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POTENTIAL OF *Acalypha wilkesiana* IN THE PHYTOREMEDIATION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM LANDFILL SOIL

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ABSTRACT
Polycyclic aromatic hydrocarbons make up a substantial portion of organic contamination in landfill soil. Environmental pollution caused by polycyclic aromatic hydrocarbons (PAHs) is of great concern because hydrocarbons are toxic to all forms of life. The research work aimed at assessing the potential of Acalypha wilkesiana plant in the phytoremediation of PAHs from landfill soil. Primarily, the research focused on identification of pollutants in the soil and plant parts. Results showed that PAHs contamination in the landfill soil is low. A. wilkesiana was planted on landfill soil and harvested after 2, 4 and 6 months. A set of A. wilkesiana were planted on uncontaminated soil. The results indicate that A. wilkesiana, when cultivated in landfill soil, exhibited a slight reduction in stem diameter when compared to the control group of plants. But the root length and stem height were lower than that of the control. The dry biomass weight of the root and leaf of exposed plant were found higher compared to the control plant. It was noticed that higher accumulation occurred of Benzo [g.h.i] perylene at 2,4- and 6-months harvesting periods with concentrations of 5.15, 23.25 and 38.54 mg/kg, respectively. Anthracene was translocated to the leaves with concentrations 9.77, 16.35 and 27.16 mg/kg, at 2-, 4- and 6-months harvesting period, respectively. The analysis of A. wilkesiana parts confirmed that PAHs were accumulated and translocated and was an evident that the plant was able to survive on the landfill soil.
Keywords: ***Acalypha wilkesiana, landfill, PAHs, phytoremediation, GC-MS***

INTRODUCTION

The chemical waste generated by industries, from agricultural practices, and poor waste management all contribute to the existence of organic contaminants into the soil. The long-term stability of these waste chemicals in the soil is responsible to human and animal health (Allamin and Shukor, 2021). The time needed for PAHs to decrease or transform to one half its initial value in contaminated soil depends on the compound, but it was reported that, some PAHs has a half-life of 8 months to 2 years, while some PAHs has ranged between 8 to 28 years. PAHs are present everywhere and highly mobile compounds that constitute a serious environmental problem, due to their occurrence, recalcitrance, bioaccumulation potential, and carcinogenic activity, PAHs becomes a serious environmental issue (Mackiewicz-Walec *et al*., 2022). Green plants, such as, *Thlaspi caerulescens*, or *Helianthus annuus*, *Brassica juncea, Salix spp*. or *Populus*, which are woody species are used in phytoremediation to reduce, absorb, or turn various environmental pollutants in water and soil, such as organic compound to harmless (Allamin and Shukor, 2021). PAHs enter into plants through leaf stomata or through the root system. PAHs accumulated in the plant part have a toxic effect (Petrova *et al.,* 2017). The plant was chosen for this research work because they provide a good root system, large biomass, and they can tolerate condition of the soil as well as greenhouse condition. A research was carried out to study the growth and metal absorption capability of *A. wilkesiana* in the phytoremediation of soil contaminated with heavy metals (Durumin-Iya *et al*., 2018). But there are few researches on the potential of the plant to undergo phytoremediation of landfill soil. *A. wilkesiana* is a plant that grows fast and different color of leaves which depends on cultivation, and it is from Euphorbiaceous family (Durumin-Iya *et al*., 2021). It was noticed that,

studies on the degradation capability of PAHs from landfill soil by *A. wilkesiana* is not enough.

Different plant species are being used to remediate a soil contaminated with hydrocarbons, but the removal capacity of plants differs from one species to another (Sun *et al.,* 2011). Limited experiments have been carried out on the phytoremediation capability of *A. wilkesiana* to absorb and accumulate PAHs in landfill soil. The objectives of this research were (a) to assess the survival of the plant grown on landfill soil and (b) to assess the absorption and transfer of PAHs to plant shoot within the research period at green house conditions.

