Songklanakarin J. Sci. Technol.
41 (6), 1413-1420, Nov. - Dec. 2019

http://www.sjst.psu.ac.th

Original Article

Effect of Triton X-100 and Tween 80 on removal of polycyclic aromatic hydrocarbons and possibility of cadmium accumulation by Siam weed (*Chromolaena odorata*)

Khanitta Somtrakoon[1*] and Waraporn Chouychai[2]

[1] *Department of Biology, Faculty of Science, Mahasarakham University, Kantharawichai, Maha Sarakham, 44150 Thailand*

[2] *Department of Biology and Biotechnology, Faculty of Science and Technology, Nakhon Sawan Rajabhat University, Mueang, Nakhon Sawan, 60000 Thailand*

Received: 9 December 2017; Revised: 27 July 2018; Accepted: 23 August 2018

Abstract

The ability of Triton X-100 and Tween 80 to stimulate phytoremediation of soil contaminated with phenanthrene (204.5 mg kg^{-1}), pyrene (253.6 mg kg^{-1}), and cadmium (81.0 mg kg^{-1}) by Siam weed (*Chromolaena odorata*) was studied. The results revealed that only 10 × critical micelle concentration of Triton X-100 added in soil planted with Siam weed could increase phenanthrene removal compared with unplanted soil that received the same surfactant at 60 days after transplantation. Also, there were no significant differences on polycyclic aromatic hydrocarbon removal in planted soil that received different surfactants. The amounts of phenanthrene and pyrene that remained in the planted soil were 7.4 and 16.7 mg kg^{-1}, respectively, while those remaining in the planted soil with surfactant addition were around 6.7–9.9 and 11.7–24.1 mg kg^{-1}, respectively. Moreover, the addition of Triton X-100 and Tween 80 tended to decrease the accumulation of cadmium in the shoot of Siam weed.

Keywords: cadmium, *Chromolaena odorata*, PAHs, phytoremediation, surfactant

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) and heavy metals, such as cadmium, are frequently found together at contaminated sites (Lyons *et al.*, 2015; Rachwał, Magiera, & Wawer, 2015) including soil samples around the railway junction of Iława Główna, Poland where the amounts of phenanthrene, pyrene, and cadmium were 0.67–5.7, 1.19–7.6, and 0.8–7.4 mg kg^{-1}, respectively (Wiłkomirski, Sudnik-Wójcikowska, Galera, Wierzbicka, & Malawska, 2011). Also the amounts of cadmium and 16 PAHs in the surface soil of a smelting area in South Central China were 1.8–248.6 mg kg^{-1} and 0.45–1.16 mg kg^{-1}, respectively (Sun, Liao, Yan, Zhu, & Ma, 2014). To our knowledge, there are no previous reports of co-contamination of phenanthrene, pyrene, and cadmium in Thai soil. Nevertheless, co-contamination of these pollutants may be possible because cadmium contamination has been extensively reported in Thai soils (Simmons, Pongsakul, Saiyasitpanich, & Klinphoklap, 2005; Sriprachote *et al.,* 2014). Mining activity is the main source of cadmium contamination in Thai soils. This can cause contamination of agricultural soil in the adjacent areas (Simmons, Pongsakul, Saiyasitpanich, & Klinphoklap, 2005). Another source of cadmium contamination in soil is the use of phosphatic fertilizers and some of them are produced from phosphorites containing high concentrations of cadmium (Mahler, Ryan, & Reed, 1987; Parkpian, Leong, Laortanakul, & Thunthaisong, 2003). These anthropogenic activities accompanied with other agricultural activities release PAHs into the environment which includes rice straw open field burning (Gadde, Bonnet, Menke, & Garivait, 2009), pre-harvest of sugar cane burning (Rangel, Henríquez, Costa, & de Lira Junior, 2018), and sewage sludge

*Corresponding author
Email address: khanitta.s@msu.ac.th

application in agricultural soil (Li & Ma, 2016). Thus, co-contamination of cadmium and PAHs could occur in the environment of Thailand.

Remediation of PAHs and cadmium from contaminated sites is an interesting task because of their ecological toxicity. Long-term exposure or the intake of PAHs in animals can cause severe disease such as carcinogenicity, teratogenicity, and genotoxicity (Dat & Chang, 2017). In addition, the long-term intake of cadmium can cause renal dysfunction (Rana, Tangpong, & Rahman, 2018). Phytoremediation is an attractive and versatile method to remove both PAHs and heavy metals from soil (Gerhardt, Gerwing, & Greenberg, 2017; Sarwar *et al.,* 2017). In general, phytoremediation can be divided into various categories (i.e. phytostimulation, phytodegradation, phytovolatilization, phytoaccumulation, and phytostabilization) based on their mechanisms of contaminant elimination and nature of contaminants (Cristaldi *et al.*, 2017). Different phytoremediation approaches are used to remove PAHs and cadmium from contaminated sites. The phytoremediation approach to remove PAHs from contaminated sites includes phytostimulation, phytodegradation, and phytoaccumulation; however, phytostimulation is the most suitable method because PAHs were completely degraded by soil microorganisms, meaning that PAHs were permanently removed (Cristaldi *et al.,* 2017). In contrast, the most suitable means to remove cadmium from soil is phytoaccumulation if the cadmium accumulates in the aerial parts of the plant because it easy to harvest the biomass of plants for further disposal (Yadav *et al.,* 2018).

The simultaneous removal of PAHs and heavy metals by plants and associated microorganisms may be difficult because plants use different mechanisms to remove PAHs and heavy metals from contaminated soil (Cristaldi *et al.*, 2017). In addition, various factors can affect phytoremediation efficiency, and these include contaminant toxicity, which adversely affects plant survival at contaminated sites, contaminant uptake by the plant, and low contaminant bioavailability (Chirakkara & Reddy, 2015). Also, the toxicity of heavy metals can inhibit the degradation of organic contaminants by soil microorganisms, thereby affecting the efficiency of the phytoremediation (Chirakkara, Cameselle, & Reddy, 2016).

