DISCUSSION

In the present work the impacts of three heavy metals – Hg, Cd and Zn on three Solanaceous plants – *Capsicum frutescens* L. *Solanum melongena* L. and *Solanum nigrum* L. were studied.

Of the three heavy metals employed, Hg proved to be the most toxic one to the plant’s overall growth, except in the case of root elongation of the plants grown in the field where Cd proved to be the most toxic one.

GERMINATION STUDIES

The seeds of the plants grown in 0.1, 1.0, 10.0 and 100.0 ppm solutions of Hg, Cd and Zn showed different degrees of inhibition in germination. In the case of Hg, and Cd treatment, the inhibition increased with the increase in the concentration of the heavy metal. At 100.0 ppm Hg there was 100% inhibition in seed germination. In all the three plants this could be seen.
Concentration dependent effects of Hg on germination and seedling growth in certain legumes were observed by Sharma (1982, 83). Edward Mrozek (1980) found that mercury and cadmium could reduce the germination rate, total performance and long term viability of seeds after an initial stimulation of germination and these were directly co-related with increase in salinity level and metal concentration. In the present study too an initial stimulation of germination could be noticed when the readings of the 1st day were recorded both in Hg and Cd, but as the exposure period increased, this effect was diminished. It is proposed that the initial stimulation by mercury may be due to the acceleration in imbibition caused by mercury. This is true for Cd too. The metal toxicity, may be a result of the break down of membrane permeability by mercury as observed by Passow and Rothstein (1960) and Sheigh and Barber (1973). Loo and Tang (1945) are of opinion that during seed germination, along with water, seeds also take up organic and inorganic substances present in the medium and these substance affect the subsequent growth of the seedlings.

In the present work, 100.0 ppm Hg had completely inhibited seed germination in all the three plants. 10.0, 1.0 and 0.1 ppm Hg affected the seed germination in direct proportion with the Hg concentration. But Cd even at 100.0 ppm did not inhibit seed germination completely, but here too a gradual increase in inhibition could be seen with the increase in the heavy metal concentration.

According to Shanti Swarup Sharma (1985), Hg at lower
concentrations may enhance the activity of hydrolytic enzyme and at higher concentrations may inhibit its activity and thus cause promotion and inhibition of seedling growth. In the present study no growth stimulation of seeds at lower Hg concentration was observed. However an initial increase in germination was noticed.

The inhibitory effect of mercury on seed germination was shown by Pathak et al. (1987). At 1000 mg/-1 concentrations of Hg 100% inhibition in germination was observed. Hinesly et al. (1971) and Singh and Mukhiya (1980) also could observe inhibition of seed germination due to metal toxicity. Mercuric chloride, when used as disinfectant has proved to affect germination and early seedling growth (Dicky and Ark, 1949; Crosier, 1950, Dempsey and Chandler 1963). Adverse effects of organic mercurials on seed germination has been reported by many (Prota, 1960; Robson and Fenn, 1961; Assache and Broeck, 1964). Mukherji and Ganguly (1974) also have studied toxic effects of mercury on seed germination. Mhatre and Chaphekar (1982) could see that the radicles could not survive after a direct contact with the toxic mercury solution, leading to germination failure. At 1000 ppm, radicle emergence itself failed to take place due to the death of the embryo. In the present study the death of embryo occurred at 100.0 ppm Hg. Vaulina et al. (1978) observed that the germination of seeds in Cadmium chloride solution resulted in impaired nuclear divisions and absence of cytokinensis. This effect of Cd ion is supposed to be associated with blocking of SH groups in contractive protein of cellular spindle or in enzymes responsible for mitosis. Mathur et al. (1987) proved that Cd and
Cr can cause inhibitory effects on the germination in *Allium cepa* seeds. They used the concentrations 1, 10, 100, 250 and 500 ppm. The higher concentrations of the metals were found to be fatal to most of the seeds tested and Cd was more toxic than Cr.

In the present work too the 10.0 and 100.0 ppm Cd concentrations proved to be detrimental to seed germination. 1.0 ppm Cd too had caused somewhat considerable decrease in germination percentage. But 0.1 ppm Cd did not produce any noticeable impact on germination. Iqbal et al. (1991) found a significant reduction in the seed germination in *Leucaena leucocephala*, *Samania saman* and *Dalbergia sissoo* when treated with higher concentration of cadmium. The activity of alpha amylase and ATP-ase showed reduced activity following Cd treatment (Mukherji and Mukherji 1990). Miles and Parker (1980) reported that addition of soil cadmium was fatal to the germination of *Andropogon scoparius*, *Monarda fistulosa* and *Rudbeckia hirta*. Soil cadmium concentrations sufficient to reduce germination by 25% were calculated to be 30 and 46 µg Cd/gm soil for *Rudbeckia* and *Andropogon* respectively.

In the case of Zn treated plants, only the 100.0 ppm concentration was found to be toxic to seed germination. At 1.0 and 10.0 ppm Zn, an enhanced rate of seed germination could be noticed. 0.1 ppm Zn did not produce any noticeable impact on seed germination. This could be seen in all the three plants. Murray and Wilkins (1986) observed that, the germination of birch seeds decreased with elevated zinc levels. The enhancement in seed germination at the lower concentrations of Zn can be
attributed to the fact that Zn serves as a micronutrient at lower concentrations. Tsui (1948) and Nason (1950) have reported that zinc is necessary for the biosynthesis of IAA. Zn can influence the hydrolysis of storage materials in germinating seeds and thus can stimulate the germination vigour (Paleg, 1961; Chrispeels and Varner, 1967; Paul et al. 1970).

**Radicle and hypocotyle elongation**

In the present study the plants treated with mercury showed the highest reduction in radicle and hypocotyle elongation at 10.0 ppm. No serious reduction in the length of the radicle and hypocotyle could be seen at 0.1 ppm Hg. But 1.0 ppm Hg had considerably reduced the lengths of radicle and hypocotyle. The cotyledonary leaves of 0.1, 1.0 and 10.0 ppm Hg treated seedlings showed signs of abnormalities like chlorosis, burnt tips, discolouration etc., the abnormalities becoming severe with the increase in mercury concentration. In all the concentrations radicles were more affected than hypocotyle.