MATERIALS AND METHODS

Soil sample collection and preparation

Landfill soil samples were collected from Matang abondaned landfill site about 16.00 km from Universiti Malaysia Sarawak (UNIMAS) with GPS position of N 01 ^{0}34' 56.6'' and E 110 ^{0} 14' 39.2''. The soil samples were collected at 5 - 15 cm depth because the top surface contains a lot of unwanted materials such as debris, broken bottles, undegraded plastics and grasses. The soil samples were collected using a stainless steel scoop and wrapped with an aluminium foil. The soil samples were kept in cooler box during transportation. Four different locations were selected with 25 meter difference from each other. Four different points were choosen in each location and the soil samples were collected.

Poly Bag Experiment

A total of sixteen (16) soil samples were spreaded and allowed to dry in a room and thoroughly mixed to ensure homogeneity and were seived through a 4-mm sieve. Approximately 500 g of dried soil were placed in poly bags and planted with plant cuttings. A total of forty eight (48) poly bags were used for this phase of the experiment. Eight (8) treated and eight (8) control poly bags were used on 2 months interval for the period of six (6) months. Pots were arranged in the greenhouse of Universiti Malaysia Sarawak, and were netted and covered with transparent polythene sheet. The plants grow under the greenhouse condition and were watered when needed.

Plants Extraction and Fractionation

The plants were harvested on 2nd, 4th and 6th months (Sheng-You *et al*., 2005). The crude extract of plant parts hydrocarbons was obtained according to the procedure explained by Smith *et al*. (2006) with slight modification. 3.0 g of powdered plant part sample was put in a thimble cellulose on a Soxhlet extractor and 350 mL of dichloromethane was used to extract for 8 hours. Crude extract was obtained by evaporating the solvent (dichloromethane) and dried in a vacuum rotary evaporator. The extract was stored in a refrigerator at 4 ^{0}C before further analysis was carried out.

An activated silica gel column chromatography was used to fractionate the crude extract by following the procedure outlined by El Nemr *et al*. (2016). A glass burette with a 1 cm diameter was utilized as the column for chromatography, and it was filled with 5.0 grams of activated silica gel ranging from 230 to 400 mesh. 2.0 mL of *n*-hexane was used to dilute the extract and was mounted on the top layer of silica gel in the column. Fractions A (AHs) and B (PAHs) were subsequently separated by washing (elute) with 70 mL *n*-hexane and 70 mL of a mixture of dichloromethane and *n*-hexane (with a ratio 1:1, v/v), respectively. B fraction was collected in a 250 mL beaker and was evaporated in a vacuum rotary evaporator to near dryness. This research was concern with fraction B which contains PAHs. B fraction was then diluted with 2.0 mL of dichloromethane and sonicated. With the help of Pasteur pipette, it was poured into 5 mL vial and a purified nitrogen gas was then used to evaporate it to dryness. 3 mL of dichloromethane (GC grade) was added to the vial and kept in a dark place at 4 ^{0}C temperature until further analysis using GC-MS.

GC-MS for PAHs Analysis

Analysis of PAHs fractions were performed on a Shimadzu Gas Chromatography-Mass Spectrometer model QP2010 Plus equipped with quadrupole mass analyser. Separation was carried out using BPX-5 fused silica capillary column coated with a 5% diphenyl and 95% dimethyl polysiloxane as stationary phase, with internal diameter of 0.25 mm, column length 30 m and film thickness of 0.25 µm. The electron ionization energy system with ionization energy of 70 eV was used for GC-MS detection. The oven temperature was initially programmed at 50 ^{o}C and held isothermal for 5 minutes. The temperature was then ramped to 300 ^{o}C at the rate of 6.5 ^{o}C/minute and held for 15 minutes at final temperature. Exactly 1 µL of sample was injected into the column using split less injection mode. Before the GC-MS analysis, 500 µL dichloromethane was used to dissolve fraction B. Identification of PAH components was performed by direct comparison of retention times of individual PAHs in a mixture of PAHs standard.

Statistical analysis

Data obtained in this research was presented as mean and standard deviation of 8 replicates for both exposed and control plants except where stated.