In recent years, the application of surfactants has emerged as a viable method to enhance the remediation of soil contaminated with both hydrophobic organic pollutants and metals (Mao, Jiang, Xiao, & Yu, 2015). Surfactants are known to desorb, solubilize or emulsify poorly water-soluble organic pollutants (Scheibenbogen, Zytner, Lee, & Trevors, 1994), and these pollutant-solubilizing properties may enhance organic pollutant degradation (Jain, Lee, & Trevors, 1992). Moreover, surfactants may increase desorption and dissolution of heavy metals from the soil and sediment and this may improve their bioavailability (Almeida, Dias, Mucha, Bordalo, & Vasconcelos, 2009; Ramamurthy & Memarian, 2012). The use of surfactants to simultaneously enhance PAH and metal phytoremediation at PAH and metal co-contaminated sites has not been widely studied. Of those studies that have been undertaken, both successes and failures have been reported. For example, the application 100 mL of the nonionic surfactant Igepal CA-720 to soil at a concentration equal to 1 x its critical micelle concentration (CMC) could improve phenanthrene (initial concentration was 100 mg kg^{-1}) phytoremediation by *Avena sativa* grown for 61 days in soil contaminated with naphthalene, phenanthrene, lead, cadmium, and chromium. However, the application of Igepal CA-720 did not enhance the removal of cadmium and lead from co-contaminated soil (Chirakkara & Reddy, 2015). In another study, the application of Triton X-100 and Tween 80 separately promoted the remediation of soil co-contaminated with 49.7 mg kg^{-1} of cadmium, 496.5 mg kg^{-1} of lead, and 495.1 mg kg^{-1} of used engine oil (Ramamurthy & Memarian, 2012). Their results revealed that the application of Triton X-100 and Tween 80 separately at concentrations higher than their respective CMC values led to an increased accumulation of cadmium and lead in the biomass of *Brassica juncea*. Tween 80 applied at concentrations ranging from 0.5–2.0 × CMC was effective at enhancing the degradation of used engine oil in rhizospheric soil planted with *Brassica juncea*. The authors suggested that surfactants may be responsible for the solubilization of cadmium, lead, and used engine oil, rendering these pollutants more bioavailable for plant roots and rhizospheric degradation (Ramamurthy & Memarian, 2012).

To the best of our knowledge, only one study by Ramamurthy and Memarian (2012) examined the ability of surfactants to enhance the simultaneous removal of mixed heavy metals and hydrocarbons in the form of used engine oil. The tested surfactants, Triton X-100 and Tween 80, were used to assist PAH phytoremediaion with mixed success by other researchers (Cheng, Lai, & Wong, 2008; Liao *et al.*, 2015). The aim of this study was to investigate the effect of two surfactants, Triton X-100 and Tween 80, on the removal of phenanthrene and pyrene by Siam weed grown in soil contaminated with these pollutants. The possibility of Siam weed to accumulate phenanthrene, pyrene, and cadmium was also investigated. Siam weed was selected for this study because this plant was reported to be a cadmium hyperaccumulator and it was reported to stimulate the removal of phenanthrene, engine oil, and total petroleum hydrocarbons from contaminated soils (Atagana, 2011; Jampasri, Pokethitiyook, Kruatrachue, Ounjai, & Kumsopa, 2016; Phaenark, Pokethitiyook, Kruatrachue, & Ngernsansaruay, 2009; Somtrakoon & Chouychai, 2018).

Triton X-100 and Tween 80 are non-ionic surfactant used as model surfactants. Both surfactants exerted positive effects in phytoremediation (Cheng *et al.,* 2017; Liao *et al.,* 2015). For example, the cost and toxicity to soil microorganisms of Tween 80 is low and it is widely used to clean up soil from hydrophobic organic compounds. Tween 80 is a versatile surfactant that can be used in phytoremediation to remove pollutants from soil (Cheng *et al.,* 2017). Even though Triton X-100 seems to be toxic to living things (Gao, Ling, Zhu, Zhao, & Zheng, 2007), it has high desorption efficiency (Gao, Ling, Zhu, Zhao, & Zheng, 2007) and was reported to stimulate the phytoremediation of PAHs (Liao *et al.*, 2015) and heavy metals (Ramamurthy & Memarian, 2012) from contaminated soil. If Tween 80 can efficiently stimulate the removal of PAH from the soil and stimulate the accumulation of cadmium than Triton X-100, Tween 80 was suggested for phytoremediation. This research studied the application of surfactants in the soil that may simultaneously improve cadmium phytoaccumulation and PAH phytostimulation by Siam weed. If soil planted with Siam weed can simultaneously remove both PAHs and metals, it can reduce the time and cost for mixed contaminant remediation of soil.

2. Materials and Methods

2.1 Preparation of phenanthrene/pyrene/cadmium-spiked soil

Soil with no previous history of PAH contamination was collected from a grassland field near the main campus of Nakhonsawan Rajabhat University, Thailand. The background level of cadmium (Cd^{2+}) was 1.1±0.2 mg kg^{-1}. Phenanthrene and pyrene were not detected in the soil as measured by gas chromatography. This soil was air-dried and ground to minimize the heterogeneity. The soil was then spiked with phenanthrene (Sigma-Aldrich, purity 98%) and pyrene (Sigma-Aldrich, purity 98%) according to the method of Somtrakoon, Chouychai, and Lee (2015). Briefly, phenanthrene and pyrene were dissolved separately in dichloromethane and added to the soil to achieve final concentrations of 204.5±41.2 and 253.6 ±33.0 mg kg^{-1} dry soil, respectively. Phenanthrene/pyrene-spiked soil was then spiked with a solution of $CdNO_3.4H_2O$ (Asia Pacific Specialty Chemicals Limited, purity 99%) to achieve a final concentration of 81.0±7.0 mg kg^{-1} dry soil for Cd^{2+}. The pH of the phenanthrene/pyrene-contaminated-soil after spiking with Cd^{2+} was 7.37. The PAH and cadmium concentrations used in this study were higher than our previous study (Somtrakoon & Chouychai, 2018).

