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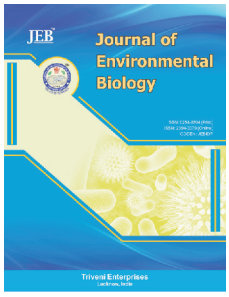

Removal of phenanthrene and cadmium from co-contaminated alkaline soil by carpet grass, Siam weed and winged bean

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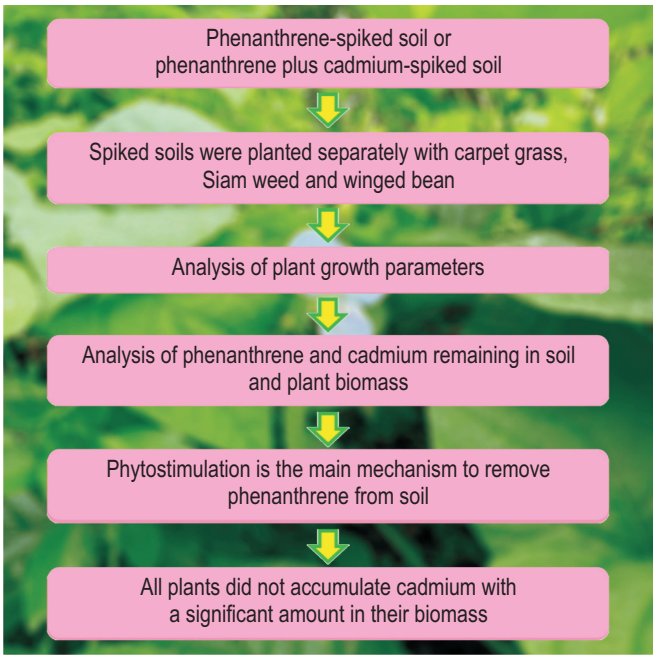
Abstract

Aim: Phytoremediation of soil co-contaminated with polycyclic aromatic hydrocarbon (PAH) and heavy metal is rarely reported. The aim of this study was to investigate the ability of carpet grass (*Axonopus compressus*), Siam weed (*Chromolaena odorata*) and winged bean (*Psophocarpus tetragonolobus*) to remove cadmium and phenanthrene concurrently from contaminated soil.

Methodology: Soil was spiked with phenanthrene alone or phenanthrene plus cadmium to give initial concentration of phenanthrene in soil with and without cadmium were 44.9 and 87.8 mg kg^{-1} dry soil, respectively. Initial concentration of cadmium in soil spiked with phenanthrene plus cadmium was 6.2 mg kg^{-1} dry soil. Carpet grass, Siam weed and winged bean were planted separately in phenanthrene-spiked soil or phenanthrene and cadmium-spiked soil for 60 days. Growth of each plant, phenanthrene remaining in soil, cadmium remaining in soil, phenanthrene and cadmium in biomass of each plant were measured on day 30 and 60 of transplantation.

Results: Carpet grass, Siam weed and winged bean grew normally in soil spiked with phenanthrene alone or phenanthrene+cadmium over the 60-day experiment. The presence of plants did not result in cadmium removal, as the amount of this metal in soil remained unchanged after 60 days. Negligible amounts of phenanthrene and cadmium were accumulated by Siam weed, carpet grass and winged bean after 60 days. All three plants could increase the removal of phenanthrene from soil. Around 6.3-12.4% and 5.1-27.1% of phenanthrene remained in planted soil in the absence or presence of cadmium in soil on day 60, respectively.

Interpretation: The results suggest that phytostimulation may be the main mechanism of phenanthrene removal from contaminated soil. The simultaneous removal of phenanthrene and cadmium in planted soil was not observe. A mild alkaline soil may decrease the accumulation of cadmium by plants.



