

CHAPTER 5

DISCUSSION

5.1 Physiological responses of plants under Stress

5.1.1 Relative Water Content (RWC) and proline accumulation

In this study the water potential of plants was not measured because of some practical problems. Instead, the RWC of the plants was determined both under normal and stress conditions. Meesilpa (1994) pointed out that either water potential or RWC may be used to indicate the water status of the plants. Minute water stress was shown by the -2 to -5 bars water potential or 90 to 92 % RWC. While the -12 to -15 bars water potential or 80 to 90 % RWC in plant tissue indicated moderate water deficit. The water potential -15 bars or lesser or RWC less than 80 % revealed severe stress. However, wilting leaves would not be observed as long as the plants maintained their RWC over 83 % . The RWCs ranging from 84.15 to 95.55 % were found in mulberries received regular irrigation, whereas all control plants in this study had more than 88 % RWC. This might be due to the hydroponically growing condition. Responding to the Cd stress, RWC of the plants were reduced. This reduction was markedly observed in the 20 ppm Cd treatment, but the minimum value was never lower than 60 % . It is possible to generalized that decreasing RWC resulted from the reduction of water potential of growing media in which the solute was added. However, only half-strength Hoagland's solution was used in this study. The change in osmolarity of the solution might have less effect on plant water status compared to plants growing in full strength solution. On the contrary, the symptoms of the plants observed during preliminary experiment have clearly shown that plants suffered the Cd stress when growing in half strength solution rather than the full strength one. Study with solution cultures, in which water ability was not limiting, showed that disorders in the water status of Cd treated plants does not depend on the water content of the substrate and that plant factors play an important role (Marchiol et al., 1996). Lettuce exposed to 0, 0.01, 0.1, 1 and 100 μM Cd for nine days showed a strongly decrease in RWC with high Cd concentrations. In spite of higher stomatal resistance,

Cd treated plants had lower water content than control plants. This indicated the decrease in water flux to the leaves (Costa and Morel, 1994). Kastori, Petrovic and Petrovic (1992) also indicated that heavy metals affect root growth, decreasing the formation of root hairs and causing structural changes which lead to increased resistance to water flow into and within roots.

Proline is accumulated by many plants under various stresses including heavy metal stress. The relationship between water deficit stress and proline accumulation has been extensively studied and well documented. In contrast, the effect of heavy metals on the accumulation of proline is less clear. Nitrate salts of Cd, Co, Zn and Pb raised proline content in seedlings of *Cajanus cajan*, *Vigna mungo* and wheat. It was found that considerable and proportionate increase in proline content rose with the concentration of heavy metals. Therefore, proline accumulation could be used as a marker to test the level of heavy metal pollution, and this accumulation was not related to osmotic adjustment (Saradhi, 1991). Twenty five days old sunflower plants exposed to 1 and 100 μM Pb, Cd, Cu or Zn for forty eight hours and leaf discs incubated in these metal solutions plus 0.5 % glucose for twelve hours showed significant increase in proline content. The author concluded that there was an interaction between water stress and heavy metal toxicity. However, the increased proline concentration in leaf discs incubated in heavy metal solutions showed that proline concentration was increased with the presence of heavy metals even in fully turgid plant cells. Thus, the effect of heavy metals on proline content most probably did not depend on their effect on water relations (Kastori et al., 1992).

In contrast, Costa and Morel (1994) reported the contrary results from the Cd-treated lettuce. Accumulation of amino acid was related to a change in leaf water status. Amino acid metabolism rose in both roots and shoots. The accumulation in root may result from amino acid transport from leaves and/or *de novo* synthesis. Proline was not accumulated in leaves but only in roots, whereas asparagine, methionine and lysine predominated. The authors suggested that Cd did not affect proline synthesis but more probably inhibited proline oxidation. Recent study investigated the effect of Cu, Cd and Zn on the accumulation of free proline in nontolerant and metal-tolerant *Silene vulgaris*. In the nontolerant ecotype these metals induced a massive

