PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON MERCURY TOXICITY IN *VIGNA MUNGO* (L.) HEPPER SEEDLINGS

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CERTIFICATE

Certified that the thesis entitled **"Physiological and Biochemical Studies on Mercury Toxicity in *Vigna mungo* (L.) Hepper Seedlings"** submitted by **Sahadevan, K.K.** in part fulfilment for the degree of ***Doctor of Philosophy*** in Botany, University of Calicut, is a bonafide record of research work undertaken by him in this department under my supervision during the period 1995-2001 and that no part of it has been submitted before, for the award of any degree.

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DECLARATION

I hereby declare that the thesis entitled "**Physiological and Biochemical Studies on Mercury Toxicity in *Vigna mungo* (L.) Hepper Seedlings**" submitted by me for the degree of ***Doctor of Philosophy*** in the Faculty of Science, University of Calicut has not been submitted for the award of any degree.

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Dedicated to my father

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INTRODUCTION

INTRODUCTION

Heavy metals are integrated components of biosphere and thus occur naturally in soil and plants. Toxicity of heavy metals have been predominantly a research subject for plant ecologist/geobotanists (Marschner, 1983). However with the report of "heavy metal flora" in mining areas and role of elevated heavy metal contents in plants, as a part of food chain, physiological aspects of heavy metal toxicity became overwhelmingly important.

There are many sources, both natural and anthropogenic from which high concentration of mercury originate in the environment. The important natural sources of mercury contamination of the environment are sedimentary rocks and volcanos. Agricultural sources of mercury contamination include pesticides and Hg-derivatives used to control diseases of fruits and seeds.

Widespread distribution of mercury causing environmental pollution results in health hazards to plants and animals. Bioaccumulation of mercury in plants results in the cycling of the element to reach food chain and ultimately causes hazardous diseases.

Plants live in diverse environment that provide essential (nutrient) as well as non-essential (non-nutrient) metals inclusive of mercury. The level of mercury in the soil ranges from low to high depending on the nature of environmental

conditions and this bio-reactive metal fluctuates the metabolites and inhibit plant growth.

In addition to nutrients, non nutrient heavy metals like mercury can persist indefinitely in the environment posing an ever increasing threat to natural and agricultural plant growth. Besides its environmental impacts, mercury is also of interest for plant physiologists because Hg related disorders often occur in plants. Toxicity of mercury, as it pertains to plants, is complex and depends on plant species, concentration and chemical form of the metal as well as composition of the soil or growth medium. However, even trace quantity of mercury can have detrimental effects on plant growth and development (Woolhouse, 1983; Lindberg, 1987; Kabata-Pendias and Pendias, 1992; Lenka *et al.*, 1993; Khanna and Rai, 1995; Syamala and Rao, 1999).

Natural and cultivated plants are exposed to mercury pollution due to natural weathering (Galloway *et al.*, 1982; Friedland, 1990; Verkleij, 1993; Ross, 1994), industrial activities (Verkleij, 1993; Syamala and Rao, 1999), mining (Foy *et al.*, 1978; Freedmann and Hutchinson, 1981; Lepp, 1981), agricultural and pesticide practices (Seaward and Richardson, 1990; Ross, 1994) and many other anthropogenic sources (Friedland, 1990; Shaw, 1990; Ross, 1994; Orcutt and Nilsen, 2000). Current rate of atmospheric deposition of mercury ranges from

< 3 μg Hg $m^{-2}y^{-1}$ in relatively unpolluted region to > 10 μg Hg $m^{-2}y^{-1}$ (Swain *et al.*, 1992; Moore *et al.*, 1995).

Toxicity and concentration of mercury found in plants may be a function of chemical form, age and/or physiological condition of the species and there will be differences in the amount of inorganic and organic (methyl) mercury in different plant tissues (Driscoll *et al.*, 1994; St Louis *et al.*, 1994; Zillioux *et al.*, 1993). According to Taiz and Zeiger (1998) the toxicity of heavy metals including mercury is mainly due to their ability to cause oxidative damage like lipid peroxidation to tissues.

There are many plants known as metallophytes which include metal accumulators, indicators and excluders (Baker and Walker, 1990). However, the survival of plants that can grow on contaminated soil is considered to be the result of tolerance rather than avoidance/accumulation (Fitter and Hay, 1983; Baker and Walker, 1990). According to these authors no plant has the ability to prevent metal uptake but can only restrict it.

Compared to the extended and systematic studies on the uptake, accumulation and inhibitory effects of cadmium (Hendry *et al.*, 1992; Bishnoi *et al.*, 1993), lead (Bhomik and Sharma, 1999) and chromium (Jana, 1988; Moral *et al.*, 1994) in different crop plants, much fewer comparative data are available on mercury toxicity. According to Woolhouse (1983) and Baker and Walker (1990), in

higher plants, accumulation of mercury is usually quite low even in heavily contaminated soils.

Earlier, Vallee and Ulmer (1972) stated that mercury is highly phytotoxic and even in very small concentration affects plant metabolism adversely and mercury toxicity has its bearing on the strong affinity to acidic and thiol groups of proteins and nucleotides, thus interfering with the function of these metabolites in plants.

One of the major symptoms of mercury toxicity is rapid inhibition of growth which has been proposed to be caused by a number of mechanisms including interactions with cell wall (Woolhouse, 1983), plasma membrane (Kocjan *et al.*, 1996), protein synthesis and enzyme inactivation (Brzyska *et al.*, 1991; Shaw, 1995) photosynthetic reactions (Harriss *et al.*, 1970; Bernier *et al.*, 1993), bioaccumulation (Barghigiani *et al.*, 1987) etc.

Although, some research has been conducted on the cellular and physiological basis of mercury toxicity, this research area is fraught with confusion and controversy. So this investigation proposes to understand physiological and biochemical aspects of mercury toxicity with emphasis on absorption, translocation accumulation, tolerance and interactions with metabolites, which may result in growth retardation.

Because mercury can exist in different forms in nature (Moore and Ramamoorthy, 1984), attempts to elucidate mechanisms of mercury toxicity have been hampered by lack of understanding and awareness of mercury formation and entry into plants. However, attempts have been made recently to demonstrate the toxicity of mercury in crop plants (Moore *et al.*, 1995; Shaw, 1995). More recently Orcutt and Nilsen (2000) reviewed the available data and literature on mercury and its apparent toxicity on wild plants in general and crop plants in particular.

It has been well established that addition of micro quantities of mercury mainly in the form of $HgCl_2$ causes growth retardation at very low concentrations especially in the nutrient medium (Shaw, 1995).

Even though, direct evidence to demonstrate the primary site of mercury toxicity is not available it is evident that the root growth retardation is the initial as well as drastic symptom of mercury toxicity in plants. However, mechanism of mercury toxicity at cellular/molecular level is not fully elucidated because mercury can interact with a number of metabolites and cellular structures especially in the root tip which is in direct contact with $HgCl_2$ solution (Shaw, 1995).

Most of the experimental studies on mercury stress have been performed on plant growth after adding known amount of mercury to the medium where different heavy metal including nutrient and non-nutrient exist in various combinations and hence the effect may be additive, antagonistic or synergestic. However, in the

present study the experimental plant, *Vigna mungo*, a common pulse crop with high nutritious and medicinal value was cultured in nutrient (Hogland) solution containing known quantities of mercury. *V. mungo*, a member of the family *Leguminosae* is grown all over India and it is cultivated in many tropical as well as sub-tropical countries. It is grown as a rainfed crop in the warm plains and cool hills upto an altitude of 6000 feet.

Although mercury is known to affect various metabolic processes (Bernier *et al.*, 1993; Subhadra *et al.*, 1991; Van Assche and Clijsters, 1990; Shaw, 1995) drastically, the effect of mercury on the metabolic changes in seedlings cultured nutrient solution is very scanty. The objective of the present investigation is to establish the relationship between mercury toxicity and changes of various metabolites, such as starch, sugars, protein, free amino acids, proline, lipids and phenolics in *Vigna mungo* seedlings. Assay of nitrogenase activity and estimation of chlorophyll pigments are also included because heavy metal toxicity responses of plants are shown by these constituents. Only very few reports are available on the formation of phytochelatins induced by mercury (Rauser, 1990) so electrophoretic study was carried out to compare the protein profile of root tissue where maximum mercury was localized compared to control as well as other tissues. More emphasis was given to establish the extend of mercury absorption, translocation and accumulation in various parts of *Vigna mungo* seedlings.

Inspite of contradictory views on mercury translocation in plants (Woolhouse, 1983; Orcutt and Nilsen, 2000) systematic studies on mercury absorption and translocation in cultivated plants grown in the presence of known quantity of mercury, that too in nutrient culture is lacking. Available literature on the effect of mercury on plant growth is based on chemical analyses/atomic absorption spectrophotometry (Moore *et al.*, 1995; Shaw, 1995). Hence in the present investigation in addition to chemical analyses of mercury in various plant parts and the residual mercury content in the nutrient solution, histochemical localisation of mercury in various plant parts by staining with dithizone (Pearse, 1972) were also included in order to pinpoint the mercury localisation in the plant body. With this approach confirmatory results were obtained to compare the metabolism and distribution/translocation of mercury in *V. mungo* seedlings.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Heavy metal pollution of soil, water and air has been accelerated in developing countries due to rapid industrialization and urbanization (Ademoroti, 1986; Tumi *et al.*, 1990; Berthelsen *et al.*, 1995; Xiong, 1998a, 1999).

Sources of heavy metal emission may be natural, lithogenic and pedogenic or anthropogenic, (Foy *et al.*,1978; Wheeler and Rolfe, 1979; Lepp, 1981; Cimino and Ziino, 1983; Moore and Ramamoorthy 1984; Adriano, 1986; Ho and Sachs, 1989; Alloway, 1990; Friedland, 1990; Filipinski and Grupe, 1990; Steffens, 1990; Seaward and Richardson, 1990, Prasad, 1997; Hopkins,1999; Orcutt and Nilsen, 2000). According to Xiong (1999) heavy metals in environment cause long-term contamination as they can not be chemically degraded.

Worldwide data on emission of heavy metals from natural sources are very scanty. Several authors suggested that windblown dusts, volcanic eruptions, marine aerosols and forest wildfires contribute much to the heavy metal contamination of the ecosystem (Zoller, 1983; Moore and Ramamoorthy, 1984; Pacyna,1986; Barghigiani *et al.*, 1987; Seaward and Richardson, 1990).

The sources of anthropogenic inputs of metals in to the environment are numerous and diverse and include mining, smelting, sewage disposal, traffic, combustion of fossil fuels (Friedland, 1990; Lenka *et al.*, 1992; Lokhande and

Kelkar, 1999) and use of pesticides and fungicides (Dickinson *et al.*, 1984, 1988; Page *et al.*, 1987).

With industrial development over the last 150 years there has been an increased release of heavy metals to the environment. Metals like Al, As, Cd, Co, Ca, Cu, Hg, Mn, Ni, Pb, Sc, Zn, Mo, Cr, Tl, U and V that released into the environment, are ultimately tend to concentrate in soils and sediments. Plants absorb ions from a complex medium, the soil, that contain not only the essential nutrient ions, but also a range of non-essential ions. If severe ionic imbalance arise in the soil/growth medium, the plant may not be able to take up nutrients efficiently, either because of direct effects of the toxic ions on root- metabolism/ function, or simply by competition or other interactions with nutrient ions (Fitter and Hay, 1983; Bhattacharyya *et al.*, 1999). The concentration of trace metals is one of the most important environmental stress factors and the extent to which a plant can survive or tolerate is determined by its sensitivity to metal toxicity.

Trace metal availability, toxicity, and adaptive strategies of plants have been excellently discussed and extensively reviewed by several authors (Foy *et al.*,1978; Hamer,1986; Baker, 1987; Roy *et al.*,1988; Barcelo and Poschenrieder, 1990; Rauser,1990; Steffens,1990; Reddy and Prasad,1990; Dickinson *et al.*,1991; Fernandes and Henriques 1991; Ernst *et al.*,1992; Robinson et *al.*,1993; Mc Neilly, 1994; Ross, 1994; Prasad, 1997; Bhowmik and Sharma,1999; Orcutt and Nilsen, 2000).

Plants adapted to metal-contaminated ecosystems could be used as indicators for exploration of metals (Brooks and Malaisse, 1985, Klerks and Weis, 1987; Baker and Proctor, 1990; Dickinson *et al.*, 1991; Mc Neilly, 1994; Berthelsen *et al.*, 1995; Kumar *et al.*, 1995). Kanerva *et al.*, (1988) identified some indicator plants for Hg, Cd, Zn, Fe and Al in south Finnish forest areas. Similarly Sarkela and Nuorteva (1987) identified a number of indicator plants in unpolluted Finnish Lapland to monitor the levels of Hg, Cd, Zn, Fe and Al. Cereals were also reported to accumulate trace metals (Malone *et al.*, 1974; Piotrowska and Dudka, 1994; Van Noordwijk *et al.*, 1995).

Recently a new approach termed phytoremediation, using plants to remove heavy metals from contaminated environment is emerged (Cunningham and Berti, 1993; Baker *et al.*, 1994; Raskin *et al.*, 1994; Salt *et al.*, 1995). Some plants known as heavy metal hyperaccumulators can extract unusually high content of heavy metals from environment *via* root system and translocate them to the above-ground parts (Brooks and Malaisse, 1985; Baker and Brooks, 1989; Brown *et al.*, 1994; Kumar *et al.*, 1995; Xiong, 1998b). According to Kumar *et al.* (1995) although some plants can accumulate unusually high concentration of heavy metals, their growth was significantly inhibited.

Heavy metals are toxic to plants and depress growth and yield of crops (Antonovics *et al.*, 1971; Foy *et al.*,1978; Lepp,1981; Sheoran *et al.*,1990; Grant *et al.*, 1998; Jindal and Kaur, 2000). Heavy metals have been reported to affect

several physiological activities during seed germination, seedling growth and several metabolic processes in plants (Singh *et al.*,1988; Kumar *et al.*,1993; Prasad, 1997; Boussama *et al.*, 1999; Chatterjee and Chatterjee, 2000; Gouia *et al.*, 2000).

Uptake and accumulation of heavy metals like Hg^{2+}, Pb^{2+} and Cd^{2+} by plants reduce qualitative and quantitative productivity of the species and cause a serious health hazard through the food chain to other life forms (Turner,1994; Blaylock *et al.*, 1997; Barman *et al.*, 1999; Petr *et al.*, 1999; Jindal and Kaur, 2000; Moreno-Caselles *et al.*, 2000).

Plants respond to heavy metals in different ways – stunted growth, chlorosis, necrosis, leaf epinasty and red-brownish discolouration are visible symptoms of severe metal phytotoxicity (Reddy and Prasad, 1992a; Mehindirata *et al.*, 2000) and it results in the inhibition/induction of different enzymes (Singh *et al.*, 1994a; Keshan and Mukherji, 1994), ion influx/efflux for ionic balance and synthesis of small peptides called phytochelatins (Rauser, 1990).

Heavy metals are known to inhibit photosynthesis, transpiration and phloem translocation (Baszynski *et al.*, 1982; Van Assche and Clijsters, 1986a,b; Arunachalam *et al.*, 1996). Trace metal toxicity reduces vigour and growth, causes death in extreme cases, interferes with photosynthesis, respiration, water relations and reproduction and causes changes in certain organelles and disruption of

membrane structure and functions (Melnichuk *et al.*, 1982; Lata, 1989; Prasad, 1997, Vanaja *et al.*, 2000).

Retardations and alterations of growth rate have been reported in *Fagus sylvatica* by cadmium and lead (Prasad, 1997) and in rice by cadmium and nickel (Moya *et al.*, 1993) and in *Vigna radiata* by lead (Tomar *et al.*, 2000). In these plants not only growth retardation, but net photosynthesis and carbohydrate distribution also are found to be inhibited considerably due to metal toxicity.

Cadmium, Ni and Cr were reported to alter the activities of hydrolyte enzymes responsible for mobilization of food resources in germinating pea seeds (*Pisum sativum*) (Bishnoi *et al.*,1993; Dua and Sawhney, 1991). Copper was known to interfere with oxidative enzymes in oat (*Avena sativa*) leaves (Luna *et al.*, 1994). Zinc inhibits RUBP carboxylase activity (Van Assche and Clijsters,1986a,b).

According to Rentsch *et al.*, (1996) the first visual symptom of metal toxicity in plant was growth retardation. It is well established that under various stress conditions transport of nitrogenous compounds is altered and specific amino acids, such as proline and γ-amino-butyric acid (GABA) accumulate (Heineke *et al.*, 1992; Breitkreuz and Shelp, 1995).

Regarding metal uptake in relation to metal tolerance, Baker and Walker (1990) suggested that different species vary in the nature of their responses to one metal

and also, tolerance to different metals vary within the same species or ecotypes. Some plants can grow in soils that contain levels of toxic ions lethal to other species. Fitter and Hay (1983) attributed this capacity of the plant species to four main mechanisms:

1. Phenological escape – where the stress is seasonal the plant may adjust its life cycle so as to grow in the most favourable season.

2. Exclusion – the plant may be able to recognize the toxic ion and prevent its uptake, and so the toxicity is not experienced.

3. Amelioration – the plant may absorb the ion but act upon it in such a way as to minimize its effects. Variously this may involve chelation, dilution, localization or even excretion.

4. Tolerance – the plant may have evolved a metabolic system which can function at potentially toxic concentrations, possibly by means of distinct enzyme molecules.

According to Fitter and Hay (1983), those species most able to resist toxic ions are found to employ more than one such mechanism, but the adoption of any one or any combination imposes important physiological and ecological constraints.

The deleterious effects of metal ions can be manifested in many ways. As pointed out by Ochiai (1987) the molecular mechanisms of metal ion toxicity can be divided into five general groups:

1. Displacing essential metal ions from biomolecules and other biologically functional units.

2. Blocking essential functional groups of biomolecules, including enzymes and polynucleotides.

3. Modifying the active conformation of biomolecules, especially enzymes and polynucleotides.

4. Disrupting the integrity of biomolecules

5. Modifying some other biologically active agents.

Berry (1986) suggested three basic strategies of response, (1)avoidance, (2)detoxification and (3) biochemical tolerance, each of which affects tissue metal concentration in different ways. According to him mechanisms which limit uptake into the root or those which limit transport at the root-shoot interface constitute the avoidance strategy. The detoxification strategy is conceptually similar to the avoidance, but the avoidance of toxicity results from internal detoxification, either by subcellular compartmentation or by binding. The third strategy, biochemical

tolerance is equivalent to Levitt's (1980) definition of tolerance, and is reflected in the presence of specialized metabolic pathways and enzymatic adaptations.

Taylor (1987) treated exclusion of metals from the symplasm as a fundamental mechanism of metal tolerance in higher plants and suggested two levels of exclusion operation: (1) avoidance or restriction of uptake and (2) restriction of transport.

According to Ho and Sachs (1989) plants develop both a strategy of avoiding uptake of toxic heavy metal ions and an ability to synthesize proteins and peptides that can tightly bind and sequester heavy metals. One mechanism that plants use to alleviate heavy metal stress is the synthesis of metal binding polypeptides, called phytochelatins (PCs). The apparent function of phytochelatin is to sequester and detoxify excess metal ions. Failure in synthesizing these peptides results in growth inhibition or cell death. The role of phytochelatins in plant metal tolerance has been reviewed by many authors (Robinson and Jackson, 1986; Tomsett and Thurman, 1988; Robinson, 1990; Rauser, 1990; Steffens, 1990).

Metal-binding polypeptides produced by higher plants, are abundant in both sulfhydryl and carboxyl groups and could have affinity for a wide range of metal ions. These metal-binding polypeptides chelate metals in the cytoplasm and thereby

reduce the concentration of cytotoxic free metal ions (Gekeler *et al.*, 1989; Grill, 1989; Robinson, 1990; De Knecht *et al.*, 1994).

Two mechanisms of metal tolerance that Baker and Walker (1990) suggested, were metal exclusion and metal accumulation. Orcutt and Nilsen (2000) suggested that precipitation, chelation and isolation of the metal in the cytoplasm are the key mechanisms for detoxification.

Phytotoxicity of heavy metals in higher plants result in the inhibition of some enzyme and induction of some other enzymes (Keshan and Mukherji, 1995). There are two predominant mechanisms of enzyme inhibition: (1) binding of the metal to sulfhydryl groups, involved in catalytic action or structural integrity of enzymes and (2) deficiency of an essential metal in metal-protein or metal-protein complexes, eventually resulting in substitution of the toxic metal for the deficient element.(Reddy and Prasad 1992a).

Heavy metals have found to change the ultra structure of plants. Thus chromium and cadmium induce structural and ultra structural changes in the vascular system of *Phaseolus vulgaris* (Vazquez *et al.*, 1987; Barcelo *et al.*, 1988). Photosynthesis is invariably affected in plants exposed to excess trace metals (Clijsters and Van Assche,1985; Stiborova *et al.*,1986; Rascio *et al.*, 1993). It has been shown that trace metals interfere with the functioning of the photosynthetic apparatus in higher plants (Baszynski *et al.*, 1980; Baszynski, 1986). In *Phaseolus*

vulgaris, zinc affected the ribulose–1,5-bisphosphate carboxylase/ oxygenase (Van Assche and Clijsters 1986a). Cadmium influenced the net photosynthesis in rice (Misra *et al.*,1989; Moya *et al.*,1993).

Peroxidase induction by trace metal was observed in *Phaseolus vulgaris* (Van Assche *et al.*, 1988). Cadmium induced peroxidase activity in roots and leaves of *Oryza sativa* and roots showed 10 to 20 fold higher activity than leaves (Reddy and Prasad, 1992b). Peroxidase induction is a general response of higher plants to uptake of toxic amount of metals such as Cd, Cu, Pb, Hg, Zn and Ni (Yi Xian *et al.*, 2000). In addition to peroxidase, the activities of other enzymes namely, malic enzyme, glucose-6-P-dehydrogenase, isocitrate dehydrogenase and glutamate dehydrogenase were also increased in *Phaeolus vulgaris* when treated with zinc and cadmium (Van Assche *et al.*, 1988). Localization of Cd in vacuoles, synthesis of phytochelatins and binding to trace metals occur mainly in roots (Grill *et al.*, 1989).

Cadmium exerts its toxicity through membrane damage and inactivation of enzyme possibly through reaction with –SH groups of proteins (Fuhrer, 1982; Fett *et al.*, 1994). Trace metals inhibit ATPase activity and also interfere with membrane integrity (Ros et al., 1990). Bhattacharyya and Choudhuri (1994) observed a significant reduction of biomass accumulation and photosynthetic pigment level in *vigna* and hydrilla. In *Vigna radiata,* Pb is observed to reduce root length, number and size of root hairs, darkness and total area of leaf, chlorophyll a, b and total

chlorophyll and dry weight, but total carotinoid and total organic nitrogen in root and leaf increased due to Pb treatment (Singh *et al.*, 1994b).

Different heavy metals at supra optimal concentration, have been shown to inhibit various metabolic processes in plants resulting in their reduced growth and development (Bala and Setia 1990; Davies 1991; Bernier *et al.*, 1993; Shaw, 1995; Lang *et al.*, 1998; Tomar *et al.*, 2000).

Mercury belongs to the common group of heavy metals that bioaccumulate in plants and reach animals through food chain. This metal causes serious health hazards throughout the world due to its widespread distribution in the environment, biotransformation and high toxicity to living system. Anonymous (1979) reported that sorbed methyl mercury is transported by the blood stream and accumulate in tissues such as liver, kidney and brain.

The first major incident involving mercury poisoning in human was reported from Minamata bay, Japan. The disease of the central nervous system, now known as 'minamata disease,' was caused by eating contaminated fish. The methyl mercury contamination was a result of water discharges from an acetaldehyde plant. Fish and shell fish concentrated mercury to the levels of 10 mg/Kg (Anonymous, 1972).

Friberg and Vostal (1972) and Suzuki (1977) classified mercurials, from the toxicological point of view, into different groups such as elemental mercury,

inorganic mercury compounds, short-chain alkyl mercurials and organo mercury compounds.

Mercury occurs in the environment as metallic Hg and as HgS. Annual production of mercury in the world is estimated to be about 9000 tons and about 50% of it is lost to the environment as pollutant (Goldberg, 1976). Data are available on the deposition of atmospheric mercury vapor especially in specific geographic areas (Lindberg, 1987; Pacyna, 1987) and by a lesser extent for the entire globe (Galloway *et al.*, 1982), but they are often excluded from global estimates of mercury cycling (Friedland, 1990).In some global estimates, the anthropogenic to natural emission ratio for mercury is quite high. However, when natural vapor emissions are included, the anthropogenic to natural ratio for mercury is very low (Galloway *et al.*, 1982; Lindberg, 1987).

It is estimated that approximately 50% of global mercury cycling is anthropogenic in origin (Miller and Buchanan, 1979). Moore and Ramamoorthy (1984) reported that natural weathering has contributed approximately 1.6×10^{10} metric tons of total mercury in to the environment throughout geological times. Total production of mercury during this century has been about 4.36×10^{5} metric tons. Mercury released in this century through human activities is almost ten times the calculated amount released due to natural weathering (Moore and Ramamoorthy, 1984).

Orcutt and Nilsen (2000) reported that total annual global emission of mercury from natural sources estimates to 0.16 x 10^6 kg. Various agricultural amendments reported to contain mercury in the range - sewage sludge – 0.1-55 $\mu g\ g^{-1}$, composted refuse – 0.09-21 $\mu g\ g^{-1}$, farmyard manure – 0.01-0.36 $\mu g\ g^{-1}$, phosphate fertilizers – 0.01-2.0 $\mu g\ g^{-1}$, nitrate fertilizers – 0.3-2.9 $\mu g\ g^{-1}$, lime – 0.05 $\mu g\ g^{-1}$ and pesticides – 0.6-6 $\mu g\ g^{-1}$.

The ratio of anthropogenic to natural sources of particulate atmospheric emission of mercury is 0.44 and the toxic level of mercury in plant leaf tissues and soil surface are 1 to 3 ppm dry weight and 0.3 to 5ppm dry weight respectively (Orcutt and Nilsen, 2000).

A wide variety of physical, chemical, biological and radiological mercury pollutants have been identified in the environment consequent to urbanization, industrialization and new technological developments (Lagerwerff, 1972; Pillai, 1989). According to these authors, sources of pollution of heavy metals such as Hg, Pb, Cd, As, Cr, Zn, Cu, Mn and Fe are mainly aquatic releases from industrial operations, atmospheric releases from fossil fuel burning, domestic sewage discharges and land run-off. Severalfold increase in the concentrations of Zn, Cd, Hg etc. have been observed in some rivers in India (Pillai, 1989; Bhattacharyya *et al.*, 1999; Lokhande and Kelkar, 1999).

Sources of Hg contamination are industry, fossil fuel burning and domestic sewage. According to Goldberg(1976), maximum consumption of Hg is in the chlor-alkali plants. The products of chlor-alkali industry can also be source of long term Hg pollution. Household bleaching solutions contain 17-24 ppb of mercury (Siegel and Eshleman, 1975). Mercuric chloride is used as catalysts in the manufacture of plastics and acetaldehyde and effluents from such plants contribute Hg into the aquatic environment. Mercury is also used in the production of batteries, street lamps, fluorescent tubes, circuit breakers etc. all of which are finally discarded as waste. Mercury compounds are used in antifouling paints, pulp and paper industry and are lost in the waste water effluents. Phenyl mercuric acetate and ethyl mercuric chloride have been used as fungicides. Other uses of mercury are in research, jewellery, moulding processes, in pharmaceuticals and in felt hat production.

Fossil fuel burning and cement manufacturing cause emission of mercury into the atmosphere (Syamala and Rao, 1999). Sewage disposal contributes to mercury contamination of aquatic environment. Mercury may get remobilized by burning of these sludges or when used as fertilizers (Moore and Ramamoorthy, 1984).

Mercury may be present in industrial wastes in the form of elemental mercury, inorganic or organic mercury compounds. Methylation of Hg to CH_3Hg^+ and $(CH_3)_2Hg$ takes place in the aquatic environment as a result of activities of

bacteria, fungi or enzyme systems. Bacteria also convert phenyl, ethyl and methyl mercury, phenyl mercuric acetate and diphenyl mercury as well as Hg^{2+} ions to elemental mercury (Pillai, 1989). According to him other possible reactions in the environment are conversion of methyl mercury into dimethyl mercury in alkaline condition, production of insoluble HgS in anaerobic conditions and oxidation of HgS to sulphate in aerobic conditions and conversion to methyl mercury.

Mercury is discharged into the aquatic environment mainly in the form of elemental mercury(Hg), divalent mercuric ions(Hg^{2+}) and phenylmercuric acetate, [$C_6H_5Hg(CH_3COO)$] (Moore and Ramamoorthy, 1984; Driscoll *et al.*, 1994). According to these authors, mercury in natural waters exist in three oxidation states: elemental mercury (0) mercurous (+1) and mercuric (+2) states. The nature of species and their distribution will depend on the pH, redox potential, nature and concentrations of anions which form stable complexes with mercury. Mercury associates strongly with suspended solids, in natural water and the extent of association is determined by water quality parameters such as pH, salinity, redox potential and presence of organic ligands. As per the stability constant, $HgCl_2$ is found mainly in the complex form in solution compared to $Hg(NO_3)_2$ which is almost completely dissociated. Then it is consistent that $Hg(NO_3)_2$ is a more potent inhibitor than $HgCl_2$ at a given concentration. Since both the salts finally caused the same maximal effect at higher concentrations, Bernier *et al.*, (1993) suggested that mercury is the inhibitory agent.

Moore and Ramamoorthy (1984) further suggested that biological and chemical cycling of mercury in the aquatic environment is a complex process involving many pathways and competing reactions depending on the nature of mercury input, physico-chemical composition of the aquatic system, and the metabolic state of various types of biota. These authors suggested that bioaccumulation of mercury is relatively high in old basal tissues of aquatic plants compared to young leaves.

In marine plankton, mercury levels are reported to be 2-10 ppb, most of which in the inorganic form, whereas at the higher trophic levels as in fish they are in the range of 0.01-2 ppm mostly in the form of methyl mercury. Reduction of photosynthesis in fresh water and marine phytoplankton are reported for organo-mercurial fungicides for concentrations as low as 0.001 ppm (Pillai, 1989)

Mercury based fungicides such as $HgCl_2$ and phenyl mercuric acetate are extensively used in seed dressing and seed preservation in many countries including India (Semu *et al.*, 1985; Maude and Bambridge, 1985). This may lead to accumulation and quantitative increase of mercury in the top soil.

Watson (1979) stated that air discharge of mercury through human activities is supposed to double between 1975 and 2025 and the amount of mercury removed from municipal sewage- plants and appearing in landfills is likely to increase in the future. The range of heavy metal concentration in the soil shows that mercury

concentration is the least, nevertheless, substantial amount of mercury is found in the soil (Wood,1974). It has been reported that only 0.2% or less than that content of mercury is passing into the plants most of which is in the form of organomercurials (Kudo et al.,1978; Sandmann and Boger, 1983).

