CHAPTER IV

RESULTS AND DISCUSSION

The capacities of *Chromolaena odorata* (siam weed) and *Vetiveria zizanioides* (vetiver grass) in removal of heavy metals from contaminated soil were investigated in this study. The study was classified into 2 sections; 1) The heavy metal removal capacity of those two plants grown in contaminated soil collected from Phra That Pha Daeng sub-district, Mae Sot district, Tak province and 2) The capacity of those two plants to removal heavy metals in the synthetic soil (uncontaminated soil adding with heavy metals (Cd, Zn, Pb and Cu) solution at the concentration of 100 mg/kg in each heavy metal). Soil and plant samples were collected at the harvest time of 30, 60, 90 and 120 days, respectively.

4.1 Heavy metals removal from contaminated soil by using *C. odorata* and *V. zizanioides*

4.1.1 Concentration of total heavy metals in contaminated soil

In this study, the concentrations of heavy metals in contaminated soil of *C. odorata* and *V. zizanioides* were investigated. The results shows that the concentration of heavy metals slightly decrease by time during the growth period (from 30-120 days) due to heavy metals in soil taken up by plants. The heavy metals concentration in contaminated soil of *C. odorata* were in ranges of 42.25-44.03, 1,976.54-2,014.24, 82.94-85.67 and 23.07-23.92 mg/kg on a dry weight basis for Cd, Zn, Pb and Cu, respectively (Appendix C.1). Alternatively, the heavy metals concentration in contaminated soil of *V. zizanioides* pot were in ranges of 41.78-42.47, 1,979.46-2,045.26, 80.06-81.01 and 23.58-24.49 mg/kg on a dry weight basis for Cd, Zn, Pb and Cu, respectively (Appendix C.2).

The highest concentration of heavy metals in contaminated soil was observed in 30 days of harvesting time in both *C. odorata* and *V. zizanioides* pot. Using ANOVA, statistical analysis, it was shown that concentration of Cd, Zn, Pb and Cu was no signification difference between the harvesting time as shown in Appendices D.1 and D.2.

Yanqun et al. (2005) reported that the concentrations of heavy metal in soil should have effect on the concentration in plants. Kabata-Pendias and Pendias (1984) also supported that the metals concentration in soils was depleted slowly by plant uptake. From the results it was found that Zn concentration was higher than Cd, Pb and Cu, possibly due to soil samples background mostly had high Zn content in its constituents (Table 3.3). Therefore, it showed the concentration of Zn highest in contaminated soil of both *C. odorata* and *V. zizanioides* pot.

4.1.2 Phytoavailability of heavy metals in contaminated soil

After sample collection in the 30, 60, 90 and 120 days of both *C. odorata* and *V. zizanioides* cultivation, contaminated soil samples were determined for the availability of heavy metals by using the DTPA extraction method. Figure 4.1 and 4.2 illustrated the concentration of availability of heavy metals in the contaminated soil. As the result shown in Figure 4.1, the availability of heavy metals in contaminated soil of *C. odorata* pot decreased by time during the growth period. The heavy metals availability in contaminated soil were in ranges of 4.69-5.43, 201.33-218.83, 5.77-6.97 and 2.03-2.19 mg/kg for Cd, Zn, Pb and Cu, respectively (Appendix C.3). The highest available metal concentration in contaminated soil was observed in the first 30 days of harvesting time. By using ANOVA, statistical analysis, it was shown that Pb availability in soil was signification difference between the harvesting time ($p<0.05$) as shown in Appendix D.3. The numbers (a and b) above the bars in Figure 4.1 are groups of the means that were significantly statistical according to the Duncan's test.

Figure 4.2 illustrated that the heavy metals availability in contaminated soil slightly decrease by harvesting time. The heavy metals availability in contaminated soil of *V. zizanioides* were in ranges of 3.87-5.52, 174.05-221.25, 5.83-6.82 and 2.04-2.08 mg/kg for Cd, Zn, Pb and Cu, respectively (Appendix C.4). The highest availability of those metals in contaminated soil was also in the first 30 days of harvesting time. From statistical analysis, the heavy metals availability and the harvesting time was no signification difference as shown in Appendix D.4.

The mentioned above concluded that heavy metals availability in contaminated soil of both *C. odorata* and *V. zizanioides* slightly decrease by time during the growth period due to heavy metals in soil taken up by plant. Zn availability was higher than Pb, Cd and Cu in the contaminated soils due to high background level of Zn. Therefore, the highest of available Zn in contaminated soil has been shown in both *C. odorata* and *V. zizanioides*. Moreover, the availability of heavy metals in contaminated soil of both *C. odorata* and *V. zizanioides* were relatively low available because the soil samples used in this experiment were slightly alkaline condition. According to the soil properties of the studied soils, the high organic matter (with the average of 3.26%) and neutral or near alkaline soil (pH range from 7.21-7.68 and 7.49-7.86 for *C. odorata* and *V. zizanioides*, respectively) might cause the low availability of metals in the studied soils. Alloway (1990) also supported to the extractable metals concentration decreased as pH increase due to the process of hydrolysis and adsorption. Organic matter is one of important in transportation and accumulation of metallic ions which affect the availability of metals due to it acts as an important sorbent (Kabata-Pendias and Pendias, 1984). Soil pH affects metal sorption and desorption on the solid phases, thus influence on availability of the metal uptake by plants. Jung and Thruton (1996) and Rosselli et al. (2003) reported that the mobility and availability of heavy metals in the soil are generally low, especially when the soil pH is high.

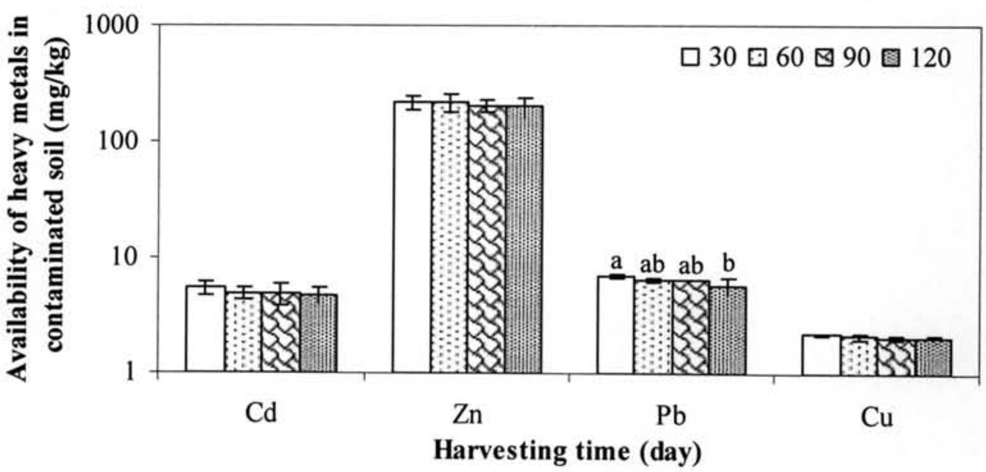

Figure 4.1 Available Cd, Zn, Pb and Cu in contaminated soil planted with *C. odorata*

