4. DISCUSSION

4.1 Seed germination

4.1.1 Effect of copper on germination

Plants normally take up nutrients from soils and sediments through their roots. It is known that accumulation of certain micronutrients in soils can be toxic for plants. In particular copper, an essential micronutrient can be toxic in excess for most plants with the exception of a few plant species that can hyper-accumulate metals (Bernal et al., 2006). Many studies have been carried out on the effect of copper on seed germination, growth of radicles, plumules or coleoptiles of plants.

The experiments of Victor et al. (2007) showed the ability of *Prosopis juliflora* to germinate in copper concentration ranging from 10 - 1280 ppm. Hameed et al. (2001) suggested that copper has different effect on different plants. According to them in *Lycopersicum esculantum* seed germination was decreased but in *Spinacea oleracea* increased by the treatment of copper chloride. Ouzounidou (1995) studied the effect of increasing copper concentration on *Minuartia hirsute, Silene compacta, Alyssum monanum* and *Thalapsi ochroleucum*. He observed that the seed germination was highly affected by higher copper concentrations, while lower concentration was necessary for the germination. There are certain plants for example *Vigna radiata* (Verma et al., 2011), *Typha latifolia* (Muller et al., 2001), *Fellopia convolvulus* (Kajaer et al., 1998), etc. have no effect of
copper application on seed germination. In *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* lower copper concentrations have no significant effect on percentage of seed germination but it had slightly reduced at higher concentration.

In several plants viz., *Albizia lebbeck*, *Leucaena leucocephala* (Shafiq *et al.*, 2008), *Spinacia oleracea* (Sharma *et al.*), *Zea mays* (Gupta and Abdulla, 2011; Patil *et al.*, 2012), *Vigna mungo* (Solanki *et al.*, 2011) *Hordeum vulgare* and *Oryza sativa* (Tariq *et al.*, 2007), *Pisum sativum*, *Phaseolus mungo* (Vig and Sharma, 2009), *Cucumis sativa* (Munzuroglu and Geckil, 2002), *Agropyron elongatum* (Saberi *et al.*, 2011) and *Triticum aestivum* (Singh *et al.*, 2007; Tariq *et al.*, 2007; Munzuroglu and Geckil, 2002; Weiwei *et al.*, 2000) reduction in seed germination was found by the application of higher copper concentrations.

In the present study the delay in seed germination was observed by the treatment of higher concentration of copper in *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia*. Similar increase in period of germination was observed in *Capsicum* species by the application of higher copper concentrations by Irina *et al.* (2011).

The inhibitory effects of heavy metal results on seed germination could be the osmotic effect (Michael *et al.*, 1972), or it could be due to decreased level of auxin resulting from enhanced destruction of auxin by metal ion (Mukherji and Das-Gupta, 1972). Reduction of seed germination can also be attributed to the alteration
of selection permeability properties of cell membrane (Muhammad et al., 2008).

4.1.2 Effect of chromium on germination

Chromium is very important for the normal growth of plants, although stimulating effects to the additions of chromium on plant growth have been observed by several researchers (Lintschinger et al., 1997), but excessive amount can leads to toxicity (Jun et al., 2009). High levels of chromium supply can inhibit seed germination and subsequent seedling growth (Zayed and Terry, 2003). The investigation of Akinci and Akinci (2010) showed that germination and viability of seeds of Cucumis melo were negatively affected by elevated concentrations of chromium. There are reports about the inhibition effect of higher concentration of chromium on seed germination for different species: chromium reduced the germination from 15 to 55 % in alfalfa at 5 to 40 mg\(^{-1}\) Cr (Peralta et al., 2001); from 2.2 to 100 % in celery at 0.01 to 10 mM Cr (Scoccianti et al., 2006); and 17 to 44 % in pea at 25 to 100 mg\(^{-1}\) Cr (Pandey and Pandey, 2008) in comparison with control applications.

The study of Jun et al. (2009) showed that the higher concentration of chromium treatments significantly inhibited germination for Vigna radiata. Similar results were obtained for Glycine max, Vigna unguiculata and Vigna aconitifolia in the present work. A result documented previously stated that the high levels (500 ppm) chromium in soil reduced germination up to 48 % in bush bean (Phaseolus vulgaris) (Parr and Taylor, 1980). Peralta et al. (2001)
found that 40 ppm of chromium reduced 23 % ability of seeds of lucerne (*Medicago sativa* cv. Malone) to germinate and grow in the contaminated medium. According to Gyawali and Lekhak (2006) the germination of different rice cultivars was effectively reduced by 0.86 % - 100 % from 10 - 800 ppm chromium concentration in culture medium.


4.1.3 Effect of ferrous on germination

Very little research has been conducted on the effect of ferrous on seed germination. According to Li *et al*. (2009) in *Medicago sativa* the rate of seed germination, plant height, weight of root and shoot and leaf area was similar to control plant at lower concentration of ferrous but affected at higher concentration. Tang *et al*. (2008) reported the inhibition of seed germination in *Phaseolus radius* at lower concentration of ferrous. Nozoe *et al*. (2009) carried out the experiments of ferrous treatment on rice and seeds of weeds growing in rice field. They reported that 100 mg/l of ferrous suppressed the germination of weed *Echinochola crcs-galli* var. crcs-galli, *Cyperus serotinus*, *Cyperus detormis* and *Monochoria korsakowii*. However it had no effect on germination of seeds of *Echinochla oryzicola*, *Schoenoplectus juncoides* and *Monochria vaginalis*. They also noticed
that ferrous had no effect on the germination of rice seed. In *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* lower concentration of ferrous has no affect on seed germination but at higher level it delayed as well as retarded seed germination. This can be attributed to the toxic effect of ferrous at higher concentration (Kuzhandaivel and Venkatesan, 2011).

4.1.4 Effect of nickel on germination

According to Brown *et al.* (1987) nickel is useful to plants as micronutrient however; excess nickel is known to be toxic. There have been several reports on the effect of nickel on seed germination in plants. In pigeon pea (Rao and Stresty, 2000), wheat (Nedhi *et al.*, 1990) and chickpea (Khan and Khan, 2010) nickel has reduced the seed germination.

This investigation has shown that higher nickel concentration has adverse effect on seed germination. It delayed and reduced percentage of germination in the investigated plants. Complete inhibition of seed germination was observed in *Glycine max* at higher nickel concentration. Maheshwari and Dubey (2007, 2008) have observed inhibition of seed germination and impaired seedling growth as well as inhibition of the activities of the hydrolytic enzymes RNases and proteases in rice cultivars in response to 200 and 400 µm nickel treatment. Nickel toxicity in plants studied by several workers. The toxicity of nickel is likely to be caused by indirect mechanisms. Chen *et al.* (2009) proposed two mechanisms of nickel toxicity in plants:
interference with other essential metal ions and induction of oxidative stress.

### 4.1.5 Effect of manganese on germination

Study of several workers indicated that manganese excess inhibits the plant growth (Feng et al., 2009; Shi and Zhu, 2008; Qing et al., 2010). In the present study, seed germination percentage decreased and germination period was delayed at higher concentration of manganese. These data are in harmony with those reported by Mumthas et al. (2010). They observed that manganese has reduced seed germination in Vigna radiata by increasing concentrations and control plants exhibited maximum percentage of germination.

Similar results were also obtained in wheat (Panda and Patra, 1997), cowpea (Lalitha et al., 1999), cotton (Shrivastava et al., 1997) and pea (Chugh and Sawhney, 1996). The reduction in germination percentage in pulses at higher concentrations may be attributed to the interference of heavy metal ion during germination metabolism (Sankar et al., 2008).

### 4.1.6 Effect of zinc on germination

Houshmandfar and Moraghebi (2011) studied the effect of heavy metal mixture of cadmium, copper, nickel and zinc on seed germination and seedling growth of safflower. According to them the germination percentage was markedly suppressed at a higher mixture level of 180 mg/Kg. Few investigators have also reported decrease in
seed germination and seedling growth due to excess level of zinc and other heavy metals (Ayaz and Kadioglu, 1997; Morzeck and Funicelli, 1982; Mahalakshmi and Vijayarangapan, 2003; Mahmood et al., 2005). In corn treated with low levels of zinc has no effect on percentage of germination (Mahmood et al., 2005).

In the current work it is found that the lower concentration of zinc has no adverse effect on period and percentage of germination which were almost similar to control. The study of Manivasagaperumal et al. (2011a) on *Cyamopsis tetragonossa* has shown that plants treated with low level of zinc (10 and 25 mg/l) showed increase in germination, seedling growth, fresh and dry weight over control. This value indicated that zinc at lower level had a beneficiary and nutritional effect (Reichman, 2002). Manivasagaperumal et al. (2011a) noticed adverse effect of zinc at higher level on *Cyamopsis tetragonossa*. Similar to this in present work *Vigna unguiculata* and *Vigna aconitifolia* also, the percentage of seed germination was reduced by higher concentration of zinc. This can be attributed to the accelerated breakdown of stored food materials in seed by the application of zinc (Houshmandfar and Moraghebi, 2011). Reduction in seed germination can also be attributed to alterations of selection permeability proportion of cell membrane (Manivasagaperumal et al., 2011a).

4.2 Growth rate and biomass

Dry matter yield decrease has generally been accepted as the standard measure for comparisons of toxicity. However, occasionally
other measures such as fresh weight, commencement of symptoms (Elamin and Wilcox, 1986), and metabolic responses have been used (Gherardi et al., 1999).

4.2.1 Effect of copper on growth rate and biomass

Copper is an essential plant micro nutrient required for the protein components of overall enzymes (Marschner, 1995). However, when present in excess quantities, copper is also toxic to plant growth potentially causing damage resulting in complete inhibition of growth (Kopittke and Menzies, 2006).

According to Kopittke and Menzies (2006) and Kopittke et al., (2007) the higher concentration of copper inhibits the growth of root and stem. The other workers Smirnoval et al. (2006), Lin et al. (2003) and Ali et al. (2002) have reported decrease in root and shoot growth due to higher amount of copper in the treatment.

In the present work the decrease in fresh weight and dry weight of root, stem and leaf of Glycine max, Vigna unguiculata and Vigna aconitifolia was observed when concentration of copper was increased in the treatment. Zhu and Alva (1993) and Kopittke and Menzies (2006) also found decrease in fresh weights due to higher concentration of copper and explained that shoot growth reduction is not due to direct toxicity of copper in the shoots, but rather to nutrient deficiencies resulting from a reduced nutrient uptake by the damaged roots. Kopittke and Menzies (2006) reported reduction in growth of cow pea due to copper treatment. In the present work also reduction in
growth of investigated plants was observed due to higher concentration of copper.

4.2.2 Effect of chromium on growth rate and biomass

Chromium plays an important role in growth and development of plants (Pratt, 1966; Bertrand and De Wolf, 1965). It is toxic to plants when present in higher concentration and affects the growth (Voelcker, 1921; Bishnoi et al., 1993; Davis et al., 2001; Gbaruko and Friday, 2007).

Chromium toxicity results in to the decrease in root and shoot length (Huffman and Allaway, 1973; Gardea-Torresdey et al., 2004; Bishnoi et al., 1993; Dube et al., 2003; Faisal and Hasaim, 2005; Rout et al., 1997; Chen et al., 2001; Iqbal et al., 2003; Sankar et al., 2006), biomass (Tripathi and Tripathi 1999; Soni, 2004), plant weight (Rout et al., 1997; Sharma and Sharma 1993), seedling height, killing of seeds (Bradshaw et al. 1965), root weight (Chen et al., 2001; Iqbal et al., 2003), fresh weight and dry weight (Sankar et al., 2006).