2.2 Experimental design

The phytoremediation experiments were set up in cylindrical plastic pots (16 cm diameter). Each pot contained 1 kg dry weight of the soil spiked with phenanthrene/pyrene/ cadmium. The young Siam weed plants used in this study were collected from a site near a rice field in Banpotpisai District, Nakhonsawan Province, Thailand. Siam weed plants were collected and transported to the laboratory. The plants were grown in unspiked soil in a nursery for 2 weeks and transplanted into the experimental pots. Next, Triton X-100 or Tween 80 was dissolved in distilled water and added separately to the soil to give final concentrations of 1 × and 10 × their respective CMC values (the CMC values for Triton X-100 and Tween 80 were 0.24 and 0.012 mmol kg^{-1}, respectively) on 53 days after transplantation. The experiments consisted of 11 treatments performed in a completely randomized design (Table 1).

Table 1. Experimental design in each treatment.

Treatment	Phenanthrene/ Pyrene/ Cadmium	Siam Weed	Triton X-100	Tween 80
T1	+	+	1 × CMC	-
T2	+	+	10 × CMC	-
T3	+	+	-	1 × CMC
T4	+	+	-	10 × CMC
T5	+	+	-	-
T6	+	-	1 × CMC	-
T7	+	-	10 × CMC	-
T8	+	-	-	1 × CMC
T9	+	-	-	10 × CMC
T10	+	-	-	-
T11	-	+	-	-

Each treatment had five replicates for a total of 55 pots and the positions of the pots were changed randomly every week to reduce positional bias. Distilled water was added every day to each pot to maintain the soil water holding capacity. The experiment was performed for 60 days. The soil samples were then collected and processed for phenanthrene, pyrene, and cadmium analysis. The roots and shoots were collected and the various parameters were measured (i.e. length and fresh and dry weights) as well as chlorophyll a, chlorophyll b, and total chlorophyll contents in the leaves. Chlorophyll contents in the leaves of the plant samples were analyzed and calculated using the equations available in Huang, El-Alawi, Penrose, Glick, and Greenberg (2004).

2.3 Phenanthrene and pyrene extraction and analysis

Soil and plant samples were subjected to Soxhlet extraction as described by Somtrakoon, Chouychai, and Lee (2015). To prepare a soil sample, 1 g dry weight of each sample was mixed with anhydrous sodium sulfate and extracted in the Soxhlet apparatus. The volumes of the extracts were reduced in an evaporator and then the soil and plant extracts were analyzed for phenanthrene and pyrene by gas chromatography with flame ionization detection (Shimadzu, Model GC-2014). The shoots (i.e. stems, branches, and leaves) and roots of Siam weed after dry weight measurement were separately cut into small pieces for the phenanthrene and pyrene analyses. Each 1 g dry weight of shoot or root was subjected to Soxhlet extraction and the extracts were analyzed for phenanthrene and pyrene as described above.

2.4 Cadmium analysis

One g dry weight of plant samples from each treatment were collected for analysis of Cd^{2+} concentrations. The plant samples were subjected to extraction according to the method described by the Land and Development Department, Thailand (2010). The Cd^{2+} content in the extracts was analyzed by inductively coupled plasma optical emission spectrometry at the Environmental Quality Examining Service Center, Faculty of Environment and Resource Studies, Mahasarakham University, Thailand.

2.5 Statistical analysis

The parameters for plant growth and the amount of each PAH are presented as mean±SE. The statistical significance of differences among treatments at $P<0.05$ was analyzed by one-way ANOVA by Microsoft Excel. Subsequent multiple comparisons of means were performed using the least significant difference test.

3. Results and Discussion

3.1 Growth of Siam weed in phenanthrene/pyrene/ cadmium-spiked soil

Growth of the Siam weed in the phenanthrene/ pyrene/cadmium-spiked soil appeared normal (Figure 1). The shoot length and root length of Siam weed grown in phenanthrene/pyrene/cadmium-spiked soil were not significantly different from those of the plants grown in unspiked soil (Table 2). In addition, the presence of both surfactants (Tween 80 and Triton X-100 at 1 × and 10 × of CMC) did not decrease the shoot length of Siam weed grown in phenanthrene/pyrene/cadmium-spiked soil compared to the plants grown in spiked soil without receiving any surfactants (Table 2). A similar trend was also seen with the shoot dry weight. The shoot dry weights of Siam weed grown in phenanthrene /pyrene/cadmium-spiked soil with or without receiving Triton X-100 and Tween 80 were not significantly different (Table 2) (Figure 1). The results in Table 2 also showed that the root fresh weight and root dry weight of Siam weed grown in phenanthrene/pyrene/cadmium-spiked soil with or without Triton X-100 or Tween 80 were not significantly different from those of the plants grown in the unspiked soil without receiving any surfactants. However, the growth of Siam weed in phenanthrene/pyrene/cadmium-spiked soil in the presence of Triton X-100 seemed to be wilted but the growth of Siam weed in unspiked soil and phenanthrene/pyrene/cadmium-spiked soil with or without Tween 80 was normal as observed by the naked eye. The results also showed that the chlorophyll a, chlorophyll b, and total chlorophyll contents in the leaves of Siam weed grown in unspiked soil was not significantly different from the plants grown in the spiked soil with Tween 80 and Triton X-100 at 1 × and 10 × CMC (Table 2).

