CHAPTER 4
RESULTS

Seed germination test

In this test, three parameters, *viz.* seed germination rate, root length, and shoot length were studied. The effects on lead treatment on these parameters are shown in Figure 7.

Effects of lead treatments on germination rates

The effects of various concentration of lead on seed germination rates are shown in Table 16 and Figure 8.

In general, the germination percentage ($P< 0.05$) of the treatments and control experiment of all plants tested were significantly different (Table 16). Significant decreases of germination rates with increasing lead concentrations were observed. The degree of response to lead treatments varied among species. Compared with the control the germination rate at low lead treatment (100 μg/ml) for *Eleusine indica*, *Euphorbia heterophylla*, and *Helianthus annuus* did not decrease significantly. For other concentrations, the germination rates were significantly decreased compared to the controls. Germination was completely inhibited only at 1000 μg/ml for all species.

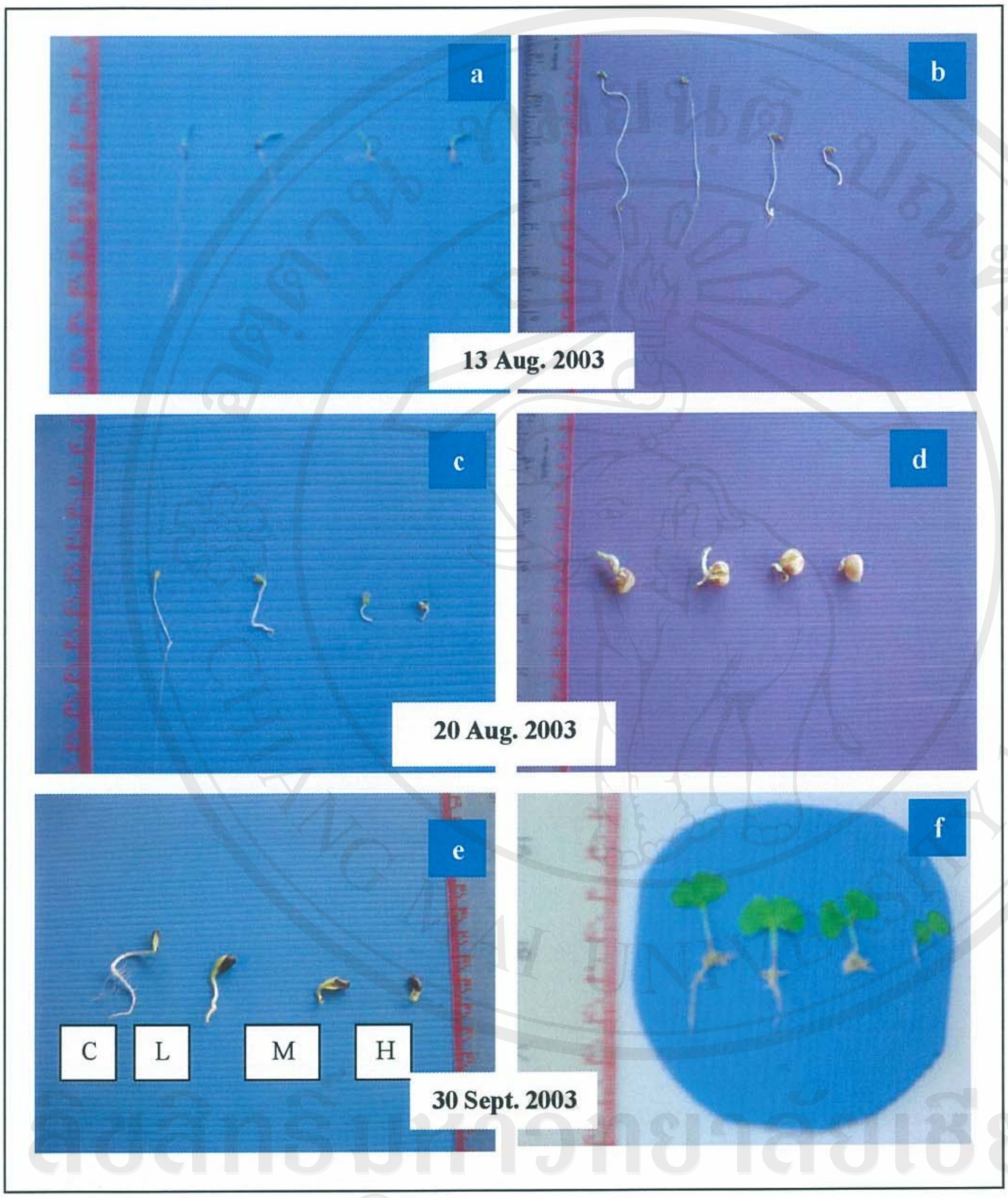

Figure 7. Effects of lead on seed germination on: a-- *Eleusine indica*, b-- *Euphorbia heterophylla*, c-- *Brassica rapa*, d-- *Pisum sativum*, e-- *Helianthus annuus*, and f-- *Lagerstroemia speciosa* (a, b, c, d, and e were after 120 hours of exposure and f was after 14 days of exposure).

Table 16. Effects of lead on seed germination rate (%)

Pb (µg/ml)	*Eleusine indica*	*Euphorbia heterophylla*	*Brassica rapa*	*Pisum sativum*	*Helianthus annuus*	*Lagerstroemia speciosa*
0	85.8 ± 9.5 [a]	58.3 ± 3.5 [a]	88.5 ±15.6 [a]	85.0 ±10.0 [a]	100.0 ± 0.0 [a]	30.0 ±8.6 [a]
100	70.0 ±17.4 [a]	55.0 ± 6.3 [a]	68.3 ± 6.2 [b]	57.5 ±15.0 [b]	95.8 ± 8.5 [a]	16.7 ±3.9 [b]
250	39.2 ±22.8 [b]	38.5 ±13.5 [b]	68.3 ±11.5 [b]	52.5 ±25.0 [b]	91.5 ± 9.8 [a]	11.7 ±3.3 [bc]
500	15.0 ±12.3 [b]	26.8 ± 9.4 [b]	66.8 ± 5.3 [b]	42.5 ±15.0 [b]	74.8 ±16.5 [b]	6.7 ±0 .4 [c]
1000	0	0	0	0	0	0
F ratio	14.878	10.743	3.923	4.504	4.439	12.429
P value	0.000	0.001	0.037	0.025	0.026	0.001

Note: Seed germination percentage results are means ± SD (n= 4). Means with different letters on the same column indicate significant differences among treatments LSD at P=0.05. F ratios are the results of one-way ANOVA analysis.

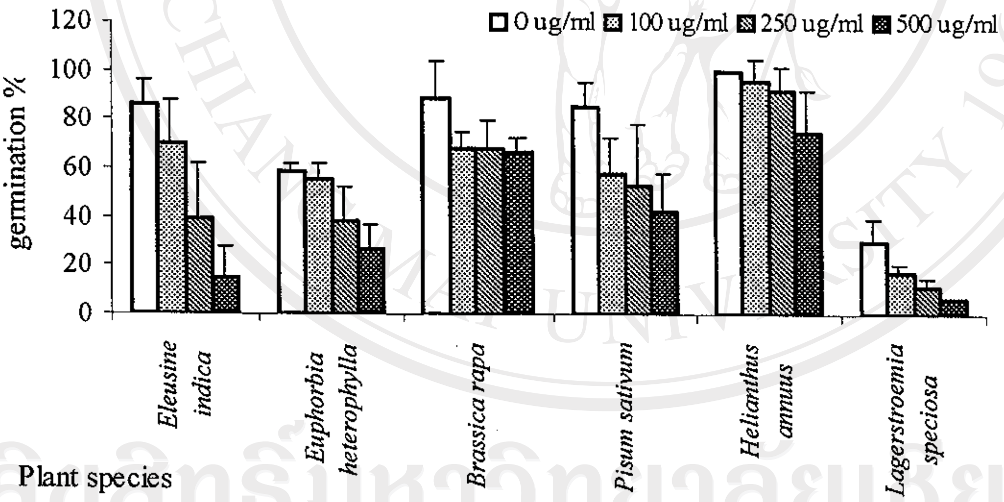

Figure 8. Effects of lead on germination rates. Error bars represent one standard deviation.

Effects of lead treatments on shoot elongation

The results of lead treatments on shoot (hypocotyl and epicotyl) elongation of six plants tested are shown in Table 17 and Figure 9.

The results showed that the differences in shoot elongation among the treatments of all plants tested were highly significant ($P \leq 0.005$). Significant decreases of shoot length with increases in lead concentrations was observed.

Table 17. Effects of lead on shoot elongation (cm)

Pb (μg/ml)	***Eleusine indica***	***Euphorbia heterophylla***	***Brassica rapa***	***Pisum sativum***	***Helianthus annuus***	***Lagerstroemia speciosa***
0	0.76 ±.05 ^{a}	8.98 ±.70 ^{a}	3.60 ±.41 ^{a}	1.50 ±.14 ^{a}	2.20 ±.24 ^{a}	1.63 ±.13 ^{a}
100	0.72 ±.04^{ab}	5.95 ±.24 ^{b}	2.07 ±.66 ^{b}	1.17 ±.26 ^{b}	1.32 ±.14 ^{b}	1.06 ±.05 ^{b}
250	0.61 ±.06 ^{b}	4.88 ±.23 ^{c}	0.61 ±.07 ^{c}	1.09 ±.11 ^{b}	0.78 ±.12 ^{c}	0.98 ±.05 ^{b}
500	0.44 ±.13 ^{c}	1.55 ±.20 ^{d}	0.42 ±.10 ^{c}	0.90 ±.20 ^{b}	0.39 ±.06 ^{d}	0.84 ± .06 ^{c}
1000	0	0	0	0	0	0
F ratio	12.458	236.140	57.384	7.183	99.557	79.138
P value	0.001	0.000	0.000	0.005	0.000	0.000

Note: Results are means ± SD (n= 4). Means with different letters on the same column indicate significant differences among treatments LSD at $P = 0.05$. F ratios are the results of one-way ANOVA analysis.

Effects of lead treatments on root elongation

The results of lead treatments on root elongation of six plants tested are shown in Table 18 and Figure 9.

The results in Table 18 show that the differences in root elongation among the treatments of all plants tested were highly significant ($P < 0.001$). Significant decreases in root growth with increases in lead concentrations was observed. At low concentrations, root length of all plants tested, except *Eleusine indica,* were 2- 6 times less than the controls. At high concentrations, root length decreased 3 times that of the control for *Eleusine indica, Euphorbia heterophylla, Brassica rapa, Pisum sativum, Helianthus annuus,* and *Lagerstroemia speciosa.*

Table 18. Effects of lead on root elongation (cm)

Pb (µg/ml)	*Eleusine indica*	*Euphorbia heterophylla*	*Brassica rapa*	*Pisum sativum*	*Helianthus annuus*	*Lagerstroemia speciosa*
0	3.62 ±.58 [a]	8.47 ±.70 [a]	3.92 ±.87 [a]	1.41 ±.24 [a]	2.99 ±.56 [a]	2.02 ±.16 [a]
100	3.12 ±.21 [a]	1.80 ±.80 [b]	0.64 ±.28 [b]	0.74 ±.38 [b]	0.54 ±.14 [b]	1.28 ±.02 [b]
250	1.42 ±.31 [b]	0.59 ±.09 [c]	0.25 ±.06 [b]	0.58 ±.04 [bc]	0.13 ±.02 [bc]	1.03 ±.04 [c]
500	0.67 ±.22 [c]	0.15 ±.04 [c]	0.14 ±.05 [b]	0.25 ±.12 [c]	0.08 ±.02 [d]	0.63 ±.09 [d]
1000	0	0	0	0	0	0
F ratio	59.229	210.129	62.143	17.971	93.488	154.155
P value	0.000	0.000	0.000	0.000	0.000	0.000

Note: Results are means ± SD (n= 4). Means with different letters on the same column indicate significant differences among treatments LSD at P= 0.05. F ratios are the results of one-way ANOVA analysis.