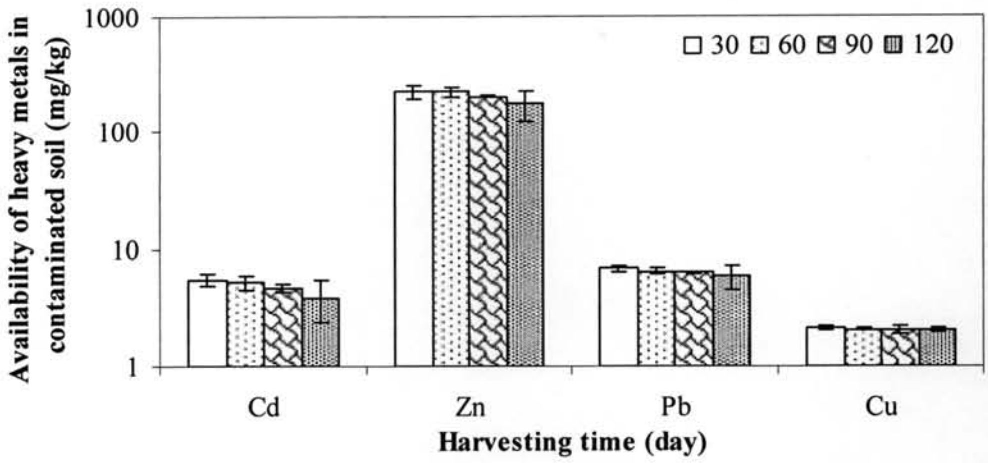

Figure 4.2 Available Cd, Zn, Pb and Cu in contaminated soil planted with *V. zizanioides*

4.1.3 Phytotoxicity of heavy metals in contaminated soil

The phytotoxicity of *C. odorata* and *V. zizanioides* grown in heavy metals in contaminated soil was also studied. The results show that both *C. odorata* and *V. zizanioides* could survive under heavy metals concentration in the contaminated soil but it was obviously exhibited abnormal characteristic in the plants as shown by a chlorosis, curly, spotted and scorching leaves (Appendix E.5).

Table 4.1 and 4.2 showed the height and relatively growth rate (RGR) of *C. odorata* and *V. zizanioides*, respectively. RGR is expressed as the percentage relative to the RGR in control soil which represent 100 %. The dry weight of both plants is given in Appendix C.33. The results showed that the heights of *C. odorata* and *V. zizanioides* were increasing with the length of harvest time. From 30.1±1.0 to 97.2±7.0 cm for *C. odorata* in contaminated soil and 29.1±2.9 to 65.0±2.6 cm. for *V. zizanioides* in contaminated soil, respectively.

Relative Growth Rate (RGR) can be expressed as the rate of plant growth with time, or the difference of plant weight increasing with time. From RGR, it was found that at harvest time of 30 days, the RGR of *C. odorata* and *V. zizanioides* decrease to 82.62% and 53.61% as control, respectively. However, it becomes increase again with the length of harvest time because all treated plant continued to produce new leave. The highest of RGR was occurred at 90 days which equal to 95.60% for *C. odorata* and 66.41% for *V. zizanioides* at 60 days of harvest time. However, RGR of *C.*

odorata and *V. zizanioides* reduced at 120 days and 90 days of harvesting time, respectively, because of the toxicity of the plant. Tanhan et al. (2007) showed the similar results as the reduction of RGR caused by the toxicity as can be seen from necrotic and cholrosis symptoms. The height of the plants increases so the weight of the plants increased which in turn cause the RGR to be increased. Sasithorn (2006) showed the similar results as the height of *V. zizanioides* increased, the weight and the RGR also increased (p<0.05).

As the results, it can be seen that both *C. odorata* and *V. zizanioides* could survive under concentration of heavy metals in contaminated soil but display symptoms of toxicity. The visible symptoms in plants may cause by the high metal concentration in the soil samples. In general, critical concentrations of trace metal in plant tissue were 5-10, 15-20 and 20-30 ppm dry weight for Cd, Cu and Zn, respectively (Kabata-Pendias and Pendias, 1987). Therefore, this high metal concentration might probably cause the toxicity to the plants.

Table 4.1 Height and Relative growth rate (RGR) of *C. odorata* in contaminated soil

Harvesting time (day)	Height (cm)		Relative growth rate (RGR)		RGR (% as control)
	control soil	contaminated soil	control soil	contaminated soil	
30	35.2±.2.1*	30.1±1.0*	0.0264	0.0217	82.26
60	68.4±2.9	45.6±2.4	0.0238	0.0218	91.68
90	82.4±2.7	68.2±2.6	0.0211	0.0202	95.60
120	101.7±4.0	97.2±7.0	0.0212	0.0194	91.41

*Mean ± Standard deviation (n = 3)

Table 4.2 Height and Relative growth rate (RGR) of *V. zizanioides* in contaminated soil

Harvesting time (day)	Height (cm)		Relative growth rate (RGR)		RGR (% as control)
	control soil	contaminated soil	control soil	contaminated soil	
30	37.1±.2.6*	29.1±2.9*	0.0270	0.0145	53.61
60	47.9±2.6	40.9±2.8	0.0170	0.0113	66.41
90	65.6±2.4	54.0±3.6	0.0162	0.0095	58.34
120	82.4±3.1	65.0±2.6	0.0161	0.0095	58.97

*Mean ± Standard deviation (n = 3)

4.1.4 Heavy metals concentration in *C. odorata* and *V. zizanioides* in contaminated soil

Heavy metals concentration in *C. odorata* and *V. zizanioides* were investigated and separated into 2 parts; various parts of plants (namely, root, stem and leaves) and the whole plants. The results were expressed in milligram per kilogram (mg/kg) of plant on a dry weight basis.

1) Heavy metals concentration in various parts of plants in contaminated soil

The result showed in Figure 4.3 indicated that Zn concentration in various parts of *C. odorata* was higher than other metals (Cd, Pb and Cu). The Zn concentration was found in ranges of 66.70-123.45, 18.77-45.28 and 72.01-98.74 mg/kg on a dry weight basis for root, stem and leave, respectively. Whereas, Cd concentration was found in ranges of 15.70-50.22, 8.93-13.07 and 11.43-14.35 mg/kg, Pb 11.38-29.07, 4.84-12.13 and 11.31-15.32 mg/kg, and Cu 9.41-31.89, 1.79-34.13 and 3.32-18.93 mg/kg on a dry weight basis for root, stem and leaves, respectively (Appendix C.5, C.7 and C.9). Figure 4.3 illustrated that Cd, Zn, Pb and Cu concentration depended on the periods of harvesting time. The highest concentration of heavy metals was occurred in root at 30 days for Zn and Cu, 60 days for Pb and 90 days for Cd. From statistical analysis, it was showed that Cd, Zn and Cu concentration in root was signification difference between the harvesting time ($p<0.05$) as shown in Appendix D.5, D.7 and D.9.