In the present work only 30 - 40 % seed germination was observed and biomass of all investigated plants was reduced at lower concentration of chromium. Adverse effects of chromium on growth rate have been reported in different plants (Hanus and Tomas, 1993; Rout et al., 1997; Joseph et al., 1995). In the investigated plants also chromium has adverse effect on the growth.
4.2.3 Effect of ferrous on growth rate and biomass

Ferrous is an essential nutrient for plants. It functions to accept and donate electrons and plays important roles in the electron-transport chains of photosynthesis and respiration (Connolly and Guerinot, 2002; Ghasemain et al., 2010). But ferrous is toxic when it accumulates to high levels, which can damage lipids, proteins and DNA. Plants must therefore respond to iron stress in terms of both iron deficiency and iron overload (Connolly and Guerinot, 2002).

Iron plays important role in numerous physiological functions. Soil condition leading to iron deficiency (such as calcareous soil), or increasing the uptake of ferrous (such as soil water logging) are widespread in nature (Snowden and Wheeler, 1993). Limited growth under ferrous deficiency or toxicity described by various parameters, i.e. biomass accumulation, shoot and root length, area and number of leaves, relative growth rate etc. has been reported for different plant species (Foy et al., 1978; Nenova and Stoyanov, 1993; Snowden and Wheeler, 1993; Dobermann and Fairhurst, 2000; De la Guardia and Alcantara, 2002; Batty and Younger, 2003).

Few workers studied the effect of various concentrations of ferrous on plants and obtained different types of results. Ghasemain et al. (2010) reported increase in biomass of soya bean by the treatment of 50 Kg / hector of iron. Singh (1995) and Naik (1984) also found similar results. These results have conformity with the results obtained by the study of Singh and Shaha (1990). According to Nenova (2008) ferrous deficiency in pea plant resulted decrease in biomass. Fageria
and Rabelo (1987) and Kuraev (1966) suggested that high internal iron concentrations in plant tissue reduce plant yield. In the current work the fresh weight and dry weight of root, stem and leaves of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* was observed lower than control due to ferrous treatment.

Nenova (2009) reported decrease in root growth of pea plant due to ferrous deficiency. Ward *et al.* (2008) observed decrease in root length under the influence of higher concentration of ferrous in *Arabidopsis*. Kuraev (1966) reported inhibition of root growth at the higher concentration of ferrous. Laan *et al.* (1991) reported that the growth of *Rumax* was decreased by increasing the concentration of ferrous ion in nutrient solution. This was accompanied by corresponding decrease in root development. In present work also decrease in growth rate of *Vigna unguiculata* and *Vigna aconitifolia* was observed by increasing the concentration of ferrous in the treatment. The inhibition of shoot and root growth due to higher ferrous concentration was due to ferrous toxicity (Nenova, 2009).

### 4.2.4 Effect of nickel on growth rate and biomass

Although nickel is natural component of the soil, human activities such as metal processing, land application of sludge and the use of certain fertilizers can lead to an accumulation of nickel at potentially toxic levels (Kabata-Pendias and Pendias, 2001). In addition to anthropogenic pollution, nickel may also accumulate in soil naturally. For instance, soils formed from serpentine minerals often contain high nickel concentrations (Batianoff and Singh, 2001).
In plants nickel is a component of the enzyme urease and is considered as an essential micronutrient for growth (Brown et al., 1987). However, excess nickel is known to be toxic and many studies have been conducted concerning nickel toxicity of various species (Mishra and Kar, 1974; Seregin and Kozhevnikova, 2006).

Few workers reported beneficial effect of nickel at lower concentration. Rao and Shantaram (2000) showed that nickel at lower level gave promotive effect on dry matter. Karagiannidis et al. (2002) found that nickel improved the yields and biomass in tomato plants. Brake et al. (2004) recorded that the tomato plant grown in soil with lower nickel concentration increased the growth and improved the fruit quality. Tripathi and Tripathi (2000) showed that nickel gave promotive effect on root and shoot growth, leaf area and biomass of Albizia lebbek. Rahmatullah et al. (2001) showed that nickel improved tomatoes, shoot and root growth. The findings of Wallace et al. (1997) were similar to results of Rahmatullah et al. (2001). Gad et al. (2007) explained that the lower concentration of nickel has beneficial effect on all growth parameters.

However, when present at higher concentration in the soil environment or experimental growth media, nickel becomes phytotoxic (Parida et al., 2003; Gajewska and Sklodowska, 2005) and many studies have been conducted concerning nickel toxicity of various species (Mishra and Kar, 1974; Seregin and Kozhevnikova, 2006). In the present work decrease in fresh weight and dry weight of root, stem and leaves was observed in Glycine max, Vigna unguiculata and Vigna aconitifolia by increasing nickel concentration in the treatment.
Kopittke et al. (2007) have also studied the effect of nickel concentration on the growth of *Vigna unguiculata* and found similar decrease in fresh weight and explained that the fresh weight of root and shoot was decreased at similar rate. The reduction in relative shoot and root mass by nickel treatment was also reported in *Lactuca sativa* L. (Heikal et al., 1989) and *Phaseolus vulgaris* L. (Piccini and Malavolta, 1992). In *Glycine max, Vigna unguiculata* and *Vigna aconitifolia* the growth rate was decreased as there was reduction in length by increasing the nickel concentration in the treatment. This was in accordance with the results of Maheshwari and Dubey (2009). They reported 50 % reduction in root length and 27 % reduction in shoot length as well as 47 % decline in fresh biomass of roots and 29 % decline in shoots compared to control by the treatment of higher concentration of nickel. A significant reduction in shoot length, root length and biomass of chick pea was reported by Khan and Khan (2010) under the effect of higher nickel content in the treatment. They also noticed the suppression of the growth of the lateral roots. A comparative study of the effect of nickel on dry matter of roots and shoots of white clover, rye grass, cabbage and maize was carried out by Yang et al. (1996). They found reduction in dry matter due to the treatment of higher concentration of nickel and explained that the root growth was more affected than shoot growth due to nickel treatment.

El-Enany et al. (2000) studied the effect of nickel on bean seedlings. They observed that the fresh and dry matter of roots and shoots of broad bean plants were adversely affected by higher nickel content in the nutrient solution. Similar reduction of the growth of root and shoot of maize was observed by L’Huiller et al. (1996) due to the
higher content of nickel in the nutrient solution and reported greater effect of nickel on growth of root than shoot. They explained that this was due to the higher level of nickel in root as shown by Cataldo et al. (1978), Lubben and Sauerbeck (1991) and Taylor (1989) as well as due to consequence of depressed mitotic activity in the root meristem by nickel content.

Few other investigations recorded decrease in fresh weight as a result of nickel toxicity (Mocquot et al., 1996; Paivoекe, 1983; Barcelo et al., 1986). They observed decrease in fresh weight as a result of change in plant water status (El-Enany et al., 2000). Nickel accumulation decreased water uptake or enhanced water loss, both of which may cause membrane damage. Plant cell membranes are generally considered primary sites of metal injury (Barcelo and Poschenrieder, 1990).

4.2.5 Effect of manganese on growth rate and biomass

Manganese is an essential micronutrient in most organisms (Millaleo et al., 2010; Marschner, 1995). In plants, it participates in the structure of photosynthetic proteins and enzymes. Its deficit is dangerous for chloroplasts because it affects the water-splitting system of photosystem II, which provides the necessary electrons for photosynthesis (Buchanan et al., 2000). However, its excess seems also to be particularly damaging to the photosynthetic apparatus (Mukhopadhyay and Sharma, 1991). Thus, manganese has two roles in plant metabolic processes: as an essential micronutrient and as a toxic
element when it is in excess (Kochian et al., 2004; Ducic and Polle, 2005).

Plant species differ considerably in their normal or adequate manganese concentrations (Clarkson, 1988) and in their susceptibility to manganese deficiency (Reuter et al., 1988; Marschner, 1995; Mengel and Kirkby, 2001).

The studies of several workers have indicated that the excess manganese inhibits the plant growth (Mukhopadhyay and Sharma, 1991; Feng et al., 2009; Shi and Zhu, 2008; Gherardi and Rengel, 2003) and decreases root, stem and leaf dry weight / biomass. This reduction in fresh / dry weight / biomass of various plant organs was observed in Lolium perenne (Rosas et al., 2007; Mora et al., 2009), Trifolium repens (Rosas et al., 2007), Juncus effuses (Najeeb et al., 2009) and Populas cathayana (Lei et al., 2007). In this study decrease in fresh weight and dry weight of root, stem and leaf of Glycine max, Vigna unguiculata and Vigna aconitifolia was observed by increasing the concentration manganese in the treatment. McQuattie and Schier (2000) noticed reduction in shoot growth and dry weight of various organs of sugar maple seedlings with increasing manganese level in the treatment. The sensitivity of other seedlings of several plant species to excess manganese was reported by Hoyle (1972), Kavvadias and Miller (1999), Keil et al. (1986), Kitao et al. (1999), Langheinrich et al. (1992), McQuattie and Schier (2000), Morrison and Armson (1968), Safford (1975) and Schweitzer et al. (1999). Excess manganese may induce nutrient deficiencies in plants by interfering with the adsorption, translocation and / or utilization of nutrient elements such
Manganese toxicity may play an important role in poor growth of plants. In several plants reduction in growth rate (Millaleo et al., 2010), decrease in plant or shoot height (Najeeb et al., 2009; Lei et al., 2007) and root length (Mumthas et al., 2010) was found due to effect of excess manganese. The reduction in growth rate was noticed in all three investigated plants by higher manganese concentration in the current work. McQuattie and Schier (2000) explained that the reduction in nutrient elements by excess manganese may affect the growth rate. The reduced growth during manganese treatment probably is due to the effect of manganese on physiological processes, for example, inhibition of DNA replication (Baranowska et al., 1977) and protein synthesis (Foy et al., 1978). The tolerance to an excess of manganese is highly dependant on the plant species and cultivars or genotypes within a species (Foy et al., 1988; Horst, 1988).

4.2.6 Effect of zinc on growth rate and biomass

Zinc is a necessary element for plants (Wang et al., 2009) and has significant role in the seed germination (Cakmak, 2008) and production of biomass (Kaya and Higgs, 2002), plant fertilization (Pandey et al., 2006) as well as it is important as a cofactor in several enzymes (Grotz and Guerinot, 2006).

Zinc can be toxic in excess for most plants with the exception of a few plant species that can hyper accumulate metals.
According to Ali et al. (1999) zinc is known to be toxic at higher concentration and several studies have been conducted concerning zinc toxicity of various species (Rauser, 1973). From these investigations it has been found that there was reduction in biomass and inhibition of the growth of various plant organs due to treatment of higher concentration of zinc. In several plants the growth of root and / or shoot has been decreased due to zinc effect. Wong and Bradshaw (1982) observed 63 % reduction of the root in response to 185 ppm of zinc. Ekaterina and Jeliazkova (2001) reported 30 % to 50 % reduction in root growth by 800 mg/l zinc treatment in Cuminum cyminum and Satvia officinalis. Patel et al. (1976) found 30% decrease in fresh and dry weight of Chrysanthemum seedling due to zinc treatment.

The other plants in which the growth of root and / or shoot decreased by zinc treatment are Nigella sativum and Triticum aestivum (EI - Ghamery et al., 2003); Phaseolus mungoo (Chaoui et al., 1997); Bacopa moniera (Ali et al., 1999) and Artemisia annua (Khudsar et al., 2004).