accumulation of proline, especially in the leaves. However, in metal-tolerant ecotype the proline concentration in the leaves of control plants was 5 to 6 times higher than in the nontolerant ecotype. The proline in the roots was about the half of that in leaves, and hardly accumulated under metal stress. Increasing the relative humidity by placing the plants under transparent polyethylene raised the leaf water content and greatly reduced metal-induced proline accumulation in the nontolerant ecotype. It also reduced metal accumulation in the leaves, but proline accumulation was absent or strongly reduced even at metal accumulation rates that were associated with a 10-fold increase of proline in uncovered control plants. Although in this 98 % relative humidity experiment, plants were exposed to 30, 60 and 120 μM Cd compared to 3.75, 7.5 and 15 μM Cd of the 75 % relative humidity treatment. For these reasons the authors concluded that proline accumulation under heavy metal exposure was induced by a metal-imposed water deficit stress rather than by a toxic heavy metal accumulation (Schat, Sharma and Vooijs, 1997).

In this study the plant also showed the marked increase in proline level, especially in the 20 ppm Cd treatment. However, *I. aquatica* should be excluded from the other species when considering the proline accumulation. This is because this plant showed insensitive response even under severe Cd stress. Furthermore, decreased RWC by Cd stress raised proline level in the same proportion as the plant faced water deficit stress. The accumulation of proline for *I. aquatica* under Cd stress is almost or entirely caused by the reduction of RWC, not by the effect of metal accumulation. It may be assumed that this plant hardly responds the water stress by accumulating proline. Proline accumulation is not detected in some drought-tolerant species of bryophytes, lichens, pteridophytes and flowering plants when subjected to severe water stress. Therefore, proline accumulation cannot be regarded as an universal response in water-stressed plants (Boonkerd, 1987). *C. barbata*, under metal stress, did not show significant correlation between RWC and proline. This plant showed relatively high RWC throughout the experiment although the wilting leaves was clearly observed. However, there was a strong correlation between RWC and proline level when the water stress had developed.

Significant correlation between RWC and proline content under water stress was also apparent in *B. oleracea* and *E. prostrata*. Furthermore, the RWC and proline content of the plants exposed to high Cd concentration had strong relationship. While Cd content and proline accumulation in the same leaves did not show significant correlation (Appendix D). These findings may lead to the conclusion that proline accumulation is triggered by the metal-induced water stress, not by the metal accumulation in the leaves. However, when considering the RWC which contributed to the accumulation of proline from the water stressed plants it is obvious that proline is rather insensitive to water stress. Marked increase in proline was observed only when plants suffered the severe water stress. It has been suggested that proline accumulation is relatively insensitive to water deficit. Sorghum and soybean did not accumulate proline until they were severely dehydrated to the point of visible wilting, at a water potential of about -20 bars (Levitt, 1980; Paleg and Aspinall, 1981). Extensive accumulation of proline over the level induced by the water stress was manifest in *B. oleracea* and *C. barbata* at day 8 of 20 ppm treatment and *E. prostrata* after transfer to normal nutrient solution for both treatments. This might be the result of an increase in Cd content in the leaves to the extent that it may cause proline accumulation. This finding is consistent with the strong correlation between shoot Cd content and proline rather than RWC.

However, if the shoot metal content is a major contribution to proline accumulation, one should detect high proline level in 5 ppm treatment samples instead. Therefore, it might come to the conclusion that metal-induced water stress is the main cause of proline accumulation. Metal itself when accumulated to a great extent may lead to proline buildup. Once these two factors coincide, the proline accumulation doubles, but the first factor prevails over the latter.

The strong correlation between root Cd content and proline or RWC may reflect the fact that water flux is impeded when roots accumulate toxic metal to some extent and this leads to proline accumulation. After elimination of Cd in culture solution, new roots developed, and plants regained high RWC. Slight proline enhancement compared with controls is due only to the effect of Cd content in leaves. According to Schat et al. (1997), the threshold exposure levels for proline accumulation coincided with the highest no-effect-concentrations for root growth. This

was not evident particularly in the case of *C. barbata* experienced 5 ppm treatment. The severe root damage was clearly observed, but no marked increase in proline content was detected.

