Chapter 5

Discussion
DISCUSSION

As the results of physiological and biochemical parameters of both experiments, laboratory and field trials, exhibited a comparable trend, the discussion and conclusion pertaining these are, therefore, combined, so as to present a coherent reading.

EFFECT OF CADMIUM, ZINC AND FLUORIDE ON GERMINATION COMPONENTS

The germination and growth of seedlings are affected by many unfavourable factors. Mayer and Mayber (1982) have discussed exhaustively the effects of environmental conditions on the germination. The germination of seed depends greatly on the uptake of water. The quality of water is an important factor controlling the germination. In the soil, heavy metals may be present or the effluents discharged from the industries into soil may contain heavy metals. The presence of some toxic heavy metals or fluoride may influence or affect the seed germination as these are not essential nutrient elements for the germination and for the subsequent seedling growth. While some other heavy metals like zinc, iron, manganese, and copper are considered to be essential for all higher plants.
In the present study, in the case of both experiments, cadmium- and fluoride-treated seedlings reduced the percent germination, shoot and root length, and fresh and dry weights. The percent moisture was also found to be reduced in the seedlings treated with either of these. Whereas zinc-treated seedlings/plants displayed the equivalent or higher growth as compared to the distilled water (DW) control (Figures 1-10, and 29-42).

The percent germination of seedlings treated with cadmium reduced considerably (Figure 1). The germination and growth of seedlings were affected by a number of unfavourable factors. Huang et al (1974) also found decreased percent germination with increasing cadmium concentration.

According to Wilkins (1978), tolerance of plant to a metal can be well assessed by measuring its root length. Breckle (1989) reviewed the effects of heavy metal on the root growth. The retarded or the complete inhibition of root growth at higher metal concentration was observed by Lyon and Beeson (1948) and by Rauser (1973). Relatively high level of cadmium significantly reduced root and shoot length in several oily seeds as was observed by Babygeya and Janardhanan (1989). The cadmium chloride treatment was highly inhibitory to the
extension growth of root, shoot, and seedling. An inhibition of root growth by heavy metals has been reported by Wilkins (1978) and Breckle (1989).

The shoot dwarfing has been reported due to heavy metals (Foy et al, 1978; Wang and Bradshaw, 1982). Delay in germination, reduced elongation of root and shoot, and low fresh and dry weight were observed due to the treatment with cadmium in mustard by Mishra and Seema (1992). The inhibition of seedling growth and its various parts by the cadmium treatment has also been reported by Snehlata (1983, 1990).

The retardation of growth maybe due to various reasons, such as change of cell wall permeability by the heavy metals (Luigi et al, 1981; Rai et al, 1981). Similar findings were also reported by Rajni et al (1989) in seeds of Triticum aestivum germinated in different concentrations of cadmium. Increase in the elongation of cell depends largely on the cell division. Cadmium chloride is known to cause impaired nuclear division and to inhibit cytokinesis, and therefore in the absence of cell division, there would not be any increment in the cell number. The result was the reduced root and shoot elongation as observed by Vaulina et al (1978).

The extension growth increased steadily in the control as well as in the zinc-treated seedlings, in both
the experiments (Figures: 2-4, 29, and 30). Tsui (1948) and Nason (1950) observed that zinc is necessary for the biosynthesis of indole acetic acid (IAA) through its involvement in the tryptophan synthesis. IAA is known to cause cellular elongation by increasing the cell wall elongation, by way of increasing the cell wall plasticity for the incorporation of cellulose molecules. However, Nag et al (1984) reported the inhibitory effects of zinc on the seedling growth.

Cadmium has been shown to be taken up by the plants and to be toxic to them (John, 1972; Page et al, 1972; Haghiri, 1973; Turner, 1973). The seedling treated with cadmium showed the reduced fresh and dry weight, and moisture content (Figures: 5-10, and 33-42). Rajni et al (1989) reported the decreased fresh and dry weight in wheat germinated in the different concentrations of cadmium. An inhibition of fresh and dry weight of maize seedlings grown in cadmium was also reported by Nassbaum et al (1988).

The emergence of root or shoot primarily depends upon the availability of water as well as on the uptake of water by the seeds. One of the causes of a reduction in seedling length is due to the decreased percent moisture as induced by the treatment with cadmium chloride. Vyas and Nagoor (1991) reported the lowering in percent germination in cadmium-treated seedlings. The
elongation growth of seedling depends on the water content. The cells have to become turgid before they can extend or expand, and only then the emergence can take place. Since the water content itself was very low, root and shoot emergence were highly inhibited (Figure: 1). As a result, fresh and dry weights reduced. Decrease in the dry weight with the increasing concentration of cadmium was reported by Someni et al (1987).

In both the experiments, seedlings treated with zinc showed higher fresh and dry weight, and percent moisture (Figures: 5-10, and 33-42). Zinc functions as a trace element in the general metabolic activities of plant. Root and shoot elongation primarily depend upon the uptake of water, which in turn depends on various osmotica such as sugars, amino acids including proline, and proteins, which are present in the cytoplasm. Zinc appeared to maintain isotonic condition and thus regulated the water uptake.

The increased fresh and dry weight reflected the metabolic status of zinc-treated seedlings. There was optimum hydrolysis of metabolites due to adequate water content and proper mobilization of food reserves and other metabolites. These metabolites were utilized for the seedling growth. Aery and Sarkar (1989) studied the effects of zinc on soyabean. The lower doses of zinc
(10-25 μg g⁻¹) were found to be promotory for the growth. Zinc is necessary for the healthy growth and reproduction of several species of algae as reported by Noda and Horiguchi (1971).