Balashouri, (1995) worked on Vigna radiata and noticed reduction in root and shoot growth due to effect of zinc. Similar results were obtained for Vigna unguiculata and Vigna aconitifolia in the present investigation. White et al. (1979) reported decrease in weight in soya bean due to effect of higher concentration of zinc. The decrease in fresh and dry weight of root, stem and leaves by increasing the concentration of zinc in the treatment in the investigated plants supports this result.
Patel (2008) found that zinc application retarded the growth of shoot and root in *Cajanus cajan* and *Trigonella foenum-graecum*. More retardation was observed in root growth than shoot growth in both plants. The reduction in growth is also consequence of zinc interference with nutrient uptake (Chaney, 1983 and Kaya *et al*., 2000), specific enzyme activities (Quariti *et al*., 1997) and certain essential metabolic events (Tripathy and Mohanthy, 1980; Van Assche and Clijsters, 1990; Alia *et al*., 1995).

### 4.3 Anatomical studies

Although a number of studies have been documented on the toxic effects of heavy metals, there are few reports concerned their effects on plant tissues and anatomical variations, especially in cultivated plants. The research work on effects of different concentrations of heavy metals was carried out on important plants viz., *Vigna unguiculata* (Kopittke and Menzies, 2006; Kopittke *et al*., 2008; Kopittke *et al*., 2009; Ratheesh Chandra *et al*., 2010), *Allium sativum* (Liu *et al*., 2009); *Origanum vulgare* subsp. *hirtum* (Panou-Filotheou *et al*., 2001), soya bean (Reis and Lavres, 2011; Lavres *et al*., 2009), *Vigna radiata* (Ratheesh Chandra *et al*., 2010), *Cicer arietinum* (Khan and Khan, 2010) and rice (Lin and Kao, 2005).

Present study has shown that different heavy metals have no effect on tissues and internal structure of plant organs at lower concentration. Certain variations or abnormalities were found at higher concentration.
Several workers have reported that the epidermis and cortex of root may rupture when exposed to aluminum (Yamamoto et al., 2001; Blamey et al., 2004; Jones et al., 2006). A number of other metals have been reported to affect plant roots in a similar manner to aluminum. Clarkson (1965) found that the results of experiments with gallium, indium and lanthanum on *Allium cepa* were similar to those with aluminum. Diatloff (1997) found that ruptures, markedly similar to those caused by aluminum, form when mung bean roots were exposed to lanthanum. Wheeler et al. (1993) reported that the visual symptoms of copper toxicity were similar to those of aluminum toxicity. Ouzounidou et al. (1995) also reported that high copper causes damage to epidermal cells of *Zea mays*. The ruptures of epidermal cells was also observed in roots of *Chloris gayana* when exposed to excess copper (Sheldon and Menzies, 2005). In the present investigation the breakdown of epidermal and outer cortical cells of root of *Glycine max* by zinc and of *Vigna unguiculata* by the treatment of copper was observed. These ruptures were markedly similar to those previously reported in *Vigna unguiculata* exposed to aluminum, copper and lanthanum by Kopittke and Menzies (2006), Kopittke et al. (2008) and Kopittke et al. (2009). According to Kopittke et al. (2009) the binding of metals like copper, aluminum and lanthanum to cell wall results increase in the cell wall rigidity and eventual cell rupturing. This is in agreement with that of Reid et al. (1996) who proposed an extracellular mechanism for the toxicity of scandium. Trace metals bind strongly to the cell wall, either through ionic binding or through covalent binding caused ruptures (Kopittke et al., 2009).
In the present study the increase in the deposition of lignin on the walls of vessel elements was found in the root of *Vigna unguiculata* and *Glycine max* due to the treatment of manganese. The higher lignification was also observed on the walls of vessel elements and xylem fibers of the stem of *Glycine max* under the influence of manganese. The xylem cells with higher lignifications on the walls were reported earlier in *Glycine max* by Reis and Lavres (2011) and Lavres *et al.* (2009). With respect to lignification, it is known that manganese along with boron, copper and ferrous is involved in the metabolism of phenol compounds and the biosynthesis of lignin (Reis and Lavres, 2011).

The treatment of manganese as well as nickel reduced the number of chloroplasts in palisade tissue of *Glycine max*. Weiland *et al.* (1975) and Reis and Lavres (2011) also observed similar reduction in number of chloroplasts in the leaves of *Glycine max* by the treatment of manganese.

From the present work it is revealed that the higher dose of manganese may results into the disorganization and deformation of xylem, especially in root of *Glycine max*. Similar disorganization of xylem cells was reported by Reis and Lavres (2011) in different varieties of *Glycine max* and suggested that the organization and ordering of the conducting tissues, as well as the integrity of the cortical cells, play a fundamental role in the nutrient absorption and transport processes.
From the survey of literature it is found that the study on the effect of heavy metals on cambial activity is scanty. In the present study it is found that nickel, copper and ferrous may reduce the cambial activity. This diminutive effect may be due to reduction of cell division by metals like copper (Liu et al., 2009).

4.4 Biochemical Studies

4.4.1 Total sugar

4.4.1.1 Effect of copper on total sugar

The study on the effect of copper on carbohydrates or total sugar content of plant organs is scanty. Normally the total sugar content decreases by the treatment of heavy metals. (Tandon and Gupta, 2002). Few workers reported decrease in total sugar in various plants by cadmium treatment. This was found in Cajanus cajan and Trigonella foenum-graecum (Patel, 2008), rice (Huang et al., 2006) and sugar beet (Greger and Lindberg, 1986). Singh et al. (2007) observed reduction in total sugar in wheat seedlings in different copper treatments. In the present work the decrease in total sugar content was observed in the root and stem of all three investigated plants except root of Glycine max and stem of Vigna aconitifolia where slightly higher total sugar content was found at lower concentration of copper. According to Singh et al. (2007) copper at lower concentration works as nutrient in plants but when its concentration increases, it causes toxicity and affects the sugar contents in plants.
4.4.1.2 Effect of chromium on total sugar

Chromium is known to be an essential element for normal carbohydrate metabolism in animal and human nutrition (Mertz, 1969; Anderson, 1989). Although chromium was found to be a stimulant for plant growth (Gericke, 1943; Pratt, 1966; Bertrand and De Wolf, 1965, 1968), several investigations carried out to determine its toxic effect on different plant species (Hasnain and Sabri, 1997; Jain et al., 2000; Ren and Gao, 2000; Peralta et al., 2001; Zeid, 2001; Manjappa et al., 2002; Dube et al., 2003; Zhou and Li, 2003, 2004; Jamal et al., 2006; Li and Yang, 2006; Karbassi et al., 2008). According to Jun et al. (2009) pulses like Lablab purpureus and Glycine max are most sensitive to chromium. In the present work three plants, Glycine max, Vigna unguiculata and Vigna aconitifolia were found sensitive to chromium where the seeds could not germinate at higher concentration of chromium and the amount of total sugar was reduced at lower chromium concentration. This was probably due to chromium toxicity which leads to decrease in enzyme activity, causes membrane damage, diminishes photosynthesis and change in chloroplast (Jain et al., 2000; Parmar et al., 2002; Du et al., 2003; Dube et al., 2003; Zayed and Terry, 2003; Scoccianti et al., 2006).

4.4.1.3 Effect of ferrous on total sugar

Although iron is essential nutrient for plants (Rutherford and Bird, 2004); its accumulation within cells can be toxic (Connolly and Guerinot, 2002). Several workers reported toxic symptoms due to the effect of higher concentration of iron (Ponnamperuma et al., 1955;
Tanaka *et al.*, 1966; Yoshida and Tadano, 1978; Laan *et al.*, 1991). No extensive study on the effect of ferrous on total sugar content has been carried out yet.

Lopez-Millan *et al.* (2000) reported decrease in sugar concentration of apoplastic fluid of sugar beet due to moderate iron deficiency. According to Achakzai (2003) ferrous reduces the oil and soluble sugar and increases starch contents in mature seeds of soya bean. From this study it has been found that ferrous has adverse effect on total sugar content of root and stem of *Glycine max, Vigna unguiculata* and *Vigna aconitifolia* at higher concentration in the treatment.

**4.4.1.4 Effect of nickel on total sugar**

Nickel is an essential micronutrient for higher plants. (Brown *et al.*, 1987; Eskew *et al.*, 1983, 1984). However, nickel at sufficient high levels may be toxic to plants (Bingham *et al.*, 1986; Farago and Cole, 1986; Foy *et al.*, 1978). Excess nickel can affect physiological or biochemical processes (Pandolfini *et al.*, 1992; Carlson *et al.*, 1975).

Very little research has been conducted on the effect of nickel on sugar contents of plants. Gad *et al.* (2007) reported increase in total soluble sugars by increasing nickel in the treatment. Moya *et al.* (1993) and Samarakoon and Rauser (1979) have found increase in the amount of carbohydrates in the leaves due to nickel toxicity. In the present study it has been observed that the lower concentration of nickel has no adverse effect on total sugar content in the root and stem of
investigated plants but at high concentration the proportion of total sugar was reduced. Few workers reported higher accumulation of carbohydrates in leaves and decrease in root by heavy metals (Moya et al., 1993; Rauser, 1978; Samarakoon and Rauser, 1979). Similar accumulation of carbohydrates was found in maize leaves by L’Huiller et al. (1996) and suggested that this was because of the inhibition of carbohydrate transport from leaves to root due to nickel toxicity. Greger et al. (1991) proposed a hypothesis whereby heavy metals have a greater effect in reducing carbohydrate transport than reducing photosynthesis.

### 4.4.1.5 Effect of manganese on total sugar

Certain workers contributed on the effect of manganese on plant growth (Feng et al., 2009; Shi and Zhu, 2008). The studies on the influence of manganese on sugar or carbohydrates is meager (Mousavi et al., 2011; Bhakuni et al., 2008). This study has shown that the total sugar content was decreased in the roots of *Vigna unguiculata* and *Vigna aconitifolia* and increased in root of *Glycine max* by higher concentration of manganese. There was reduction in the amount of total sugar due to influence of manganese treatment in the stem of all three investigated plants. Normally increase in manganese in the treatment results decrease in plant nutrients (McQuattie and Schier, 2000). This may be due to phytotoxic effect of excess manganese which reduces photosynthesis and causes biochemical disorders (Millaleo et al., 2010) and retards the plant growth (Mousavi et al., 2011).
4.4.1.6 Effect of zinc on total sugar

The total sugar content was reduced and observed lower than control in root and stem of investigated plants due to zinc treatment except the roots of *Glycine max* where total sugar content was higher than control. Few workers also found decrease in total sugar and / or carbohydrates by zinc treatment. This was observed by Cvetanovska and Spasenoski (2001) in root, stem and leaves of *Lycopersicon esculentum*; by Lanaras *et al.* (1993) in the leaves of wheat plant and by Manivasagaperumal *et al.* (2011a) in the seedlings of *Cyamopsis tetragonoloba*. The observed decline with respect to the high level of zinc may be due to its role on the enzymatic reactions related to the cycles of carbohydrate metabolism (Rabie *et al.*, 1992) as well as due to photosynthetic inhibition or stimulation of respiration rate (Tzvetkova and Kolarov, 1996; Zengin and Kirbag, 2007).

4.4.2 Protein

4.4.2.1 Effect of copper on protein

Increased doses of heavy metals reduce protein contents (Tandon and Gupta, 2002). Guo *et al.* (2007) carried out experiments by adding aluminum, copper and cadmium in nutrient solutions on barley plants. They had reported decrease in protein content in roots and leaves by the combine effect of these three metals in Shang 70 - 119 variety of barley.
Llorens *et al.* (2000) studied copper exposure upon tissue cultured *Vitis vinifera*. They found dramatic changes in nitrogen metabolism, which was reflected in total nitrogen, amino acids and protein contents in both roots and leaves.