The growth of tomato, corn and beans were inhibited by 0.01 ppm and 0.05 ppm Methyl mercury (Vallee and Ulmer, 1972). Both Hg and methyl derivatives of mercury can strongly interact, with -SH and -s-s group in proteins and other biological molecules (Vallee and Ulmer 1972). This will bring about growth retardation. Pathak et al. (1987) observed that average lengths of root and shoot were reduced by increasing the concentration of mercury. Lipsey (1975) found that 5 and 50 ppm methyl mercury treated seedlings of maize were significantly shorter, than the control. At 5 ppm MMH, 4% of the seeds failed to germinate and at 50 ppm, 21% failed. Mercury induced growth reduction was more
pronounced in roots than in shoots (Mukherji and Ganguly, 1974). As reported by Gould et al. (1961) the growth reduction may be due to the break down of meristematic tissue as a result of mercury toxicity. Decrease in the growth of rice seedlings grown in different concentrations of saturated extract of the discharge from a chlor-alkali factory was reported by Misra and Misra (1984). Misra et al. (1985) found that toxicity of a pollutant is directly proportional to the exposure period of the organism to the pollutant. In the present study, the radicle elongation was found to be more affected than hypocotyle elongation. In Pisum sativum, Shanti Swarup Sharma (1985) too could observe such a condition. Loo and Tang (1945) are of opinion that mercury can inhibit the mobilization of food reserve from the cotyledons to the radicle, which account for the inhibition of growth. Mercury can also inhibit the activity of hydrolytic enzymes and thus can cause inhibition of seedling growth (Shanti Swarup Sharma, 1985). Truzuki and Yamada (1979) proved that mercury could suppress the activity of sucrose-6-phosphate in yeast cells. The same suppression may be operating in the seedling growth too. In general the toxicity of mercury may be due to its affinity for -SH groups of amino acids (Jerome and Ferguson, 1972; Korn et al. 1979). Morgan et al. (1966) are of opinion that an increase in IAA oxidase, and Stonier et al. (1968) the destruction of auxin products, may be the cause of the growth changes in seedlings due to Hg toxicity.

The phytotoxicity of mercury compounds is mainly due to the tight binding of Hg to SH - groups of sugar and aminoacid
transport carriers (Bernd and Ulrich, 1983). When the uptake of these solutes are affected, the overall plant growth too may be affected. Lipsey (1975) is of opinion that the inhibition of shoot and root growth of maize seedlings due to mercury toxicity, may be a result of the competitive binding for sulphhydryl groups of mitochondrial protein and not a denaturing of mitochondrial protein.

In the present work, cadmium too had affected the radicle and hypocotyle elongation of all the three plants. 0.1 ppm Cd did not produce much considerable impact on radicle and hypocotyle elongation, but 1.0, 10.0 and 100.0 ppm Cd had caused considerable reduction in the elongation of radicle and hypocotyle. The phytotoxicity was found to increase with the increase in the concentration of cadmium. The cotyledonary leaves had shown signs of chlorosis. Radicle region of 100.0 ppm Cd treated seedlings had become brown in colour. Cotyledonary leaves had burnt tips and margins.

Mahelar et al. (1987) reported that Cd when taken up, can cause growth reduction in plants. Page et al. (1972); Mullenber and Time (1985) also have observed growth reduction in plants like corn and barley. In Capsicum annuum L., 50% reduction in plant growth could be seen at 4.36 mg/lit. of Cd (Estan et al. 1988). Shrotriya and Singh (1988) could observe that cadmium can inhibit root growth in all phases of life span of the plants. Root elongation of Spruce was severely inhibited by Cd treatment (Rilobus and Buczeek, 1985). Cadmium inhibited the shoot elongation and leaf development of Pisum sativum seedlings (Wong et al. 1988).
Cadmium is able to inhibit the cell wall metabolism related to growth, by binding to the concerned enzymes. This produces a drastic change in the structural composition of the cell wall (Francis, 1991). According to Sneh Lata (1991) cadmium restricts the availability of substances necessary for growth and thus suppresses the activity of hydrolytic enzymes. Another view is that Cd can stop cytokinesis and this will bring about growth retardation (Vaulina et al. 1978). Effect of Cd ion is supposed to be associated with SH group blocking. Burton and Morgan (1984) observed a reduction in plant shoot yield and root length when Cd in soil exceeded certain threshold values.

The impact of zinc on radicle and hypocotyle elongation was such that, in all the three plants, 100.0 ppm Zn proved to be toxic for radicle and hypocotyle growth. But 10.0 ppm Zn was very much favourable for the elongation of radicle and hypocotyle. At 1.0 ppm Zn also, a slight increase in the growth of radicle and hypocotyle over the control could be observed. But 0.1 ppm Zn did not produce any considerable impact.

This increase in growth performances of the seedlings treated with 1.0 and 10.0 ppm Zn may be a result of its micronutrient value. Rotheneberger and Balitz (1977) reported that low concentrations of cadmium and zinc can slightly stimulate the seedling growth of some selected grass but retarded growth in all species at higher concentrations. Aery and Sarkar (1991) too share the same view. Maze (1919) and Sommer (1928) are of opinion that zinc is essential for plant growth. Kanwar (1964), Saxena and Singh (1970) and Patel et al. (1976) observed
destruction of the wall of the chloroplast due to mercury toxicity. Mercury can cause a reduction in the photosynthetic pigment in *Amaranthus* sp. (Mukhiya et al., 1983). This reduction in photosynthetic capacity in turn leads to reduction in growth. Mohapatra and Panigrahi (1991) reported that at high concentrations of HgCl₂ a significant decline in chlorophyll content occurred in mulberry plant, which in turn led to depletion in primary production and total production. Mohapatra et al. (1990) also have observed a drastic browning of the leaves, and considerable inhibition of photosynthetic pigment content of mulberry plants due to metal toxicity. Misra et al. (1985) could observe complete inhibition of photosynthesis within 50 minutes due to Hg toxicity. They are of opinion that, mercury acted by destroying the pigment and thus reducing the rate of photosynthesis. As reported by Vallee and Ulmer (1972) mercury and its derivatives can strongly interact with -SH and -S-S groups in protein and other biological membranes. Destruction of protein structure will indirectly influence the synthesis of photosynthetic pigments also. Some researchers like De Filippis (1979), De Filippis et al. (1981) suggest that, metals affect the levels of protochlorophyll and carotenoid content and they do not cause the degradation of chlorophyll.