Mercury has always been reported to be more toxic compared to other heavy metals like cadmium (Nordberg, 1976;Rai *et al.,* 1981; Fergusson, 1990; Kneer and Zenk, 1992; Gadallah, 1994; Shaw, 1995), chromium (Chandra and Garg, 1992; Garg *et al.*, 1994) and lead (Huang *et al.*, 1987; De Grado *et al.*, 1999; Xiong, 1999; Orcutt and Nilsen, 2000; Jindal and Kaur, 2000).

Toxicity of mercury has been reported in many plants and in very low concentrations mercury causes hazards to plant growth (Vallee and Ulmer,1972; Sandmann and Boger, 1983; Kagi and Hapke, 1984; Baker *et al.*, 1985; De *et al.*, 1985). These authors suggested that mercury is phytotoxic even in small quantity and the toxicity has bearing on its strong affinity to acidic and thiol groups of protein and nucleotides, thus interfering with the functions of these compounds/organelles. In addition, mercury competes with other metals such as Cu or Zn within the cell (Marschner, 1983). Mercury enters plants as inorganic forms from the soil or water or from the process of methylation that occurs in plants. According to Woolhouse (1983) mercury after being absorbed from the soil remains deposited mostly in the root tissues.

In higher plants concentration of mercury is normally quite low even in heavily contaminated soils and content of mercury varies among plant groups (Foy *et al.*, 1978). Moore *et al.*, (1995) reported that the consequences of mercury distribution is in the order, herbs < trees and shrubs < aquatic macrophytes < sphagnum < mosses < lichens < fungi. According to these authors, grasses and herbs contain 20 μg kg^{-1}, trees and shrubs 29 μg kg^{-1}, aquatic mesophytes 40 μg kg^{-1}, sphagnam moss 69 mg kg^{-1}, lichen 170 μg kg^{-1} and fungi 280 μg kg^{-1} of mercury. Considerable amount of mercury and methyl mercury is reported to be present in vegetables and the range of mercury is 3 – 139 μg/Kg and that of methyl mercury is 0.3 – 30 μg/Kg (Cappon, 1987). According to Orcutt and Nilsen (2000) critically toxic level in most plants is considered to be 1-8 ppm.

Various forms of growth retardation and physiological changes have been reported in plants by mercury toxicity (Nag *et al.*, 1980). In *Cyperus* and *Chloris* root growth inhibition has been reported due to mercury treatment and the rate of inhibition increased with the increase in concentration of mercury (Lenka *et al.*, 1993). Maitani *et al.* (1996) observed a reduction in the relative root elongation of *Rubia tinctorum* in root cultures when treated with 10μM Hg^{2+} whereas relative root elongation registered an enhancement with 100μM Pb^{2+}. However the total γ-Glu-Cys content in root cultures exposed to Hg^{2+} (10 μM), Pb^{2+}, Cd^{2+}, As^{3+} and Ag^{+} (100 μM) for 3 days were found to be more than 30μmolg^{-1}.

Mercury is highly toxic to vascular plants and the toxic effects of mercury have been observed in young barley plants at levels of 3 ppm dry weight while volatile forms of mercury have been reported to inhibit growth in sensitive plants at 0.5-1 ppm. Earlier investigators suggested that growth inhibition by mercuric chloride may occur at concentrations of 0.002 – 0.25 mg L^{-1} (Hollibaugh *et al.*,1980). According to Axelsson and Axelsson (1987) the lethal concentration of Hg for *Laminaria digitata* is <5μM. Mercury(II) has a tremendously high affinity for sulfur and hence it can coordinate to essential functional groups of proteins and render them inactive.

Mercury forms stable complexes with a variety of organic ligands and has exceptional affinity for sulfhydryl groups of proteins (Falchuk *et al.*, 1977; Nath *et al.*, 1993). The strongest covalent complexes are formed with S-containing ligands such as cysteine, the next strongest with amino acids and hydroxy carboxylic acids. Mercury (II) is so selective for sulfur that it is commonly used in biochemical analyses to determine the amount of cysteine present in proteins. The binding constant of Hg(II) to S^- is 1×10^{24} (Borovik,1990).

Eventhough mercury belongs to the same group II b of the periodic table of elements along with zinc and cadmium, the mechanism underlying mercury – tolerance in higher plants is less known in comparision with that of the other two elements(Godbold and Huttermann, 1985). While Davies *et al.* (1991) suggested a

vacuolar compartmentation mechanism for Zn-tolerance and a phytochelatin – induction mechanism for Cd –tolerance (Steffens, 1990). Lenka *et al.* (1993) attributed mercury tolerance in *Chloris barbata* to either one or both of the above mechanisms.

According to Maitani *et al.* (1996) phytochelatin (PC_2) is the most abundant class III metallothionein produced in higher plants due to Hg^{2+} exposure. The authors suggested that, since mercury (II) has a linear configuration in co-ordination compounds, PC_2 can effectively protect plants against the Hg^{2+} toxicity.

As reported earlier (Vallee and Ulmer,1972) mercury is an inhibitor of enzymes and proteins. The extreme class b character of Hg^{2+} and R-Hg^{+} provides high affinity towards thiol groups and enhanced covalence resulting in increased bio-transport, distribution and toxicity to biological systems (Moore and Ramamoorthy, 1984).

Interaction of mercuric chloride with primary amino group of membranes containing phosphatidyl serine and phosphatidyl ethanolamine was studied by Delnomdedien (1989). Their results revealed that the neutral, positively and negatively charged phospholipids are strongly affected by mercury. This mercury-lipid interaction suggested new mechanism for biological effect of mercury at membrane level. Studies on the effect of heavy metals Zn and Hg on growth and biochemical constituents of *Vigna radiata* seedlings (Pratima *et al.*, 1989) showed a

decline in the respiratory rate of seedlings. The levels of total nitrogen, total sugars and malic acids declined in the embryo with a concomitant accumulation in cotyledons.

Mercury is, an enzyme and protein inhibitor in biological systems and all mercurial compounds are highly toxic to plants in general and aquatic plants in particular (De Fillippis, 1979; Baker and Walker, 1989; Reed and Gadd, 1990). Brzyska *et al.* (1991) reported that mercury decreased both the soluble and immobilized enzyme activity even at low concentration (0.2 mM) by 20 to 50 % and an addition of magnesium ions to the soluble forms of endopolygalacturonase counteracted the inhibitory effect of Hg. According to Jeana and Choudhuri (1982) Hg increase permeability of tissues due to membrane damage in some aquatic plants.

Prasad and Prasad (1987), Jain and Puranik (1993) and Prasad (1997) have reported decline in chlorophyll content due to mercury toxicity and according to them this decline is linked to the photosynthetic productivity. According to Jain and Puranik (1993), one of the mechanisms by which mercury exerts its toxic effects is by interaction with essential –SH group of enzymes and structural proteins. The accumulation and thereby the toxicity of the mercury may be reduced by the supply of thiols. Reduced glutathione (GSH) is the predominant, free thiol present in plants. The concentration of GSH in plant cell is modified by developmental and environmental factors such as heavy metals and cell culture studies have indicated

that GSH is a substrate for the synthesis of heavy metal binding phytochelatins (Rauser, 1987; Scheller *et al.*, 1987; Obata *et al.*, 1994). According to Tukendorf and Rauser (1990) and De Vos *et al.* (1992) accumulation of phytochelatins is associated with decline in GSH

Jain and Puranik (1993) reported that supply of 0.01 to 0.1 mM reduced glutathione (GSH) to excised greening maize leaf segments prevented the inhibitory effect of mercury on chlorophyll biosynthesis and the supply of other thiols such as dithiothreitol (DTT), cysteine and mercaptoethanol also reduced the inhibition of chlorophyll formation by mercury.

Bernier *et al.* (1993) investigated in detail the effect of mercury on photosystem II sub-membrane fraction of barley leaves (*Hodeum vulgarae*) and reported that mercury is an environmental contaminant that strongly inhibits photosynthetic electron transport, photosystem II being the most sensitive target. Oxygen evolution was strongly inhibited and chlorophyll fluorescence was severely quenched by mercury. These authors further observed that chloride, an inorganic cofactor known to be essential for the optimal functioning of photosystem II, significantly reversed the inhibitory effect of mercury at the donor side of photosystem II and also that cysteine residue(s) have implications in maintaining structural and functional integrity of photosystem II.

The molecular mode of action of mercury in the oxygen evolving complex of PS II is described by Bernier and Carpentier (1995). Incubation of thylakoid membranes in the presence of mercury cause depletion of an extrinsic polypeptide of molecular weight 33 kDa, without affecting other two closely related extrinsic polypeptides of 16 and 23 kDa. This indicates the existence of an intrinsic binding site for EP 23. According to these authors, the mercury inhibition of PS II might be due to this release of EP 33 and the Cl^- reduction in mercury toxicity might be due to reduction in the extent of EP 33 depletion.

Murthy and Mohanty (1995) suggested that mercury inhibit photosynthetic electron transport at various sites in *Spirulina patensis* and the photosystem II is more susceptible to heavy metal ions compared to photosystem I.

Mishra and Choudhuri (1996) investigated the membrane damage caused by Pb^{2+} and Hg^{2+} in two rice cultivars and observed an increase in the activity of lipogenase and malondialdehyde content due to treatment with both the heavy metals. Mercury increased the activity of peroxidase and the level of H_2O_2 while decreased the activities of SOD and catalase. An increase in leakage of electrolytes was also observed. The authors concluded that, Hg^{2+} and Pb^{2+} caused membrane damage in *Oryza sativa* was mediated by reactive oxygen species and hydrogenperoxide induced by these metals.

Several mechanisms of heavy metal tolerance in plants have been proposed which include (1) production of intracellular metal binding compounds (2) alteration of metal compartmentation patterns (3) alteration of cellular metabolism and (4) alteration of membrane structure (Verkleij and Schat, 1990).

Although tolerance to metals particularly to mercury has been reported for bacteria (Nakamura *et al.*, 1986), tolerance by plant population to Hg is less known (Chaney and Strickland, 1984; Godbold and Huttermann, 1985). Deshkar *et al.* (1990) reported that modified *Hardwickia binata* bark can adsorb Hg (II) from wastewater up to a capacity of 21 mg/g and thus it can be used for purifying wastewater. In a biomonitoring study by Lenka *et al.*,(1993) two mercury tolerant grasses- *Chloris barbata* and *Cyperus rotundus* were isolated from a mercury polluted locality near a chlor-alkali plant, where mercury contamination was as high as 557 mg Kg^{-1} soil and according to them tolerance to Hg was more in *C. barbata* than *C. rotundus*.

Setia and Bala (1994), reported that mercury do not cause any cellular disorganization in the roots of *Triticum aestivum* but it induce secretion of mucilagenous substance on the epidermal surface and suppress the differentiation of vascular tissues. Mercury cause considerable decrease in root diameter and transectional area of cortical cells and enhance cell wall thickness considerably.

Khanna and Rai (1995) investigated the mercury induced inhibition of *Raphanus sativus* seedling growth and this could be reversed in the presence of L-proline, L-histidine and L-methionine, but L-phenylalanine, L-alanine and L-aspartic acid had no effect on mercury toxicity. The reduced level of mercury in *R. sativus* tissues when supplemented with exogenous proline indicate that effects of proline on amelioration of mercury toxicity are related more to its inhibitory effects on mercury uptake rather than on mercury toxicity itself. These authors further suggested that stress induced accumulation of amino acids within the plant have significant effect on the ion uptake or ionic balances of plants which in turn would help the plant in mitigating the stress.

According to Shaw (1995), Hg and Cd significantly inhibited seed germination and seedling growth in *Phaseolus aureus* but had little primary damaging effect on membranes. According to him lipid peroxidation occurs in *Phaseolus* by Hg treatment and it is indicated that guaiacol and ascorbate peroxidases, and catalases are actively involved in scavenging cellular H_2O_2 and other free radicals. The lipid peroxidation induced by these metals is a consequence rather than the primary cause of toxicity.

Inhibitory effect of $HgCl_2$ and some organomercurials on protein molecules is widely used in molecular biology for the elucidation of protenaceous nature of biomolecules such as a membrane protein inclusive of aquaporins (Borgnia *et al.*, 1999).

The major impact of mercury is that it has a high affinity for sulfhydryl group and thus can inactivate many proteins and enzyme systems in plants. It appears to be easily mobilized in plants, hence it can be accumulated in plant parts that could be consumed by humans or other animals. Consequently, like other heavy metals that can be accumulated in plants, it is a concern relative to animal and human health (Orcutt and Nilsen, 2000).

Tolerance to mercury has been reported in plants, although the exact mechanism or mechanisms are not known. Some plants form insoluble S-rich proteins, probably similar to phytochelatins, while other plants may form volatile organic derivatives to remove it from tissues (Orcutt and Nilsen, 2000).

MATERIALS AND METHODS

MATERIALS AND METHODS

1. Plant material

Viable seeds of Black Gram (*Vigna mungo* L. Hepper) var. T1 were procured from the Tamil Nadu Agricultural University, Coimbatore, India. Germplasm of the variety was maintained and cultivation for seed multiplication was done in the field laboratory of the department of Botany, throughout the course of this investigation.

2. Chemicals

Either AR or GR grade chemicals were purchased from E. MERCK/BDH, SRL and/or Glaxo Laboratories Ltd. Some standards and rare chemicals were purchased from Sigma chemical company, St. Louis, USA.

3. Container for culture

Good quality plastic trays of size 37 x 27 cm and having a depth of 7 cm were used for the preparation of hydroponic system. Plastic trays of size 36.5 x 26.5 cm and 5 cm depth having holes of 5 x 5 mm^2 were placed inside the above trays. Plastic twines of diameter 1 mm were tied interweavingly lengthwise and breadthwise the trays forming a mesh to provide mechanical support to the seedlings. The smaller trays were placed inside the bigger trays in such a manner

that there was a gap of 3 cm height in between the two trays. This gap provided room for growth of the root system.

Nutrient culture studies were conducted in the field laboratory of the department keeping the trays on a wooden table of size 192 x 75 x 99 cm with a roof of colourless polythene sheet having thickness of 0.2 mm.

4. Composition and preparation of nutrient solution

A modified Hoagland's solution after Epstein (1972) as described by Taiz and Zeiger (1991) was employed in the present study. Composition of the modified solution is shown in Table-1. Stock solution of each nutrient was prepared separately and appropriate volumes were mixed together to make up nutrient solution of final volume and concentration. pH of the solution was adjusted to 6.8 using 1N HCl or NaOH as the case may be.

5. Treatment with mercury

Mercury was added to the nutrient solution as Mercuric chloride ($HgCl_2$). Ten micro molar stock solution of $HgCl_2$ was prepared and appropriate volume mixed with required volume of nutrient stock solution and final volume was made up to 2000 ml.

Three concentrations of $HgCl_2$, 1μM, 5μM and 10μM were selected for the present study. These concentrations were selected based on a preliminary study

Table 1. Composition of modified Hogland nutrient solution employed in the present investigation

Compound		Concentration of stock solution	Concentration of stock solution	Volume of stock solution per litre of final solution
		mM	gL^{-1}	ml
Macronutrients	KNO_3	1000	101.10	6.0
	$Ca(NO_3)_2.4H_2O$	1000	236.16	4.0
	$NH_4H_2PO_4$	1000	115.08	2.0
	$MgSO_4.7H_2O$	1000	246.49	1.0
Micronutrients	KCl	25	1.864	2.0
	H_3BO_3	12.5	0.773	
	$MnSO_4.H_2O$	1.0	0.169	
	$ZnSO_4.7H_2O$	1.0	0.288	
	$CuSO_4.5H_2O$	0.25	0.062	
	H_2MoO_4	0.25	0.04	
	Fe EDTA	53.7	30.0	0.3

Adopted from Taiz and Zeiger (1991)

conducted, in which percentage reduction in root elongation was taken as the criteria for determining the degree of toxicity.

6. Surface sterilization and germination

Seeds of black gram (*Vigna Mungo* L. Hepper) var TI having uniform size, colour, and shape were selected and surface sterilized with 0.1% (w/v) aqueous solution of mercuric chloride for three minutes with frequent shaking and then washed thoroughly in double distilled water. Seeds were sown in petri dishes of diameter 9 cm and height 1.5 cm, lined with filter paper. Thirty seeds were sown in each Petri dish and sufficient double distilled water was added. Petri dishes containing seeds were kept in darkness for germination.

7. Plant culture

For culture studies 48 hrs old uniform, healthy seedlings of *Vigna mungo* were selected from the petri dishes and transplanted to nutrient solution containing 0 μM, 1 μM, 5 μM or 10 μM $HgCl_2$. Nutrient solution without $HgCl_2$ (i.e. 0 μM) served as control. In the present investigation the 48 hours old seedlings are referred to as seedlings of zero hour interval. Eighty seedlings were planted in each tray and the distance between the seedlings were 3 cm. Each tray contained 2000 ml nutrient/treatment solution and this solution was replaced with fresh solution at every 24 hrs interval.

8. Sampling

Random samplings were done at an interval of 12 hr, 24 hr, 48 hr, 72 hr, and 168 hr regularly. Seedlings collected were thoroughly washed and separated as root, stem, leaf and cotyledon and used for various analyses.

PHYSIOLOGICAL STUDIES

9. Visual symptoms

Samples collected were observed for visual symptoms of toxicity, such as chlorosis, colouration of plant parts etc.

10. Morphological measurements

Growth of seedlings was assessed in terms of root length, stem length, leaf area, fresh weight and dry weight.

10.1. Root length

The sampled seedlings of 0 hr, 12 hr, 24 hr, 48 hr, 72 hr and 168 hr were washed in distilled water, blotted and root length was measured in millimeters manually , using a graduated scale of make 'Sharp'. Measurements of not less than 6 seedlings were recorded each time.

10.2. Stem length

The portion of the seedlings in between the tip of its epicotyl and the region where the hypocotyl joins with its radicle was taken as the length of stem.

Stem length was measured in millimeter using the same method used for measuring root-length.

10.3. Leaf area

Leaf area was measured in mm^2 using the drawings obtained by superimposing on graph paper.

10.4. Secondary root number

Number of secondary roots emerged at each interval was noted.

11. Root/Shoot ratio

Root/Shoot ratio on the basis of both tissue elongation (ie. root length and shoot length) and tissue dry weight per plant were calculated from the data obtained.

12. Fresh weight

For fresh weight determination, seedlings were carefully taken from the nutrient solution, washed in distilled water and blotted to dryness. Seedlings were

separated as root, stem, leaf and cotyledon and then weighed using Shimadzu electronic balance.

13. Dry weight

Samples after determining the fresh weight were transferred to pre-weighed containers and placed in a hot air oven at 100° C for 1hr and then at 60° C till the weight became constant. Per-plant dry weight, dry weight percentage as well as moisture content were calculated.

14. Relative growth rate (RGR)

Relative growth rate of root, stem and leaf in terms of dry weight were calculated using the formula described by Kozlowski and Pallardy (1997).

$$RGR\ (mg\ mg^{-1}\ h^{-1}) = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

where W_1 and W_2 are the dry weights at the beginning and end of the sampling period, t_1 and t_2 are the hours of sampling.

15. Net assimilation rate (NAR)

Net assimilation rate on the basis of leaf dry weight was calculated using the formula described by Koglowski and Pallardy (1997).

$$NAR\ (mg\ mm^{-2}\ h^{-1}) = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\ln LA_2 - \ln LA_1}{LA_2 - LA_1}$$

where W_1 and W_2 are the leaf dry weights at the beginning and end of the sampling period, t_1 and t_2 are the hours of sampling and LA_1 and LA_2 are leaf area present at sample time t_1 and t_2 respectively.

16. Tolerance index percentage

Tolerance index percentage was calculated according to the method of Turner (1994).

$$TI\ \% = \frac{\text{Observed value in solution with metal}}{\text{Observed value in solution without metal}} \times 100$$

17. Stomatal studies

Stomatal index was calculated according to the method of Meidner and Mansfield (1968). Stomatal density on abaxial and adaxial sides of the leaf were computed under a light microscope, preparing impressions using a nail enamal. Stomatal length and width were also been measured using a micrometre.

Stomatal index was calculated as follows:

$$\text{Stomatal index} = \frac{\text{Number of stomata per unit area}}{\text{Number of stomata per unit area} + \text{number of epidermal cells per unit area}} \times 100$$

BIOCHEMICAL STUDIES

18. Estimation of starch

Starch was estimated according to the method of Putcher *et al.*, (1948) as described by Whelan (1955). Potato starch was used as the standard.

Weighed, fresh samples of tissues from seedlings of different treatments and control were homogenized with 30% (v/v) perchloric acid (PCA), using a clean mortar and pestle. Acid washed sand was added as an abrasive. After centrifugation for five minutes at 4500 rpm the supernatant collected. The residue was washed thrice with 15% PCA and the supernatant pooled together. The final volume of the supernatant was noted. A known quantity of supernatant was taken in triplicate and equal volume of iodine potassium iodide reagent was added. The samples were kept aside undisturbed for half an hour and then centrifuged. The supernatant was decanted with extreme care to avoid loss of precipitate. The precipitate was then washed with alcoholic sodium chloride and centrifuged again. The residue was further washed with alcoholic sodium hydroxide. Tubes were gently shaken and tapped till all the blue colour was discharged. It was then centrifuged and washed again with alcoholic sodium chloride. The precipitate was dissolved in 5 ml of 6N sulphuric acid. Suitable aliquots were taken in duplicate after centrifugation for colour development. Colour development was done using the method of Montgomery (1957) by adding 0.1 ml of 80% phenol and 5 ml of

concentrated sulphuric acid using a burette. The absorbance was read at 540 nm using a Shiumadzu – UV-1601 UV-Visible spectrophotometer.

19. Estimation of total sugars

Weighed fresh samples each of the control and experimentals were homogenized in hot 80% (v/v) ethanol. The homogenate was centrifuged and supernatant was collected in china dishes. The residue was washed three to four times with hot 80% ethanol and the pooled supernatant in china dishes were evaporated by keeping it on a water bath at 80° C. The dried material was dissolved in a known volume (10 ml) of distilled water and suitable quantity of aliquots were taken for colour development. Colour development was done using alkaline copper tartrate and arsenomolybdic acid reagents according to the method of Somogyi (1952). Absorbance of blue colour was read at 620nm using a Shimadzu–UV-1601 UV-Visible spectrophotometer.

20. Estimation of protein

Protein content was estimated using Folin-Ciocalteau reagent according to the method of Lowry *et al.*, (1951). Bovine serum albumin was used as the standard.

Weighed fresh tissues of treatments as well as control were homogenized, using pre-chilled glass pestle and mortar, in chilled distilled water. Volume of the

homogenate was measured. A known quantity of the homogenate was taken in triplicate and equal volume of 10% (w/v) trichloro acetic acid (TCA) was added and kept undisturbed in a refrigerator for two hours for flocculation. It was then centrifuged for five minutes and the supernatent decanted off. The residue was washed twice with 3% (w/v) TCA followed by 80% (v/v) acetone and then by anhydrous acetone to remove pigments. The precipitate was dissolved in 5 ml of 0.1 N sodium hydroxide by placing it in a boiling water bath. Suitable aliquots were taken in duplicates after centrifugation and volume was made up to 1 ml with double distilled water. Colour development was done using Folin-Ciocalteau reagent after 10 minutes of adding 5 ml alkaline copper reagent to the aliquot. Absorbance was read at 750 nm in a Shimadzu-UV-1601 UV-Visible spectrophotometer after 30 minutes of colour development.

21. Estimation of total free amino acids

Total free amino acids were estimated according to the method of Moore and Stein (1948) as suggested by Sadasivam and Manikam (1992). L-leucine was used as the standard.

Weighed quantity of fresh samples were homogenized with a small quantity of acid washed sand using a clean mortar and pestle and then 5ml of 80% (v/v) ethanol was added to it. The homogenate was centrifuged and supernatant collected. This extraction was repeated twice with the residue and all supernatant

pooled. From this supernatant 0.1ml of aliquot in triplicate were taken and added 1ml of 'ninhydrin solution' [prepared by disolving 0.8g stannous chloride in 500ml of 0.2M citrate buffer (pH5.0) and adding this solution to 20g of ninhydrin in 500ml of methyl cellosolve]. Volume was then made up to 2ml with distilled water and tubes were heated in a boiling water bath for 20 minutes. To this mixture 5ml of 'diluent solvent' [prepared by mixing equal volumes of water and n-propanol] was added and stirred well. Intensity of the purple colour was read after 15 minutes at 570nm using a colorimeter of Systronics make.

22. Estimation of proline

Proline was estimated according to the method of Bates *et al.*, (1973) as described by Sadasivam and Manikam, (1992). L. proline from E-MERK was used as the Standard.

Weighed fresh samples each of the experimental and control were homogenized in 10 ml of 3% (w/v) aqueous sulphosalicylic acid and the homogenate was filtered through Whatman No.2 filter paper. Two milliliter of the filtrate each were taken in triplicate and 2 ml of glacial acetic acid and 2 ml acid-ninhydrin were added to the filtrate. Tubes were heated in a boiling water bath for one hour and then the reaction was terminated by placing the tubes in ice bath. For colour development 4 ml toluene was added to the reaction mixture and stirred well for 20-30 seconds. Then the toluene layer was separated and brought to room

temperature. Red colour intensity was measured at 5.20 nm using a Shimadzu-UV-1601 UV-Visible spectrophotometer .

Proline content was calculated using the formula

$$\text{Proline } (\mu\text{moles } g^{-1} \text{ tissue}) = \frac{\mu g \text{ proline / ml x ml toluene}}{115.5} \times \frac{5}{\text{g sample}}$$

where 115.5 is the molecular weight of proline.

23. Nitrate reductase activity (NRA)

Nitrate reductase activity was measured according to the method suggested by Hageman and Reed (1980) as described by Sadasivam and Manikam (1992) . Sodium nitrite was used as the standard.

Weighed quantity of fresh sample was homogenized in a known volume of medium containing 1mM EDTA, 10mM cysteine and 25mM potassium phosphate adjusted to a final pH 8.8 and filtered through four layers of cheese cloth and then centrifuged for 20 minutes at 20,000g. The supernatant decanted through glass wool and used for assay.

The reaction was initiated by adding 0.2ml enzyme extract to a reaction mixture containing 0.5ml of 0.1M phosphate buffer (pH 7.5) 0.2ml of 0.1M potassium nitrate solution, 0.4ml 2mM NADH solution and O.7ml water and

incubated at 30°C for 15 minutes. The reaction was terminated by the rapid addition of 1ml of 1% suplhanilamide prepared in 2.4 N HCl followed by 1ml of 0.02% N-(1- napthyl) ethylenediamine dihydrochloride reagent. Absorbance was read at 540 nm after 30 minutes.

Calculation was made with the help of a standard graph prepared with sodium nitrite and activity was expressed as quality of nitrite formed per hour per gram fresh tissue.

24. Estimation of total lipid

Total lipid was estimated gravimetrically. Weighed dry samples of the different treatments and control were homogenized in chilled concentrated diethyl either. The homogenates were centrifuged and supernatant was collected in pre-weighed china dishes. The residue was washed 3-4 times with chilled concentrated diethyl either and the pooled supernatant in the china dishes were kept overnight in a hot air oven at 60°C. The china dishes with dried samples were kept in a desiccator for bringing it into room temperature and then the weight of the china dishes were noted. The difference in weight gave the weight of total lipid.

25. Estimation of total phenolics

Total phenolics was estimated according to the method of Malick and Singh (1980) as described by Sadasivam and Manikam (1992). Catechol was used as the standard.

Five hundred milligram of fresh sample was homogenized using a mortar and pestle in 10ml of 80% ethanol (v/v) and the homogenate was centrifuged at 10000 rpm for 20 minutes. The supernatant was collected. Extraction was repeated with 5 ml of 80% ethanol and the supernatants pooled. The pooled supernatant was evaporated to dryness and the residue obtained was dissolved in a known volume of distilled water. Suitable aliquots in triplicate were pipetted out and the volume in each tube was made up to 3ml with double distilled water. 0.5ml of Folin-Ciocalteau reagent was added to the tubes and after 3 minutes, 2ml of 20% Na_2CO_3 solution (w/v) was also added. The reagents were mixed thoroughly and the tubes were placed in boiling water for exactly one minutes. Then the tubes were cooled and absorbance was measured at 650nm against a reagent blank.

26. Estimation of chlorophyll

Chlorophyll estimation was done according to the method of Arnon (1949) as described by Jayaraman (1981). Fresh leaves of experimentals and control seedlings were washed with distilled water and blotted between sheets of filter papers.

Weighed quantities of fresh tissue were homogenized with a clean mortar and pestle in 80% (v/v) acetone. The extract was centrifuged for five minutes and the supernatant collected. The residue was washed again with 80% acetone and centrifuged. This process of washing was repeated till the pellets become

colourless. The final volume of the pooled supernatant was noted. absorbance of the extract was read at 663 nm and 645nm using a Shimadzu UV-1601 UV- Visible spectrophotometer.

Chlorophyll-a, b and total chlorophyll were calculated as follows:

Chlorophyll-a mg g^{-1}tissue = $12.7(A_{663})-2.69(A_{645})$ x V/(1000 x W)

Chlorophyll-b mg g^{-1} tissue = $22.9(A_{645})-4.68(A_{663})$ x V/(1000 x W)

Total chlorophyll. mg g^{-1}tissue = $20.2(A_{645})+8.02(A_{663})$ x V/(1000 x W)

Where A= absorbance at specific wavelength,

V= final volume of leaf extract in 80% acetone,

W= fresh weight of tissue extracted.

From the data obtained chlorophyll a/b ratio was also been computed.

27. Electrophoretic study

Protein profile of the root tissue of both mercury treated and control seedlings of *Vigna mungo* were prepared by SDS PAGE.

Fresh, root tissues collected at each interval were washed thoroughly in double distilled water and homogenized in Tris-HCl buffer of pH 8.4 under ice cold condition. Homogenate was filtered through four layers of cheese cloth and then centrifuged at 8000 g for 10 minutes (4°C).

SDS PAGE was performed in a Bio Rad Mini gel Electrophoretic system according to the method of Laemmli (1970). The separating gel and stacking gel used were of 10.5% and 5% strength respectively. Running buffer used was Tris-glycine of pH 8.4. After washing the wells with double distilled water followed by sample buffer, 30 μL of samples and standards in sample buffer were loaded in each well, and a constant current of 4 volts per well was applied for 2 hours. After running, the gels were stained with Coomassie brilliant ,Blue R-250 and distained in a mixture of methanol (5%), acetic acid (6.5%) and water.

28. Estimation of mercury

Mercury content in root, stem, leaf and cotyledon as well as in the growth medium was analysed. Samples were prepared according to the method of Allan (1969). Oven dried plant materials were wet digested by reflexing in a 10 ml mixture of nitric acid, and perchloric acid in the ratio of 10:4 for 3 to 4 hours until the solution became colourless. Then the samples were transferred to standard flasks and made upto 100 ml. Mercury was analysed using a Mercury Analyser – MA 5800 E of make Electronics Corporation of India Ltd. The reaction mixture contained 8 ml of 10% (v/v) HNO_3 2 ml of 20% (w/v) $SnCl_2$ in concentrated HCl and 2 ml alequote. Aqueous solution of $HgNO_3$ was used as standard.