In this work the protein content of roots and stem was studied where the treatment of higher copper concentration resulted in reduction of protein in these two organs of investigated plants. In wheat also the reduction in protein was observed in response to copper (Lanaras *et al.*, 1993). During the seedling growth of wheat, there was decline in protein content in leaf tissues by the higher copper concentrations (Singh *et al.*, 2007). Tripathi and Tripathi (1999) also found decrease in protein content in *Albizia lebbak* and explained that this was either due to reduced *de novo* synthesis of proteins or increased decomposition of proteins into amino acids. According to Samantary (2000) the decrease in protein content may be due to inhibition of protein synthesis by heavy metal.

### 4.4.2.2 Effect of chromium on protein

Plant growth is inhibited by chromium toxicity (Citterio *et al.*, 2003; Karbassi *et al.*, 2008). This work showed that the seeds were failed to germinate by chromium treatment except at 0.2 mM chromium concentration. At this treatment protein content in root and shoot of investigated plants was reduced. Similar decrease in protein content was reported by Sankar *et al.* (2007) in *Glycine max* by increased chromium concentrations.
Chromium can degrade protein (Panda and Choudhury, 2005). According to Sankar et al. (2007) during the transport of heavy metals into the plants, it can act at different sites to inhibit a large number of enzymes having functional sulphydryl groups. It results in the deleterious effect in the normal protein form (Dua and Sawhney, 1991) by disrupting in the pathways and protein synthesis (Nagoor, 1999).

4.4.2.3 Effect of ferrous on protein

The information regarding the impact of ferrous on protein content in the plant organ is scarce available. Shainberg et al. (2000) observed no differences in fresh weight, dry weight, chlorophyll and soluble protein content between ferrous treated and control plants. In the root and stem of Glycine max, Vigna unguiculata and Vigna aconitifolia ferrous has no adverse effect on protein content at lower concentration but it has reduced protein content when concentration was increased in the treatment. Kuzhandaivel and Venkatesan (2011) suggested that increase in ferrous may cause changes in the biochemical constituents and disturbs the enzyme activity. According to Batty and Younger (2003) ferrous toxicity affects the nutrient contents and inhibits the plant growth.

4.4.2.4 Effect of nickel on protein

El-Enany et al. (2000) studied the nickel toxicity in bean seedlings. According to them nickel inhibits protein contents by about 50% at low and higher loses. In the roots of Glycine max and Vigna aconitifolia protein content was slightly higher than control at lower
concentration of nickel. In the root of *Vigna unguiculata* and stem of all three investigated plants protein content was decreased by increasing the nickel concentration in the treatment. Similar results were obtained by Singh and Pandey (2011). They reported increase in protein content by lower concentration of nickel in the leaves of *Pistia stratiotes* which decreased at higher concentrations. Reduction in protein content in plants by nickel can be attributed to effect on nitrate reductase activity (Vajpayee *et al*., 2000).

### 4.4.2.5 Effect of manganese on protein

Lidon (2002) contributed on the lipid, carbohydrate and protein accumulation in manganese treated rice. He noticed decrease in protein by the effect of higher concentration of manganese. According to Qing *et al*. (2010) manganese excess increased root, stem and leaf manganese content but decreased soluble protein content in leaves and roots of *Citrus grandis* seedlings. From the present study it is revealed that protein content in root and stem was not adversely affected by lower manganese treatment but reduced when concentration was increased. This can be, probably due to the direct effect of manganese on physiological processes, for example inhibition of DNA replication (Baranowska *et al*., 1977) and protein synthesis (Foy *et al*., 1978).

### 4.4.2.6 Effect of zinc on protein

In higher plant under condition of zinc deficiency several metabolic processes are impaired, such as RNA metabolism and protein synthesis (Sharma *et al*., 1981; Kitagishi and Obata, 1986),
control mechanism of generation and detoxification of the oxygen derived radicals (Cakmak and Marschner, 1988) as well as structural and functional integrity of cell membrane (Welch, et al., 1982).

Cakmak et al. (1989) found decrease in soluble protein in the leaves of *Phaseolus vulgaris* due to zinc deficiency. According Wang et al. (2009) excess zinc can damage plants and inhibits the growth. They reported decrease in chlorophyll content and reduction in soluble protein in the leaves of rapeseed seedlings due to excess zinc. Similar reduction in protein content was also found in stems of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* by increasing zinc concentration in the treatment. There was decrease in total soluble protein in leaves and roots of *Artemisia annua* under the influence of higher zinc concentration (Khudsar et al., 2004). Manivasagaperumal et al. (2011a) reported decrease in amino acids and protein content in *Cyamopsis tetragonoloba* by excess of zinc. They explained that the inhibition of excess zinc in amino acids and protein might be due to binding of metals with the sulphhydryl group of protein and causing deleterious effect in the normal protein form.

Wang et al. (2009) noticed increase in total soluble protein in the rapeseed roots in contrast to stem under excess zinc. Similar to these results in the roots of *Glycine max* and *Vigna aconitifolia* the protein content was higher than control due to zinc treatment. Rout and Das (2003) suggested that an increased number of nucleoli resulted in the synthesis of new protein in roots in response to zinc stress.
4.4.3 Potassium

4.4.3.1 Effect of copper on potassium content

There are few reports on induced deficiency of various mineral contents under copper toxicity (Mocquot et al., 1996; Bouazizi et al., 2010; Lequeux et al., 2010). It was noted that the concentrations of potassium tended to decrease slightly - particularly in the low copper solutions in which growth was more rapid (Kopittke and Menzies, 2006). These results were in consequence with other studies (Ali et al., 2002; Lidon and Henriques 1993; Ouzoundou et al., 1995; Panou-Filotheou and Bosabalidis, 2004) and is most likely due to a nonspecific effect of copper on ion absorption and translocation due to an impairment of root function (for example, membrane leakiness, reduced ion uptake, or a reduction in radial transport or xylem loading). Increased soil copper concentration further resulted in a high decrease of potassium bioaccumulation, particularly at the upper part of the Oregano (Origanum vulgare subsp. hirtum) stem (Panou-Filotheou et al., 2006).

Kopittke and Menzies (2006) reported that plants growing in high copper solutions were found to have reduced shoot root concentrations of potassium. They found 50 % and 45 % decreases in potassium content of Vigna unguiculata root and stem respectively. Similar results were obtained for Vigna unguiculata in the present study.
In *Thlaspi ochroleucum*, high concentrations of copper inhibited potassium bioaccumulation (Ouzounidou *et al.*, 1992), while in *Oryza sativa*, potassium concentrations in stem tissues did not reveal any correlation with increasing copper level (Lidon and Henriques, 1993). Sheldon and Menzies (2004) showed that the shoot concentration of nutrient cations calcium, potassium, magnesium and manganese decreased in rhodes grass (*Chloris gayana*) as the concentration of copper in the nutrient solution increased. According to them at high copper concentrations, severe root damage was observed which reduced uptake of these elements due to breakdown of membrane function.

Murphy *et al.* (1999) reported a slight reduction in potassium concentration in *Arabidopsis* roots. According to them this was due to potassium efflux as part of a mechanism of copper tolerance.

In the present investigation decrease in potassium content was observed in *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* by higher copper concentration in the treatment. Similar decrease was also found in green gram by Lidon and Henriques (1993). The study of Ouzounidou (1994) also showed decrease in potassium content by the elevated level of copper in green gram. The decrease in potassium content of green gram due to copper may be attributed to the toxic effect of copper on plant growth or competition by other ions, which in turn exercised a regulatory control on potassium uptake (Lidon and Henriques, 1993; Ouzounidou, 1994).
4.4.3.2 Effect of chromium on potassium content

In the present study decrease in potassium content in roots and shoots of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* was recorded due to the treatment of lower concentration of chromium.

Turner and Rust (1971) found that 0.1 ppm of chromium in nutrient culture resulted in the decrease in concentration and contents of potassium, calcium, ferrous and manganese in shoots and potassium, magnesium, phosphorous, ferrous and manganese in roots as compared to the untreated soya bean plants. They also observed that in soil culture, chromium treatment significantly decreased concentrations of potassium, calcium, potassium, magnesium, boron and copper in shoots of soya bean. From these studies they suggested that chromium interfered with the ability of the plants to obtain these mineral elements from soil.

4.4.3.3 Effect of ferrous on potassium content

Lopez-Millan *et al.* (2004) studied sugar beet plant for ferrous deficiency conditions and concluded that the concentrations of inorganic cations potassium, calcium, and magnesium in apoplastic fluid of sugar beet leaves increased with iron deficiency with a similar trend to that found for the organic anions. The largest increase in apoplastic fluid concentrations with iron deficiency was 20-fold for calcium, followed by 3.5-fold for magnesium, and 1.7-fold for potassium. The concentrations of potassium and magnesium were higher in xylem sap than in the apoplastic fluid. In the present study at
lower concentration (0.2 mM ferrous content) increase in potassium content in roots of *Glycine max* and *Vigna unguiculata* and in stems of *Vigna unguiculata* was reported. At higher concentrations of ferrous reduction in potassium content was recorded in roots and shoots of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia*.

### 4.4.3.4 Effect of nickel on potassium content

Kopittke *et al.* (2007) noticed that an increase in nickel content caused a decrease in potassium, calcium and magnesium content in the shoot tissue. In the present investigation a decrease in potassium content was observed in roots and shoots of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* due to treatment of various concentrations of nickel. These observations are in general agreement with those reported for nickel toxicity in white clover, ryegrass, and cabbage (Yang *et al.*, 1996), tomato (*Lycopersicon esculentum* L.) (Palacios *et al.*, 1998) and fenugreek (Parida *et al.*, 2003).

### 4.4.3.5 Effect of manganese on potassium content

According to McQuattie and Schier (2000), content of all nutrients decreased with increase in manganese in sugar maple. They also concluded that manganese significantly reduced foliar concentrations of nutrient elements, except phosphorous, in newly germinated non-mycorrhizal seedlings of sugar maple.

In the present study an increase in potassium content was reported due to treatment of lower concentrations of manganese in
roots and shoots of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia*. However, higher concentrations of manganese showed decrease in potassium content in these plants.

Excessive manganese concentrations in plant tissues can alter various processes, such as enzyme activity, absorption, translocation and utilization of other mineral elements causing oxidative stress (Millaleo *et al.*, 2010). These may result reduction in nutrients or minerals.

### 4.4.3.6 Effect of zinc on potassium content

An increase in zinc supply resulted in a decrease in the concentrations of potassium, calcium, magnesium and phosphorous in the roots of pea plant (Stoyanova and Doncheva, 2002). They also observed that potassium content of the stems and leaves was not significantly affected by higher concentrations of zinc. The root and stem of all three experimental plants treated with zinc showed decrease in potassium values in the present study. An excess of zinc has been reported to have a negative effect on mineral nutrition (Chaoui *et al.*, 1997).

### 4.4.4. Phosphorous

#### 4.4.4.1 Effect of copper on phosphorous content

According to Lexmond and Van der Vorm (1981) the excess of copper resulted in lowering of phosphorous content in green gram.
Kopittke and Menzies (2006) studied the effect of copper on mineral contents in root and shoot of *Vigna unguiculata*. They found the different effects of copper on different types of mineral elements. They noticed the decrease in content of potassium, magnesium, ferrous and manganese by increasing the copper concentration in the treatment while the proportion of other elements viz., sulphur, phosphorous and boron remains unaffected or increases slightly. Similar increase in phosphorous content was observed in the root and stem of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia*.

4.4.4.2 Effect of chromium on phosphorous content

Turner and Rust (1971) investigated that the lower concentration of chromium in culture medium has adverse effect on mineral contents and their amount is reduced in root and shoot of soya bean. They also observed decrease in the minerals by chromium treatment in the soil culture. Similar adverse effect of chromium was observed in present study. A decrease in phosphorous content in roots and shoots of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* was found due to the treatment of chromium. This may be due to the effect of chromium on the uptake of minerals from the soil.