In the present study the cotyledonary as well as the mature leaf chlorophyll contents of the Cd treated plants showed much variation from the control. All the 4 concentrations of Cd had caused a reduction in the cotyledonary chlorophyll content. Except *Capsicum frutescens* L., the other two plants showed more
toxicants like Zn at higher concentrations act upon the germinating seeds, a diminished level and decreased efficiency of phytase enzymes involved in food and energy utilisation result, which in turn may lead to growth inhibition.

HISTOCHEMICAL ANALYSIS

Chlorophyll content

The Chlorophyll-a, Chlorophyll-b and Total chlorophyll contents of the mercury treated plants were found to be affected. This could be noticed both in the cotyledonary and mature leaves of all the three plants.

In the case of cotyledonary leaves all the 3 concentrations of Hg (0.1, 1.0 and 10.0 ppm) considerably decreased the chlorophyll content. But in the mature leaves, 0.1 ppm Hg did not produce considerable reduction of the chlorophyll content. In the cotyledonary leaves, Chl-a showed more reduction than Chl-b. In mature leaves too Chl-a was more inhibited than Chl-b by mercury except in a few cases.

Nag et al. (1981) assumed that impaired chlorophyll development by heavy metals may be due to the interference with synthesis of proteins, the structural components of chloroplast. Treatment with heavy metals may block either the synthesis or the activity of the enzyme proteins responsible for chlorophyll biogenesis. It was observed by Nanda et al. (1986) that Phaseolus aureus Roxb. when grown on higher concentrations of the solid waste extract from a chlor-alkali plant, the chlorophyll pigment content was very much reduced. Hanery and Lipsey (1973) and Chaphekar and Kulkarni (1979) have reported the
the micronutrient value of Zn to plant growth at lower concentration. Zinc is needed for the biosynthesis of IAA through its involvement in the synthesis of the precursor of auxin (tryptophan) by influencing the activity of the enzyme tryptophan synthetase (Tsui, 1948; Nason, 1950). Zinc acts as an activator of several enzymes like carbonic anhydrase (Keilin and Mann, 1940), alcohol dehydrogenase and pyridine nucleotide dehydrogenase (Hooh and Vallee, 1958).

It was reported that zinc at higher concentrations can cause leakage of metabolites in de-embryonated rice half seeds. Abnormal mitosis in onion root-tip cells due to zinc toxicity was reported by Nag et al. (1980). Nag et al. (1984) observed that root growth and shoot growth of rice seedlings was highly affected by elevated levels of zinc sulphate, root growth was more affected. Halevy (1962) reported that the growth inhibition by growth retardants was associated with increased peroxidases and IAA Oxidase activities. Then the auxin destruction may be enhanced, which in turn affects the plant growth. Nag et al. (1984) also share the same view. In their work, it was observed that application of ZnSO₄ produced a remarkable inhibition of - amylase in rice endosperm, the degree of inhibition increasing with the increase in the concentration of the metal. This may either be due to inactivation of enzyme protein or due to the interference with hormonal control of hydrolase formation. Koller et al. (1962); Mukherji et al. (1971) are of the opinion that, the inorganic phosphates from phytin – (the primary reserve phosphate in ungerminated seeds) – is hydrolysed in the presence of the catalysing enzyme phytase, during seed germination. When
reduction in the cotyledonary Chlorophyll- a content than cotyledonary Chlorophyll- b content. The inhibition found to increase with the increase in the concentration of Cd.

The chlorophyll content of the mature leaves was also affected by cadmium treatment, throughout the experiment period. In *C. frutescens* L. Chlorophyll-b was more affected by the heavy metal than Chlorophyll-a. In *S. melongena* L. and *S. nigrum* L. Chlorophyll-a showed more inhibition than Chlorophyll-b. Here too 100.0 ppm Cd caused the most severe reduction in chlorophyll content, followed by 10.0 ppm Cd. 1.0 and 0.1 ppm Cd did not produce any noticeable decrease. Workers like Baszynski (1986) and Becerril et al. (1988) are of opinion that cadmium can affect the concentration and composition of pigments and inhibit the activities of PS I and PS II. Inhibition of photophosphorylation by cadmium was reported by Lucero et al. (1976). Weigel and Jager (1980) reported that even low Cd\(^{2+}\) concentration can strongly inhibit RUBP-carboxylase/oxygenase activity of C3 plants. Iglesias and Andreo (1984) observed an inhibition of PEB-carboxylase in C4 plants due to cadmium toxicity. Many scientists could observe Cd dependent reduction in the photosynthetic rate in plants (Bazzaz et al., 1974; Huang et al., 1974; Bazynski et al., 1980).

As reported by Nag et al. (1981) Cd may be interfering with the proteins, which are the structural components of chloroplast and it may be blocking either the synthesis or the activity of enzyme proteins responsible for chlorophyll biogenesis. Stobart et al. (1985) and Prasad and Prasad (1987) are of opinion that heavy metals like cadmium, mercury etc. can inhibit the enzymes
responsible for the biosynthesis of chlorophyll. As reported by Vallee and Ulmer (1972) heavy metal toxicity is mainly due to their reaction with proteins which are rich in cystein, methionine, histidine etc. Muthuchelian et al. (1988) could observe a decreased chlorophyll biosynthesis in *Vigna sinensis* as a result of Cd$^{2+}$ and Cu$^{2+}$ toxicity. They opined that this might have occurred due to the interference of Cu$^{2+}$ and Cd$^{2+}$ with the sulphydril site of the enzymes involved in the chlorophyll biosynthesis. Loss of chlorophyll and other symptoms such as chlorosis and necrosis have been reported by many (Haghiri 1973; Root et al., 1975; Singh 1988) as a result of Cd$^{2+}$ and Cu$^{2+}$ toxicity. Maria and Erling (1991) studied the effect of Cd$^{2+}$ on photosynthesis in sugar beet. They observed that plants cultivated in Cd$^{2+}$ at a concentration above 5 µm contained low chlorophyll content. This may be due to an impairment in the supply of Mg and Fe to the leaves (Greger and Windberg 1987), because these elements are necessary for the formation of chlorophyll.

According to Weigel (1985) Cd$^{2+}$ inhibits photosynthesis by interaction with different sites of the Calvin cycle. Krupa et al. (1987) are of opinion that cadmium reduces chloroplast and chlorophyll content by delaying the formation of thylakoid membrane.