Figure 4.4 shows that the heavy metals concentration in *V. zizanioides* depended also on the periods of harvesting time. The Cd concentration in *V. zizanioides* was found in ranges of 3.24-11.24, 1.64-10.53 and 5.07-25.94 mg/kg on a dry weight basis for root, stem and leave, respectively. Whereas, Zn concentration was found in ranges of 85.41-163.18, 35-56.04 and 26.53-63.78 mg/kg, Pb 16.32-40.63, 6.39-13.71 and 1.56-9.77 mg/kg, and Cu 5.67-15.62, 2.23-21.20 and 3.86-13.69 mg/kg on a dry weight basis for root, stem and leave, respectively (Appendix C.6, C.8 and C.10). The highest concentration of heavy metals was occurred in root at 90 days for Cu and 120 days for Cd, Zn and Pb, respectively. From statistical analysis, it was shown that heavy metals concentration in various parts of plant was signification difference between the harvesting time ($P<0.05$) accepted Zn and Pb concentration in stem which was no signification difference between the harvesting time as shown in Appendix D.6, D.8 and D.10.

The heavy metals concentration in various parts of *C. odorata* and *V. zizanioides* were found to be highest in as compared to other parts (leave and stem) because root cell plasma membrane is closer to the soil surface and hold much more solutions than in the other parts. Plants have a propensity to uptake and/or selectively absorb the metals. Metals may absorb, retained and accumulated in root rather than translocated to other parts. Somsanguan (2003), Sampanpanish (2005), Sasithorn (2006) and Tanhan et al. (2007) showed the similar results as concentration of Cd, Zn, and Pb in roots of *V. zizanioides*, *V. nemoralis* and *C. odorata* were higher than in other parts.

Adriano (2001) reported the distribution of trace elements in plants can be categorized into three group: (1) more uniformly distributed between roots and shoots, e.g., Zn, Mn, Ni and B, (2) more in roots than in shoots, which moderate to sometimes large quantities in shoot, e.g., Cu, Cd, Co, and Mo and (3) mostly in roots with very little in shoots, e.g., Pb, Sn, Ti, Ag, Cr, and V. Most of this experimental plants, Zn was found in relatively high concentration in root which could possibly due to high total and bioavailability Zn contents in contaminated soils.

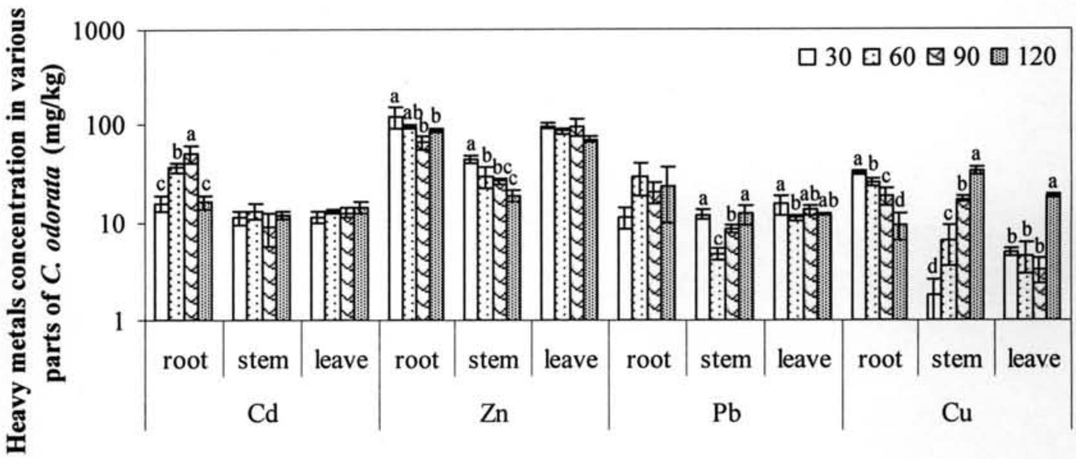

Figure 4.3 Heavy metals concentration in various part of *C. odorata* in contaminated soil

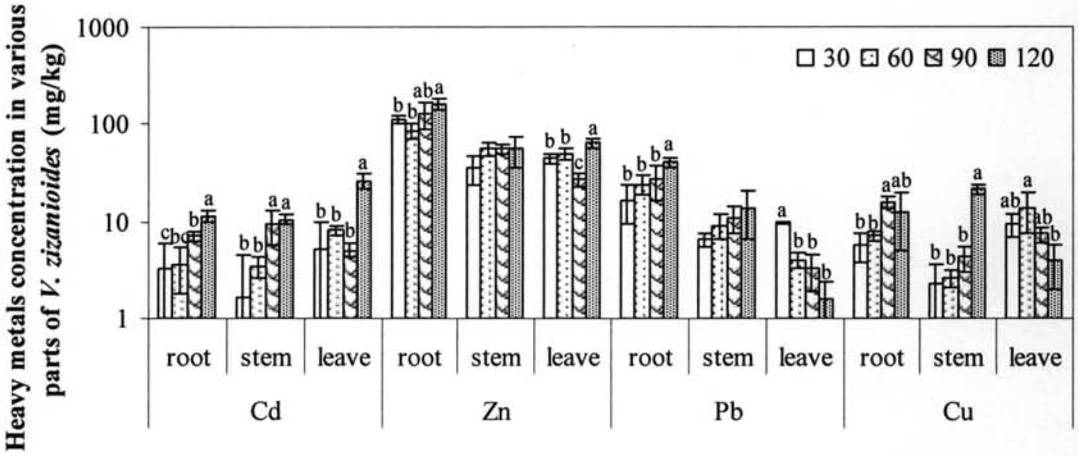

Figure 4.4 Heavy metals concentration in various part of *V. zizanioides* in contaminated soil

2) Heavy metals concentration in the whole plants in contaminated soil

In order to compare the heavy metals uptake from two different plants, average concentration of each metal were calculated for the plants at the same age. Figure 4.5 and 4.6 showed heavy metals concentration in whole plants of *C. odorata* and *V. zizanioides*, respectively in relative to the periods of harvesting time. Cd, Zn, Pb and Cu concentration in the whole plants of *C. odorata* were in ranges of 11.91-15.88, 54.60-84.07, 11.12-14.16, 7.66-24.44 mg/kg on a dry weight basis, respectively (Appendix C.11). As a result, the amounts of Cd, Pb and Cu concentration in the whole of plant tend to increase by time during the growth period, exceptional for Zn which show negative concentration. The highest of Cu and Pb concentration were found in 120 days while the highest of Cd and Zn concentration were found in 90 and 30 days, respectively. From statistical analysis, it was shown that the Cd, Zn and Cu concentration was signification difference between the harvesting time (p<0.05) as shown in Appendix D.11.

Figure 4.6 indicated that the heavy metals concentration in the whole of *V. zizanioides* increased slightly by the periods of harvesting time. The heavy metals concentration were in ranges of 2.46-14.79, 61.13-93.48, 9.95-25.24 and 4.99-13.36 mg/kg on a dry weight basis for Cd, Zn, Pb and Cu, respectively (Appendix C.12). The highest concentration of heavy metals in *V. zizanioides* was found in 120 days. From statistical analysis, the results show significance difference for heavy metals in all harvesting time (p<0.05), except Zn concentration as shown in Appendix D.12.