Bioaccumulation percentage of mercury in different tissues were also been calculated from the data obtained.

29. Histochemical localization of mercury

For histochemical studies, root and stem tissues of both control and treatments were collected at every intervals. Root tissues of 1 cm length from the root tip region and stem tissues of 1 cm length from the region below the primary leaves were fixed in a Formaldehyde-Acetic acid-Alcohol mixture (FAA) dehydrated through TBA series, and paraffin infiltrated blocks were prepared. Sections were cut at 10 μm thickness. De-paraffinized sections were stained with dithizone to localize mercury according to the method of Pearse (1972). Photographs were taken using a Carl Zeiss Axiolab microscope.

30. Statistical analyses

All experiments were repeated a minimum of six times and the mean values are given in tables and figures. Standard deviation and standard error were also calculated. The values in tables were mean value $\pm$ standard error. Test of significance was done using Fisher's 't' test.

RESULTS

RESULTS

PHYSIOLOGICAL STUDIES

1. Morphological variations

Toxicity of mercury resulted in many visual morphological changes in *Vigna mungo* seedlings. Reduced root growth was the first observed symptom of toxicity. Reduction of root elongation was least in seedlings treated with 1μM mercuric chloride solution and highest in 10μM solution (Plate 1-3). Root of mercury treated seedlings showed brown colouration and its intensity increased proportional to the concentration of mercury. At higher concentration (10μM) the roots showed deterioration after 7 days of treatment. Roots of 5μM and 10μM mercury treated seedlings appeared to be hard and brittle.

After 48 hrs. of growth in medium containing Hg^{2+}, the basal portion of hypocotyl, where it joins with radicle showed swelling and it was more in 5μM and 10μM concentrations (Plate 2, 3). Adventitious roots which were also hard and brittle, found to emerge from this swollen area.

There was no significant visual morphological signs of mercury toxicity in the stem, other than its stunted elongation. Stem of seedlings in higher mercury concentration was more thicker and brittle than that of control.

Mercury toxicity affected leaf colour also. Leaves of treated seedlings were pale green and this discolouration was more pronounced in 5μM and maximum in 10μM treatments, compared to the lower concentration as well as control.

2. Morphological parameters

2.1 Root length

Variation in root length due to mercury treatment is given in Plate 1-3 and Table 2. Inhibitory effect of mercury in root growth was very evident within a period of 12 hr (Plate 1, Table 2) in all the concentrations and the rate of growth retardation increased with increase in concentration of mercury. After 24 hr, root growth was less than half in 1μM while root growth was completely arrested in 10μM Hg treatment in comparison with the control. The root length at 10μM was only 12% of that of control.

2.2 Stem length

Stem length was also found to be reduced due to mercury toxicity (Plate 1-3, Table 2). Generally a gradual reduction in stem growth was observed and this reduction was increasing proportional to the increase in concentration of mercury .

Nevertheless, at the highest concentration of mercury (10 μM), the stem length reached about only one half of the control after 168 hr of growth.

Table 2. Effect of mercury on root length, stem length, leaf area and secondary root number of *V. mungo* seedlings during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root length (mm)	Control	7.67 ± 0.89	16.71 ± 0.31	29.13 ± 1.29	53.50 ± 2.06	70.80 ± 1.17	115.40 ± 9.69
	1 μ M		15.00 ± 0.58	19.42 ± 1.43	26.17 ± 1.11	33.17 ± 2.34	56.17 ± 2.11
	5 μ M		13.32 ± 0.70	14.20 ± 0.96	14.50 ± 0.96	16.17 ± 0.86	19.14 ± 1.25
	10 μ M		11.13 ± 0.93	12.10 ± 0.86	12.67 ± 0.94	14.00 ± 0.29	14.14 ± 0.83
Stem length (mm)	Control	12.13 ± 0.85	15.25 ± 0.43	29.60 ± 1.32	46.00 ± 1.58	66.80 ± 3.87	90.00 ± 3.16
	1 μ M		13.22 ± 0.92	21.90 ± 1.22	36.90 ± 1.42	51.80 ± 2.84	67.00 ± 3.72
	5 μ M		14.57 ± 0.18	23.00 ± 1.45	31.67 ± 1.05	44.00 ± 2.10	53.20 ± 2.82
	10 μ M		14.38 ± 0.87	22.90 ± 0.80	25.71 ± 1.83	41.20 ± 2.06	51.17 ± 2.41
Leaf area (mm^2)	Control	8.67 ± 0.94	19.25 ± 1.17	34.13 ± 1.46	51.34 ± 2.09	163.67 ± 6.18	821.83 ± 11.14
	1 μ M		16.17 ± 0.37	23.50 ± 1.63	47.92 ± 2.81	146.17 ± 4.27	579.67 ± 9.22
	5 μ M		12.58 ± 0.61	21.33 ± 1.30	42.33 ± 2.09	97.33 ± 3.82	257.17 ± 6.87
	10 μ M		12.25 ± 0.18	17.17 ± 1.86	38.10 ± 1.62	80.36 ± 2.28	181.67 ± 5.34
Secondary root number	Control	a	a	a	4.17 ± 0.57	12.60 ± 0.36	39.80 ± 2.40
	1 μ M		a	a	2.04 ± 0.03	3.80 ± 0.75	9.80 ± 1.83
	5 μ M		a	a	a	1.02 ± 0.02	7.50 ± 0.22
	10 μ M		a	a	a	a	3.83 ± 1.61*

a - absent

Values are mean of six replicates ± SE

* Adventitious roots

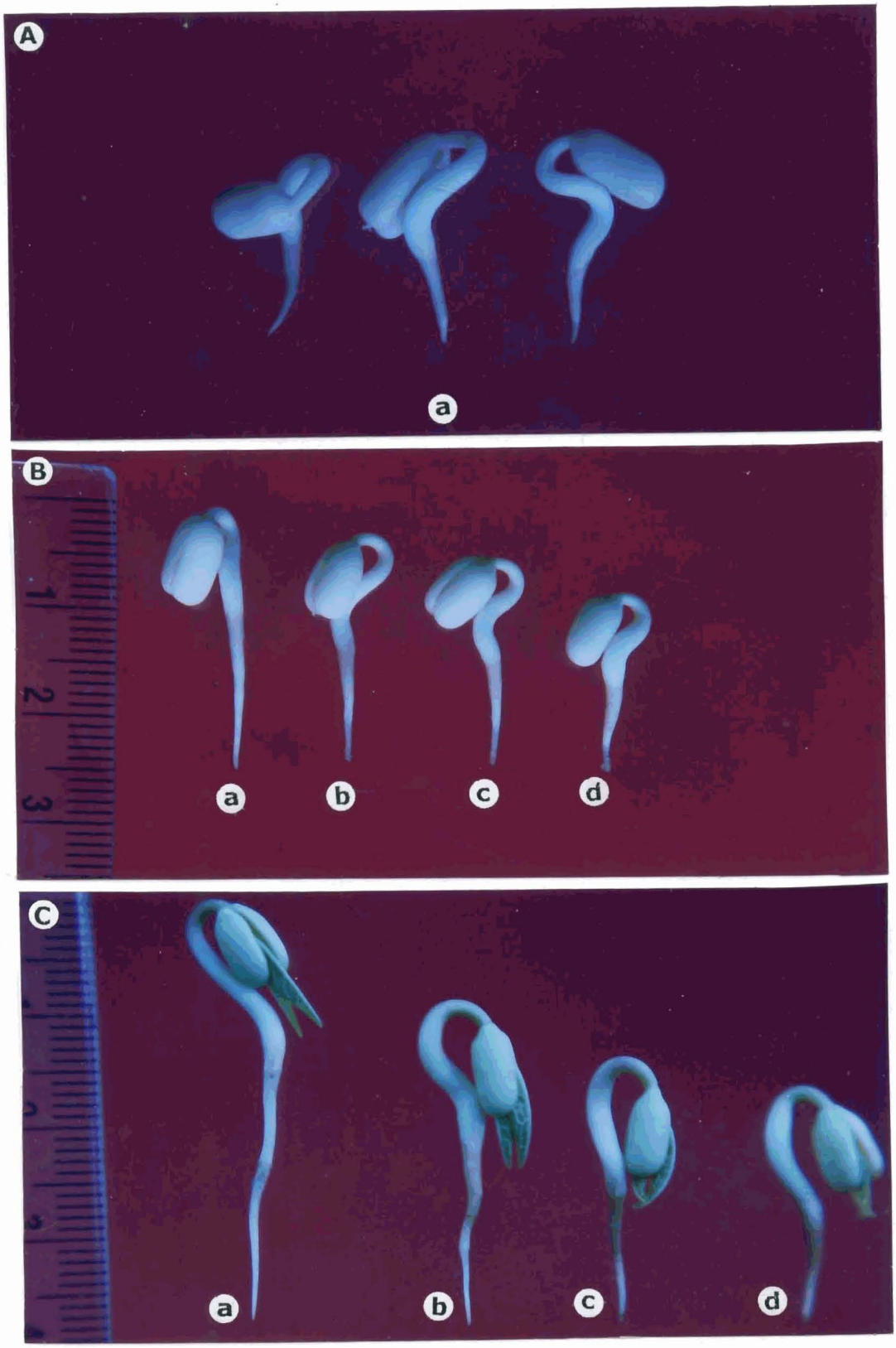

PLATE 1. **Morphology of mercury treated *V. mungo* seedlings.**
(A) 0 hr (B) 12 hr (C) 24 hr
(a) Control (b) 1 μM (c) 5 μM (d) 10 μM

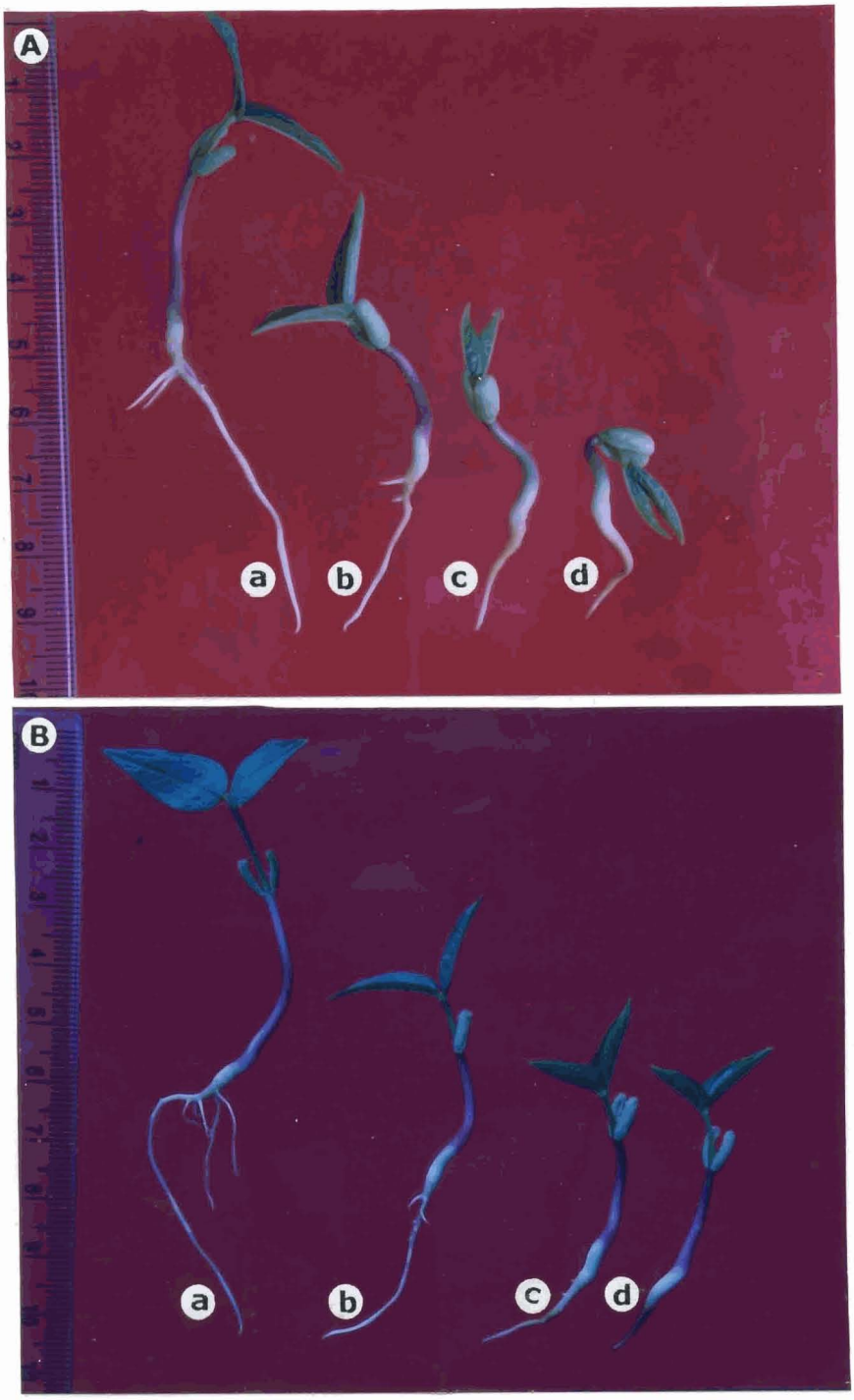

PLATE 2. **Morphology of mercury treated *V. mungo* seedlings**
(A) 48 hr (B) 72 hr
(a) Control (b) 1 μM (c) 5 μM (d) 10 μM

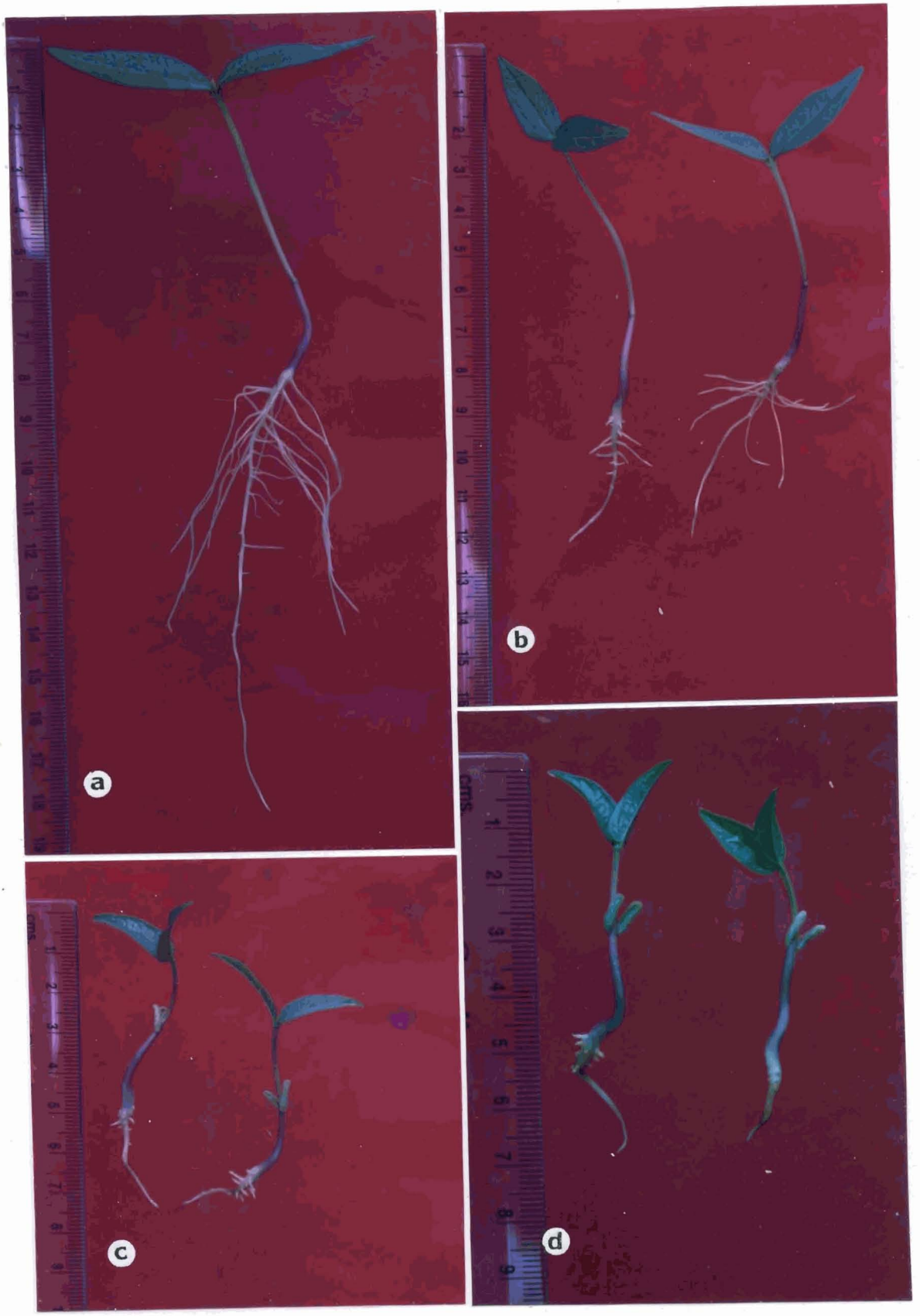

PLATE 3. **Morphology of mercury treated *V. mungo* seedlings – 168 hr**
(a) Control (b) 1 μM (c) 5 μM (d) 10 μM

2. 3 Leaf area

A general reduction of leaf area was observed in all treatments compared to the control. But in the case of 1 μM concentration the leaf area did not decrease significantly up to 72 hr while in 5 and 10μM, significant reduction of leaf growth was observed at all intervals. After 168 hr of growth, in 10 μM mercury treatment the leaf area was only 22% in comparison with the control value.

2.4. Secondary root number

Vigna mungo seedlings started production of secondary roots after 48 hr of growth in hydroponics and a large number of secondary roots were observed after 168 hr of growth (Table 2). Under mercury toxicity secondary root formation was completely absent at all concentrations up to 48 hr and later, only a few secondary roots were formed in all treatments. As reported earlier, in 10μM concentration, the "secondary roots" are originated from the swollen hypocotyl region so they can be considered as adventitious roots (Plate 3).

3. Root /Shoot ratio

Table 3 shows the values of root/shoot ratio of *V. mungo* seedlings during growth under mercury toxicity at different intervals. Root/shoot ratio of control plants showed an increase upto 72 hr and then decreased slightly as growth advanced to 168 hr, while that of treatment plants registered a gradual decrease

Table 3. Effect of mercury on root / shoot ratio (based on length and dry weight) in *V. mungo seedlings* during growth

Treatment	Interval (hour)					
	0	12	24	48	72	168
Control	0.6323 (0.1382)	1.0258 (0.1657)	1.0304 (0.1887)	1.1630 (0.1747)	1.0599 (0.1189)	1.2822 (0.0975)
1μM	-	1.1021 (0.2138)	0.8868 (0.1773)	0.7092 (0.1412)	0.6403 (0.1226)	0.8384 (0.1397)
5μM	-	0.8826 (Q.1947)	0.6174 (0.2004)	0.4578 (0.1552)	0.3675 (0.0837)	0.3598 (0.0756)
10μM	-	0.7740 (0.1925)	0.5284 (0.2274)	0.4928 (0.1778)	0.3398 (0.0617)	0.2763 (0.0391)

values in parenthesis are on dry weight basis

during growth and it was more in higher concentrations. At every interval the control plants have the highest value of root/shoot ratio and it decreased with increase in metal concentration.

4. Dry weight per plant

Dry weight distribution of *V. mungo* plant is given in table 4. Values are on per plant basis.

4.1 Root

Dry weight of roots in control seedlings showed a gradual and steady increase during growth up to 168 hr (Fig. 1A). 1μM mercury(II) chloride treated plants showed the same pattern as that of control at all intervals. But when concentration of Hg increased to 5μM significant reduction was observed from 48 hr onwards and at 168 hrs only less than half value was there. The change in the root dry weight per plant in 10μM treatment was different from other treatments, in that, up to 48 hr dry weight registered a gradual increase but then it decreased drastically at 72 hr and 168 hr intervals by 63% and 81 % respectively of the control.

4.2 Stem

Only insignificant differences were observed in the dry weight distribution of stem of *V. mungo* seedlings grown in various concentrations of $HgCl_2$ up to 24 hr of growth (Fig. 1B), afterwards a considerable reduction in dry weight of

Table 4. Effect of mercury on dry weight per plant of *V. mungo* seedlings (mg plant^{-1}) during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	0.30 ± 0.02	0.60 ± 0.01	1.24 ± 0.04	1.74 ± 0.12	2.47 ± 0.15	3.98 ± 0.09
	1 µM		0.65 ± 0.02	1.03 ± 0.05	1.44 ± 0.03	2.27 ± 0.08	4.05 ± 0.23
	5 µM		0.51 ± 0.01	1.03 ± 0.03	1.31 ± 0.04	1.37 ± 0.04	1.60 ± 0.02
	10 µM		0.51 ± 0.02	1.03 ± 0.03	1.40 ± 0.05	0.91 ± 0.03	0.78 ± 0.02
Stem	Control	2.04 ± 0.02	3.11 ± 0.13	5.23 ± 0.25	6.57 ± 0.13	12.14 ± 0.63	23.04 ± 1.59
	1 µM		2.54 ± 0.04	4.60 ± 0.19	6.65 ± 0.30	11.69 ± 0.43	16.70 ± 0.89
	5 µM		2.32 ± 0.04	4.12 ± 0.13	5.61 ± 0.12	9.71 ± 0.42	14.86 ± 1.18
	10 µM		2.35 ± 0.11	3.72 ± 0.09	5.47 ± 0.08	10.51 ± 0.28	14.53 ± 0.67
Leaf	Control	0.13 ± 0.003	0.51 ± 0.007	1.34 ± 0.02	3.93 ± 0.12	8.63 ± 0.27	17.80 ± 0.93
	1 µM		0.50 ± 0.009	1.21 ± 0.02	3.55 ± 0.05	6.82 ± 0.17	12.30 ± 0.75
	5 µM		0.30 ± 0.005	1.02 ± 0.02	2.83 ± 0.06	4.68 ± 0.09	6.29 ± 0.20
	10 µM		0.30 ± 0.007	0.81 ± 0.02	2.40 ± 0.05	4.24 ± 0.11	5.43 ± 0.29
Cotyledon	Control	42.40 ± 0.91	27.80 ± 0.44	14.80 ± 0.48	9.15 ± 0.33	6.70 ± 0.29	3.36 ± 0.06
	1 µM		28.27 ± 0.55	16.61 ± 0.64	14.17 ± 0.33	9.80 ± 0.34	6.10 ± 0.08
	5 µM		29.26 ± 0.86	20.56 ± 0.76	15.55 ± 0.43	11.58 ± 0.13	10.29 ± 0.24
	10 µM		28.94 ± 0.85	23.27 ± 0.48	16.61 ± 0.50	12.38 ± 0.37	12.40 ± 0.33

Values are mean of six replicates ± SE

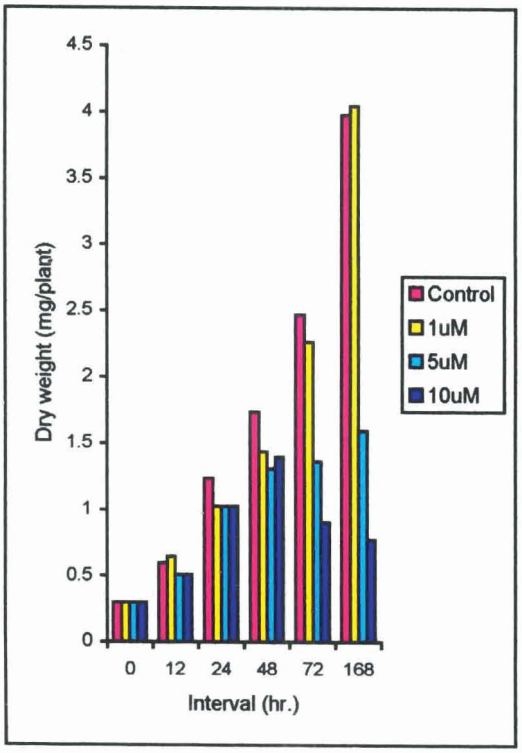

Fig. 1A. Effect of mercury on dry weight/plant of root tissue of *V. mungo* seedling.

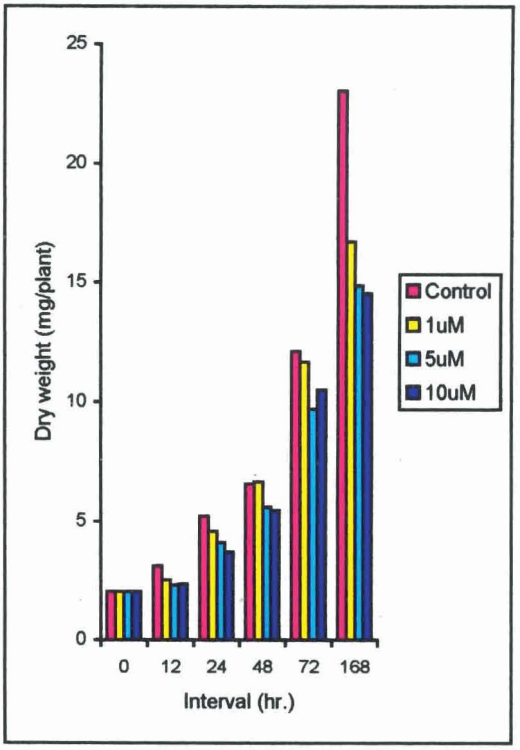

Fig. 1B. Effect of mercury on dry weight/plant of stem tissue of *V. mungo* seedling.

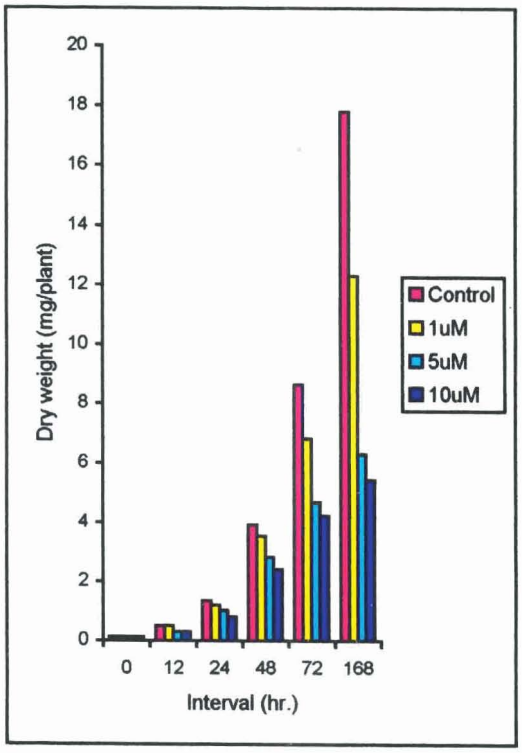

Fig. 1C. Effect of mercury on dry weight /plant of leaf tissue of *V. mungo* seedling.

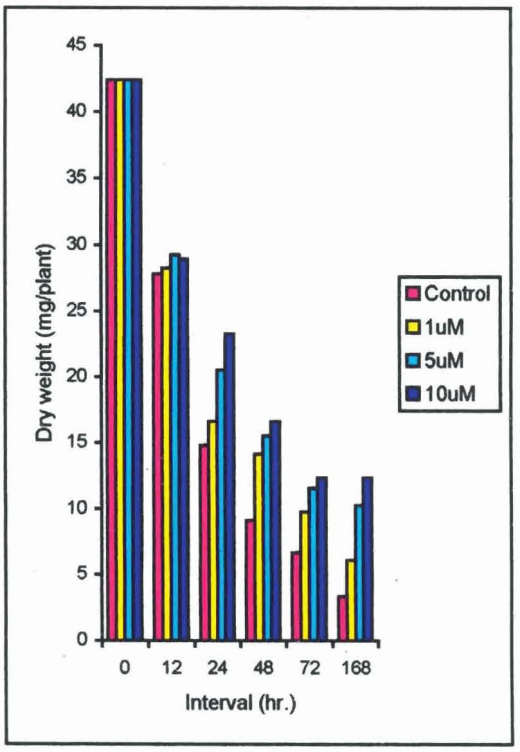

Fig. 1D. Effect of mercury on dry weight/plant of cotyledon tissue of *V. mungo* seedling.

stem occurred in 1, 5 and 10μM Hg treatment in comparison with respective control but the difference in dry weight between the different concentrations of Hg were negligible. Reduction in stem dry weight was very significant at 168 hr compared to respective control ($P < 0.01$).

4.3 Leaf

Leaf dry weight on per plant basis did not show significant changes in treatments compared to the control upto 48 hr. But 72 hr onwards it was reduced in 5 and 10μM concentrations ($P<0.01$ and $P < 0.01$) and at 168 hr the dry weight content of leaf was less than half in 5 and 10μM concentrations compared to control.

4.4 Cotyledon

Dry weight reduction was very significant during growth and only 9% of biomass was left behind in the cotyledons of control plants after 168 hr of treatment. Though a general reduction in biomass occurred in the colyledons of all experimental plants in all concentrations, significant amount of biomass was retained in all experimentals compared to the control.

5. Water content percentage

Generally water content percentage was more in root and stem tissues, compared to leaf and cotyledons (Table 5). In root and stem, the control seedlings

Table 5. Effect of mercury on water content percentage in various tissues of *V. mungo* seedlings during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	92.46	93.32	92.45	93.68	95.04	94.61
	1 µM		92.83	92.88	92.48	93.72	92.02
	5 µM		93.75	92.31	91.46	91.63	91.79
	10 µM		93.75	91.64	90.25	91.88	92.32
Stem	Control	88.81	89.82	91.08	91.98	93.07	93.23
	1 µM		90.73	91.14	92.08	93.18	92.94
	5 µM		90.43	90.68	91.65	92.63	92.66
	10 µM		89.91	90.77	91.76	91.75	91.14
Leaf	Control	93.53	83.18	83.71	83.76	87.20	89.78
	1 µM		83.39	84.93	84.31	87.81	89.62
	5 µM		84.94	83.29	83.70	87.58	88.41
	10 µM		84.89	83.89	84.39	87.09	87.18
Cotyledon	Control	68.39	77.34	84.74	87.82	89.61	90.25
	1 µM		77.49	83.66	82.64	86.02	85.74
	5 µM		77.03	79.98	81.47	84.42	85.10
	10 µM		77.03	77.07	80.75	84.24	83.55

have the maximum water content and it showed a slight reduction with increase in concentration of mercury in the growth medium. Water content in leaf tissue showed no considerable change among control and treatments. Cotyledonary tissues of control seedlings possessed more water content percentage when compared with that of treatments. Water content in cotyledons of treatment seedlings showed a gradual reduction with increase in mercury concentration.