In the present work the chlorophyll contents of the plants treated with zinc showed various degrees of variation over the control. This was observed both in the cotyledonary leaves and mature leaves. In the cotyledonary leaves all the 4
concentrations of Zn produced some effects on the chlorophyll content.

10.0 ppm Zn had caused the chlorophyll content to increase considerably over the control. 1.0 ppm Zn too had caused an increase in the cotyledonary chlorophyll content but to a lesser degree. Though feeble, there was an increase in the chlorophyll content of the cotyledonary leaves of the seedlings treated with 0.1 ppm Zn. Chlorophyll-a showed more increase than Chlorophyll-b in the majority of cases.

But 100.0 ppm Zn had greatly reduce the chlorophyll content of the cotyledonary leaves. Chlorophyll-b was more affected than Chlorophyll-a.

In the case of mature leaves too 10.0 ppm Zn caused a considerable increase in the chlorophyll content. Chlorophyll-a showed more increase than chlorophyll-b except in C. frutescens L. 1.0 ppm Zn too caused an increase in the chlorophyll content, but to a lesser degree. Chlorophyll-a and b did not show much variation in the degree of enhancement. Only negligible variation from the control was produced by 0.1 ppm Zn.

As reported by Arey and Sunanda Sarkar (1991) in the present study too, lower concentration of Zn produced beneficial effects on the plant growth. 10 µg/g⁻¹ added zinc caused a substantial percent increase in all the parameters studied, over the control (Aery and Sunanda Sarkar, 1991). Reddy and Rao (1978) reported that small concentration of Zn (0.011, 0.11, and 0.22 ppm) is beneficial to plant growth. They are of opinion that 0.11 ppm Zn is an optimum concentration for the growth of Cicer arietinum. But in the present study 10.0 ppm Zn caused a better growth.
At higher concentrations, zinc can inhibit the various metabolic processes in the plant body. Baishnab and Mohanty (1980) reported that, the presence of 2 millimolar ZnSO₄ inhibited the electron transport activities of photosystem II in isolated barley chloroplasts. Severe chlorosis and stunting of corn, grown in zinc rich soil was observed by Judith et al. (1978). Excess zinc may interfere with iron metabolism in the plant. According to Shive (1941) the toxic doses of a particular element may cause the deficiency of other elements. The toxicity of zinc at higher levels may be a result of its interference with the uptake of other elements or it may be inhibiting certain enzyme system causing changes in metabolites.

According to Agarwala et al. (1977) the leaf chlorosis caused by Zn toxicity may be an after effect of the inhibition of Fe translocation from root to shoot. Amber et al. (1970) and Chapman et al. (1939) also share the same view. This will lead to a decrease in the leaf chlorophyll content as observed in the present study. According to Goldschmidt (1962), because of the almost identical radii of the hydrated ions of Zn²⁺ and Fe²⁺ an Fe/Zn competition for an iron - requiring step in the biosynthesis of chlorophyll may occur and when Zn wins the race, chlorosis results. Hsu and Miller (1965); Machold and Stephan (1969); Duggan and Bassman (1974) also share the same view. Fredman (1977) observed a stunted growth and chlorosis of corn grown in an abandoned zinc mining area.

In the present study Hg caused the highest reduction in chlorophyll content, followed by Cd. The effect of Zn was of a
dual nature.

Carotenoid

The carotenoid pigment of the cotyledonary as well as mature leaves of the heavy metal treated plants showed much variation from the control. Mercury caused the highest reduction followed by Cd. Zn at lower concentrations was beneficial but became toxic at the higher concentration (100.0 ppm).

The carotenoid is a very important pigment. It is believed that the light energy absorbed by the carotenoids is transferred to Chlorophyll-a for photosynthesis. So its destruction causes many serious growth abnormalities.

In the cotyledonary leaves 0.1, 1.0 and 10.0 ppm Hg caused considerable reduction in the carotenoid content. In the mature leaves, 0.1 ppm Hg did not cause a serious reduction of the carotenoid content. But 1.0, 10.0 and 100.0 ppm Hg had highly reduced the carotenoid pigment content. The effect of 100.0 ppm Hg was the most severe followed by 10.0, 1.0 and 0.1. In all the three plants, this condition was noticed.

In the cadmium treated plants too, carotenoid content was highly affected. In the cotyledons, 0.1, 1.0, 10.0 and 100.0 ppm Cd produced considerable reduction in the carotenoid pigment. But in the mature leaves, only the 10.0 and 100.0 ppm Cd caused a noticeable reduction in the carotenoid content.

As a result of 10.0 ppm Zn treatment, the carotenoid content of all the three plants showed much increase over the control, both in the cotyledonary and mature leaves. 0.1 and 1.0 ppm Zn too had caused the carotenoid content to increase over the
control, but to a lesser degree.

At the same time 100.0 ppm Zn had highly reduced the carotenoid content of the cotyledonary as well as the mature leaves of all the three plants studied.

The ability of the heavy metal to affect the membrane structure and their permeability (Epstein, 1972) may account for the reduction in the carotenoid content at higher concentrations of the heavy metals.

**Total Carbohydrate**

In all the plants studied, the total carbohydrate content of the mature leaves of the mercury treated plants showed reduction over the control. The inhibition found to increase with the increase in the concentration of Hg. 100.0 ppm Hg caused the highest reduction followed by 10.0 and 1.0 ppm Hg. 0.1 ppm Hg did not produce any considerable inhibition of the total carbohydrate content. Qualitative studies too had exhibited reduced contents of carbohydrate metabolites (starch and carbohydrate) in the leaves treated with higher concentration of Hg.

Misra and Misra (1982) observed that as a result of Hg toxicity, the total carbohydrate, protein and nucleic acid content and nitrogen fixation showed a considerable reduction. It is proposed by Nielson (1971) that, the reduced rate of the accumulation of assimilatory products may be due to the inhibiting action of mercury on cell division. Mercury can reduce the photosynthetic rate which in turn will affect the total carbohydrate content (Pfluger and Widemann, 1977). Beauford et al. (1977) are of opinion that mercury can adversely affect
the metabolism of the plants.

