INTRODUCTION

The moment a green plant sprouts from the seed, it becomes the concern of nature to look after its further growth. With the unfolding of the first two green leaves and the development of independent photosynthetic machinery it gets the green signal to start its active life. The tiny sapling amid the surrounding eternity gradually finds that its free life is largely influenced at every step by the interplay of nature. It is in the form of environment that nature spreads its network of control over the baby plant.

The controlling factors of the environment are chiefly dispersed through two agents, viz., sun and the soil. One offers the food and the other provides water and mineral matters and thus guiding the overall management in the nutritional makeup. The idea that plants obtain their nutrients from atmosphere, water and soil is less than 200 years old. During fourteenth and fifteenth centuries the ideas about plant nutrition that gained momentum from the ancient Greek Science were based on the view that soil represents the medium in which the "nourishing juices" were prepared. It was de Saussure (1804), the eminent French plant physiologist, who first postulated that the growth of the plant depends on the absorption of nitrogen and certain other elements by plant roots (Noggle and Fritz, 1977). This discourse put a significant milestone towards a long way of further research on the nutritional status of plants.

With the switchover to rapid industrialization everywhere the atmosphere is polluted to an enormous extent. Industrial wastes in the form of harmful chemicals are being continuously emitted into rivers. The ashes of nuclear explosion tests are released in the sea water and thus rendering the coastal areas inhabitable for the marine organisms. The sewages coming out along with the water of cultivated fields and charged with mercurial insecticides find their outlet into the river for disposal. Chimneys of the factories emit smokes and poisonous gases like CO, CO_2, NO_2, SO_2 and NH_3 in the atmosphere. Exhaust pipes of the automobiles liberating unhealthy gases also pollute the atmosphere. The filthy oils released by ships in the ocean form a layer on sea water which makes the marine animals and phytoplanktons extremely difficult to survive. Gases and chemicals liberated from oil refineries are the other areas of pollution. The poor sanitary system that exists particularly in the underdeveloped countries coupled with the heaping up of sullages and dirty drain water are all that contribute to making the environment unhygienic for human habitation. The dangerous effects of such environmental pollution can be seen in the gradual diminution and final total extinction of some biological objects.

The chemical compounds disposed into water from the factories gradually deposit at the bottom of lakes, streams and sea water. Most of the heavy metals become bound to the cell walls of phyto-planktons and algae leading to the death of these microflora and the organisms which eat upon them. The water of lakes in some parts of India like Bharatpur and Raigarh of Rajasthan state has become polluted by chlorine and insecticides to such an alarming extent that it has become impossible to make safe sojourn by the visiting migratory birds flying from Siberia during the chilling winter days prevailing there.

Frequent attempts were made from time to time to protect the environment from pollution but none of these has been materialised nor any norms have been formulated in this regard. It was in the year 1283 during the regime of King Edward the First in England, the first law to keep the environment clean through the imposition of certain restrictions was enacted. In 1306 people became so concerned that a Royal Proclamation was signed, prohibiting the burning of coal in London (Clayton, 1958). The first authentic book relating to the evil effects of pollution was published during 1661 by John Avelin, a British Journalist. He formulated certain disciplines and proposed to follow them for keeping the environment habitable by the living beings. Nowadays in several countries, while mooting any programme of industrial development, it has been made imperative to make a strict vigil on the measure to prevent environment pollution.

The pollution of environment with heavy metals mainly stems from their hazardous effects as contaminants of soil, plants, animals and human beings. Although a lot of work has been done with regard to the toxic effects of heavy metals on the human body, comparatively little study has been made so far in relation to the physiological changes operating in plants particularly in germinating seeds. Recent work done by Mukherji and his coworkers in our laboratory on the characterization of heavy metal toxicity like those of Cu, Pb, Hg and Cr has thrown some light on the physiological and biochemical changes of germinating rice, wheat, mungbean and lettuce seeds (Mukherji and DasGupta, 1972; Mukherji and Ganguly, 1974; Mukherji and Maitra, 1976, 1977; Mukherji and Mukherji, 1977; DasGupta and Mukherji, 1977; Mukherji and Roy, 1977 and Mukherji and Nag, 1977). They found different degree of inhibition of seedling growth in the metalliferous media and also most of the physiological processes showed a decline in rate.

The ability of plants to grow in soil rich in metals has been mainly attributed to their inherent tolerance to them (Antonovics et al., 1971 and Ernst , 1974). These observations reveal that toxic immunity developed against one metal is specific for that metal only. According to Gartside and McNeilly (1974) the capacity for metal tolerance is species specific. This has prompted Ernst (1976) to summarize the suggested mechanism for heavy metal tolerance as - (1) Avoidance and (2) Tolerance. Avoidance involves the development of an exclusion mechanism through which metal uptake is prevented (Levitt, 1958). In the mechanism of tolerance uptake occurs and the resistance offered is due to exclusion of metals from the metal sensitive sites, production of specific metal-resistant enzymes or alteration of metabolic pathways (Levitt, 1958). It has been reported that in the process of uptake organo-metallic compounds exhibit a slower rate than the ionic forms of the heavy metals (Turner and Gregory, 1967; Ernst, 1968 and Schiller, 1974).