In comparison, it is quite obvious that per equal mass of whole plant *C. odorata* uptake more Cd than *V. zizanioides*. On the other hand, *V. zizanioides* was found to uptake higher Pb at the later stage of harvesting.

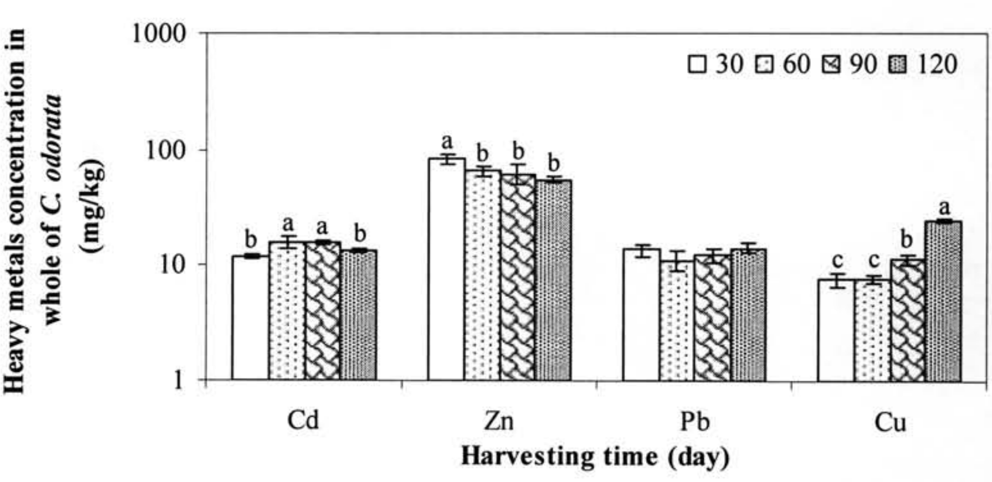

Figure 4.5 Heavy metals concentration in *C. odorata* in contaminated soil

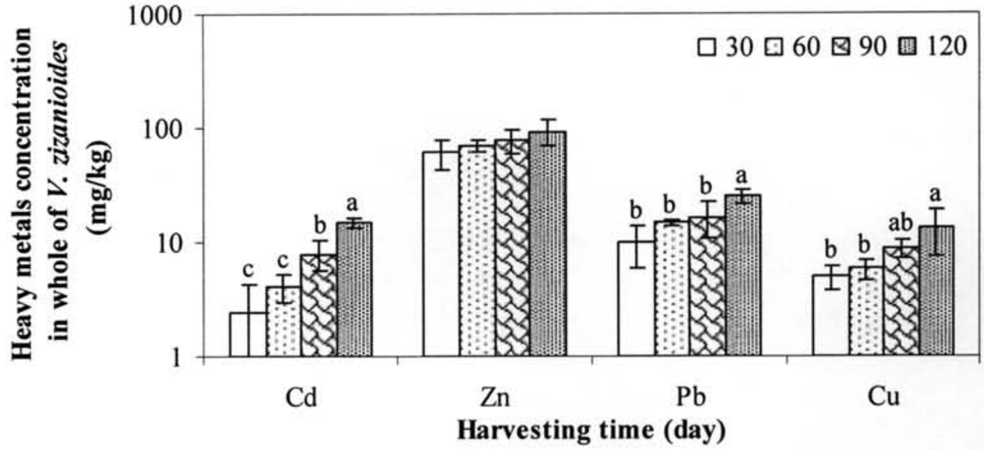

Figure 4.6 Heavy metals concentration in *V. zizanioides* in contaminated soil

3) Metals accumulated in plants grown in contaminated soil

To apply these two plants for Cd removal from contaminated site, the accumulated of Cd in the plants at different time of harvesting are important. So, the Cd accumulated as mass in each parts of the two plants were calculated as shown in Table 4.3 and 4.4 for *C. odorata* and *V. zizanioides*, respectively. In practice, aboveground parts (stem and leave) are of interest since it can be easily harvest from the area and leave the plant root to regrow.

As illustrated in Table 4.3 and 4.4, the results indicated that the accumulation of Cd in aboveground and root of both plants were increased by increasing harvesting time. Moreover, the results also indicated that Cd accumulated in aboveground part of *C. odorata* were higher than those in *V. zizanioides* at any time of harvesting. Figure 4.7 showed the comparison of Cd accumulated in aboveground part of the two plants at different time of harvesting. The result showed obviously that *C. odorata* can uptake more Cd than *V. zizanioides*, hence *C. odorata* may be suitable to grow in Cd contaminated area as compared to *V. zizanioides*. However, the further treatment for the plant after harvesting should be considered

Table 4.3 Cd accumulations in aboveground and root of *C. odorata* grown in contaminated soil

Harvesting time (day)	Leave (mg)	Stem (mg)	Root (mg)	Aboveground (stem+leave) (mg)
30	0.012±0.005*	0.007±0.002*	0.004±0.002*	0.019±0.007*
60	0.023±0.013	0.018±0.010	0.017±0.014	0.041±0.022
90	0.033±0.002	0.024±0.008	0.040±0.008	0.057±0.007
120	0.046±0.008	0.059±0.018	0.031±0.005	0.105±0.025

*Mean ± Standard deviation (n = 3)

Table 4.4 Cd accumulation in aboveground and root of *V. zizanioides* grown in contaminated soil

Harvesting time (day)	Leave (mg)	Stem (mg)	Root (mg)	Aboveground (stem+leave) (mg)
30	0.002±0.002*	0.001±0.001*	0.003±0.003*	0.003±0.001*
60	0.004±0.001	0.008±0.005	0.005±0.003	0.012±0.006
90	0.005±0.003	0.021±0.003	0.011±0.003	0.026±0.003
120	0.046±0.012	0.025±0.008	0.023±0.009	0.071±0.019

*Mean ± Standard deviation (n = 3)

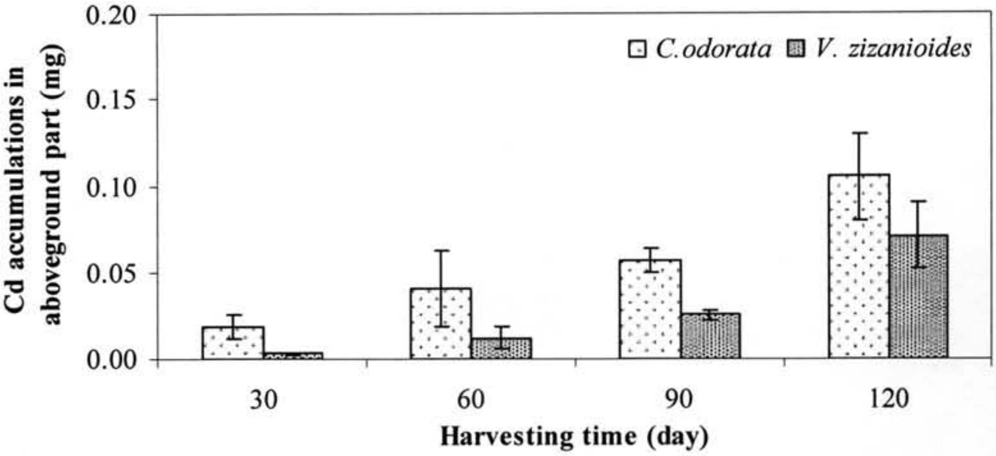

Figure 4.7 Comparison of Cd accumulated in aboveground part of *C. odorata* and *V. zizanioides* grown in contaminated soil