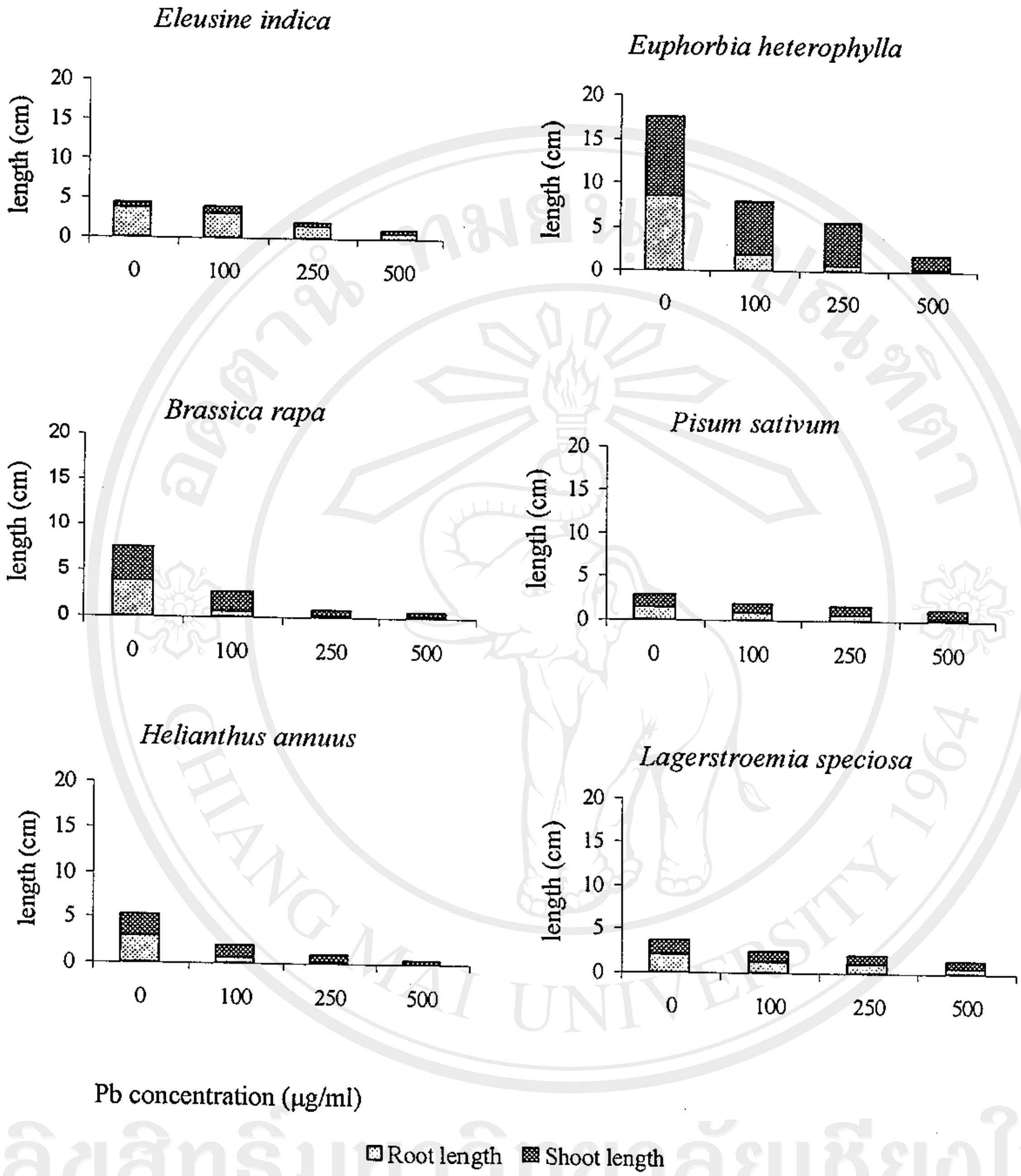

Figure 9. Effects of lead on shoot and root elongation.

Inhibitory effects of lead on seed germination

In order to compare the tolerance to lead between all the plants tested, all measured data were transformed to the relative values of the controls. The parameter used for seed germination rate was the percent DFC value and for shoot and root elongation the percent phytotoxicity was calculated.

Table 19 and Figure 10 show that the % DFC value increased with increased lead concentration. The r^2 for all species tested was 0.86 -0.99. The highest % DFC value was in *Eleusine indica* which indicates its relatively greater susceptibility to lead. The lowest % DFC value was recorded in *Helianthus annuus*. *Brassica rapa* was the only species that responded to all lead concentrations equally. In order to investigate lead concentrations which had deleterious effects on the plants tested, the EC_{50}: effective concentration for 50 % inhibition relative to the control was determined.

Table 19. Percent DFC values of the plants tested

Pb (µg/ml)	***Eleusine indica***	***Euphorbia heterophylla***	***Brassica rapa***	***Pisum sativum***	***Helianthus annuus***	***Lagerstroemia speciosa***
100	18.4	5.6	22.9	32.4	4.3	44.4
250	54.4	33.9	22.9	38.2	8.5	61.1
500	82.5	54.1	24.6	50.0	25.3	77.8
r^2	0.95	0.94	0.86	0.99	0.96	0.98
EC_{50}	<250	>500	>500	500	>500	>100

Table 20 and Figure 10 show that the % phytotoxicity values for shoot growth increased with increased lead concentration. The r^2 values for all species tested were 0.71 -0.99. The highest value was found in *Brassica rapa*, while the lowest was in *Pisum sativum*.

Table 20. Percent phytotoxicity for shoot length

Pb (μg/ml)	***Eleusine indica***	***Euphorbia heterophylla***	***Brassica rapa***	***Pisum sativum***	***Helianthus annuus***	***Lagerstroemia speciosa***
100	5.5	33.7	42.4	21.8	40.3	34.9
250	19.8	45.7	83.1	27.3	64.5	40.0
500	41.8	82.7	88.3	40.0	82.3	48.2
r^2	0.99	0.98	0.71	0.99	0.95	0.99
EC_{50}	>500	>250	>100	>500	<250	>500

Table 21 and Figure 10 show that the % phytotoxicity values in root growth increased with increased lead concentration. The r^2 values for all species tested were 0.72 -1.00. The highest value was found in *Euphorbia heterophylla*, while the lowest was in *Eleusine indica*.

Table 21. Percent phytotoxicity for root length

Pb (μg/ml)	***Eleusine indica***	***Euphorbia heterophylla***	***Brassica rapa***	***Pisum sativum***	***Helianthus annuus***	***Lagerstroemia speciosa***
100	13.8	78.8	83.6	47.6	82.0	36.9
250	60.8	93.1	93.6	58.8	95.6	48.9
500	81.6	98.3	96.4	82.3	97.4	68.7
r^2	0.87	0.84	0.81	0.99	0.72	1.00
EC_{50}	<250	<100	<100	>100	<100	>250

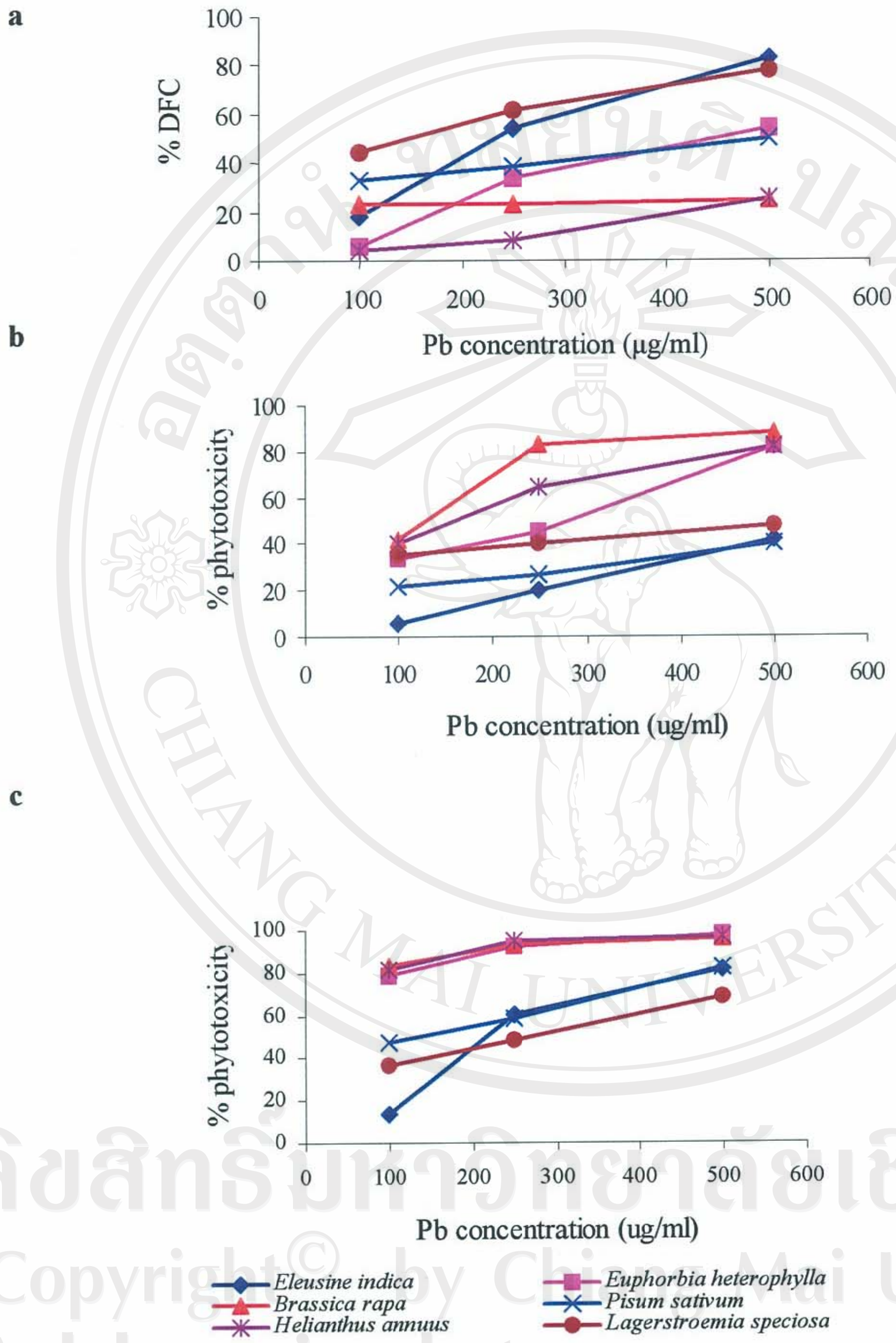

Figure 10. Inhibitory effects of lead on seed germination: a-- % DFC, b-- % phytotoxicity of shoot elongation, and c-- % phytotoxicity of root elongation.

Seedling growth test

This test was undertaken to follow the growth, development, and morphology of seedlings grown in a lead-enriched medium. The plants tested and lead concentrations were the same as those in the seed germination test. In this test, the exposure period of all species was the same. The degree that different plant species respond to lead toxicity is different. In order to obtain accurate toxicity levels and suitable exposure periods for different plants, exposure times were 4, 5, and 8 weeks for herbaceous cultivated crops, herbaceous weeds, and trees, respectively. Besides the growth parameters, leaf morphological characters and fluctuating asymmetry (FA) were also studied.

Effects of lead treatments on shoot length

The initial shoot length (epicotyl and hypocotyl), before lead treatment, did not differ among control and different treatment levels (Table 22). The final shoot height differed significantly among treatments (Table 23 and Figure 10).

Table 22. Initial shoot lengths (cm)

Species	Mean height ± SD
Eleusine indica	4.76 ± 1.98
Euphorbia heterophylla	16.47 ± 1.01
Brassica rapa	12.06 ± 1.43
Pisum sativum	13.89 ± 1.11
Helianthus annuus	4.68 ± 0.72
Lagerstroemia speciosa	4.65 ± 1.27
Shorea roxburghii	10.63 ± 1.92

Table 23. RGR for shoot length

Pb (μg/g)	*Eleusine indica*	*Euphorbia heterophylla*	*Brassica rapa*	*Pisum sativum*	*Helianthus annuus*	*Lagerstroemia speciosa*	*Shorea roxburghii*
0	39.5±22 [a]	145.7±33[a]	60.3±15[a]	118.3±24[a]	73.5±14[a]	15.0±10[a]	6.4±4[a]
100	31.0±15[ab]	105.8±63[ab]	36.2±10[b]	73.5±19[b]	74.8±27[a]	14.4± 4[a]	6.0±6[a]
250	23.2±16[ab]	60.4±42[bc]	21.3±19[b]	60.7±33[b]	25.2±28[b]	6.5± 4[ab]	4.9±3[a]
500	12.3± 3[b]	28.4± 8[c]	3.9± 5[b]	56.1±31[b]	17.1±13[b]	2.5± 4[b]	5.0±3[a]
F ratio	2.185	6.048	12.844	4.308	8.045	4.289	0.143
P value	0.143	0.009	0.000	0.028	0.003	0.028	0.982

Note: Results are means ± SD (n= 4). Means with different letters on the same column indicate significant differences among treatments LSD at P =0.05. F ratios are the results of one-way ANOVA analysis.