Introduction

A variety of sources are known to contribute to polycyclic aromatic hydrocarbon (PAH) and heavy metal contamination in the environment. Among these, the major sources are coal and wood burning, vehicle emissions and industrial activities such as petroleum refining, coke production and steel metallurgy (Hussain *et al*., 2015; Rachwał *et al*., 2015; Subramanian *et al*., 2015). Many PAH-contaminated sites also contain heavy metals such as arsenic, aluminium, cadmium, chromium, copper, lead, nickel and zinc. Moreover, the concentrations of total PAHs and metals may vary from site to site (Kuppusamy *et al*., 2016; Lyons *et al*., 2015; Rachwał *et al*., 2015). Removal of PAHs and heavy metals from the environment can be challenging. Phytoremediation has proved to be successful in the removing both PAHs (Somtrakoon *et al*., 2014 a, b; Xiao *et al*., 2015) and heavy metals (Khaokaew and Landrot, 2015; Tanhan *et al*., 2007; Waranusantigul *et al*., 2008) from contaminated soil. During phytoremediation, PAHs are mostly removed by competent rhizospheric microorganisms whose catabolic activities are stimulated by their association with the plant roots (Shahsavari *et al*., 2015; Tejeda-Agredano *et al*., 2013). In contrast, heavy metals are typically removed from contaminated sites by phytoaccumulation (Khaokaew and Landrot, 2015; Tanhan *et al*., 2007; Waranusantigul *et al*., 2008) or phytostabilization (Hu *et al*., 2013).

Success in phytoremediation depends on various factors such as type and concentration of contaminants, type of plants used and environmental variables. Among the latter, soil pH has been identified as a critical factor for plant growth (Willscher *et al*., 2017). Several soil series distributed in various parts of Thailand such as Takhli soil (pH 7.5-8.0), Kamphaeng Saen sub soil (pH 7.5-8.0), Tha Chin soil (pH 7.0-8.0) and Lop Buri soil (pH 7.0-8.0) are alkaline (Srithawat Na Ayuthaya, 1995). Soil alkalinity conditions have been reported to impede pollutant bioremediation (Anh *et al*., 2013). Soil pH may affect the bioavailability of metals and this may in turn affect their uptake by plants (Anh *et al*., 2013; McBride, 2002). In general, a lower soil pH renders metals more soluble and likely more bioavailable (Anderson and Nilsson, 1974). While it is expected that alkaline soil may pose a problem for phytoremediation of PAH and metal co-contaminated soils, very few studies have examined this.

Thus, the aim of the present study was to compare the ability of three plants from different families cultivated alone to remove phenanthrene and cadmium from alkaline soil contaminated with both pollutants. One of the plants used, winged bean, is widely cultivated as a food plant in Thailand, but it does not grow well in acidic soil with pH below 5.5 (National Park Board, 2013). Winged bean was reported to enhance anthracene and fluorene biodegradation in alkaline soil (pH 8.1) (Somtrakoon *et al*., 2014 b); however its propensity for metal accumulation is not known. The other two plants were carpet grass and Siam weed. Carpet grass is a common lawn grass in Thailand and it can be grown in different soil types with varied pH. Siam weed is also widely distributed throughout Thailand (Tanhan *et al*., 2007) and it was found to grow in areas with cadmium contamination which occur naturally in the zinc mining area in Mae Sot district, Tak province, Thailand (soil pH was 7.05-7.85) (Phaenark *et al*., 2009). Both Siam weed and carpet grass were reported to accumulate lead (at pH ranging from 7.05-7.85) and cadmium (at pH ranging from 5.50-6.50), respectively. To our knowledge, carpet grass has not been reported to enhance PAH removal, unlike Siam weed which has from soil (at pH 7.1) (Jampasri *et al*., 2016). To our knowledge, winged bean, Siam weed and carpet grass have never been tested before for the removal of both PAH and cadmium concurrently from alkaline soil.

Materials and Methods

Soil collection and analysis: Soil was collected from Khaorad Agricultural Station, Faculty of Agricultural Technology and Industrial Technology, Nakhonsawan Rajabhat University in January, 2016 during the dry season in Thailand. The soil was air dried at room temperature (28-31ºC) for 72 hrs to constant dry weight before use. Concentration of total cadmium in soil and selected physical and chemical characteristics of the soil were analyzed at the Environmental Quality Examining Service Center, Faculty of Environment and Resource Studies, Mahasarakham University, Thailand. The soil contained 47.3% sand, 18.9% silt and 33.8% clay, and had a cationic exchange capacity of 15.1 cmol kg^{-1} and a pH of 8.9. Soil was alkaline in nature. The background level of cadmium in form of Cd^{2+} was 1.9 g kg^{-1}, while phenanthrene was not detected in this soil.