The treatment with sodium fluoride, in both the experiments, reduced shoot, root, and seedling length considerably (Figures: 2-4, and 29-30). The percent moisture was also lowered in the treated seedlings. Perhaps due to very low uptake of water, shoot and root emergence and elongation were affected negatively.

Since one of the conditions for the germination and an important step in germination is the hydration of water by the imbibing seeds; when the particular threshold is reached in moisture content in seed, then and then only the cellular processes in the seedling are activated. Sodium fluoride had a two-fold toxic effect, one that of sodium and other that of fluoride. Both show a negative relationship with the water content. The solution became hypertonic at the concentration used, and thus, retarded the water uptake.

The negative influence of fluoride on plant has been discussed by many workers (McCune, 1969; Treshow, 1970; Ballantine, 1984; Mejstrik, 1985). Ballantine et al (1979) reported a decrease in fresh weight of bean leaf and pea epicotyl treated with fluoride. The data on
fresh weight of seedling also reflected the percent moisture, which was reduced by sodium fluoride (Figures 5-10, and 33-42). The fresh weight of any seedling or plant organ depends upon the uptake of water. Since the uptake of water was highly reduced, the fresh weight too reduced. This findings are supported by those reported by Ballantine (1984), who showed a decrease in water uptake, water content, fresh weight, and stem elongation of 17-day old pea shoots treated with 1 mM potassium fluoride for 24 hours.

The reduction in dry weight in sodium fluoride-treated seedling indicated a disturbed metabolism, as the dry weight reflects an overall metabolic status of a seedling. Synthesis of any compound during germination is one of the contributory factors to the dry matter accumulation which, in case of sodium fluoride, seem to be at a lower level and so very low dry weight was recorded. The results are in agreement with those reported by others (Treshow, 1970; Murray, 1984; Sakurai et al, 1986; Pandey, 1991).

The reduction in fresh and dry weight is directly reflected in the percent moisture in the seedling. The reduction in moisture may be due to the reduced water uptake. The diminished water uptake in the potassium fluoride-treated pea shoots was reported by Ballantine (1984).
Vijaywargiya and Pandey (1991) pointed out disturbances in the growth of sorghum seedlings treated with sodium fluoride as indicated by decreased root and shoot length, as well as fresh and dry weight. Chang and Thompson (1966) also showed that fluoride accelerated ageing in corn roots, and inhibited the root growth. Lai and Ambasht (1981) have shown a decreasing trend in the germination of *Leonatis nepatifolia* due to the harmful effects of sodium fluoride.

**EFFECT OF CADMIUM, ZINC, AND FLUORIDE ON CARBOHYDRATE METABOLISM**

*Total Sugar, Reducing Sugar, and Invertase Activity:*

During the germination, the stored energy sources such as starch, lipids and protein are broken down to provide the energy as well as the basic components for the anabolic activities. The growth and development of radicles and primary leaves, for example, depend on the hydrolysis of these energy-releasing compounds. Carbohydrates are the most important energy sources present in the plants. They act as the storage material in cereals and legumes. Sugar, which is one of the readily available polysaccharides, is found very commonly in all living beings.
The total sugar content of cotyledon and of embryo axis increased with the germination hours (Figures: 13-14). Similarly, the total sugar content increased with the growth period, but fell after 60 days (Figure: 58).

In both the experiments, seedlings/plants treated with cadmium showed higher sugar content than that of control. Seedlings require sugar for the maintenance of their water balance; uptake of water is mainly because of the solutes present in the cell and sugars, being osmotica, are involved in the osmo-regulation. Under the adverse environmental condition, like CdCl$_2$, the imbibition may be possible due to the presence of such substances as sugar. The sugars are also required to provide the substrates for the increased respiration to survive and grow under the metallic stress. So, the gradual increase in total sugar content in cotyledon and embryo axis of all seedlings was observed (Figures: 13-14).

Once the root or shoot emerges, for the further elongation, auxin-induced cell elongation requires an incorporation of cellulose microfibrils. These are available only from the glucans and other polysaccharides. An increased total sugar and decreased reducing sugar content in seedlings/plants treated with
cadmium suggested no release of reducing sugars from the total sugars. The lower level of reducing sugars in cotyledon and embryo axis of seedlings treated with cadmium during germination and seedling growth resulted in the reduction of seedling length.

The activity of invertase increased with the germination hours in treated as well as untreated control seedlings/plants. A decrease in the invertase activity in seedlings/plants treated with cadmium may be on account of the metal stress, an utilization of sugar in the preceding germination hours. Alternatively, it may also be suggestive of accumulation of total sugar due to the depressed invertase activity so that these can be utilized during further seedling/plant growth. The heavy metals are known to be inhibitory to the enzymes; a lowered invertase activity indicate its inhibition and corresponding accumulation of the total sugars not yielding reducing sugars.

Huang et al (1974) studied the inhibition of metabolism by cadmium in soyabean. Rajni et al (1989) reported the decreased invertase activity in wheat seedlings germinated in cadmium. Similar results were also reported by Beri et al (1989). The α-amylase activity in Phaseolus aureus was inhibited by cadmium (Snehlata, 1991). Vyas and Nagoor (1991) also noted the lowered amylase activity and high sugar content in maize.
seedlings treated with cadmium chloride. They also noted that starch content was more in the seedlings treated with higher concentrations of cadmium. The heavy metals suppressed glucose-6-phosphate dehydrogenase activity in yeast (Truzuki and Yamada, 1979). Setia et al. (1989) reported the influence of cadmium nitrate on the growth and reproductive behaviour of pea plants. The biochemical analysis of harvested pea plants indicated the reduced level of total sugar and starch by cadmium nitrate.

