CHAPTER 5
DISCUSSION

Seed germination test

The effects of lead on seed germination were tested as short-term tests and reliable results were obtained within a short experimental period. Wilkins in 1957 suggested that normal treatment times of these tests are 2-8 days (Kohl and Losch, 1999). In my research, exposure times were 5 days (120 hours), except for *Lagerstroemia speciosa*, which took 14 days for the seeds germinate. This method is a more suitable test for herbaceous plants which can be germinated within a week. Germination test should not run longer than 10 days.

Inhibitory effects on seed germination

In this test, the inhibition percentages relative to the controls were calculated to compare the inhibitory effects between the plants tested. All values of inhibition percentage; % DFC, and % phytotoxicity, clearly revealed the relative inhibitory effects of lead on seed germination on all plants tested (Figure 10). The results, included in Tables 16, 17, and 18, show that lead inhibited both the seed germination rate, as well as shoot and root elongation of the six species tested. The r^2 values 0.71-1.00 indicate that all seed germination test parameters were highly inhibited by the lead treatments.

Percent DFC

Helianthus annuus had the highest germination rate and *Lagerstroemia speciosa* the lowest which also took three times longer to germinate than the other species (120 hours). Germination rates are an important criterion for species selection in phytoxicity tests. All seeds did not germinate at 1000 μg /ml lead concentration. The tolerance of plants to lead toxicity can be determined by the percent DFC at high lead concentrations. The lower the DFC % the more tolerant the plant is. The results show that *Eleusine indica* was the most vulnerable to lead toxicity for germination, while *Brassica rapa* was the most tolerant one. EC_{50} values were much lower for *Eleusine indica* and *Lagerstroemia speciosa* than the others.

Percent phytotoxicity of shoot elongation

The results in Table 20 and Figure 10 show that all lead concentrations were toxic to shoot elongation for all plants tested. According to the values of percent phytotoxicity at high concentrations on shoot length, *Brassica rapa* and *Helianthus annuus* were more sensitive to lead than the others. The cultivated crop species showed their sensitivity to stress in a shorter time than the other plants. The EC_{50} values were much lower for *Brassica rapa* and *Helianthus annuus* than the other species.

Percent phytoxicity of root elongation

The results in Table 21 and Figure 10 show that all lead concentrations caused the reduction of root growth in all plants tested. Compared with the controls, the root length of lead treated plants was significantly lower. Similar results have also been observed in *Brassica pekinensis* (Lour.) Rupr. (Cruciferae), see page 11 (Xiong, 1998). Numerous authors have proved that heavy metals in soil inhibit root growth and tolerance measured by root growth capacity at a high treatment levels (Kohl and Losch, 1999). According to the percent phytotoxicity values at high lead concentrations on root length, *Brassica rapa*, *Euphorbia heterophylla*, and *Helianthus annuus* were most sensitive to lead. EC_{50} values were much lower for *Brassica rapa*, *Euphorbia heterophylla* and *Helianthus annuus* than the other species.

At high concentration (500 μg/ ml), the inhibition of root growth was 3-56 times lower than the control roots, depending on the species. Obroucheva *et al.* (1998) reported that the inhibition of root growth at lead concentrations of 10^{-2}-10^{-6} M or soil content above 10 mg/ kg. Primary root growth of maize was more inhibited than lateral roots by lead (Obroucheva *et al.*, 1998). The main morphological changes in roots observed in my work was decreased root growth, which was more clearly affected than root dry biomass weight of the seedlings.

Sensitivity of seed germination test parameters and indices

One aim of my study was to identify sensitive parameters and indices for biomonitoring lead pollution. In order to evaluate their sensitivity, a ranking system was used. Inhibition percentages at low treatment were assigned 4 ranks (Table 44). The lower the rank the higher the sensitivity.

Table 44. Ranking system of inhibition percentage

Inhibition percentage	Rank
< 10	4
10 - 25	3
25 - 50	2
> 75	1

Based on this ranking system, the rating of germination test indices were compared (Table 45). The results clearly reveal that the order of sensitivity of parameters and indices: root elongation > shoot elongation > seed germination and % root phytoxicity > % shoot phytoxicity > % DFC, respectively.