6. Relative growth rate (RGR)

Relative growth rate pattern of *V. mungo* seedlings on the basis of root length, stem length and leaf area is given in table 6. Relative growth rate was calculated and represented as mg $mg^{-1}h^{-1}$.

6.1 RGR of root

Control plants exhibited an enhanced relative growth rate during 12 to 24 hr and there after growth rate was slightly reduced. Seedlings treated with Hg at 1, 5 and 10 μM concentrations showed almost the same pattern of relative growth, but the values were slightly reduced compared to the control. At concentrations 5μM and 10μM, the growth rate was reduced at a significant level particularly during 72 and 168 hr of growth. In 10 μM the growth rate obtained was negative.

Table 6. Effect of mercury on relative growth rate (RGR) of V.mungo seedlings (mg mg^{-1} h^{-1}) during growth

Tissue	Treatment	Interval (hour)				
		12	24	48	72	168
Root	Control	0.0578	0.0605	0.0141	0.0146	0.0050
	1 μM	0.0644	0.0384	0.0140	0.0190	0.0060
	5 μM	0.0442	0.0586	0.0100	0.0019	0.0021
	10 μM	0.0442	0.0586	0.0116	-0.0179	-0.0016
Stem	Control	0.0351	0.0433	0.0095	0.0256	0.0067
	1 μM	0.0183	0.0495	0.0154	0.0235	0.0037
	5 μM	0.0107	0.0479	0.0129	0.0229	0.0044
	10 μM	0.0118	0.0383	0.0161	0.0272	0.0037
Leaf	Control	0.1139	0.0805	0.0448	0.0328	0.0075
	1 μM	0.1123	0.0736	0.0448	0.0272	0.0061
	5 μM	0.0697	0.1020	0.0425	0.0210	0.0031
	10 μM	0.0697	0.1831	0.0453	0.0237	0.0026

6.2 RGR of stem

Relative growth rate of stem also showed a significant increase during 12 – 24 hr in the experimentals compared to the control. In all treatments, relative growth rate of stem exhibited reduction at 12 hr interval itself and then a significant increase occurred at 24 hr followed by a sharp reduction during further growth period. At 10 μM Hg concentration the, relative growth rate of stem was very low compared to other treatments as well as control.

6.3 RGR of leaf

Relative growth rate of leaves in control plants showed linear reduction during all intervals. Even though experimental plants also exhibited almost similar pattern of growth, at higher concentrations such as 5 and 10 μM, the growth rate reduced significantly compared to the control as well as their respective values of previous intervals.

7. Net assimilation rate (NAR)

NAR values of control and treatments in terms of mg $mm^{-2}h^{-1}$, calculated from the data of leaf area and dry weight per plant at various interval are given in Table 7.

In the control and 1 μM mercury treated plants, the net assimilation rate increased upto 24 hr and then reduced considerably during 48 to 168 hr. The net

Table 7. Effect of mercury on net assimilation rate (NAR) of *V.mungo* seedlings (mg mm^{-2} h^{-1}) during growth

Treatment	Interval (hour)					
	0	12	24	48	72	168
Control	0	0.0024	0.0027	0.0026	0.002	0.0023
1 µM		0.0026	0.0030	0.0028	0.0015	0.0013
5 µM		0.0014	0.0036	0.0025	0.0012	0.0010
10 µM		0.0014	0.0029	0.0025	0.0014	0.0001

assimilation at 5 and 10 μM concentrations exhibited very low rate at 12 hr and increased at 24 hr. But during 48 to 168 hr of intervals significant reduction was occurred and the least value of net assimilation rate was shown by 10 μM treated plants during 168 hr of growth. Increase in NAR of 5 and 10 μM treated plants from 12 to 24 hr was more pronounced than that of 1 μM and control.

8. Tolerance index percentage

Tolerance index percentage with respect to root growth, stem growth and leaf enlargement is given in Table 8.

8.1 Root

When tolerance index on the basis of root growth is considered (Fig. 2A), there was a marked decrease in 1, 5 and 10μM mercury concentrations at 12 hr interval. In all treatments tolerance index decreased with increase in Hg concentration and also with growth period. The maximum decrease in tolerance index of root throughout the experimental period was in the order of 10 μM > 5 μM >1 μM > 0 μM.

8.2 Stem

It is interesting to note that the tolerance index percentage in respect of the stem growth showed two distinct phases (Fig. 2B). At 12 and 24 hr intervals plants of higher concentrations exhibited more tolerance index compared to lower

Table 8. Effect of mercury on tolerance index percentage of *V.mungo* seedlings during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	100	100	100	100	100	100
	1 µM		87.2 ± 16.34	63.66 ± 4.67	48.91 ± 3.95	46.85 ± 3.30	48.67 ± 1.83
	5 µM		76.94 ± 17.86	46.56 ± 6.44	27.11 ± 1.79	22.84 ± 2.63	16.59 ± 1.08
	10 µM		66.58 ± 5.55	39.67 ± 2.82	23.68 ± 1.76	19.77 ± 1.82	12.26 ± 0.72
Stem	Control	100	100	100	100	100	100
	1 µM		81.16 ± 5.63	73.99 ± 7.51	80.22 ± 5.25	77.54 ± 8.75	74.44 ± 7.47
	5 µM		89.45 ± 7.23	77.70 ± 4.89	68.84 ± 4.47	65.87 ± 3.14	59.11 ± 4.24
	10 µM		88.24 ± 11.46	77.36 ± 2.70	55.9 ± 3.98	61.67 ± 4.58	56.85 ± 4.90
Leaf area	Control	100	100	100	100	100	100
	1 µM		83.98 ± 7.14	68.85 ± 4.79	93.33 ± 5.46	89.31 ± 5.06	70.54 ± 1.49
	5 µM		65.37 ± 8.67	62.51 ± 9.67	82.46 ± 6.02	59.47 ± 2.94	31.29 ± 0.84
	10 µM		60.17 ± 5.89	50.3 ± 5.46	74.21 ± 7.06	49.10 ± 1.39	22.11 ± 0.65

Values are mean of six replicates ± SE

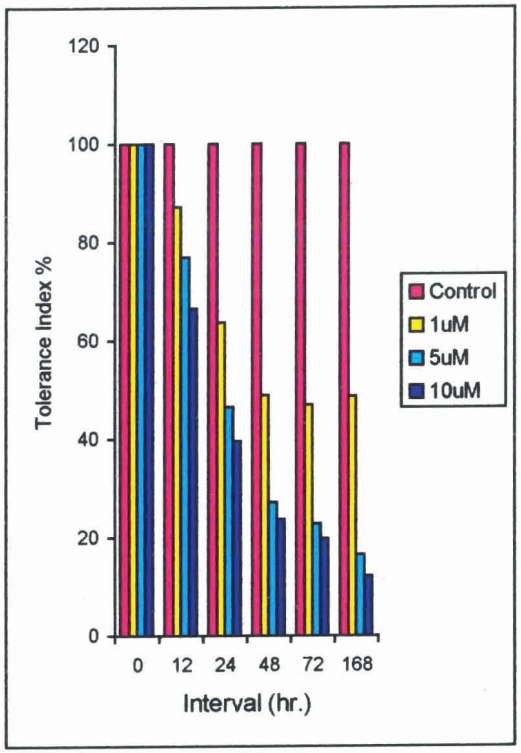

Fig. 2A. Effect of mercury on tolerance index % of root tissue of *V. mungo* seedling.

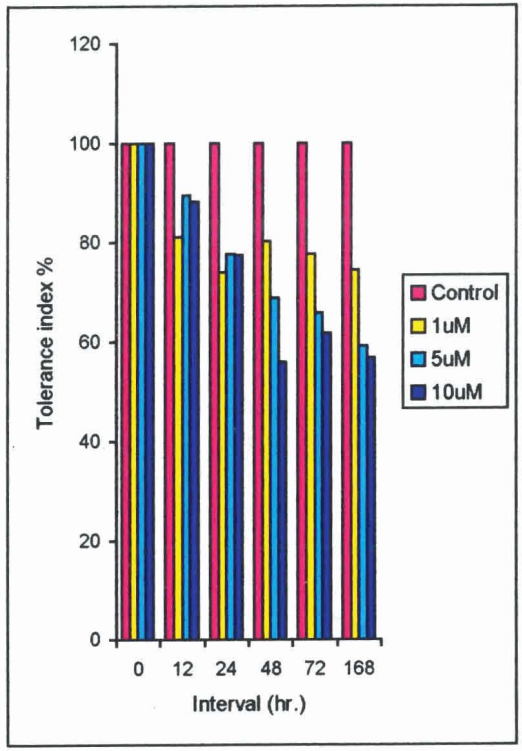

Fig. 2B. Effect of mercury on tolerence index % of stem tissue of *V. mungo* seedling.

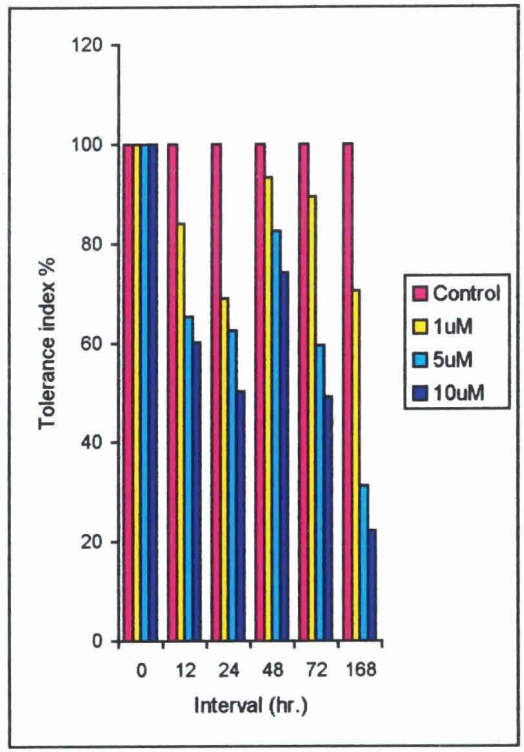

Fig. 2C. Effect of mercury on tolerance index % of leaf area of *V. mungo* seedling.

concentration. This character changed during 48, 72 and 168 hr, where higher concentrations exhibited lesser tolerance index values and the reduction in tolerance index was directly proportional to the concentration of mercury.

8.3 Leaf area

Tolerance index with respect to leaf area (Fig. 2C) showed a general reduction in the values proportional to the increasing concentrations of mercury. However, at 24 hr interval, the tolerance index of all treatments showed a significant reduction compared to the previous as well as next intervals. Then the values decreased gradually during growth up to 168 hr. Maximum reduction in tolerance index was for 10 μM treatment at 168 hr.

9. Stomatal studies

Table 9 shows the effect of mercury on stomatal density, size and index of *Vigna mungo* seedlings after 168 hr of growth. Stomatal index of lower and upper side of mercury treated *Vigna mungo* leaves registered a gradual but insignificant increase with increase in mercury concentration in the treatment solution. However increase in stomatal index at 10 μM mercury treatment was significant ($P < 0.01$) when compared to that of the control.

Stomatal density on both adaxial and abaxial side of leaf increased significantly with mercury treatment and the highest value observed was in 10 μM

TABLE 9. Effect of mercury on stomatal density, size and stomatal index of *V. mungo* seedlings

Interval (hr)	168							
Treatment	Stomatal density (mm^2)		Stomatal length (μm)		Stomatal width (μm)		Stomatal index %	
	Lower side	Upper side	Lower side	Upper side	Lower side	Upper side	Lower side	Upper side
Control	133.67 ± 1.87	75.36 ± 1.02	37.10 ± 1.08	34.33 ± 1.51	22.38 ± 1.36	20.75 ± 0.66	41.01 ± 1.08	37.09 ± 1.23
1 μM	142.51 ± 2.13	83.12 ± 1.39	34.86 ± 1.26	31.95 ± 2.23	17.21 ± 0.76	14.14 ± 0.90	41.94 ± 1.33	38.52 ± 1.04
5 μM	161.38 ± 2.44	99.47 ± 1.36	30.14 ± 1.09	27.40 ± 0.06	14.59 ± 0.06	10.29 ± 0.26	44.06 ± 1.15	40.24 ± 1.82
10 μM	175.72 ± 2.73	108.30 ± 2.81	26.86 ± 0.89	24.14 ± 1.68	12.57 ± 0.35	9.86 ± 0.11	45.29 ± 1.02	41.36 ± 1.07

Values are mean of six replicates ± SE

mercury concentration. Number of epidermal cells per unit area also increased with increase in mercury concentration. While stomatal length and width reduced significantly with mercury treatment. Stomatal width in 10 μM concentration was only half of the value for respective control.

10. Dry weight percentage

Table 10 shows the dry weight percentage of *V. mungo* plants grown under various concentrations of mercury.

10.1 Root

The difference in dry weight percentage of roots in control and treatment plants up to 24 hr were insignificant, but thereafter the experimental plants showed an increase compared to the corresponding control plants. From 48 hr onwards all treated plants showed dry weight increase in roots irrespective of the concentrations of mercury. In 10 μM treatment the root dry weight percentage showed a progressive increase upto 48 hr and decreased thereafter by 17% and 21% at 72 and 168 hr respectively compared to the value for 48 hr.

10.2 Stem

In control stem, dry weight percentage was decreased during growth upto 168 hr. In all treatments, stem tissues exhibited a slight higher biomass content in comparison with their respective controls. However, this increase was insignificant

statistically in most cases. But at 10μM concentration, the dry weight increase in the stem was found to be significant compared to the control ($P < 0.01$) at 72 and 168 hr.

10.3. Leaf

Dry matter percentage of leaf was reduced gradually during growth in the control as well as experimentals. When a comparison is made between the values of control and experimentals, the change in dry weight percentage was negligible in all cases irrespective of the mercury concentration, except at 168 hr. At this interval, an increase in dry weight percentage was observed for 5 and 10 μM treatment compared to the control.

10.4 Cotyledon

The dry weight percentage of cotyledons of control as well as experimental plants decreased significantly during the period of growth up to 168 hr. Among the treatments, reduction in dry weight percentage was in the order of control > 1μM > 5 μM > 10 μM. In comparison with the control, increase in dry matter of cotyledon was significant only after 24 hr and maximum dry matter was present in the plants grown in 10μM mercury after 168 hr of growth and the value was almost double that of control at the same interval.

Table 10. Effect of mercury on dry weight percentage of various tissues of *V. mungo* seedlings during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	7.54 ± 0.39	6.68 ± 0.26	7.55 ± 0.18	6.32 ± 0.27	4.96 ± 0.19	5.39 ± 0.14
	1 µM		7.17 ± 0.18	7.12 ± 0.33	7.52 ± 0.16	6.28 ± 0.17	7.98 ± 0.23
	5 µM		6.25 ± 0.17	7.69 ± 0.20	8.54 ± 0.21	8.37 ± 0.15	8.21 ± 0.22
	10 µM		6.25 ± 0.23	8.36 ± 0.18	9.75 ± 0.27	8.12 ± 0.17	7.68 ± 0.21
Stem	Control	11.19 ± 0.12	10.18 ± 0.39	8.92 ± 0.33	8.02 ± 0.19	6.93 ± 0.13	6.77 ± 0.21
	1 µM		9.27 ± 0.18	8.86 ± 0.27	7.92 ± 0.27	6.82 ± 0.26	7.06 ± 0.15
	5 µM		9.57 ± 0.20	9.32 ± 0.24	8.35 ± 0.18	7.37 ± 0.17	7.34 ± 0.28
	10 µM		10.09 ± 0.40	9.23 ± 0.17	8.24 ± 0.14	8.25 ± 0.17	8.86 ± 0.16
Leaf	Control	6.47 ± 0.15	16.82 ± 0.17	16.29 ± 0.25	16.24 ± 0.13	12.80 ± 0.32	10.22 ± 0.13
	1 µM		16.61 ± 0.33	15.07 ± 0.23	15.69 ± 0.26	12.19 ± 0.19	10.38 ± 0.19
	5 µM		15.06 ± 0.18	16.71 ± 0.39	16.30 ± 0.22	12.42 ± 0.24	11.59 ± 0.19
	10 µM		15.11 ± 0.26	16.11 ± 0.29	15.61 ± 0.17	12.91 ± 0.25	12.82 ± 0.17
Cotyledon	Control	31.61 ± 0.43	22.66 ± 0.13	15.26 ± 0.21	12.18 ± 0.21	10.39 ± 0.22	9.75 ± 0.12
	1 µM		22.51 ± 0.30	16.34 ± 0.32	17.36 ± 0.16	13.98 ± 0.23	14.26 ± 0.21
	5 µM		22.97 ± 0.08	20.02 ± 0.29	18.53 ± 0.23	15.58 ± 0.26	14.90 ± 0.18
	10 µM		22.97 ± 0.11	22.93 ± 0.21	19.25 ± 0.18	15.76 ± 0.18	16.56 ± 0.19

Values are mean of six replicates ± SE

BIOCHEMICAL STUDIES

11. Starch content

Starch content in the tissue of *V. mungo* seedlings grown under various concentrations of mercury is given in table 11.

11.1 Root

Starch content in root tissue showed only slight variation and the variation increased during growth. In 1μM mercury treatment, a slight reduction was observed during all intervals compared to the control. This trend of reduction was shown in the next concentration *i.e.*, 5 μM at all intervals in general and during 72 hr and 168 hr, this reduction was very significant ($P < 0.01$). Only very low starch content was present in the root tissues in 10μM concentration at all intervals. In comparison with the control, the roots of 10μM concentration contained only less than one fourth of the starch during 72 – 168 hr of treatment.

11.2 Stem

Stem tissue of *V. mungo* seedlings contained less starch than the roots in the control as well as in the experimentals upto 24 hr and during growth from 72 – 168 hr, starch content registered a significant increase. Compared to the control in 1 μM mercury treatment, only marginal reduction was observed up to 48 hr but in 72 and 168 hr the reduction was significant. Starch content of stem tissue at 5 μM

concentration also showed a reduction compared to 1 μM and the reduction was very significant at 168 hr ($P < 0.01$). At 10 μM treatment also, there occurred further significant reduction particularly at 72 and 168 hr.

11. 3 Leaf

Starch content of leaves showed a linear increase during growth. But under mercury stress of 1μM concentration, starch content in leaf tissue reduced significantly only at 168 hr ($P < 0.02$). In 5 μM concentration also the same trend was observed. In 10 μM concentration, at all intervals except 24 and 48 hr, there was significant reduction compared to that of other concentrations as well as control. In comparison with the control, at 168 hr, only less than half of the starch content was present in 10 μM treatment.

11.4 Cotyledon

Cotyledons of *V. mungo* seedlings contained considerable amount of starch and during growth significant reduction was occurred. At 1 μM concentration of mercury, the starch content of cotyledon reduced gradually and at all intervals starch content was significantly more when compared to the control. Similarly at 5 μM concentration the starch content present in the cotyledon at all intervals was more in comparison with the control as well as that of 1μM. At 10μM concentration also starch content was very high. In other words, starch mobilization from

Table 11. Effect of mercury on starch content in various tissues of *V. mango* seedlings (mg g^{-1} dry tissue) during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	39.28 ± 1.34	29.37 ± 1.19	24.57 ± 0.32	22.25 ± 0.90	32.26 ± 1.61	36.40 ± 0.78
	1 µM		20.48 ± 0.77	23.96 ± 0.95	22.23 ± 0.73	24.66 ± 0.23	27.36 ± 0.38
	5 µM		17.20 ± 0.93	19.27 ± 0.21	21.10 ± 0.40	22.59 ± 0.51	16.74 ± 0.29
	10 µM		13.32 ± 0.61	10.62 ± 0.27	17.74 ± 0.85	15.34 ± 0.22	8.59 ± 0.30
Stem	Control	18.98 ± 1.70	22.93 ± 0.53	17.33 ± 0.13	24.89 ± 0.12	56.77 ± 1.73	60.95 ± 1.36
	1 µM		19.80 ± 0.97	17.92 ± 0.61	21.92 ± 0.52	34.40 ± 1.59	50.82 ± 1.55
	5 µM		12.10 ± 0.41	12.94 ± 0.72	20.96 ± 0.44	34.00 ± 0.71	43.35 ± 1.18
	10 µM		16.85 ± 0.45	19.87 ± 0.95	21.87 ± 0.43	24.12 ± 0.33	29.16 ± 0.47
Leaf	Control	6.01 ± 0.88	14.33 ± 0.43	24.38 ± 0.84	24.09 ± 0.34	48.05 ± 1.02	81.76 ± 2.17
	1 µM		12.31 ± 0.44	23.73 ± 0.79	27.04 ± 0.27	45.87 ± 1.30	71.14 ± 2.79
	5 µM		11.35 ± 0.26	29.13 ± 0.31	26.93 ± 0.61	39.50 ± 0.89	46.44 ± 1.24
	10 µM		6.45 ± 0.24	28.60 ± 0.80	33.81 ± 1.75	38.17 ± 1.10	35.23 ± 1.25
Cotyledon	Control	103.09 ± 3.80	81.24 ± 1.29	56.47 ± 1.51	39.65 ± 2.01	23.99 ± 0.58	11.78 ± 0.43
	1µM		84.49 ± 1.09	65.59 ± 1.22	51.22 ± 1.35	43.29 ± 1.75	27.21 ± 0.72
	5µM		86.86 ± 2.52	72.59 ± 1.10	66.33 ± 2.19	58.43 ± 1.54	39.90 ± 1.57
	10µM		89.86 ± 2.38	77.90 ± 2.40	70.87 ± 1.51	63.89 ± 1.73	51.67 ± 2.15

Values are mean of six replicates ± SE

cotyledons was highly inhibited by various concentrations of mercury in comparison with the control and the rate of inhibition was progressively increased with the concentration of mercury.

12. Sugar content

Table 12 shows the variation in sugar content in different parts of *V. mungo* seedlings due to mercury toxicity.

12.1 Root

Sugar content in the root system of control plants exhibited general increase as growth advanced to 168 hr. In 1μM mercuric chloride, roots showed only slight increase in sugar content except at 72 and 168 hr when compared to that of control and at 168 hr, the sugar content was less than that of the control. Almost the same trend was shown by roots of plants grown at 5 μM also. However at higher concentration (10 μM) sugar content increased further and maximum sugar content was observed at 168 hr.

12.2 Stem

Stem tissues of control plant exhibited a general increase in sugar content. Plants grown in 1 μM $HgCl_2$ solution, showed a slight increase when compared to the control in all intervals. The increase was significant at 72 and 168 hr ($P < 0.01$). Further increase of sugar content was observed in the stem tissue of plants grown in

5μM and 10 μM concentrations. However at 168 hr the difference was negligible in comparison with that of 5 μM. When a comparison is made between the control and each treatment, sugar content was increased proportional to the concentration of $HgCl_2$ in the growth medium at all intervals except 168 hr of 10μM concentration.

12. 3 Leaf

Leaf tissue of *V. mungo* seedlings treated with 1μM concentration of mercury showed only slight increase in sugar content compared to that of the control except at 72 and 168 hr, where the increase was significant ($P < 0.01$). At 5 μM concentration also leaf tissue exhibited same trend in the distribution of sugars, but comparatively sugar content was more in samples of 5 μM compared to 1 μM. However the difference was not in a significant level.

Again at 10μM concentration a significant (but insignificant when compared with 5 μM) increase was observed in sugar content of leaf tissue at all intervals in comparison with the control.

12.4 Cotyledon

Cotyledons of *Vigna mungo* contained considerable amount of sugars and the content increased slightly during growth. However samples treated with different concentrations of mercury registered slight reduction compared to that of

Table 12. Effect of mercury on sugar content in various tissues of *V. mungo* seedlings (mg g^{-1} dry tissue) during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	22.55 ± 2.39	20.81 ± 1.35	30.07 ± 1.06	51.27 ± 2.53	69.17 ± 2.18	86.83 ± 1.48
	1 µM		21.05 ± 1.09	36.38 ± 1.83	74.60 ± 1.06	67.83 ± 2.07	72.06 ± 2.38
	5 µM		21.44 ± 1.28	57.22 ± 1.95	75.41 ± 1.76	94.03 ± 1.08	86.06 ± 2.38
	10 µM		26.72 ± 2.08	66.51 ± 1.56	91.08 ± 1.33	99.75 ± 1.85	110.29 ± 1.95
Stem	Control	72.03 ± 1.43	50.00 ± 0.88	40.47 ± 1.79	56.61 ± 1.12	78.93 ± 2.31	107.39 ± 2.22
	1µM		66.88 ± 1.73	51.35 ± 1.41	73.11 ± 1.90	127.57 ± 1.91	134.42 ± 2.83
	5 µM		85.16 ± 1.36	78.54 ± 0.97	101.40 ± 1.80	135.14 ± 2.04	166.49 ± 5.04
	10 µM		92.67 ± 2.08	120.90 ± 1.63	117.48 ± 0.97	149.21 ± 1.94	158.80 ± 1.47
Leaf	Control	52.86 ± 2.47	12.43 ± 0.48	19.33 ± 0.80	39.66 ± 0.92	31.88 ± 1.48	67.51 ± 0.78
	1 µM		12.28 ± 0.78	23.03 ± 0.53	42.83 ± 0.51	58.08 ± 1.97	79.87 ± 1.45
	5 µM		18.13 ± 0.53	28.01 ± 0.48	45.15 ± 0.49	50.72 ± 1.77	95.94 ± 1.64
	10 µM		22.37 ± 0.53	39.48 ± 0.81	44.46 ± 1.02	73.51 ± 0.62	97.50 ± 0.70
Cotyledon	Control	5.28 ± 0.60	8.61 ± 0.40	16.71 ± 0.52	25.86 ± 1.07	38.31 ± 0.87	45.64 ± 1.95
	1 µM		9.46 ± 0.71	17.01 ± 1.16	20.79 ± 0.92	33.12 ± 0.93	31.84 ± 1.12
	5 µM		9.66 ± 0.57	13.17 ± 0.75	21.48 ± 0.49	31.19 ± 0.96	29.53 ± 1.34
	10 µM		11.89 ± 0.35	12.34 ± 0.35	20.21 ± 0.68	26.46 ± 0.57	24.34 ± 0.48

Values are mean of six replicates ± SE

the control. In 1 μM treatment, the reduction was insignificant at some intervals and same trend was followed in the cotyledons of plants grown in 5 and 10 μM. But in 10 μM concentration reduction in sugar content was significant at all intervals except at 12 hr.

13. Protein content

Data on the effect of mercury on protein content in *V. mungo* seedlings are given in Table 13.

13.1. Root

Protein content of *V. mungo* seedlings grown in 1 μM mercuric chloride solution registered only negligible reduction in root tissue during the period of growth upto 72 hr and afterwards at 168 hr, there occurred a significant reduction compared to the control. When the concentration was 5 μM, at 24 hr interval, the reduction in protein was significant ($P < 0.01$).

Protein content in root tissue of the seedlings at 5 μM concentration also exhibited a gradual reduction during growth up to 168 hr and the reduction was significant compared to that of the control as well as that of 1 μM concentration. Almost the same trend of reduction was observed in the protein content of root tissues at 10 μM concentration. However, comparatively very low protein content was present in the root tissue of plants grown in 10 μM solution of $HgCl_2$ during

the period 24 – 168 hr and when compared with control this reduction was very significant.

13.2 Stem

Stem tissue also exhibited same variation in protein content as that of root under different concentrations of mercury. At 1 μM concentration, protein content reduction was negligible at all intervals except at 168 hr compared to the control. In comparison with the control, significant decrease in protein content was observed in the stem tissues of 5 μM and 10 μM concentrations of mercury.

13.3 Leaf

Leaf tissue also exhibited almost similar trend in protein content as that of the stem. Only insignificant reduction was occurred in 1 μM treatment compared to the control. But at 5 and 10 μM solution, the protein content was reduced significantly at all intervals and more reduction was observed in 10 μM concentration during 24 – 168 hr.

13.4 Cotyledon

The cotyledon showed a reduced protein content when the growth advanced to 168 hr in the control itself. In 1 μM treatment, protein content was decreased significantly during the period of 48–168 hr ($P < 0.01$). Almost the same trend of reduction in protein content was shown in cotyledons of 5 μM and the reduction

Table 13. Effect of mercury on protein content in various tissues of *V.mungo* seedlings (mg g^{-1} dry tissue) during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	47.48 ± 1.86	58.53 ± 1.80	41.59 ± 1.99	38.77 ± 1.58	35.69 ± 2.82	37.11 ± 1.32
	1 μM		58.58 ± 2.51	42.13 ± 1.26	36.45 ± 2.13	34.55 ± 2.23	21.05 ± 0.90
	5 μM		60.00 ± 1.92	35.50 ± 1.43	24.36 ± 1.64	16.97 ± 1.55	13.52 ± 0.86
	10 μM		57.76 ± 0.16	30.14 ±1.32	17.13 ± 1.44	15.15 ± 1.72	12.24 ± 1.56
Stem	Control	38.61 ± 0.71	44.89 ± 1.08	44.93 ± 1.46	40.27 ± 1.67	36.36 ± 1.59	34.42 ± 1.45
	1 μM		44.77 ± 0.97	45.15 ± 0.90	43.18 ± 1.77	32.11 ± 2.05	26.77 ± 1.56
	5 μM		39.60 ± 1.46	35.73 ± 1.50	31.02 ± 1.32	29.72 ± 2.44	18.39 ± 1.63
	10 μM		39.54 ± 1.19	34.02 ± 1.19	32.89 ± 2.43	23.39 ± 1.21	11.34 ± 0.95
Leaf	Control	78.05 ± 2.47	33.47 ± 0.71	30.02 ± 0.80	26.29 ± 0.52	25.94 ± 1.09	28.67 ± 1.37
	1 μM		31.31 ± 0.78	30.39 ± 0.93	24.54 ± 0.85	24.53 ± 0.64	24.08 ± 1.16
	5 μM		29.75 ± 0.06	27.95 ± 0.66	20.55 ± 0.86	18.04 ± 0.81	12.60 ± 0.73
	10 μM		30.31 ± 0.86	23.15 ± 0.93	19.67 ± 0.83	16.96 ± 1.08	9.91 ± 0.55
Cotyledon	Control	39.99 ± 0.63	49.65 ± 1.19	63.50 ± 1.31	59.69 ± 0.59	64.00 ± 2.02	34.04 ± 1.54
	1 μM		52.73 ± 0.76	50.37 ± 0.55	31.22 ± 0.92	34.84 ± 0.93	15.50 ± 0.77
	5 μM		45.49 ± 1.09	31.97 ± 0.65	20.13 ± 1.19	18.74 ± 0.90	9.53 ± 0.81
	10 μM		41.27 ± 0.87	26.78 ± 0.52	12.10 ± 0.51	11.23 ± 0.55	8.70 ± 0.66

Values are mean of six replicates ± SE

was significant in all samples compared to that of 1 μM. Further reduction at a significant level was occurred in the protein content of cotyledons of 10 μM solution at all intervals and after 48 hr only very low amount of protein was present in these samples.