Table 23 shows that shoot length in high lead concentrations was significantly shorter than in other treatments in all species tested except *Shorea roxburghii*. Although the shoot length of low and medium treatments was shorter than the control, the difference was not significant in all species except *Brassica rapa* and *Pisum sativum*.

P values (>0.05) in *Eleusine indica* and *Shorea roxburghii* showed that the differences between treatments were not significant at the 0.05 level. Apart from these species, the others were significantly different.

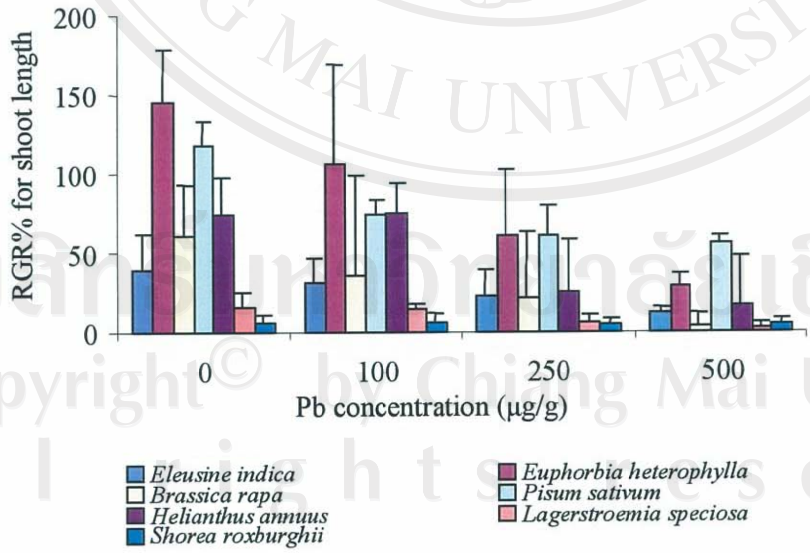

Figure 11. Effects of lead on shoot length. Error bars represent one standard deviation.

In order to investigate differences between treatments, interaction over time with repeated ANOVER tests were done on shoot length (Table 24 and Appendix 2).

Table 24. *P* values of repeated analyses of variances on shoot length

Source of variation	*Eleusine indica*	*Euphorbia heterophylla*	*Brassica rapa*	*Pisum sativum*	*Helianthus annuus*	*Lagerstroemia speciosa*	*Shorea roxburgii*
Between subjects							
Treatment	0.484	0.016	0.003	0.173	0.012	0.848	0.999
Intercept	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Within subjects							
Time	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Treat*Time	0.112	0.000	0.000	0.003	0.003	0.000	0.991

Note: *P* value is results of repeated measure analyses ANOVERs.

There was a significant time by treatment interaction for shoot length in all species tested except *Eleusine indica* and *Shorea roxburghii* (P>0.05). Growth patterns (Figure 12) indicate that the shoot length of all species tested at the time of lead addition were not different among treatments, but the growth rates were different within one week after treatment. The slope for increasing shoot length in the control plants was steeper than in the others.

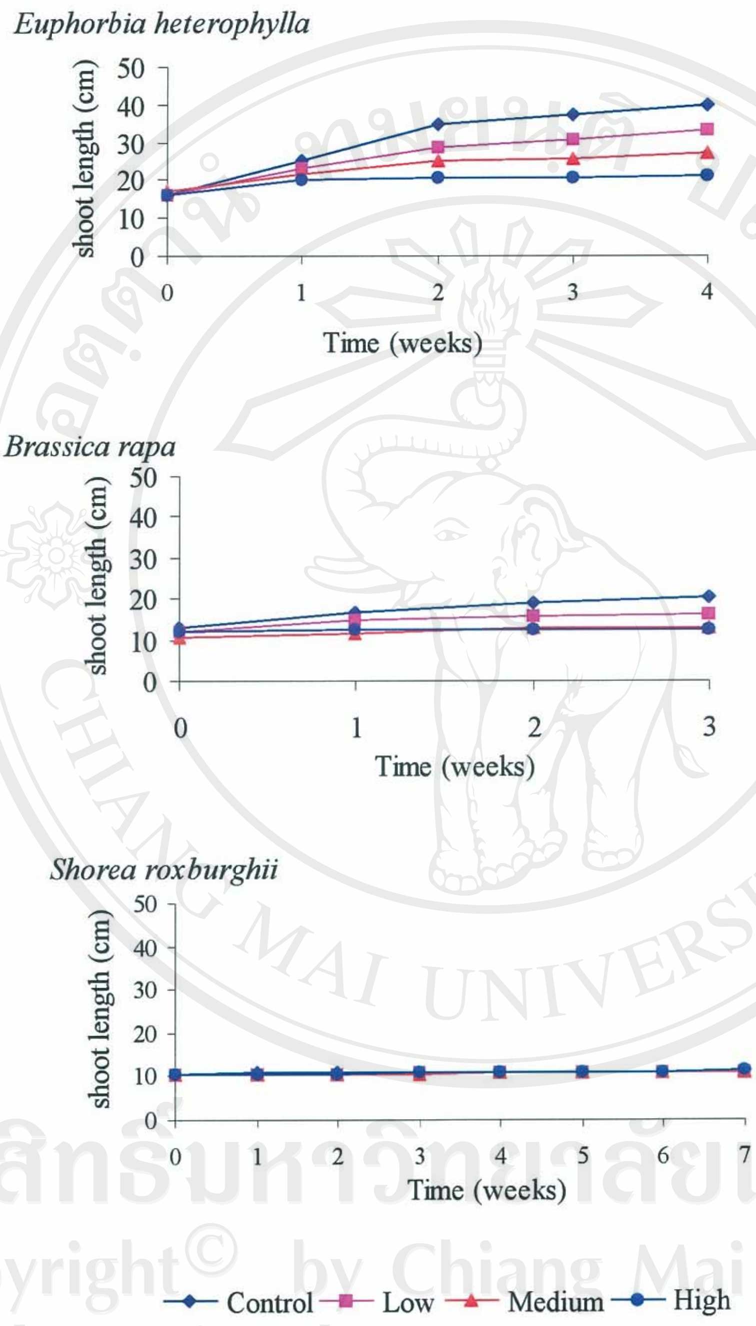

Figure 12. Shoot length plotted across the time for all lead treatments. 0 week shown on the (x) axis means one day before adding lead.

Effects of lead treatments on leaf number

The results showed that leaf number in high concentrations was significantly less than in the other treatments in all species tested. The control plants had more leaves than the others, but the difference was not significant in some species. The differences between treatments in *Shorea roxburghii* and *Pisum sativum* showed no significant difference at the 0.05 level. Apart from these two species, the others were significantly different (Table 25 and Figure 13).

Table 25. Number of leaves

Pb (μg/g)	***Eleusine indica***	***Euphorbia heterophylla***	***Brassica rapa***	***Pisum sativum***	***Helianthus annuus***	***Lagerstroemia speciosa***	***Shorea roxburghii***
0	5.5±1.0[a]	9.0±2.5[a]	8.0±1.4[a]	14.3±0.9[a]	10.0±0.8[a]	6.3±1.9[a]	6.3±0.9[a]
100	5.3±0.5[a]	5.8±1.5[ab]	6.5±1.3[ab]	11.5±2.1[a]	9.8±0.9[a]	5.0±1.2[ab]	6.3±2.4[a]
250	3.8±0.9[b]	4.8±4.5[bc]	5.8±0.5[b]	11.7±2.6[a]	7.5±1.7[b]	3.3±1.7[b]	5.8±0.9[a]
500	1.0±0.0[c]	1.5±1.0[c]	3.5±1.9[c]	11.0±2.5[a]	6.8±1.3[b]	1.5±0.6[b]	5.0±1.4[a]
F ratio	31.538	5.175	7.418	1.853	6.811	8.408	0.522
P value	0.000	0.016	0.005	0.191	0.006	0.003	0.675

Note: Results are means ± SD (n= 4). Means with different letters on the same column indicate significant differences among treatments LSD at P =0.05. F ratios are the results of one-way ANOVA analysis.

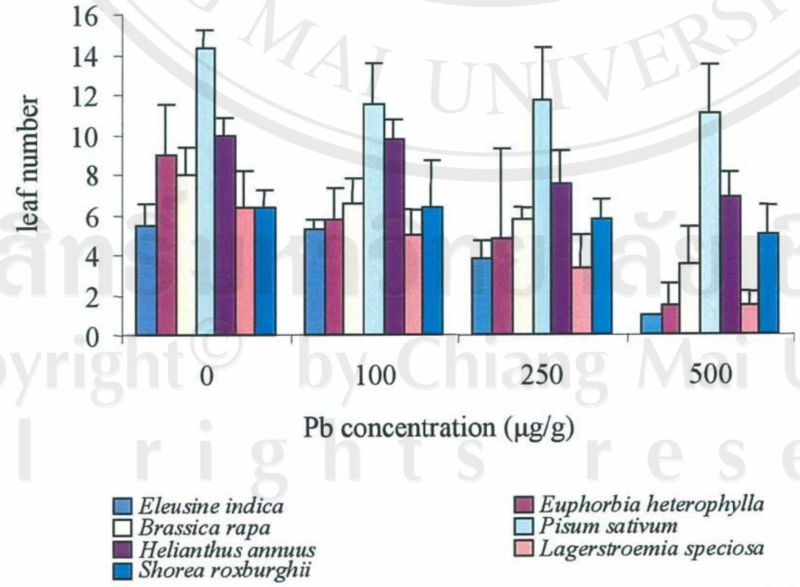

Figure 13. Effects of lead on leaf number. Error bars represent one standard deviation.

In order to investigate differences between treatments, interaction over time with repeated ANOVER tests were done on leaf number (Table 26 and Appendix 2).

There was a significant time treatment interaction for leaf number in all plants tested except *Shorea roxburghii*. For *Pisum sativum*, the control plants did not have significantly more leaves than the lead treatments at harvest time, but, *P* value <0.05 indicates that there was a significant time by treatment interaction. Growth patterns (Figure 14) indicate that all plants initially had the same number of leaves, but after treatments the leaf number of treatments were lower than those of the controls with longer exposure times. The fluctuation of slope was found in lead treatment plants, especially at high concentrations due to leaf shedding.

Table 26. *P* values of repeated analyses of variances on leaf number

Source of variation	*Eleusine indica*	*Euphorbia heterophylla*	*Brassica rapa*	*Pisum sativum*	*Helianthus annuus*	*Lagerstroemia speciosa*	*Shorea roxburgii*
Between subjects							
Treatment	0.002	0.009	0.002	0.482	0.254	0.016	0.838
Intercept	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Within subjects							
Time	0.000	0.059	0.000	0.000	0.004	0.000	0.913
Treat*Time	0.000	0.002	0.008	0.031	0.000	0.008	0.586

Note: *P* value is from repeated ANOVERs analyses.