Table 45. Comparison of germination test sensitivities

Plant species	% DFC	% shoot phytotoxicity	% root phytotoxicity
Eleusine indica	3	4	3
Euphorbia heterophylla	4	2	1
Brassica rapa	3	2	1
Pisum sativum	2	3	2
Helianthus annuus	4	2	1
Lagerstroemia speciosa	2	2	2
Average rank	3	2.5	1.7

I found root elongation to be the most sensitive parameter and percent root phytotoxicity the most sensitive index for biomonitoring. Seed germination and %DFC are the least sensitive ones. This is consistent with Baker *et al.* (1983), who suggested that seed germination is not a sensitive parameter for assessing metal tolerance.

Among seed germination test parameters, root elongation is an obvious indicator of toxic concentrations. This can be used as an early warning indicator in biomonitoring of lead pollution.

Germination tolerances

Another aim of my study was to determine the tolerance or sensitivity of plants to lead. Tolerance is measured at high levels of lead addition and sensitivity is measured at low levels. The ability of all plants tested was determined at low and high lead concentrations. In order to compare their tolerance or sensitivity, the % root phototoxicity, the most sensitive index, was used (Table 46 and Figure 26).

Table 46. Tolerance and sensitivity according to % root phytotoxicity

Order of tolerance	**Order of sensitivity**
Lagerstroemia speciosa > Eleusine indica > Pisum sativum >Brassica rapa > Helianthus annuus> Euphorbia heterophylla	*Brassica rapa >Helianthus annuus >Euphorbiaheterophylla > Pisum sativum > Lagerstroemia speciosa >Eleusine indica*

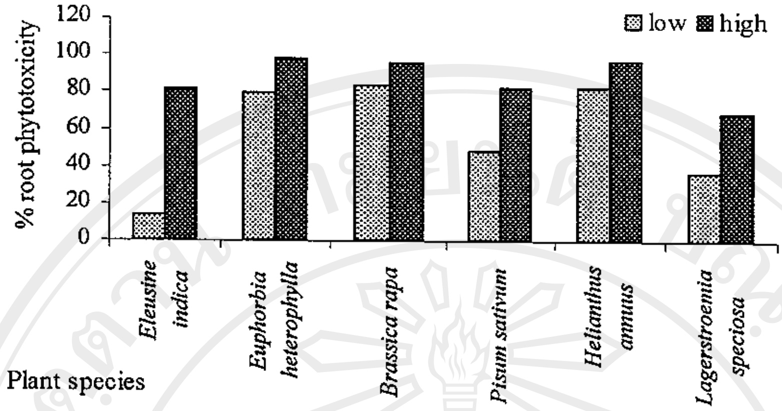

Figure 26. Comparison of tolerance and sensitivity in terms of % root phytotoxicity.

According to the orders of sensitivity and tolerance of the plants tested, herbaceous plants are more sensitive than tree species and dicots are more sensitive than monocots (*Eleusine indica*). In this test, all seed germination parameters were inhibited by lead, however, complete growth inhibition, <10 % of control, (Kohl and Losch, 1999) was found in *Brassica rapa*, *Helianthus annuus*, and *Euphorbia heterophylla* at medium and high treatments.

Seedling growth test

The effects of lead on seedling growth were tested as a long- term growth test. Normally, the exposure time of this test depends on the growth media, plants tested, and test conditions. Generally, 2- 8 weeks are needed.

Inhibitory effects on seedling growth

The inhibition percentages relative to the controls were calculated to compare the inhibitory effects between the plants tested. The inhibition percents of all growth parameters clearly show the relative inhibitory effects of lead on seedling growth for all plants tested (Table 34).

Growth parameters

Lead negatively affected all growth parameters, however, the magnitude of lead toxicity depends on the plant species. Complete growth inhibition was found only in *Brassica rapa* at high treatment level for shoot length. There was no complete inhibition for the other parameters. Depending on the growth parameter and lead concentration, the inhibition percentage varied from 1.8-93.5 %. Among growth parameters, biomass and shoot length were more inhibited by lead leaf number and leaf length. EC_{50} values were much higher for leaf length than the others.