RESULTS AND DISCUSSION

Plant Survival and Growth on Landfill Soil

Plants grew up for a harvesting period of 2, 4 and 6 months in pots on landfill soil. The leaves biomass of *A. wilkesiana* were not affected by the landfill soil significantly. The rate of plant growth increased a little but it can be compared with unexposed plant (control).

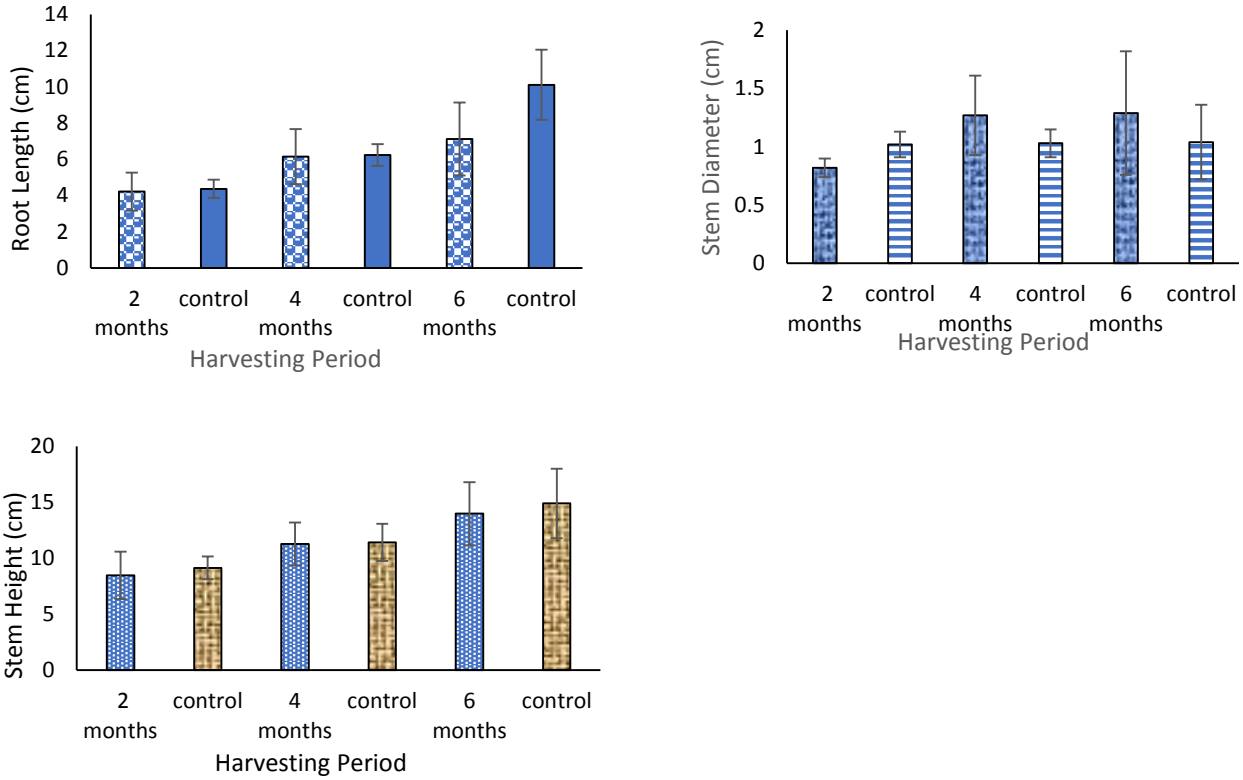

Figure 1: Root length, stem diameter and stem height in (cm), were plotted against the harvesting period

The *A. wilkesiana* root length on 2 and 4 harvesting periods were close to their controls, but lower on 6 months harvesting period when compared to the control. The stem diameter was found to be lower than the control, but was found to be higher than the controls on 4 and 6 harvesting periods. However, the PAHs concentration present in the landfill soil could be the cause of slow development of the plant. In terms of plant root growth, the 2 months and 4 months propagation of the plant on landfill soil did not have much influence on the plant development. At the end of the 6 months experimental period, the weight of *A. wilkesiana* plants at control soil increased relative to the initial fresh plant weight. Though the PAHs concentration in landfill soil caused slight increase in the root and stem heights as well as the diameter of the stem. The plant growth slowdown may be due to the excess calcium, iron, and PAH that are present in the soil (Alkio *et al.*, 2005).