Cell walls play a remarkable role by acting as amulet against the heavy metals entering the protoplasm. Ernst (1972) observed that a major bulk of lead and chromium are bound to cell walls of shoots and roots allowing very little to enter the plasma membrane thus avoiding any damage that might have been caused.

In microorganisms the tolerance of heavy metal has been accomplished by a pool of sulphydryl groups. It is also possible that at least a part of the toxicity can be overcome by metal chelation resulting in the formation of metallo-proteins (Uchida *et al.*, 1973). Ernst *et al.* (1975) found an accumulation of larger amount of malic acid in some Zn-tolerant plants than the corresponding amount in Zn-intolerant ones. Copper excess can be brought down to a tolerable range by its complexing with phenolic compounds during manganese stress as found in *Rumex tianschanicus* (Pershukova and Levanidov, 1973). In *Aspergillus niger* mercury tolerance has to be accompanied by an increase in non-protein sulphydryl groups (Ashworth and Amin, 1964) and the same for copper tolerance occur in *Saccharomyces cerevisiae* (Ashida and Nakamura, 1959). However, maintenance of high energy is required to combat the onslaught of metal excess and its tolerance (Ernst, 1976).

In plants inhibition of growth is a marked feature that characterize metal toxicity. Growth rate of *Chlorella pyrenoidosa* has been remarkably reduced by the application of Hg^{++} in the medium (Hannan and Potouillet, 1972). In *Citrus* seedlings, roots showed the symptoms of Cu-toxicity and the growth of the meristem is arrested due to accumulation of copper at that region (Smith, 1953). Seed germination is largely affected by the toxic concentrations of heavy metals (Agarwala et al., 1961; Berducou and Phipps, 1961; Mukherji and DasGupta, 1972; Mukherji and Ganguly, 1974 and Mukherji and Nag, 1977). However Singh (1961) found some encouragement of germination in wheat seeds in presence of Zn at lower concentrations. Rooting of cuttings are also inhibited in presence of heavy metals (Robbins and Harvey, 1972). Grain yields are decreased by the application of excessive amount of heavy metals (Fuehring, 1966; Spencer, 1966; Nikolov, 1966 and Boawn and Rasmussen, 1971). Pollution by heavy metals may induce antimitotic action, abnormalities in cell division, absence of spindle formation and even inhibition of cytokinesis leading to the development of binucleate cells (Bielecki and Wasko, 1971; Venot and Giacchero, 1971; Bielecki, 1974; and Mukherji and Maitra, 1976). Respiration is remarkably affected by heavy metals. Severe inhibition of respiration amounting to block of hexose monophosphate shunt has been noticed in cells of Chlorella sp. pretreated with $CuSO_4$ (Hassall, 1965).

Heavy metal toxicity also affects the nitrogen metabolism. Augustaitene (1966) reported that concentration of most of the amino acids is decreased by Zn^{++}. Nitrogen uptake by Fusarium culmorum from the culture medium is reduced by the presence of $HgCl_2$ (Tolba and Saleh, 1958). However, copper in combination with other metals like Co, Fe, B, Mn, Zn and Mo has been shown to stimulate N_2-fixation in Mycobacterium roseo-album (Il'ina, 1966). Nucleic acid metabolism is also the victim of metal toxicity. Viral m-RNA is degraded in presence of heavy metal ions (Frankel-Conrat, 1957 and Huff _et al._, 1964). Santilli and Katz (1962) demonstrated a bathometric shift of RNA from TMV in ultraviolet spectrum when treated with $HgCl_2$. Addition of Zn^{++} in _Chlorella_ resulted in parallel increase of labelled m-RNA (Altmann _et al._, 1968). Comparatively DNA degradation is rather slow in presence of metal ions like Cu^{++} and Fe^{++} (Erlenmeyer _et al._, 1968; Schweitz, 1969). DasGupta and Mukherji (1977) found an increase in the amount of alkali soluble protein in both embryo and endosperm of germinating rice seeds under all the growth inhibitory concentrations of copper which resulted in reduced RNA/protein and DNA/protein ratios.