According to the results of repeated analysis on shoot length and leaf number, a significant time by treatment interaction could be found in all plants tested except *Eleusine indica* for shoot length and *Shorea roxburghii* for both parameters. The results from this analysis should be considered in determination of plant tolerance and exposure duration. In *Eleusine indica*, this indicates tolerance, but in *Shorea roxburghii* both tolerance and insufficient exposure time. Figures 12 and 14 indicate that lead effects growth development over time. Shoot height and leaf numbers in the control plants were higher and faster than those of all lead treatments, especially at high concentrations.

Some necrotic symptoms in leaves could be found plant at high lead concentrations in *Shorea roxburghii* and *Euphorbia heterophylla* before shedding their leaves. Most leaves turned yellow after 2-3 weeks of treatment and then gradually dried and were shed. Figure 18 clearly indicate that root parts were more affected than shoots. Greger (1999) noted that higher plants take up metals from water or air by the shoot and their roots take up metals from the soil, sediment, or from the solution in which they are cultured.

Percent dry biomass weight

Table 35 and Figure 22 indicate that the percent of dry biomass weight increased with higher lead concentrations. This means that lead treatments decreases water loss from plants. Numerous studies about the relationship between the heavy metal toxicity and water use efficiency in plants have been done. Becerril found that lead caused a drastic reduction of water use efficiency (Poschenrieder and Barcelo, 1999). It means that lead inhibits plant physiological processes related to water. Since water is the major component of plants, studies about this parameter are reliable indicators for biomonitoring.

Relative biomass yields

Table 37 and Figure 23 show that relative yield, relative to total dry biomass weight of the control, decreased with increased lead concentrations. Quantifying biomass is a basic and common measurement to determine growth changes due to stress conditions and biomass weight is used to compare growth responses between different plant species. Plant tolerance according to relative yield is discussed on p.77.

Absolute FA

Tables 31 and 32 indicate that FA values of the controls decreased significantly compared to the lead treatments in all plants. Figure 20 shows that the greater the FA value the smaller the total dry biomass value in all plants tested. Tarun *et al.* (2002) pointed out that FA may have a direct relationship with fitness in many organisms. Evans and Marshall (1996) found that FA was greater in populations that had lower biomass accumulation and flower production in *Brassica campestris* L. (Cruciferae). As FA is an easily visible morphological symptom, it can be used an early waning sign in biomonitoring. Limitations of this index are that linear relationships between FA and lead treatments were not found and in narrow leaf bladed plants, *e.g. Eleusine indica*, where FA can not be measured.

Sensitivity of seedling growth test parameters and indices

In order to identify the most sensitive seedling growth parameter, the ranking system shown in Table 44 was used. Then the sensitivity of seedling growth test parameters were compared (Table 47). The results show that the order of sensitivity of parameters is total biomass> shoot height> leaf number> leaf length.

Table 47. Comparison of seedling growth test parameters for lead sensitivity

Plant species	Shoot length	Leaf number	Leaf length	Total biomass
Eleusine indica	3	4	4	3
Euphorbia heterophylla	2	2	3	2
Brassica rapa	2	3	3	4
Pisum sativum	2	3	4	2
Helianthus annuus	4	4	4	4
Lagerstroemia speciosa	4	3	3	2
Shorea roxburghii	4	4	4	2
Average rank	3	3.3	3.6	2.7

Biomass can be used as the most sensitive growth indicator for biomonitoring. Kohl and Losch (1999) suggested that the tolerance of plants to metal pollution could be quantified by measuring the plant biomass after the treatment period.

According to the seed germination test results, root elongation and % root phytotoxicity were the most sensitive parameter and index. In this test, only dry root biomass weight was done. Average rank values of root dry biomass and % root phytotoxicity were compared in order to find the most sensitive unit.

The results show that root length is more sensitive than root biomass because the average ranks of % root phytotixicity and root biomass were 1.7 and 2.7, respectively. Baker and Walker (1990) proved that root length is more sensitive to metal treatment than dry root weight. It is difficult to measure root length without damaging taproots planted in soil or sand because the growth media in pots is compacted and some taproots penetrate the basal hole in pots at harvest time. Root elongation is usually measured in short-term seed germination tests.