4.4.4.3 Effect of ferrous on phosphorous content

Certain workers contributed on the impact of iron on various aspects of growth and development of plants in past several years. They studied the effects if iron deficiency (Jiang *et al.*, 2007; Næve and Rehm, 2006; Wang *et al.*, 2008; Zocchi *et al.*, 2007; Kurepa *et al.*, 2008;

From the published literature it appears that the study on the effect of ferrous on phosphorous content in the organs of angiospermic plants is not carried out. Present work showed that phosphorous content of root and stem of investigated plants slightly reduced at lower concentration of ferrous which was increased by increasing concentration of ferrous in the treatment. From this it can be assumed that ferrous deficiency may reduce phosphorous contents in roots and stem.

### 4.4.4.4 Effect of nickel on phosphorous content

Nickel has different effects on roots of different plants. Phosphorous content increased in the root of cabbage but proportion of sulphur decreased by higher concentration of nickel (Sagner *et al*., 1998). In root of raye grass as well as maize both phosphorous and sulphur content was increased by higher concentration of nickel (Yang
et al., 1996). In root and shoot of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* the amount of phosphorus was increased by increasing nickel concentration in the treatment. Increase in phosphorus content by nickel in *Vigna unguiculata* was also reported by Kopittke *et al.* (2007).

### 4.4.4.5 Effect of manganese on phosphorous content


Soil, foliar application or seed treatment of manganese is essential for better crop yield and quality (Mousavi *et al.*, 2011). Bansal and Nayyar (1994) investigated the effect of manganese foliar applications on 10 cultivars of soyabean and observed a significant increase in economic and biological yield of soyabean. In the present work the treatment of manganese was found beneficial on phosphorous content in soya bean (*Glycine max*) as well as on *Vigna unguiculata* and *Vigna aconitifolia* as the amount of phosphorous was increased by increasing manganese concentration.
4.4.4.6 Effect of zinc on phosphorous content

The study on the effect of zinc on phosphorous content of plant organs is scanty. Jiang et al. (2007) have shown that the external application of phosphorous protects chloroplasts from zinc toxicity by forming P - Zn complexes. From the present work it is revealed that the lower concentration of zinc in the treatment has reduced phosphorous content in Glycine max, Vigna unguiculata and Vigna aconitifolia as reported by Wang et al. (2009) in Brassica nepus and Menser and Sidle (1985) in soya bean but higher concentration of zinc in the treatment has increased phosphorous content in roots and stems. Zinc reduced the total phosphorous contents in Psium sativum (Paivoke, 2003) and Brassica compestris (Chatterjee and Khurana, 2007). The experimental studies of Stoyanova and Doncheva (2002) showed that zinc decreases inorganic phosphorous in pea plant and suggested that the uptake and distribution of phosphorous is influenced by zinc concentration.

4.4.5 Calcium

4.4.5.1 Effect of copper on calcium content

Many studies have been carried out on the effect of copper excess on growth, mineral nutrition and metabolism of plants. Copper excess reduces growth (Maksymiec et al., 1995), photosynthetic activity (Lidon and Henriques, 1993) and the quantum of yield of PS II photochemistry assessed by chlorophyll fluorescence (Maksymiec and Baszynski, 1999). In several plants, the reduction in calcium content
was observed due to the influence of higher copper concentration. This was reported in *Chloris gayana* (Sheldon and Menzies, 2004), *Rosamainus officinalis* (El-Said Deef, 2007), *Vigna radiata* (Manivasagaperumal *et al*., 2011b) and *Cucumis sativus* (Alaoui-Sosse *et al*., 2004). The experimental studies of Kopittke and Menzies (2006) and Kopittke *et al*. (2007) have shown that the plant growth was decreased by copper excess and also reduced shoot concentration of calcium and other minerals in *Vigna unguiculata*. In this study also the decrease in calcium content was observed in root and stem of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia*. Similarly Ouzounidou (1994) observed a sharp decline in calcium content in the roots and shoots of *Alyssum montanum*, when copper was applied in higher concentration.

The decrease in concentration of calcium is typical of copper toxicity (Ali *et al*., 2002; Lidon and Henriques 1993; Ouzounidou *et al*., 1995; Panou-Filotheou and Bosabalidis, 2004). According to Kopittke and Menzies (2006) this copper toxicity is most likely due to a nonspecific effect of copper on ion absorption and translocation due to an impairment of root function (for example, membrane leakiness, reduced ion uptake, or a reduction in radial transport or xylem loading).

### 4.4.5.2 Effect of chromium on calcium content

Chromium interferes with several metabolic processes, causing toxicity to the plants as exhibited by reduced root growth and phytomass, chlorosis, photosynthetic impairing, stunting and finally
plant death (Huffman and Allaway, 1973; Kocik and Ilavsky, 1994; Gardea - Torresdey et al., 2004).

Several investigators found that chromium was toxic to plants (Koenig, 1911; Vonscharrer and Schorpp, 1935; Lyon et al., 1970; Wallace et al., 1976; Watanabe, 1984; Moral et al., 1993; Samantaray et al., 1996a, 1996b). In this study it was observed that the lower concentration of chromium reduced calcium content in roots and stems of investigated plants. Turner and Rust (1971) found that 0.1 ppm of chromium in nutrient culture resulted in the decrease in contents of calcium, potassium, ferrous and manganese in shoots of soya bean. The inhibitory effect of chromium on plant growth was thought to be the result of specific interaction between chromium and phosphorous (Robinson et al., 1935; Soane and Saunder, 1959; Spence and Millar, 1963; Moral et al., 1995) or ferrous (Cannon, 1960; De Kock, 1956; Hewitt, 1963) in plant nutrition.

The decrease in calcium content and other mineral nutrients and inhibitory effect of chromium was also reported by Azmat and Khanum (2005) in Vigna radiata. They explained that this effect was mainly due to the decrease in levels of auxins resulting from its enhanced destruction by metal ions as suggested by Ae et al. (1990).

4.4.5.3 Effect of ferrous on calcium content

contributed on the impacts of ferrous sulphate on mineral contents of *Vitis vinifera*. De Dorlodot *et al.* (2005) studied the ferrous toxicity in rice plant. In the present investigation at lower concentration of ferrous (0.2 mM) calcium content was increased in roots of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* and stem of *Vigna aconitifolia*. Higher concentration of ferrous had decreased calcium contents in both roots and stems of these experimental plants. This may be due to toxicity resulted by the elevated concentration of ferrous within plants or because of nutrient deficiency under influence of nutrient up take by the higher ferrous concentration (Batty and Younger, 2003).

### 4.4.5.4 Effect of nickel on calcium content

Physiological role of nickel and its toxic effect on higher plants have been studied by Seregin and Kozhevnikova (2006) and Khan and Khan (2010). Certain workers explained that nickel at higher concentrations retards the plant growth (Baccouch *et al.*, 1998; Gonnelli *et al.*, 2001; Wang *et al.*, 2001). The study of Yang *et al.* (1996) on mineral nutrients suggested that the higher concentration of nickel reduces the influx and transport of calcium and other minerals in white clover, ryegrass and maize. In the present work decrease in calcium content in roots and increase in stem was observed in the experimental plants due to higher nickel concentration. El - Enany *et al.* (2000) found inhibitory effect of nickel and notice decrease in calcium content in root and shoot of bean plant. Heavy metals generally reduce root growth more intensively that shoot growth decreasing the root/shoot ratio, but the amount of metal uptake and
intensity of root growth inhibition may vary with the plant species and the growth conditions (Foy et al., 1987; Barcelo and Poschenrieder, 1990).

### 4.4.5.5 Effect of manganese on calcium content

The study on the effect of manganese on calcium content of various plant organs is scanty. Orhue and Nwaoguala (2010) reported that the amount of calcium and other mineral increases in *Telfairia occidentalis* by the effect of increased concentration of manganese. In the root of *Glycine max* and *Vigna unguiculata* calcium content was found higher than control at lower concentration which was decreased by elevated concentration of manganese. In stem of all three experimental plants there was decrease in calcium content by increasing concentration of manganese. Clark (1982) explained that manganese at higher concentration interferes with the absorption and utilization of other mineral elements.

### 4.4.5.6 Effect of zinc on calcium content

The information regarding the effect of zinc on calcium content of plant organs is meager. Zinc has reducing effect on calcium content in the roots and stems of *Glycine max; Vigna unguiculata* and *Vigna aconitifolia*. Stoyanova and Doncheva (2002) observed a different pattern for calcium accumulation in zinc treated pea plants in the above ground parts and the roots. They reported increased calcium concentration in the leaves and stem whereas 42 % reduction in calcium content in roots by highest zinc treatment. Chaoui et al. (1997)
have suggested that an excess of zinc has negative effect on mineral nutrition. Excess zinc also affects the vital processes of plants (Doncheva et al., 2001; De Vos et al., 1991).

4.4.6 Magnesium

4.4.6.1 Effect of copper on magnesium content

Few workers in their investigations on the effect of copper on minerals included the study of magnesium content in plant parts. Sheldon and Menzies (2005) reported that the shoot concentration of magnesium was reduced by higher copper concentration in solution culture experiments on Chloris gayana. In copper stressed cucumber plants Alaoui - Sosse et al. (2004) reported decrease in the magnesium content in leaves and observed no change in the amount of root magnesium. In spinach plants, Ouzounidou et al. (1998) found declined in magnesium content under copper treatment. In the roots and stems of Glycine max, Vigna unguiculata and Vigna aconitifolia similar decline in magnesium content was observed under the influence of higher concentration of copper in the treatment. Manivasagaperumal et al. (2011) indicated that magnesium content in the leaves of green gram increased at low copper level and decreased at high copper levels. Ouzounidou (1994) and Lequeux et al. (2010) explained that copper toxicity induces magnesium deficiency. Kopittke and Menzies (2006) also noticed reduction in magnesium and other minerals under the impact of higher copper concentration in the shoot of Vigna unguiculata and explained that the reduction in the minerals and shoot growth was not result of direct copper toxicity to the shoots but due to
nutrient deficiencies caused by damage to the roots by the higher copper concentration.

### 4.4.6.2 Effect of chromium on magnesium content

Chromium inhibits the growth of root and shoot (Citterio et al., 2003; Gbaruko and Friday, 2007). Experimental studies have showed that chromium treatment reduced magnesium content in root of soya bean (Turner and Rust, 1971) and in root and stem of Vigna radiata (Azmat and Khanum, 2005). Similar decrease was observed in roots and stems of three experimental plants in this investigation. This may be due to phyotoxic effect of chromium (Jun et al., 2009).

### 4.4.6.3 Effect of ferrous on magnesium content

According to Batty and Younger (2003) the higher ferrous concentration inhibits the plant growth. Nenova (2008) reported decrease in magnesium, manganese and copper concentration in the roots of pea plant due to excess iron supply. Yogeesha (2005) observed no influence of ferrous on magnesium content in the petiole of grape plant. There was decrease in magnesium content in sorgham (Singh and Yadav, 1980) and rice plants (Venkatasubrahmanyam and Mehta, 1975) by the application of excess ferrous. In the roots as well as stem of the plants studied the amount of magnesium was decreased by various concentration of ferrous. Ward et al. (2008) explained that ferrous toxicity is the cause of inhibition of the growth of roots.
4.4.6.4 Effect of nickel on magnesium content

Nickel at higher concentration has toxic effect on plants (Gonnelli et al., 2001; Rao and Sresty, 2000). It was toxic to Phaseolus vulgaris grown with 1 to 2 µg Ni/l in nutrient solution but had no effect on the content of magnesium (Piccini and Malavolta, 1992). Kopittke et al. (2007) found an increase in magnesium content in root and decrease in stem of Vigna unguiculata by increasing nickel concentration. Similar changes in magnesium content were observed in the roots and stems of Glycine max, Vigna unguiculata and Vigna aconitifolia by nickel treatment in the present work. These results were in general agreement with those reported for nickel toxicity in the white clover, ryegrass and cabbage (Yang et al., 1996), tomato (Palacios et al., 1998) and fenugreek (Parida et al., 2003).