14. Free amino acids content

Table 14 shows the distribution of free amino acids of *V. mungo* seedlings grown under various concentration of mercury.

14.1 Root

The free amino acids content in root tissues were comparatively more than that of other tissues. During growth up to 168 hr, there was only negligible change in the free amino acid content. In 1 μM mercury treatment, there was a slight increase compared to the control, but the increase was seen insignificant except at 48 and 168 hr ($P < 0.1, 0.1$). The other concentrations of mercury *i.e.*, 5 μM and 10 μM also showed only slight increase at all intervals. But when a comparison is made with the control, root tissue contained significantly more free amino acids in 5 and 10 μM concentrations.

14.2 Stem

Free amino acid spectrum of stem tissue also registered almost similar trend of distribution as that of root tissue. In 1 μM concentration, the increase of free

amino acid was insignificant at all stages compared to the control and similar pattern was shown at 5 μM and 10 μM concentrations also. Even though the increase in free amino acid content of the stem tissue at 5 and 10 μM concentrations are not significant when compared with each other as well as that of 1 μM, the increase in free amino acid content in 10 μM concentration was significant when compared with respective control values except at 72 hr interval. In 168 hr of growth the free amino acid content was found to decrease in control and all treatments compared to the corresponding values of their previous interval.

14. 3 Leaf

Distribution of free amino acids content of leaf tissue showed almost similar pattern to that of shoot tissue. Free amino acid content of leaf tissue at 1 μM concentration was slightly higher than the control and during 48 - 168 hr, the increase was significant compared to the control ($P < 0.01$). But at 5 μM concentration, though further increase was observed, the changes were insignificant in comparison with lower concentration (1 μM). Significant enhancement was occurred in the free amino acid content at 10 μM concentration during all intervals when compared with the other concentrations *i.e.*, 1 and 5 μM as well as with the control.

Table 14. Effect of mercury on total free amino acid content in various tissues of *V.mungo* seedlings (mg g^{-1} dry tissue) during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	8.49 ± 0.17	12.78 ± 0.30	11.66 ± 0.20	13.41 ± 0.47	13.89 ± 0.40	12.78 ± 0.13
	1 µM		11.99 ± 0.18	13.31 ± 0.55	15.69 ± 0.27	14.93 ± 0.36	14.66 ± 0.15
	5 µM		17.92 ± 0.32	17.17 ± 0.09	15.57 ± 0.15	17.51 ± 0.11	16.17 ± 0.10
	10 µM		20.32 ± 0.24	17.01 ± 0.16	14.56 ± 0.10	18.47 ± 0.12	15.36 ± 0.10
Stem	Control	4.20 ± 0.12	6.97 ± 0.08	11.15 ± 0.22	11.60 ± 0.25	16.16 ± 0.29	9.45 ± 0.15
	1 µM		8.20 ± 0.22	12.35 ± 0.15	16.16 ± 0.25	14.22 ± 0.44	12.46 ± 0.28
	5 µM		7.57 ± 0.16	14.16 ± 0.22	14.01 ± 0.12	16.55 ± 0.27	12.81 ± 0.41
	10 µM		9.32 ± 0.26	15.49 ± 0.29	17.35 ± 0.24	17.09 ± 0.00	13.21 ± 0.09
Leaf	Control	14.68 ± 0.02	5.59 ± 0.00	7.73 ± 0.05	6.34 ± 0.04	11.33 ± 0.23	9.33 ± 0.20
	1 µM		6.38 ± 0.40	9.82 ± 0.09	9.50 ± 0.13	13.04 ± 0.25	12.24 ± 0.10
	5 µM		7.33 ± 0.05	8.56 ± 0.12	9.20 ± 0.06	10.50 ± 0.24	12.25 ± 0.09
	10 µM		9.31 ± 0.09	10.18 ± 0.08	10.51 ± 0.13	12.24 ± 0.15	13.42 ± 0.08
Cotyledon	Control	3.48 ± 0.020	5.33 ± 0.13	6.66 ± 0.41	9.93 ± 0.25	6.93 ± 0.19	3.38 ± 0.10
	1 µM		5.21 ± 0.06	6.98 ± 0.16	6.34 ± 0.04	6.65 ± 0.70	4.84 ± 0.42
	5 µM		5.83 ± 0.04	5.89 ± 0.10	6.85 ± 0.05	8.41 ± 0.13	6.91 ± 0.13
	10 µM		5.14 ± 0.03	5.80 ± 0.11	7.75 ± 0.04	8.95 ± 0.06	7.31 ± 0.18

Values are mean of six replicates ± SE

14.4 Cotyledon

Comparatively low amount of free amino acids were present in the cotyledons of *V. mungo* and during growth the content was increased considerably upto 48 hr and then decreased. In 1 μM mercury treatment the free amino acid content remained unchanged except at 168 hr, where a slight increase was observed. Similar pattern of free amino acid distribution was shown by cotyledons at 5 μM concentration also. At 10 μM concentration the free amino acid content was almost similar to that of other treatments, but slightly increased during 48–168 hr. However, when a comparison is made with the control, cotyledons of 5 and 10 μM concentrations showed significantly higher content at 72 and 168 hr, while during 12 and 24 hr no change was occurred. In general, in all treatments amino acid content showed an increase at 168 hr of growth.

15. Proline content

Proline content of various sample tissues of *V. mungo* treated with various concentrations of $HgCl_2$ are given in table15.

15.1 Root

When a comparison is made between the control and treatments, at 1 μM concentration proline content was significantly increased during all intervals except at 12 hr. At 5 μM concentration, proline content was again increased significantly. At 10 μM concentration also there occurred significant increase over the other

treatments as well as control. Proline content in all treatments and control increased considerably as growth advanced from 0-168 hr.

15.2 Stem

Proline content of stem tissue increased progressively during growth in the control. In 1 μM mercury treatment there was significant increase in proline content at all intervals compared to their respective control. 5 μM mercury treatment also showed increased accumulation of proline at all intervals. Significant increase of proline was shown by the stem tissue of 10 μM concentration compared to the previous concentration (5 μM) as well as to the control.

15.3 Leaf

Proline content of leaf tissue showed a progressive increase compared to the control at 1 μM concentration ($P < 0.01$). However, when the concentration was increased to 5 μM, there was only slight increase in proline and similar trend of increase in proline was shown by the leaf tissue of 10 μM concentration also. However, compared to the control the proline content in leaf tissue of all the concentrations were significantly high.

15.4 Cotyledon

Cotyledon showed only very low content of proline compared to other tissues. Mercury treatment at 1,5 and 10 μM concentrations resulted only in slight

Table 15. Effect of mercury on proline content in various tissues of *V. mungo* seedlings (μ moles g^{-1} dry tissue) during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	39.39 ± 0.54	38.77 ± 1.50	46.89 ± 1.46	49.74 ± 0.51	45.53 ± 1.31	52.77 ± 0.50
	1μM		39.75 ± 1.40	54.90 ± 0.84	56.32 ± 0.82	55.09 ± 0.74	60.85 ± 1.30
	5μM		47.04 ± 0.64	58.91 ± 2.08	58.64 ± 0.93	62.74 ± 2.55	68.33 ± 1.22
	10μM		54.24 ± 1.92	60.81 ± 0.84	59.18 ± 1.74	66.51 ± 2.02	64.47 ± 1.17
Stem	Control	30.92 ± 0.18	22.20 ± 0.98	37.53 ± 0.45	54.04 ± 1.12	53.54 ± 1.59	52.64 ± 1.03
	1μM		27.72 ± 0.76	43.43 ± 1.00	53.99 ± 2.49	62.06 ± 1.21	65.72 ± 1.98
	5μM		27.38 ± 0.73	54.97 ± 2.28	69.64 ± 2.47	69.21 ± 0.88	67.72 ± 1.13
	10μM		35.78 ± 0.50	71.24 ± 1.70	74.97 ± 0.76	71.23 ± 1.63	78.61 ± 1.23
Leaf	Control	40.03 ± 0.93	10.64 ± 0.37	14.43 ± 0.68	17.36 ± 0.31	28.28 ± 0.78	33.55 ± 0.98
	1μM		16.62 ± 0.36	18.71 ± 0.60	24.67 ± 0.32	38.47 ± 0.41	45.66 ± 1.25
	5μM		16.87 ± 0.46	19.21 ± 0.42	26.75 ± 0.55	40.82 ± 0.89	47.97 ± 0.69
	10μM		19.32 ± 0.06	22.47 ± 0.43	29.02 ± 0.38	41.05 ± 1.32	49.61 ± 1.40
Cotyledon	Control	6.83 ± 0.22	10.77 ± 0.31	19.99 ± 0.66	26.03 ± 0.33	25.89 ± 0.48	19.18 ± 0.82
	1μM		11.95 ± 0.22	20.07 ± 0.43	20.33 ± 0.40	24.75 ± 0.93	20.48 ± 0.35
	5μM		12.19 ± 0.22	17.78 ± 0.20	20.35 ± 0.59	23.56 ± 0.45	23.42 ± 1.14
	10μM		12.80 ± 0.48	16.53 ± 0.39	20.78 ± 0.47	22.60 ± 0.70	21.38 ± 0.97

Values are mean of six replicates ± SE

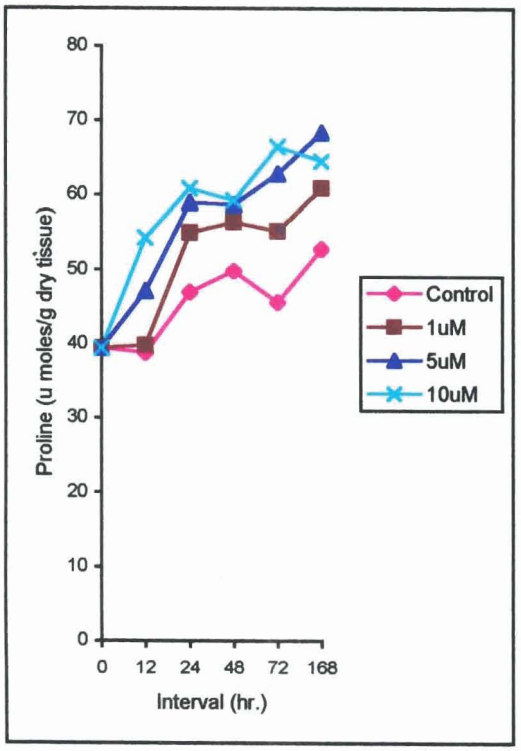

Fig. 3A. Effect of mercury on proline content of root tissue of *V. mungo* seedling.

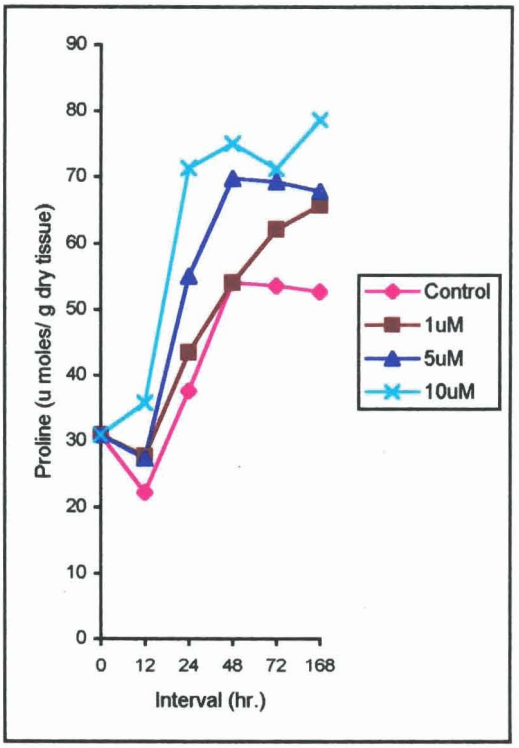

Fig. 3B. Effect of mercury on proline content of stem tissue of *V. mungo* seedling.

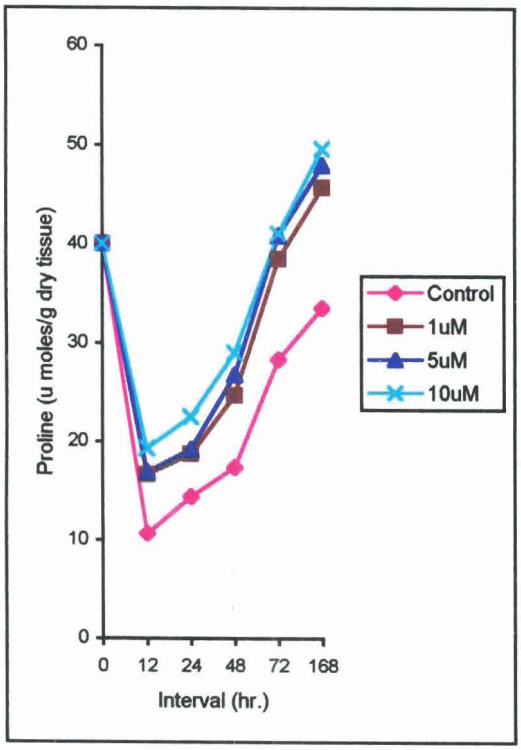

Fig. 3C. Effect of mercury on proline content of leaf tissue of *V. mungo* seedling.

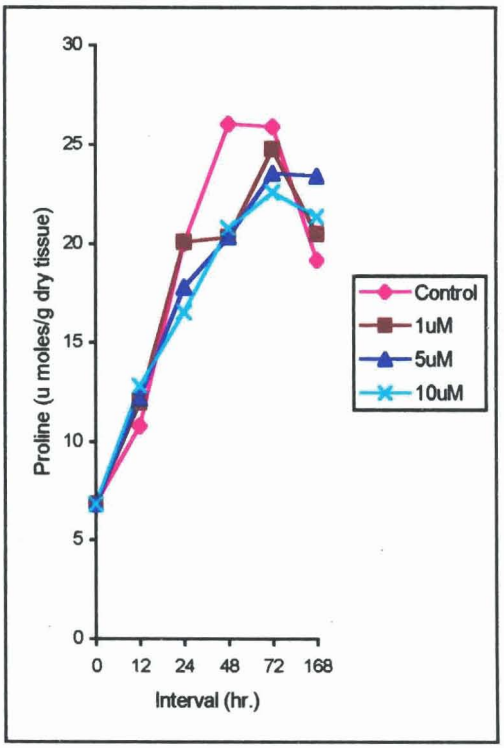

Fig. 3D. Effect of mercury on proline content of cotyledon tissue of *V. mungo* seedling.

changes in the proline content. The peroline content was found to be increased from 0-72 hr duration in control and treatments but showed a slight decrease in 168 hr.

16. Nitrate reductase activity (NRA)

Nitrate reductase activity in various tissues of control and mercury treated *V. mungo* seedlings are given in table 16.

16.1 Root

Root of control *V. mungo* seedlings showed almost uniform activity of nitrate reductase during growth up to 168 hr. Under mercury stress at 1 μM level, NR activity was significantly reduced at all intervals compared to the control.

In 5 μM and 10 μM mercury treatments, nitrate reductase activity was decreased more in comparison with respective control and in each interval the least activity observed was in 10 μM concentration. Nitrate reductase activity showed a linear decrease in mercury treated seedlings during growth from 0 to 168 hr.

16.2 Stem

Nitrate reductase activity in the stem tissue of control plants was progressively increasing up to 48 hr and gradually declined thereafter. In 1μM mercury treatment, NR activity reduced slightly after 72 hr. But significant reduction of NR activity was observed in the stem of plants grown in 5μM up to

168 hr. In 10μM, the nitrate reductase activity of stem was very feeble and in comparison with the control the activity was almost less than one third at all intervals except at 12 hr.

16.3 Leaf

NR activity of leaf tissue of *V. mungo* was comparatively higher than that of other tissues during growth up to 168 hr and the activity was almost uniform throughout the experimental period. In 1 μM concentration, the activity was slightly reduced during 12 and 24 hr and afterwards there occurred significant decrease upto 168 hr compared to the control. NR activity became very feeble in the leaf samples of 5 and 10μM concentration at 12 and 24 hr itself and afterwards highly reduced in comparison with the control.

16.4 Cotyledon

Cotyledons of *V. mungo* seedlings showed moderate NR activity during growth up to 48 hr and then decreased gradually. But mercury treatment with 1 μM solution of $HgCl_2$ resulted in a significant reduction of enzyme activity compared to the control. At 5μM concentration, NR activity was almost similar to that of 1μM up to 24 hr, and later the activity did not show any change. In 10 μM concentration, cotyledons registered only very feeble NR activity at all intervals, compared to the control as well as other samples.

Table 16. Effect of mercury on nitrate reductase activity in various tissues of *V. mungo* seedlings (μ moles NO_2 $h^{-1}g^{-1}$ fresh tissue) during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	2.04 ± 0.04	2.44 ± 0.04	2.15 ± 0.02	2.81 ± 0.04	1.97 ± 0.02	2.12 ± 0.02
	1μM		1.85 ± 0.05	1.71 ± 0.01	2.01 ± 0.03	0.98 ± 0.05	1.09 ± 0.04
	5μM		1.42 ± 0.03	1.38 ± 0.02	1.20 ± 0.02	0.78 ± 0.03	0.83 ± 0.02
	10μM		1.16 ± 0.04	0.99 ± 0.04	1.01 ± 0.04	0.86 ± 0.02	0.57 ± 0.01
Stem	Control	1.72 ± 0.07	1.54 ± 0.05	2.08 ± 0.06	2.39 ± 0.03	1.95 ± 0.04	1.73 ± 0.04
	1μM		1.24 ± 0.01	1.48 ± 0.03	1.42 ± 0.03	1.24 ± 0.04	0.94 ± 0.04
	5μM		1.07 ± 0.04	1.38 ± 0.05	1.20 ± 0.02	0.92 ± 0.02	0.76 ± 0.01
	10μM		0.90 ± 0.04	0.73 ± 0.01	0.93 ± 0.03	0.67 ± 0.04	0.51 ± 0.03
Leaf	Control	2.69 ± 0.09	2.30 ± 0.03	3.04 ± 0.01	2.67 ± 0.04	2.18 ± 0.09	2.41 ± 0.01
	1μM		2.07 ± 0.05	2.14 ± 0.04	1.87 ± 0.04	1.63 ± 0.02	1.26 ± 0.05
	5μM		1.90 ± 0.01	1.76 ± 0.04	1.26 ± 0.00	1.20 ± 0.02	1.00 ± 0.06
	10μM		1.72 ± 0.07	1.51 ± 0.02	0.95 ± 0.05	0.92 ± 0.05	0.76 ± 0.01
Cotyledon	Control	1.86 ± 0.04	2.29 ± 0.04	1.84 ± 0.04	2.07 ± 0.06	1.44 ± 0.04	0.66 ± 0.04
	1μM		1.56 ± 0.01	1.27 ± 0.03	1.33 ± 0.03	1.11 ± 0.05	1.12 ± 0.01
	5μM		1.48 ± 0.06	1.16 ± 0.06	0.94 ± 0.01	1.03 ± 0.07	1.03 ± 0.02
	10μM		0.98 ± 0.03	0.58 ± 0.03	0.80 ± 0.02	0.78 ± 0.01	0.71 ± 0.05

Values are mean of six replicates ± SE

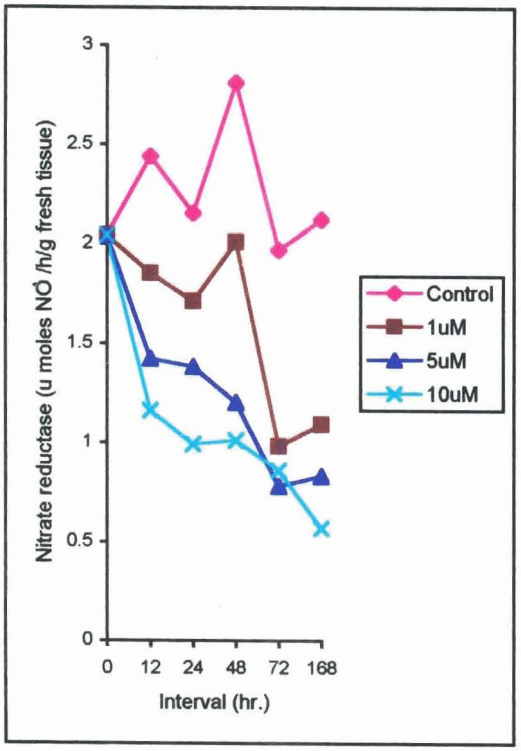

Fig.4A. Effect of mercury on nitrate reductase activity in root tissue of *V. mungo* seedling.

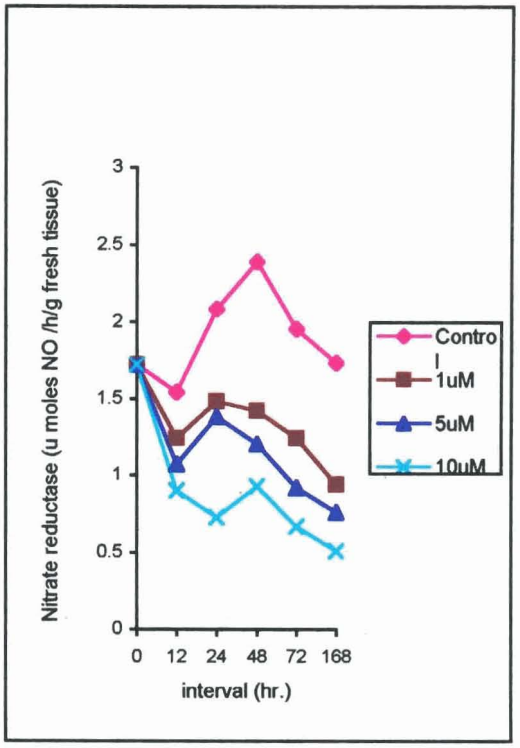

Fig. 4B. Effect of mercury on nitrate reductase activity in stem tissue of *V. mungo* seedling.

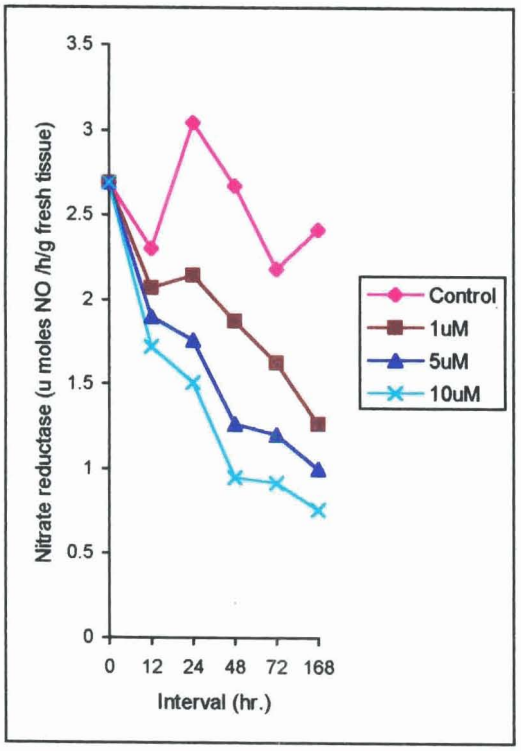

Fig. 4C. Effect of mercury on nitrate reductase activity in leaf tissue of *V. mungo* seedling.

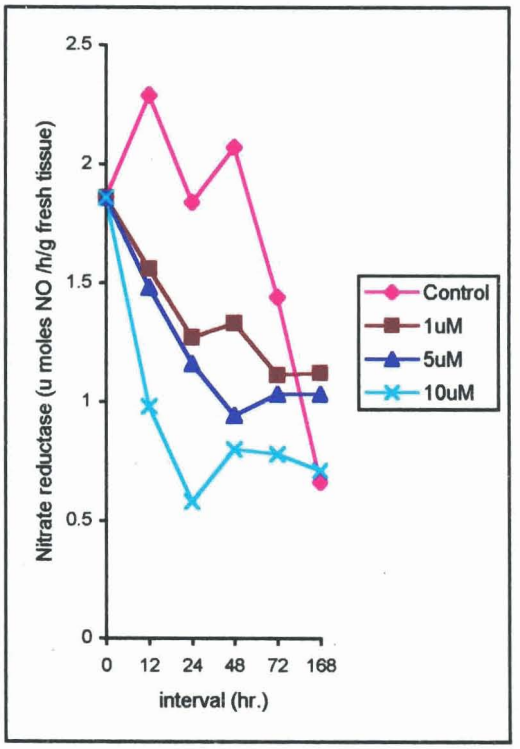

Fig. 4D. Effect of mercury on nitrate reductase activity in cotyledon tissue of *V. mungo* seedling.

17. Lipid content

Table 17 shows the distribution of lipid content in different plant parts of *V. mungo* seedlings.

17.1 Root

Root tissue showed slight reduction of lipid content in samples treated with 1,5 and 10 μM concentrations of mercury. The reduction of lipid content in all concentrations at 72–168 hr were significant compared to the control.

17.2 Stem

Lipid content of stem tissue did not show much significant changes when the plants were grown in various concentrations of mercury. However, when the concentration of mercury was high (10μM), lipid content was reduced significantly during all intervals except 72 and 168 hr compared to that of control

17. 3 Leaf

Leaf contained more lipid than root and stem and during growth there was no much changes. In various concentrations of mercury only negligible changes were observed at all intervals. Minimum lipid content was present in the leaves of plants grown in 10μM mercury at 168 hr.

Table 17. Effect of mercury on total lipid content in various tissues of *V. mungo* seedling (mg g^{-1} dry tissue) during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	4.00 ± 0.21	4.98 ± 0.07	7.00 ± 0.33	4.02 ± 0.25	6.02 ± 0.54	5.00 ± 0.27
	1 µM		3.00 ± 0.14	3.99 ± 0.05	5.99 ± 0.63	3.98 ± 0.18	3.98 ± 0.15
	5 µM		4.01 ± 0.32	5.00 ± 0.47	3.02 ± 0.22	2.99 ± 0.112	3.02 ± 0.14
	10 µM		4.03 ± 0.61	3.04 ± 0.24	3.00 ± 0.37	3.01 ± 0.11	2.01 ± 0.06
Stem	Control	5.00 ± 0.70	3.00 ± 0.04	5.00 ± 0.49	4.99 ± 0.70	5.97 ± 0.26	3.99 ± 0.22
	1 µM		3.98 ± 0.23	3.98 ± 0.02	5.00 ± 0.53	3.98 ± 0.13	3.01 ± 0.19
	5 µM		3.99 ± 0.02	4.02 ± 0.43	3.23 ± 0.18	3.20 ± 0.21	4.00 ± 0.25
	10 µM		2.02 ± 0.53	3.03 ± 0.13	2.98 ± 0.04	5.04 ± 0.39	3.00 ± 0.15
Leaf	Control	5.95 ± 0.35	7.52 ± 0.53	5.52 ± 0.62	6.51 ± 0.61	5.09 ± 0.33	6.99 ± 0.44
	1 µM		8.49 ± 0.39	6.51 ± 0.47	5.51 ± 0.39	7.50 ± 0.51	5.50 ± 0.37
	5 µM		7.99 ± 0.84	4.97 ± 0.39	6.46 ± 0.44	4.49 ± 0.122	6.51 ± 0.49
	10 µM		7.49 ± 0.44	5.45 ± 0.22	6.55 ± 0.82	6.12 ± 0.18	4.01 ± 0.21
Cotyledon	Control	12.15 ± 0.32	11.12 ± 0.75	9.49 ± 0.54	13.02 ± 0.50	7.97 ± 0.37	1.99 ± 0.01
	1 µM		9.80 ± 0.36	10.59 ± 0.73	12.02 ± 0.64	10.42 ± 0.85	3.50 ± 0.16
	5 µM		11.94 ± 0.63	13.84 ± 0.82	12.62 ± 0.36	11.76 ± 0.57	6.51 ± 0.45
	10 µM		12.89 ± 0.58	11.28 ± 0.55	13.13 ± 0.94	12.75 ± 0.64	8.99 ± 0.52

Values are mean of six replicates ± SE

17.4 Cotyledon

Cotyledon of *V. mungo* showed the presence of more lipid content in comparison with all other tissues of the seedlings and during growth up to 168 hr, lipid content was reduced gradually and at all internals the reduction was significant. When the plants were subjected to treatment with various concentrations of mercury, the rate of reduction during growth was slower than that of the controls, so that more lipid was retained in the cotyledons after 168 hr. Concentration of 5 and 10μM treatments, also resulted in almost same trend in the utilisation of lipid as that of the 1 μM. However in these concentrations, more lipid was retained at all stages particularly during 72-168 hr compared to the control.

18. Phenolics

Total phenolic content of *V. mungo* seedlings are given in Table 18.

18.1 Root

Root tissue of control seedlings showed only very low quantity of phenolics. But the plants grown in various concentrations of mercury contained more phenolics in their roots. During growth up to 168 hr, there occurred slight but insignificant increase in phenolic content. There was no significant changes in the phenolics of roots when the concentration of mercury increased from 1μM to 10μM.

Table 18. Effect of mercury on total phenolics in various tissues of *V. mungo* seedlings (mg g^{-1} dry weight) during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	0.77 ± 0.02	0.73 ± 0.05	0.82 ± 0.007	0.81 ± 0.008	0.93 ± 0.02	1.23 ± 0.01
	1μM		1.05 ± 0.02	1.36 ± 0.01	1.36 ± 0.01	1.75 ± 0.02	2.40 ± 0.01
	5μM		1.46 ± 0.03	1.20 ± 0.01	1.36 ± 0.009	1.67 ± 0.006	2.56 ± 0.04
	10μM		1.38 ± 0.04	1.14 ± 0.02	1.37 ± 0.01	1.85 ± 0.01	1.69 ± 0.007
Stem	Control	0.30 ± 0.01	0.35 ± 0.01	0.55 ± 0.01	0.67 ± 0.01	0.72 ± 0.01	1.33 ± 0.01
	1μM		0.37 ± 0.01	0.47 ± 0.009	0.73 ± 0.02	1.03 ± 0.007	2.83 ± 0.02
	5μM		0.34 ± 0.02	0.61 ± 0.009	0.77 ± 0.01	1.24 ± 0.01	2.59 ± 0.03
	10μM		0.40 ± 0.02	0.73 ± 0.005	1.33 ± 0.01	1.70 ± 0.01	2.37 ± 0.06
Leaf	Control	1.34 ± 0.02	0.50 ± 0.005	0.55 ± 0.005	0.62 ± 0.005	0.78 ± 0.006	1.08 ± 0.01
	1μM		0.52 ± 0.01	0.62 ± 0.003	0.83 ± 0.006	1.31 ± 0.004	2.02 ± 0.009
	5μM		0.61 ± 0.008	0.63 ± 0.005	1.04 ± 0.003	1.61 ± 0.007	2.33 ± 0.01
	10μM		0.65 ± 0.01	0.63 ± 0.005	1.21 ± 0.003	1.70 ± 0.006	2.50 ± 0.01
Cotyledon	Control	0.54 ± 0.004	0.73 ± 0.01	0.98 ± 0.005	0.99 ± 0.007	0.87 ± 0.01	0.21 ± 0.02
	1μM		0.76 ± 0.009	0.98 ± 0.005	0.75 ± 0.005	0.72 ± 0.01	0.56 ± 0.006
	5μM		0.74 ± 0.004	0.80 ± 0.002	0.81 ± 0.003	0.96 ± 0.006	0.81 ± 0.003
	10μM		0.76 ± 0.005	0.70 ± 0.003	0.83 ± 0.003	0.95 ± 0.008	0.91 ± 0.002

Values are mean of six replicates ± SE

18.2 Stem

Stem tissue also contained very small quantity of phenolics in control plants and registered a slight and gradual increase up to 168 hr. Stem of mercury treated plants also contained only low quantity of phenolics. More phenolic content was occurred in the stem tissue of plants grown in 5 and 10μM concentrations of mercury during 72-168 hr of growth.