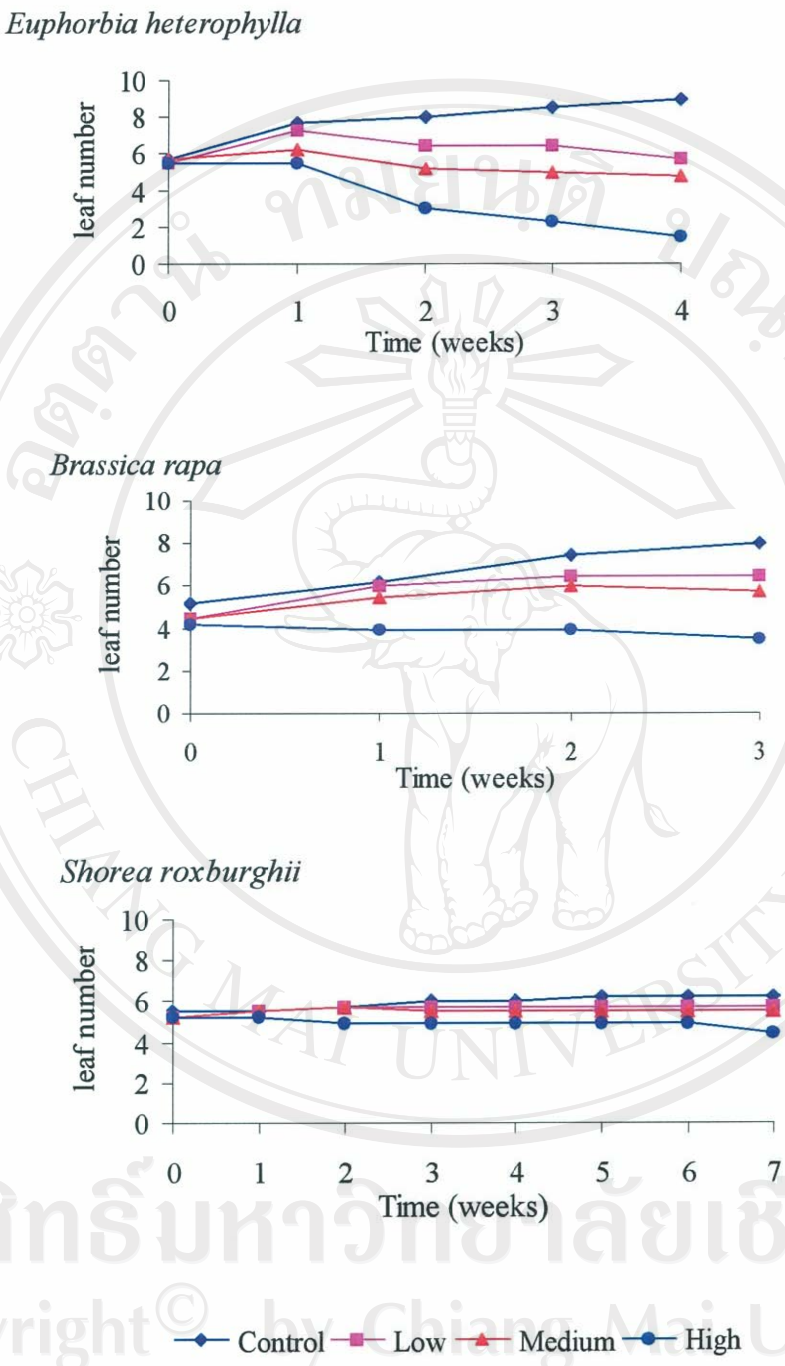

Figure 14. Leaf number plotted across time for all lead treatments. 0 week shown on the (x) axis means one day before adding lead.

Effects of lead treatments on leaf length

The leaf length of three mature leaves per plant was measured to minimize the influence of measurement error. The results showed that leaf length differences among treatments were significant in *Euphorbia heterophylla, Brassica rapa, Pisum sativum,* and *Helianthus annuus*. The other species were not significantly different (P>0.05) (Table 27 and Figure 15).

Table 27. Leaf length (cm)

Pb (µg/g)	*Eleusine indica*	*Euphorbia heterophylla*	*Brassica rapa*	*Pisum sativum*	*Helianthus annuus*	*Lagerstroemia speciosa*	*Shorea roxburghii*
0	16.4±6.4[a]	6.0±0.5[a]	8.4±1.1[a]	24.1±1.7[a]	5.0±0.4[a]	4.0±1.4[a]	4.7±1.4[a]
100	17.7±2.3[a]	5.2±0.1[b]	7.4±0.9[a]	22.0±2.1[a]	4.9±0.2[ab]	3.3±0.8[a]	4.2±0.5[a]
250	12.4±0.9[a]	3.9±0.7[c]	5.4±0.3[b]	21.6±1.8[a]	4.5±0.3[bc]	3.4±0.2[a]	4.0±1.1[a]
500	12.4±2.9[a]	3.4±0.7[c]	5.8±0.6[b]	17.9±2.7[b]	4.2±0.2[c]	3.0±0.4[a]	4.2±0.4[a]
F ratio	2.128	19.689	12.355	5.940	6.883	1.053	0.352
P value	0.150	0.000	0.001	0.010	0.006	0.405	0.788

Note: Results are means ± SD (n= 12). Means with different letters on the same column indicate significant differences among treatments LSD at P =0.05. F ratios are the results of one-way ANOVA analysis.

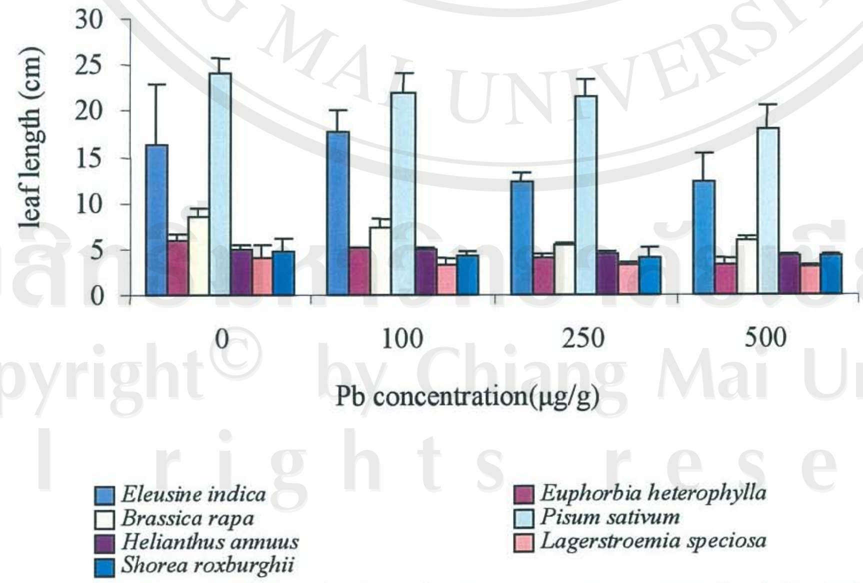

Figure 15. Effects of lead on leaf length. Error bars represent one standard deviation.

Effects of lead treatments on biomass

The effects of lead on biomass are shown in Table 28 and Figure 16. The total biomass dry weight at high concentrations was lower than that of the control in all plants tested, but significant decreases were found only in *Euphorbia heterophylla, Pisum sativum,* and *Helianthus annuus* (P<0.05).

Table 28. Total dry biomass (g)

Pb (μg/g)	***Eleusine indica***	***Euphorbia heterophylla***	***Brassica rapa***	***Pisum sativum***	***Helianthus annuus***	***Lagerstroemia speciosa***	***Shorea roxburghii***
0	$0.20\underline{+}.17^{a}$	$0.75\underline{+}.28^{a}$	$0.50\underline{+}.26^{a}$	$0.80\underline{+}.09^{a}$	$0.41\underline{+}.10^{a}$	$0.29\underline{+}.26^{a}$	$0.64\underline{+}.47^{a}$
100	$0.17\underline{+}.05^{a}$	$0.43\underline{+}.12^{b}$	$0.47\underline{+}.21^{a}$	$0.60\underline{+}.12^{ab}$	$0.38\underline{+}.03^{a}$	$0.15\underline{+}.08^{a}$	$0.47\underline{+}.14^{a}$
250	$0.11\underline{+}.02^{a}$	$0.33\underline{+}.15^{b}$	$0.31\underline{+}.06^{a}$	$0.49\underline{+}.16^{b}$	$0.36\underline{+}.09^{a}$	$0.09\underline{+}.05^{a}$	$0.54\underline{+}.33^{a}$
500	$0.13\underline{+}.07^{a}$	$0.24\underline{+}.06^{b}$	$0.27\underline{+}.07^{a}$	$0.47\underline{+}.19^{b}$	$0.24\underline{+}.05^{b}$	$0.11\underline{+}.03^{a}$	$0.40\underline{+}.08^{a}$
F ratio	0.812	6.835	1.679	4.563	4.016	1.567	0.477
P value	0.511	0.006	0.224	0.024	0.034	0.249	0.704

Note: Results are means ± SD (n= 4). Means with different letters on the same column indicate significant differences among treatments LSD at P =0.05. F ratios are the results of one-way ANOVA analysis.

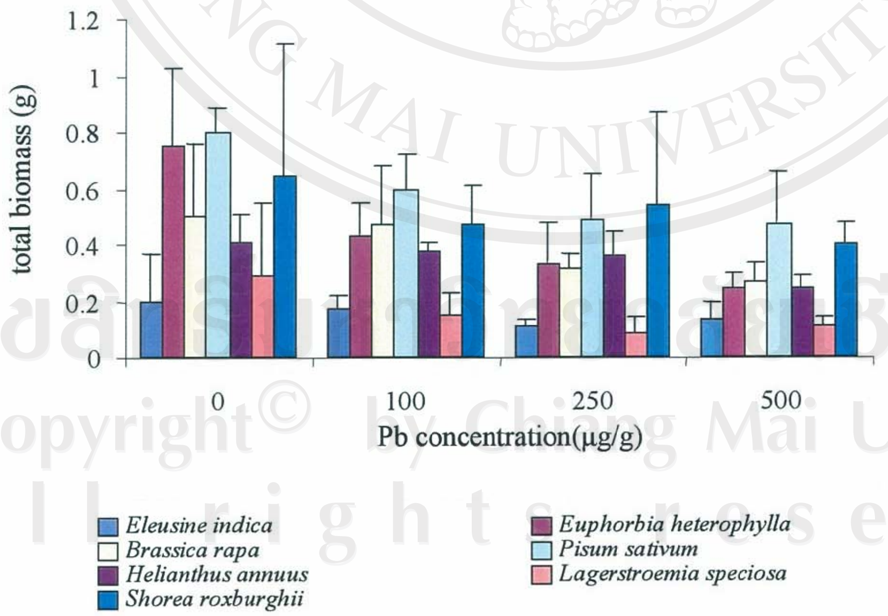

Figure 16. Effects of lead on total biomass. Error bars represent one standard deviation.

In order to study the most affected plant part, shoots (aerial parts) and root (underground) parts were separately harvested and weighed (Figure 17). The shoot biomass dry weight of *Eleusine indica*, *Lagerstroemia speciosa,* and *Shorea roxburghii* treated with lead were not significantly different from the controls as well as from each other (P >0.05) (Table 29). The biomass of *Euphorbia heterophylla, Brassica rapa, Pisum sativum,* and *Helianthus annuus* were significantly lower than those of the controls (P<0.05).

Table 29. Shoot biomass (g)

Pb (μg/g)	***Eleusine indica***	***Euphorbia heterophylla***	***Brassica rapa***	***Pisum sativum***	***Helianthus annuus***	***Lagerstroemia speciosa***	***Shorea roxburghii***
0	$0.16 \pm .14^{a}$	$0.66 \pm .25^{a}$	$0.53 \pm .07^{a}$	$0.68 \pm .08^{a}$	$0.35 \pm .10^{a}$	$0.16 \pm .14^{a}$	$0.37 \pm .23^{a}$
100	$0.13 \pm .05^{a}$	$0.36 \pm .11^{b}$	$0.42 \pm .19^{ab}$	$0.52 \pm .10^{ab}$	$0.33 \pm .02^{a}$	$0.08 \pm .04^{a}$	$0.31 \pm .11^{a}$
250	$0.07 \pm .02^{a}$	$0.29 \pm .14^{b}$	$0.27 \pm .06^{bc}$	$0.42 \pm .12^{b}$	$0.30 \pm .05^{ab}$	$0.05 \pm .02^{a}$	$0.30 \pm .15^{a}$
500	$0.09 \pm .05^{a}$	$0.20 \pm .05^{b}$	$0.24 \pm .06^{c}$	$0.40 \pm .15^{b}$	$0.21 \pm .06^{b}$	$0.07 \pm .03^{a}$	$0.28 \pm .09^{a}$
F ratio	0.837	6.580	6.189	5.103	4.148	1.705	0.216
P value	0.499	0.007	0.009	0.017	0.031	0.219	0.884

Note: Results are means ± SD (n= 4). Means with different letters on the same column indicate significant differences among treatments LSD at P =0.05. F ratios are the results of one-way ANOVA analysis.