4.4.6.5 Effect of manganese on magnesium content

The experimental work to investigate the role of manganese on magnesium in plant organs is scanty. According to Orhue and Nwaoguala (2010) manganese treatment has no pronounced influence on the soil chemical properties but has impact on mineral nutrient content and uptake by plants. Their green house and field studies have shown that the content of various nutrients viz., calcium, manganese, ferrous, copper increases in the stem of Tefairia occidentalis by elevated level of manganese. In the roots of sugar maple seedlings content of all nutrients decreased with increase in manganese concentration (McQuattie and Schier, 2000). In the roots and stems of experimental plants in this work also the decrease of magnesium was
observed by higher manganese concentration in the treatment. According to Abou et al. (2002) manganese toxicity is intensified when other available elements such as calcium, potassium, ferrous and silicon are in a low quantity.

4.4.6.6 Effect of zinc on magnesium content

According to Cvetanovska and Spasenoski (2001) in *Lycopersicon esculantum* increased zinc concentrations in nutrient media of experiments caused the most increasing of micronutrient contents in root than in stem and the little in leaves. In the roots and stem of investigated plants magnesium content was decreased by increasing zinc concentration in the treatment. The study of Wang et al. (2009) on rapeseed seedlings also reported decrease in magnesium content by higher concentration of zinc. They noticed more decrease in root than leaves. Magnesium content was decreased with increased zinc accumulation in ryegrass leaves (Bonnet et al., 2000). Stoyanova and Doncheva (2002) found that magnesium decreased in root under zinc stress. Bernhard et al. (2005) showed that magnesium content decreased in *Agrostis stolonifera* shoots with increased zinc exposure. These findings indicated that excess zinc might suppress the uptake of these elements due to the competition among metal elements (Sinha et al., 2006).

4.5 Metal content in plant organs

4.5.1 Copper uptake
This study has shown that copper contents in the roots and stems of Glycine max, Vigna unguiculata and Vigna aconitifolia were increased by copper treatment.

Kopittke and Menzies (2006) have also found similar increases in the roots and stem of Vigna unguiculata by copper application. There are several other plants where an increase in copper content was observed by copper treatment. They are Vigna radiata (Manivasagaperumal et al., 2011b), Alyssum montannum (Ouzounidou, 1994), Zea mays (Mocquot et al., 1996), Glaucium flavum (Cambrolle et al., 2011), Brassia juncea (Cu, 2008), Origanum vulgare subsp. hirtum (Panou-Filotheou et al., 2006) and Elsholtzia splendens (Houng - Yan et al., 2005).

In the present study more increase in copper content was found in root than in stem. Kopittke and Menzies (2006) reported approximately 10-fold greater copper content in the roots than the shoots of Vigna unguiculata. Houng-Yan et al. (2005) also reported more accumulation of copper in roots than stem and leaves after copper application. Studying the effect of copper on the accumulation of copper in banana plantlets Nassar (2004) found an increase in copper levels in both shoots and roots similar to the findings of Lidon et al. (1993) and Moya et al. (1993). The increase was more pronounced in roots suggesting an immobilization of copper from root to shoot (Romeu-Moreno and Mas, 1999). This immobilizing mechanism resulted in high content of copper in root, which has a stronger effect on this organ than on others (Llorens et al., 2000).
4.5.2 Chromium uptake

A number of studies were made to investigate the chemistry of chromium in soil and its uptake by plants (Desmet et al., 1975; Cary et al., 1977a, 1977b; Lahouti and Peterson, 1979; Ramachandran et al., 1980; Samantary and Das, 1997). Cary et al. (1977a, 1977b) and Lahouti and Peterson (1979) reported that chromium (VI) was taken up by plants because of its mobile nature in soil while chromium (III) was not. Yongpisansang et al. (2005) explained that chromium accumulation increased slightly as the supply level increased. In the roots and stem of investigated plants slight increase in chromium content was noticed under the influence of lower chromium concentration. More increase was in roots than stems. Sankar et al. (2007) also found more accumulation of chromium in root than shoot of soya bean and water lettuce and explained that this may be due to the fact that the chromium can be slowly transported from root to shoot. There are two explanations about the slow transport of chromium to shoot. Sankar et al. (2007) suggested that it is due to sequesterization of the most chromium in the vacuoles of the root cells to render it non-toxic which may be a natural toxicity response of the plant. Sigel (1973) pointed out that chromium (III) is formed due to the reduction and forms complexes with COOH groups in the roots thus preventing it from passing into shoot system.

4.5.3 Ferrous uptake

Certain workers studied various aspects about ferrous uptake in the plants (Kim and Guerinot, 2007; Hell and Stephan, 2003; Vert et
al., 2002; Batty and Younger, 2003; Achakzai, 2003). Brown et al. (1961) have found that roots of chlorotic *Glycine max* develop the capacity to reduce and absorb ferric more rapidly than the roots of green plants. In the roots and stems of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* ferrous content was increased by increasing the ferrous concentration in the treatment. Batty and Younger (2003) studied the effects of external iron concentrations upon the uptake of ferrous by the seedlings of *Phragmites australis* and showed that ferrous uptake by seedlings did increase with increasing ferrous supply and suggested that there is not a clear relationship between uptake of ferrous and concentration of ferrous in plant organs.

According to Sliman (1990) excess zinc has adverse effect on ferrous uptake and translocation in soya bean. Lingle *et al.* (1963) also observed similar effect of zinc and suggested that zinc may be acting as a metabolic poison to some key essential to ferrous absorption. Wang *et al.* (2009) found decrease in ferrous, copper, magnesium and manganese in the roots and leaves of *Brassica nepus* treated with zinc. They reported more decrease in roots than leaves. Ambler *et al.* (1970) found that zinc interfered with ferrous translocation from roots to above ground parts of soya bean plants. These findings indicated that excess zinc might suppress the uptake of ferrous and other elements due to the competition among metal elements (Sinha *et al*., 2006).

### 4.5.4 Nickel uptake

According to Khan and Khan (2010) nickel concentration was increased in the various organs of chick pea by increasing application
of nickel solution. Highest concentration of nickel was recorded in the leaves. A strong linear relationship was recorded with the concentration of nickel applied and its accumulation in the leaves by them. The concentration of nickel in the roots was less than leaves. Lowest concentration was recorded in stem. Maheshwari and Dubey (2009) noticed increase in nickel content in the seedlings of rice with increasing levels of nickel in the growth medium. Concentration of absorbed nickel was greater in roots than in shoots. Lin and Kao (2005) observed increased nickel content in root by the effect of increasing nickel concentration on rice seedlings. They noticed that this had no effect on shoot growth and nickel content in shoot. In Glycine max, Vigna unguiculata and Vigna aconitifolia as the nickel concentration increased in the treatment, nickel content was also increased in roots and stems. The increase in root nickel was higher than that observed in stem nickel. These results were similar to the findings of Kopittke et al. (2007) on Vigna unguiculata.

Singh and Pandey (2011) reported more accumulation of nickel in roots than leaves in Pistia stratiotes. According to them the tissue concentration of nickel in both leaves and roots was dose dependent. The maximum nickel accumulation was observed in the roots than leaves at 10.0 ppm nickel - exposure, which showed low translocation of nickel towards aerial parts of plants. The rate of accumulation of nickel by plants is dependent on its concentration in soil solution, and that the transfer of nickel from root to shoot is regulated by the root (Cataldo et al., 1978). The roots of plants act as a barrier against heavy metal translocation possibly as a result of potential tolerance mechanism (Ernst et al., 1992). Toxic metals are typically sequestered
in roots with limited translocation of such metals to shoots (Rao and Sresty, 2000; Shah et al., 2001; Verma and Dubey, 2003; Parida et al., 2003; Sharma and Dubey, 2007). The prominent sequestration of nickel in root like many other heavy metals, suggests that certain mechanisms exist in roots that immobilize and sequestrate nickel thereby preventing its translocation to shoots (Cataldo et al., 1978).

4.5.5 Manganese uptake

Several workers in their experimental studies found that manganese treatment results the increase in manganese content in plant organs. This increase was observed in shoots of *Oryza sativa* (Lidon and Teixeira, 2000) and *Brassica napus* (Moroni et al., 2003), leaves of *Hordeum vulgare* (Demirevska-Kepova et al., 2004), *Vigna unguiculata* (Fuhrs et al., 2008, 2009) and *Populus cathayana* (Lei et al., 2007); and root, stem and leaves of *Citrus grandis* (Qing et al., 2010). In few plants more manganese content was found in leaves than root under the influence of manganese. They were *Glycine max* (Lavres J. et al., 2009), *Acer saccharum* (McQuattie and Schier, 2000) and *Oriza sativa* cv. Safari (Lidon, 2001).

In the investigated plants more increase in manganese content was observed in roots than stems by increasing manganese concentration in the treatment. Similar higher accumulation of manganese content was observed in the roots of *Lolium perenne* (Rosas et al., 2007). Farasova and Beinrohr (1998) found more accumulation of manganese in shoot of *Sinapis alba*. The distribution of manganese excess in both roots and shoots is dependent on plant
species and genotype (Millaleo et al., 2010). Early research associated manganese tolerance in some plants with a greater retention of manganese excess in roots, as mentioned by Andrew and Hegarty (1969) in regard to tropical and temperate legume species. The root retention of heavy metals has been attributed to the formation of metal complexes in roots (Foy et al., 1978). Metals with high electro-negativity accumulate in roots in larger amounts than metals with low electro-negativity (Millaleo et al., 2010).

4.5.6 Zinc uptake

Ambler et al. (1970) studied the growth of soya bean in nutrient solution and found that the uptake of zinc into roots was fairly rapid, although the upward transport of zinc was slow. Plants removed approximately 80 % of the original applied zinc from the nutrient solution in 48 hours. Sliman (1990) reported increase in zinc content in the roots and tops by increasing zinc level in the nutrient solution in two varieties of soya bean. In the current work also increase in zinc content was observed by increasing zinc concentration in the treatment. Zinc content was higher in roots compared to stem. Zinc concentration was increased in roots and leaves of Brassica napus with increased zinc supply (Wang et al., 2009). Zinc concentration also increased in leaves and roots of pea plants under zinc stress (Stoyanova and Doncheva, 2002). Jiang and Wang (2008) found increased zinc in roots and leaves of Phragmites australis and that zinc content in roots were 10 times higher than in leaves. They suggested that zinc appeared to accumulate preferentially in roots relative to upper plant parts.
4.6 *In Vitro* heavy metal removal by *Pseudomonas* species

Heavy metals are groups of pollutants, which are not biodegradable and tend to accumulate in living organisms (Kobya et al., 2005). These metals have the property of bio-magnification and accumulate in the food chain from unspecific compounds inside the cells causing toxicity at cellular level (Gupta and Mohapatra, 2003; Rajendran et al., 2003).