Spiking of soil with phenanthrene and cadmium (Cd^{2+}): PAH and heavy metal used in this study was phenanthrene and cadmium. Phenanthrene-spiked soil was prepared according to the method described in the previous publication of Somtrakoon *et al*. (2015). The initial measured concentrations of phenanthrene in soil with and without cadmium were 44.9 and 87.8 mg kg^{-1} dry soil, respectively. Cadmium nitrate [$Cd(NO_3)_2.4H_2O$] (Asia Pacific Specialty Chemicals Limited, New South Wales, Australia; purity 99%) soluble in distilled water was added to the phenanthrene-spiked soil to get a final concentration of about 5 mg kg^{-1} d.wt. of soil in each batch of one kg soil. The measured concentration of cadmium (Cd^{2+}) in soil at the beginning of the experiment was 6.2 mg kg^{-1} d.wt.

Plant preparation: Naturally grown plants of Siam weed were collected from a field which was previously used for farming in Chumsang District, Nakhonsawan Province, Thailand. The plants were transported to laboratory and were grown in non-contaminated soil in a nursery for one week. Carpet grass plants were purchased from a retail store in Nakhonsawan District, Nakhonsawan Province, Thailand and plants of similar weight and height were used in the phytoremediation experiment.

Finally, seedlings of winged bean (commercial seeds of Chuayongseng Ltd., Bangkok, Thailand) were prepared by immersing them in distilled water before planting the seedlings into the soil. The date of seedling transplantation was considered day 0 of the experiment.

Pot experiment: Phytoremediation experiments were performed in the early summer of 2016 following the protocol described in the previous publication of Somtrakoon *et al*. (2015). Each of experimental pots contained one kg d.wt. of soil spiked with either phenanthrene+cadmium or phenanthrene alone. The spiked soils were separately planted with carpet grass, Siam weed or seedlings of winged bean. The pots containing unspiked soil and planted with one of carpet grass, Siam weed or winged bean were set for plant growth. The other two pots were soil spiked with phenanthrene+cadmium or phenanthrene alone but without any plants for measuring PAH loss under plant-free conditions. Five independent replicates of each treatment were prepared in a completely randomized design. Each pot was watered regularly with distilled water to maintain the water holding capacity of soil at approximately 60% during 60-day experiment.

On day 30, soil and plant samples were collected from the first set of 55 experimental pots. From each pot, one gram d.wt. of soil was collected for analysis of phenanthrene concentration by GC-MS and another two grams of soil were collected for analysis of cadmium concentration. The plant from each pot was used to measure the length, fresh weight and dry weight of shoots and roots and chlorophyll a, chlorophyll b and total chlorophyll contents in leaves. Chlorophyll contents in plant leaves were analyzed according to the method described in the previous publication of Huang *et al*. (2004). On day 60, another set of 55 pots were subjected to various measurements as above. In addition, samples of plant tissues were sent for analysis of phenanthrene and cadmium contents. Extractions of phenanthrene from soil and plant tissues were done by soxhlet apparatus, as well as analysis by GC-MS were done according to the condition for PAH separation which was described by Somtrakoon *et al*. (2015).

Cadmium (Cd^{2+}) analysis: Two gram dry weight of soil and one gram dry weight of whole plant sample from each treatment were collected at the end of the experiment (60 days) to analyze the concentration of Cd^{2+} in soil and plant biomass. Soil and plant samples were subjected to extraction according the method described by Land Development Department, Thailand (2010). Cadmium content was analyzed by atomic absorption spectroscopy at the Environmental Quality Examining Service Center, Faculty of Environment and Resource Studies, Mahasarakham University. The detection limit of cadmium analysis was 0.5 mg kg^{-1}.

Statistical analysis: Plant growth parameters and the amount of phenanthrene remaining in soil were expressed as means ± SE. One-way ANOVA was used to assess statistical significance among treatments for plant growth and phenanthrene biodegradation. Subsequent multiple comparisons of means were performed by the LSD method. Considering significance at $p<0.05$.