Reduction in photosynthetic rate will lead to reduced accumulation of the assimilatory products (Harris et al., 1970). Mercury compounds including mercuric chloride are taken up into cells and inhibit metabolism and growth (Beauford et al., 1977). As reported by Frossard and Moeri (1986) about Cd, Hg too may be causing reduction of the assimilatory products as a result of the inhibition of the photosynthetic rate. Boyacka and Wojtezak (1979) reported about the impact of Hg on the permeability of biomembranes, which in turn may lead to a reduced accumulation of assimilatory products. Many have reported about the inhibition of photosynthesis and photosynthetic pigments as a result of Hg toxicity. (Harris et al., 1970; Kamp Nielson, 1971; Haney and Lipsey, 1973; Bazzaz et al., 1974; Huang et al., 1974; Overnell, 1975; De Filippis and Pallaghy, 1976; Lucero et al., 1976; Geike, 1977; Chaphekar and Kulkarni, 1979; De Filippis, 1979; Nag et al., 1981; Mukkiya et al., 1983; Nanda et al., 1986; Mohapatra et al., 1990; Mohapatra and Panigrahi, 1991). When the photosynthesis and photosynthetic pigment contents are adversely affected, naturally the photosynthetic process will be reduced, and this may account for the reduced content of total carbohydrate in the Hg treated plants.

Cd treated plants too had exhibited a reduction in the carbohydrate content. The impact was highly pronounced in 100.0 ppm Cd treated plants followed by 10.0 ppm Cd treated plants. 0.1 and 1.0 ppm Cd did not cause any considerable reduction in the carbohydrate content. The effect of cadmium on the
photosynthetic products was well visible in the qualitative localisation of these metabolites. At 100.0 ppm Cd a very low content of starch and carbohydrate could be seen.

Pfluger and Widemann (1977) could observe a loss of sugar content in potato due to cadmium toxicity. Cadmium decreased the content of soluble carbohydrate and starch. Lee et al. (1976) are of opinion that plant metabolism may be affected by cadmium in different ways. Cadmium can accumulate in cell nuclei and it can influence the biochemical processes of the plants (Das et al., 1988). Gregor and Lindberg (1986) reported that young sugar beets grown on cadmium containing media showed a decreased rate of CO₂ fixation, reduced carbohydrate content and growth. Greger et al. (1991) could observe reduction in the carbohydrate content of sugar beet, when the concentration of cadmium was full in the medium. Cadmium can inhibit photophosphorylation and CO₂ fixation and it can inhibit the chlorophyll content (Hampp et al., 1976; Bazynski et al., 1980; Dudka et al., 1983). These effects in turn affect the carbohydrate synthesis. According to Gregar et al. (1986) synthesis of sugar and its accumulation in roots were affected by Cd treatment. They are of the opinion that a reduced photosynthetic rate as a result cadmium toxicity can lead to this result. The inhibition of photophosphorylation by Cd is well clear (Lucero et al., 1976). Bazzaz and Govindjee (1974); Liand Miles (1975) are some others who could observe on inhibition of photosystem II reaction in maize and spinach chloroplasts. There are various researchers who proved beyond doubt that Cd²⁺ can affect the photosynthetic processes in higher plants (Carlson et al., 1975; Bazynski et al., 1980; Jastrow and
reported an inhibition of whole plant photosynthetic CO₂ fixation. This will lead to reduced production of assimilatory products. Greger and Lindberg (1986) observed that in both roots and shoots of sugar beets, the contents of sucrose, glucose and fructose were low in the presence of Cd²⁺. In their opinion, this may be an effect of reduced rate of photosynthesis. The sugar accumulation in roots also decreased in the presence of Cd. This can either be related to inhibition of sugar transport or to diminished sugar formation which might have led to decreased phloem loading which in turn caused a decrease in sugar accumulation. Many have reported the inhibiting effect of Cd on photosynthesis and photosynthetic pigments (given elsewhere). This also will lead to a reduced synthesis of the assimilatory products.

Reduced rate of photosynthesis in phytoplankton due to mercury pollution is reported by Harris et al. (1970). Reduction in the growth rate of algae as a result of reduction in chlorophyll pigment, photosynthetic oxygen evolution and photosynthetic chemical yield has been observed by Overnell (1975). Kamp Nielson (1971) could notice a reduction in the chlorophyll content of phytoplankton due to HgCl₂ toxicity which in turn led to inhibition of growth and reduction in the primary production. DeFilippis and Pallaghy (1976) and Geike (1977) could observe an inhibition of chlorophyll synthesis by mercury. Hota and Misra (1982) and Sathapathy and Misra (1982) reported a reduction in the total cellular carbohydrate content, protein, nucleic acid and total nitrogen fixation due to mercury toxicity.
The dual nature of zinc was very clear in its effect on the total carbohydrate content. In all the three plants studied 1.0 ppm and 10.0 ppm Zn produced a considerable increase in the total carbohydrate content. But 100.0 ppm Zn caused a reduction in the total carbohydrate content. 0.1 ppm Zn did not produce much impact on the total carbohydrate content. These results were observed in the qualitative studies too.

Zinc serves as a micronutrient for plant growth. Zn is involved in the synthesis of tryptophan, a precursor of auxin. It activates the enzyme tryptophan synthetase (Skoog, 1940; Tsui, 1948; Nason, 1950). Many enzymes like carbonic anhydrase, alcohol dehydrogenase, pyridine nucleotide dehydrogenase etc. are also activated by zinc. (Keilin and Mann, 1940; Sibly and Wood, 1951; Hooh and Vallee, 1958; Vallee and Wacker, 1970).

An increase in the photosynthetic process as a result of zinc application is reported by various workers (Voica, 1969; Porokhneivich and Vakulchuk, 1975). According to Misra and Biswal (1980) IAA can inhibit the destruction of Chl-a and b. All these processes caused an increase in the photosynthesis of the plants, which in turn led to a higher carbohydrate content in the plants treated with lower concentrations of zinc.

But at 100.0 ppm Zn concentration, the total carbohydrate content of the leaves showed a marked reduction over the control. This may be a result of toxicity of zinc at this concentration. As reported by Baishnab and Mohanty (1980) zinc can inhibit the electron transport activities of photosystem II. When
photosynthesis is thus affected, naturally the synthesis of assimilatory products will also be affected. Many researchers like Chapman et al. (1939); Goldschmidt (1962); Hsu and Miller (1965); Machold and Stephan (1969); Amber et al. (1970); Duggan and Gassman (1974); Agarwala et al. (1977); Fredman (1977) have reported zinc dependent reduction in photosynthesis and photosynthetic pigment content. This reduction in the overall photosynthesis will ultimately lead to the decrease in the content of the assimilatory products.