Based on the values presented in Table 2, Triton X-100 and Tween 80 at 1 × and 10 × CMC did not affect any shoot or root growth of Siam weed in this study. Tween 80 is generally reported to be non-toxic to plant growth. For example, Agnello, Huguenot, van Hullebusch, and Esposito (2015) showed that Tween 80 at concentrations of 0.5 ×, 1 × and 3 × CMC did not affect *Medicago sativa* germination. Likewise, the application of Tween 80 at 100 mg kg^{-1} soil did not cause any significant harmful effect on the biomass of *Agropyron elongatum* (Cheng, Lai, & Wong 2008). However, the leaves and shoot of Siam weed that received 1 × and 10 × CMC of Triton X-100 appeared to be dwarfed and wilted upon visual inspection. Triton X-100 was reported to be toxic to plant root growth. For example, Triton X-100 at concentrations 5.0, 2.5, and 1.25 g L^{-1} inhibited the root growth of *Allium cepa* L. (Ferruzan, Güden, Halide, Fatmanur, & Sinem, 2012). A significantly negative impact of either Tween 80 or Triton X-100 on chlorophyll content in the leaves of Siam weed was not observed in this study. The effect of the surfactant on chlorophyll contents in the leaves of the plants was variable that depended on the type of surfactant (Agnello, Huguenot, van Hullebusch, & Esposito, 2015; Caux, Weinberger, & Carlisle, 1988). For example, Triton (alkyl aryl polyether alcohols) decreased chlorophyll contents in *Lemna minor* (Caux, Weinberger, & Carlisle, 1988); however, Tween 80 did not have any significant effects on chlorophyll contents in the leaves of the plant (Agnello, Huguenot, van Hullebusch, & Esposito, 2015).

3.2 Phenanthrene and pyrene removal from soils

The addition of both surfactants (Triton X-100 and Tween 80) at 1 × and 10 × CMC did not significantly improve the removal of pyrene from the planted or unplanted pots compared to the pots without surfactant treatments (Table 3). Only 10 × CMC of Triton X-100 improved the removal of phenanthrene from the soil planted with Siam weed compared

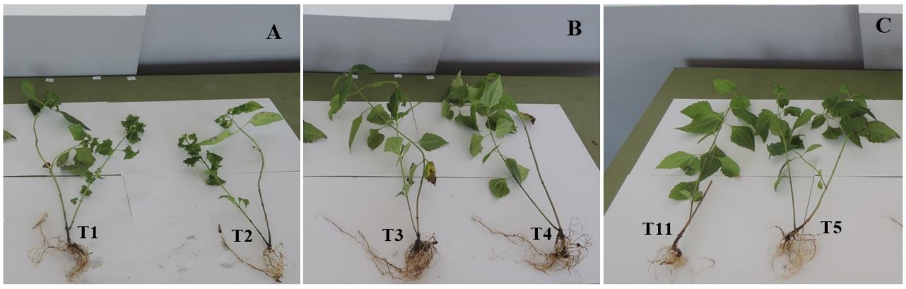

Figure 1. Physical appearance of Siam weed grown in phenanthrene/pyrene/cadmium-spiked soil with Triton X-100 (A), Tween 80 (B) or without treatment (C). Abbreviations: T = treatment.

Table 2. Growth of shoot, growth of root, and chlorophyll content in the leaves of Siam weed vegetated in phenanthrene/pyrene/ cadmium-spiked and unspiked soil for 60 days.

Treatment	Shoot			Root			Chlorophyll		
	Length (cm)	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight (g)	Dry weight (g)	Chlorophyll a (mg ml^{-1})	Chlorophyll b (mg ml^{-1})	Total chlorophyll (mg ml^{-1})
T1	35.0±3.7a	4.5±0.5b	1.5±0.2a	18.1±3.2a	1.7±0.3a	0.6±0.2a	23.9±5.1b	13.2±3.1a	37.1±8.2b
T2	37.6±4.4a	3.7±0.3b	1.6±0.2a	19.8±3.4a	1.4±0.4a	0.4±0.2a	38.2±5.2b	21.4±5.6a	59.6±9.6ab
T3	34.4±5.6a	3.8±0.3b	1.8±0.2a	14.8±4.2a	1.5±0.5a	0.7±0.3a	62.2±7.8a	25.8±1.5a	88.0±9.1a
T4	32.9±2.3a	4.6±0.8b	1.2±0.5a	15.2±4.6a	1.7±0.4a	0.5±0.1a	47.4±5.7ab	24.0±3.7a	71.4±9.3ab
T5	35.7±4.8a	7.1±0.9a	1.6±0.2a	14.7±2.3a	1.8±0.5a	0.5±0.1a	36.0±8.4b	18.4±2.2a	54.4±10.2b
T11	31.5±4.7a	5.1±0.4b	1.3±0.5a	11.0±2.5a	1.7±0.2a	0.4±0.1a	44.8±7.7ab	26.2±1.9a	71.0±7.1ab

Different lower case letters denote significant differences (P<0.05) between plant growth parameters in the same column.
Abbreviations: T = treatment.

Table 3. Amounts of phenanthrene and pyrene remaining in the soil vegetated with and without Siam weed in phenanthrene/ pyrene/cadmium-spiked soil for 60 days.

Treatment	Phenanthrene (mg kg^{-1})	Pyrene (mg kg^{-1})
T1	7.2±0.5ab	11.7±1.6b
T2	6.7±0.8b	12.2±1.8b
T3	8.5±0.8ab	24.1±4.3ab
T4	9.9±3.1ab	12.1±1.9b
T5	7.4±1.4ab	16.7±5.4b
T6	12.7±0.8ab	31.4±1.5ab
T7	15.3±0.8a	19.3±1.0ab
T8	23.2±7.0a	33.9±7.1a
T9	13.1±2.2ab	27.2±7.8ab
T10	16.9±2.6a	26.1±0.7a

Different lower case letters denote significant differences ($P<0.05$) between each parameter in the same column. Abbreviations: T = treatment.

to the unplanted soil which received the same surfactants. The amounts of phenanthrene and pyrene remaining in the soil planted with Siam weed and received surfactant treatments on day 60 were around 6.7–9.9 mg kg^{-1} and 11.7–24.1 mg kg^{-1}, respectively. In comparison, the amounts of phenanthrene and pyrene that remained in the soil planted with Siam weed in the absence of any surfactants in the soil on day 60 were 7.4 mg kg^{-1} and 16.7 mg kg^{-1}, respectively. In the unplanted soil, the amounts of phenanthrene and pyrene that remained on day 60 of the experiment were around 12.7–23.2 mg kg^{-1} and 19.3–33.9 mg kg^{-1}, irrespective of whether the soil received a surfactant or not (Table 3).