A gradual increase in the reducing sugar content was observed in control and treated seedlings (Figures: 14-15). The reducing sugar content in control as well as treated plants increased with the growth, declining after 60 days (Figure: 59). The reducing sugar content in the seedlings/plants treated with cadmium was always less than that of the control. These observations are supported by the findings of Greger and Lindbergh (1986). As explained earlier, a decrease in reducing sugar content and an increase in the total sugar content suggest that total sugars were not hydrolyzed optimally in order to maintain the osmotic relation, turgor pressure, and water balance in the plant cells so as to allow optimum solute level for the cellular growth and development under the stressed condition. The total sugars are hydrolyzed into glucose and fructose, from
which the cellulose synthesis takes place, especially from glucose. Glucose may also be oxidized to provide the respiratory substrates and, thereby, energy to the cells and the tissues as already discussed.

Zinc plays an important role in the carbohydrate metabolism (Singh, 1969; Kapur et al, 1977; Sakal et al, 1980). The total sugar content, reducing sugar content, and invertase activity increased in seedlings and plants treated with zinc (Figures 13-16, 23-24, 58-59, and 65). Initially the total sugar content was high in both seedlings and plants, as compared to other treatments. But, as germination/growth period advanced, it reduced. An initial high total sugar content in the zinc-treated seedlings/plants indicate its requirement for the initiation of germination. A decreased content afterwards indicate its utilization by various metabolic processes.

An increased invertase activity in the zinc-treated seedlings and plants indicated that more and more total sugars are converted into the reducing sugars. Higher levels of reducing sugar were found in the zinc-treated seedlings/plants.

The seedlings/plants treated with sodium fluoride showed the similar pattern as that of cadmium. Fluoride inhibits the metabolism of carbohydrates.
Yu et al (1988) suggested that fluoride exerts a strong influence on the energy metabolism of germinating seeds. They, therefore, mentioned that seedlings exposed to fluoride contain more sugars than the control. It may be, thus, concluded that the observed accumulation of sugar may be due to an impaired utilization of glucose produced during the events of germination. This may also be considered as an adaptive mechanism executed by the plants in order to maintain the osmo-regulation. The present observations are in agreement with the above findings. In contrast to this, Lustinec et al (1962) and Ordin and Shoe (1963) showed a decreased sugar content in the HF-treated plants.

A decrease in the activity of invertase in NaF-treated seedlings/plants might also imply a good convergence with the reduced content of reducing sugar.

Vora and Bhatnagar (1986) studied the sugar content in the leaves of nine tree plants growing in high and low polluted industrial areas of Ahmedabad. The team observed less total and reducing sugar contents in the leaves of plants growing in highly polluted areas. They observed that the low levels of sugars in the leaves was due to the low rate of photosynthesis.
The control as well as the treated plants showed a decline in their total and reducing sugar contents during the terminal growth stage (senescence) (Figures: 58-59). The invertase activity also reduced. A decrease in the total sugar might be due to higher respiration rate during the senescence (Kao, 1988). Nooden and Leopold (1978) suggested that the senescence may be correlated with a lowering in the hydrolyzing enzyme activity like invertase. The source of sugar is photosynthesis, which is lower in the senescing leaves (Nooden, 1986). The reduced chlorophyll level, i.e., the photosynthetic activity, might decrease the reducing sugars in the leaves. A decrease in the reducing sugar content also suggests the nutrients being diverted from leaf to the reproductive structures, developing fruits, etc.

In all the treated and untreated seedlings, higher content of total sugar, reducing sugar, as well as higher invertase activity were found in their embryo axis than the cotyledon. This is because embryo axis is a growing system requiring, at each stage of its growth, energy. This rising demand for energy is met with by a higher turn over of carbohydrate metabolism. But cotyledons being storage organs, their turnover is less and, eventually, metabolites pass on or translocate to the growing embryo axis.
Protein, Amino Acids, Protease Activity:

Proteins are the basis of all life activities because protoplasm being 90% of any cell, and also all the reactions of living systems are catalyzed by enzymes which are protein in nature. An extremely wide variety of proteins found in nature and are made up of sequences of amino acids linked together by peptide bonds. The source of amino acids for protein synthesis varies from tissue to tissue and according to the physiological status or condition of the tissue in which protein synthesis is taking place. In the developing seedlings, some amino acids are translocated to the growing tips from the storage tissue of endosperm or cotyledons. Much protein synthesis takes place in developing as well as in meristematic tissues.

In both the experiments, protein content increased with increase in germination/growth period in all, treated and control seedlings/plants (Figures: 11-12, and 56). Under the field conditions, protein content declined after 60 days of growth.

Green gram is a pulse crop. A higher protein content in control seedlings indicated more of its synthesis. The better seedling growth might be
correlated with the higher protein content.

The cadmium-treated seedlings showed less protein content as compared to the control. A reduction in protein content due to the cadmium treatment has also been observed by many workers (Siegel, 1974; Maitra and Mukherji, 1979; Jastrow and Koepp, 1980). A reduction in the growth of seedlings treated with cadmium may be due to the reduction in metabolic activities such as protein synthesis and others which were necessary for the growth and development of the seedlings. Nag et al (1989) reported a decrease in the soluble proteins in the leaves of pea plants treated with cadmium. Beri et al (1989) reported a decreased protein content in lentil seeds germinated in different concentrations of cadmium.