Total protein content

Mercury caused the highest reduction in the total protein content of all the three plants. The inhibition was found to increase with the increase in the concentration of the heavy metal. All the 4 concentrations of Hg had caused considerable decrease in the total protein content. When the protein content of the 100.0 ppm Hg treated plants was localised in leaves by qualitative histochemical studies in all the three plants studied, a reduced protein content could be observed. The competitive binding for sulphydryl groups by mercury must have occurred (Lipsey, 1975) which accounts for the change in the protein content. Sardhar and Chowdhari (1984) could observe a decrease in the chlorophyll, protein, DNA and RNA content and dry weight of plants due to heavy metal pollution. The inhibitory action of Hg$^{2+}$ ions on the metabolism of plants may be due to the irreversible binding of Hg with -SH and -S-S- groups of enzymatic proteins (Vernanzi et al. 1991). Jerome and Ferguson, (1972) Korn et al. (1979) also share the same view. Bernd and
Ulrich (1983) are of opinion that the phytotoxicity of Hg compounds is mainly due to the tight binding of mercury to the essential SH-groups of sugar and aminoacid transport carriers. The destructive effects of mercury on the photosynthetic apparatus, may be an after effect of the inhibitory activity of Hg on the synthesis or activity of enzyme proteins responsible for the biogenesis of chlorophyll pigments (Nag et al. 1981). Others like Siegel (1974); Maitra and Mukherji (1979); Jestro and Koepppe (1980); Singh and Shrotiya (1987) also are of opinion that protein is susceptible to metal toxicity. Hota and Misra (1982) reported the effect of Hg Cl₂ on the contents of protein, nucleic acid and total nitrogen fixation rate of Cyindropersimum sp. As explained by Singh and Shrottiya (1987) of Cd, Hg toxicity may also be causing a reduction in the synthesis of enzymes and may lower the level of nucleases. In this case the enzymatic degradation by nucleases may be slow, but the rate of RNA synthesis also will be slow. RNA reduction may be a result of the decrease in the synthetic capacity of the tissue. Due to the low levels of RNA, amino acids may not get incorporated into proteins.

Cadmium toxicity also caused a reduction in the protein content, in all the plants studied. 10.0 and 100.0 ppm Cd. caused highly considerable reduction in the protein content, but to a lesser degree than caused by Hg treatment. 0.1 and 1.0 ppm Cd did not cause much decrease in the protein content. When the protein content of 100.0 ppm Cd treated plants was studied qualitatively, the decreased activity of protein could be observed.
According to Wickliff et al. (1980) Cd can cause an inhibition in nitrogen fixation, which in turn may affect the protein synthesis. Because of the high affinity of Cd for metallothioneins it can substitute other metals in metallothioenzymes. Others like Siegel (1974); Maitra and Mukherji (1979); Jastrow and Koepppe 1980; Nag et al. (1981) Singh and Shrotriya (1987) are of opinion that protein is susceptible to metal toxicity. In their studies Singh and Shrotriya (1987) could observe that at 10.0 ppm Cd concentration, inhibition of protein started to occur. According to Bartolf and Price (1980) Cd influence the activity of enzymes by binding with the metallothioneins or cadmium binding proteins. Sneh Lata (1991) reported that Cd inactivates the pre-existing enzymes and does not affect their synthesis. This view was based on the observation that control and Cd treated seeds had nearly similar protein content. But in the present study the candidate could observe considerable reduction in the protein content of the 10.0 and 100.0 ppm Cd treated plants.

Cadmium may affect the fluidity of biological membranes (Langey, 1963; Sorensen et al; 1985) and this may be a reason for the reduced content of protein, because similar changes may occur in membrane bound enzymes.

According to Wickliff et al. (1980) the amount of nitrogen fixed by Alum rubra decreased with the increase in Cd
concentration. Cadmium is able to form complexes with certain proteins. Its affinity for metallothioneins helps it to substitute other metals in metalloenzymes. Siegel (1974); Maitra and Mukkerji (1979); Jastrow and Koeppe (1980); Nag et al. (1981) and Singh and Shrotriya (1987) also share the view that protein is susceptible to metal toxicity. Singh and Shrotriya (1987) reported that at 10.0 ppm Cd, protein inhibition occurred. Nag et al. (1981) reported that heavy metals can interfere with the synthesis of protein and this will lead to the impaired chlorophyll development, since proteins are the structural components of chloroplast. Heavy metals may block either the synthesis or the activity of the enzyme proteins responsible for chlorophyll biogenesis. It is proposed by Singh and Shrotriya (1987) that the general decline in metabolism due to Cd toxicity may be a result of the lower levels of nucleic acid synthesising enzymes and nucleases. As a result of decrease in nucleases, enzymatic degradation by nucleases will be slow but the rate of RNA synthesis also may be slow. RNA content reduction may be a result of the decreasing synthetic capacity of the tissue. It is proposed that due to low levels of RNA the aminoacids may not get incorporated into protein.

Kesseler and Monselize (1959) reported that zinc is needed for protein synthesis. Workers like Fujiwara and Tsutsumi (1959); Naik and Asna (1961); Prakash (1965); Cocucci and Rossi (1972); Agarwala et al. (1978) could observe a reduced protein content in zinc deficient plants. Ribosome content of soybean tissue showed a reduction as a result of zinc deficiency, since zinc is
a critical component of ribosome structure (Koeppe, 1977). Singh and Shrotriya (1987) reported that 58.7% increase in protein content over the control could be seen in seedlings grown in 50 ppm Zn but higher concentrations of zinc produced a decrease in the protein content.

In the present work the leaf protein content of all the three plants studied was affected by zinc treatment. Zinc showed a dual effect on protein content. 1.0 and 10.0 ppm Zn caused considerable increase in the protein content of the treated plants. But 100.0 ppm Zn caused an inhibition of the protein content. No considerable change in the protein content was produced by 0.1 ppm zinc. Sadaphal and Das (1956) could observe an increase in the protein content of wheat as a result of zinc application.

FOLIAR EPIDERMAL STUDIES

Stomatal index, frequency and abnormalities

The plants treated with different concentrations of Hg did not show much variation in the stomatal index and frequency over the control except the 100.0 ppm Hg treated plants. In *G. frutescens* L. treated with 100.0 ppm Hg, 62.67% decrease in the frequency of normal stomata could be observed. Simultaneously with this, 643.72% increase in the frequency of abnormal stomata, like stomata with single guard cell, development arrested stomata, disintegrated stomata etc. could be seen. In *G. melongena* L. treated with 100.0 ppm Hg also a decrease of 65.57% in the frequency of normal stomata followed by considerable
increase in the frequency of abnormal stomata could be observed.