4.2 Heavy metals removal from synthetic soil by using *C. odorata* and *V. zizanioides*

4.2.1 Concentration of total heavy metals in synthetic soil

The concentration of heavy metals in synthetic soil after planting of *C. odorata* and *V. zizanioides* decreased with increasing time of plant lifes. The heavy metals concentrations in synthetic soil of *C. odorata* were in the ranges of 92.09-93.37, 129.02-131.36, 103.17-105.76 and 101.72-103.44 mg/kg on a dry weight basis for Cd, Zn, Pb and Cu, respectively (Appendix C.13), and concentration of heavy metals in synthetic soil of *V. zizanioides* pot were 92.03-93.96, 124.80-126.12, 104.37-107.83 and 103.64-106.64 mg/kg on a dry weight basis for Cd, Zn, Pb and Cu, respectively (Appendix C.14). The highest concentration of heavy metals was in 30 days of harvesting time in both *C. odorata* and *V. zizanioides* pot. According to the results, it was found that the concentration of heavy metals in synthetic soil both *C. odorata* and *V. zizanioides* pot decrease slightly by time during the growth period due to heavy metals in soil taken up by those plant. After calculation using ANOVA, statistical analysis, it was shown that concentration of heavy metals in synthetic soil was no significant difference between the harvesting time as shown in Appendix D.13 and D.14.

4.2.2 Phytoavailability of heavy metals in synthetic soil

After sample collection in the 30, 60, 90 and 120 days of both *C. odorata* and *V. zizanioides* cultivation, soil samples were determined for the availability of heavy metals by using the DTPA extraction method. Figure 4.8 and 4.9 illustrated the concentration of availability of heavy metals in the contaminated soil. As the result shown in Figure 4.8 and 4.9 indicated that the availability of heavy metals in synthetic soil of both *C. odorata* and *V. zizanioides*, decreased by time during the growth period. The heavy metals availability in synthetic soil of *C. odorata* were in the ranges of 65.91-87.74, 55.04-72.09, 23.59-42.97 and 57.15-74.66 mg/kg for Cd, Zn, Pb and Cu, respectively (Appendix C.15), where the heavy metals availability in synthetic soil of *V. zizanioides* pot were 65.64-83.94, 49.24-73.58, 38-69.41 and 57.77-72.89 mg/kg for Cd, Zn, Pb and Cu, respectively (Appendix C.16). The highest availability of heavy metals was in 30 days of harvesting time in both plants. After calculation using ANOVA, it was shown that heavy metals availability in soil was no significant difference between the harvesting time as shown in Appendix D.15 and D.16

Moreover, high metals concentration in both plants (*C. odorata* and *V. zizanioides*) was observed in synthetic soil as the metals are in the readily form for the plants uptake. In general, the solubility and availability of metals in plants are related the metal concentration in soil (Adriano, 2001) as the amount of metal uptake by plant would reveal the metal availability in soil. Therefore, as the results, it may be implied that the availability of heavy metals in soil of *C. odorata* and *V. zizanioides* pot decrease slightly by time during the growth period due to heavy metals in soil taken up by plant the soil. Environment, nutrition, growth stage and some other factors controlling plant growth may also indirectly affect the metal levels in plants (Xian, 1989)

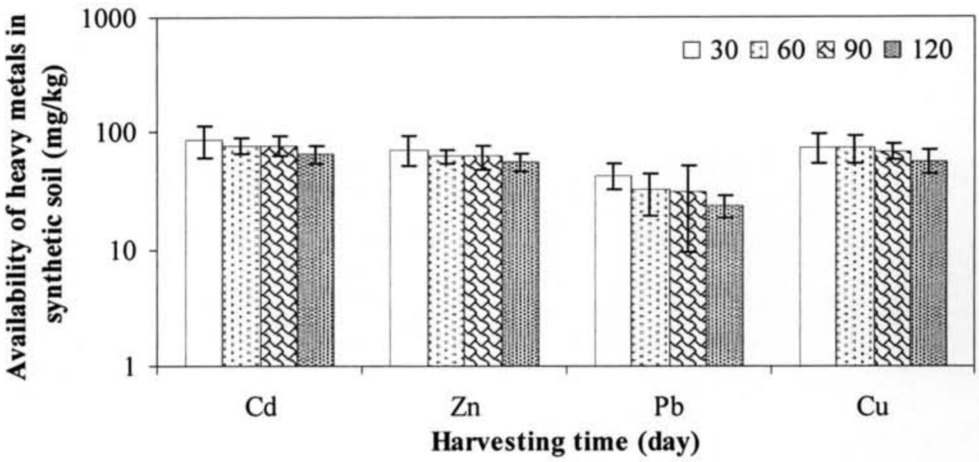

Figure 4.8 Available Cd, Zn, Pb and Cu in synthetic soil planted with *C. odorata*

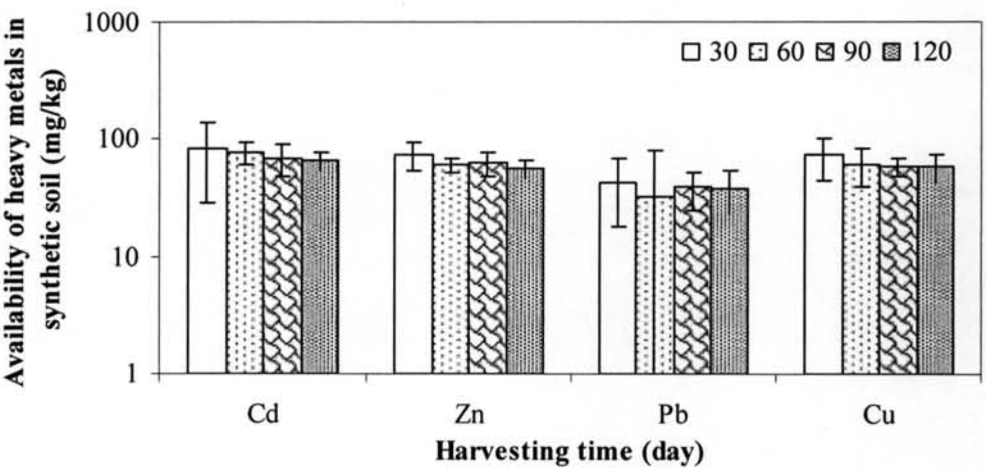

Figure 4.9 Available Cd, Zn, Pb and Cu in synthetic soil planted with *V. zizanioides*

4.2.3 Phytotoxicity of heavy metals in synthetic soil

Phytotoxicity of heavy metals in synthetic soil of *C. odorata* and *V. zizanioides* was investigated. The height and relative growth rate (RGR) are shown in Table 4.5 and 4.6, for *C. odorata* and *V. zizanioides*, respectively. Table 4.5 showed the lowest RGR of *C. odorata* was observed in the 90 (70.83%) days of harvesting time possibly due to the phytotoxicity of the plants. However, the lowest RGR of *V. zizanioides* was found at 120 days of the harvesting time (Table 4.6). Again, the growths of both *C. odorata* and *V. zizanioides* could survive under heavy metals concentration in the synthetic soil as shown by increased in the height but it was obviously exhibited abnormal characteristic (chlorosis, curly, spotted and scorching leaves) (Appendix E.5). The height of *C. odorata* and *V. zizanioides* still increased as increased the harvesting time from 24.9±1.0 to 76.2±6.6 and 28.6±2.0 to 62.9±2.2 cm. for *C. odorata* and *V. zizanioides*, respectively.

