in response to increasing concentrations of Hg, the ratio increased significantly with increase in the concentrations of Cd. In SST Hg also caused a significant increase in the levels of carotenoids, but the increase was comparatively less than that in GST as is evident from the comparison of chl to carotenoid ratios in the two cases. Thus, the response of the test species was dependent clearly on its stage of development. This is also evident in the case of Cd which resulted in a significant decrease in the levels of carotenoids in SST in comparison to the control, unlike its effect in GST, and consequently higher ratios of chl to carotenoid than that in GST. While Hg stimulated the synthesis of carotenoids both in SST and GST, Cd had little stimulatory effect on their synthesis in SST, although some stimulatory effect in GST may not be ruled out. Reports on the influence of metals on the levels of carotenoids, particularly in terrestrial plants, are scant. Krupa et al. (1993a) recently reported an increase in chl to carotenoid ratio in Phaseolus vulgaris in response to Ni similar to that observed for Cd in the present study. Shaw et al. (1989) working on a blue-green alga reported a decrease in the ratio in response to mercury-contaminated effluent. Thus, the effect seems to be metal-specific rather than species-specific.

The ability of plants to alter the levels of carotenoids is important in the development of tolerance against a stress since they protect the photosynthetic tissues against photosensitized oxidation. While the carotenes are responsible for de-excitation of singlet oxygen and the triplet excited state of chlorophylls, the oxygenated forms of carotenoids, the xanthophylls undergo interconversion from one species to another in a cyclic manner leading to non-photochemical quenching and thermal dissipation of the excess excitation energy (Demmig-Adams and Adams III 1992). Although an increase in non-photochemical quenching of excitation energy has been reported in terrestrial plants under metal stress (Krupa et al. 1993a, b), the involvement of xanthophyll cycle in such dissipation has not been established. The inability of Cd, unlike Hg, to induce enhanced synthesis of carotenoids, at least enough to replace the pigments destroyed during their interaction with singlet oxygen and/or the excited chl molecules, was possibly the reason for the significant decrease of their levels in SST in response to 20-μM Cd when compared to the

control, and also the increase in chl to carotenoid ratio as a whole in GST. The death of the seedlings in SST in 30 μM or higher Cd treatments may have been due to the unavailability of sufficient amounts of carotenoids to cope with the enhanced generation of singlet oxygen and accumulation of excited chl molecules in comparison to that under lower concentration treatments. The same deficiency probably had also exposed the chl a molecules to toxic singlet oxygen in the case of Cd treatment leading to their destruction and consequently a decrease in chl a to chl b ratio (Table 1). Some stimulatory effects of the metal on the synthesis of these antioxidant pigments during germination stage might be protecting the seedlings in GST from excess oxidative injury at higher concentrations that would have otherwise proved lethal.

In conclusion, the study revealed that in toxicity testing involving plants, the stage of their development should be given due consideration. And secondly, for establishing the degree of toxicity of a metal in relation to other through phytoassay, the overall responses of the test plant should be taken into account rather than considering only a single endpoint.

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