18. 3 Leaf

The distribution of phenolics in leaf tissue was almost similar to that of the stem tissue in the control as well as experimental plants. In this case also maximum phenolic content was present in the leaf tissue of plants subjected to 5 and 10μM mercury treatment during the period 72-168 hr.

18.4 Cotyledon

Phenolic content in cotyledons was very low in control seedlings and there was a gradual increase up to 48 hr and then decreased in 72 and 168 hr. But in plants treated with mercury, the reduction during various intervals was very slow, so more phenolics was present in the tissues of mercury treated plants at 168 hr compared to the control plants.

19. Chlorophyll content

Table 19 shows the chlorophyll content of *V. mungo* leaves during growth up to 168 hr. In control after 12 hr of growth significant reduction in chlorophyll-a,

Table 19. Effect of mercury on chlorophyll content in *V. mungo* seedlings (mg g^{-1} tissue) during growth

Interval (hour)	Treatment											
	Control			1µM			5µM			10µM		
	Chl.a	Chl.b	Total Chl.	Chl. a	Chl.b	Total Chl.	Chl. a	Chl. b	Total Chl.	Chl. a	Chl. b	Total Chl.
0	1.14 ± 0.01	2.10 ± 0.01	3.28 ± 0.02									
12	3.33 ± 0.06	6.36 ± 0.01	9.75 ± 0.24	3.37 ± 0.06	6.44 ± 0.12	9.93 ± 0.18	3.12 ± 0.13	6.04 ± 0.33	9.23 ± 0.46	2.12 ± 0.13	4.04 ± 0.20	6.22 ± 0.33
24	2.70 ± 0.0002	1.47 ± 0.04	4.24 ± 0.06	2.72 ± 0.007	1.46 ± 0.07	4.25 ± 0.07	2.21± 0.12	1.26 ± 0.18	3.47 ± 0.18	2.98 ± 0.02	1.74 ± 0.01	4.78 ± 0.006
48	5.05 ± 0.01	3.14 ± 0.006	8.25 ± 0.006	4.33 ± 0.32	2.54 ± 0.19	6.95 ± 0.45	3.19 ± 0.12	1.78 ± 0.06	5.03 ± 0.18	3.14 ± 0.06	1.67 ± 0.03	4.87 ± 0.06
72	12.03 ± 0.03	5.63 ± 0.03	17.81 ± 0.008	12.96 ± 0.08	4.84 ± 0.04	18.05 ± 0.08	12.00 ± 0.008	4.99 ± 0.003	17.07 ± 0.32	9.45 ± 0.08	4.34 ± 0.02	14.03 ± 0.15
168	16.14 ± 0.04	7.24 ± 0.01	21.92 ± 0.05	15.70 ± 0.004	5.97 ± 0.009	21.97 ± 0.01	13.11 ± 0.004	5.69 ± 0.005	19.07 ± 0.003	11.70 ± 0.03	3.59 ± 0.03	15.44 ± 0.05

Values are mean of six replicates ± SE

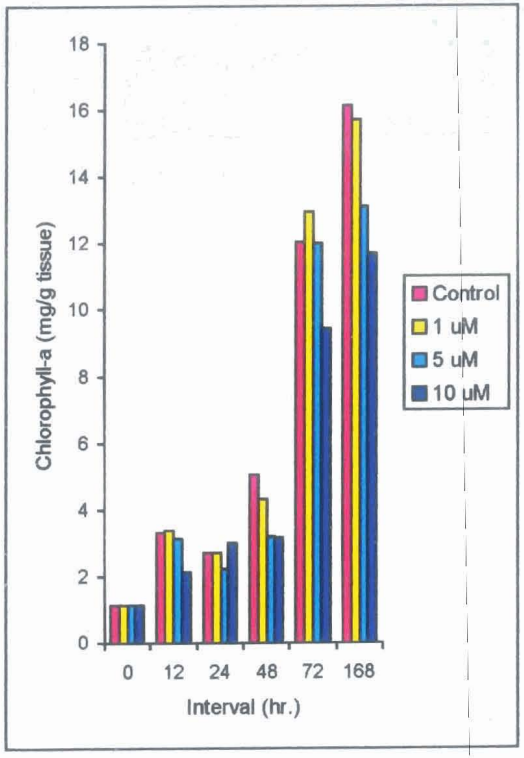

Fig. 5A. Effect of mercury on chlorophyll-a content in *V. mungo* seedling.

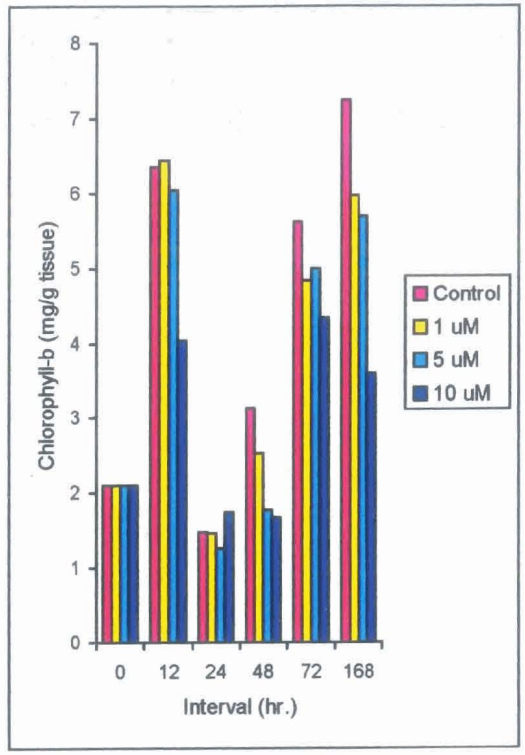

Fig. 5B. Effect of mercury on chlorophyll-b content in *V. mungo* seedling.

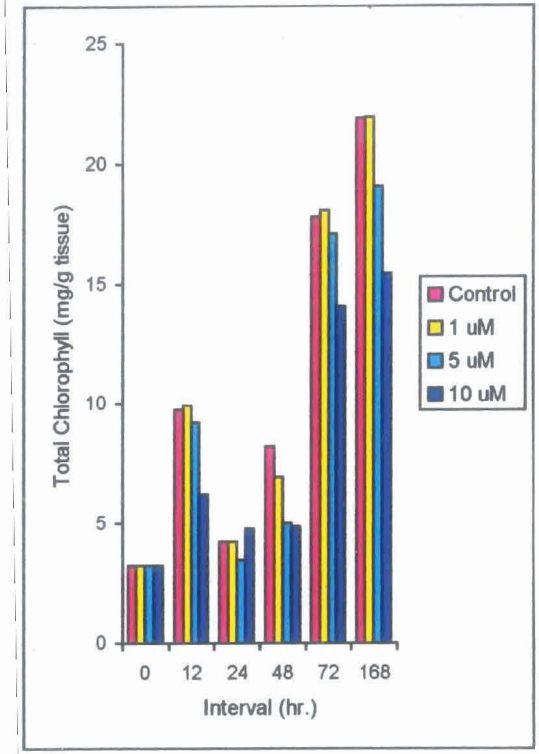

Fig. 5C. Effect of mercury on total chlorophyll content in *V. mungo* seedling.

b and total were occurred. Both chlorophyll-a, b as well as total chlorophyll were reduced to less than one half at 24 hr. Afterwards chlorophyll content was again increased to a maximum value at 168 hr.

Leaves of plants grown in 1μM mercury contained almost equal amount of chlorophyll-a, b and total after 12 hr. Similar to the control, at 24 hr, all types of chlorophyll were found to reduce significantly. After 24 hr, a progressive increase of chlorophyll content was observed up to 168 hr.

At 5 μM concentration also after 12 hr, the chlorophyll a, b and total were reduced similar to that of the control plants and at 24 hr, chlorophyll contents were highly reduced compared to other treatments as well as control.

Chlorophyll a, b and total chlorophyll contents of leaf tissue at 10μM concentration were significantly reduced even after 12 hrs compared to the control and this trend was followed at all intervals.

20. Chlorophyll a/b ratio

Table 20 shows the chlorophyll a/b ratio of the leaves of *V. mungo* seedlings grown in different concentrations of mercury. During leaf growth, a/b ratio was increased gradually. Mercury did not show any effect on the a/b ratio in treatments at any interval. However, at 168 hr, the plants treated with 10μM concentration showed an increased a/b ratio.

Table 20. Effect of mercury on chlorophyll a/b ratio of *V. mungo* seedlings

Treatment	Interval (hour)					
	0	12	24	48	72	168
Control	0.54	0.52	1.81	1.62	2.16	2.24
1 μM	-	0.52	1.84	1.71	2.66	2.62
5 μM	-	0.52	1.85	1.77	2.39	2.31
10 μM	-	0.54	1.73	1.85	2.16	3.34

21. Electrophoretic study

Plate 4 shows the protein bands obtained in the electrophoretic analysis of root tissues at 12 and 24 hr intervals. Since the results of 48-168 hr intervals were almost similar to that of 12 and 24 hr, only the result of the first two intervals were included in the text. SDS PAGE electrophoretic study of protein profile in the root tissues revealed some additional bands due to mercury treatment. These bands were of molecular weight 28, 35 and 44 kDa. No such bands were present in control tissue. These bands were more conspicuous in 5 and 10 μM mercury treated seedlings.

22. Bioaccumulation of mercury

Table 21 shows the data on bioaccumulation of mercury in various tissue of *V. mungo* seedlings treated with 1, 5 and 10μM $HgCl_2$ solution.

22.1 Root

Root of *V. mungo* seedlings, grown in different concentrations of mercury contained considerable quantity of the metal. At 1μM concentration the mercury content was comparatively low and increased gradually during growth up to 168 hr. When the concentration of mercury in the growth medium was 5μM, the accumulation also was increased considerably and more accumulation was observed in the samples at all intervals. In 10μM, the root contained considerable

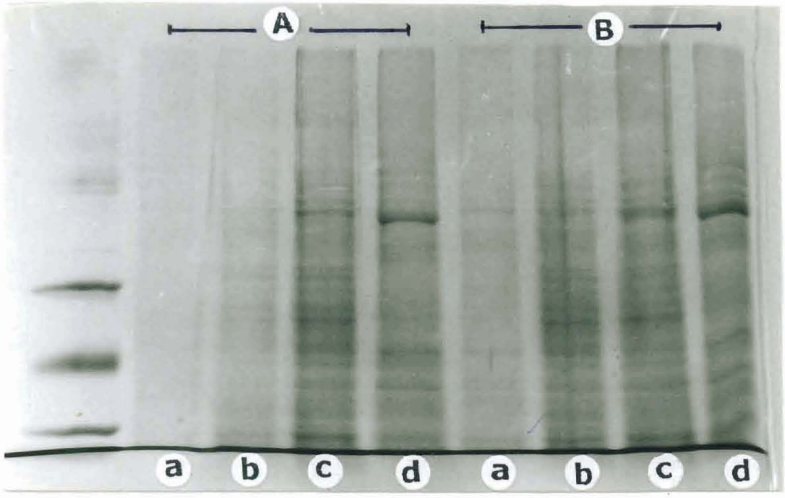

PLATE 4. **Protein profile of root tissues of mercury treated *V. mungo* seedlings**
(A) 12 hr (B) 24 hr
(a) Control (b) 1 μM (c) 5 μM (d) 10 μM

amount of mercury which increased significantly in accordance with the period of growth.

22.2 Stem

Mercury accumulation in the stem tissues of *V. mungo* seedlings was very low in comparison with root tissues. However, there was a gradual increase in mercury content during growth up to 168 hr in all treatments. Mercury content of the stem tissue showed a corresponding increase in accumulation with increase in metal concentration in the growth medium. At 10μM, the mercury content in stem increased considerably and at 168 hr, there was a significant increase in the accumulation.

22. 3 Leaf

Leaf tissue showed very low quantity of mercury when compared to the stem and root tissues. However there was a gradual increase in the quantity of mercury in the leaves at all concentrations during growth.

22.4 Cotyledon

Cotyledon showed the presence of mercury at all stages of growth and the content was increased during growth up to 168 hr. There was increase in the rate of accumulation of mercury when the concentration of the mercury in the culture medium increased from 1 μM to 10μM.

Table 21. Bioaccumulation of mercury in different tissues of *V. mungo* seedlings (μg g^{-1} dry tissue) during treatment

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	ND	ND	ND	ND	ND	ND
	1μM		0.64 ± 0.017	0.71 ± 0.004	1.10 ± 0.0034	3.09 ± 0.052	6.0 ± 0.051
	5μM		2.53 ± 0.031	5.35 ± 0.0032	11.13 ± 0.044	17.36 ± 0.019	35.41 ± 0.47
	10μM		11.15 ± 0.046	15.94 ± 0.058	21.27 ± 0.118	30.96 ± 0.017	78.65 ± 0.063
Stem	Control	ND	ND	ND	ND	ND	ND
	1μM		0.017 ± 0.00073	0.047 ± 0.00051	1.11 ± 0.0063	1.15 ± 0.033	1.29 ± 0.065
	5μM		0.36 ± 0.015	0.45 ± 0.003	1.26 ± 0.0053	2.84 ± 0.073	3.02 ± 0.0074
	10μM		0.65 ± 0.019	0.67 ± 0.0085	2.19 ± 0.0062	3.02 ± 0.0047	21.39 ± 0.074
Leaf	Control	ND	ND	ND	ND	ND	ND
	1μM		0.13 ± 0.044	0.187 ± 0.0012	0.197 ± 0.0093	0.203 ± 0.0083	0.321 ± 0.0014
	5μM		0.059 ± 0.002	0.146 ± 0.00096	0.40 ± 0.0042	0.776 ± 0.0014	1.02 ± 0.0081
	10μM		0.25 ± 0.019	0.516 ± 0.0028	1.06 ± 0.028	1.87 ± 0.039	3.20 ± 0.032
Cotyledon	Control	ND	ND	ND	ND	ND	ND
	1μM		0.11 ± 0.0028	0.48 ± 0.0013	0.38 ± 0.0091	0.36 ± 0.0045	0.31 ± 0.0062
	5μM		0.094 ± 0.004	0.37 ± 0.0017	0.506 ± 0.011	0.916 ± 0.024	1.12 ± 0.0088
	10μM		0.12 ± 0.0014	0.50 ± 0.0008	0.95 ± 0.0115	1.52 ± 0.012	1.78 ± 0.039

Values are mean of six replicates ± SE ND – not detected

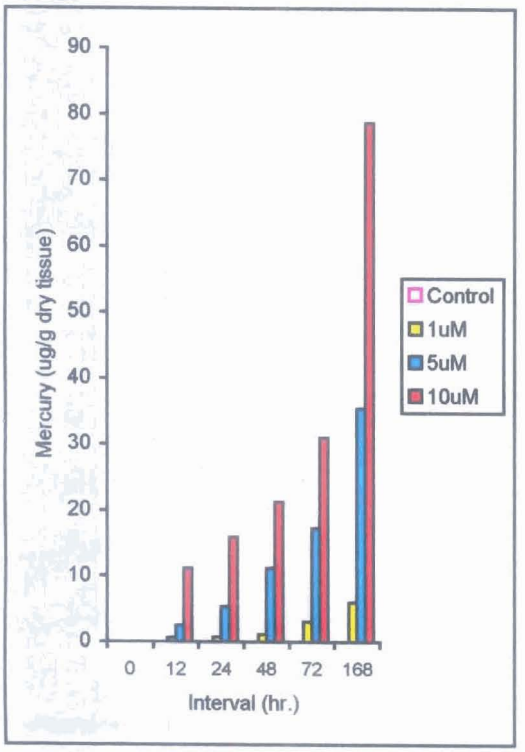

Fig. 6A. Mercury bio-accumulation in root tissue of *V. mungo* seedling.

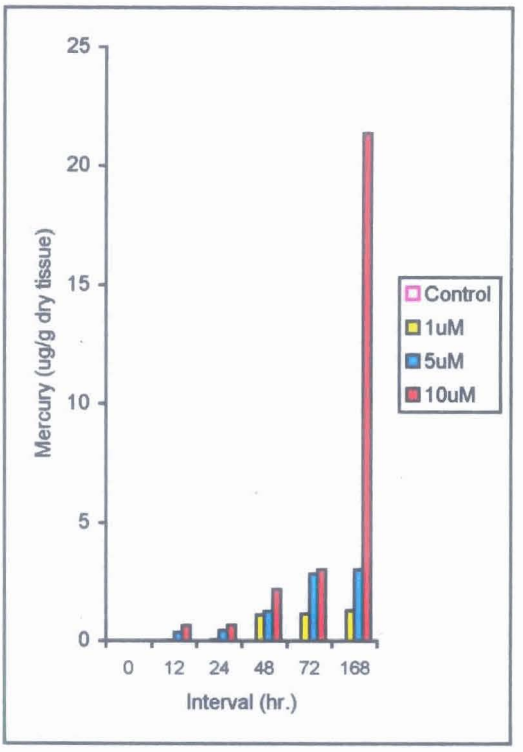

Fig. 6B. Mercury bio-accumulation in stem tissue of *V. mungo* seedling.

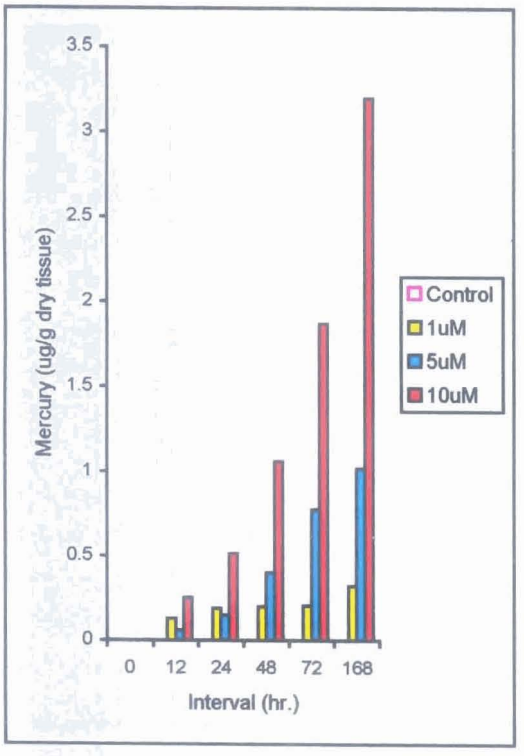

Fig. 6C. Mercury bio-accumulation in leaf tissue of *V. mungo* seedling.

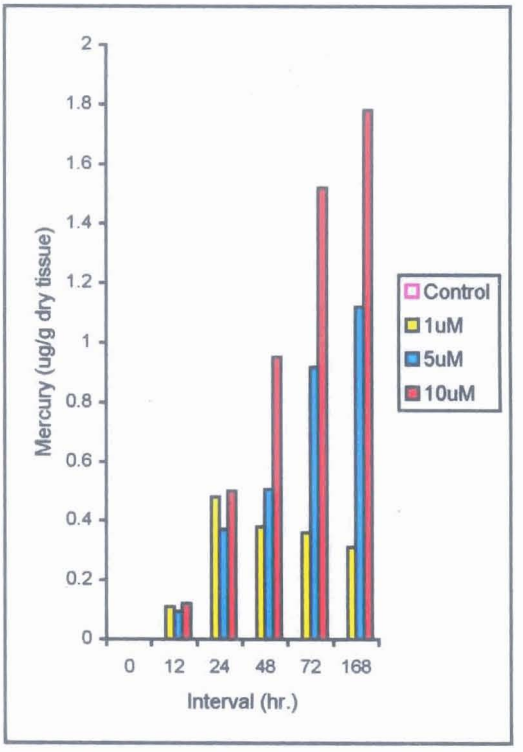

Fig. 6D. Mercury bio-accumulation in cotyledons of *V. mungo* seedling.

Table 22. Bioaccumulation percentage of mercury in different tissues of *V. mungo* seedlings during growth

Tissue	Treatment	Interval (hour)					
		0	12	24	48	72	168
Root	Control	0	0	0	0	0	0
	1μM		71.35	49.86	39.47	64.33	75.75
	5μM		83.14	84.71	83.71	79.30	87.28
	10μM		91.62	90.43	83.51	82.85	74.89
Stem	Control	0	0	0	0	0	0
	1μM		1.90	3.30	39.83	23.94	16.29
	5μM		11.83	7.12	9.48	12.97	7.44
	10μM		5.34	3.80	8.60	5.26	20.37
Leaf	Control	0	0	0	0	0	0
	1μM		14.49	13.13	7.07	4.23	4.05
	5μM		1.94	2.31	3.01	3.54	2.51
	10μM		2.05	2.93	4.16	3.26	3.05
Cotyledon	Control	0	0	0	0	0	0
	1μM		12.26	33.71	13.63	7.50	3.91
	5μM		3.09	5.86	3.81	4.18	2.76
	10μM		0.99	2.84	3.73	2.65	1.69

TABLE 23. Mercury content in nutrient solution after sample collection at each interval ($\mu g\ L^{-1}$)

Treatment	Interval (hour)					
	0	12	24	48	72	168
Control	0	0	0	0	0	0
1 µM	0	178.65 ± 3.14 (91.45)	161.43 ± 2.72 (108.15)	169.29 ± 2.86	159.13 ± 4.14	160.56 ± 3.21
5 µM	0	935.94 ± 5.52 (416.97)	874.63 ± 3.11 (474.05)	901.33 ± 3.46	912.09 ± 2.43	876.74 ± 7.35
10 µM	0	1932.64 ± 5.59 (765.19)	1906.23 ± 6.81 (786.14)	1898.27 ± 4.66	1839.61 ± 8.20	1881.42 ± 6.84

Values are mean of six replicates ± SE (values in parenthesis shows the difference between added mercury content in the nutrient solution and sum of the mercury absorbed by the plant and that retained in the nutrient solution – details are given in Materials and Methods.

23. Bioaccumulation percentage of mercury

Bioaccumulation percentage of mercury in different tissues of *Vigna mungo* seedlings at different intervals is given in Table 22. Maximum percentage of accumulation was observed in root tissues and least in leaf and cotyledons.

Table 23 shows mercury content in the nutrient solution after the sample collection at each interval. Since the nutrient solution was replaced with fresh solution at each 24 hr the quantity of mercury remained in the nutrient solution registered an increase with sampling period.

24. Histochemical studies

Eventhough histochemical staining was done on root and stem tissues of all the three concentrations and control at all intervals, dithizone positive staining was evidently seen in root tissues at higher concentrations of 5 and 10 µM mercury treatments. So the data on histochemical observations of root at all intervals and stem sections of 168 hr interval only are included in this study. Results are given in plates 5-11.

24.1. Root

One micromolar concentration did not show any dithizone positive materials up to 72 hr of treatment. After 12 hr, the root section of mercury treated seedlings showed yellow coloured material along the outer surface of the piliferous layer

which was absent in the control (Plate 5). In root sections of 5 and 10 μM concentrations after 24 hr, the yellow colour along the outer side of piliferous layer appeared more thick and yellow stained patches were observed inside the stelar region of 10 μM treatment indicating the mercury deposition (Plate 6). After 48 hr, yellow coloured patches were seen localized in the differentiating conducting tissues of 5 and 10 μM mercury treatment (Plate 7).

Roots of 1 μM mercury treatment showed distinctly yellow stained patches of mercury localization in the vessel cells at 72 hr. This stained mercury patches were increased progressively as mercury concentration increased from 1 μM to 10 μM. The ground tissue in mercury treated roots were completely distorted.

After 168 hr of growth in nutrient solution the roots of control seedlings showed the normal structure. There was not any yellow patches observed and also the cells of ground tissue or vascular bundles were not thick walled. In the case of 1 μM treated roots the piliferous layer cells were distorted and coloured layers were present outside the piliferous layer. Roots treated with 5 μM mercury showed increased number and size of the yellow patch and again the number and size of coloured patches increased when concentration of mercury increased to 10 μM. In 5 and 10 μM concentration of mercury the piliferous layers of roots were more distorted and the yellow stained lining became more denser and thicker.

In all the treatments, localization of mercury was observed only in the stelar region and it increased as growth advanced from 24 to 168 hr as well as the concentration of mercury increased from 1 μM to 10 μM. The xylem vessels as well as ground tissues in 168 hr old mercury treated roots were highly thick walled and distorted also.

24.2. Stem

When a comparison was made between the structure of stem tissues of control and mercury treated seedlings, no sign of mercury localization was observed (Plate 10, 11). The control as well as treatment stems were lobed in shape but the lobing was more pronounced and uniform in control.

In addition to the difference in the size and shape (lobing) of stem between the control and experimentals, some interesting anatomical variations also were observed. It is observed that some cells of the epidermal layer, particularly near the lobed region appear enlarged and projected outwards (Plate 10, 11). These trichome-like structures are mostly consisted of more than one cell and their shape also was different from the other epidermal cells. This type of trichome-like structures were observed in all concentrations of mercury. Even though no dithizone-stained masses of mercury were observed in the stem, yellowish lining was observed on both inner and outer surface of the trichome walls.

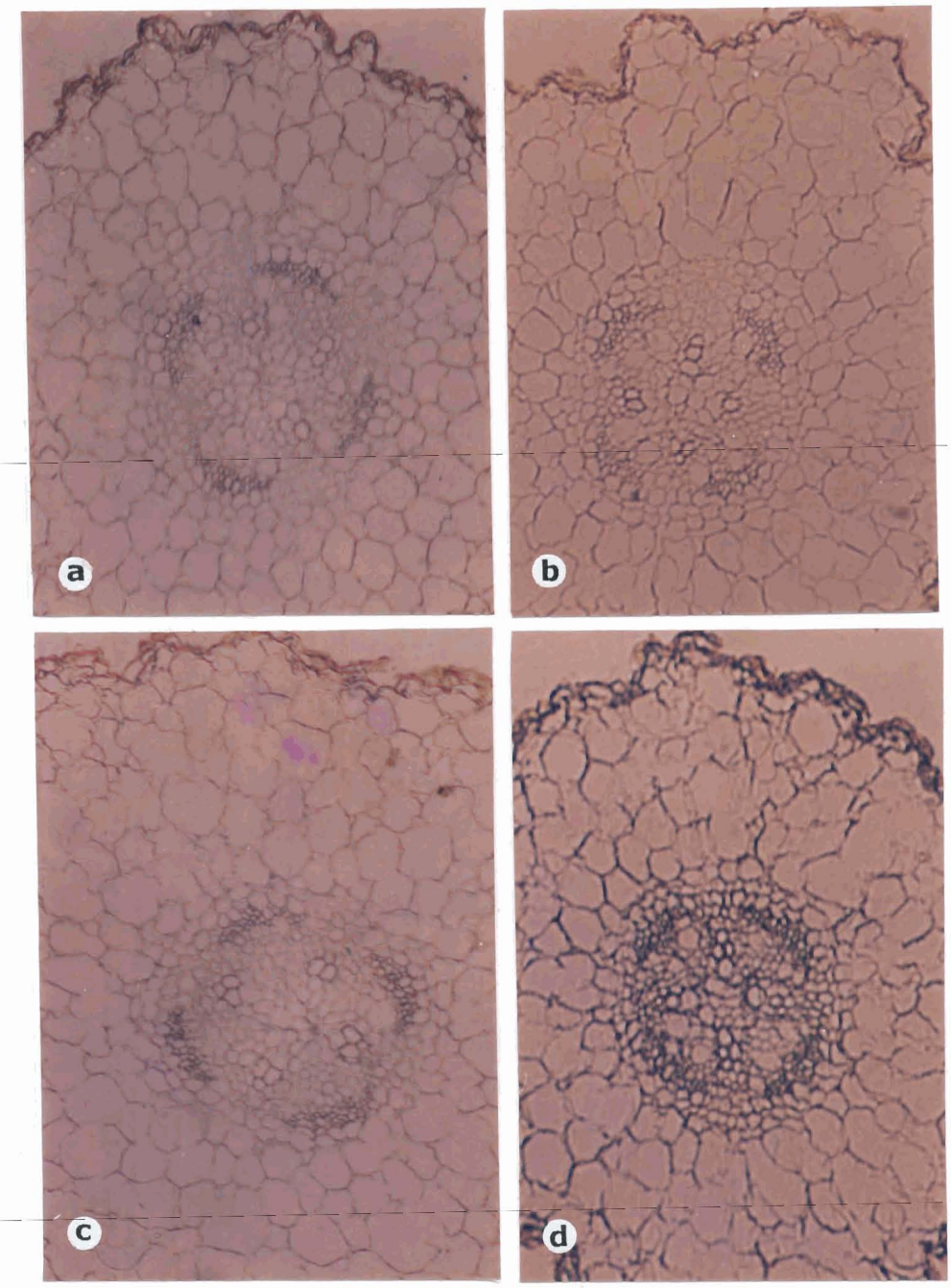

PLATE 5. **Transverse section of root of *V. mungo* seedlings after 12 hr Hg^{2+} treatment – stained with dithizone**
(a) Control (b) 1 μM (c) 5 μM (d) 10 μM