Normal and phytotoxic concentrations of metals were reported by Levy et al. (1999), which were 0.5-10 and 30-300 mg/kg for Pb, 3-30 and 20-100 mg/kg for Cu and 10-150 and >100 mg/kg for Zn. According to the results both plants (*C. odorata* and *V. zizanioides*) showed higher heavy metal concentrations than the normal or already in the range of phytotoxic levels. These may implied that plant species growing on the pot contaminated with heavy metals could tolerate for high metals concentration levels. Restriction of upward movement from roots into shoots can be considered as one of the tolerance mechanism (Verkleij and Schal, 1990).

Table 4.5 Height and Relative growth rate (RGR) of *C. odorata* in synthetic soil

Harvesting time (day)	Height (cm)		Relative growth rate (RGR)		RGR (% as control)
	control soil	synthetic soil	control soil	synthetic soil	
30	35.2±.2.1*	24.9±1.0*	0.0264	0.0238	90.10
60	68.4±2.9	33.6±2.6	0.0238	0.0228	95.96
90	82.4±2.7	55.4±1.8	0.0211	0.0149	70.83
120	101.7±4.0	76.2±6.6	0.0212	0.0193	91.02

*Mean ± Standard deviation (n = 3)

Table 4.6 Height and Relative growth rate (RGR) of *V. zizanioides* in synthetic soil

Harvesting time (day)	Height (cm)		Relative growth rate (RGR)		RGR (% as control)
	control soil	synthetic soil	control soil	synthetic soil	
30	37.1±.2.6*	28.6±2.0*	0.0270	0.0236	87.28
60	47.9±2.6	41.6±3.1	0.0170	0.0157	92.55
90	65.6±2.4	52.2±4.2	0.0162	0.0154	94.79
120	82.4±3.1	62.9±2.2	0.0161	0.0138	85.46

*Mean ± Standard deviation (n = 3)

4.2.4 Heavy metals concentration in *C. odorata* and *V. zizanioides* in synthetic soil

Heavy metals concentration in *C. odorata* and *V. zizanioides* grown in synthetic soils were also investigated and separated into 2 parts; various parts of plants (root, stem and leaves); and the whole plants. The results were expressed in milligram metal per kilogram (mg/kg) of plant on a dry weight basis.

1) Heavy metals concentration of in various parts of plants in synthetic soil

Determination of Cd concentration in *C. odorata* was found in the ranges of 117.04-326.63, 86.47-126.53 and 69.26-149.88 mg/kg on a dry weight basis in root, stem and leaves, respectively. Whereas Zn concentration was found in the ranges of 93.20-162.43, 68.15-103.61 and 141.65-228.24 mg/kg on a dry weight basis, Pb 22.37-98.50, 3.32-25.73 and 11-33.78 mg/kg on a dry weight basis and Cu 27.62-90.17, 8.74-43.81 and 8.42-13.47 mg/kg on a dry weight basis for root, stem and leaves, respectively (Appendix C.17, C.19 and C.21). Figure 4.10 illustrated that Cd, Zn, Pb and Cu concentration depended on the harvesting time. The highest concentration of heavy metals in root was found in 30 days for Pb and in 90 days for Cd, Zn and Cu. From statistical analysis, it was shown the heavy metals concentration in various parts of plant was significant difference with the harvesting time ($p<0.05$) as shown in Appendix D.17, D.19 and D.21.

The heavy metals concentration in the various parts of *V. zizanioides*, Cd concentration was found in the ranges of 159.28-337.01, 140.32-216.58 and 57.56-220.31 mg/kg on a dry weight basis for root, stem and leave, respectively. Whereas, Zn concentration was found in the ranges of 249.59-375.60, 115.16-250.98 and 69.34-300.17 mg/kg on a dry weight basis, Pb 138.21-232.02, 23.84-111.42 and 46.67-83.30 mg/kg on a dry weight basis, and Cu 103.06-295.86, 54.23-95.48 and 51.48-78.92 mg/kg on a dry weight basis for root, stem and leave, respectively (Appendix C.18, C.20 and C.22). Figure 4.11 also illustrated Cd, Zn, Pb and Cu concentration depended on the harvesting time. The highest concentration of heavy metals in root was found in 120 days for Cd and Pb and in 90 days for Zn and Cu. From statistical analysis, it was shown that heavy metals concentration in various parts of plant was significant difference between the harvesting time ($p<0.05$) as shown in Appendix D.18, D.20 and D.22.

The concentration of heavy metals in various parts of *C. odorata* and *V. zizanioides* slightly increases with harvesting time. The highest concentration of heavy metals in both *C. odorata* and *V. zizanioides* in root was higher than leave and stem. On the other, the concentration of Zn in *C. odorata* was leave>root>stem. This may be due to Zn is an essential element for plant nutrition which involve in a number of metallo-enzymes, protein synthesis and the transformation of carbohydrates (Kochian, 1993; Romhels and Marschner, 1991). Therefore, Zn may be needed for these processes, and taken up more in the leaves as compared to the root, hence the higher Zn concentration levels was observed in the leaves of *C. odorata*. Plant distributes metals internally in many different ways. They may localize selected metals mostly in roots and stems, or they may accumulate other metals in nontoxic form for latter distribution and use. A mechanism of tolerance or accumulation in some plants apparently involves binding potentially toxic metals at cell walls of roots and leave, away from sensitive sites within the cell or storing them in a vacuolar compartment storing them in a vacuolar compartment.