Results and Discussion

PAHs and metals are often found together in the environment. Remediation efforts must be taken this into account. However, most of the phytoremediation studies have focused on either PAH or metal remediation, but not both. The present study examined three plants that might be able to tolerate metals while enhancing PAH removal from PAH and metal co-contaminated alkaline soil. In this study, the overall growth of winged bean, Siam weed and carpet grass appeared normal in phenanthrene+cadmium-spiked alkaline soil. The presence of phenanthrene or phenanthrene+cadmium in soil did not adversely affect the shoot growth of carpet grass and winged bean (Table 1). For shoot growth of plant, the shoot fresh weights and shoot dry weights of carpet grass and winged bean in soil spiked with phenanthrene were not significantly different from those of plants grown in soil spiked with phenanthrene+cadmium on both 30 and 60 days of experiment. In comparison, the shoot lengths of carpet grass grown in soil spiked with phenanthrene and phenanthrene+cadmium (57.6 and 48.8 cm, respectively) were longer than that of plants grown in unspiked soil (23.6 cm). In contrast to carpet grass, growth of Siam weed shoots was decreased in the presence of phenanthrene or phenanthrene+cadmium in soil. The fresh and dry weights of Siam weed shoots on day 60 were 10.3-14.0 g and 1.76-2.39 g in the presence of phenanthrene and phenanthrene+cadmium in soil, respectively. In comparison, the shoot fresh and dry weights of Siam weed grown in the absence of any phenanthrene or cadmium were 22.9 and 5.61 g, respectively (Table 1).

Table 2 shows the results of root growth of carpet grass, Siam weed and winged bean in soil spiked with phenanthrene or phenanthrene+cadmium. The root of carpet grass grown in phenanthrene-spiked soil for 60 days was longer than that of plant grown in unspiked soil or phenanthrene+cadmium spiked soil. In contrast, the roots of winged bean grown in unspiked soil or soil spiked with phenanthrene+cadmium for 60 days were longer than that of plant grown in phenanthrene-spiked soil. However, the differences in root lengths for both plants grown under different treatments were not statistically significant. For Siam weed, none of the experimental treatments affected the root length as compared to the unspiked control. The root fresh weight and root dry weight of carpet grass grown in phenanthrene-spiked soil for 60 days were higher than those of plant grown in unspiked soil or soil spiked with phenanthrene+cadmium, but the differences were not statistically significant (Table 2). Similarly, the root fresh weight and root dry weight of winged bean grown for 60 days in soil spiked with phenanthrene+cadmium were higher than those

of plant grown in unspiked soil or soil spiked with phenanthrene. Again, the differences were not statistically significant (Table 2). The root fresh weight of Siam weed grown for 60 days in unspiked soil and soil spiked with phenanthrene or phenanthrene+ cadmium were around 1.0-1.4 g. The values were not statistically significantly different from one another. In contrast, the root dry weight of Siam weed grown in soil spiked with phenanthrene+cadmium (0.08 g) was significantly lower than that of plant grown in phenanthrene-spiked (0.25 g) or unspiked soil (0.14 g).

Also, the presence of phenanthrene or phenanthrene+ cadmium in soil did not adversely affect the chlorophyll contents in leaves of carpet grass, Siam weed and winged bean (Table 3). The contents of chlorophyll a, chlorophyll b, total chlorophyll and ratio of chlorophyll a and b of these plants grown in soil spiked with phenanthrene or phenanthrene+cadmium were not significantly different from those of plants grown in unspiked soil on both days 30 and 60 of experiment. This is not surprising because these plants are found throughout Thailand and they may have adapted to growth in various soil types, perhaps with different contaminants.