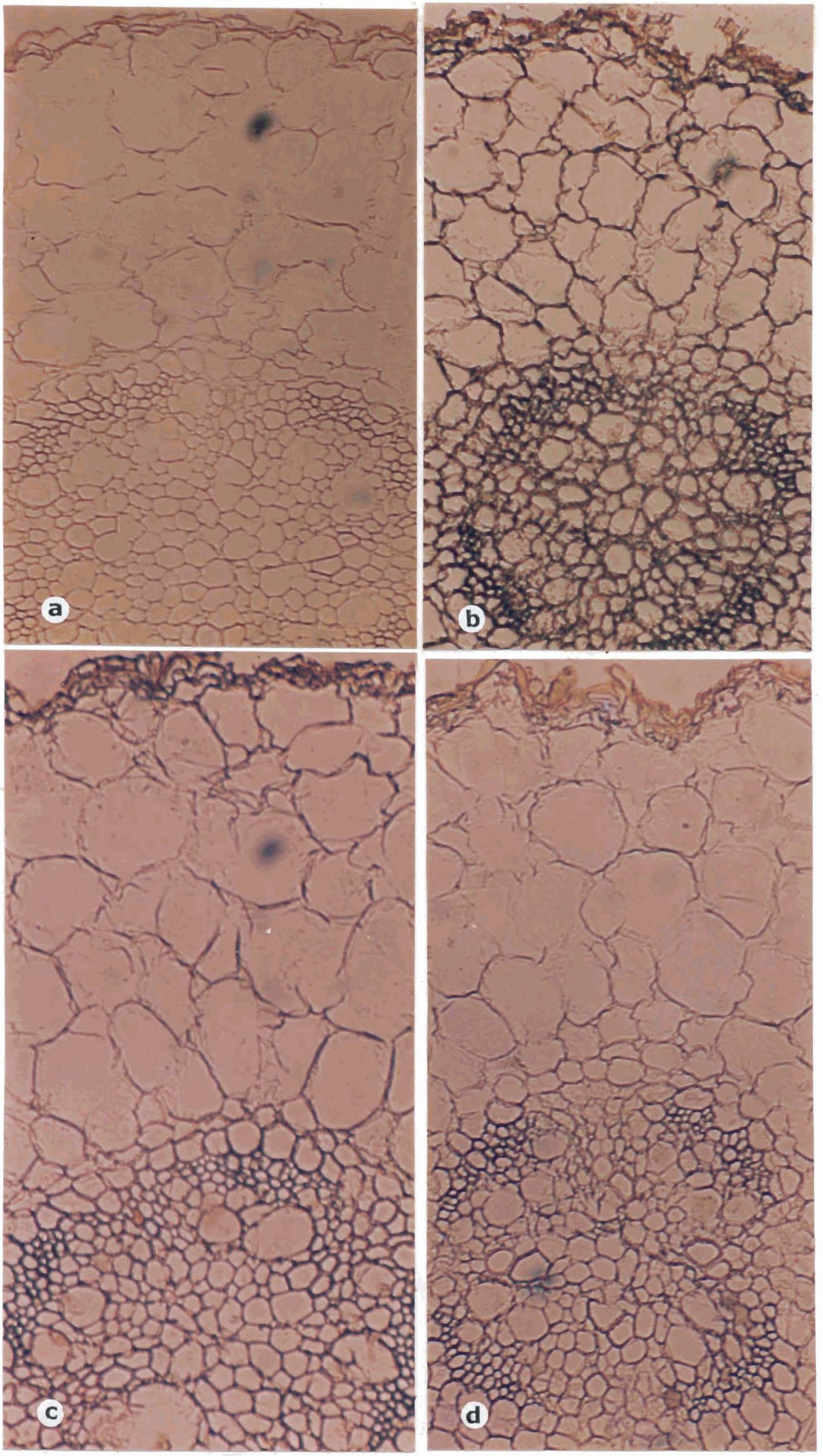

PLATE 6. **Transverse section of root of *V. mungo* seedlings after 24 hr Hg^{2+} treatment – stained with dithizone**
(a) Control (b) 1 μM (c) 5 μm (d) 10 μM

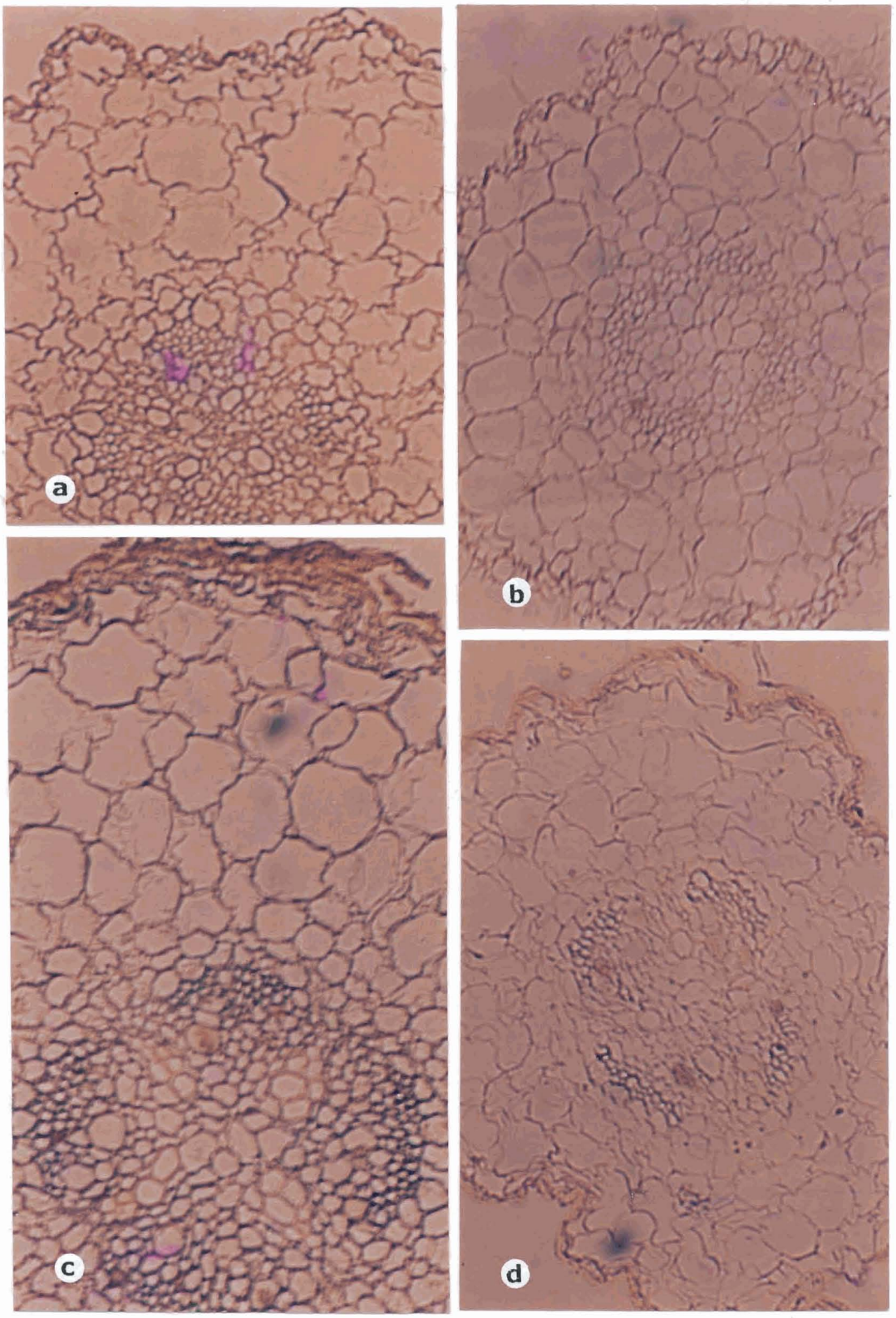

PLATE 7. **Transverse section of root of *V. mungo* seedlings after 48 hr Hg^{2+} treatment – stained with dithizone**
(a) Control (b) 1 μM (c) 5 μM (d) 10 μM

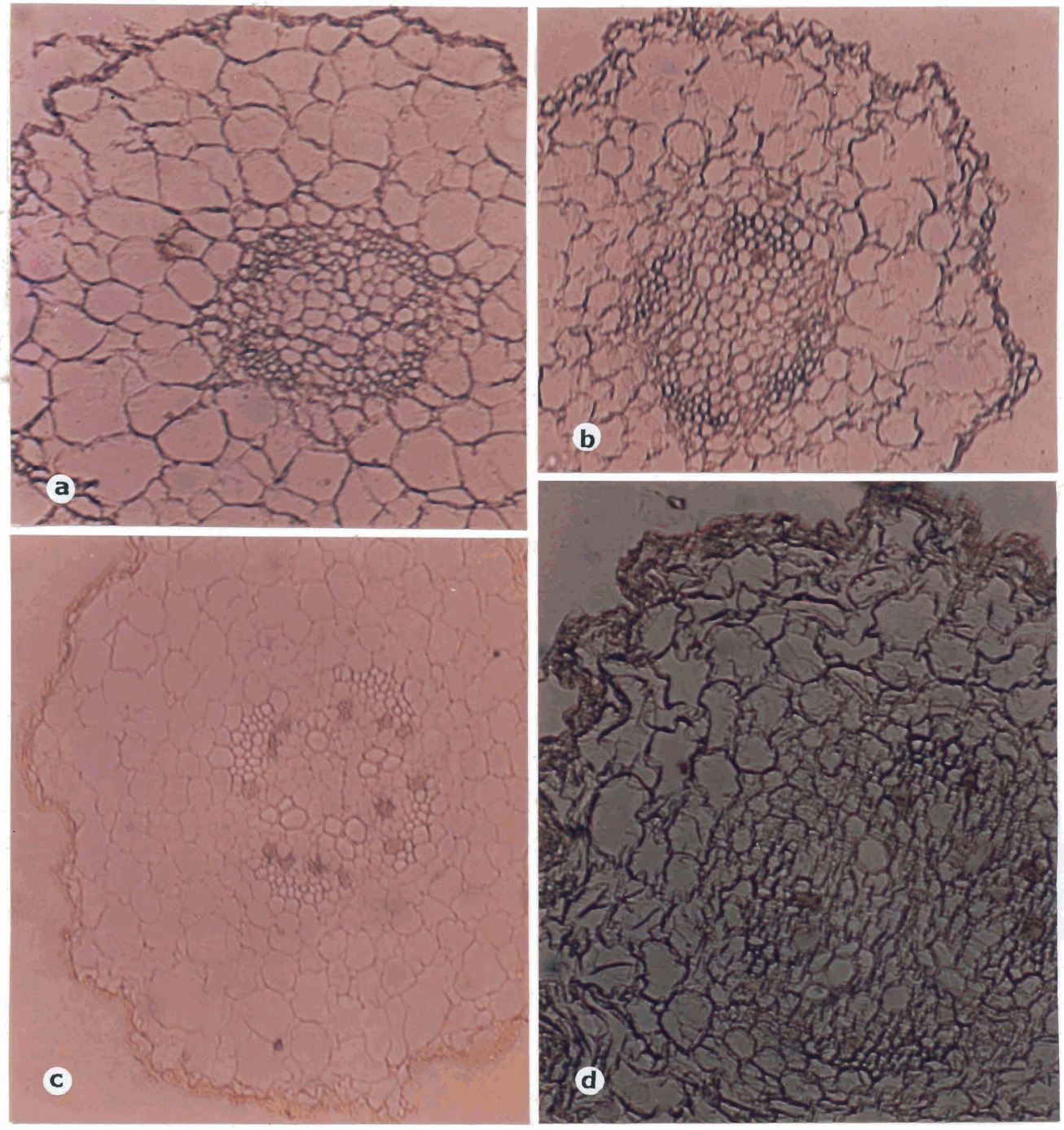

PLATE 8. **Transverse section of root of *V. mungo* seedlings after 72 hr Hg^{2+} treatment – stained with dithizone**
(a) Control (b) 1 μM (c) 5 μM (d) 10 μM

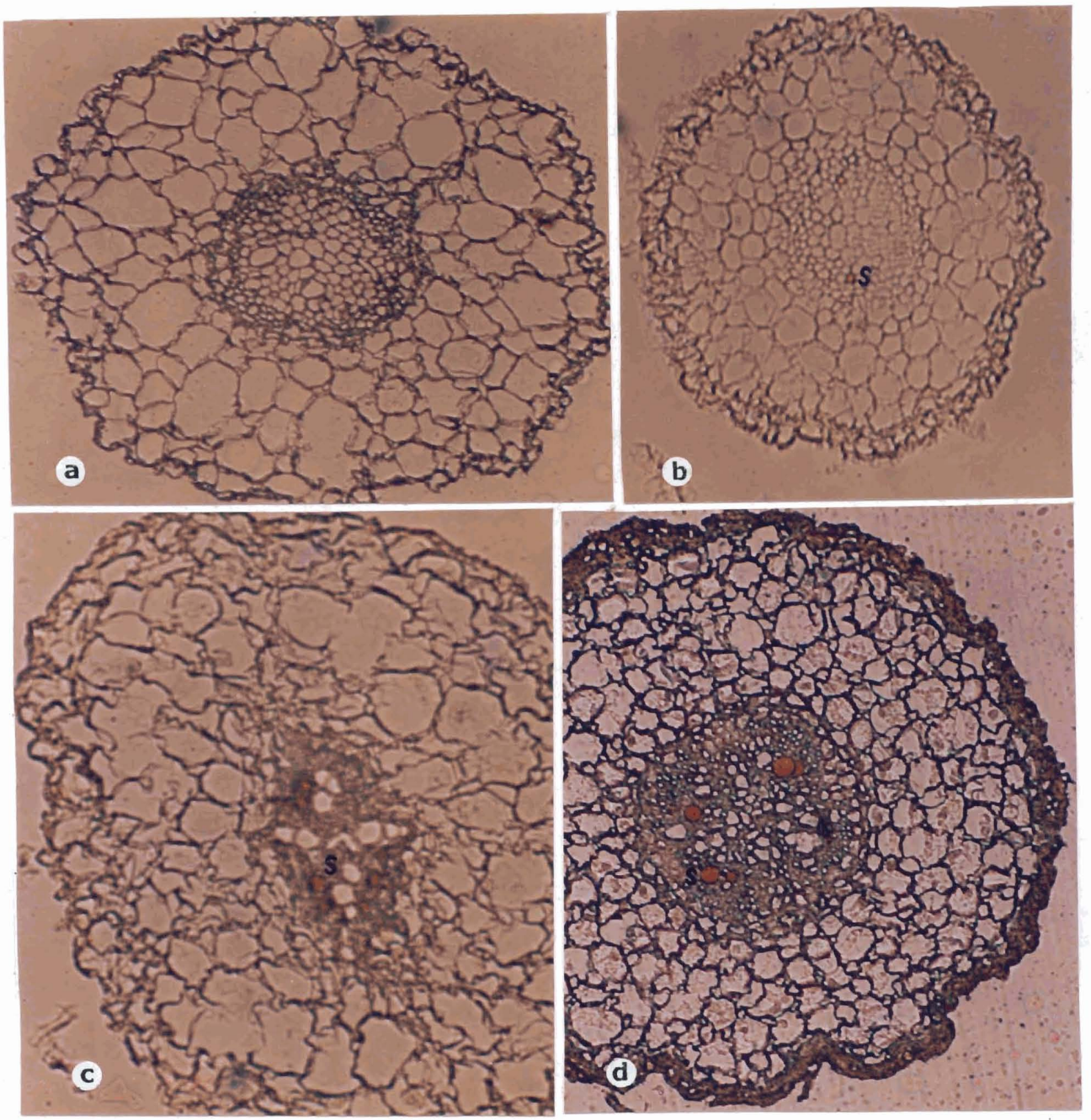

PLATE 9. **Transverse section of root of *V. mungo* seedlings after 168 hr Hg^{2+} treatment – stained with dithizone**
(a) Control (b) 1 μM (c) 5 μM (d) 10 μM
s : stained mass

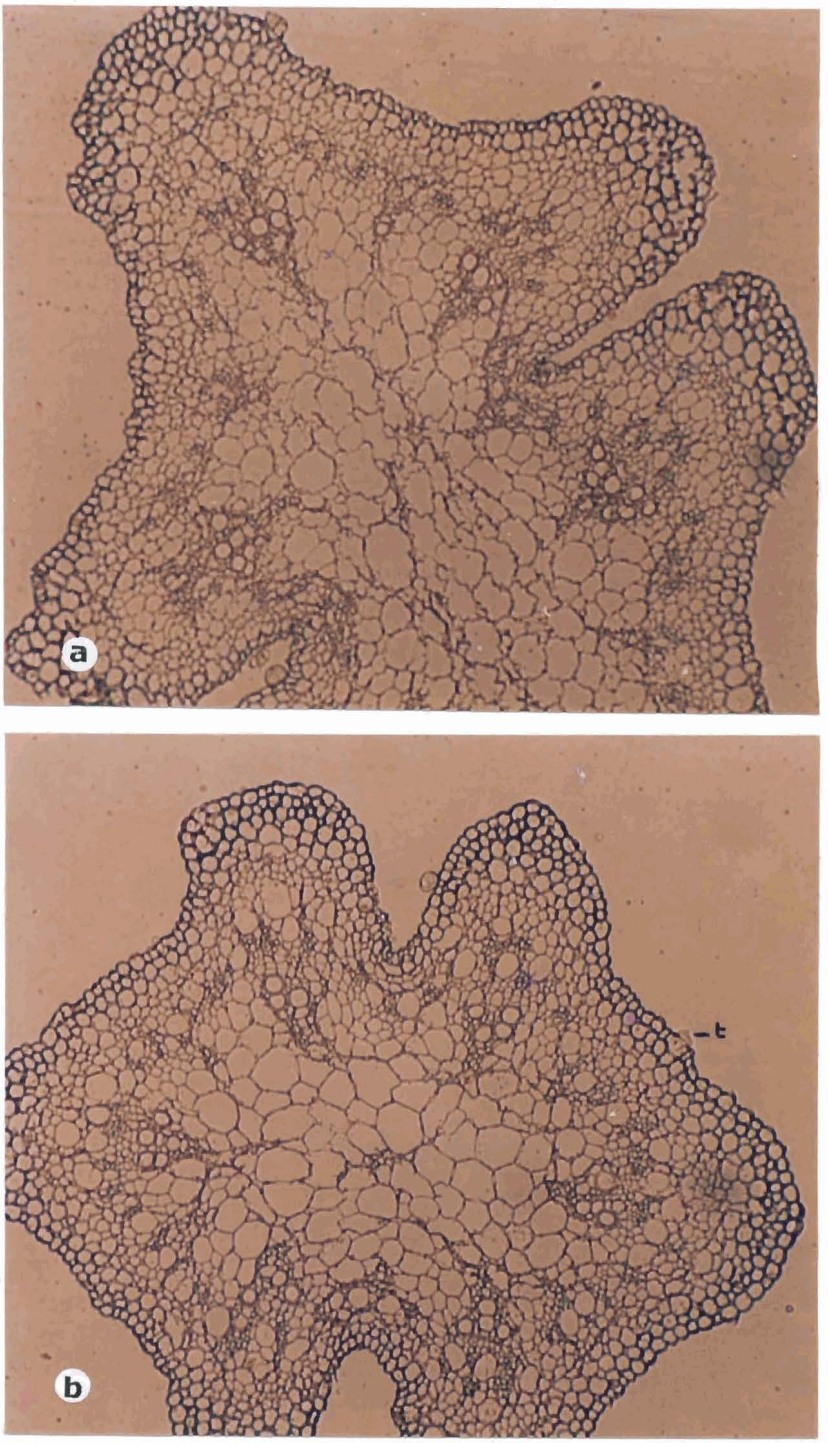

PLATE 10. **Tranverse section of stem of *V. mungo* sedlings after 168 hr Hg^{2+} treatment – stained with dithizone**
(a) Control (b) 1 μM
t : trichome

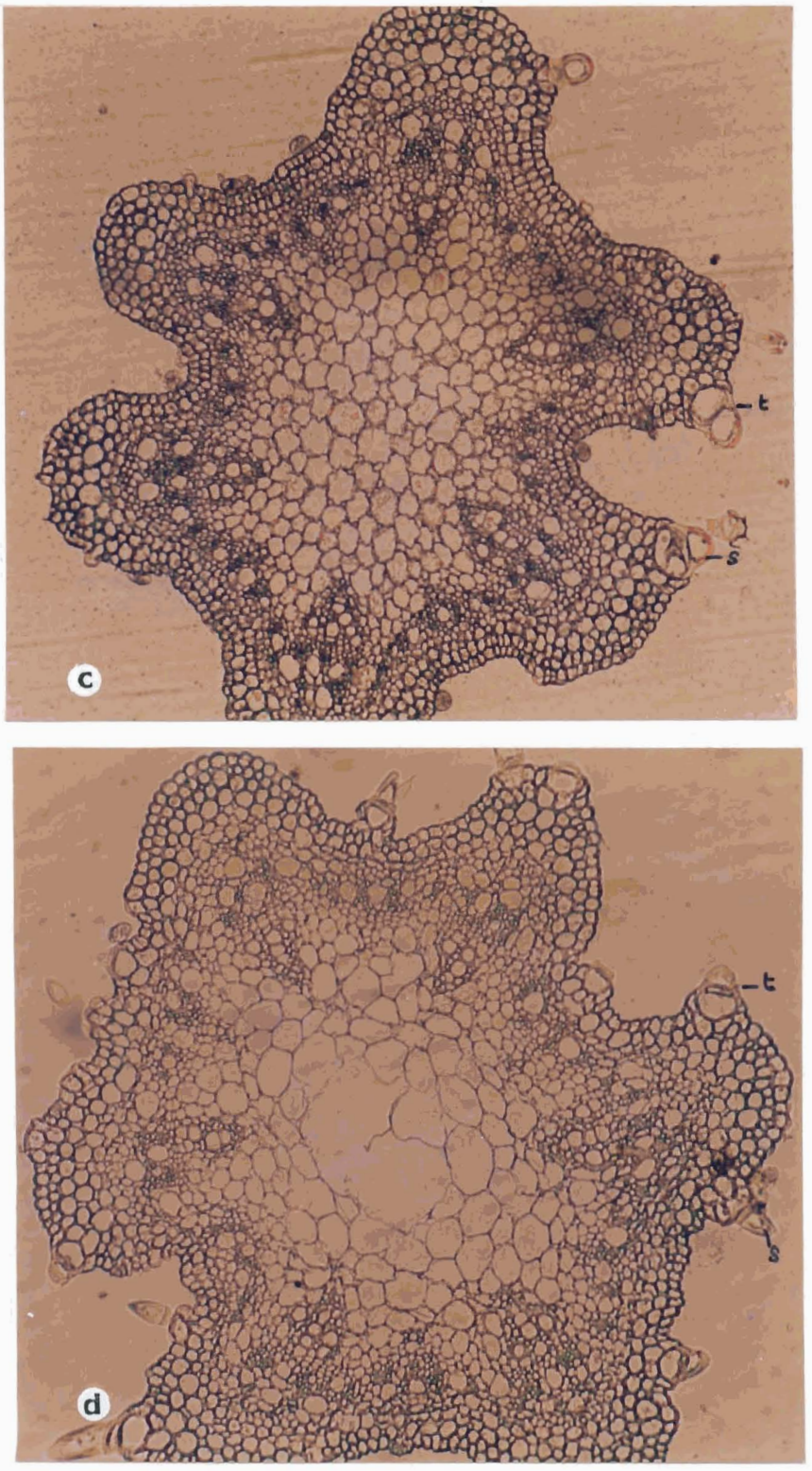

PLATE 11. **Transverse section of stem of *V. mungo* seedlings after 168 hr Hg^{2+} treatment – stained with dithizone**

(c) 5 μM (d) 10 μM

t : trichome s : stained patch

DISCUSSION

DISCUSSION

Toxic effects of mercury on *Vigna mungo* seedlings were expressed morphologically as reduced root and stem growth, leaf area and inhibition of secondary root formation (Plate 1-3). Relative growth rate of root also was significantly reduced under mercury toxicity at 10 μM and this concentration was critical to inhibit growth (Table 6). According to Orcutt and Nilsen (2000) a critically toxic level of mercury in most plants to affect growth is considered to be 1-8 ppm. Similarly, mercury has been reported to inhibit growth in many sensitive plants like *Raphanus sativus* (Khanna and Rai, 1995) at 0.5-1 ppm, *Phaseolus aureus* (Shaw, 1995) at 0.5 to 20 μM. Nevertheless, mercury tolerant plants like *Chloris barbata* exhibited inhibition of root growth only at 500 μgL^{-1} and *Cyperus rotundus* at 100 μgL^{-1} (Lenka *et al.*, 1993).

Growth retardation by heavy metals, including mercury have been reported in many plants such as *Cyperus* (Lenka *et al.*, 1993), *Sesamum* (Singh *et al.*, 1994a), *Rubia tinctorium* (Maitani *et al.*, 1996). These authors also suggested that stunted growth of root and shoot may be due to auxin regulation interference with heavy metals.

In *Vigna mungo*, inhibition of seedling growth in general and root growth in particular was shown by all parameters including growth in length (Table 2),

root/shoot ratio (Table 3), leaf area, dry weight per plant (Table 4) and relative growth rate of root, stem and leaf (Table 6). These observations are in consistent with the observations of Shaw (1995) who reported inhibition of root growth in *Phaseolus aureus* treated with 0.5 to 20 µM mercury and this toxicity was more compared to cadmium toxicity.

Lack of secondary root production was found to be a characteristic feature of mercury toxicity in *V. mungo*. But at high mercury concentration 'secondary roots' which are adventitious in origin, were observed developing from the hypocotyl region (Plate 3), probably as an adaptation for survival, since the original root system become almost non functional at higher concentration of Hg. Initial visual symptoms of Cu toxicity in rice plants reported were decreased root and shoot elongation, leaf chlorosis, and inhibition of secondary root formation (Lidon and Henriques, 1993). The decrease in leaf area in Cd-treated plants was not only due to the reduced cell size but also due to decreased intercellular space (Prasad, 1997). Root growth inhibition was reported in *Chloris barbata* by mercury (Lenka *et al.*, 1993) and shoot growth in barley due to the toxicity of Cu, Cd and Pb (Stiborova *et al.*, 1986).

Data on dry weight per plant basis (Table 4; Fig. 1 A,B,C) showed reduction in root, stem and leaf dry weight at 5 µM and 10 µM mercury treatments. Kalyanaraman and Sivagurunathan (1993) reported that the dry matter yield of root,

stem and leaf of *Vigna mungo* decreased with increasing level of Cd and Cu in the growth medium. Further, the elemental accumulation in tissue was directly proportional to that applied in soil. The maximum amount of Cd, Cu and Zn accumulation occurred in the leaves. According to these authors, controlled application of Cu and Zn were found to increase the dry weight, whereas application of Cu and Zn beyond the rate of 50 mg/kg of soil and 100 mg/kg of soil respectively decreased the yield. In the case of Cd, even low concentration was harmful to *Vigna mungo* resulting in significant yield reduction. The responses of *V. mungo* towards Cd, Cu and Zn are different from that of mercury observed in the present study. Minimum accumulation of Cd, Cu and Zn occurred in the leaves whereas mercury accumulation was maximum in the roots indicating poor translocation of Hg in *V. mungo* (Table 21).

Kumar *et al.* (1993) and Bharti and Singh (1993) suggested that heavy metals such as cadmium, lead etc. caused dry weight increase in leaves of *Sesamum indicum* seedlings probably due to metal-stress-induced enhanced translocation of reserves from seeds. However, contradictory to this, in the present study leaf dry weight was reduced by mercury and cotyledon retained most of their dry matter contributing to their own increased dry weight. Xiong (1999) reported that lead (Pb) treatment in *Brassica chinensis* showed 24% reduction in shoot biomass and young seedlings were highly sensitive to Pb toxicity.

Cotyledon of *V. mungo* showed significant reduction of biomass in control and only 9% was retained at 168 hr of growth (Fig. 1D), while, mercury treated cotyledons retained significant quantity of biomass compared to the control presumably due to the inhibition of hydrolytic enzymes of reserve mobilisation as suggested by Bishnoi *et al.* (1993).

When the growth analyses were carried out in terms of relative growth rate (RGR) of root, stem and leaf separately (Table 6), it is evident that comparatively minimum RGR value was obtained in the case of roots as a result of maximum inhibition of root growth by mercury toxicity. Net assimilation rate (NAR) showed maximum reduction at 72 and 168 hr (Table 7) compared to the earlier stages. So the inhibitory effect of mercury on leaf was not only reflected in leaf growth (area) but in the biomass of the leaf also. In other words, leaf growth as well as assimilation rate were subjected to almost similar inhibition by mercury.

Mercury interfere plant's physiology through interaction with biochemical constituents or with other essential elements. Based on the ability of ions to interact with ligands of biological origin, metals have been classified into Class A, B and Border line (Woolhouse, 1983). According to him mercury comes under Class B and this group of metals bind preferentially with ligands containing S, N or I as donor atoms. Also Class B metals strongly bind with sulfur-containing amino acids. With all the above characteristics, mercury adversely affects the general metabolism which may be detrimental to the plant.

Data on biochemical analyses of metabolites in *Vigna mungo* cultured under mercury toxicity showed significant fluctuations in their distribution during growth. Starch content was reduced significantly in roots and the least amount was present in the root tissues of 10 μM during 72-168 hr (Table 11). Stem and leaf tissues exhibited reduced starch content in all treatments at all intervals. This observation is plausibly because of reduced photosynthesis under mercury toxicity as already reported by Jain and Puranik (1993) in maize, Bernier *et al.* (1993) in barley and other heavy metals like lead in spinach (Stefanov *et al.*, 1995), copper in rice (Lidon *et al.*, 1993) and cadmium in maize (Prasad, 1995). Starch content of cotyledon was more in all treatments because mobilization of starch from the cotyledon to seedling axis was highly inhibited by mercury in all concentrations. Mercury was found to enter cotyledons (Table 21) and hence the amylase and protease activities were inhibited and so more dry matter was retained in the cotyledons. Almost similar observation in *sesamum* was made by Singh *et al.* (1996) and Kumar *et al.* (1993). Depleted starch content (Table 11) and enhanced sugar content (Table 12) in the roots of *V. mungo* seedlings as a result of mercury treatment, reveal that metabolisable carbohydrates are mobilised neither from the cotyledons which are the storage organs of seedlings nor from the leaves as photosynthate, probably owing to the inhibitory effects of mercury on translocation as suggested by Bishnoi *et al.* (1993) in Pigeon pea in which cadmium suppressed

the activity of amylase and protease and so is responsible for retardation of dry matter mobilization from the cotyledons.

In comparison with control, sugar content in *V. mungo* root and stem tissue under mercury toxicity was significantly more in all concentrations and the rate of increase was proportional to increase in concentration of mercury (Table 12). Leaf tissue of mercury treated plants also contained more sugar compared to the control. Since inhibition of photosynthesis due to mercury toxicity is an established fact (Bernier *et al.*, 1993; Moore *et al.*, 1995), in *V. mungo* occurrence of increased soluble sugar in leaves may probably be due to inhibited translocation of sugars already synthesised, to stem and root. However, occurrence of increased sugar content in the root and stem may be the result of impaired respiration due to mercury inhibition. The reduced sugar content of cotyledons in mercury treated *V. mungo* plants may be the result of feeble amylase activity as described earlier in Pigeon pea (Bishnoi *et al.*, 1993).

Root protein content significantly reduced in all experimental samples compared to the control (Table 13). Protein content reduction in stem and leaf tissue followed the same trend of root tissue. A similar observation of reduced protein content was reported in *Zea mays* leaves treated with cadmium (Ferretti *et al.*, 1993). Cotyledons also showed reduction in protein content due to mercury treatment (Table 13) indicating protein degradation and mobilization to root and shoot systems. Protein hydrolysis due to heavy metals like lead has been reported in

sunflower, where higher concentration of Pb resulted in water stress leading to inhibition of protein synthesis (Kastori *et al.*, 1996). Stiborova *et al.* (1986) reported retardation in shoot and root growth and a decline in protein and glutathione content in barley due to exposure to copper, cadmium and lead.