The application of a surfactant to soil may increase both PAH and metal phytoremediation capacity as shown by some previous research. However, the behavior of the surfactant may vary depending on the soil characteristics, type of plant, type of pollutants, and other environmental factors (Cheng, Lai, & Wong, 2008; Ramamurthy & Memarian, 2012; Chirakkara & Reddy, 2015; Liao *et al.*, 2015; Liao *et al.*, 2016). In comparison with soil without surfactant, Triton X-100 and Tween 80 did not enhance the phenanthrene and pyrene removal from soil in this study. The inhibitory and stimulatory effects of Triton X-100 and Tween 80 on phenanthrene and pyrene removal from soil were not detected in either the planted or unplanted soil. Most of the soil planted with Siam weed with or without receiving surfactants did not significantly increase the phenanthrene and pyrene removal from the soil compared to soil without Siam weed planting. However, only phenanthrene removal in the soil planted with Siam weed and received 10 × CMC of Triton X-100 was higher than the unplanted soil which received the same surfactant. This meant that the presence of Triton X-100 and Tween 80 at concentrations of 1 × and 10 × CMC did not retard microbial degradation of PAHs in the unplanted soil. In general, soil microorganisms usually adapt to degrade the contaminants in soil. The interaction between plant root and rhizospheric microorganisms helped to increase the pollutant degradation rate (Alagić, Maluckov, & Radojičić, 2015).

There were uncertain results concerning the use of surfactants in phytoremediation and rhizoremediation of PAH and petroleum hydrocarbons from contaminated soil. For example, the application of 10,000 mg L^{-1} of Triton X-100 accelerates the initial phenanthrene and pyrene removal from soil planted with *Zea mays*. More than 80% of phenanthrene and 30% of pyrene were removed in the first 15 days of the experiment. However, at the end of the experiment the amounts of phenanthrene and pyrene that remained in the soil were not significantly different between the planted soil with or without receiving a surfactant (Liao *et al.*, 2015). In another study, the addition of Tween 80 at 100 mg kg^{-1} in soil planted with *Agropyron elongatum* increased the removal of pyrene (initial concentration 294±3 mg kg^{-1}) from the soil to 79% compared to only 61% in soil without Tween 80 after 60 days. Tween 80 probably caused a positive effect on pyrene removal through enhancing the bioavailability of pyrene in the soil (Cheng *et al.*, 2008). However, the addition of 200 mL for 10,000 mg L^{-1} of Tween 80 every half month could remove only 47% of the total petroleum hydrocarbons from the soil planted with *Zea mays* while 52% of the total petroleum hydrocarbons was removed in planted soil without surfactants (Liao *et al.*, 2016).

3.3 Phenanthrene and pyrene accumulation in Siam weed

Negligible amounts of phenanthrene and pyrene accumulated in the Siam weed tissues (Table 4). The root of Siam weed seemed to accumulate both phenanthrene and pyrene to a higher extent than the shoot. In the soil without any surfactants added, the amount of phenanthrene accumulated was about 15.9 µg per g dry weight of shoot and 180.3 µg per g dry weight of root of Siam weed grown in soil spiked with phenanthrene/pyrene/cadmium on day 60. About 37.7 µg of pyrene per g dry weight of shoot and 496.9 µg of pyrene per g dry weight of root were detected in Siam weed grown in soil spiked with phenanthrene/pyrene/cadmium on day 60 of the experiment. Moreover, the addition of Triton X-100 and Tween 80 at 1 × and 10 × CMC to the soil did not improve either phenanthrene or pyrene accumulation in the root or shoot of Siam weed. The amounts of phenanthrene in the root and shoot of Siam weed in the presence of Triton X-100 and Tween 80 were around 126.3–168.8 and 23.3–33.4 µg per g dry weight, respectively, on day 60. The amounts of pyrene in

Table 4. Accumulation of phenanthrene, pyrene, and cadmium in shoot (i.e. stems, branches, and leaves) and root of Siam weed vegetated in phenanthrene/pyrene/cadmium-spiked soil with or without surfactants for 60 days.

Treatment	Phenanthrene (µg g^{-1})	Pyrene (µg g^{-1})	Cadmium (µg g^{-1})
Shoot			
T1	23.3	24.9	18.1
T2	12.2	15.5	9.4
T3	18.3	38.9	14.2
T4	33.4	60.6	12.8
T5	15.9	37.7	36.9
Root			
T1	126.3	398.7	43.5
T2	139.5	352.1	42.1
T3	N.D.	N.D.	43.0
T4	168.8	428.8	33.5
T5	180.3	496.9	39.5

Abbreviations: N.D. = not detected, T = treatment.

the biomass of Siam weed root were 398.7 μg per g dry weight and 428.8 μg per g dry weight grown in phenanthrene/pyrene/cadmium-spiked soil and received 1 × CMC of Triton X-100 or 10 × CMC of Tween 80, respectively (Table 4).

The accumulation of phenanthrene and pyrene in Siam weed biomass was negligible. In the presence or absence of Triton X-100 and Tween 80, the amounts of phenanthrene and pyrene in Siam weed biomass seemed similar. Thus, the main mechanism of phenanthrene and pyrene removal may be from microbial degradation of phenanthrene and pyrene in the soil (Alagić, Maluckov, & Radojičić, 2015). Results from the use of surfactant to improve PAH accumulation in the plant also varied. For example, the accumulation of PAHs, such as fluorene, phenanthrene, pyrene, acenapthene, and fluoranthene, was inhibited in maize leaves planted in soil that received Tween 80 (Liao *et al.,* 2016). In this study, the accumulation of phenanthrene and pyrene did not improve in the presence of Triton X-100 and Tween 80. A study by Liao *et al.* (2015) also reported that the accumulation of phenanthrene and pyrene by maize plants was not significantly influenced by the addition of Triton X-100, saponin, or rhamnolipid. Another report revealed that using a surfactant may inhibit PAH accumulation. For example, the application of a surfactant, such as 100–200 mg $kg^{-1}$of cetyltrimethylammonium bromide, to 20.5 mg kg^{-1} of phenanthrene- and 40.3 mg kg^{-1} of pyrene-contaminated soil significantly reduced the uptake of phenanthrene and pyrene by *Chrysanthenum coronarium*, *Brassica campestris*, and *Lactuca sativa* by about 51–66%, 62–71%, and 34–53%, respectively (Lu & Zhu, 2009).