5.1.2 Chlorophyll contents

Most of the plants subjected to Cd stress had lower chlorophyll contents. These contents reached the lowest point after the plants responding to the stress for a long time even when Cd in solution was removed. However, *I. aquatica* did not behave like other species. The decrease of chlorophyll contents were maximum at the last day of Cd exposure (20 and 8 days of exposure to 5 and 20 ppm treatment, respectively). In addition, *C. barbata* at day 8 showed similar result. This is because the senescent leaves were included while determining the chlorophyll contents. *I. aquatica* was the most susceptible species under Cd stress. Its leaves turned yellow and dropped, remaining few unhealthy leaves near the tip, whereas *C. barbata*'s leaves died downward from the tip of leaf blade, and immature leaves were also died after this plant subjected to severe Cd stress.

Chlorophyll contents reduction may be derived from Cd which had been applied by numerous ways. Three days old seedlings of *Triticum aestivum* germinated from the seeds which had been soaked in CdCl₂ solution for twelve hours showed a reduction in chlorophyll a and b contents (Kalita, Devi and Bhattacharyya, 1993). Seedlings of *Vigna catjang* and twigs of the aquatic plant *Hydrilla verticillata* treated with 10 µM Cd for six days decreased the chlorophyll a, b and total chlorophyll contents. However, the chlorophyll a/b ratio showed slight increase by the metal treatments. The authors suggested that Cd inhibited chlorophyll biosynthesis at the protochlorophyllide stage by interference with the enzyme protochlorophyllide reductase. In addition, chlorophyll level might also be decreased by enzymatic degradation (Bhattacharyya and Choudhuri, 1994). Moreover, twenty one days old *Vigna radiata* sprayed with 40, 60, 80 and 100 µM CdSO₄ once a week until grain filling was complete decreased both chlorophyll a and b contents with increasing concentrations. While chlorophyllase activity showed an increasing trend (Keshan and Mukherji, 1992).

Boonfahprathan (1983) found the reduction in chlorophyll content in *Brassica chinensis* and rice (*Oryza sativa* L.) exposed to various Cd concentration for nine and fifteen days. Active and total iron reduction was also detected in *B. chinensis*. Cadmium impeded the Fe uptake and translocation by inhibiting root growth. In contrast, at the 40 ppm treatment for rice, active and total iron increased. Therefore, the chlorosis did not result from Fe deficiency, but from Fe toxicity. The symptom of chlorosis was indirectly caused by metal-induced changing in Fe status rather than metal itself.

In contrast, Shaw (1995) reported an increase in chlorophyll contents under Cd stress. This increase might be due to an increase in the number of chloroplasts per cell and/or a decrease in the cells' volume leading to increase in the number of cells per unit weight. However, there were a decrease chlorophyll a to chlorophyll b ratio resulted from the destruction of chlorophyll, preferable of chlorophyll a, as a result of their reaction with singlet oxygen which is produced when the acceptor side of PSII is inhibited.

B. oleracea leaves did not show any symptoms of chlorosis until this plant was transferred to normal nutrient solution and the new leaves developed. In fact, the plant had dark greyish green –metallic like color-when the stress proceeded. *E. prostrata*'s leaves at the 8th day treatment also had dark green appearance. From observation these samples were more likely to have more chlorophyll contents compared with controls. In addition, higher chlorophyll contents might have been detected from the metal stressed plants which had lower RWC because more plant tissues were obtained when the determination was based on the weight unit, like the experiment conducted by Shaw (1995). However, the increase was not detected. This may be due to the lower water content in the samples impeded the chlorophyll extraction by the method described in Chapter 3. The fluctuation of chlorophyll contents in *C. barbata* was also due to practical problem with the extraction. *C. barbata*'s leaves with increasing age have thick and large midrib which were difficult for chlorophyll extraction. Nevertheless, *E. prostrata* at the 11th day of 5 ppm treatment had symptom of chlorosis observed in the new leaves and necrosis developed from the lower leaves. These symptoms proceeded to the end of the treatment. These samples showed distinct difference in chlorophyll contents from the controls.

There is a correlation between shoot Cd and chlorophyll a and a+b contents. This is obvious in *B. oleracea* and *E. prostrata* which have high Cd contents in leaves together with the symptom of chlorosis. To sum up, leaf Cd content other than RWC determines the fate of chlorophyll.