The seedlings as well as plants treated with zinc showed higher protein content compared to the control (Figures: 11-12, and 56). The protein content increased with germination/growth period, which could be correlated with a gradual increase in their fresh and dry weights. This could also be correlated with a gradual increase in the root and shoot length with the growth period in both the experiments. The extension growth of seedlings and plants requires a continuous supply of the cellular skeletons including protein, amino acids, sugars, etc.
Gangadhara et al (1989) reported an elevated protein content in the capitulum of sunflower grown in the soil supplemented with zinc sulphate. Zinc in low doses showed an enhancement in protein contents. Srivastava (1979) reported similar results in barley seeds treated with 400 ppm zinc sulphate. A lower level of zinc increased protein content up to some extent (Singh and Shrotriya, 1989).

Agrawala et al (1978) observed decreased concentration of soluble protein in leaf tissue extracts deficient in zinc. In the zinc-deficient plants, a decrease in protein content was also observed (Fujiwara and Tsutsumi, 1959; Naik and Asna, 1961; Prakash, 1965; Cocucci and Rossi, 1972).

The protease activity increased with the germination/growth period (Figures: 21-22, and 62) under the laboratory and the field conditions, both in control and the treated seedlings/plants.

The proteases are the enzymes which react with protein to break the peptide bonds and so release free amino acids. The higher protease activity in the control seedlings as well as plants during the early growth period, suggested a higher metabolic activity or turnover. A promotion in their growth may be correlated with their higher protease activity. The higher protease
activity might provide more amino acids, which in turn may account for the better growth of seedlings as evident from the seedling length and weight.

A reduced protease activity during the early growth period with as induced by the treatment with cadmium suggest a reduction in metabolic activity. As a result of which, growth and development were affected adversely. Shehlata (1990) showed that cadmium suppressed the activity of hydrolytic enzymes, protease and amylase, in Pisum sativum. Rajhi et al. (1989) reported an inhibition in protease activity in wheat seedlings germinated in cadmium. Similar results were also reported by Beri et al. (1989).

As the germination period proceeded, a higher protease activity was found in the cadmium-treated seedlings and plants as compared to the control. Vyas and Nagoor (1991) reported a higher protease activity in the seedlings treated with different concentrations of cadmium chloride. A higher protease activity indicated more breakdown of protein, hence, the lower levels of protein were found in the cadmium-treated seedlings/plants than that of control.

The seedlings treated with zinc showed higher protease activity during the initial germination stages compared with that of control. A higher protease
activity resulted in the enhanced liberation of free amino acids, which were then available for the growing embryo axis resulting in the better and the rapid seedling growth.

The protease activity declined after some period of time, indicating then less breakdown of protein resulting in more protein content in such seedlings treated with zinc.

There was a gradual rise in amino acid content in cotyledons and embryo axes of all seedlings grown in laboratory (Figures: 19-20). In the field, a progressive ascent was also seen (Figure: 60), but after 60 days it declined.

Initially, the amino acid content was higher in the control seedlings than that of cadmium-treated one. In green gram, protein is a major seed reserve. During the germination, proteins in cotyledon are hydrolyzed and the product, i.e., amino acids, are used up for the growth of embryo axis. A decreased protease activity in the cadmium-treated seedlings during an early germination period led to a less breakdown of protein, hence the amino acid content remained very low, as a result of which seedling growth was affected. Whereas, owing to a higher protease activity in the control seedlings during these periods, higher amino acids levels were maintained
leading to a healthy or a normal seedling growth. The promotion of seedling growth might, thus, be correlated with more nutrients like amino acids. Beri et al. (1989) reported a decrease in amino acid content in lentil seedlings germinated in various concentrations of cadmium. Setia et al. (1989) also reported comparable results.

During the later stages of growth, the amino acid content of cadmium-treated seedlings/plant rose above that of control. There was a concurrent rise in the protease activity in the cadmium-treated seedlings. This indicates an inadequate utilization of free amino acids which are generated in more quantity by the increased protease activity. An accumulation of amino acids also point out to their osmo-regulatory role under the metal stress. Leonard et al. (1966) reported an accumulation of putrescine in cadmium-treated oat and bean leaves. Such accumulations may be considered as the induction of stress in the cadmium-treated plants. The accumulation of amino acids in comparatively old seedlings reflect the stress condition and it might be the cause of survival of seedlings under the stress condition. The reduced amino acid content in control seedlings and plants implied their proper utilization in the cellular activities.
A similar trend in the content of amino acid was also noticed in the seedlings and plants treated with zinc as seen in the control. An increased amino acid content during the early germination/growth period in the zinc-treated seedlings/plants was observed. An increase in amino acid content with zinc treatment showed its promotory effect by increasing the synthesis of amino acids. The favourable influence of zinc on growth and yield of soyabean and lentil was studied by Kapur et al (1977) and Sakal et al (1980).

The increased amino acid content in the zinc-treated seedlings/plants might be advantageous to the seedlings/plants, as some of these amino acids, like proline, are known to cause or increase the hydration of protoplasm. The sulphur-containing amino acids, like methionine, cystine and cysteine, maintain the redox potential. Secondly, tryptophan may be readily available for the IAA synthesis, because zinc is known to be involved in the IAA synthesis. The synthesis of protein and an activation of oxidative processes and the enzymes are also known to be affected by zinc as a micro-nutrient. Kapur et al (1977) and Sakal et al (1980) reported the increased amino acid in zinc-treated seedlings.