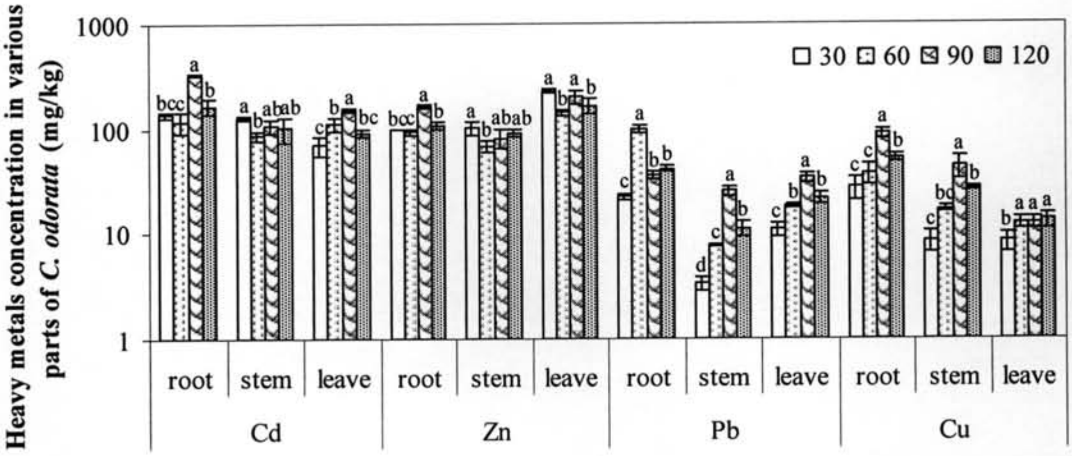

Figure 4.10 Heavy metals concentration in various part of *C. odorata* in synthetic soil

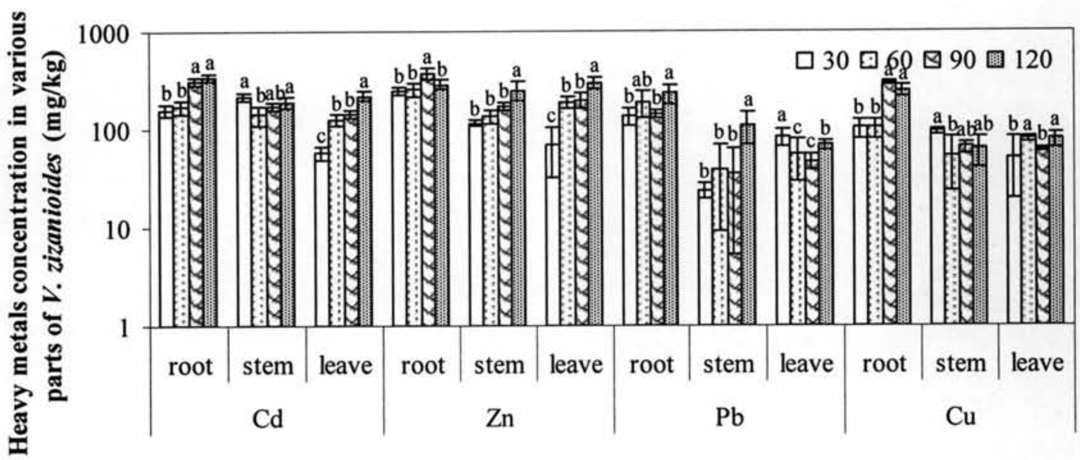

Figure 4.11 Heavy metals accumulation in various part of *V. zizanioides* in synthetic soil

2) Heavy metals concentration in the whole plants in synthetic soil

The heavy metals concentration in whole of *C. odorata* were in the ranges of 98.49-156.04, 106.19-165.78, 10.15-31.47, and 11.79-34.19 mg/kg on a dry weight basis for Cd, Zn, Pb and Cu, respectively (Appendix C.23). Figure 4.12 illustrated to Cd, Zn, Pb and Cu concentration that depended on the periods of harvesting time. As a result, the amounts of Cd, Pb and Cu concentration in the whole plant increased by time during the growth period, except for Zn concentration. The highest concentration of Cd, Zn and Cu were found in 90 days while the highest concentration of Pb was found in 60 days. From statistical analysis, it was shown that the heavy metals concentration was significant difference with the harvesting time ($p<0.05$) as shown in Appendix D.23.

Figure 4.13 showed that the heavy metals concentration in *V. zizanioides* were in ranges of 149.70-261.95, 156.75-311.32, 77.36-193.92, 81.27-153.42 mg/kg on a dry weight basis for Cd, Zn, Pb and Cu, respectively (Appendix C.24) which depended on the periods of harvesting time. The highest concentration of heavy metals was found in 120 days, except Cu accumulation in 90 days. From statistical analysis, it was shown that the heavy metals concentration was significant difference with the harvesting time ($p<0.05$) as shown in Appendix D.24.