5.1.3 The growth of plants

The number of leaves

The number of leaves of control plants increased with time and reached a maximum when the plant aged. This is because senescence prevails over the development of new leaves. Plants exposed to toxic Cd had lower number of leaves both by limiting development of new leaves and causing senescence of the old ones. These effects were evident in the 20 ppm treatment. Decrease in the number of leaves may be due to the metal burden in the leaves together with water deficit stress induced by the metal. Having been transferred to normal nutrient solution, plants regained high RWC, and new leaves developed. *C. barbata* which accumulated most of the Cd at lower leaves could recover and produce new leaves rapidly. *E. prostrata* which had more ability to translocate Cd to shoots suffered the metal stress. Like *E. prostrata*, *I. aquatica* could translocate Cd to the upper part, but this plant produced new leaves and grew rapidly. This is because the plant alleviates the stress by branching new shoots at the first node from the root and the main shoot gradually died from lower part toward the tip. However, this was not observed in the 20 ppm treatment at which the plant suffered severe stress.

Leaf area

Leaf area is related to the number of leaves. However, intense reduction of leaf area under Cd stress was obvious. This is because leaf area is more affected by decreasing RWC. This is evident when plants facing water deficit stress (Saradadevi, Padmasree and Raghavendra, 1996). It is an adaptive mechanism of plants under such stress (Meesilpa, 1994). Wilting leaves of all plant species was observed especially at 20 ppm treatment. *C. barbata* folded its leaves to

minimize water loss and the growth of new leaves was severely inhibited. *B. oleracea* covered its leaves with silver powder-like substance. Having been transferred to normal nutrient solution, the plants developed new roots, and increased leaf area. Marked expansion and extension of the leaf blade of *C. barbata* were observed. This may be due to the longer life span of *C. barbata*, while other species were nearly fully mature.

Root to Shoot Ratio

Study on birch (*Betula pendula*) exposed to 0 and 2 μM CdCl_2 for six days revealed that root to shoot dry weight ratio was increased. This indicates a change in biomass distribution between roots and shoots. A reduced life span of the roots, due to exposure to toxic metals, may act to increase the allocation of carbohydrates to the root system which, in turn, eventually may reduce shoot growth (Gussarsson et al., 1996).

The increase in root to shoot ratio of plants under drought stress was observed. Shoot growth is dramatically reduced rather than root growth (Meesilpa, 1994). Lower leaf area reduces the use of photosynthates. Consequently, they are translocated to root, thereby encouraging root growth (Taiz and Zeiger, 1991).

Increased root to shoot ratio was also observed in *I. aquatica* and *B. oleracea* under Cd stress. The highest ratio was obtained from the day 8 samples. This indicates a maximum shoot growth inhibition when plants exposed to high concentration of Cd for a maximum period.

In contrast, weeds showed contrary results under metal stress. Lowest root to shoot ratios were found for the longest exposure time (at day 20 and day 8 for 5 and 20 ppm Cd treatment). This reveals severe inhibition of root rather than shoot growth. As for the control plants, high root to shoot ratios were observed. A root system that fills more of the soil volume or is large relative to the shoot can be important in maintaining leaf water status (Meesilpa, 1994).

5.1.4 Cadmium accumulation

Cadmium accumulation in root

A number of authors reported that most of the Cd absorbed by the treated plants was retained in the root system (Costa and Morel, 1994; Gussarsson et al, 1996; Marchiol et al., 1996). Cataldo, Godbold and wildung (1983) stated that at concentration more than 0.5 μM Cd (the concentration used in this study was 44.5 and 177.9 μM or 5 and 20 ppm Cd, respectively), passive processes including adsorption and diffusion began to predominate. That is, higher concentration ($>0.5 \mu\text{M}$ Cd) resulted in a marked increase in the proportion of Cd irreversibly bound to cell wall compared with the metabolically absorbed fraction. The use of 90 μM Cd with excised roots may have resulted in a disruption of metabolic processes due to Cd toxicity.