The amino acid content of zinc-treated seedlings was lower as compared to that of the control.
during the later phase of germination, which indicate a proper utilization of amino acids in the various metabolic processes as a result of which an enhanced growth was recorded in such seedlings or plants.

The seedlings/plants treated with sodium fluoride showed a reduction in their protein content when compared to that of the control. The decreased protein content due to the NaF treatment might be on the account of hydrolysis of protein into amino acids providing them to a reserve pool under the condition of stress. Initially, protease activity was low and increased during the later stage of germination, this resulted into more breakdown of protein into amino acids.

These findings are supported by the results of Godzik and Linskens (1974). A higher protease activity during the later stages of germination resulted into increased de novo synthesis of new type of proteins. It has been proved that the plants acquire resistance to the stress by way of synthesis of new or novel proteins (Genkel, 1970). A less protein content in the NaF-treated seedlings might be connected with a higher activity of protease at the later stages of germination, and that enhanced protease activity would be responsible for the breakdown of the proteins into amino acids.
Vijaywargiya and Pandey (1991) have reported that an increase in NaF concentration as well as an increase in the exposure time of sorghum seedlings would decrease the amount of protein. Chang (1970) also showed that NaF caused a decrease in the size of both, free and bound, ribosomes, as well as a decrease in their protein and RNA contents. Pandey and Rao (1980b) expressed the similar views. They showed that the amounts of carbohydrate and protein were considerably reduced in the HF-exposed *Gladiolus* plants. Cecil and Wake (1962) suggested that the reduction in the protein content is due to an inactivation of enzymes responsible for the protein synthesis.

Bhatnagar (1986) indicated the possibility that the reduced photosynthetic activity might alter the protein accumulation in plants. Constantinidou and Kozlowski (1979) also concluded that the decreased protein content was due to the lowered photosynthesis.

Reddy (1984) reported an accumulation of amino acids in the seeds of bajra (pearl millet) under the adverse conditions. Arndt (1970) showed the destruction of enzyme responsible for the protein and amino acid synthesis in the HF-treated plants.

The leaves of control and the treated plants displayed a decline in their protein and amino acid
contents during the terminal (senescence) growth stage. The activity of protease also reduced considerably. The leaf protein breakdown in the senescing plant has been reported by a number of workers (Wareing and Phillips, 1978; Thimann, 1986; Sexton and Woolhouse, 1984; Nooden, 1986). The reduction in protein level may be the result of the reduced protein biosynthesis in the senescing leaves (Nooden and Leopold, 1978).

ELECTROPHORETIC STUDIES

The protein and amino acids are essential biomolecules for the various cellular structures and the physiological processes as well as for the enzymatic reactions. Seeds have the reserve of protein, which is largely depleted during the germination and early seedling growth, in order to supply proteins and amino acids for the de novo synthesis of various proteins. Hence, it is not expected of protein levels to remain apparently steady during germination and early seedling growth. The free amino acids in cell constitute a 'pool' from which they may be drawn out and used for the synthesis of new polypeptides, and eventually the proteins as and when required.

Although, the total protein content of seed/seedling may not change, yet the synthesis of new
proteins may go on, and hence the type and kind of proteins may change from time to time.

Thus, the quantification of total protein content will not reveal such subtle qualitative as well as quantitative changes in the proteins taking place during the growth and development of young seedlings. In fact, the total protein content may remain constant, allowing qualitative changes to take place. And here electrophoresis may come in the picture as a tool to decipher or comprehend the qualitative variations in protein during the growth and development of seedlings as well as among the treated seedlings.

Hence, keeping these points in mind, an attempt was made to study the qualitative changes occurring in proteins during the growth and development of seedlings grown in the presence of test solutions employing the disc gel electrophoresis.

The Rf value of separated protein bands so obtained were calculated and based on this they were approximately categorized as: higher molecular weight (45.0-66.0 kD*), high molecular weight (29.0-45.0 kD), low molecular weight (14.2-29.0 kD), and lower molecular weight (below 14.2 kD) by comparing the Rf values of each

* kD = kilo Dalton
band with that of the standards, i.e., proteins of known molecular weight. A distinct pattern of distribution of proteins emerged with the progress in germination hours and with respect to the distilled water (DW) control and treatments of cadmium, zinc and fluoride.

As evident from the data (Tables: 5-9, Plates: 3 and 4), there was variation in the number of protein bands with regard to the germination hours. A gradual increase in the number of bands was clearly seen in all four cases.

The seedlings treated with distilled water (control) and with zinc showed increase in the number of bands initially, but at the later stages of germination, the number of bands remained steady. While those seedlings treated with cadmium and fluoride showed much more difference in their number of protein bands, some in number increment did take place especially during the later phase of germination. This might be due to a delayed germination and growth in the cases of cadmium and fluoride treatments.

The high molecular weight (HMWt) proteins fall under the peptide polymer group being a mixture of more than one polypeptide chain aggregates stabilized by various inter- and intra-chain interactions. These proteins have typically low electrophoretic mobility (Rf)
as they offer more resistance during the separation because of their high molecular weight and bulky size.

The low molecular weight (LMWt) proteins are characterized by more solubility and high Rf due to smaller size and the less resistance they offer during the electrophoretic run. An increase in number of bands, therefore, indicate that there were such polypeptide aggregates (polymeric polypeptides) composed of LMWt polypeptides.