Conventional techniques aimed at removal of heavy metals usually include chemical precipitation (USEPA, 2000), ion exchange (Riley and Taylor, 1968), some adsorption process (Bhattacharyya and Venkobachar, 1984), membrane process (Kapoor and Viraraghavan, 1995), crystallization, and electrochemical treatment (Schiewer and Volesky, 1995; Wilde and Benemann, 1993). Conventional techniques for removing dissolved heavy metals include chemical precipitation, carbon adsorption, electrolytic recovery, ion-exchange, chelation and solvent extraction or liquid membrane separation (Vasudevan et al., 2003; Lodeiro et al., 2005; Liu et al., 2006; Sharma et al., 2006; Samarghandi et al., 2007; Malakootian et al., 2009) all exhibit several disadvantages, such as high cost, incomplete removal, low selectivity, high energy consumption (Panjeshahi and Ataei, 2008) and generation of toxic slurries that are difficult to be eliminated (Celaya et al., 2000; Okafor and Opuene, 2007).

Biological process for removal of metal ions from solution can be divided into three general categories: (i) biosorption (adsorption) of metal ions onto the surfaces of a microorganism, (ii) intracellular
uptake of metal ions, and (iii) chemical transformation of metal ions by microorganisms. The latter two processes require living organisms (Veglio and Beolchini, 1997; Corder and Reeves, 1994; Darnell et al., 1986). The microorganisms respond to heavy metals by several processes; including transport across the cell membrane, biosorption to the cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation-reduction reactions (Rai et al., 1981; Macaskie and Dean, 1989; Huang et al., 1990; Avery and Tobin, 1993; Brady et al., 1994; Veglio et al., 1997).

Biosorption is either metabolism independent, such as physical or chemical sorption onto the microbial cell walls, or metabolism associated, such as transport, internal compartmentalization and extracellular precipitation by metabolites. In addition, an important aspect of biosorption is that it can be carried out either with metabolically active or inactive cells (Kurek et al., 1982; Brierley, 1990; Volesky, 1994; Chen et al., 2000).

4.6.1 Residual metal concentration

Silva et al. (2009) reported that Pseudomonas aeruginosa AT18 removed 95 % of the copper ions with an uptake capacity of 86.95 mg of copper/gm of biomass. In the present study also 95 % copper removal was observed.

Parameswari et al. (2009) studied the bioaccumulation of chromium and reported 93 % of chromium accumulation by Pseudomonas and only 7 % of chromium was left in residual solution.
In this work 11% of chromium was accumulated in *Pseudomonas* cells and 89% of chromium was found in the residual metal solution.

Langley and Beveridge (1999) studied *Pseudomonas aeruginosa* PA01 biofilms (attached to Sepharose surfaces) subjected to dissolved iron (III) and reported that most iron was removed from solution within 25 hours. Similar to these results in the present investigation also 95% ferrous removal was observed within 330 minutes of contact time.

The removal of nickel from metal solution by *Pseudomonas* was investigated by few workers. According to Kumar *et al.* (2010) *Pseudomonas* can remove 65% of nickel. Pandian and Mahendradas (2011) reported 86% removal of nickel by *Pseudomonas aeruginosa*. In this work also 60% nickel removal was observed after 330 minutes of experiment.

Present study showed that about 67% of manganese was removed by *Pseudomonas* from the metal solution. This was in accordance with the results of Silva *et al.* (2009).

Certain workers carried out investigations on zinc removal by *Pseudomonas*. Ahemad and Malik (2011) reported 29 mg/l zinc uptake by *Pseudomonas* species from 0.4 to 1.6 mM concentrations. Shakibaie *et al.* (2004) and XinCai *et al.* (2006) suggested that *Pseudomonas* can remove zinc up to 38.4% from the metal solution. The results obtained in the present work support these workers.
4.6.2 Heavy metal adsorption on cells of *Pseudomonas* species

Biosorption is a passive process of metal uptake using biomass (Volesky and Holan, 1995). It is a non-directed physico-chemical complexation reaction between dissolved metals and charged cellular components, which involves sorption and/or complexing of metals to living or dead cells (Kamaludeen *et al*., 2003).

Biosorption is responsible for metal concentration by non-living biomass owing to the absence of metabolic activity necessary for intracellular metal accumulation (Godlewska-Zyłkiewicz, 2006). The advantage of biosorption process is that desorption of the metal is possible if desired. Further, it can be used for the metabolic processes of different microorganisms like bacteria, algae, fungi and plants.

The feasibility of using inert microorganisms as potential biosorbents, has been studied widely in last years (Tsezos *et al*., 1995; Hu *et al*., 1996; Singleton and Simmons, 1996; Hu and Reeves, 1997; Riordan *et al*., 1997), as the process offers several advantages like the low operating cost, minimization of the volume of disposable chemical and/or biological sludge, high detoxifying efficiency of very dilute effluents and no nutrient requirement (Kratochvil and Volesky, 1998).

A wide variety of fungi, algae, and bacteria are now under study or are already in use as biosorbents for heavy metal remediation (Gadd, 1992; Macaskie and Dean, 1989; Volesky and Holan, 1995). Algae, fungi, yeast, and bacteria can remove heavy metals from aqueous solutions by binding the cationic metals onto negatively charged
functional groups distributed on their cell walls, such as carboxyl and phosphoryl groups (Ehrlich, 1997; Gadd, 1990).

4.6.2.1 Adsorption by bacteria

Bacterial cell walls are negatively charged under circumneutral pH conditions and they can passively adsorb positively charged metal ions. This electrostatic complexation is followed by further deposition of dissolved metals and then development of biominerals at the cell surfaces (Beveridge and Murray, 1980; Doyle et al., 1980; Beveridge et al., 1982; Ferris and Beveridge, 1986; Ferris, 1989).

*Pseudomonas* species is Gram negative organism. Gram negative cell walls are more complex than Gram positive cell walls, both structurally and chemically. It has higher amount of lipopolysaccharides on its cell membrane with several membrane proteins (Lins et al., 1999).

The heavy metal ions tend to bind with the different functional groups like phosphoryl, carboxyl, carbonyl, sulphydryl and hydroxyl groups of lipopolysaccharides and form organometallic compounds. The metal ions act as lewis acid (an electron acceptor) and the ligand acts as lewis base (an electron donor). A monomer of lipopolysaccharide can attach to one or more metal ions (Voet and Voet, 2004). This attachment is temporary as physico-chemical agents like heat, acetone or alcohol treatment dissolves lipopolysaccharide molecules from the Gram negative cell membranes of *Psedomonas* species and hence patches are created on cell surface. However, the
patch formation is not uniform and hence there will not be total cell rupturing. Because of this the materials present in the cytoplasm are retained in the cell (Voet and Voet, 2004; Lehninger et al., 2005; Stryer et al., 2002; Gram, 1884).

4.6.2.1.1 Copper adsorption

Certain bacterial species have been reported for copper biosorption. They are *Enterobacter* species (Lu et al., 2006), *Bacillus cereus*, *Bacillus sphaericus* and *Bacillus subtilis* (Da Costa and Duta, 2001), *Bacillus sphaericus* (Tuzen et al., 2007), *Desulfovibrio desulfuricans* (Chang et al., 1997), *Pseudomonas putida* (Pardo et al., 2003; Wong et al., 1993; Sar et al., 1999; Chen et al., 2005), *Pseudomonas* species (Shetty and Rajkumar, 2009) *Pseudomonas aeruginosa* (Silva et al., 2009). Few other workers were also reported biosorption of copper (Andrezza et al., 2010 and Parungao et al., 2007). In the present study biosorption of copper on cell walls of *Pseudomonas* species have been recorded. This adsorption was due to copper (II) in the form of the divalent cation so higher protonation of the cell wall components decreased the metal uptake of the biomass, whereas on increasing pH, the negative charge density increases, due to the deprotonation of the metal binding sites (Shetty and Rajkumar, 2009).

4.6.2.1.2 Chromium adsorption

A number of chromium adsorbing microorganisms have been reported including *Pseudomonas* species (Mondaca et al., 1998),
Microbacterium (Pattanapipipaisal et al., 2001), Desulfovibrio (Michel et al., 2001), Enterobacter species (Wang et al., 1990), Escherichia coli (Shen and Wang, 1993), Bacillus species (Campos et al., 1995) and several other bacterial isolates (Holman et al., 1999). The bacterial biomass of Bacillus coagulans can be used to bind dissolved chromium (VI) and for the biosorption of chromium (VI) in different matrices (Srinath et al., 2003; Kratochvil et al., 1998; Gadd and White, 1993; Hu and Reeves, 1997).

Numerous bacterial genera viz., Pseudomonas, Bacillus, Enterobacter, Deinococcus, Desulfovibrio, Rhodobacter, Shewanella, Microbacterium and Escherichia were reported for chromium adsorption under both aerobic and anaerobic conditions (Tuzen et al., 2007; Wong et al., 1993; Sar et al., 1998; Chang et al., 1997). Desulfovibrio vulgaris was used for chromium (VI) reducing activity by its soluble and membrane fractions (Lovely and Phillips, 1994). In the present work chromium adsorption was observed by Pseudomonas species. Similar adsorption of chromium was reported by Singh et al. (2005) and explained that there was reduction of chromium (VI) in to insoluble chromium (III) on the cell surface of Pseudomonas species causing biosorption. He also suggested that the formation of chromium (III) also provides protection to Pseudomonas cells from the toxicity of chromium (VI).

4.6.2.1.3 Ferrous adsorption

The studies on the adsorption of ferrous by bacteria are scanty. Tuzen et al. (2007) reported Bacillus sphaericus as iron biosorbent.
Lee and Beveridge (2001) also reported adsorption of iron by *Pseudomonas aeruginosa* cells. Similar to their work ferrous adsorption was noticed in the present investigation.

**4.6.2.1.4 Nickel adsorption**

The major sources of nickel contamination to water comes from industrial process such as electroplating, mine producing, metal finishing and porcelain enameling (Hussein *et al*., 2004; Xiaocun *et al*., 2011). Therefore several workers contributed on the removal of nickel from waste water or aqueous solution using *Pseudomonas* and other bacteria (Zaidi and Musarrat, 2004; Rodriguez *et al*., 2006; Asthana *et al*., 1995). Kaewchai and Prasertsan (2002) studied nickel and cadmium adsorption by dried cells of *Enterobactor agglomerans* SM38 and found that at optimum pH their removal reached 25.2 % and 32 % respectively. The experimental studies of Ansari and Malik (2007) showed that the biosorption of nickel was increased from 6.96 to 55.31 mg/g of cells at a concentration ranging from 50 to 400 µg/ml after two hours of incubation in a single metal solution. A further increase in incubation time has no significant effect on the biosorption of metals. Yanli *et al*. (2010) studied biosorption characteristics of *Pseudomonas fluorescens* and suggested that *Pseudomonas fluorescens* biomass may be used as an inexpensive, effective and easily cultivable biosorbent for the removal of nickel (II) ions from environmental and industrial waste water.

According to Sar *et al*. (1999) *Pseudomonas aeruginosa* cells showed significant sorption of nickel (265 mg/g) and explained that
*Pseudomonas aeruginosa* biomass can be used as an efficient nickel removal system. The results obtained in this study are in favour of these investigators suggesting utility of *Pseudomonas* species for nickel removal. The other investigators also support the use of *Pseudomonas* cells for nickel removal. Narasimhulu and Setty (2012) from their investigation concluded that nickel biosorption was between 36 to 90 % by *Pseudomonas* species.

Gialamouidis *et al.* (2009) reported maximum uptake capacity of nickel 508 mg/g for *Pseudomonas* species and explained that 87 % nickel can be removed from *Pseudomonas* and suggested that nickel can be easily and quantitatively recovered from biomass. Xiaocun *et al.* (2011) studied nickel adsorption from synthetic waste water using *Pseudomonas alcaligenes* biomass (PA-2). According to them the maximum nickel adsorption capacity of *Pseudomonas alcaligenes* (PA-2) was 88.23 mg/g PA-2 at pH 5.0. Their study pointed to the potential to the new use *Pseudomonas alcaligenes* biomass as an effective biosorbent for the removal of nickel from environmental and industrial waste water.