It is thought that the divalent ions are taken up by a common uptake system and this system is not responsive to ion influx or net uptake, unlike other uptake systems such as phosphate, sulphate and nitrate where carrier synthesis is under the control of feedback mechanisms (Meharg, 1993).

This study reveals that plant root could absorbed Cd to a great extent within two days. Hart et al. (1998) reported that unidirectional Cd^{2+} influx into the wheat root symplast occurred for at least 75 minutes. The rate of accumulation began to decline after two to four hours of uptake. In this study the highest value was obtained from samples at the final day of exposure. The plant root were severely damage, except *B. oleracea* which was capable of developing new roots after removal of Cd. At this stage, *E. prostrata* had at least 50 % of rotten root, but then these plant could also produced new root from the living portion. All species intensely produced new roots from the upper part of stem adjacent to the old roots when transferred to new Cd-free culture solution. However, maximum metal loading capacity of plant roots is not known.

After the plants had been transferred to normal nutrient solution, the root Cd content decreased. This is because the metal may translocate to the shoot or new developing roots which were low in Cd content cause a dilution effect when the Cd in roots were extracted.

Cadmium accumulation in shoot

There is a strong correlation between shoot and root Cd contents. That is, Cd translocation rate is a function of the root content because Cd mainly enters the stele through leakage (Arduini, Godbold and Onnis, 1996). Large variations in root-to-shoot Cd distribution occur among plant species (Hart et al., 1996). Cadmium itself was mainly accumulated by roots and only a small portion was transported to the shoot (Gussarsson et al., 1996). *B. oleracea* was capable of accumulating high Cd concentration compared to other species. However, *E. prostrata* was an effective species in translocating Cd from roots to the above ground parts. At day 2 of the 20 ppm treatment all plant species had lower percentage of Cd translocation compared to the 5 ppm treatment at the same period. This may due to the reduction of transpiration of which the reduction under high Cd supply has been suggested by Barcelo and Poschenrieder (1990). Movement of Cd from roots to shoots is likely to occur via the xylem and to be driven by transpiration from the leaves (Hart et al., 1998).

5.2 Selection of metal tolerant species

5.2.1 Selection by determining metal accumulation

Among the possible tolerant mechanisms of plants adapted to contaminated soils, limited root uptake is considered by far less important than shoot exclusion (Arduini et al., 1996). Ebbs et al. (1997) used hydroponic techniques to screen the candidate species for phytoextraction of Cd and Zn from a contaminated soil. The nutrient solution was supplemented with 100 $\mu\text{M Zn}^{2+}$, 5 $\mu\text{M Cu}^{2+}$ and 1 $\mu\text{M Cd}^{2+}$. These metal concentrations were sufficiently toxic to inhibit germination and growth of many species. Several *Brassica* spp. were more tolerant to high levels of metal in the solution. Cadmium and Zn concentrations in the shoots of these *Brassica* spp. were similar,

approximately 40 and 2,000 mg/kg, respectively. However, these plant species were stunted and chlorotic after four weeks of growth. In contrast, *Ipomoea purpurea* and *Vicia villosa* which grew fairly well with little or no evidence of toxicity had significantly lower shoot Cd and Zn contents than those for the *Brassica* spp., suggesting that these plants may exclude heavy metal. Two grass species, *Agrostis capillaris* L. and *Festuca rubra* L., which were endemic to metal polluted soil, also tolerated the heavy metal by exclusion and showed no evidence of metal hyperaccumulation. Consequently, selection of tolerant species based on metal accumulation falls into two categories. The first is to select the accumulator like *Thlaspi caererulescens* which is a well known Zn and Cd hyperaccumulator. The second is to select the metal excluder.