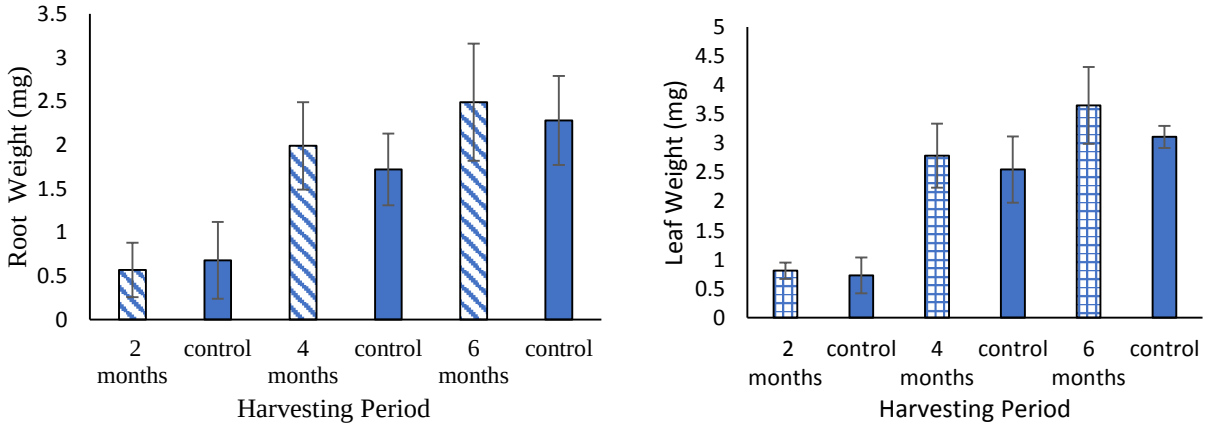

Figure 2: Root weight and leaf weight of the exposed plant and control were plotted against the harvesting period.

Phytoremediation study of PAHs from Landfill Soil

Several landfill sites are usually located at less populated or non-urban areas, where leachate is partially treated or not treated at all, the leachate drops and contaminate the environment, therefore, remediation is very important. Phytoremediation methods utilise the ability of the natural or actively managed soil–plant relationship to remove toxic substances, degrade and inactivate potentially harmful hydrocarbons from landfill soil (Jones *et al*., 2006).

GC Chromatograms of PAHs in Landfill Soil

Figure 3.0 shows the GC chromatogram of PAHs obtained from landfill soil. An important part of landfill remediation scheme is to identify and quantify the available PAHs present in the soil.

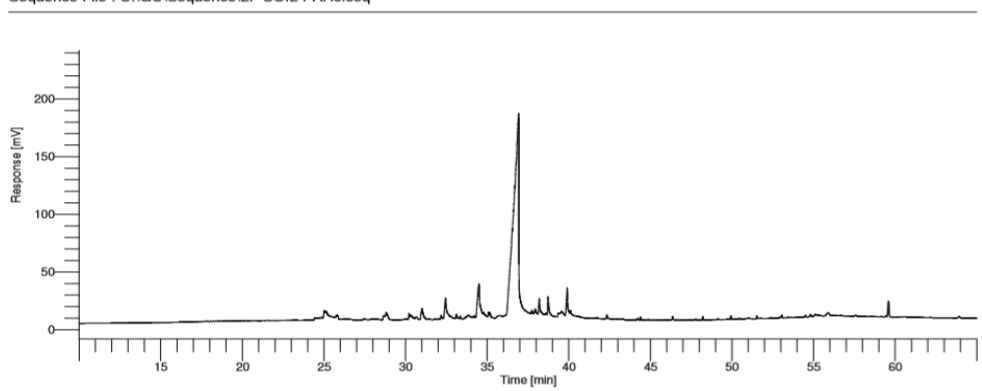

Figure 3**:** GC chromatograms of PAHs present in Landfill soil. The high peak was the response of Eicosene as an internal Standard

Initial Concentration of PAHs in Landfill Soil

The presence of vegetation helps a lot in the dissipation of PAHs in the landfill soil by phytodegradation or biodegradation. Sixteen targetted PAHs have been determined in landfill soils samples collected from Matang abandoned landfill. Table 1.0 shows the concentation of PAHs detected from landfill soil. A significant high concentration occurred in the landfill soil for anthracene and pyrene with with concentration 56.25 and 55.91 mg/kg, respectively.

