DISCUSSION
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It is believed that the present study has helped in understanding the toxicity of cadmium in the early stages of growth and development of the plant. The influence of Cd on seed germination appears to be minimal up to a level of 10 m.Eq/l. Further growth and development of plant are severely affected by Cd, and the degree of inhibition of growth is dependent on the concentration of Cd in the culture solution. Though the growth of plant is inhibited, the root growth offered the most sensitive reflection of Cd toxicity over a range of concentration.

The plants absorbed the Cd from the culture solution and accumulated more Cd in roots than in shoots. A positive correlation between root and culture solution metal concentration was observed. Pigment analysis of the leaves revealed that chl a synthesis was more affected than chl b synthesis. Chlorophyll levels were more reduced than carotenoid levels. In general, the synthesis of photosynthetic pigments were inhibited. Studies on carbohydrate metabolism revealed that starch hydrolysis was inhibited by Cd during germination and early phases of growth.
It is evident from the present study that the supply of Cd to plant produced severe adverse effects on the growth and development. However, the influence of Cd on the germination appears to be minimal to a level up to 10 m.Eq/l in Phaseolus aureus Ræmb. Edward (1980) has reported that Cd and Hg reduce the germination rate in Spartina alternifolia. Further, Lane and Martin (1980) have observed that Pb supply up to a level of 10 m.Eq/l had little influence on the germination of Raphanus sativus seeds. However, at higher supply levels, they have observed a marked reduction in the germination rate. In the present study the minimal effect of Cd on seed germination in P. aureus may be attributable either to the higher ability of the embryo to combat Cd toxicity or to a physical or chemical barrier to its entry into the seed. However, in lead treated seeds histochemical studies provided evidence for the latter mechanism. Such a mechanism may also be operative for Cd in P. aureus seeds.

Observations on the root and shoot growth were made in 0.01, 0.1, 0.3, 0.6 and 1.0 m.Eq/l of CdCl₂ solution as the growth of these organs was found inhibited at higher concentrations exhibiting the toxic nature. It may be
noted that Cd has not inhibited the hypocotyl growth at lower concentrations (0.01 m.Eq/l). A retardation in shoot elongation was noted at a higher concentration (1.0 m.Eq/l) besides affecting the root growth severely (50%).

The action of Cd on elongation of radicle and on formation of root hairs was conspicuous from early stages of development. And it may be important in a way that the absence of root hairs at higher concentration (1.0 m.Eq/l) reduced the root surface area causing serious decrease in absorption abilities. This is important with regard to the subsequent inhibition of root development.

Vaulina et al. (1973) have observed certain disturbances in nuclear divisions and cessation of cytokinesis in *C. capillaris* treated with CdCl$_2$ solutions. The effect of Cd ions is supposed to be associated with the SH-groups blocking in contractile protein of cellular spindle or in enzymes responsible for mitosis. This is also may be a reason for the reduced growth at higher concentration of CdCl$_2$.

Keck (1976) has demonstrated that the reductions in respiration and concomitant ATP levels in segments of
roots by Cd ions, leads to decreased growth rate of root. Cd decreased the respiration rate and increased the senescence, leading to reduced growth of aquatic angiosperms (Sardhar and Choudhuri 1982). Similarly the reduced root growth in the present study may be due to inhibition of respiration by Cd.

Brown and Martin (1981) have observed that Cd pretreatment to the roots of Holcus lanatus had resulted in significantly greater root growth in the plants than the plants given no Cd pretreatment. Enhanced tolerance to Cd was restricted only to the actual roots that were given Cd pretreatment and does not extended to the latter developed roots, that were not exposed to Cd pretreatment. From these observations it is understood that root growth studies in metal solutions are important to understand the tolerance mechanism.

Besides these alterations in growth, certain morphological changes at higher concentrations were well observed in roots. The roots are thick and stunted. The root tips were apparently dark brown and the hypocotyl also showed stunted growth in plants treated with 1.0 m.Eq/l of Cd. The hypocotyl regions of plants in low concentrations (0.01 m.Eq/l) were greenish white in colour. The cotyledons were also brown and swollen in 1.0 m.Eq/l
as the age of plant increased, while in control and 0.01 m.Eq Cd treated plants the cotyledons decreased in their size and withered of from the plant. Chlorosis in cotyledons and a characteristic dark banding at the junction of root and stem, stem and cotyledons were observed in 1.0 m.Eq Cd treated plants. Marked swelling of the cotyledons was one of the effects associated with the growth inhibition.