This study found that soil cropped separately with winged bean, Siam weed and carpet grass exhibited improved phenanthrene removal either in the presence or absence of cadmium in alkaline contaminated soil compared to unplanted soil. In unplanted soil without cadmium, the percentage of phenanthrene remaining on days 30 and 60 were 97.6 and 52.1%, respectively (Table 4). Phenanthrene removal increased on day 30 in soil without cadmium but planted with carpet grass, Siam weed or winged bean in that the percentage of phenanthrene remaining were 59.0, 45.5 and 7.9%, respectively. The results showed that winged bean removed phenanthrene at the fastest rate and to the highest extent in this soil. By day 60, high amount of phenanthrene was removed from this soil by all three plants. In particular, lower percentage of phenanthrene remained in soil planted with carpet grass (6.6%) and Siam weed (6.3%) as compared to winged bean (12.4%) (Table 4). In soil without plants, 52.1% of phenanthrene remained on day 60.

The percentage of phenanthrene remaining in cadmium-spiked unplanted soil on day 30 was 60.9%, while that in soil planted with carpet grass, Siam weed and winged bean were 35.5, 31.4 and 23.8%, respectively. By day 60, more phenanthrene was removed, such that the amount remaining in unplanted soil was not significantly different from those in planted soil. The percentage of phenanthrene remaining in cadmium-spiked soils was around 5.1-27.1% (Table 4). Of the three plants, winged bean led to the largest percentage of phenanthrene removal in this cadmium-spiked soil.

The alkalinity condition has been reported to limit PAH remediation in several studies (Ke *et al*., 2003; Bentancur-Galvis *et al*., 2006). Ke *et al*. (2003) reported that remediation of pyrene-contaminated sediment planted with *Kandelia candel* for six months was inhibited under alkaline conditions. In the present study only 34.9% of pyrene was removed from contaminated sediment at pH 8.70-8.95, while 70.9% of pyrene was removed at pH 6.56-7.05 (Ke *et al*., 2003). In current study, the 3 plants were found to accumulate negligible amounts of phenanthrene in their tissues (Table 5). Only 1.0 and 1.2 mg cadmium kg^{-1} dry plant were detected in the whole plant of Siam weed and winged bean, respectively, grown for 60 days in phenanthrene+cadmium spiked soil. Cadmium was not detected in carpet grass biomass. The amount of cadmium remaining in soil planted with carpet grass, Siam weed and winged bean were not significantly different from those in unplanted soil. The amount of cadmium

Table 1: Shoot growth parameters of three plants grown in soil spiked with phenanthrene or phenanthrene plus cadmium for 60 days

Plant	Day 30			Day 60		
	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Carpet grass						
Unspiked soil	7.7 ± 0.3[a]	0.3 ± 0.1[a]	0.04 ± 0.00[a]	23.6 ± 2.2[b]	2.5 ± 0.5[a]	0.43 ± 0.1[a]
Phenanthrene	15.5 ± 4.4[a]	1.0 ± 0.3[a]	0.13 ± 0.04[a]	57.6 ± 5.1[a]	3.8 ± 0.6[a]	0.73 ± 0.2[a]
Phenanthrene+Cd	16.2 ± 10.8[a]	1.0 ± 0.9[a]	0.11 ± 0.09[a]	48.8 ± 6.7[a]	3.4 ± 0.8[a]	0.65 ± 0.1[a]
Siam weed						
Unspiked soil	52.7 ± 5.0[a]	28.1 ± 6.5[a]	11.5 ± 2.1[a]	52.0 ± 9.9[a]	22.9 ± 1.2[a]	5.61 ± 1.4[a]
Phenanthrene	45.0 ± 2.3[a]	32.9 ± 2.1[a]	11.4 ± 2.2[a]	36.8 ± 5.8[a]	10.3 ± 1.6[b]	1.76 ± 0.2[b]
Phenanthrene+Cd	40.8 ± 4.4[a]	12.6 ± 4.0[b]	3.8 ± 1.5[b]	48.3 ± 0.9[a]	14.0 ± 3.8[b]	2.39 ± 0.8[b]
Winged bean						
Unspiked soil	40.7 ± 3.3[a]	1.6 ± 0.1[a]	0.23 ± 0.01[a]	59.0 ± 5.2[a]	1.5 ± 0.3[a]	0.28 ± 0.0[a]
Phenanthrene	55.1 ± 10.7[a]	1.6 ± 0.3[a]	0.25 ± 0.06[a]	56.9 ± 9.7[a]	1.2 ± 0.1[a]	0.35 ± 0.1[a]
Phenanthrene+Cd	48.7 ± 9.9[a]	1.0 ± 0.2[a]	0.25 ± 0.06[a]	61.4 ± 9.3[a]	1.8 ± 0.5[a]	0.44 ± 0.1[a]