Another reason for reduced protein content in the roots of mercury treated *V. mungo* may be inhibition of protein synthesis (Reddy and Prasad, 1992a), unavailability of essential elements (Reddy and Prasad, 1992c; Prasad, 1997) or inhibition of reserve (amino acids) mobilization from cotyledons (Bishnoi *et al.*, 1993; Bernier and Carpentier, 1995).

Protein hydrolysis resulted in its reduced quantity and concomitant raise in free amino acids (Table 14) including proline (Table 15) were found to be characteristic feature of toxic effects of mercury in *V. mungo.* Synthesis and activities of many enzyme-proteins were known to be inhibited by increased heavy metal concentration. Well known examples are nitrate reductase activity inhibition by lead (Kastori *et al.*, 1995; Singh, 1995; Singh *et al.*, 1996; Keshan and Mukherji, 1994) and glutamate dehydrogenase and γ-keto glutarate amino transferase by lead and cadmium (Kastori *et al.*, 1993; Van Assche and Clijsters, 1990).

Plant cells after a heavy metal exposure, synthesize small sulphur-rich peptides that bind heavy metals. They are composed of three amino acids, namely L-cysteine, L-glutamic acid and glyciene. Glutamic acid is linked to each cysteine

by a γ-peptide linkage; the general structure of this peptide is $(\gamma\text{-Glu-Cys})_n$–Gly where n = 2-11. These peptides are called Phytochelatins (PCs) (Grill *et al.*, 1985, 1986, 1989).

According to Grill *et al.* (1987), Rauser (1990), De Knecht *et al.* (1994) and Leopold *et al.* (1999) phytochelatins are induced by Cd, Pb, Zn, Ag, Au, Cu and Hg. Not withstanding elucidation, purification and characterisation, mercury-induced phytochelatins are not specifically known to occur in higher plants.

As mentioned earlier, depletion in protein synthesis due to heavy metal toxicity has been reported in higher plants (Prasad and Prasad, 1987; Singh *et al.*, 1994a; Kastori *et al.*, 1996). However, in *V. mungo* despite the reduction of protein content, production of phytochelatin in the prsence of mercury can not be ruled out because electrophoretic study of protein profile of root tissue revealed additional bands of protein in mercury treated tissues compared to the control (Plate 4). Inspite of protein depletion, the additional bands might probably be the indications of phytochelatin synthesis induced by mercury. Binding of heavy metal ions to phytochelatin molecules start very quickly (Leopold *et al.*, 1999). According to whom in *silene* and tomato after 20-30 minutes of application, Cu and Cd induce metal-phytochelatin complex formation. According to Orcutt and Nilsen (2000) phytochelatin synthesis is a detoxification process and mechanism of detoxification should start immediately as the metal enters the cell, otherwise direct binding of metals to key enzymes of metabolism will lead to adverse effect of toxicity.

Synthesis of phytochelatins triggered by many heavy metals were reported in *Silene cucubalus* (Grill *et al.*, 1987), *Pisum sativum* (Klapheck *et al.*, 1995), *Vigna cajan* (Bhattacharyya and Choudhuri, 1995), *Silene vulgaris* (Verkleij *et al.*, 1990; Leopold *et al.*, 1999) and *Armoracia rusticana* (Kubota *et al.*, 2000). According to Borovik (1990) toxic metals were well known for their capacity to co-ordinate with essential functional group of proteins which can render the proteins inactive. This is especially true of Hg(II) which has tremendously high affinity for sulfur (binding constant of Hg(II) to sulfur is 1×10^{-24}) and Hg(II) is very selective for sulfur. However, role of mercury in inducing phytochelatin synthesis has been investigated by only a few authors (Rauser, 1990).

Phytochelatin synthesis in *V. mungo* was indirectly evident from electrophoretic study and hence detoxification of mercury might occur to some extend. Neverthless this plant cannot be considered as mercury-tolerant because metal tolerance does not appear to involve mechanisms as simple as phytochelatin production as suggested by Turner (1994).

In vivo binding of cadmium and copper to produce phytochelatins has been demonstrated elaborately (Robinson, 1990; Grill *et al.*, 1987; Rees and Wagner, 1987) and only indirect evidence are available to show that mercury binds to polypeptides in plants (Grill *et al.*, 1987). Nevertheless, *in vitro* studies (Maroni, 1990) showed the capacity of mercury to induce metallothioneins and this capacity was very high compared to many other heavy metals.

Remadevi and Prasad (1998) reported that in *Ceratophyllum* glutathione (GSH) increased upto 48% corresponding to tolerance index in plants treated with 2 μM copper. When the concentration was raised to 4 μM, inspite of increased lipid peroxidation, GSH was reduced because at this level, the toxicity was increased to lethal level.

Kastori *et al.* (1996) reported that in sunflower lead (Pb) stimulated proline synthesis as well as protein hydrolysis leading to an accumulation of proline. According to these authors this proline participate in osmotic regulation, stabilises enzymes and proteins and conserve energy and nitrogen for post stress period. In sunflower comparatively more proline accumulation was observed in leaves because lead (Pb) caused water deficit. However, in *Vigna mungo* in the present study more proline was observed in roots than on leaves (Table 15, Fig. 3A,C) and presumably water deficit may be prevailed in the roots due to more ions present in the nutrient solution and toxicity of mercury. Comparatively less proline accumulation was observed in leaf and stem tissues of *V. mungo* where mercury accumulation also was comparatively lesser (Table 22). However, in cotyledons proline content was very low (Fig. 3D) and not significantly changed. Cotyledonary tissues are not very active metabolically, and mercury toxicity may not induce much stress and so proline accumulation may not be necessary.

Nitrate reductase activity (NRA) of root, stem, leaf and cotyledon tissues of mercury treated *V. mungo* seedlings showed a general reduction and the activity was very feeble at higher concentration (10 μM) of mercury (Table 16).

Eventhough the seeds of *V. mungo* contain sufficient nitrogen pool, like *Sesamum* (Singh *et al.*, 1994a), the reduced NRA in mercury treated tissues indicate the inhibition of reserve mobilization from the cotyledons (Table 16). Nitrate reductase catalyses the reduction of nitrate in plants and it is believed to be a rate limiting step in nitrate assimilation and this enzyme activity is often correlated with nitrogen status and growth of plants. Several plants were reported to be affected by metal toxicity expressed as inhibited nitrate reductase as in *Sesamum* (Singh *et al.*, 1991; Kumar *et al.*, 1993), sunflower (Kastori *et al.*, 1996) and mung bean (Keshan and Mukherji, 1994).

There are several reasons for inhibition of nitrate reductase activity by heavy metals in plants. According to Gengenbach *et al.* (1973) the inhibition of NRA may be because of decrease in NADP supply to the enzyme. Rebechini and Hanzley (1974) suggested that disorganised chloroplast as a result of toxicity of heavy metal may be the cause for reduced NRA. Lesser NO_3^- supply is considered to be another reason for NRA inhibition (Singh *et al.*, 1988).Water stress created by heavy metal stress and/or direct effect of the metal on nitrate reductase enzyme synthesis (Shaner and Boyer, 1976; Srivastava, 1980) as the metal has strong affinity for

binding with functional SH groups of the enzyme (Srivastava, 1980; Prasad and Prasad, 1987) were also considered as reasons for NRA inhibition by heavy metals. According to Singh *et al.* (1994a) pronounced decrease in *in vivo* NRA due to heavy metal was indirect, possibly through the inhibition of NADH and NO_3^- supply as *in vitro* enzyme was positively affected by many heavy metals when NO_3^- and NADH were supplied exogenously to the assay media.

In *Vigna mungo* the inhibition of nitrate reductase activity in root tissue (Fig.4A) may be due to reduced NO_3^- availability since the absorption of these radicals by root was inhibited by the presence of mercury, inspite of sufficient amount of NO_3^- present in the nutrient medium. Another possibility of the unavailability of NO_3^- radical because of mercury induced inhibited mobilization of NO_3^- from the cotyledon cannot be ruled out because *V. mungo* cotyledons contain substantial amount of protein and/or nitrogen rich metabolites (Table 13, 14). Nitrate reductase activity in the cotyledonary tissue exhibited significant reduction at all intervals compared to their respective controls confirming the inhibitory effect of mercury on nitrate reductase which is in consistent with the view of Singh *et al.* (1994a) and Kastori *et al.* (1996).

Reduced lipid content in the root, stem and leaf tissue of mercury treated plants (Table 17), especially at higher concentration (10 μM) is a characteristic feature of heavy metal toxicity on plants as described by Stefanov *et al.* (1993).

Significant reduction of lipid (membrane lipid) in spinach leaves, exposed to copper (Maksymiec *et al.*, 1997), in tomato plants exposed to cadmium (Krupa and Baszynski, 1989) and in spinach exposed to lead (Stefanov *et al.*, 1993) are comparable to the reduction of lipid in *V. mungo* treated with mercury. According to Stefanov *et al.* (1993) the lipid content of spinach leaves subjected to lead treatment showed more lipid content in the thylakoid membrane probably to protect photosynthetic apparatus. But in *V. mungo* lipid content of leaves did not undergo any significant change by mercury (Table 17). While in root, lipid content was significantly reduced. However, in cotyledons lipid content was more in mercury treated plants compared to the control. A probable reason for this observation was cotyledons being storage organs contain considerable amount of lipid and due to the effect of mercury, reserve mobilization was retarded as described earlier, and retained more lipid in the cotyledons, while in control the lipid content was reduced significantly due to mobilization to the growing seedlings.

Chlorophyll a, b and total in 5 μM mercury treatment showed significant reduction after 24 hr (Table 19). But in 10 μM mercury treatment, high reduction of all types of chlorophyll occurred from 12 hr onwards (Fig. 5A,B,C). Chlorophyll a/b ratio showed increased value only at 168 hr in 10 μM mercury concentration (Table 20).

Inhibition of chlorophyll synthesis/photosynthetic processes has been reported in many plants such as *Vigna radiata* by cadmium (Keshan and Mukherji,

1992), maize by cadmium (Prasad, 1995), *Oryza sativa* by zinc (Ajay and Rathore, 1995), spinach by lead (Stefanov *et al.*, 1995) and Cucumber by cadmium and lead (Lang *et al.*, 1995, 1998).

In the present investigation total chlorophyll, chlorophyll-a and chlorophyll-b have been found reduced significantly due to mercury toxicity. According to Jain and Puranik (1993) in maize chlorophyll synthesis was inhibited by mercury due to the interference of cytoplasmic protein synthesis. Eventhough the rate of photosynthesis was not investigated in the present study, distribution of sugars and starch can be correlated with this process. Sugar content in leaf (Table 12) of experimental plants exhibited an increase while starch content decreased significantly (Table 11). This observation indicates that photosynthetic function was only partially inhibited by mercury. Inhibitory effect of Cd resulting in RUBP-carboxylase and PEP-carboxylase activities have been reported by the interaction of Cd with SH groups of the enzymes (Malik *et al.*, 1992).

Syamala and Rao (1999) reported that plants growing near a cement factory in Andhra Pradesh where the soil was contaminated with mercury, showed reduced photosynthesis, chlorophyll, protein and reducing sugars. This finding is in consistent with the observations of chlorophyll analyses in *V. mungo*. Remadevi and Prasad (1998) reported that high copper content in *Ceratophyllum demersum* showed loss of chlorophyll due to peroxidation of chloroplast membrane mediated by copper toxicity.

Based on the observations of mercury inhibited photosynthetic electron transport activity even at very low concentration in intact cells of cyanobacterium *Spirulina platensis* by altering spectral properties of plastocyanin, Murthy and Mohanty (1995) reported that mercury can interact with oxygen evolving complex plastocyanin, Ferrodoxin and iron-sulphate cluster on the acceptor side of photosystem II. According to these authors the ability of mercury to form organometalic complexes with amino acids occurring in the proteins of photosynthetic centres was due to its strong affinity to C=O, C–N, C–S and C–SH groups.

Reduction of net photosynthesis in Cd treated *Solanum melongena* was reported by Mehindirata *et al.* (1999). Investigations available on toxic effect of mercury on photosynthetic apparatus and processes are on isolated chloroplasts (Sersen *et al.*, 1998). So the inhibitory effects were many fold and explained at molecular level. However, a direct correlation of these findings cannot be drawn with the inhibitory effect of mercury on *V. mungo* in the present study because of reasons such as (1) the translocation of mercury to leaf was comparatively low, (2) though the tissue contain mercury (Table 21) in small quantity, their interference does not inhibit the photosynthetic process completely.

At higher concentration (10 μM), chlorophyll synthesis, in general (Fig. 5C) and chlorophyll-a in particular (Fig. 5A) were reduced in the leaves, on the other

hand sugar content was increased. Even though mercury was reported to inhibit photosynthetic electron transport (Bernier *et al.*, 1993) and photosystem II was the most sensitive target (Bernier and Carpentier, 1995), by electrophoretic studies in *Hordium vulgare* treated with mercury revealed that an extrinsic polypeptide of molecular weight 33 kDa of oxygen evolving complex was depleted probably because of high affinity of mercury for sulfhydryl groups of proteins. This protein was similar to many other intrinsic proteins.

Despite the inhibitory effect of mercury on photosynthetic processes and occurrence of the metal in the leaf tissue, in the present study the plants show the ability for photosynthesis to some extent and grow probably by adopting the synthesis of photochelatins to detoxify the mercury toxicity.

In *Vigna mungo* stomatal size (width and breadth) was reduced in both lower and upper sides of the leaves of mercury treated plants (Table 9). Even though references on the effect of mercury on stomatal development/activity is scanty in the literature, increased stomatal resistance in Cd-treated plants has been reported (Bazzaz and Govindjee, 1974). Interference with stomatal function is considered to be a primary mode of action of Cd and several other metals (Becerril *et al.*, 1989). The inhibition of stomatal opening in plants exposed to Cd may depend not only on Cd concentration and exposure time but also on the degree of toxicity suffered by the plants (Barcelo *et al.*, 1986). Mehindirata *et al.* (1999) reported a reduction in stomatal conductance, stomatal length,width, density and stomatal index in Cd

treated *Solanum melongena*. Adverse effect of gas exchange in lead and cadmium treated *Medicago sativa* and *Trifolium pratense* was reported by Becerril *et al.* (1989).

Transpiration and stomatal conductance were negatively affected by heavy metals as a result of interaction with Ca^{2+} mediated signals in guard cells and their well known antagonistic effect on Ca^{2+} at the membrane level (Orcutt and Nilsen, 2000). According to Lu *et al.* (1998) and Taiz and Zeiger (1998) stomatal conductance and yield of plants are positively correlated because higher stomatal conductance increase high CO_2 diffusion into the leaf leading to high photosynthetic rate which in turn increase productivity. In *Vigna mungo* occurrence of low stomatal size indicate comparatively reduced rate of photosynthesis which has already been reported as a direct as well as important effect of heavy metals such as cadmium (Misra *et al.*, 1989; Moya *et al.*, 1993), lead (Thapa *et al.*, 1988; Bharti and Singh, 1993), copper (Lidon and Henriques, 1991) and zinc (Kastori *et al.*, 1992). So in *V. mungo* mercury affect photosynthesis adversely which is shown by depleted synthesis of chlorophyll and starch. Effect of mercury on photosynthesis was shown indirectly by reduced stomatal size which in turn reduce stomatal conductance leading to reduced productivity or biomass as described earlier.

Histochemical studies by staining transverse sections of root and stem with dithizone showed considerable amount of mercury localized in the conducting

tissues of root (Plate 5-9). The coloured masses of mercury-dithizone complex appeared as yellow patches. The absence of dithizone stained masses in the stem confirms the scanty translocation of mercury from the roots (Plate 10, 11). This observation is in conformity with the observation of Woolhouse (1983) and Khanna and Rai (1995), according to whom the translocation of mercury from roots to aerial parts is not so rapid and mostly the absorbed metal remains deposited in the root tissues. However, contradictory to this statement Orcutt and Nilsen (2000) suggested that mercury is easily mobilised in plants.

After a very short period of (24 hr) mercury treatment, an yellow coloured material was observed along the outer surface of the piliferous layer of roots in *V. mungo* (Plate 6) and the intensity of this layer increased progressively during further growth. In *Triticum aestivum*, due to the effect of cadmium, lead and nickel a mucilagenous layer was observed in the roots (Setia and Bala, 1994). According to Woolhouse (1983) the presence of mucilage on the epidermal surface is exclusively seen in mercury treated plants and this might be an adaptive response of plants, since mucilage is known to facilitate uptake of water and ions into the roots by ion-exchange processes. Mucilage secreted from meristem and other plant parts was also reported to act as a ligand for toxic metals like Al, Pb, Cd and Cu (Horst *et al.*, 1982; Morel *et al.*, 1986). According to this view the dithizone positive yellow layer on the root may reduce the absorption of mercury by acting as ligand for this metal.

Woolhouse (1983) suggested that the increased cell wall thickening in epidermal and hypodermal cells of mercury treated stem indicate an early maturity of these tissues and the alteration in the stem structure could be correlated with the structural change *vis-à-vis* disfunctioning of the root system following mercury treatment. Since heavy metals interfere with cell division and cell elongation (Davies, 1991) differentiation is inhibited in plants. Although cell wall thickening was not evident in Hg treated root cells, partial disfunctioning can be attributed because some conducting cells, are occluded with mercury content. Similarly, inhibition of cell division and elongation is apparent owing to the stunted nature of roots in *V. mungo.*

In a histochemical study in Lemna minor, Kocjan *et al.* (1996) showed lead localisation on the outer side of endodermis cell walls of the root and when more time was given deposit also increased. Lead was deposited on the cell walls of deeper cell layers of root indicating the involvement of apoplasm in lead translocation. Lead penetration to the apoplast in *Allium cepa* (Wierzbicka, 1987) reached the entire region of plant body. Since lead deposits are observed in the vacuoles also, it is reported that lead can be transported symplastically also in *Lemna minor* (Kocjan *et al.*, 1996). However, despite the occurrence of mercury in stem and leaf, the mode of translocation is not distinct in the histochemical study which showed mercury deposits in some conducting cells of roots and all other cells are devoid of any such inclusion. So it is apparent that transport of mercury is

through apoplast (cell wall) which is an open lattice of polysaccharides through which mineral nutrients diffuse readily and because all cells are separated by cell walls, ions can diffuse across a tissue entirely through the cell wall space without entering the cytoplasm (Taiz and Zeiger, 1998). The involvement of cell wall in metal uptake was confirmed by Morishita and Boratynski (1992) who suggested that more than 90% of the heavy metal ions are located in cell walls.

According to Prasad (1997) cadmium induced decrease of water stress resistance in *Phaseolus vulgaris*. Cell wall elasticity also was reduced reslting in low water stress tolerance. Xylem obstruction by cadmium induced cell wall degradation causes reduced water translocation and reduced water absorption occurs because of reduced root growth. In the roots of *V. mungo*, some vessels were found fully filled with dithizone-stained inclusions (mercury) and this may block water translocation and may be one of the reasons for reduced growth. Impaired water uptake and reduced water stress resistance by mercury also result in growth retardation. The mercury deposition was in the conducting tissues of the root alone. Similarly, ultrastructural details of cells affected by mercury absorption were not clearly visible for the mercury deposits occupied the whole space of the cells and appeared as masses of coloured material (Plate 9).

In *V. mungo* the transverse section of stem (Plate 10, 11) showed some epidermal cells specially developed into a gland like structure and these structures were completely absent in the control (Plate 10a). The anatomical appearance of

these appendages were almost similar to the secretary trichomes explained by Fahn (1982). According to him in *Phaseolus multiflorus* secretary trichomes of leaf or stem consist of a uniseriate stalk and a multicelled oval head. Between the cellulose layer of wall and cuticle at the top of the gland a subcellular space was formed during secretion. When pressure reaches a certain value, pores in the cuticle open and droplets appear on the surface (Fahn, 1982). The size and distribution of the trichomes on stem epidermis of *V. mungo* vary in different concentrations of mercury. Trichomes in 1 μM concnetration were only very few in number and smaller in size (Plate 10b), while in 5 μM concentration, number of secretary trichome increased compared to the control (Plate 11c) and, 10 μM concentration showed maximum number and size of secretary trichome on the stem epidermis (Plate 11d). Hence the apparent function of these structures may be secretion of some mercury containing substances or salts through these trichomes to expel mercury content particularly at more toxic concentrations *i.e.* at 5 and 10 μM concentrations.

Bioaccumulation of mercury was maximum in roots (Table 21, Fig. 6A) and the quantity increased with increasing metal concentration and progressive growth. Since the roots were directly in contact with the mercury applied in the nutrient medium, maximum mercury concentration was observed in root tissue (Fig. 6A). Singh *et al.* (1994b) reported that in *Vigna radiata* seedlings the absorbed lead was accumulated in the root and leaf tissues.According to Morishita and Boratynski

(1992) more than 90% of the heavy metal ions were located in the cell wall fraction of the roots. Neverthless, some heavy metal ions can penetrate into the above-ground parts of plants. Significant accumulation of lead in roots has been reported (Ernst *et al.*, 1992; Stefanov *et al.*, 1995) in leaves of spinach plant.

Heavy metals such as Cd and Pb were reported to accumulate 5 to 10 times in the roots than the other plant parts and thus was in the oldest leaf also (Lang *et al.*, 1998).

Stem tissue of *V. mungo* contained considerable quantity of mercury (Fig. 6B), neverthless, histochemical staining with dithizone did not show coloured mercury complex in stem tissues (Plate 10, 11). This may be because of the insufficient amount of mercury to complex with dithizone, or due to the limited sensitivity of dithizone to form coloured complex. Mercury content of stem tissue increased corresponding to the increased concentration as well as growth period (Table 21). According to Woolhouse (1983) and Khanna and Rai (1995) mercury translocation from root to stem was very slow and most of the absorbed mercury was get deposited in the root tissue. In the present investigation the reduced content of Hg in stem of *V. mungo* was in consistent with the views of these authors.

Formaldehyde treated bark of *Hardwickia binata* was reported to adsorb considerable amount of mercury (Deshkar *et al.*, 1990). In the present study at

highest concentration (10 μM) of mercury, roots of *V. mungo* contained comparatively large quantity of mercury. At this concentration, the root tissue become hard and stunted probably due to adsorbed mercury.

According to Wheeler and Power (1995) the solution toxicity of an ion is dependent on the rate that the plant takes the ion up and on the toxicity of that ion within the plant. Based on this statement the mercury absorbed in the root causes maximum toxicity to the root system because the absorbed Hg^{2+} ions are not distributed or translocated evenly to all parts of the plant.

Xiong (1999) reported that in *Brassica chinensis*, lead accumulation was dependent on the lead content in the growth medium. This view is in conformation with the findings of the present study in *Vigna mungo* that when minimum mercury content was accumulated in the samples of 1 μM concentration (Table 21) the quantity progressively increased proportional to the increase of mercury in the nutrient medium.

Data on the residual mercury analysis in the nutrient solution (Table 23) after sample collection at 12 and 24 hr and comparison against the total uptake by the plant showed that about 1/3 of the total mercury applied in each treatment was lost during growth. One of the important causes for loss of mercury may be the occurrence of trichome-like secretary structures on the stem (Plate 10, 11) and apparent escape of mercury through them. This observation and concept are in

conformation with the suggestion of Orcutt and Nilsen (2000) who stated that plants may form volatile organic derivatives of mercury to remove it from tissues. Even though stained masses are not seen inside the cavities of these glands yellowish stained patches are observed on both inner and outer surface of the trichome confirming the association of mercury with the trichomes.

Since one transverse section of 10 μm thickness showed roughly 10 to 15 trichomes innumerable number of trichomes may be present throughout the stem surface. In addition to this secretary trichomes in the stem, leaves also may consist of trichomes/ hydathodes for the secretary purpose because mercury accumulation was observed in the leaves (Table 21) also.

Data on mercury accumulation in various plant parts upto 168 hr of growth (Table 21) indicate that absorption of mercury was proportional to the concentration of the medium *i.e.* availability of Hg^{2+} ions as well as growth period. Neverthless, *V. **mungo*** plants cannot be called as tolerant to mercury because at 10 μM concentration survival of the plant was only for limited period. Even though substantial amount of mercury was absorbed and translocated to various tissues it has also been observed that considerable amount of mercury was presumed to be lost from stem tissues as described earlier, through trichome-like structures. Progressive increase of mercury content in the plant tissues and concomitant depletion in the residual nutrient solution indicate continuous absorption and

translocation of mercury. However, when a comparison is made between the mercury content, added to the nutrient medium on one hand (Table 21) and sum of the balance amount in the residual solution and accumulated mercury content on the other (Table 23), it is obvious that significant amount of mercury was lost during growth of the seedlings. This disparity may be correlated with or adjusted against the apparent loss of mercury due to the escape through the trichome-like structures on the stem. Hence some sort of 'cycling' of mercury occurs from the nutrient medium to the plant and from the plant to the atmosphere. So *V. mungo* plant is neither an excluder (Baker, 1981; Fitter and Hay, 1983; Baker and Walker, 1990; Turner, 1994; Orcutt and Nilsen, 2000), nor an accumulator of mercury (Baker and Walker, 1990; Turner, 1994). Similarly, this plant never show strategies like avoidance, internal detoxification or biochemical tolerance (Berry, 1986) because mercury enters the plant and internal detoxification or biochemical tolerance are not apparent. However, the strategy of response shown by *V. mungo* towards mercury may be considered as amelioration (Fitter and Hay, 1983) according to whom amelioration means plant absorb the toxic ion and act upon it in such a way as to minimise the effect variously and this may involve chelation, dilution, localisation or excretion. The cycling of mercury between growth media, plant and atmosphere involves absorption chelation – to some extent by phytochelatin formation, localisation in roots and finally excretion through trichome like structures.

If the growth medium is soil contaminated with mercury, the metal may enter the plant and get returned to the atmosphere by this type of cycling and finally reach the soil from the atmosphere. So the cycling of mercury can be considered as soil-plant-atmosphere continuum similar to the SPAC concept of water relation in plants.

In the present investigation, since roots were in direct contact with mercury containing nutrient solution, growth retardation is initiated in the roots and more pronounced effect was manifested on roots than on stem or leaf. Since the higher concentration (> 10 μM) of mercury results in injury and death, *V. mungo* is not tolerant to mercury. The non tolerance of *V. mungo* was confirmed from the tolerance index analyses also. Tolerance index reduced significantly with increase in mercury concentration in the growth medium.

According to Moore and Ramamoorthy (1984), mercury absorption and accumulation in diatom and green algae was taken place by both active and passive absorption. However it is not clear that how much mercury is bound inside and on the cell surface or externally by metabolites. Further, these authors suggested that if mercury was internally bound, Hg^{2+} reesidues would likely bioaccumulate and enter food chain but in the case of external binding, external sequestration could remove Hg^{2+} from the cells. In *Vigna mungo* the absorption and translocation of mercury was observed throughout the growth period irrespective of its toxicity

resulting in growth retardation and trichomes present all over the stem are indicative of expelling mercury. So it can be assumed that mercury absorption in *V. mungo* is mostly by passive process.

Since the cell wall of root hairs are in direct contact with essential ions and mercury, if the mercury ions enter the membranes, toxic changes may be initiated in the roots immediately. However, *V. mungo* plants survive at lower concentration of mercury, despite the direct contact of the root hairs with toxic Hg ions. As described earlier cell wall protects the membrane and their metabolism preferably by its appoplastic translocation of this metal (Taiz and Zeiger, 1998). Considerable quantity of mercury may be adsorbed by cell wall (Deshkar *et al.*, 1990) and mucilagenous material present on the root surface (Woolhouse, 1983). Hence the chance for direct entry of mercury into cell membrane is meagre and if at all Hg enter cytoplasm phytochelatin synthesis is triggered immediately (Rauser, 1990) to detoxify the effect. According to Cumming and Tomsett (1992) once the heavy metal has encountered the plasmalemma, it enters the cell *via* transport mechanisms similar to those of essential elements. Antagonism and synergism between metals can have considerable impact on ionic balance which can cause membrane degradation, affect electrical charge potential across membranes and disrupt pH compartmentation resulting in imbalance of energy and metabolic activity, disturbance in ionic balance in membranes, organelles and cytoplasm. The ionic antagonism contribute to detrimental effect on growth and development of plants.

Ionic imblanace also causes distortion of organelles and cytoplasm leading to cell division inhibition and growth retardation, and many metabolic pathways such as photosynthesis, respiration and enzyme activity etc. are inhibited. As a result of organelle disruption mercury ion binds with SH or SS bonds and replace essential ions, prosthetic group, co-enzyme etc., and all these lead to primary indirect injury to plant growth (Orcutt and Nilsen, 2000).

CONCLUSION

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Vigna mungo seedling growth is very sensitive to low levels (1 μM) of $HgCl_2$ in nutrient culture. Growth retardation, was shown in 1μM $HgCl_2$ and progressively increased with increase in mercury concentration. Root system showed maximum growth inhibition followed by leaf area and stem elongation. Biomass accumulation and stomatal conductance were also affected adversely due to mercury stress.

Starch content of root, stem and leaf were depleted with a concomitant raise in sugar. Reduced chlorophyll content and starch depletion indicate inhibited photosynthesis. Despite this inhibition of photosynthesis, total sugar accumulation in Hg treated plants may be due to the reduced rate of respiration and other catabolic activities. Protein content depletion resulted in an enhanced amino acid accumulation particularly proline content.

Reduced nitrate reductase activity was indicative of retarded nitrate content absorbed from the nutrient solution and cotyledonary reserve. Proline content was observed maximum in high concentration of Hg where growth retardation was maximum. Reduction of stomatal conductance as a characteristic of metal toxicity was shown by reduced stomatal size in *V. mungo* plants grown under Hg stress.

Histochemical studies showed localisation of Hg in roots as dithizone-mercury complex in some conducting cells, completely filling the lumen of these cell. However, this stained contents were totally absent in stem sections. But some of the epidermal cells of the stem were appeared as modified trichome-like structures containing some slightly stained material indiating the presence of mercury. The distribution of this trichome-like structure was very high owing to the occurrence of many numbers in a single transverse section. Since mercury is reported to escape as organic derivatives from plant tissues, these trichomes aparently served as exit for the disposal of Hg from the plant. The presence of trichome-like structures and translocated Hg content present in stem obtained by chemical analysis were evidences to correlate Hg toxicity and anatomical modifications in stem of *V. mungo* seedlings.

Mercury accumulation occurred maximum in root tissue and found to be translocated to stem and leaves also. The absorbance and translocation were found to be continuous and probably the translocation was by apoplastic means. Passive uptake by adsorption on root surface and by mucilagenous secretion of the root surface also presumed to be occurred.

Vigna mungo can not be considered as tolerant to mercury because beyond 10 μM concentration the seedlings do not survive. Neverthless, *V. mungo* absorb and accumulate considerable amount of mercury from the nutrient solution and

some absorbed Hg is lost to the atmosphere through the trichome like structures present on the stem. Despite the "cycling" of Hg occur from nutrient medium to plant and from the plant to the atmosphere, *V. mungo* plant is not at all tolerant to mercury.

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