The root biomasses of the plants tested were quite low (Table 30). The root biomass in most of the species tested decreased with lead increased concentrations, but the decreases were not significantly different from the controls as well as from each other (P>0.05). The only species that root biomass significantly decreased was *Euphorbia heterophylla* (*P<0.05*).

Although the root biomass weight of the controls and treatments were not significantly different, root deformation was recorded in all plants (Figure 18). The main deformation symptoms were reduction of primary roots length and number and length reduction of secondary roots.

Table 30. Root biomass (g)

Pb (µg/g)	*Eleusine indica*	*Eurphorbia heterophylla*	*Brassica rapa*	*Pisum sativum*	*Helianthus annuus*	*Lagerstroemia speciosa*	*Shorea roxburghii*
0	0.05±.03[a]	0.09±.03[a]	0.06±.01[a]	0.12±.02[a]	0.06±.01[a]	0.13±.12[a]	0.28±.24[a]
100	0.05±.02[a]	0.06±.00[b]	0.05±.02[ab]	0.08±.02[ab]	0.05±.01[a]	0.06±.05[a]	0.16±.05[a]
250	0.04±.02[a]	0.04±.01[b]	0.04±.01[b]	0.08±.04[ab]	0.06±.05[a]	0.04±.03[a]	0.24±.18[a]
500	0.04±.02[a]	0.04±.01[b]	0.03±.02[b]	0.07±.04[b]	0.03±.01[a]	0.04±.04[a]	0.12±.02[a]
F ratio	0.335	7.985	3.305	2.355	1.036	1.409	0.868
P value	0.800	0.003	0.057	0.123	0.412	0.288	0.484

Note: Results are means ± SD (n= 4). Means with different letters on the same column indicate significant differences among treatments LSD at P =0.05. F ratios are the results of one-way ANOVA analysis.

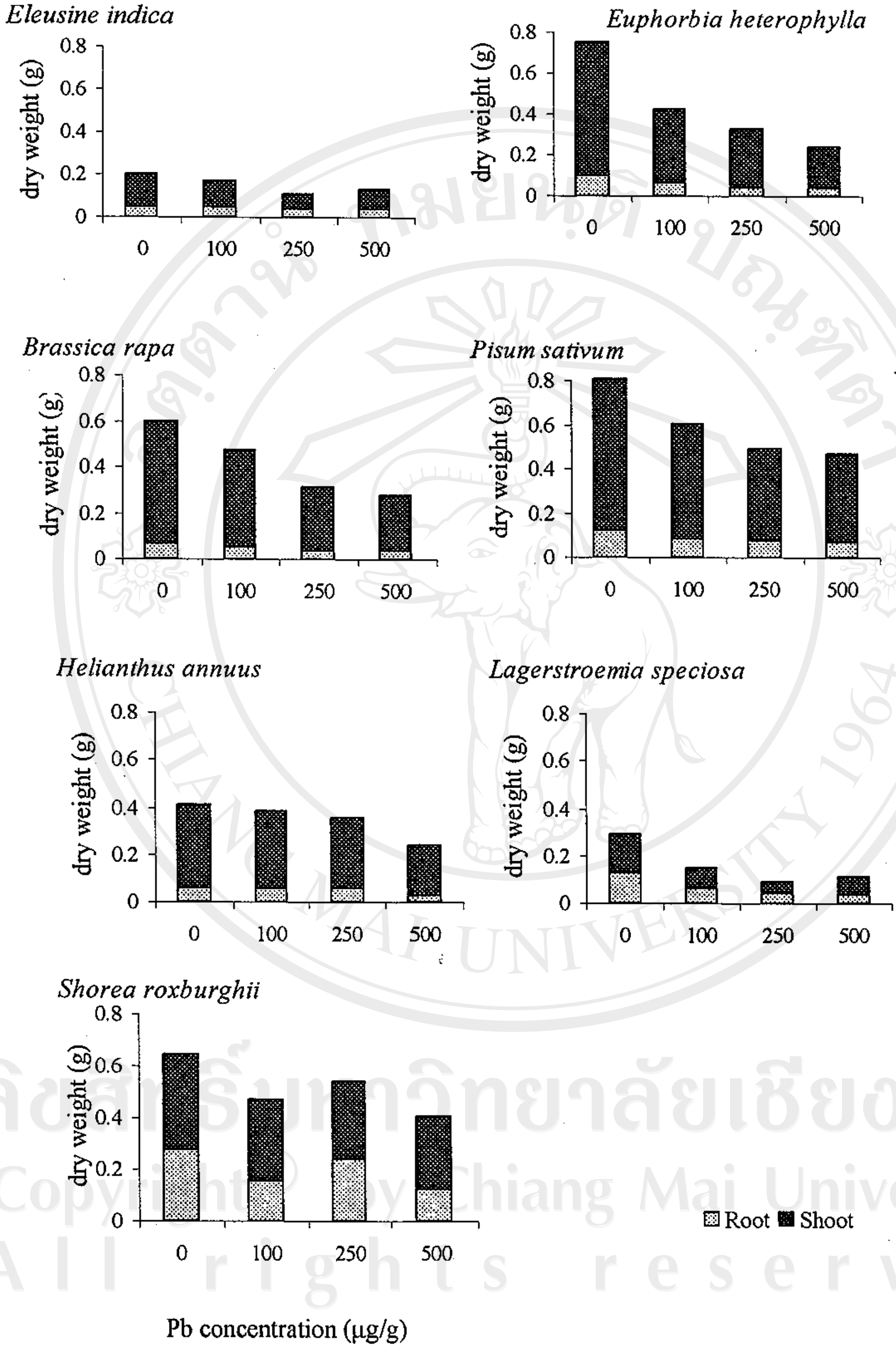

Figure 17. Relation of biomass weight of shoots and roots.

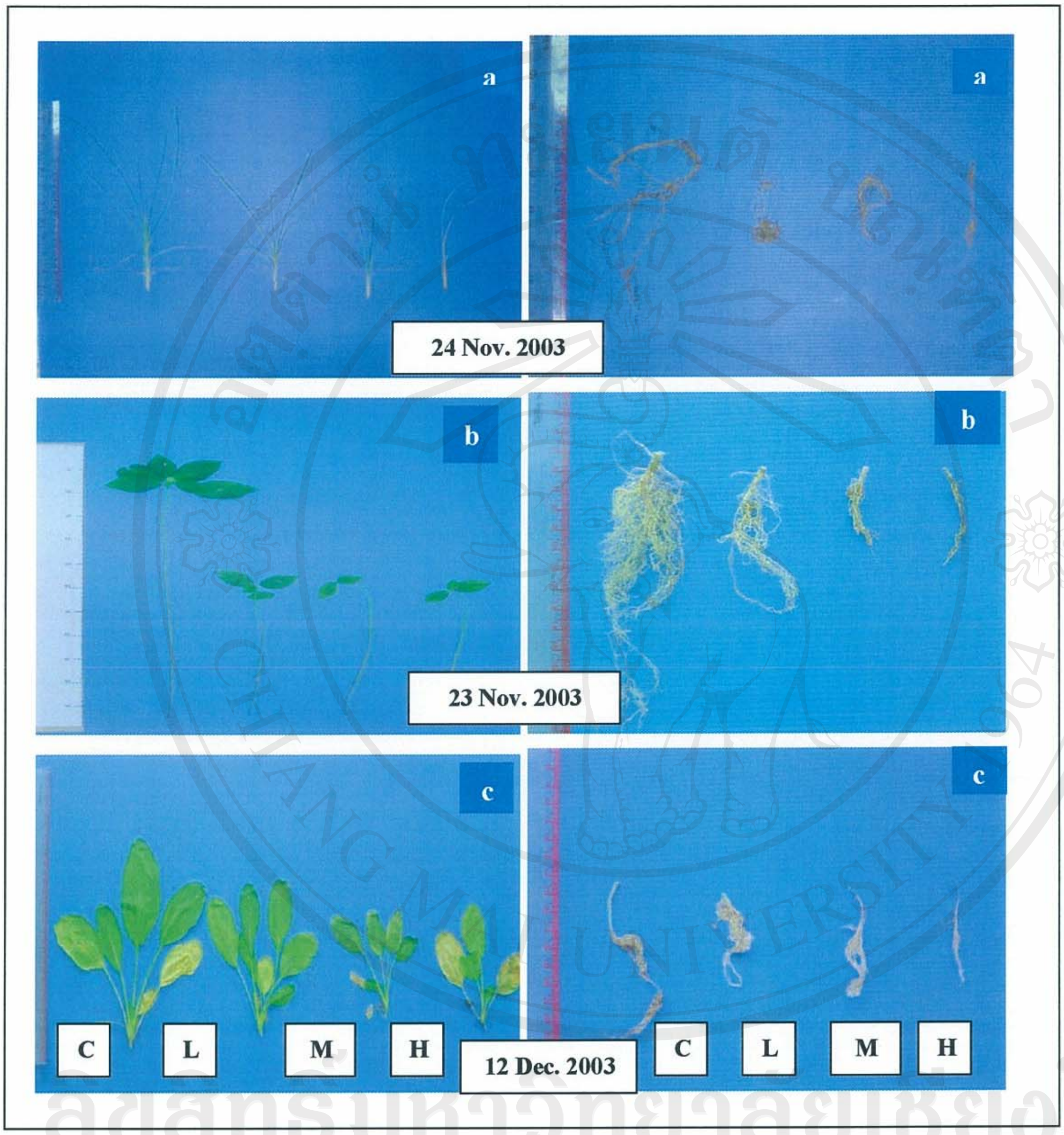

Figure 18. Effects of lead on seedling growth (from left to right-control, low, medium, and high treatment): a-- *Eleusine indica*, b-- *Euphorbia heterophylla*, c-- *Brassica rapa* (130, 70, and 42 days after germination, respectively).

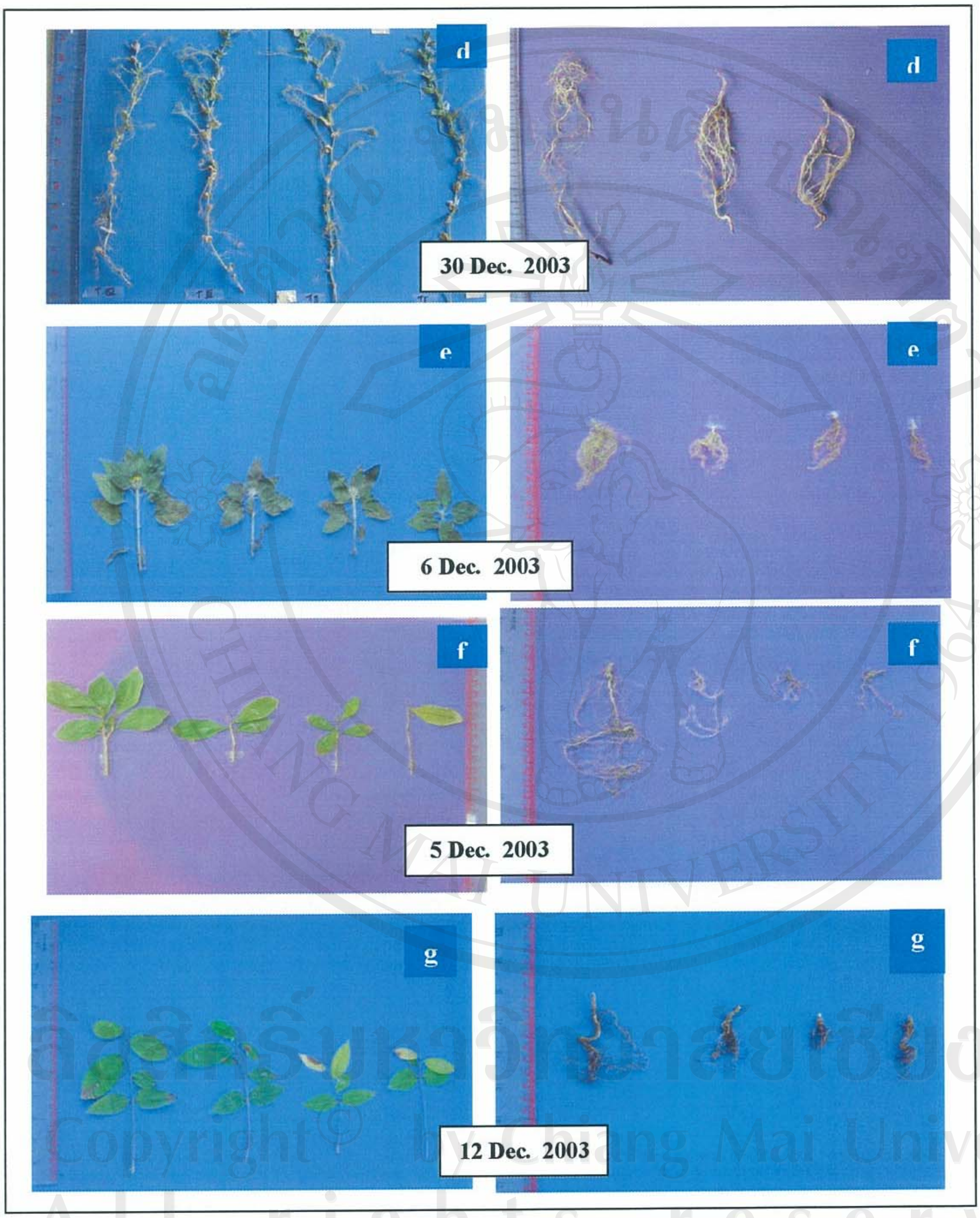

Figure 18. continued: d-- *Pisum sativum*, e-- *Helianthus annuus*, f-- *Lagerstroemia speciosa* g-- *Shorea roxburghii* (45, 40, 150, and 160 days after germination, respectively).