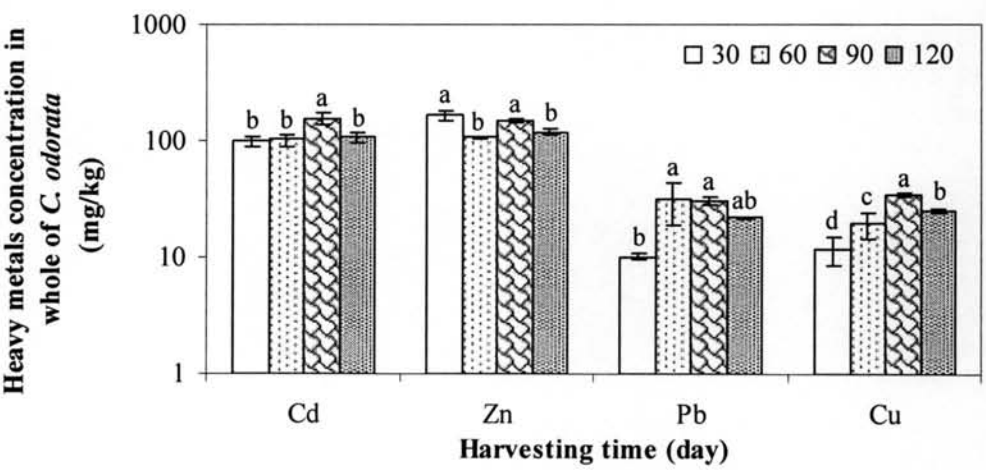

Figure 4.12 Heavy metals concentration in *C. odorata* in synthetic soil

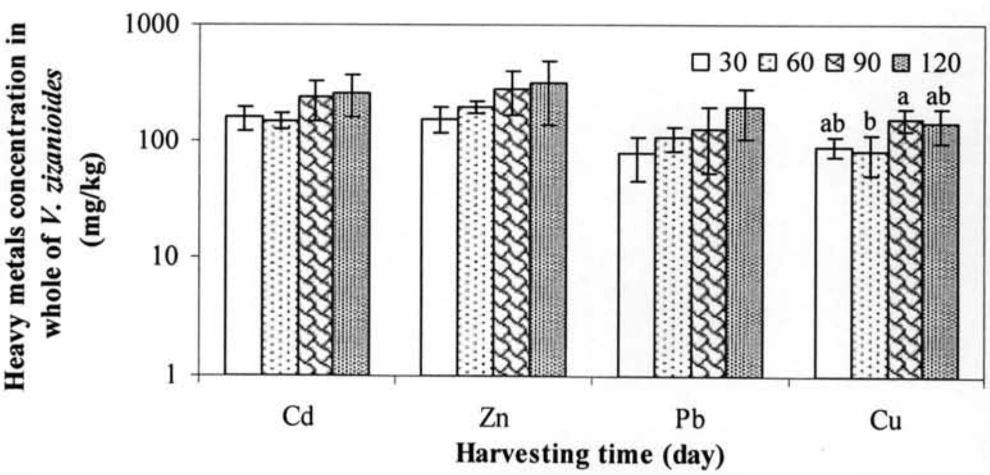

Figure 4.13 Heavy metals concentration in *V. zizanioides* in synthetic soil

3) Metals accumulated in plants grown in synthetic soil

In the same manner, the Cd accumulated in each part of the two plants grown in the synthetic soil were calculated as shown in Table 4.7 and 4.8. The same trend were found in the synthetic soil that Cd accumulation in various parts of plant was increased by increasing harvesting time and at 120 days of harvesting time showed the highest Cd accumulated in aboveground part for both *C. odorata* and *V. zizanioides.* In addition, the Cd accumulated in both plants were found much higher than the ones grown in contaminated soil i.e. 0.105 mg Cd and 0.785 mg Cd for aboveground part of *C. odorata* at 120 day harvesting from contaminated and synthetic soil, respectively. This result was not surprising since Cd in synthetic soil were readily available form and hence easily to be uptake by the plants. However, this fact reveals that both *C. odorata* and *V. zizanioides* have capability to uptake Cd not only in their early stage of growing but continue uptaking until their maturity stage.

Alternatively, in synthetic soil, *V. zizanioides* appeared to be more efficiency in heavy metal removal than *C. odorata* (See Figur 4.14). This may be due to *V. zizanioides* could grow in highly contaminated soils without the perceived phytoxicity symptoms. In addition, *V. zizanioides* is also able to tolerate a variety of pollutants in soil and water (Pinthong et al., 1998; Truong, 2000). As *V. zizanioides* is tolerance to heavy metals, there is a great potential to use this plant in phytoextraction strategy although *V. zizanioides* is not a hyper-accumulator (Chen et al., 2004). Chen et al. (2000) also found that the total Cd uptake in the aboveground parts of *V. zizanioides* was even greater than that of the hyperaccumulator *T. carelessness* owing to the farmer's high biomass. Moreover, *V. zizanioides* grows very fast with a fine, deep and penetrating root system, which is capable of reaching down to 3-4 m in the first year (Truong, 2000).

Table 4.7 Cd accumulations in aboveground and root of *C. odorata* grown in synthetic soil

Harvesting time (day)	Leave (mg)	Stem (mg)	Root (mg)	Aboveground (stem+leave) (mg)
30	0.068±0.012*	0.089±0.009*	0.042±0.025*	0.157±0.021*
60	0.208±0.150	0.123±0.105	0.072±0.047	0.331±0.255
90	0.291±0.057	0.142±0.065	0.163±0.056	0.434±0.086
120	0.396±0.053	0.389±0.316	0.330±0.223	0.785±0.368

*Mean ± Standard deviation (n = 3)

Table 4.8 Cd accumulations in aboveground and root of *V. zizanioides* grown in synthetic soil

Harvesting time (day)	Leave (mg)	Stem (mg)	Root (mg)	Aboveground (stem+leave) (mg)
30	0.065±0.047*	0.308±0.149*	0.331±0.389*	0.373±0.168*
60	0.143±0.051	0.320±0.182	0.325±0.225	0.463±0.211
90	0.461±0.322	0.470±0.049	0.867±0.243	0.931±0.370
120	0.558±0.609	0.613±0.134	1.339±0.713	1.171±0.608

*Mean ± Standard deviation (n = 3)

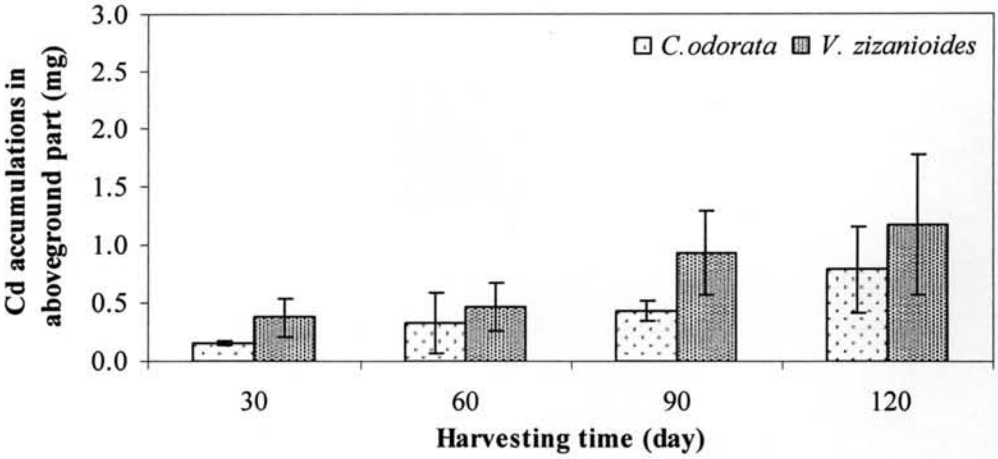

Figure 4.14 Comparison of Cd accumulated in aboveground part of *C. odorata* and *V. zizanioides* grown in synthetic soil

4.3 The application of *C. odorata* and *V. zizanioides* for the heavy metals removal

The application by using *C. odorata* and *V. zizanioides* is to remove heavy metals in contaminated area at Mae Sot district, Tak Province. This contaminated area is clay-loam soils which have the background metals concentrations in the ranges of 46.24, 2,124.26, 87.96 and 25.89 mg/kg of Cd, Zn, Pb and Cu, respectively. It was shown that metal concentrations (Cd, Zn, Pb and Cu) in soils are varied where Zn, Cu and Pb were lower than standard level, except for Cd. Cd concentration was higher than the standard levels for habitat and agriculture (not exceed 37 mg/kg) set by Pollution Control Department (PCD). Thus, this work intends to give a helpful contribution in this field. Cd was an interest metal in terms of the management as it is non essential and can be accumulated in the soil to the levels that is highly toxic to plant and animals. The results revealed that *C. odorata* may be more suitable to be used in this contaminated area to remove Cd in the soil.

By using *C. odorata* as the Cd removal, the aboveground plants should be cut off at 120 days of harvesting time due to the highest Cd accumulation was found and it (dicot species) also produces new branches and leave. The removed parts can be further managed by burning or other techniques for safety environment and human health. Moreover, in every 120 days of harvesting time, the aboveground plants can be repeated cut off in order to remove Cd in soils. This possibly one way to remove the Cd contaminated in this particular area.

From the results obtained, it can be concluded that *C. odorata* had more heavy metal removal efficiency than *V. zizanioides*. This probably due to the fact that *C. odorata* is a native plant in which widely distributed within a pronounced dry season throughout the country in both heavy metal contaminated and non-contaminated areas. *C. odorata* was also found commonly growing on the disturbed zinc contaminated soils around disused zinc mines in Mae Tao district, Tak province. The importance of using the native plant for phytoremediation was reported by Yoon et al. (2006). They stated that these plants are better in terms of survival, growth and reproduction under environmental stress than plants introduced from other environment. Therefore, this may cause *C. odorata* to be more preferable to remove heavy metals in contaminated soil rather than *V. zizanioides*.