The fresh weight and dry weights of both shoot and root were decreased in treated plants. It was also observed that dry mass accumulation both in shoots and roots was decreased as CdCl₂ supply was increased. At higher CdCl₂ concentrations (1.0 m.Eq/l) the reduction was up to 50%. Smilde (1981) has studied the phytotoxic metal doses in oats, maize, rye grass, lettuce and french beans and found that they vary with metal and plant species increasing in the order of Cd < Ni < Zn < Cr < Pb. The Cd was proved to be the most toxic element of the metals investigated. At low dosages (100 mg/Kg of substrate) Cd significantly depressed dry weights of sensitive crops such as spinach. The higher dosages (300 mg/Kg substrate) reduced the dry weights of all crops other than oats. Similarly a decrease in drymass yield in plants by Cd treatment was reported (Haghir 1973,
In lower concentrations 0.01, 0.1, and 0.3 m.Eq of Cd, the percentage of moisture content was comparatively higher in the roots and shoots of treated plants than that of control. This increase in moisture content may be due to the metal increase in cell size (i.e. volume), accumulation of metal ions and thus leading to uptake of more water.

The fresh weight to dry weight ratio was increased in 2 day to 4 day old shoots as Cd concentration increased, indicating high moisture content. However in 6 day old plants the ratio tends to decrease. In roots the fresh weight to dry weight ratio remained almost constant. But at 1.0 m.Eq/1 of Cd the ratio was decreased indicating low moisture levels of roots.

A common feature observed was decrease in dry mass accumulation and moisture levels. The decrease in ratio with increasing Cd supply indicates a progressive lessening of water content at high concentration 1.0 m.Eq/1 (70%).

The observed loss of water may be due to reduced water uptake, which may be due to the arrest of root development. Decrease in dry weight and tissue permeability
was observed in aquatic angiosperms in Cd polluted industrial waste (Saradnar and Choudhuri 1982). Both fresh weight and dry weight showed a reduction with increasing Cd supply. The fresh weight to dry weight ratio provides an indication of water content of the tissue. This is not only a reflection of the turgor of the tissue but also provides a measure of plants ability to maintain metabolic function.

The fresh weight to dry weight reductions were also supported by visual examination of the plants which appeared distinctly flaccid under 1.0 m.Eq/l conditions. The reduction in fresh weight and dry weight also reflected in the reduction in the growth of root and shoot. The toxic influence of Cd was reflected in reduction in the growth of the plant.

The concentration dependent Cd toxicity observed in the present investigation was also reported by Mitchell et al. (1973) and Bingham et al. (1975 and 1976).

Roots have accumulated more Cd than shoots; the accumulation of Cd both in roots and shoots was increased with Cd concentration and time. Cd accumulation was very high in roots and shoots of 1.0 m.Eq/l treated 6 day old plants.
It was also reported by Taylor and Allinson (1981) that the soil amended with 250 ppm of Cd depressed the alfalfa yield and harvest tissue had high Cd concentrations. Several investigators have indicated the increased plant tissue concentrations of Cd as a result of Cd amendments to the root medium (Jarvis et al. 1976, Munshower 1977, Brinkhuis et al. 1980, and Redecap et al. 1981).

Grasses were found to have relatively low metal contents. Simon (1977) reported that monocotyledonous plants take up less Cd than dicotyledons; consequently dicotyledonous weed species acquire a greater burden of Cd than grasses. According to Page et al. (1972) analysis of whole plants has shown that the roots (rye grass and yorkshire fog) provide the site of highest metal burden. This finding corresponds with those based on solution culture techniques when 83% Cd was localised within the root tissue.

Following the root absorption of Cd it was also reported that Cd is strongly retained by roots; while with only 2% of the accumulated Cd being transported to leaves as much as 8% was transported to seed during seed filling. Once accumulated in the root and leaf tissues, Cd rapidly equilibrated between the insoluble, soluble
and organelle fractions (Cataldo et al., 1981). However in water hyacinth, *Eichhornia crasipes* Chabo et al. (1982) have reported high concentrations of Cd in leaves. Further Weigel and Jager (1980) have observed the accumulation of Cd in the subcellular fractions of leaves and roots of Cd treated bean plants.