Different lower case letter denote significant difference ($p<0.05$) between the same plant in the same column

Table 2: Root growth parameters of three plants grown in soil spiked with phenanthrene or phenanthrene plus cadmium for 60 days

Plant	Day 30			Day 60		
	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Carpet grass						
Unspiked soil	8.7 ± 1.1[a]	0.1 ± 0.0[a]	0.05 ± 0.01[a]	15.5 ± 3.1[a]	0.4 ± 0.0[a]	0.05 ± 0.0[a]
Phenanthrene	7.2 ± 1.0[a]	0.3 ± 0.0[a]	0.05 ± 0.01[a]	18.2 ± 0.5[a]	0.6 ± 0.2[a]	0.09 ± 0.0[a]
Phenanthrene+Cd	7.2 ± 1.8[a]	0.1 ± 0.0[a]	0.05 ± 0.01[a]	15.8 ± 2.7[a]	0.3 ± 0.1[a]	0.06 ± 0.0[a]
Siam weed						
Unspiked soil	9.1 ± 2.4[a]	0.2 ± 0.1[a]	0.10 ± 0.07[a]	8.7 ± 3.5[a]	1.2 ± 0.6[a]	0.25 ± 0.1[a]
Phenanthrene	5.1 ± 1.5[a]	0.01 ± 0.0[a]	0.01 ± 0.00[a]	9.8 ± 2.0[a]	1.4 ± 0.7[a]	0.14 ± 0.0[a]
Phenanthrene+Cd	6.3 ± 0.7[a]	0.5 ± 0.0[a]	0.02 ± 0.01[a]	9.3 ± 3.2[a]	1.0 ± 0.9[a]	0.08 ± 0.0[b]
Winged bean						
Unspiked soil	9.5 ± 1.3[a]	0.4 ± 0.0[a]	0.04 ± 0.01[a]	14.5 ± 3.4[a]	0.6 ± 0.1[a]	0.06 ± 0.0[a]
Phenanthrene	12.1 ± 1.3[a]	0.5 ± 0.1[a]	0.09 ± 0.02[a]	9.9 ± 1.3[a]	0.5 ± 0.2[a]	0.06 ± 0.0[a]
Phenanthrene+Cd	14.2 ± 1.6[a]	0.2 ± 0.0[a]	0.03 ± 0.01[a]	14.9 ± 1.7[a]	0.8 ± 0.1[a]	0.08 ± 0.0[a]

Different lower case letter denote significant difference ($p<0.05$) between the same plant in the same column

Table 3: Chlorophyll content in leaves of each plant grown in soil spiked with phenanthrene or phenanthrene plus cadmium for 60 days