Table 1: Initial concentration (mg/kg) of PAHs with their respective Retention time from Landfill Soil

PAHs	Retention time n=8	Concentration (n = 8
Naphthalene	13.94±0.03	4.78±0.02
Acenaphthylene	19.74±0.06	0.47±0.01
Acenaphthene	16.42±0.03	1.02±0.01
Fluorene	20.34±0.01	6.82±0.03
Phenanthrene	22.31±0.07	10.48±0.02
Anthracene	25.90±0.09	56.25±0.58
Fluoranthene	26.09±0.06	28.72±0.33
Pyrene	30.36±0.01	55.91±0.62
Benzo[a]anthracene	31.19±0.04	7.96±0.02
Chrysene	35.69±0.05	32.51±0.17
Benzo[b]fluoranthene	35.83±0.01	15.81±0.04
Benzo[k]fluoranthene	39.46±0.02	17.98±0.05
Benzo[a]pyrene	39.55±0.01	4.63±0.02
Dibenzo[a,h]anthracene	40.52±0.06	13.82±0.05
Benzo[g,h,i]perylene	44.78±0.01	36.74±0.34
Indeno[1,2,3-cd] pyrene	44.82±0.04	38.65±0.11

Absorption and Accumulation of PAHs in Plants

The concentration of PAHs absorbed by the plant was obtained from the standard calibration curve of PAHs obtained from GC-MS analysis. The hydrophobic characteristics of PAHs made it available for adsorption by organic particles in the soil (Tipmanee *et al*., 2012). Several PAHs have been detected from soil samples collected from areas far away from human settlements and industrial activites (Perra *et al*., 2011). Concentration of several PAHs in the root of *A.*

wilkesiana grown on landfill soil are presented in Table 2.0. The level of naphthalene, acenaphthene and acenaphthylene are regarded as insignificant impact because of their high volatile nature (Gworek *et al.,* 2014).

Table 2: Concentration (mg/kg) of PAHs in *A. wilkesiana* root grown on Landfill soil within three different harvesting period (n=8)

Compound name	2 months	Control 2	4 months	Control 4	6 months	Control 6
Naphthalene	0.18±0.01	4.78±0.02	0.64±0.01	2.11±0.01	1.08±0.01	1.32±0.01
Acenaphthylene	0.25±0.01	0.47±0.01	0.74±0.01	0.21±0.00	1.52±0.13	0.05±0.00
Acenaphthene	0.86±0.01	1.02±0.01	1.07±0.01	0.42±0.01	1.96±0.16	0.01±0.00
Fluorene	1.17±0.02	6.82±0.03	1.85±0.25	3.91±0.03	1.47±0.02	2.57±0.03
phenanthrene	1.79±0.04	10.48±0.02	2.64±0.16	7.16±0.12	3.82±0.18	4.36±0.16
Anthracene	3.51±0.12	56.25±0.58	9.35±1.22	33.70±2.11	16.75±2.35	21.54±2.11
Fluoranthene	3.65±0.03	28.72±0.33	8.24±0.46	17.34±0.51	11.67±1.41	9.68±1.36
Pyrene	1.13±0.01	55.91±0.62	6.53±0.23	29.44±3.01	11.61±1.03	22.37±1.25
Benzo[a]anthracene	1.32±0.01	7.96±0.02	3.11±0.16	4.75±0.83	5.26±0.92	2.11±0.01
Chrysene	1.87±0.3	32.51±0.17	5.24±0.17	25.16±1.06	9.36±0.68	14.59±0.68
Benzo[b]fluoranthene	0.15±0.01	15.81±0.04	1.92±0.01	11.63±1.06	2.52±0.21	6.37±0.14
Benzo[k]fluoranthene	3.64±0.06	17.98±0.05	6.06±0.81	9.12±0.27	9.98±0.67	5.84±0.13
Benzo[a]pyrene	0.61±0.01	4.63±0.02	0.73±0.01	2.24±0.33	0.85±0.01	1.36±0.01
Dibenzo[a,h]anthracene	0.32±0.01	13.82±0.05	5.26±1.06	8.09±1.08	11.37±2.03	4.82±0.67
Benzo[g,h,i]perylene	5.15±1.04	36.74±0.34	23.25±2.68	19.46±1.72	38.54±2.99	13.29±1.08
Indeno[1,2,3-cd]pyrene	0.88±0.01	38.65±0.11	5.98±1.43	20.07±1.81	13.54±1.07	11.51±1.35