Studies on pigmentation levels in control and treated plants indicated that chlorophyll levels were decreased in Cd treated plants. The decrease in levels was more pronounced at increased supply of Cd.

The reduced levels of photosynthetic pigments in Cd treated plants and greening of leaf disks, may be due to inhibitory effect of Cd on the synthesis of these pigments. Similarly Hampp and Landsian (1972), have observed the Pb inhibition of chlorophyll synthesis in greening oat leaf disks. They have also observed an increase in chlorophyll a/b ratio indicating the inhibitory effect of Pb more on chl b synthesis than on chl a synthesis. But in the present investigation a decrease in chl a/b ratio in Cd treated plants was observed. This indicates that Cd inhibitory effect was more on chl a synthesis than on chl b. Thus the influence of Cd ions differed from that of Pb ions as reported by
Hanpp and Landszien (1972). Further the inhibition of the activities of the chlorophyll biosynthetic enzymes by Pb was also demonstrated in isolated chloroplast (Hanpp and Zeigler 1974).

In Euglena Defilippis et al. (1981 a) have observed a decrease in total chlorophyll and carotenoid levels in Cd, Hg and Zn treated cells. Further they have also observed a decrease in chl a/b ratio in Cd treated Euglena cells.

The decrease in total chlorophyll levels in Cd treated plants may be due to either inhibitory effect of Cd on the reduction steps of chlorophyll biosynthesis or due to enhanced oxidative steps. The former explanation may be possible, since Cd inhibition of reductive reactions in plant metabolism were reported (Bassas et al. 1974 b, Koepppe and Miller 1970 and Koepppe et al. 1973).

Cd inhibition of Photosynthetic generation of reducing power, NADPH, was demonstrated in isolated chloroplasts and intact cells of some fresh water algae (Miles et al. 1972). Defilippis et al. (1981b) and Vanduijven Dijk and Desmit (1975) have demonstrated that photosystem II associated electron transport is more inhibited than photosystem I activity in Euglena. NADPH oxidoreductase
inhibition by Cd was also observed. This results in the reduction of NADPH levels needed for reductive steps in photosynthesis.

DeFilippis et al. (1981 c) and Keck (1973) have demonstrated that the levels of ATP decreased while those of ADP levels are elevated by Cd treatment in Euglena. These results indicate that Cd inhibits the ATP forming reaction. This leads to the retardation of all ATP dependent processes.

Cd is known to cause other effects that directly or indirectly reduces the chlorophyll levels ultimately by inhibiting photosynthesis.

In general, inhibition of net photosynthesis in detached leaves and algae by heavy metals was well documented. It was suggested that the site of inhibition may be at carboxylation and reductive steps of Calvin cycle. Further Tripathy and Prasanna (1981) have also made confirmatory evidence by observing Cd damage, altering the structure of the thylakoid membranes of chloroplasts which results in the loss of chloroplast function.
It is interesting to note in the present investigation, an increase in chlorophyll to carotenoids ratio in Cd treated plants. This indicates that carotenoid synthesis is more inhibited than chlorophyll synthesis. However Defilippis et al. (1981 a) have observed a decrease in chlorophyll to carotenoid ratio in Euglena. The chlorophylls to carotenoid ratio may be better interpreted by observed Cd effects on photosystems and the distribution of pigments between photosystem I and photosystem II. Carotenoids as accessory pigments for trapping the solar energy are more associated with photosystem II than photosystem I.

From the literature it is evident that photosystem II activity is more affected by Cd than photosystem I, although both the systems exhibited reduced photochemical activities (Overnell 1975 a, Bazzaz and Govindjee 1974). So the carotenoid pigments more associated with PS II are more affected than chlorophylls (Overnell 1975 b).

The pigment synthesis may also be disturbed due to the reduced levels of chlorophyll synthesizing enzymes i.e. reduction in aminolevulinic acid dehydrase activity by Cd ions which compete with their active sites (Abdulla and Haeger Aronsen 1971). Heavy metal ions compete with
iron for a site on chlorophyll biosynthetic enzymes or may alter the subcellular or cellular distribution of iron in the leaf. The decrease in iron content of leaves by heavy metals including Cd may also reduce the total chlorophyll of leaves.