Plant	Day 30				Day 60			
	Chlorophyll a (mg ml^{-1})	Chlorophyll b (mg ml^{-1})	Total chlorophyll (mg ml^{-1})	a/b ratio	Chlorophyll a (mg ml^{-1})	Chlorophyll b (mg ml^{-1})	Total chlorophyll (mg ml^{-1})	a/b ratio
Carpet grass								
C	28.8 ± 2.9[a]	22.3 ± 12.7[a]	51.1 ± 15.6[a]	1.8 ± 0.9[a]	20.4 ± 9.3[a]	14.5 ± 0.3[a]	34.9 ± 9.2[a]	1.4 ± 0.6[a]
Phe	31.8 ± 0.4[a]	22.7 ± 0.1[a]	54.5 ± 0.3[a]	1.4 ± 0.0[a]	15.2 ± 1.2[a]	19.0 ± 4.9[a]	34.2 ± 5.1[a]	0.9 ± 0.2[a]
Phe+Cd	31.5 ± 0.4[a]	26.0 ± 0.9[a]	57.5 ± 1.3[a]	1.2 ± 0.0[a]	21.8 ± 1.7[a]	22.8 ± 5.6[a]	44.6 ± 7.3[a]	1.0 ± 0.2[a]
Siam weed								
C	30.0 ± 0.7[a]	13.2 ± 1.4[a]	43.2 ± 2.1[a]	2.3 ± 0.3[a]	22.7 ± 5.5[a]	9.9 ± 3.0[a]	32.7 ± 8.6[a]	2.4 ± 0.2[a]
Phe	29.4 ± 2.7[a]	17.6 ± 5.3[a]	47.0 ± 7.7[a]	1.9 ± 0.4[a]	22.3 ± 2.1[a]	9.4 ± 1.0[a]	31.8 ± 3.1[a]	2.4 ± 0.0[a]
Phe+Cd	20.5 ± 8.2[a]	11.6 ± 5.9[a]	32.1 ± 13.8[a]	2.0 ± 0.3[a]	19.3 ± 1.8[a]	8.0 ± 0.9[a]	28.3 ± 2.7[a]	2.4 ± 0.0[a]
Winged bean								
C	23.3 ± 5.7[a]	38.0 ± 10.4[a]	61.2 ± 16.1[a]	0.6 ± 0.0[a]	13.2 ± 1.2[a]	18.8 ± 3.0[a]	32.0 ± 2.2[a]	0.8 ± 0.2[a]
Phe	23.2 ± 5.7[a]	37.9 ± 10.5[a]	61.2 ± 16.2[a]	0.6 ± 0.0[a]	12.7 ± 1.9[a]	19.9 ± 3.4[a]	32.6 ± 2.3[a]	0.7 ± 0.2[a]
Phe+Cd	23.1 ± 5.6[a]	37.9 ± 0.4[a]	61.0 ± 16.1[a]	0.6 ± 0.0[a]	6.5 ± 3.1[a]	22.4 ± 10.1[a]	28.9 ± 10.4[a]	0.4 ± 0.2[a]

Different lower case letter denote significant difference ($p<0.05$) between the same plant in the same column. Abbrevation; Phe = Phenanthrene; C = Unspiked soil

remaining in soil ranged from 4.7-8.1 mg kg^{-1} dry soil in unplanted soil and soil planted with the test plants on day 60 (Table 5). This suggests that phytostimulation may be the mechanism by which phenanthrene was removed from soil, as have been reported for PAH removal by other plants in slightly alkaline soil without (Liu *et al*., 2013) or with some metal (zinc, cadmium and lead) co-contaminants (Wang *et al*., 2014).

The presence of cadmium at the concentration tested did not inhibit phenanthrene removal in planted alkaline soil. The results also showed that cadmium were not accumulated by these plants. Low concentration of phenanthrene was detected in whole plant biomass. The amount of phenanthrene found in Siam weed and winged bean plants grown in soil without cadmium addition were only 15 and 17 μg g^{-1} d.wt. of plant biomass. Carpet grass accumulated 49 and 19.1 μg g^{-1} of phenanthrene in its biomass when grown in soil without cadmium and soil spiked with cadmium, respectively (Table 6). These were higher than the other two plants. This result is not surprising for winged bean because to our knowledge, this plant has not been reported to be capable of metal accumulation. The results showed that winged bean can survive in soil spiked with phenanthrene+cadmium for 60 days. A previous study also showed that this plant can remove other PAHs such as anthracene and fluorene from alkaline soil

Table 4: Percentage of phenanthrene remaining in soil spiked with phenanthrene or phenanthrene plus cadmium and planted with carpet grass, Siam weed and winged bean for 60 days

Plant	Percentage	
	Day 30	Day 60
Phenanthrene-spiked soil		
No plant	97.6 ± 10.5[a]	52.1 ± 3.7[a]
Carpet grass	59.0 ± 21.5[ab]	6.6 ± 0.8[b]
Siam weed	45.5± 6.3[b]	6.3± 0.6[b]
Winged bean	7.9 ± 4.1[c]	12.4± 3.9[b]
Phenanthrene+cadmium (II)-spiked soil		
No plant+Cd	60.9 ± 13.1[a]	18.9 ± 12.9[a]
Carpet grass+Cd	35.5 ± 3.3[b]	27.1± 10.6[a]
Siam weed+Cd	31.4± 7.8[b]	13.2 ± 5.7[a]
Winged bean+Cd	23.8± 2.6[b]	5.1 ± 2.5[a]