3.4 Cadmium accumulation in Siam weed biomass

Cadmium concentration in the phenanthrene/pyrene/cadmium-spiked soil at the beginning of the experiment was 81 mg kg^{-1}.The accumulation of cadmium by Siam weed was negligible (Table 4). Only 36.9 μg and 39.5 μg of cadmium per g dry weight of shoot and root, respectively, were detected in the biomass of Siam weed grown in phenanthrene/pyrene/cadmium without any surfactant treatments. The addition of Triton X-100 and Tween 80 tended to decrease the accumulation of cadmium in the shoot of Siam weed. At 10 × CMC of Triton X-100 and Tween 80, the amount of cadmium accumulation was lower in the shoot biomass of Siam weed than with the 1 × CMC. Only 14.2–18.1 μg of cadmium and 9.4–12.8 μg of cadmium per g dry weight of shoot were detected in Siam weed that received 1 × and 10 × CMC of both surfactants, respectively. However, the addition of Triton X-100 and Tween 80 at both concentrations did not affect the cadmium accumulation in the root of Siam weed.

The application of Triton X-100 and Tween 80 at 1 × of CMC tended to increase the cadmium accumulation only in the biomass of Siam weed root; however, the accumulation of cadmium in the biomass of Siam weed shoot did not improve. Increasing the heavy metals in the root biomass in the presence of surfactants could be due to several mechanisms, such as the surfactants changing the membrane permeability of the root cells or surfactants promoting the desorption of metals. These mechanisms may accelerate the metal uptake by plant functions (Gregorio *et al.,* 2006; Mao, Jiang, Xiao, & Yu, 2015). However, the exact mechanisms by which Triton X-100 and Tween 80 promoted the accumulation of cadmium in the biomass of Siam weed root was not confirmed in this study. There have been reports that surfactant addition could promote metal accumulation. For example, combined applications of Triton X-100 at 5 × and 10 × CMC increased the permeability of the root membrane and increased the lead phytoextraction efficiency to 56%. The application of Triton X-100 to soil with *Sinorhizobium* sp. Pn002 was crucial for the survival of the plant in the presence of lead and Triton X-100 (Gregorio *et al.,* 2006). Triton X-100 at 0.25 mM also favored copper solubility and favored the accumulation of copper in the root of *Halimione portulacoides* (Almeida, Dias, Mucha, Bordalo, & Vasconcelos, 2009). In turn, there were reports of Tween 80 stimulating the accumulation of cadmium from contaminated soil. For example, the combined application of 5 mmol of Tween 80 per kg with 1 mmol of gibberellic acid (GA_3) per kg promoted cadmium accumulation in the shoot of *Tagetes patula* by 0.46 times compared to the control without GA_3 or Tween 80 (Sun *et al.,* 2013). The use of Triton X-100 and Tween 80 in combination was also reported. The application of Triton X-100 and Tween 80 in sandy soil (pH 6.6) at concentrations higher than their CMCs enhanced the cadmium and lead accumulation in the roots of Indian mustard (*Brassica juncea*) (Ramamurthy & Memarian, 2012).

4. Conclusions

Triton X-100 and Tween 80 did not improve either phenanthrene or pyrene removal from soil planted with Siam weed in this study. Only the addition of 10 × CMC of Triton X-100 could improve phenanthrene removal from the planted soil. The accumulation of PAHs in the Siam weed biomass was not significant. Degradation of phenanthrene and pyrene by competent microorganisms already present in the soil was possibly the main mechanism of PAH removal in this study. A slight increase in the accumulation of cadmium in the biomass of Siam weed root was observed in this study; however, the amount of cadmium accumulation was negligible. Siam weed was not suitable for simultaneous phytoremdiation of soil contaminated with both PAH and cadmium.

Acknowledgements

The authors gratefully acknowledge financial support from the Faculty of Science, Mahasarakham University (Financial Year 2017). We would like to thank Dr. Jolyon Dodgson, Faculty of Science, Mahasarakham University for his help in correcting the English of this manuscript.

References

Alagić, S. Č., Maluckov, B. S., & Radojičić, V. B. (2015). How can plants manage polycyclic aromatic hydrocarbons? May these effects represent a useful tool for an effective soil remediation? A review. *Clean Technology Environment Policy, 17*(3), 597-614. doi:10.1007/s10098-014-0840-6

Almeida, C. M. R., Dias, A. C., Mucha, A. P., Bordalo, A. A., & Vasconcelos, M. T. S. D. (2009). Influence of surfactants on the Cu phytoremediation potential of a salt marsh plant. *Chemosphere, 75*(2), 135-140. doi:10.1016/j.chemosphere.2008.12.037

Agnello, A. C., Huguenot, D., van Hullebusch, E. D., & Esposito, G. (2015). Phytotoxicity of citric acid and Tween®80 for potential use as soil amendments in enhanced phytoremediation. *International Journal of Phytoremediation, 17*(7), 669-677. doi:10.1080/15226514.2014.964837

Atagana, H. I. (2011). The potential of Chromolaena odorata (L) to decontaminate used engine oil impacted soil under greenhouse conditions. *International Journal of Phytoremediation*, *13*(7), 627-641. doi:10.1080/15226514.2010.525551

Caux, P. Y., Weinberger, P., & Carlisle, D. B. (1988). A physiological study of the effects of Triton surfactants on Lemna minor L. *Environmental Toxicology and Chemistry, 7*(8), 671-676. doi:10.1002/etc.5620070808

Cheng, K. Y., Lai, K. M., & Wong, J. W. C. (2008). Effects of pig manure compost and nonionic-surfactant Tween 80 on phenanthrene and pyrene removal from soil vegetated with Agropyron longatum. *Chemosphere, 73*(5), 791-797. doi:10.1016/j.chemosphere.2008.06.005

Cheng, M., Zeng, G., Huang, D., Yang, C., Lai, C., Zhang, C., & Liu, Y. (2017). Advantages and challenges of Tween 80 surfactant-enhanced technologies for the remediation of soils contaminated with hydrophobic organic compounds. *Chemical Engineering Journal, 314*, 98-113. doi:10.1016/j.cej.2016.12.135.