Effects of lead treatments on FA

The FA was measured before lead addition and at harvest time. To minimize measurement error, three mature leaves at the nearly same distance from the terminal bud per plant were measured. Before treatment, FA could be found in some plant species: *Brassica rapa* and *Helianthus annuus,* but, the differences between before and after treatments were significant in all species tested (Table 31 and Figure 19). FA symptoms appeared within two weeks after lead treatments in all species especially in young leaves. This symptom appeared more early than defoliation symptoms. FA values in low and medium lead treatments were higher than those in high lead treatments. A linear relationship of FA among treatments was not found (Table 32).

Table 31. FA differences before and after treatments

Species	Mean	SD	t	df	Sig (2 tailed)
Euphorbia heterophylla	-0.085	0.076	-4.80	15	0.000
Brassica rapa	-0.130	0.065	-7.99	15	0.000
Pisum sativum	-0.152	0.138	-4.40	15	0.001
Helianthus annuus	-0.099	0.064	-6.20	15	0.000
Lagerstroemia speciosa	-0.099	0.116	-3.41	15	0.004
Shorea roxburghii	-0.077	0.056	-5.50	15	0.000

Note: t values are the results of paired t test and n = 48.

Table 32. FA differences between lead treatments

Pb (µg/g)	*Euphorbia heterophylla*	*Brassica rapa*	*Pisum sativum*	*Helianthus annuus*	*Lagerstroemia speciosa*	*Shorea roxburghii*
0	0.01±.01[a]	0.07±.02[a]	0.03±.03 [a]	0.03±.04[a]	0.02±.04[a]	0.00±.00[a]
100	0.11±.06[b]	0.21±.04[b]	0.11±.01[ab]	0.13±.05[b]	0.22±.10[b]	0.11±.04[b]
250	0.12±.04[b]	0.18±.04[b]	0.25±.21[b]	0.09±.05[ab]	0.18±.12[b]	0.09±.03[b]
500	0.11±.08[b]	0.18±.03[b]	0.23±.08[b]	0.17±.04[b]	0.16±.03[b]	0.11±.05[b]
F ratio	4.205	14.419	3.426	7.840	4.304	8.738
P value	0.030	0.000	0.052	0.004	0.028	0.002

Note: Results are means ± SD (n=12). Means with different letters on the same column indicate significant differences among treatments LSD at P =0.05. F ratios are the results of one-way ANOVA analysis.

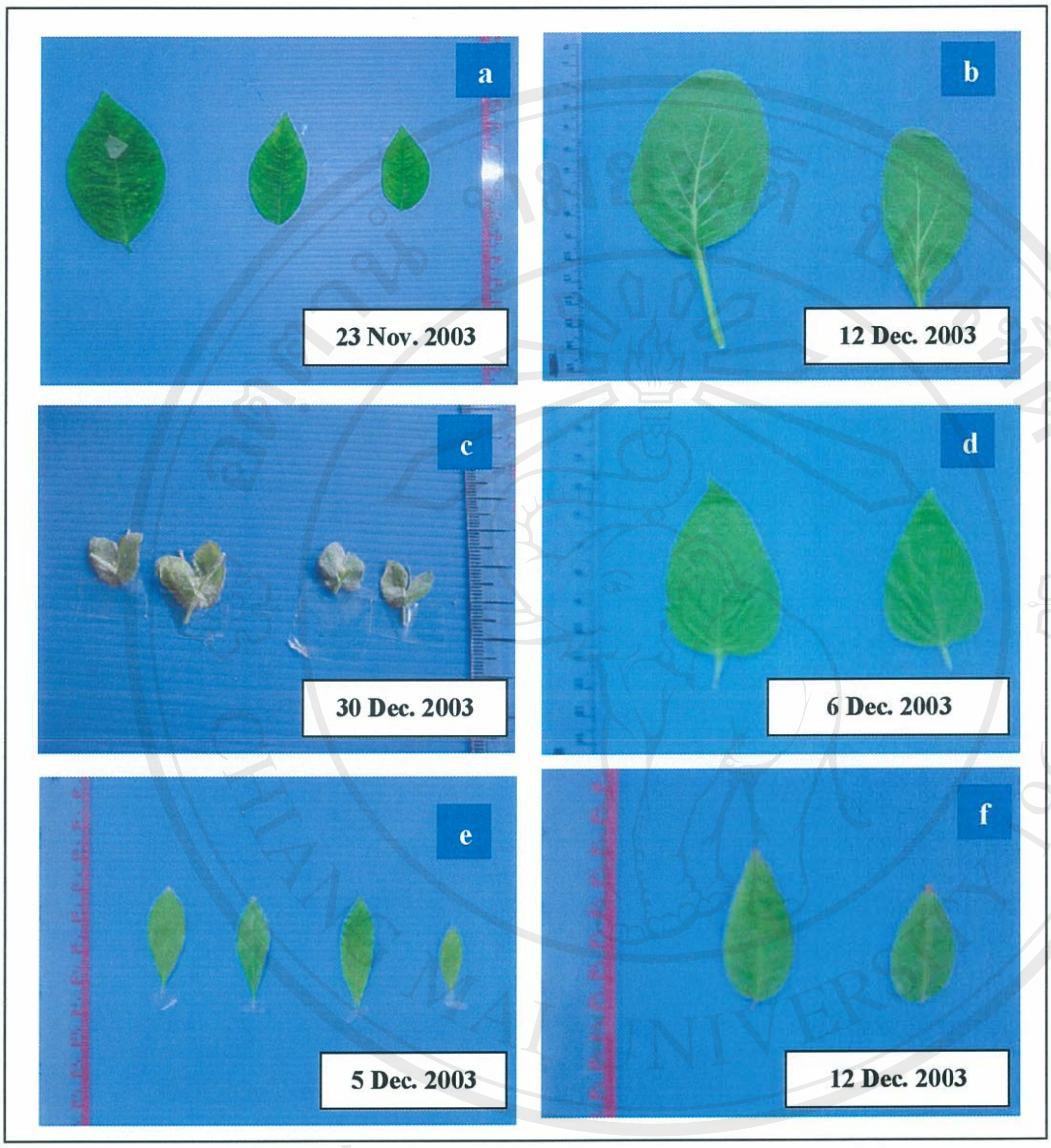

Figure 19. FA symptoms found in leaves in control (left) and lead treated plants (right): a-- *Euphorbia heterophylla*, b-- *Brassica rapa*, c-- *Pisum sativum*, d-- *Helianthus annuus*, e-- *Lagerstroemia speciosa*, and f-- *Shorea roxburghii* (70, 42, 45, 40, 150, and 160 days after germination, respectively).

Relative absolute FA (relative to the controls) was calculated to compare between the plants tested (Table 33). The higher the values indicate the more sensitivity to lead. Figure 20 shows that in contrast to total biomass, FA increased after lead treatment in all plants tested. The FA in all lead treatments was not significantly different.

Table 33. Relative absolute FA values

Pb (μg/g)	***Euphorbia heterophylla***	***Brassica rapa***	***Pisum sativum***	***Helianthus annuus***	***Lagerstroemia speciosa***	***Shorea roxburghii***	**Average**
100	18.1	3.0	3.3	4.3	10.1	0	6.6
250	20.7	2.5	8.0	2.9	8.2	0	7.0
500	18.5	2.6	7.2	5.8	7.5	0	7.0

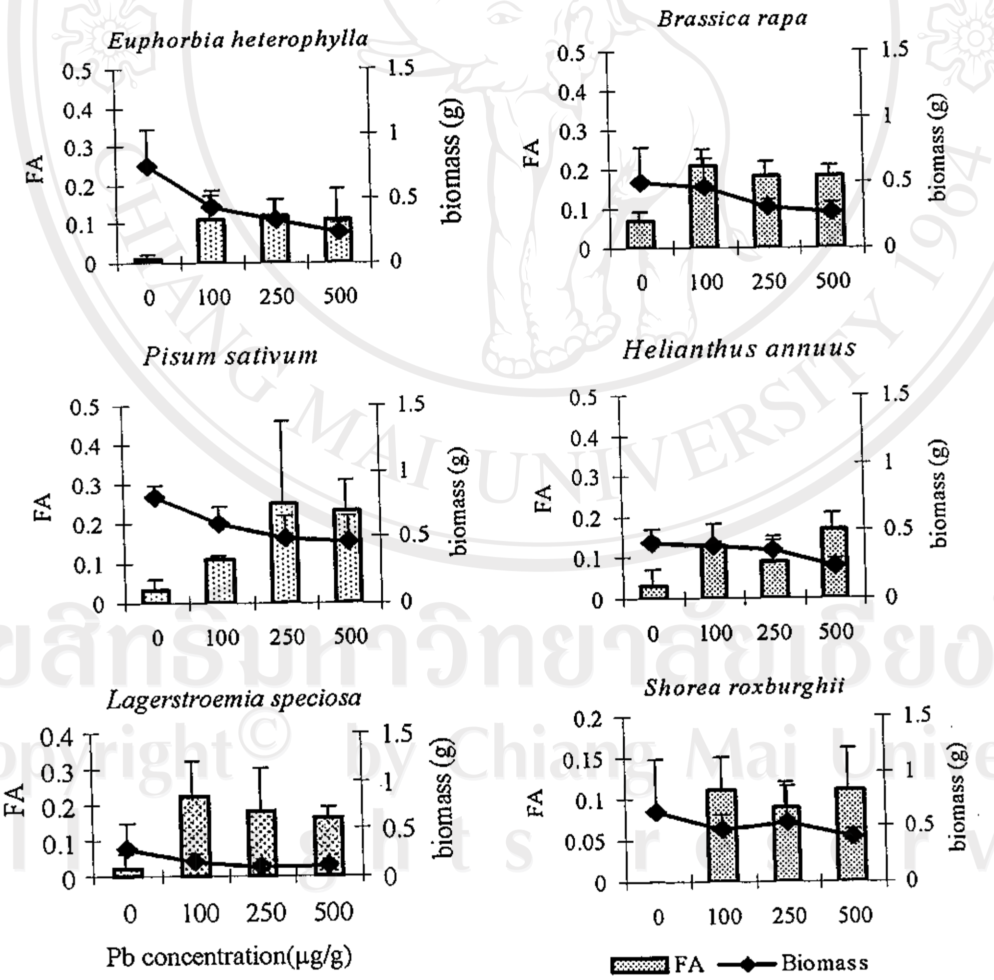

Figure 20. Comparison of the effects of lead on changes in total biomass and FA).