The initial phenanthrene concentration in soil was 87.8 and 44.9 mg kg^{-1} in soil without and with Cd, respectively, and the initial concentration of cadmium (where relevant) was 6.2 mg kg^{-1}; Different lower case letter denote significant difference ($p<0.05$) between the same plant in the same column

Table 5: Amount of cadmium remaining in soil and accumulation of cadmium in tissues of plant grown in soil spiked with phenanthrene plus cadmium for 60 days

Treatment	Cd (mg kg^{-1})
Unplanted soil	8.1 ± 0.8[a]
Soil planted with carpet grass	7.5 ± 3.3[a]
Soil planted with Siam weed	4.7 ± 0.4[a]
Soil planted with winged bean	6.0 ± 3.6[a]
Biomass of carpet grass	B.D.
Biomass of Siam weed	1.0
Biomass of winged bean	1.2

B.D. = below detection limit; the initial cadmium concentration found in soil was 6.2 mg kg^{-1}

Table 6: Phenanthrene accumulated in tissues of each plant grown in soil spiked with phenanthrene or phenanthrene plus cadmium for 60 days

Plant	µg g^{-1} dry weight whole plant
Soil spiked with phenanthrene	
Carpet grass	49.0
Siam weed	15.0
Winged bean	17.0
Soil spiked with phenanthrene+ cadmium	
Carpet grass	19.1
Siam weed	17.0
Winged bean	17.0

(pH 8.1) (Somtrakoon *et al*., 2014 b). Negligible amount of cadmium was accumulated by Siam weed and carpet grass in this study, in contrast to other studies which reported the ability of Siam weed (Phaenark *et al*., 2009; Tanhan *et al.*, 2007) and carpet grass (Sao *et al*., 2006) to accumulate cadmium. However, in the earlier studies, Siam weed and carpet grass were planted in soil with lower pH as compared to the present study. Phaenark *et al*. (2009) reported that Siam weed was grown in soil (pH range 7.05-7.85) contaminated with zinc and cadmium. The plant was reported to accumulate cadmium in their shoot to the levels exceeding 100 mg kg^{-1} d.wt. Siam weed grown in Hoagland's solution (pH 5.5) containing 5 mg l^{-1} of cadmium ($CdSO_4$) for 15 days accumulated 0.10 and 1.44 g of cadmium per kg d.wt. in the shoot and root, respectively (Tanhan *et al*., 2007) while Siam weed planted in a cadmium-contaminated agricultural field in the Mae Sot district (Tak province, Thailand) was reported to accumulate only 4.3 mg and 2.8 mg of cadmium per kg of shoot and root, respectively (Khaokaew and Landrot, 2015). As for carpet grass, this plant was reported to accumulate cadmium when grown in zinc- and cadmium-contaminated soil (pH 5.5-6.5). The amount of cadmium found in the shoot and root of carpet grass were 669 and 1965 mg kg^{-1} d. wt., respectively, on day 30 of experiment (Sao *et al*., 2006). They also reported that small amount of cadmium was accumulated in both Siam weed and carpet grass.

In the current study, the amount of cadmium accumulated by Siam weed and carpet grass was lower than all the previous studies mentioned above. The previous studies measured cadmium accumulation in hydroponic solutions or in soil with lower pH than the current study. In contrast, the soil used in the current study was alkaline (pH 8.9) and the pH of soil after cadmium addition was about 0.1 pH unit below the initial soil pH. It is likely that the alkalinity of soil decreased the bioavailability of cadmium to the tested plants. In support of this view, Yanai *et al*. (2006) reported that higher pH (pH>6) resulted in lower cadmium bioavailability in soil and led to decreasing cadmium accumulation by the hyperaccumulator plant *Thlaspi caerulescens*.

In summary, winged bean was found to accelerate phenanthrene removal more than the other two plants on day 30. By day 60, all planted treatments showed comparable phenanthrene removal. The main mechanism of phenanthrene removal in this study may be phytostimulation. The alkalinity of soil used in this study likely reduced cadmium accumulation in plant biomass in alkaline soil (Yanai, 2006).

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