In the cases of cadmium- and fluoride-treated seedlings during the germination period, it was noticed that the number of HMWt proteins bands decreased, and these were not found at, the terminal phase of germination, while the LMWt proteins increased in number. This may be explained in terms of partial breakdown of HMWt proteins into the LMWt proteins to meet the requirements of growing seedlings under the condition of metal stress.

These findings support the fact that continuous turnover of HMWt and LMWt proteins takes place during the germination and early seedling growth. Further, these also imply that the turnover of HMWt and LMWt proteins was influenced considerably by extension of the period of germination.
The data (Tables 5-9, Plates 3 and 4) show that there were less number of total protein bands during the early germination phase, which indicates that the seed had very few protein types as the reserve. The subsequent increase in the number conveys that the synthesis and accumulation of new proteins and/or the alterations of protein chains took place actively and these accumulated proteins would be utilized during the approaching germination hours.

Steward et al. (1965) studied the extractable soluble proteins from pea seedlings through electrophoresis in relation to different organs of seedlings and plants. They learnt that the changes occurred in the protein complements of the same plant organs as they developed. Similarly, Moore (1989) inferred that the observed differences in the enzyme proteins were the cause of differentiation.

Nebu (1989)), through electrophoretic separation of soluble proteins, showed that the total soluble proteins decreased with the advancement in germinating and growing radish (Raphanus sativa L.) seeds and seedlings. Based on these observations, he suggested that the larger protein molecules were being broken down to a degree into the smaller proteins increasing the concentration of LMWt proteins and thus maintained a continuous turnover of both HMWt and LMWt proteins.
Rathod (1992) showed that the treatment of Rhizobium inoculum in combination with GA$_3$ and IAA to alfalfa plants changed the protein assembly or its buildup in the leaves during the different stages of growth and development.

Thanki (1992) demonstrated with the help of electrophoresis that the treatments of seeds with different plant hormones distinctly resulted in the altered characteristics of proteins indicating the influence of externally supplied hormones on the turnover of proteins during the germination of cereal seeds. While Thanki (1993) evinced that the developing and maturing seeds of pigeonpea (*Cajanus cajan*) displayed a distinct pattern of protein bands separated during electrophoretic run.

Thus, the electrophoretic separation of the soluble proteins elucidated the major changes in enzyme proteins which were not adequately reflected in the biochemical quantifications of the total protein during the course of study.
EFFECT OF CADMIUM, ZINC AND FLUORIDE ON PHENOL, AND PEROXIDASE AND IAA OXIDASE ACTIVITIES

Phenol:

The phenol content of control and treated seedlings/plants increased with germination/growth period (Figures: 17-18, and 57). The control and zinc-treated seedlings showed higher phenol content during the initial germination stages, only a slight increase took place in the case of cadmium and fluoride at the later stage.

There are mainly two types of phenols participating in various metabolic activities: the inhibitory phenols and the promotory phenols, depending upon whether they have inhibitory or promotory effects on the growth and development. The o-diphenols enhance the growth by reducing IAA oxidase activity (Nitsch and Nitsch, 1962); on the other hand, phenol like p-coumaric acid is a cofactor for IAA oxidase, which thus may enhance the activity, and thereby may inhibit the growth (Gortner et al, 1958).

The phenols are the secondary metabolites which influence the growth by altering growth stimulating activities of phytohormones such as gibberellins, auxins, and cytokinins.
A high phenol content during an early growth period in the control and zinc-treated seedlings/plants may be due to a higher metabolic activity and growth promotory effect of phenols. It has been clearly demonstrated that certain heavy metals are capable of causing the reduction in activity of several enzymes and the stimulation of some others in a germinating seed (Mukherji and Roy, 1978; Naq et al, 1981).

A high phenol content during the terminal growth phases in the cadmium- and fluoride-grown seedlings/plants may demonstrate the inhibitory effects of phenols. This could be correlated with the seedling/plant length, which was greatly reduced in these cases (Figures: 2-4, and 29-30).

An influence of the aerial pollution on an accumulation of phenols in the leaves has been reported by several workers (Koukol and Dugger, 1967; Menser and Chaplin, 1969; Howell et al, 1971; Howell and Kramer, 1973; Howell, 1974; Agrawal et al, 1982). Tingey (1974) suggested that an increase of phenols in the leaves was associated with a concomitant increase in the level of soluble carbohydrates, which was in partial agreement with the results obtained in the present study as evident from the content of total sugars (Figure: 58).
Peroxidase:

The peroxidase activity increased with the germination/growth period (Figures: 25-26, and 64). The activity was high in the seedlings/plants treated with zinc and distilled water as compared to that of treated with either cadmium or fluoride.

Peroxidase is one of the several important enzymes involved in the process of differentiation with multiple roles. Its primary function is to oxidize hydrogen peroxide, which, if allowed to accumulate, is toxic. It is associated, directly or indirectly, with the various physiological processes, including the IAA degradation, lignin formation, respiration, differentiation, ripening, and the senescence (Gasper et al, 1982). A reduction in the peroxidase activity in cadmium and fluoride treated ones resulted into the poor or less differentiation and growth.

The peroxidase activity is important for the seed germination and seedling growth, as it degrades H$_2$O$_2$ formed during many biochemical processes. A high peroxidase activity in the control and zinc-treated seedlings indicated an instant degradation of H$_2$O$_2$, and peroxide could not accumulate, thus seedlings grew in a better way. While the reduced peroxidase activity in the cadmium- and fluoride-treated seedlings was evident of the accumulation of H$_2$O$_2$, an inarguable sign of the
stress, and the subsequent damages caused by $H_2O_2$, including the impaired seedling growth.