The 100.0 ppm Hg treated S. nigrum L. showed a reduction of 37.66% in the frequency of normal stomata, over the control. Here the increase produced in the frequency of abnormal stomata was 118%.

100.0 ppm Cd also had considerably increased the frequency of abnormal stomata, together with a reduction in the frequency of normal stomata. In C. frutescens L., S. melongena L. and S. nigrum L., the reduction produced in the frequency of normal stomata was respectively 59.19%, 56.97% and 36.08%. In these plants, the frequency of abnormal stomata showed an increase of 610.4%, 490.07% and 136.74% respectively over the control.

But none of the concentrations of Zn, even the 100.0 ppm, did not produce any variation in the stomatal frequency of normal or abnormal stomata.

Though many scientists have done much work on the effect of air pollution on the stomatal structure, the effect of soil pollution on stomatal behaviour is not dealt with in detail. Inamdar and Chaudhari (1984) have worked on the effect of environmental pollution on the stomata of Peristrophe bicalyculata Nees. They have reported that stomata with single guard cell, unequal or displaced guard cells and arrested stomatal development are more frequent in polluted leaves than in the non-polluted leaves. Sharma and Butler (1973) suggested that these modifications have ecotypic significance for plants growing in polluted areas. Sharma and Tyree (1973), Yunus and Ahmad
(1978, 1979), Garg and Varshney (1980) Prasad and Inamdar (1990) are some others who have done a deep work on the effect of environmental pollution on leaf epidermis. Increase in stomatal frequency in the plants exposed to pollutants is reported by Jafri et al. (1979) and Chakraborty and Gupta (1981).

**Palisade ratio**

The palisade ratio value was found to vary by Hg treatment. In *C. frutescens* L. 0.1 ppm Hg did not produce any variation in the palisade ratio value, but 1.0, 10.0 and 100.0 ppm Hg had considerably decreased the palisade ratio value. In 100.0 ppm Hg treatment 63.92% reduction over the control could be seen. In *S. melongena* L. too, Hg caused a reduction in palisade ratio value at 10.0 and 100.0 ppm concentrations. At 100.0 ppm, there was 57.47% reduction in palisade ratio value.

The palisade ratio value of *S. nigrum* L. also was affected by 1.0 10.0 and 100.0 ppm Hg treatments. At 100.0 ppm, there was 69.89% reduction in palisade ratio value.

Due to Cd treatment also a variation in the palisade ratio value could be seen. The effect of 1.0, 10.0 and 100.0 ppm Cd on the palisade ratio was considerable, but to a lesser degree when compared with Hg. Even at 100.0 ppm Cd, only 11.08% reduction in palisade ratio value could be observed, in *C. frutescens* L. In *S. melongena* L. the palisade ratio value was found to decrease considerably due to 10.0 and 100.0 ppm Cd treatment. 40.89% reduction was produced by 100.0 ppm Cd. The palisade ratio value of *S. nigrum* L. was affected by Cd treatment to such an extent.
that at 10.0 and 100.0 ppm concentrations, the palisade ratio values exhibited 57.31% and 61.89% reduction over the control.

0.1 and 1.0 ppm Zn treatment did not produce much variation in palisade ratio value. 10.0 ppm Zn had produced a slight increase in the palisade ratio value. In C. frutescens L. 26.75% increase over the control could be seen at 10.0 ppm. In S. melongena L. an increase of 10.16% in palisade ratio value was produced by 10.0 ppm Zn treatment. It is observed that 10.0 ppm Zn caused an increase of 11.56% in the palisade ratio of S. nigrum L. But 100.0 ppm Zn produced considerable reduction in the palisade ratio value of all the three plants, but to a lesser degree than the reduction caused by 100.0 ppm Hg and Zn. Though it is proposed that, palisade ratio is a comparatively constant character (Thara, 1993), in the present study, a considerable reduction in the palisade ratio value could be observed at higher concentrations of Hg, Cd and Zn. The intense toxicity of the heavy metals may be causing a change even in such a constant character.

GROWTH, PHYTOMASS, PRODUCTIVITY AND YIELD

The various growth parameters like height of the plants, length of the roots, number of branches and number of leaves were studied both in the control as well as in the various heavy metal treated plants.

The plants treated with Hg showed considerable reduction in the height of the plants throughout the experiment period.
inhibition increased with the increase in the concentration of the heavy metal. 100.0 ppm Hg produced the most severe reduction in plant height followed by 10.0 and 1.0 ppm. 0.1 ppm did not produce any considerable reduction in height.

Mercury treatment had caused a noticeable decrease in the root length of the plants. Here also, the reduction increased with the increase in the concentration of Hg. The number of branches and leaves too had exhibited high reduction over the control as a result of Hg treatment. In G. frutescens L. and S. nigraum L. branching was much delayed as a result of 100.0 ppm Hg treatment S. melongena L. treated with 100.0 ppm Hg failed to produce any branches.

Leaf number was highly reduced due to Hg toxicity. Here too the reduction was in direct proportion with the concentration of the heavy metal. In all the plants treated with 100.0 ppm Hg the leaf number was very few. 10.0 and 1.0 ppm Hg too had caused a considerable reduction in the number of leaves. Chlorosis of the leaves was very clear.

When the various morphological parameters were highly reduced, the phytomass and productivity were also affected adversely. The inhibition occurred in all these characters found to increase with the increase in the Hg concentration.

The flowering and fruiting of the plants were adversely affected by the various concentrations of Hg. None of the plants treated with 100.0 ppm Hg showed any sign of flowering. Though flowering was noticed in 10.0 and 1.0 ppm Hg treated plants, the
number of flowers was very few. A delay in flowering also could be observed. On the plants treated with 10.0 ppm Hg only a few fruits could be seen. Even on the 1.0 ppm Hg treated, plants, the fruiting was very poor.