Chirakkara, R. A., & Reddy, K. R. (2015). Biomass and chemical amendments for enhanced phytoremediation of mixed contaminated soils. *Ecological Engineering, 85*, 265-274. doi:10.1016/j.ecoleng.2015.09.029

Chirakkara, R. A., Cameselle, C., & Reddy, K. R. (2016). Assessing the applicability of phytoremediation of soils with mixed organic and heavy metal contaminants. *Reviews in Environmental Science and Bio /Technology, 15*(2), 299-326. doi:10.1007/s11157-016-9391-0

Cristaldi, A., Conti, G. O., Jho, E. H., Zuccarello, P., Grasso, A., Copat, C., & Ferrante, M. (2017). Phytoremediation of contaminated soils by heavy metals and PAHs. A brief review. *Environmental Technology and Innovation, 8*, 309-326.

Dat, N. D., & Chang, M. B. (2017). Review on characteristics of PAHs in atmosphere, anthropogenic sources and control technologies. *Science of the Total Environment, 609*, 682-693. doi: 10.1016/j.scitotenv.2017.07.204

Ferruzan, D., Güden, Y., Halide, A., Fatmanur, ö., & Sinem, L. (2012). Phytotoxic effects of non-ionic surfactant octylphenol series (Triton X-100, Triton X-114, Triton X-405) on onion. *Asian Journal of Chemistry, 24*(12), 5746-5748. Retrieved from http://eds.a.ebscohost.com/eds/detail/detail?vid=0&sid=b2c60bb1-d3af-4ca3-aae7555647fdafb5%40sessionmgr4008&bdata=JnNpdGU9ZWRzLWxpdmU%3d#AN=89561587&db=a9h

Gadde, B., Bonnet, S., Menke, C., & Garivait, S. (2009). Air pollutant emissions from rice straw open field burning in India, Thailand and the Philippines. *Environmental Pollution, 157*(5), 704-713. doi:10.1016/j.envpol.2009.01.004

Gao, Y. Z., Ling, W. T., Zhu, L. Z., Zhao, B. W., & Zheng, Q. S. (2007). Surfactant-enhanced phytoremediation of soils contaminated with hydrophobic organic contaminants: Potential and assessment. *Pedosphere, 17* (4), 409-418. Retrieved from https://ac.els-cdn.com/S1002016007600502/1-s2.0-S1002016007600502-main.pdf?_tid=93ab4c77-bbd4-4269-8239-249562fb8233&acdnat=1531309426_2ae638285433a39c2376d7c305174471

Gerhardt, K. E., Gerwing, P. D., & Greenberg, B. M. (2017). Opinion: Taking phytoremediation from proven technology to accepted practice. *Plant Science, 256*, 170-185. doi:10.1016/j.plantsci.2016.11.016

Gregorio, S. D., Barbafieri, M., Lampis, S., Sanangelantoni, A. M., Tassi, E., & Vallini, G. (2006). Combined application of Triton X-100 and Sinorhizobium sp. Pb002 inoculum for the improvement of lead phytoextraction by Brassica juncea in EDTA amended soil. *Chemosphere, 63*(2), 293-299. doi:10.1016/j.chemosphere.2005.07.020

Huang, X.-D., El-Alawi, Y., Penrose, D. M., Glick, B. R., & Greenberg, B. M. (2004). Responses of three grass species to creosote during phytoremediation. *Environmental Pollution, 130*(3), 453-463. doi:10.1016/j.envpol.2003.12.018

Jain, D. K., Lee, H., & Trevors, J. T. (1992). Effect of addition of Pseudomonas aeruginosa UG2 inocula or biosurfactant on biodegradation of selected hydrocarbons in soil. *Journal of Industrial Microbiology, 10*(2), 87-93. Retrieved from https://link.springer.com/article/10.1007/BF01583840

Jampasri, K., Pokethitiyook, P., Kruatrachue, M., Ounjai, P., & Kumsopa, A. (2016). Phytoremediation of fuel oil and lead co-contaminated soil by Chromolaena odorata in association with *Micrococcus luteus*. *International Journal of Phytoremediation, 18*(10), 994-1001. doi:10.1080/15226514.2016.1183568

Li, H., & Ma, Y. (2016). Field study on the uptake, accumulation, translocation and riskassessment of PAHs in a soil-wheat system with amendments of sewage sludge. *Science of the Total Environment*, 560-561, 55-61. doi:10.1016/j.scitotenv.2016.04.017

Liao, C., Liang, X., Lu, G., Thai, T., Xu, W., & Dang, Z. (2015). Effect of surfactant amendment to phytoremediation by maize (*Zea mays* L.). *Ecotoxicology and Environmental Safety, 112*, 1-6. doi:10.1016/j.ecoenv.2014.10.025

Liao, C., Xu, W., Lu, G., Deng, G., Liang, X., Guo, C., & Dang, Z. (2016). Biosurfactant-enhanced phytoremediation of soils contaminated by crude oil using maize (Zea mays L.). *Ecological Engineering, 92*, 10-17. doi:10.1016/j.ecoleng.2016.03.041

Lu, L., & Zhu, L. (2009). Reducing plant uptake of PAHs by cationic surfactant-enhanced soil retention. *Environmental pollution, 157*(6), 1794-1799. doi:10.1016/j.envpol.2009.01.028