The level of activity of peroxidase may, thus, be correlated with the instantaneous degree of seedling growth taking place. Siegel and Galston (1967) verified this and demonstrated a positive correlation between the peroxidase activity and the growth rate of the stems of pea plant. Rai et al (1981) demonstrated that many of the physiological and biochemical processes, viz, respiration, photosynthesis, protein synthesis, chlorophyll synthesis, etc., were severely affected at high metal concentration.

Altman et al (1966) observed that the higher rate of peroxidase activity might be due to the higher rate of respiration. One of the most important functions proposed for peroxidases was their ability to oxidize IAA (Stonier et al, 1979). Malik and Singh (1980) stated that the peroxidases are widely distributed in the plant tissues and are physiologically very important owing to their association with numerous catalytic functions.

Lamport (1986) established that the peroxidases are important components of cell walls engaged in different plant mechanisms. The peroxidases are also involved in the induction of new cell wall synthesis (Gasper et al, 1982; Sequeira, 1983). This might be an
important defensive response to the attack by pathogens, or impart resistance to any kind of stress.

Lhoste (1979) revealed the fluoride-induced increase in peroxidase activity in tobacco leaves, and increase in the activity as the leaves aged. Lee et al (1965) also stated that a number of enzymes were inhibited by fluoride, while others, such as glucose-6-phosphate dehydrogenase, catalase, peroxidase, and cytochrome oxidase, were stimulated.

**IAA Oxidase:**

The activity of IAA oxidase increased with the germination/growth period (Figures: 27-28, and 65). Initially, a high IAA oxidase activity was found in the seedlings/plants treated with zinc and in control, as compared to that of the cadmium- and fluoride-treated ones.

IAA (indole-3-acetic acid), being a plant hormone, plays a substantial role in the early seedling growth and the extension growth of all plant organs. It is required to enhance the incorporation of cellulose particles. It is, therefore, a prime need of any extending tissues for their extension or elongation. IAA oxidase, a polyphenol oxidase, catalyzes the oxidation of IAA or its synthesis.
IAA is essential for the elongation during the initial phases of germination. A higher IAA oxidase activity in the control and in zinc-treated seedlings/plant signifies higher IAA content. The initial low IAA oxidase activity in the cadmium- and fluoride-grown seedlings/plants, and its subsequent rise with the growth are indicative of lower IAA level, as it remained unoxidized and was allowed to be resynthesized so that it can be utilized during the subsequent growth processes. This may be an adaptive strategy for the seedlings.

The substrate for IAA oxidase activity is IAA. It is known that the stress condition reduces the levels of growth promoters like IAA, gibberellins, etc., and enhances the levels of growth inhibitors like abscisic acid. Initially, cadmium and fluoride induced the stress condition, which in turn decreased the IAA synthesis and, therefore, its concentration, and as IAA was inadequate, the activity of IAA oxidase then decreased. Thus, a decreased seedling growth may be a result of lower IAA content.

It was postulated that some heavy metals, which are known to inhibit enzyme activities by way of forming a metal-protein complex, particularly in vitro system, actually induce or activate some new enzymes in the
Nag et al. (1984) showed that the activities of peroxidase, IAA oxidase, and ascorbic acid oxidase in seedlings increased in response to the addition of zinc.

IAA oxidase, being protein in nature, is highly influenced by the metal treatments. A higher IAA oxidase activity was found in the seedlings exposed either to cadmium or to fluoride during the late germination. This suggests more breakdown of IAA causing lesser growth, as the incorporation of cellulose microfibrils lowered in response to the reduced IAA level. This was evident from the reduced shoot and root length.

GROWTH INDICES

The Net Assimilation Rate (NAR, Figure: 43), an index of the productive efficiency of plant in relation to the total leaf weight, was high initially, in the control and the zinc-treated plants than that of cadmium- and fluoride-treated plants. An increased NAR for such plants indicate their higher ability to assimilate the photosynthates, as zinc is a micro-nutrient and a cofactor for a variety of enzymes.

The rate of apparent photosynthesis of the whole plant over a long period can be estimated
indirectly from the rate of dry matter accumulation by the whole plant and from the rate of growth in the leaf area. The term, Net Assimilation Rate, refers to this indirect estimate; it is an important part of the growth analysis approach (Watson, 1952).

The NAR, on a leaf area basis provides a measure of the apparent photosynthesis of the leaves minus the respiratory losses from the rest of the plant. Friend (1966) showed that the assimilates are translocated from the leaves to other parts of plant and are then used for the growth of leaves and tiller production.

Singh and Rao (1986) found a distinct relationship between the NAR and the chlorophyll content while evaluating the growth and productivity of *Phaseolus aureus*. Newman (1962) also noted a decreased NAR in the HF-exposed plants due to a reduced photosynthesis and an enhanced respiration rate.

In the present study, too, there was a reduction in the chlorophyll content in the cadmium- and fluoride-treated plants. Hence, it could be argued that lowering the assimilative capacity of the cadmium- and fluoride-exposed plants was primarily due to a decreased chlorophyll content of the leaves.
A higher NAR was recorded in the plants grown in the presence of either cadmium or fluoride during the terminal phase of vegetative growth. This might be considered to be an adaptive strategy on the part of a plant to survive under a strong metal stress as more sugar, i.e., a hexose, is produced by the photosynthesis, which can be a source of energy.