In this study *B. oleracea* (*Brassica oleracea*) seems to be the metal accumulator. This plant accumulated high concentration of Cd compared to other species. By visual symptoms, *C. barbata* seemed to be severely affected, but from the preliminary experiment it could tolerate the toxic Cd to some extent. Moreover, this plant lowered the metal burden by accumulating it in the lower leaves. Cadmium was eliminated by senescence of these leaves which had high metal content. Unfortunately, the Cd concentration used in this study are extremely high, leading to predomination of passive uptake process. The metal accumulator especially hyperaccumulators have different pattern of uptake. High shoot metal concentrations described for hyperaccumulator grown in uncontaminated soils suggest an unusually active mechanism for metal uptake (Brown et al., 1995). The necessity of using low Cd concentration to determine accumulators also reported by Costa and Morel (1994). At Cd concentrations in the solution higher than 0.1 μM , the Cd content differed only slightly among lettuce cultivars and the ratio between the highest and the lowest Cd contents in shoots was low. However, at low Cd concentrations in the medium, sharp differences could be observed. In such conditions, this ratio represented a valuable parameter to screen vegetable cultivars which were not the metal accumulators.

5.2.2 Selection by root growth determination

One of the most conspicuous symptoms of metal toxicity is inhibition of root growth which, in turn, has led to the widespread use of this character to assess metal tolerance of plants (Davies, 1994). Root growth is the results of two different mechanisms, cell division at the root tip and cell elongation in the extension zone. Both cell elongation and division are affected by the presence of Cd, but the effect on cell elongation is predominant. The methods for measurement of lateral root, mitotic index, root density and the estimation of root elongation rate by marking with Indian ink and measuring the increased distance later were described by Arduini et al. (1994). However, Schat et al. (1997) measured root growth inhibition under metal stress by staining the roots in a suspension of finely powdered charcoal and measuring the length of the unstained apical root segments later.

Although this study did not focus on the measurement of root growth, the symptom and degree of root damage was observed. Roots of four plant species were affected by Cd of both concentrations during the initial two days of exposure. The Cd-treated root turned yellow, whose intensity increased with Cd supply. Moreover, the damage was observed at approximately 0.5 to 1 centimeter from the root tips. *I. aquatica*'s roots were the most susceptible to Cd, whereas only *B. oleracea*'s root did not rot and was capable of developing new growth. Therefore, *B. oleracea* was the most Cd tolerant species among the plant materials based on root growth determination.

However, an increase in root growth as a response of plants to low levels of Cd or other cations has been reported by Arduini et al. (1994). This indicates that selection by this method is limited to the concentration of Cd which leads to root growth inhibition. Other effective methods are the study of cellular sequestration, cellular compartmentation and biochemical detoxification and complexation by phytochelatins which are most effective at chelating Cd and Cu. Tolerant mechanism occurs via limiting of net accumulation of a toxic ion, either by suppression of influx, increased efflux, preservation of membrane integrity, or by the development of highly specific uptake systems which do not transport the toxic ion concerned (Meharg, 1993).

5.2.3 Selection by using proline accumulation

Costa and Morel (1994) suggested that proline accumulation in plants under Cd stress is induced by a Cd-imposed decrease of the plant water potential. Therefore, proline-mediated alleviation of water deficit stress could substantially contribute to the Cd tolerance of the plant. In addition to performing in the water balance maintenance, proline is conceivable to have other significant functions such as scavenging of hydroxyl radicals and involving in metal chelation in the cytoplasm especially Zn and Ni which are poor inducers of phytochelatin synthesis (Schat et al., 1997).

Schat et al. (1997) investigated the free proline accumulation in a metal-tolerant and a nontolerant ecotype of *Silene vulgaris*. It was found that in the tolerant ecotype the constitutive proline concentration in the leaves was 5- to 6-fold higher than the nontolerant ecotype. However, Cd effectively induced proline accumulation in the nontolerant ecotype at much lower exposure levels compared with the metal tolerant *S. vulgaris*. Proline accumulation did not occur until metal-imposed growth reduction was apparent. In other words, proline accumulation can at most alleviate, but not prevent metal stress. It is unlikely that any proline mediated alleviation of metal-imposed water deficit stress would greatly contribute to metal tolerance. The authors suggested that adaptive tolerance to metals associated with a strongly decreased tendency to accumulate proline upon heavy metal exposure by preventing the development of metal-induced water deficit stress. Under drought stress some plants such as sunflower, sorghum and soybean did not accumulate proline until severely dehydrated to the point of visible wilting. Proline is not a sensitive indicator of stress-induced dehydration. Its accumulation is a sign of injury (Levitt, 1980).