There are many indices to compare plant biomass, such as relative yield, shoot and root biomass ratio, tolerance index *etc*. In my study, the relative yield and percent dry weight were used as seedling growth indices. These two indices are opposite trends because the greater relative yield value means less inhibitory effect of lead on plants.

In contrast with relative yield, the higher the percent of dry biomass weight means a greater inhibitory effect. These results indicate that percent of dry biomass weight provides a reliable indicator of the phytotoxic effects of heavy metals.

Apart from these two growth parameter indices, one morphological parameter index, absolute FA, was also studied. This index is similar to the percent of dry biomass weight. The FA values of lead treatments were higher than those of the controls in all plants tested. Average relative values were calculated to compare their sensitivities (Tables 33, 36, 37 and Figure 27).

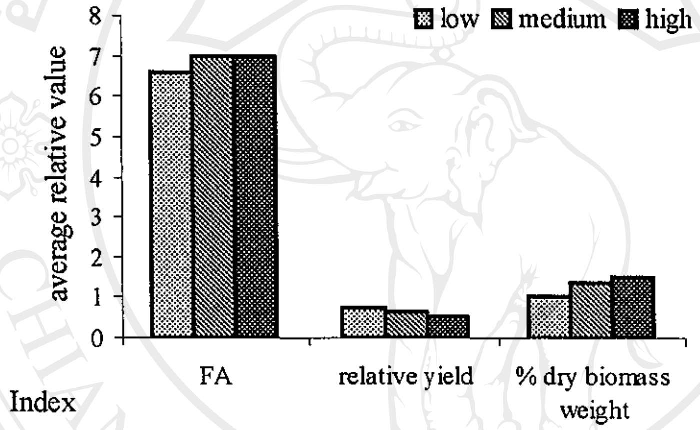

Figure 27. Comparison of FA and two growth parameter indices.

Figure 27 clearly reveals the sensitivity of FA. Because of its sensitivity, it has recently gained the attention of scientists. Tracy in 1995 proved that FA may be sensitive to stress levels that are too low to affect other factors such as growth, reproduction, survival, *etc.* (Tarun *et al.*, 2002).

Ability for seedling growth

In this test, a plant's ability for seedling growth by using relative yield, the most common and basic growth index, at low and high lead treatments is measured. For sensitivity, ability was measured at low treatment and for tolerance ability was measured at high treatment levels (Table 48 and Figure 28).

Table 48. Comparison of plant ability in terms of relative yield

Order of tolerance	Order of sensitivity
Shorea roxburghii > Eleusine indica > Helianthus annuus> Pisum sativum > Brassica rapa> Lagerstroemia speciosa > Euphorbia heterophylla	*Lagerstroemia speciosa> Euphorbia heterophylla> Shorea roxburghii > Pisum sativum > Eleusine indica > Helianthus annuus> Brassica rapa*

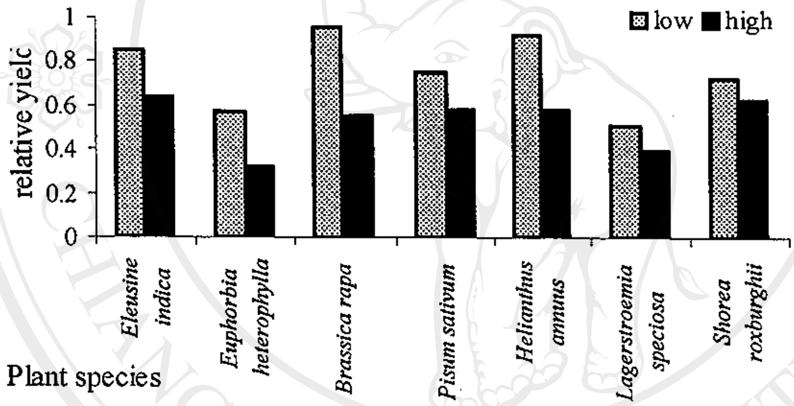

Figure 28. Comparison of tolerance and sensitivity in terms of relative yield.