The Leaf Weight Ratio (LWR, Figure: 44), an index of leafiness of the plant on a dry weight basis, and a measure of the 'productive investment' of the plant as it deals with the relative expenditure on potentially photosynthesizing organs, declined uniformly as the growth advanced. The LWR represents the degree of distribution of photosynthates between the leaves and the rest of the plant.

The plants treated with cadmium and fluoride had a lowered NAR in comparison with the other two. This growth index points out that less photoassimilates were available, as a result of which, the LWR also remained low during the partitioning of energy resources for the production of new shoots, for the expansion of new leaves, for the elongation and development of stem.

Although the NAR was high in the cadmium- and fluoride-grown plants, the RGR was less as these plants were under the chemical stress, a diversion of
assimilates for the dry matter accumulation did not take place; instead it may be that hexoses were used up in the respiration.

The Relative Growth Rate (RGR, Figure 45), Blackman's 'efficiency index', an expression of growth in terms of a rate of increase in size per unit of size, declined with the growth period. Cadmium and fluoride reduced the rate slightly initially and remarkably in the end as compared to others. This reduction in the RGR could be attributed to the chlorophyll degradation. The RGR provides a measure of comparing the efficiency of growth of plants of widely different size and can be separated into a morphological and physiological components (Hughes, 1962). Both, RGR and NAR, always decline with the time periods.

The RGR decreased progressively, because the photosynthates were being diverted for the various metabolic activities. Several workers have reported a fluctuating trend in the RGR (Friend, 1966; Ryle, 1966; Vora et al, 1975). They also observed a steady decline in the RGR as also observed in the present case.

The Biomass Duration (BMD, Figure 46), an approximate measure of the stands vitality, which takes into account how much dry weight develops, and how long it lasts, increased with the growth substantially. The
treatment with zinc enhanced RGR significantly and consistently. In contrast, cadmium and fluoride decreased it. The former caused much decrease.

The partitioning of dry matter (DM) biomass accumulated as a result of the photosynthesis into leaf, stem, or root organs of plant. A higher BMD in the zinc-treated and the control plants indicate their enhanced photosynthetic activity. As a result of this, accumulation of DM was higher and the DM was retained longer. While the plants treated with either cadmium or fluoride displayed a lower BMD indicating a reduced photosynthetic activity of these plants. Srivastava (1979) found that the DM accumulation and the synthesis of various amino acids increased when zinc was supplied externally.

**YIELD PERFORMANCE**

The variations in the yield of a number of crops have been evaluated by a number of workers, and the yield parameters vary greatly from species to species. The response of a plant to a metal is dependent on the concentration of metal, the environmental factors, and the stage of growth/development, as well as on the resistance characteristics of that particular species; the response may range from the phenomena of a
stimulation to the marked reduction in the productivity.

In the present study, plants treated with zinc displayed the best yield performance. The fruit length (Figure: 47), the number of fruits per plant (Figure: 48), and the weight of fruit per plant (Figure: 53) were highest in the case of treatment with zinc. The harvest index (Figure: 55) was also maximum.


The better yield in the zinc-treated plants may be attributed to increased chlorophyll content of the plant due to zinc application. The enzyme, carbonic anhydrase, which is involved in the $\text{CO}_2$-fixation, contains zinc as a cofactor. In confirmation to this, the decreased photosynthetic activity and the decreased growth were observed in the cotton leaves deficient in zinc (Poincelot, 1979).

The 100-seed weight (Figure: 54) was higher in the case of zinc treatment. Gleadow et al (1982) observed that an increase in the grain/seed weight was due to the increased cell number of the endosperm and the increased plastid number per cell, resulting in a greater
capacity to accumulate protein and/or starch.

The plants treated with fluoride recorded less yield as compared to that of control and zinc. Rao and Pal (1978b) showed that atmospheric fluoride could cause hidden and visible injuries to the maize plant and, hence, affected the yield. It is widely known that fluoride inhibits the respiratory and photosynthetic processes and also induce the necrotic lesions. Therefore, other related parameters like growth and yield would also be affected adversely.

The reduced yield may be associated with the decreased chlorophyll level. The fluoride pollution is known to reduce the chlorophyll content in plants (Turck and Mathe, 1975, 1976; Czuchajowska and Przybylski, 1978). Vijaywargiya and Pandey (1991) showed that sodium fluoride caused a marked reduction in chlorophyll-a, chlorophyll-b, and the total chlorophyll in sorghum seedlings. Lal and Ambasht (1981) also found that chlorophyll content was reduced greatly in Diospyros melanoxylon due to fluoride.

The plants treated with cadmium recorded the least yield. Huang et al (1974) reported that root, shoot, leaf, and nodule dry weights were affected by the cadmium treatment. Ganesan and Manoharan (1983) reported that the root length, shoot length, leaf area, and dry
matter were inversely proportional to the cadmium concentration. An inhibition of plant growth leads to the reduction in the dry mass, and subsequently to the reduced yield as a result of treatment with cadmium salts (Ormord, 1977; Burton et al, 1983; Dubey and Dwivedi, 1987; Mathur et al, 1987).

The plants exposed to cadmium recorded the lowest harvest index (Figure: 55). The weight of 100 seeds was also minimum (Figure: 54). The lower level of chlorophyll content in the cadmium-treated plants might be responsible for the less yield. The leaves of plants grown in the presence of cadmium displayed the reduced chlorophyll and the reduced Hill activity (Snehlata, 1990).

It may, thus, be concluded that the lowered chlorophyll in the plants grown in the presence of either cadmium or fluoride led to severely affected photosynthetic activity. As a result, the assimilative capacity of plants decreased, which in turn contributed to the reduced yield.