Inhibitory effects of lead on seedling growth

All seedling growth parameters, *viz*. shoot length, leaf number, leaf length, and biomass were negatively affected by lead treatments. The inhibitory symptoms of lead treatments on the plants tested are shown in Figures 19 and 21 indicate that the degree of inhibition by lead depends on the plant species. Visible inhibition symptoms appeared more clearly with longer exposure times. The main inhibition symptoms found in plants at high lead treatment were defoliation; chlorosis, especially in young leaves, and stunting.

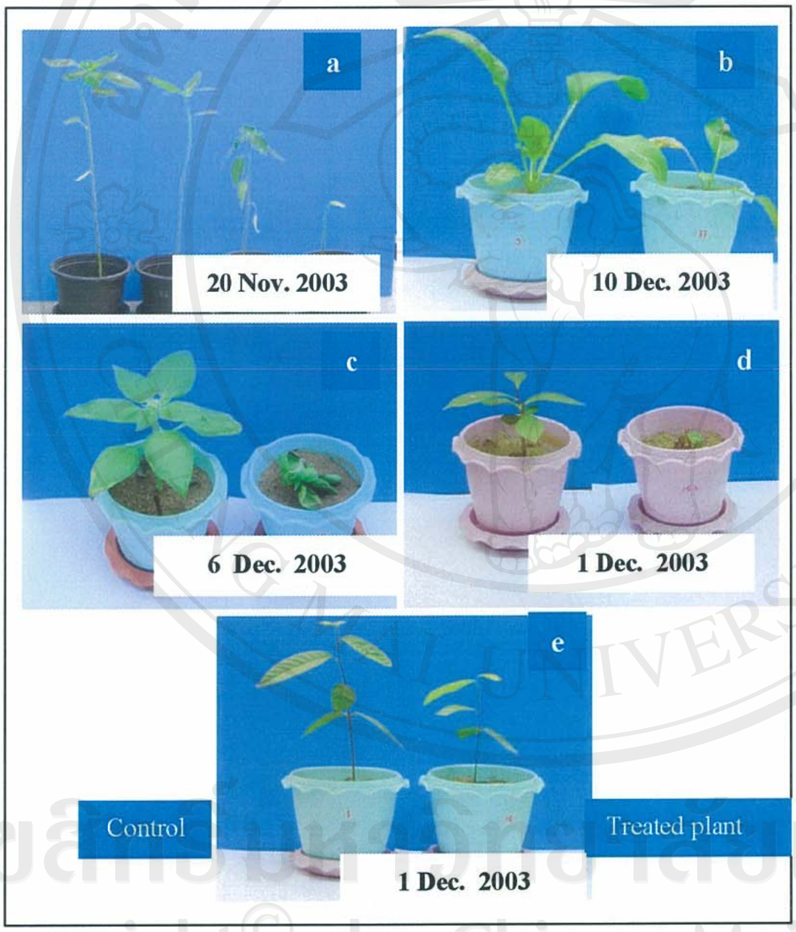

Figure 21. Lead phytotoxicity symptoms compared with control plants; a-- *Euphorbia heterophylla*, b-- *Brassica rapa*, c-- *Helianthus annuus*, d-- *Lagerstroemia speciosa*, and e-- *Shorea roxburghii* (70, 40, 40, 150, and 139 days after germination, respectively).

In order to compare tolerance to lead toxicity, many biomonitoring indices such as inhibition percentage, tolerance index, shoot and root biomass ratio, *etc.* have been used. In my study, inhibition percentage was calculated to identify the most sensitive growth parameter and indices related to biomass, *viz.* percent biomass dry weight and relative yields were calculated to identify tolerance.

Inhibition percentage

Inhibition percentages were calculated for all growth parameters in all plants tested. Based on the inhibition percentages shown in Table 34, sensitivity of growth parameters and EC_{50} values could be identified.

Table 34. Inhibition percentages in the plants tested

Parameters/ Pb treatment	*Eleusine indica*	*Euphorbia heterophylla*	*Brassica rapa*	*Pisum sativum*	*Helianthus annuus*	*Lagerstroemia speciosa*	*Shorea roxburghii*
Shoot length							
Low	21.7	27.4	40.0	37.9	1.8	4.1	5.1
Medium	41.3	58.5	64.7	48.7	65.7	56.6	23.4
High	68.8	80.5	93.5	52.6	76.8	83.5	20.9
EC_{50}	>250	<250	>100	<500	<250	<250	>500
Leaf number							
Low	4.5	36.1	18.8	19.3	2.5	20.0	-19.0
Medium	31.8	47.2	28.1	17.5	25.0	48.0	-9.5
High	81.8	83.3	56.3	22.8	32.5	76.0	4.8
EC_{50}	>250	>250	<500	>500	>500	>250	>500
Leaf length							
Low	7.5	13.7	12.4	8.6	2.6	17.3	9.7
Medium	24.5	35.0	35.3	10.4	11.4	15.3	14.2
High	24.6	42.9	31.6	25.6	17.13	25.1	10.8
EC_{50}	>500	>500	>500	>500	>500	>500	>500
Biomass							
Low	15.1	43.3	4.8	25.4	8.4	49.4	27.4
Medium	46.0	56.6	37.4	38.6	13.8	67.2	15.8
High	36.5	68.3	44.3	42.2	41.6	61.0	37.4
EC_{50}	>500	<250	>500	>500	>500	>100	>500

Percent of dry biomass weight

It is interesting to note that the dry and fresh biomass weights decreased with increased lead concentrations. This is in contrast with the percent of dry biomass weight, which is the ratio of dry and fresh weights, which increased with higher lead concentrations (Table 35 and Figure 22). Statistical differences were not found except in *Eleusine indica.*

Table 35. Percent of dry biomass weight

Pb (µg/g)	*Eleusine indica*	*Eurphorbia heterophylla*	*Brassica rapa*	*Pisum sativum*	*Helianthus annuus*	*Lagerstroemia speciosa*	*Shorea roxburghii*
0	51±15[a]	26± 3[a]	7±3[a]	15±2[a]	16± 1[a]	29± 9[a]	52±5[a]
100	55± 7[ab]	24± 1[a]	9±2[a]	16±4[a]	14± 2[a]	34± 4[a]	48±3[a]
250	68±11[b]	42±28[a]	13±2[ab]	16±2[a]	25±11[a]	32±12[a]	50±2[a]
500	74± 7[c]	50±33[a]	18±9[b]	16±1[a]	19± 9[a]	41± 7[a]	50±2[a]
F ratio	4.199	1.333	3.305	0.164	1.722	1.437	1.160
P value	0.030	0.310	0.058	0.919	0.215	0.281	0.365

Note: Results are means ± SD (n= 4). Means with different letters on the same column indicate significant differences among treatments LSD at P =0.05. F ratios are the results of one-way ANOVA analysis.

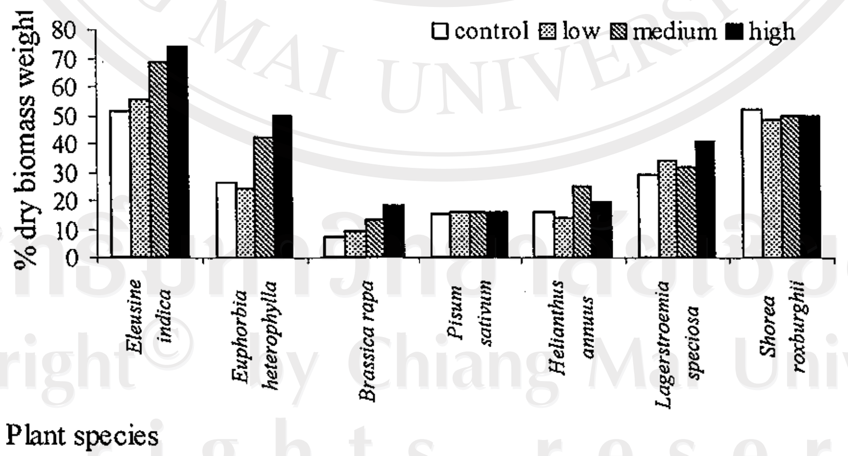

Figure 22. Effects of lead on the percent of dry biomass weights.

The relative percent of dry biomass weight (relative to the controls) was calculated to compare between the plants tested (Table 36). Higher relative percent of dry biomass weight values indicate more sensitivity to lead.

Table 36. Relative percent of dry biomass weight

Pb (µg/g)	***Eleusine indica***	***Euphorbia heterophylla***	***Brassica rapa***	***Pisum sativum***	***Helianthus annuus***	***Lagerstroemia speciosa***	***Shorea roxburghii***	**Avg**
100	1.08	0.92	1.29	1.06	0.88	1.17	0.92	1.04
250	1.33	1.62	1.86	1.06	1.56	1.10	0.96	1.36
500	1.45	1.92	2.57	1.06	1.19	1.41	0.96	1.51

Relative biomass yield

In order to study the correlation between total biomass weight, the most sensitive parameter, and different lead treatments, relative biomass yield can be used as a growth index (Table 37 and Figure 23). The higher the value of relative biomass yield indicates more tolerance to lead. r^2 values of the herbaceous plant group, except *Eleusine indica*, indicate that the relative yield and lead treatments were highly negatively correlated (0.472- 0.051), but those of the other species correlated poorly (0.003- 0.009).

Table 37. Relative biomass yields

Pb (µg/g)	***Eleusine indica***	***Euphorbia heterophylla***	***Brassica rapa***	***Pisum sativum***	***Helianthus annuus***	***Lagerstroemia speciosa***	***Shorea roxburghii***	**Avg**
100	0.849	0.567	0.952	0.746	0.916	0.506	0.726	0.75
250	0.540	0.434	0.626	0.614	0.862	0.328	0.842	0.61
500	0.635	0.317	0.557	0.578	0.584	0.390	0.626	0.53
r^2	0.003	0.277	0.051	0.111	0.472	0.009	0.008	

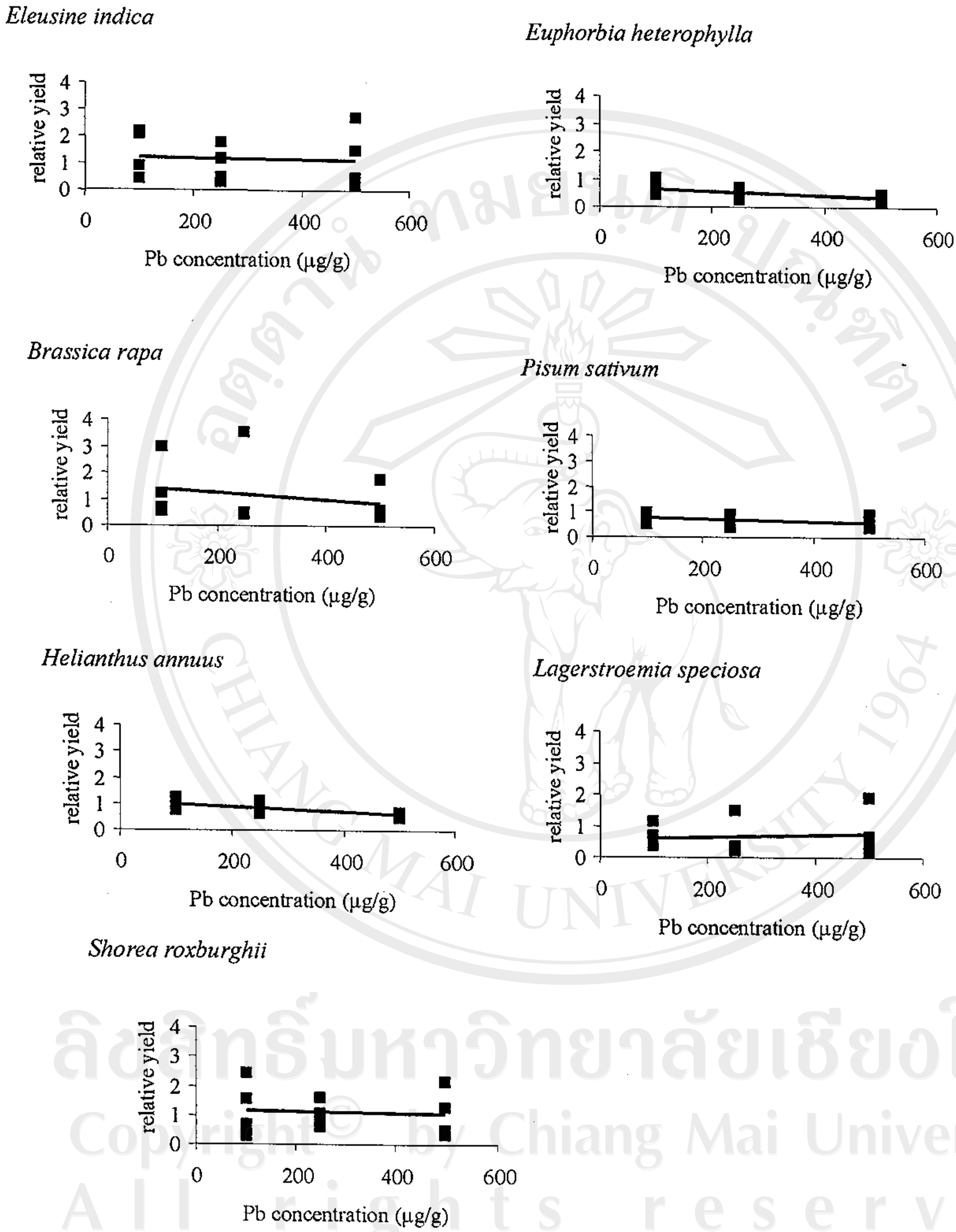

Figure 23. Correlation between relative yield and lead treatments.