Lyons, B. P., Barber, J. L., Rumney, H. S., Bolam, T. P. C., Bersuder, P., Law, R. J., & Al-Sarawi, C. (2015). Baseline survey of marine sediments collected from the State of Kuwait: PAHs, PCBs, brominated flame retardants and metal contamination. *Marine Pollution Bulletin, 100*(2), 629-636. doi:10.1016/j.marpolbul.2015.08.014

Mahler, R. J., Ryan, J. A., & Reed, T. (1987). Cadmium sulfate application to sludge-amended soils: Effects on yield and cadmium availability to plants. *Science of the Total Environment, 67*(2-3), 117–131. doi:10.1016/0048-9697(87)90205-1

Mao, X., Jiang, R., Xiao, W., & Yu J. (2015). Use of surfactants for the remediation of contaminated soils: A review. *Journal of Hazardous Materials, 285*, 419-435. doi:10.1016/j.jhazmat.2014.12.009

Parkpian, P., Leong, S., Laortanakul, P., & Thunthaisong, N. (2003). Regional Mornitoring of lead and cadmium contamination in a tropical grazing land site, Thailand. *Environmental Monitoring and Assessment, 85*(2), 157-173. Retrieved from https://link.springer.com/content/pdf/10.1023%2FA%3A1023638012736.pdf

Phaenark, C., Pokethitiyook, P., Kruatrachue, M., & Ngernsansaruay, C. (2009). Cd and Zn accumulation in plant from the Padaeng zinc mine area. *International Journal of Phytoremediation, 11*(5), 479-495. doi:10.1080/15226510802656243

Rachwał, M., Magiera, T., & Wawer, M. (2015). Coke industry and steel metallurgy as the source of soil contamination by technogenic magnetic particles, heavy metals and polycyclic aromatic hydrocarbons. *Chemosphere, 138*, 863-873. doi:10.1016/j.chemosphere.2014.11.077

Ramamurthy, A. S., & Memarian R. (2012). Phytoremediation of mixed soil contaminants. *Water, Air, and Soil Pollution, 223*(2), 511-518. doi:10.1007/s11270-011-0878-6

Rana, M. N., Tangpong, J., & Rahman, M. M. (2018). Toxicodynamics of Lead, Cadmium, Mercury and Arsenic- induced kidney toxicity and treatment strategy: A mini review. *Toxicology Reports, 5*, 704-713. doi:10.1016/j.toxrep.2018.05.012

Rangel, M. G. L., Henríquez, J. R., Costa, J. A. P., & de Lira Junior, J. C. (2018). An assessment of dispersing pollutants from the pre-harvest burning of sugarcane in rural areas in the northeast of Brazil. *Atmospheric Environment, 178*, 265-281. doi:10.1016/j.atmosenv.2018.02.006

Sarwar, N., Imran, M., Shaheen, M. R., Ishaque, W., Kamran, M. A., Matloob, A., & Hussian, S. (2017). Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. *Chemosphere, 171*, 710-721. doi:10.1016/j.chemosphere.2016.12.116

Scheibenbogen, K., Zytner, R. G., Lee, H., & Trevors, J. T. (1994). Enhanced removal of selected hydrocarbons from soil by *Pseudomonas aeruginosa* UG2 biosurfactants and some chemical surfactants. *Journal of Chemical Technology and Biotechnology, 59*(1), 53-59. doi:10.1002/jctb.280590109

Simmons, R. W., Pongsakul, P., Saiyasitpanich, D., & Klinphoklap, S. (2005). Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice grain downstream of a zinc mineralized area in Thailand: Implications for public health. *Environmental Geochemistry and Health, 27*(5-6), 501-511. doi:10.1007/s10653-005-7857-z

Somtrakoon, K., Chouychai, W., & Lee, H. (2015). Removal of anthracene and fluoranthene by waxy corn, long bean and okra in lead-contaminated soil. *Bulletins of Environmental Contamination and Toxicology, 95* (3), 407-413.doi:10.1007/s00128-015-1587-4

Somtrakoon, K., & Chouychai, W. (2018). Removal of phenanthrene and cadmium from co-contaminated alkaline soil by carpet grass, Siam weed and winged bean. *Journal of Environmental Biology, 39*(6), (In press). doi:10.22438

Sriprachote, A., Pengprecha, S., Pengprecha, P., Kanyawongha, P., Ochiai, K., & Matoh, T. (2014). Assessment of cadmium and zinc contamination in the soils around Pha Te Village, Mae Sot District, Tak Province, Thailand. *Applied Environmental Research, 36*(4), 67-79. Retrieved from http://www.tci-thaijo.org/index.php/aer

Sun, L., Liao, X., Yan, X., Zhu, G., & Ma, D. (2014). Evaluation of heavy metal and polycyclic aromatic hydrocarbons accumulation in plants from typical industrial sites: potential candidate in phytoremediation for co-contamination. *Environmental Science and Pollution Research, 21*(21), 12494-12504. doi:10.1007/s11356-014-3171-6

Sun, Y., Xu, Y., Zhou, Q., Wang, L., Lin, D., & Liang, X. (2013). The potential of gibberellic acid (GA_3) and Tween-80 induced phytoremediation of co-contamination of Cd and Benzo[a]pyrne (BaP) using Tagetes patula. *Journal of Environmental Management, 114*, 202-208. doi: 10.1016/j.jenvman.2012.09.018

Wiłkomirski, B., Sudnik-Wójcikowska, B., Galera, Wierzbicka, M., & Malawska, M. (2011). Railway transportation as a serious source of organic and inorganic pollution. *Water, Air, and Soil Pollution, 218*(1-4), 333-345.doi:10.1007/s11270-010-0645-0

Yadav, K. K., Gupta, N., Kumar, A., Reece, L. M., Singh, N., Rezania, S., & Khan, S. A. (2018). Mechanistic understanding and holistic approach of phytoremediation: A review on application and future prospects. *Ecological Engineering, 120*, 274-298. doi:10.1016/j.ecoleng.2018.05.039