4.6.2.1.5 Manganese adsorption

The study on biosorption of manganese by bacteria is scanty. Troshanpy (1969) contributed on the ability of microorganisms of reduction of iron and manganese from ore-bearing lakes.

Silva *et al.* (2009) studied the sorption of chromium, copper, manganese and zinc by *Pseudomonas aeruginosa* AT18 from a site
contaminated with petroleum and heavy metals. Their results showed that *Pseudomonas aeruginosa* AT18 has the capacity for biosorption of chromium, copper and zinc in solutions although its capacity for the sorption of manganese was low (22.39 mg Mn$^{2+}$/g of biomass) in comparison to chromium, copper and zinc. In the present investigation also lower (0.40 ppm) manganese adsorption by *Pseudomonas* cells was found after 330 minutes of contact time. Gialamouidis *et al.* (2010) contributed on the biosorption of manganese by *Pseudomonas* species, *Staphylococcus xylosus* and *Blakeslea trispora* cells and suggested that these microorganisms can act as biosorbents for manganese.

4.6.2.1.6 Zinc adsorption

Celaya *et al.* (2000) studied the capacity of *Thiobacillus ferroxidans* for zinc adsorption. Tuzen *et al.* (2008) reported biosorption of zinc (II) by *Bacillus thuringiensis*.

Silva *et al.* (2009) investigated *Pseudomonas aeruginosa* AT18 biomass for zinc uptake. They found that pH 7.0 was optimum and at this pH 87.7 % of the metal removed with a maximum biosorption capacity of 77.5 mg of zinc/gm of *Pseudomonas aeruginosa* AT18 biomass. Vullo *et al.* (2008) concluded that the optimum pH for zinc (II) biosorption was achieved at pH 7.5 by *Pseudomonas veronii* 2E. The effect of pH was also investigated by Satiroglu *et al.* (2001). In the present investigation adsorption of zinc by cell wall of *Pseudomonas* species was recorded which was in accordance with the other investigations. Bhagat and Srivastava (1993) concluded that in
Pseudomonas stutzeri RS34, the zinc resistance mechanism involves sequestration of the metal ion in the cell wall. Chen et al. (2005) noticed longer biosorption time for zinc on Pseudomonas putida which was followed by constant time.

4.6.3 Heavy metal accumulation in cells of Pseudomonas species

Microbial biomass can be used to decontaminate metal bearing wastewaters as well as to concentrate metals. The nature of biological surfaces is such that different functional groups form complexes with metal ions, resulting in chemical complex as an uptake mechanism (Huang et al., 1990). Accumulation of metals in microbial biomass also proceeds by different processes such as uptake by transport, entrapment in extracellular capsules, precipitation, and oxidation-reduction reactions (Brady and Duncan, 1994). Heavy metal resistance in microorganisms can occur by a variety of mechanisms, including physical sequestration, exclusion and/or efflux, reduced uptake, detoxification, and synthesis of binding proteins (Mago and Srivastava, 1994).

4.6.3.1 Copper accumulation

Saxema et al. (2001) studied removal of copper ion in wastewater by Pseudomonas putida S4 and obtained more than 80 % of copper ion removal present in the wastewater. Similar results were obtained in the present study where 93 % copper was removed. Savvidis et al. (2003) also studied the bioaccumulation of copper ions in the Pseudomonas isolated from the environment contaminated with
heavy metals. Koedam et al. (1994) found highly fluorescent isolates belong to *Pseudomonas* species have high ability to resist and accumulate one or more of the metal mixture chromium (VI), copper (II), cadmium (II), and nickel (II).

Some other studies have been conducted on accumulation of copper in *Pseudomonas aeruginosa* (Philip et al., 1998; Chang et al., 1997; Sar et al., 1999), *Pseudomonas putida* (Wong et al., 1993; Pardo et al., 2003) and *Pseudomonas stutzeri* (Mattuschka et al., 1994). They suggested that various species of *Pseudomonas* are useful for the removal of copper by bioaccumulation. Chen et al. (2005) investigated the characteristics of living and nonliving cells of *Pseudomonas putida* for removal of copper (II) and zinc (II) from aqueous solution. Few other studies were also suggested *Pseudomonas* species for copper removal by bioaccumulation (Hassen et al., 1998; Sar et al., 1999; Chen et al., 2005; Shetty and Rajkumar, 2009; Zaki and Farag, 2010).

### 4.6.3.2 Chromium accumulation

Several bacterial strains *Desulfovibrio vulgaris, Enterobacter cloacae* HO-1, *Alcaligenes eutrophus, Dinococcus radiodurans* R1, *Desulfomicrobium norvegicum* (Michel et al., 2003), *Bacillus* (Camargo et al., 2003), *Bacillus coagulans* (Srinath et al., 2003, Kratochvil et al., 1998; Gadd and White, 1993; Hu and Reeves, 1997), *Shewanella* (Guha et al., 2001; Viamajala et al., 2003, 2004; Middleton et al., 2003), *Desulfovibrio* (Chardin et al., 2002), *Escherichia coli* (Puzon et al., 2002), *Alcaligenes* (Peitzsch et al., 1998), *Enterobacter cloacae* (Ohtake et al., 1990), *Escherichia coli,*
Pseudomonas, Shewanella oneidensis and Aeromonas species (Wang, 2000), Enterobacter cloacae (anaerobic) (Wang et al., 1989), Pseudomonas ambigua (aerobic) (Suzuki et al., 1992), Pseudomonas putida (aerobic) (Ishibashi et al., 1990), Bacillus species (aerobic and anaerobic) (Campos-Garcia et al., 1997) Pseudomonas fluorescens (aerobic and anaerobic) (Bopp and Ehrlich, 1988) and Escherichia coli (aerobic and anaerobic) (Shen and Wang, 1993) have been described for their ability to reduce hexavalent chromium into insoluble low valence form chromium (III) both aerobically and aerobically. Parameswari et al. (2009) recorded maximum accumulation 93 % at 25 ppm chromium concentration in Pseudomonas fluorescens. They further reported that within 18 hour Pseudomonas fluorescens showed 100 % accumulation at pH 6.5. Tarangini et al. (2009) reported 0.78 mg/gm chromium uptake by Pseudomonas aeruginosa at pH 3.0 within 20 minute contact time.

Several other workers investigated the use of various species of Pseudomonas viz., Pseudomonas ambigua (Suzuki et al., 1992), Pseudomonas putida (Ishibashi et al., 1990) and Pseudomonas fluorescens (Bopp and Ehrlich, 1988) for the removal of chromium by bioaccumulation. In this investigation 8.66 mg/l chromium accumulation in Pseudomonas cells was observed at pH 6.0 supporting the work of above investigators.

4.6.3.3 Ferrous accumulation

Troshanpv (1969) reported that some of bacterial isolates could reduce the transition metal iron as ferrous (III) aerobically, although he
found that bacterial iron reduction was frequently more sensitive to oxygen than manganese (IV) reduction. Langley and Beveridge, (1999) studied *Pseudomonas aeruginosa* PAO1 biofilm for iron uptake and suggested that biofilm conditions are more conducive to metal deposition, especially with dissolved ferrous. Ottow (1970) concluded that increased soluble iron (II) in biofilms was likely due to metabolism during which dissolved iron (III) serves as an electron acceptor. Although *Pseudomonas aeruginosa* is not considered to be a dissimilatory iron reducer, it can still reduce iron (III) nonspecifically and can use iron (III) during respiration instead of oxygen. Larger amount of iron (475 ppm after 330 minutes of contact time) was removed from experimental solution in the current study which is in accordance with the results obtained by Langley and Beveridge, (1999).

4.6.3.4 Nickel accumulation

Nickel is an important environmental inorganic pollutant, with allowed levels under 0.04 mg/l in human consumption water (Rodriguez *et al.*, 2006). The significance of *Pseudomonas* for nickel removal from contaminated materials was explained by several workers (Sar *et al.*, 1998, 1999; Wang *et al.*, 2003; Rodriguez *et al.*, 2006; Yanli *et al.*, 2001). Patel *et al.* (2006) studied nickel accumulation in nickel resistant bacterial isolates from industrial effluent. The isolate was closely related to *Pseudomonas fragi* in which maximum accumulation of nickel was 0.59 mg/g (dry weight of bacterial cells). Abdel-Monem *et al.* (2010) investigated bioaccumulation of nickel by two bacterial species, *Bacillus subtilis*
117S and *Pseudomonas cepacea* 120S. The maximum uptake of nickel was 234.4 mM/ml by *Pseudomonas cepacea* 120S (living and dead biomass). According to them nickel removal increased significantly during contact time from 1 to 8 hours and then remained constant until 24 hours.

In this study 198.56 ppm nickel accumulation in *Pseudomonas* cells was found when it was treated with 500 ppm nickel after 330 minutes of contact time. Results obtained in the present study indicated that *Pseudomonas* cells may be useful in removal of nickel up to certain extent from waste water. Wang *et al.* (2003) also have suggested that *Pseudomonas putida* can be utilized for nickel and copper removal from electroplating waste water.

Zaidi and Nusarrat (2004) investigated that live cells of *Bacillus* sp. SJ-101 exhibited both the extracellular and intracellular accumulation of multiple heavy metals in order as nickel > zinc > lead > copper. Rodriguez *et al.* (2006) suggested that *Pseudomonas* has a lower number of wall binding sites to interact with nickel ions, but has a stronger binding affinity to this metal. According to Zaidi and Musarrat (2004) the enhanced biosorption and bioaccumulation capacity of a bacterial strain depends upon the anionic nature of its surface. Because of this it could be exploited as a valuable bioinoculant for nickel bioremediation.

### 4.6.3.5 Manganese accumulation

The research work pertaining the removal of manganese by uptake in bacteria is less. Pavani and Gayathramma (2011) studied
bioaccumulation of manganese by *Bacillus circulans* from aqueous solutions. They reported maximum uptake of manganese in the cells grown with 4.0 mM concentration and further increase in the metal concentration was found to decrease in uptake. Hakeem and Bhatnagar (2010) contributed on heavy metal reduction of pulp and paper effluent by *Staphylococcus, Streptococcus* and *Pseudomonas*. They studied the bioaccumulation of manganese, copper, cadmium and mercury in these bacteria and observed better remediation of cadmium, copper, manganese and mercury with *Pseudomonas* species when pH was in the range of 7-8.5. The investigation of Lopez *et al.* (2000) suggested that heavy metals accumulation occur very rapidly in *Pseudomonas fluorescens* 4F39. In the present study also higher accumulation of manganese (339 ppm after 330 minutes of contact time) was observed in *Pseudomonas* cells. From this it is suggested that *Pseudomonas* cells can be useful in remediation of manganese.

### 4.6.3.6 Zinc accumulation

The studies on zinc accumulation in bacterial cells is scanty. Sodeberg *et al.* (1990) explained that Gram positive bacteria were the most susceptible bacterial group to zinc ion but Gram negative aerobic bacteria like *Pseudomonas* were usually not inhibited even at the highest concentration. McEldowney (1994) carried out research on cadmium and zinc attachment and detachment interactions of *Pseudomonas fluorescens* H2 with glass. He observed more accumulation of cadmium and zinc in *Pseudomonas fluorescens* H2 when the concentration of these metals was increased in the culture medium. Joo *et al.* (2010) studied the zinc uptake by *Pseudomonas*
*aeruginosa* and *Bacillus cereus*. They reported faster rate of zinc accumulation in the initial stage of experiments. In this work higher zinc accumulation (484 ppm after 330 minutes of contact time) in *Pseudomonas* cells was observed.