When exposed to Cd stress, *B. oleracea* and *E. prostrata* markedly accumulated proline, but the former prevailed over the latter. *B. oleracea* was also found to be the most Cd tolerant species compared to other plant materials. This is contrary to the Schat et al.(1997)'s study. Therefore, it is no doubt that proline should not be used as an interspecies assessment of Cd stress or other stresses because each species differs in proline accumulation under stress. Moreover, it

is uncertain that proline accumulation in Cd tolerant cultivar of *B. oleracea* under stress will be lower than the susceptible cultivar. High level of proline were detected in *E. prostrata*'s leaves when randomly collected from the susceptible plants and the more tolerant ones which did not show any symptom of wilting under prolonged Cd stress. As can be seen, proline is insensitive and not specific to Cd stress. For these reasons, proline is relatively unsuitable method for screening tolerant species.

5.2.4 Selection based on yield reduction

Shoot dry weight is supplemented with the metal content in plants for determining metal tolerance. Intensity is greater when plants exposed to high Cd concentration and/or for a long duration. During the onset of the 5 ppm Cd exposure, *B. oleracea* is more tolerant based on the lower percentage of yield reduction (Table 5.1).

Table 5.1 Percentage of shoot dry weight of stressed plants relative to the controls

Duration	<i>I. aquatica</i>	<i>B. oleracea</i>	<i>E. prostrata</i>	<i>C. barbata</i>
5 ppm Cd				
2d	-11.50	-4.11	-12.21	-37.96
11d	-40.67	-8.10	-24.18	-43.17
20d	-46.93	-23.16	-50.60	-54.82
7d (27)	-16.74	-54.72	-56.91	-41.67
14d (34)	-35.09	-49.59	-74.36	-58.56
20ppm Cd				
2d	-10.05	+0.98	+1.61	-16.14
5d	-21.10	-29.67	-21.72	-6.29
8d	-45.37	-54.11	-21.07	-28.31
7d (15)	-67.83	-73.94	-37.97	-52.32
14d (22)	-26.65	-44.87	-54.96	-72.46

E. prostrata decreased its yield with time even when Cd-containing solution was removed. This was accompanied by the chlorosis of newly developed leaves. Although this plant could blossom under Cd stress, it failed to develop seeds. *I. aquatica* had relatively low yield reduction after having been transferred to Cd-free solution. This is probably due to the control plants nearly fully matured, leading to slight difference between controls and metal-stressed plant. The increased yield resulted from variation of samples, not by the growth stimulation by Cd.

5.3 Possibility of using plant materials for phytoremediation

Metal tolerant plants especially the hyperaccumulators are currently used to remediate contaminated sites. One of the characteristics of hyperaccumulator species are that they exhibit shoot/root ratios of metal content greater than one. This indicates a specific internal pumping system of metals from roots to shoot tissue. Cadmium hyperaccumulator species are defined as those whose leaves contain more than 100 mgCd/kg dry weight when grown in metal-rich soils (Brown et al., 1995). Citing in Watanabe's report (1997), the hyperaccumulators should have the following traits: high contaminant accumulation rate even at low concentration in the environment; ability to accumulate very high levels of contaminants; ability to accumulate several metals; fast growth; high biomass production; and resistance to diseases and pests.

As can be seen from the shoot Cd content, *B. oleracea* and *E. prostrata* had sufficiently high shoot Cd content. However, phytoextraction of contaminants depends on shoot biomass production (Ebb et al., 1997) because total metal translocation is achieved by multiplication of shoot metal concentration by shoot dry weight (Brown et al., 1994). Comparable total metal translocation was observed in *B. oleracea* and *E. prostrata* because both species did not differ in shoot Cd content and shoot biomass (see Table B-1) though *E. prostrata*, not being exposed to Cd, had greater biomass. *E. prostrata* developed strong root system, but had slower rate of Cd uptake relative to *B. oleracea*. Nevertheless, this plant was more likely to accumulate more Cd with time. However, this study concerned the Cd exposure of plants directly from nutrient solution. If this plants were grown in the contaminated soil, less Cd in shoot would be observed. Further investigation should focus on the use of low concentration of metal to determine the efficiency of uptake process.