Determination of lead tolerance ability

According to the seed germination and seedling growth tests; two indices, *viz.* percent root phytotoxicity and relative yield, two sets of tolerant and sensitive plant species tested, are listed in Tables 46 and 48. These orders are summarized in Table 49.

Table 49. Sensitivity and tolerance orders

Plant species	Sensitivity		Tolerance	
	% root phytotoxicity	relative yield	% root phytotoxicity	relative yield
Eleusine indica	6	5	2	2
Euphorbia heterophylla	3	2	6	7
Brassica rapa	1	7	4	5
Pisum sativum	4	4	3	4
Helianthus annuus	2	6	5	3
Lagerstroemia speciosa	5	1	1	6
Shorea roxburghii	not tested	3	not tested	1

Based on Table 49, three plant groups can be made:

1. Sensitive group-- *Euphorbia heterophylla, Lagerstroemia speciosa,*
2. Intermediate group-- *Brassica rapa* , *Pisum sativum, Helianthus annuus,* and
3. Tolerant group -- *Eleusine indica* , *Shorea roxburghii.*

Lead analysis

In this test, three plants were selected to study lead uptake and translocation. These species were *Euphorbia heterophylla, Brassica rapa,* and *Shorea roxburghii* from representatives of the three ability groups: sensitive, intermediate, and tolerant, respectively. These species are from three plant types, *viz.* herbaceous weed species, cultivated crops, and tree species.

Lead concentrations in plant tissues

Tables 41 and 42 show that lead concentrations in plant tissues varied depending on the plant species, plant parts, and lead levels in the growth media. Among the plants tested, lead concentrations increased in the following order: *Euphorbia heterophylla*

> *Brassica rapa,* and > *Shorea roxburghii.* Lead contents in root parts were higher than those in aerial plant parts. Lead content in plant tissues increased with increases in lead treatment levels in: high > medium> low, respectively.

According to the growth test results the order of lead tolerance is *Shorea roxburghii* > *Brassica rapa* > *Euphorbia heterophylla*. This order is totally different from that of lead concentration in plant tissues (Figure 29). This means that lead accumulation in plant tissues is not related to lead tolerance. The results from correlation tests supported this finding since there was no significant correlation between lead in plant tissues and biomass weight, which is the basic parameter to determine plant tolerance.

This finding agrees with previous research. Greger (1999) showed that the differences in accumulation of a metal were not correlated with tolerance to that metal. Wierzbicka (1997) also found that there was no relationship between the degree of tolerance to lead and the amount of lead in a plant's tissues.

Lead concentrations in roots were higher than those in above ground plant parts. Many authors have shown that when the soil is contaminated with heavy metals, plants take up the metal by the roots. Xiong (1998) found that the lead hyperaccumulator, *Brassica pekinensis* (Lour.) Rupr. (Cruciferae), had a root lead concentration of 33,647 μg/g at lead treatment 1000 μg/g while the shoot was 7,358 μg/g.

I found that lead concentration in roots was higher than those in aerial plant parts, except in *Euphorbia heterophylla* at high levels. Lead concentrations in aerial plant parts exceeded than roots at high levels. Many plants can accumulate significant amounts of heavy metals in their roots, but few have the ability to translocate appropriate levels of heavy metals to their shoots (Weatherford *et al.*, 1997). Wenzel and Jockwer (2000) found that a lead hyperaccumulator, *Biscutella laevigata* L. (Cruciferae), had shoot lead concentration of >1000 mg/kg. They proved that plants, in which shoot metal concentrations exceeded those in roots, were very useful for phytoremediation, because they could be mowed and removed like agricultural crops. Reeves and Brooks (1983) discovered another lead hyperaccumulator species, *Thlaspi* *rotundifolium* L. subsp. *cepaeifolium* (Cruciferae) in a mining area in northern Italy, where the lead concentration in dried leaves was 8,200 µg/g (0.82%). This concentration is the highest record known in flowing plants.