The peculiarity in the action of heavy metals lies in the fact that when all the major metabolic activities show a general inhibition, activities of some enzymes are encouraged. Activities of catalase and peroxidase generally show increments by treatment with metals like Zn, Fe, Co, Ni, Cr and Cu (Wu and Tsul, 1959; Dekock _et al._, 1960; Agarwala et al., 1961 and Vardya, 1964). According to Mukherji and Maitra (1976) and Mukherji and Nag (1977) reduced growth is generally associated with increased enzyme activity. Findings of Tang and Maretzki (1970) revealed that RNase from sugarcane leaves was strongly inhibited by Cu^{++} and Zn^{++} ions. Inhibition of RNase activity in germinating seeds by $CuSO_4$, $HgCl_2$ and Pb-acetate has been shown to be reversed by the simultaneans application of hormones (Mukherji and Ganguly, 1974; Mukherji and Maitra, 1976 and DasGupta and Mukherji, 1977). IAA oxidase activity showed a rise in lettuce seedlings treated with $CuSO_4$ (Mukherji and DasGupta, 1972).

The injurious effect of heavy metal toxicity is also extended to photosynthesis. Mercurial compounds remarkably inhibit the rate of photosynthesis. It has got a far-reaching effect on the marine phytoplanktons and the fishes living on them. Toxicity produced by copper on photosynthetic organism is also very significant (Greenfield, 1942; Macdowell, 1949). Reduction in the rate of photosynthesis may be due to suppression of any of the intricate biochemical reactions at any stage or the locus of inhibition may involve either the failure to synthesize necessary photosynthetic pigments or by restraining them to act normally. Addition of $HgCl_2$ results in the loss of activity of spinach chloroplasts (Katoh, 1972). Godnev and Lipskaya (1965) found an inhibition of chlorophyll-a formation in sugerbeet by the addition of Co and Zn. Even the rate of CO_2 incorporation may be reduced (Squire and Jones 1971). By the process of photosynthesis a large bulk of CO_2 is removed from the atmosphere. But if these pollutant sinks are crammed by debarring the photosynthetic machinery from continuing its normal operation it certainly leads to the piling up of pollutants and thereby causing health hazards.

The entire gamut of literature surveyed on heavy metal toxicity reveal that metals are essential to carry on growth, metabolism and cellular activities of plants. But when the metal ions are in excess they inflict toxic injury endangering the life of plant. The toxic effects find their manifest in stunted growth, poor germination rate, delay in sprouting, inhibition of shoot and root growth, reduction in grain yield, inhibition of photosynthesis and respiration and abnormalities in cell division. Under natural condition plants are able to evolve certain immunity against metal poisoning by their inherent growth pattern in the metalliferous soils. But the situation is different when the plants or plant parts are exposed to the toxic concentrations of metal ions in laboratory condition. No mechanisms, viz., "tolerance" and "avoidance" would be operative here which could offer a full scope safeguard against the toxicity. This is in sharp contrast with the tolerant plants, growing in metalliferous area which are prone to get a liberal discount from the lethal influence of heavy metal toxicity and which they do get to some extent by evolving certain adaptive resistance.

The most telling answer at this moment to save the seedlings imbibing metal ions at toxic concentrations is to explore the possibility of overcoming the injurious effects by applying growth hormones or any other metabolic intermediates that would be efficaceous to expunge the toxicity. Most of the work done on plant pollution are mainly based on the studies of either higher plants growing on metalliferous areas or of microbes in cultured condition which include bacteria, fungi, diatoms, phytoplanktons and green algae of which Chlorella pyrenoidosa has been extensively studied.

Keeping in mind that the major bulk of studies relating to plant pollution has been made with intact angiospermic plant or plant parts and with algae or bacteria and also comparatively little work has been done with germinating seeds, the present work has been undertaken with a view to making further discourse on the behaviour of germinating seeds affected by heavy metal toxicity. The test materials selected include the seeds of rice (Oryza sativa), mungbean (Phaseolus aureus), wheat (Triticum sativum), lettuce (Lactuca sativa), potato (Solanum tuberosum) discs, leaf discs of Cephalandra indica and root tips of onion (Allium cepa).

In the present study three metallic compounds have been selected viz., $HgCl_2$, $CuSO_4$ and $ZnSO_4$. Different concentrations of these salts have been applied to seeds during germination with a view to ascertain primarily the degree of inhibition of seedling growth caused by them. With the samplings made out of the affected seedlings, assays were made which included respiration, weight changes, pigment changes, electrolyte leakage, heat damage, water uptake; carbohydrate, nitrogen, DNA, RNA and protein contents and enzyme activities. Gel electrophoresis of buffer-soluble protein, basic protein and peroxidase isoenzymes was also studied. Cytological studies of onion root tip, pigment content and Hill reaction of leaf discs and assay of leachates from metal affected potato discs and Cu-uptake have also been included. The second objective of this work has been confined to test the possibility of protecting the plant parts grown in the toxic medium by the simultaneous application of GA_3, IAA, kinetin and cyclic-AMP. It would be interesting to observe whether the growth encouraging property of hormones can act as an amulet against the toxic influence produced by the metals or conversely to what extent the heavy metal toxicity can thwart the growth promoting activity of hormones.