Starch is the most important and widespread reserve carbohydrate in seeds. This polysaccharide is mobilised into an available form for embryo. Two catabolic pathways of starch including hydrolytic process involving enzymes α-amylase and β-amylase, and phosphorolytic pathway involving the enzyme starch phosphorylase. Starch breakdown into simple sugars is very important during germination and growth of the seedling. In the present investigation control plants recorded decreased starch levels with an increase in reducing and non reducing sugar levels from 2 day to 6 day old plants indicating that starch is being hydrolysed into simple sugars. In Cd treated plants also a little increase in reducing and non reducing sugar levels was observed in 2 day to 5 day old plants when compared to control. This is also evident from the starch levels of treated plants. However the reduced levels of reducing and non reducing sugars in treated 6 day old plants may be due to the inhibitory effect of Cd on photosynthesis. The low levels of reducing and
non reducing sugars may also be responsible for the reduced growth rates in Cd treated plants.

Cd was found to have inhibitory effect on starch hydrolysis in Cd treated plants and the inhibition in breakdown of starch is dependent on Cd concentration. In 1.0 m.Bq Cd treated plants the breakdown of starch is more reduced than in control plants. This reduction is breakdown of starch may be due to the inhibition of amylase activity by Cd as heavy metals are known to inhibit the amylase activity. Zn\(^{2+}\) was found to inhibit the \(\alpha\) amylase activity in \textit{in vitro} studies of maize, wheat, barley and \textit{Bacillus subtilis} (Irshad et al. 1981). So the starch levels are high in treated plants than the controls.

Proteins are the most important and major reserve food material in the seeds. Proteins are hydrolysed into their constituent amino acids by proteases, and they are mobilised to the germinating seeds and utilised for the protein synthesis or to provide energy by oxidative deamination. So the proteolysis following the germination of seed is important for further growth of the seedling.
The protein content of cotyledons is very important because they are the primary sites of protein reserve. In the present investigation the protein content of cotyledon of 3 day old Cd treated plants was not much altered when compared to control. In 5 day and 7 day old plants the protein content of Cd treated plants was higher than the control though there was a common decreasing trend from 3 day to 7 day old plants. In the present investigation the protein levels were very high in 1.0 m.Eq/l of Cd treated plants over controls on the 7th day.

The same trend was observed in roots and leaves also. In roots the protein content was higher in 1.0 m.Eq of Cd treated 3 day old plants. The protein content decreased as the age of plant increased, but the protein levels were high in treated plants especially in 1.0 m.Eq, when compared to control. Protein levels in roots were 50% higher in 1.0 m.Eq of Cd treatments over control in 7 day old plants.

The common phenomenon observed in leaf protein content, was a decrease in protein levels in control and treated plants as the age of plant increases; but percentage of decrease was low in control over the treated plants, except in 7 day old plant at 1.0 m.Eq/l of Cd.
This type of significant increase in total protein levels were also observed by DeFilippis et al. (1981 a) in *Euglena gracilis*. Where the heavy metals Zn, Cd and Hg caused an enhancement in protein levels. Similarly Cd accumulation in protein fractions of soybean seed has been reported by Braude et al. (1980). This accumulation of Cd in protein may reduce the hydrolysis of protein during the germination of seed. Further the decrease in protein hydrolysis may be due to the inhibitory effect of Cd ions on the protein hydrolytic enzymes like proteases, and hence the higher levels of protein in the cotyledons of treated plants. Further, the reduced growth rates in roots may also be due to the decrease in protein hydrolytic reactions and decreased metabolic activities like respiration.

Cd binding proteins with properties similar to mammalian metallothionine have been isolated from the roots of Cd treated tomato plants (Bartolf et al. 1980) and from soybean tissues (Casterline and Barnett, 1977). This is similar to effects of Zn on *Chlorella* (DeFilippis and Pallaghy 1976) and Pb on mouse kidney tissue (Choi and Richter 1974), where heavy metals increased the levels of protein as well as the DNA and RNA contents of the cells.
From this it may be suggested that the Cd ions inhibit the activity of proteolytic enzymes like proteases, thus causing the reduction in amino acid production and protein synthesis which ultimately decrease the plant growth.