Lead analysis in plant tissues

Based on the seedling growth test results, the descending order to lead tolerance in terms of relative yield were *Euphorbia heterophylla, Lagerstroemia speciosa, Brassica rapa, Pisum sativum, Helianthus annuus, Eleusine indica,* and *Shorea roxburghii.* In order to study lead translocation in plant tissues, three species that were the most, intermediate, and least sensitive to lead were selected for lead analysis. *Euphorbia heterophylla, Brassica rapa,* and *Shorea roxburghii* were selected as the most, intermediate, and least sensitive species, respectively.

Microwave digestion

In order to validate the extraction efficiency of the microwave system, 100 ppm of lead standard solution was added to all samples including the controls and lead treated plant tissues before digestion. The percent recovery was calculated by comparing the spiked amounts of lead and lead concentrations in the control samples. The percent recovery of lead extraction from plant tissue was higher than 85% (Table 38).

Table 38. Percent recovery of lead extracted by microwave digestion

Plant species	Number	Percent recovery (%)	Mean	SD
Euphorbia heterophylla	1	87	86.5	2.5
	2	85		
Brassica rapa	1	92	90.0	8
	2	88		
Shorea roxburghii	1	87	85.0	8
	2	83		

Lead analysis

Calibration curve of lead

The calibration curve of lead was constructed by dilution of different concentrations of standard lead solution (10-50 ppm). Absorbance of each concentration was measured (Table 39). Standard lead concentrations were plotted against AAS absorbance (Figure 24).

Table 39. Absorbance of different concentrations of lead by AAS

Concentration (ppm)	Absorbance
10	0.101
20	0.193
30	0.240
40	0.336
50	0.433

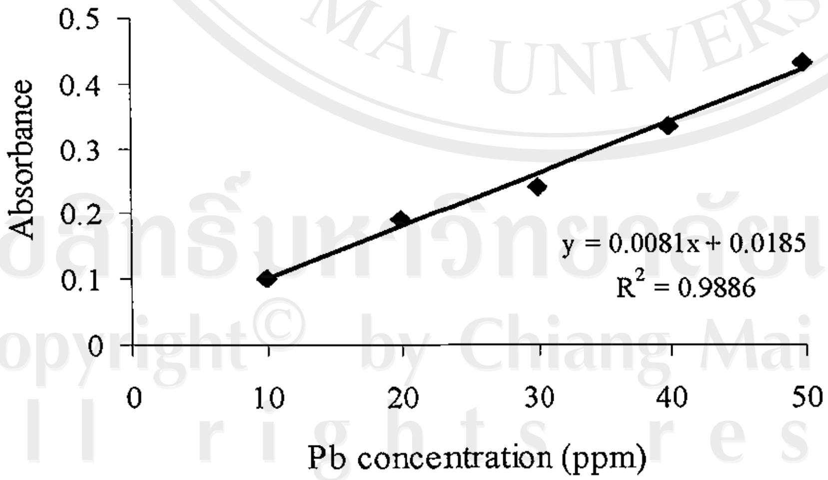

Figure 24. Calibration curve of lead standards

Precision test for quality control

The analytical methods applied for lead analysis in plant tissues was controlled by a precision test. The results of four replications were calculated for the coefficient of variation of analysis (CV) and are listed in Table 40. CV values indicate that the difference between repeatability of lead content in plant tissues was quite high.

Table 40. Precision test for analytical methods (CV %)

Pb treatment (µg/g)	***Euphorbia heterophylla***	***Brassica rapa***	***Shorea roxburghii***
100	11.9	8.9	15.4
250	5.1	7.8	7.1
500	7.0	2.7	2.6

Lead concentration in plant tissues

The lead concentrations in roots and shoots of three plant species are listed in Table 41 (Appendix 3). In all the roots and shoots, the mean lead concentrations increased with more lead added. Lead concentrations in *Euphorbia heterophylla* were higher than the others.

Table 41. Lead content in roots and shoots (µg /g)

Pb (µg/g)	***Euphorbia heterophylla***		***Brassica rapa***		***Shorea roxburghii***	
	Shoot	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
0	0.0$\pm$0.0^{a}	0.0$\pm$0.0^{a}	0.0$\pm$0.0^{a}	0.0$\pm$0.0^{a}	0.0$\pm$0.0^{a}	0.0$\pm$0.0^{a}
100	112$\pm$16^{a}	316$\pm$35^{a}	0.0$\pm$0.0^{a}	112$\pm$10^{b}	5$\pm$4^{a}	52$\pm$8^{b}
250	1143$\pm$8^{b}	2100$\pm$157^{b}	31$\pm$10^{b}	664$\pm$44^{c}	7$\pm$5^{a}	232$\pm$12^{c}
500	3240$\pm$175^{c}	2875$\pm$254^{c}	165$\pm$16^{c}	872$\pm$12^{d}	107$\pm$10^{b}	930$\pm$17^{d}
F ratio	581.487	169.508	140.783	653.253	131.002	2755.37
P value	0.000	0.000	0.000	0.000	0.000	0.000

Note: Results are means $\pm$ SD (n=2). Means with different letters on the same column indicate significant differences among treatments LSD at P =0.05. F ratios are the results of one-way ANOVA analysis.

Lead concentration in plants is greatly affected by both lead treatments and the plant tissue as well as by the interaction between these two factors ($P<0.05$) in all plant tested, except *Euphorbia heterophylla* ($P > 0.05$) (Table 42). This means that lead concentrations in plants depends on not only lead levels in the growth medium and plant tissues, but also the interaction of these two factors ($P \leq 0.006$).

The lead concentrations in roots was significantly higher than in shoots except for *Euphorbia heterophylla* at high lead levels. The correlation between lead concentrations and lead content in plants was also different between different plant species (r^2 values: 0.958, 0.763 and 0.697).

Table 42. Summary of multivariate tests for lead concentrations in plant tissues

Source	df	MS	F ratio	*P* value	r^2
		Euphorbia heterophylla			
Treatment	3	8051873	84.01	0.000	0.958
Plant tissue	1	158587	1.66	0.225	
Intercept	1	23943895	249.83	0.000	
error	11	95840			
		Brassica rapa			
Treatment	3	240590	6.80	0.007	0.763
Plant tissue	1	527366	14.96	0.003	
Intercept	1	852113	24.17	0.000	
error	11	35258			
		Shorea roxburghii			
Treatment	3	231097	5.90	0.012	0.697
Plant tissue	1	299958	7.66	0.018	
Intercept	1	445976	11.39	0.006	
error	11	39163			

Lead uptake and translocation in plants

In order to investigate lead uptake, the transfer coefficient (K), which is the ratio of lead concentration in shoots and in the sand was calculated. To study lead translocation in plant tissues (lead translocation from root to shoot), the translocation factor (TF) was calculated.

The results are shown in Table 43 and Figure 25. The K values and TF of *Euphorbia heterphylla* at all lead concentrations were highest, followed by those of *Brassica rapa* and *Shorea roxburghii.* These values increased with increased lead concentrations in the sand, except for *Shorea roxiburghii.*

Table 43. K values and TF

Pb (µg/g)	K value			TF		
	Euphorbia heterophylla	*Brassica rapa*	*Shorea roxburghii*	*Euphorbia heterophylla*	*Brassica rapa*	*Shorea roxburghii*
100	1.12	0.00	0.05	0.35	0.00	0.09
250	4.57	0.12	0.03	0.55	0.05	0.04
500	6.48	0.33	0.22	1.13	0.19	0.12

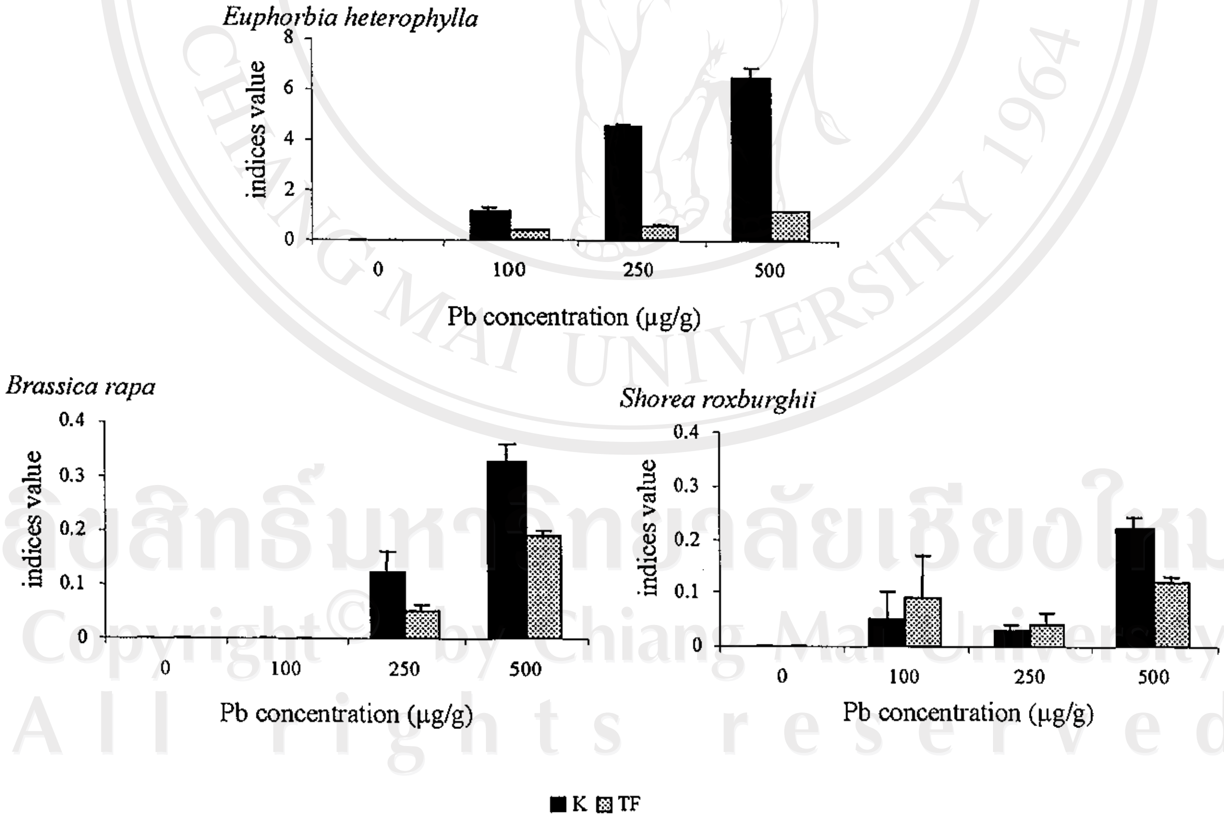

Figure 25. K value and TF of a-- *Euphorbia heterophylla*, b-- *Brassica rapa*, c-- *Shorea roxburghii*.

Correlation of lead content in plant tissues and biomass weight

Correlation tests were done to find out the correlation of lead contents in plant tissues and biomass weight. The results from these tests show that there were no significant correlation between lead contents in different plant parts and biomass weight (Appendix 4).