The results from lead concentrations in plants show that lead can accumulate up to 3,240 µg/g (0.32 %) in the above ground parts of *Euphorbia heterophylla* while in *Brassica rapa,* and *Shorea roxburghii* it was 0.017 % and 0.011 %, respectively. The level of lead accumulation in *Euphorbia heterophylla* qualifies this species for phytoremediation programs and phytotoxicity tests in biomonitoring programs.

Lead concentration in plant tissues significantly increased with higher lead concentrations in a sand growth medium. Numerous investigations show that lead concentrations in plant tissues is significantly related to the lead levels in the growth medium. This concerns the K value and TF, which are discussed next.

Lead uptake and translocation in plants

The data in Table 41 is plotted in Figure 29. Based on these patterns, it can be seen that the differences in lead uptake by plants depended on the plant species rather than the lead concentrations in the growth medium. Greger (1999) noted that the phytoavailability of metals depends on the form of the metal and on the plant species tested. Even within the same species, different genotypes differ in metal uptake. Due to this fact, a mapping metal pollution using different genotypic plants needs to be considered. Results from botanical identification and metals amounts in plant tissues should be compared with metals amounts in soil in order to obtain reliable monitoring results.

In my research, lead uptake and translocation in plant tissues were compared using the K value and TF. According to the results shown in Table 43, it can be concluded that the K value and TF increased with more lead in the growth medium. The K and TF values of *Euphorbia heterophylla* were higher than those of the other two species, while *Shorea roxburghii* was the lowest.

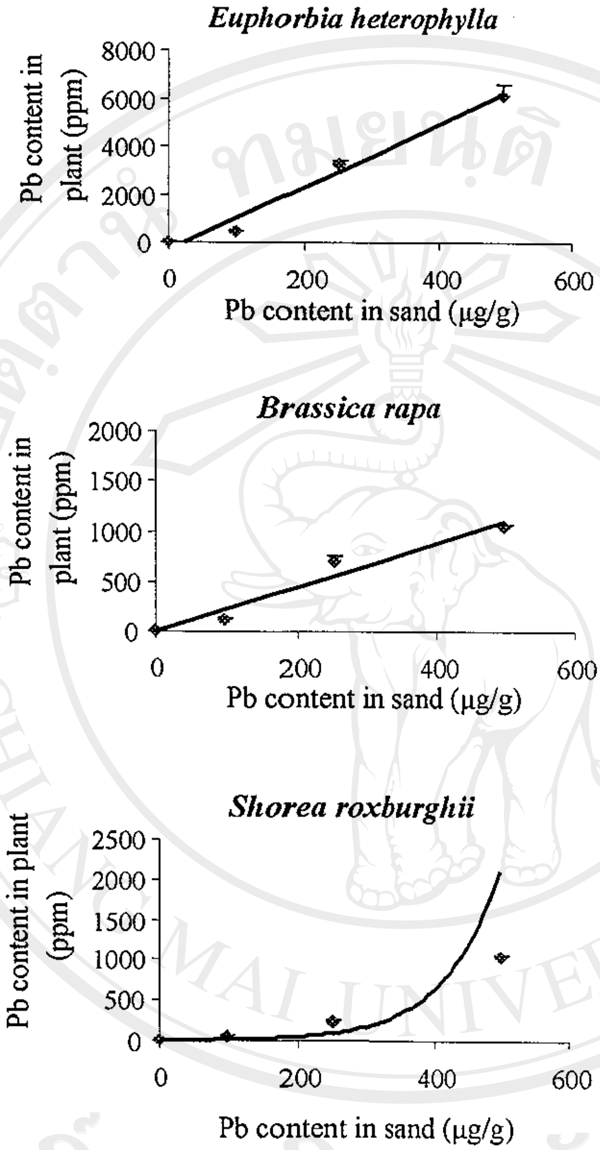

Figure 29. Lead content in sand and plant relationships.

Plants can be divided into three categories based on their responses to increasing metal concentrations in soil (Greger, 1999). *Euphorbia heterophylla* and *Brassica rapa* can be considered as indicators species in which the relationship between lead uptake and lead contents in the growth medium is linear. *Shorea roxiburghii* can be considered as an excluder species because it is able to restrict lead uptake from the growth medium.