Accumulation of PAHs in the plant root are presented in Table 2.0. High accumulation of benzo[g.h.i] perylene and fluoranthene was noticed in plant root on 2 months harvesting period with quantity 5.15 and 3.65 mg/kg, respectively. High accumulation of PAHs in the root has occurred on Idenol[1,2,3-cd] pyrene, anthracene and benzo[g,h,i]perylene and on 6-months with values 13.54, 16.75 and 38.54 mg/kg, respectively. The accumulation of PAHs in the root of exposed plant are lower than the concentration in the plant grown on control soil except for some few PAHs (see Table 2.0). The accumulation and transfer of PAHs in plant root followed the trend pattern of 6 months > 4 months > 2 months. The plants recorded an increased in PAHs uptake into the root on all the harvesting period.

Translocation of PAHs to Plants Leaves

Concentrations of PAHs translocated to the leaf of *A. wilkesiana* for the 3 harvesting periods are presented in Table 3.0. Translocation of anthracene and pyrene to the leaf has occured on 2 months with concentration 9.18 and 9.78 mg/kg, respectively. Accordingly, an increased in PAHs transfer to the leaf of the plant on 4 months was noticed from anthracene, pyrene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene with values 16.35, 15.33, 13.80 and 11.16 mg/kg, respectively. The plant translocated several PAHs such as anthracene, chrysene, pyrene, benzo[g,h,i]perylene, flouranthene and indeno[1,2,3-cd]pyrene to plant leaf on 6 months with high quantity (see Table 3.0). *A. wilkesiana* translocated PAHs to the leaves by following the trend pattern 6 months > 4 months > 2 months.

The differences in the PAH concentrations level were found to be large, which arises from the harvesting period. Generally, the concentrations of PAHs in plant leaves increased as the harvesting time increases. It was noticed that, the concentration of PAHs present in unexposed plant leaves were detectable, which could be due to leaves absorption from the atmosphere. And this could probably be via the retention of vapor phase of hydrocarbons on the waxy leaf cuticle (Petrova *et al.,* 2017). And this phenomenon was presented in a number of previous researches (Gao and Zhu, 2004). "Previous researches showed that hydrocarbon (PAHs) enters the plants through the stomata of the leaves, via the cuticles then into the epidermis to form a residue on the cell walls" (Kvesitadze *et al*., 2009). The important principles for the absorption of hydrocarbons by the roots and the plant vascular behavior for its metabolic degradation have been studied in different researches (Oleszczuk and Baran, 2005). For instance, it was indicated that the uptake of PAHs has a positive correlation between *n*-octanol and the water partition coefficient (Kow) (Petrova *et al*., 2017). Previous studies showed that PAHs with molecular weight that is low or medium are transferred to the shoots (Kipopoulou *et al*., 1999). But, PAHs with high molecular weight are not transferred to the shoot easily due to their weak solubility in water

with high Henry's constant value and a Kow, therefore, it firmly confined to root of the plant (Kang *et al*., 2010). The transfer of PAHs to plant leaves was correlated with the solubility, which shows more effectiveness to PAHs with an intermediate polarity (Briggs *et al*., 1982). More so, some researchers suggest that composition of the root (such as lipid contents) can be correlated with the plant root absorption of lipophilic organic compounds lipid contents (Chiou *et al.,* 2001)