According to Hota and Misra (1982) mercury can inhibit the total carbohydrate, protein and nucleic acid content and total nitrogen fixation. Hg can inhibit the cell division of plants (Nielson, 1971). Hg at high concentrations is capable of decreasing the dry weight, carotenoid and chlorophyll content and the photosynthetic rate. Beauford et al. (1977) are of opinion that, inhibition caused by Hg on plant growth may be a result of its adverse effects on the plant metabolism. Greenfield (1942) and Harris et al. (1970) are of opinion that, Hg can cause specific inhibition of photosynthesis. When the photosynthetic process is inhibited, the overall growth performance of the plant will be affected as in the present study. It is found that heavy metals are capable of decreasing the chlorophyll content, DNA, RNA and protein and dryweight (Sardhar and Chowdhari, 1984), which in turn will lead to a decrease in the overall growth performance of the plant. Puerner and Siegel (1972) reported growth inhibition in cucumber as a result of mercuric chloride poisoning. A reduction in drymatter content of Hg treated plants could be observed by Pfluger and Widemann (1977). Many researchers have reported morphological and physiological disorders of plants caused by mercury—(Strognonv, 1964; Levitt, 1972; Mayber and Gale 1975; Misra, 1984). According to Mhatre and Chaphekar (1982) low concentrations of Hg have a slight stimulatory effect.
on the shoot growth, but at higher concentrations, growth was either reduced or failed to take place. Pathak et al. (1987) reported that shoot elongation was inhibited by mercury and it has an inhibitory effect on the plant’s dry weight.

The inhibitory effect of Hg on photosynthesis and photosynthetic pigment in turn can cause a considerable inhibition in the overall growth of the plants. The inhibitory effect of Hg on photosynthetic pigment and photosynthesis has been worked out by many (given elsewhere), which in turn will lead to growth reduction.

Cd treatment also caused an inhibition in the morphological parameters. 100 ppm and 10.0 ppm Cd caused a very considerable reduction in the height of the plants. The effect of 1.0 and 0.1 ppm Cd was not very considerable. Root inhibition was more pronounced in Cd treated plants than Hg treated ones. Number of branches and leaves was also adversely affected, as a result of these the phytomass and productivity of the plants also showed considerable decrease over the control. All these effects were concentration dependent.

Flowering and fruiting of the treated plants showed considerable reduction over the control. A delay in flowering and fruiting could be observed in 100.0 ppm Cd treated plants. 10.0 ppm Cd too had produced considerable inhibition in flowering and fruitset. The effect of 0.1 and 1.0 ppm Cd was not well pronounced. According to Aery and Sunanda Sarkar (1991) above 5 μg/g⁻¹, Cd caused a decrease in all growth parameters. They are
of opinion that, roots were more severely affected than shoots. As a result of cadmium treatment considerable reduction in all growth parameters studied had occurred. Scientists like Lagerwerff and Biersdorf (1972), Turner (1973), John and Laerhoven (1976) and David (1977) also could observe considerable reduction in different growth parameters due to Cd toxicity. According to Aery and Sunanda Sarkar (1991), the reduction observed in the different growth parameters due to higher levels of heavy metals may be due to the reason that the stressed plants have to spend more energy for their survival in the hostile environment, which otherwise would be available for their growth process. As in the case of mercury, here also the photo synthetic inhibition caused by cadmium, toxicity may in turn will be inhibiting the proper growth of the plant. Singh (1993) reported a decrease in the fresh and dry weights of roots and shoots as a result of Cd toxicity. Zinc treatment had a dual effect on the growth of the plants. 1.0 and 10.0ppm Zn treated plants showed a considerable enhancement in the various morphological parameters studied. The overall growth of 1.0 and 10.0ppm Zn treated plants exhibited more vigour over the control. The height of the plants, length of the roots, and number of branches and leaves showed much increase over the control when treated with 1.0 and 10.0ppm Zn. The phytomass and productivity of the plants were much more than that of the control. Flowering and fruiting too had increased over the control.

But 100.0ppm Zn was unfavourable for the plant growth. All the morphological parameters of the treated plants were adversely
affected by 100.0 ppm Zn. Phytomass and productivity too had exhibited reduction over the control. Flowering and fruiting were delayed, and only a few flowers and fruits could be seen on the treated plants.

Rothenberger and Galitz (1977) are of opinion that, low concentrations of cadmium and zinc can slightly stimulate the growth of plants, but higher concentrations retard the growth. Many other like Maze (1919); Sommer (1928); Kanwar (1964); Saxena and Singh (1970); Patel et al. (1976) are of opinion that low amounts of zinc can increase the yield of the plant. Aery and Sunanda Sarkar (1991) reported that when the zinc concentrations in the soil increased beyond 25 Mg/g⁻¹, a steady drop up to 90% in different parameters could be observed. Yield reduction as a result of high zinc concentration is reported by many (Gupta and Singh, 1972; Patel et al., 1976; Ohki, 1975, 76, 78). As reported by Aery and Sunanda Sarkar (1991) the stressed plants may be utilising their energy for the survival in the hostile environment, which otherwise could be used for their growth process. At such a situation the overall growth of the plants will be reduced, which in turn will reflect on the productivity and yield of the plants.

SEM STUDIES OF SEEDS

The SEM photographs of the seeds of the heavy metal treated plants exhibited serious abnormalities. 10.0 ppm Hg treated plants of all the three plants produced seeds with many abnormalities. 100.0 ppm Cd too had drastically changed the seed
structure. The effect of 100.0ppm Zn on seed structure was not so serious.

When the overall growth of the plants is seriously affected by the heavy metals, the fruit development also will be affected. This will reflect on the seed output. Since the metabolic processes of the plants are not normal, the nutrient elements necessary for the fruit and seed development will be very scanty, and this may account for the disfigured appearance of the seeds.

Yield reduction as a result of Cd toxicity is reported by many (Turner 1973; Williams and David 1977). Zn also can cause a reduction in plants at higher concentrations (Gupta and Singh, 1972; Patel et al. 1976; Ohki 1975, 76 and 78). Besides inhibiting the metabolic processes, Hg can even cause a disturbance in cell division (Ramel; 1974). All these inhibiting factors may be acting upon the seed development, and as a result the seeds may become highly affected.

In the present study, it was observed that mercury caused the highest inhibition in all the parameters studied, followed by Cd. Except in the case of root elongation of mature plants. In the case of root growth, Cd produced more inhibition than Hg. Zn had a dual affect on almost all the parameters studied. Lower concentrations of Zn produced an enhancing effect on the various plant characters, whereas 100.0ppm Zn proved to be toxic to the plant growth.
V. SUMMARY AND CONCLUSION