Table 3: Concentration (mg/kg) of PAHs in *A. wilkesiana* leaves grown on landfill soil at three different harvesting period (n=8)

Compound name	2 months	Control 2	4 months	Control 4	6 months	Control 6
Naphthalene	0.21± 0.01	4.78± 0.02	0.55± 0.01	2.16± 0.01	0.86± 0.01	1.32± 0.01
Acenaphthylene	0.06± 0.01	0.47± 0.01	0.22± 0.01	0.19± 0.02	0.41± 0.01	0.05± 0.00
Acenaphthene	0.23± 0.01	1.02± 0.01	0.46± 0.01	0.73± 0.02	0.69± 0.01	0.01± 0.00
Fluorene	0.19± 0.01	6.82± 0.03	0.78± 0.01	3.95± 0.02	1.62± 0.01	2.57± 0.03
Phenanthrene	0.25± 0.01	10.48± 0.02	0.51± 0.01	7.27± 0.01	0.83± 0.01	4.36± 0.16
Anthracene	9.77± 1.2	56.25± 0.58	16.35± 1.33	34.11± 0.27	27.16± 1.68	21.54± 2.11
Fluoranthene	2.19± 0.01	28.72± 0.33	7.34± 0.24	15.38± 1.06	11.67± 2.06	9.68± 1.36
Pyrene	9.38± 0.96	55.91± 0.62	15.33± 0.85	32.02± 2.13	22.61± 2.57	14.37± 1.25
Benzo[a]anthracene	0.86± 0.01	7.96± 0.02	2.04± 0.06	3.88± 0.31	4.11± 0.12	2.11± 0.01
Chrysene	4.8± 0.07	32.51± 0.17	9.84± 1.00	18.27± 0.44	15.26± 0.38	14.59± 0.68
Benzo[b]fluoranthene	1.65± 0.01	15.81± 0.04	4.33± 0.32	9.36± 0.25	8.32± 0.31	6.37± 0.14
Benzo[k]fluoranthene	1.75± 0.02	17.98± 0.05	3.69± 0.05	11.03± 0.21	7.98± 0.09	5.84± 0.13
Benzo[a]pyrene	0.12± 0.00	4.63± 0.02	0.19± 0.01	2.91± 0.63	0.35± 0.01	1.36± 0.01
Dibenzo[a,h]anthracene	1.38± 0.01	13.82± 0.05	3.71± 0.6	8.39± 0.26	5.24± 0.13	4.82± 0.67
Benzo[g,h,i]perylene	5.93± 0.04	36.74± 0.34	11.16± 1.23	24.05± 0.12	17.02± 1.52	13.29± 1.08
Indeno[1,2,3-cd] pyrene	7.3± 0.16	38.65± 0.11	13.8± 1.71	19.33± 1.27	18.43± 1.47	11.51± 1.35

CONCLUSION

A. wilkesiana have been evaluated for its potential to survive and grow on landfill soil. The plant is capable to extract and accumulate PAHs in the root, and then transfer to the leaf on different harvesting period. Phytoremediation may give a viable solution to the issues of pollution and will draw a significant attention of researchers. Remediation of contaminated soil using plants has drawn great values, because it is an alternative to the removal of soil, replacement soil and other soil remediation techniques. The study had examined the growth and the responses of the plant grown on landfill soil and the accumulation of hydrocarbons in its parts. Hydrocarbons were initially adsorbed to the cell walls of the plant and diffuse into subcellular tissues gradually. The extent of lipophilic compound absorption can be determined by the contents of the lipid of intracellular components, and the diffusion rate is related to the concentration gradient over a period of cultivation which was established between cell walls and organelles inside cells. This study provided data on the PAHs concentrations in the plant. The concentration of PAHs in the roots of *A. wilkesiana* varied between 1.34 – 11.34 mg/kg. The high accumulations of *A. wilkesiana* could be attributed to the capability to absorb more hydrocarbons. The concentration of hydrocarbons accumulated by the plant was

higher compared to the concentrations in the control soil and control plants.

Conflict of Interest

Authors declare that there is no conflict of interest.

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