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Screening and Identification of Plants at a Petroleum Contaminated Site in Malaysia For Phytoremediation

ABSTRACT

There is lack of sufficient data that describe which plants can be used in phytoremediation for petroleum and heavy metal contaminated sites, especially in the tropical climate region. The aim of the study was to identify native plants growing on a petroleum contaminated site in Malacca, Malaysia, which have a phytoremediation potential on petroleum. The second aim was to identify native plants at the same contaminated site for phytoremediation of heavy metal contaminants or hyper accumulation plants. In the initial screening of contaminated sites, some of the native plants were found to have the capability to grow in very high concentration of total petroleum hydrocarbon (TPH). This indicates that some of these plants have high potential to act as a phytoremediator. Paspalum vaginatum Sw, Paspalum scrobiculatum L. varbispicatum Hack, Eragrostis atrovirens (Desf.) Trin. exSteud, Cayratia trifolia (L.) Domin, Chloris barbata (L.) Sw, Pycreus polystachyos (Rottb.) Beauv and Ischaemum timorense Kunth were found to be potential phytoremediatory of TPH in contaminated soil. These plants were chosen based on thier high rate of survival in contaminated sites and in terms of uptake or in degrading contaminants. The Biological Accumulation Coefficient (BAC) has been used as a guideline to choose potential plants for heavy metal phytoremediation. In the study, the plants were screened based on BAC values for arsenic (As) and lead (Pb). The selected plants, Melochia corchorifolia L., Ludwigia octovalvis (Jacq.) P. H. Raven, P. vaginatum, Cyperus sphacelatus Rottb., are potential as phytoremediators while L. octovalvis and Melastoma malabathricum L. are potential Pb phytoremediators.

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INTRODUCTION

Phytoremediation is the technology that uses green plants to remediate various media (soil, water or sediment) contaminated with different types of contaminants (organic and inorganic) and interacted with microorganisms (*ITRC 2001; Ghosh and Singh 2005*). Green remediation technology shows the ability to remove pollutants such as organic contaminant, Total Petroleum Hydrocarbon (TPH) and heavy metal was absent in Taiwan (*Lai et al. 2014*). Phytoremediation is a cost-effective, environmentally friendly and engineering-economical alternative to remediate arsenic-contaminated soils suitable for use in developing countries (*Yang et al. 2012; Ghosh and Singh 2005; Lasat 2002*). However, there are several disadvantages in implementing phytoremediation: only surface contamination can be removed or degraded; clean-up is restricted to areas that are amenable to plant growth; and most importantly, it may take a long time for site remediation to be effective (*Marchetti 2003; Ghosh and Singh 2005*). The harvested plant biomass from phytoremediation may be classified as a hazardous waste hence disposal should be proper and must take into consideration that climatic conditions could be potential limiting factors.

Plants have been shown to encourage organic contaminant reduction principally by providing an optimal

environment for microbial proliferation in the root zone (rhizosphere) (*Kruger et al. 1997*). This degradation process is influenced not only by rhizosphere microorganisms, but also by unique properties of the host plant (*Chaudhry et al. 2005*). Based on *ITRC* (*2009*), phytodegradation, also called phytotransformation, refers to the uptake of organic contaminants with the subsequent breakdown, mineralization, or metabolization by the plant itself through various internal enzymatic reactions and metabolic processes.

A plant may act as a heavy metal hyperaccumulator via uptake and accumulation of heavy metals in various parts of the plant. There are numerous references concerning hyperaccumulating plants. The hyperaccumulator must have a relatively large ratio of biomass concentration of the contaminant to the concentration of contaminant in the soil (*Brooks 1998*). Hyperaccumulating plants that are often found growing in affected areas naturally accumulating more concentration of heavy metals/metalloids in their shoots than in their roots (*Ozturk et al. 2003*). A hyperaccumulator has been defined as a plant that can accumulate, >100 mg kg^{-1} of Cd, >1,000 mg kg^{-1} of Ni, Pb, As and Cu, or >10,000 mg kg^{-1} of Zn and Mn, in their shoot dry matter (*Abou-Shanab et al. 2007*; *Gonzaga et al. 2006*). In hyperaccumulating plants, the metal concentrations in shoots are invariably greater than that in the roots, demonstrating a special ability of the plant to absorb and transport metals and store them in their aboveground components (*Baker and Brooks 1989*; *Baker et al. 1994*; *Brown et al. 1994*; *Wei et al. 2002*). Also, a hyperaccumulator is regarded as a plant in which the concentration of heavy metals in its above ground components is 10 to 500 times more than that in normal plants (*Shen and Liu 1998*). The first hyperaccumulators to be characterized were members of the Brassicaceae and Fabaceae families (*Salt et al. 1998*). Therefore, it will be useful to identify plants having the ability to hyperaccumulate heavy metals, especially in tropical climate region. It is important to use native plants for phytoremediation because these plants are often better in terms of survival, growth and reproduction under environmental stress than plants introduced from other environment (*Yoon et al. 2006*).

At present, there is lack of sufficient data that describe which plant can be used in phytoremediation especially in tropical climate areas. The first aim of the study was to identify native plants growing on a petroleum contaminated site in Malacca, Malaysia, which have potential to be used in phytoremediation to remediate petroleum. Total Petroleum Hydrocarbon (TPH) levels of the soil sludge area representative plant have grown were measured and assessed for this study. TPH had historically been the primary criteria to assess environmental management in the oil and gas industry. The second aim was to identify native plants at the same contaminated site for phytoremediation of heavy metal contaminants or hyper accumulation plants. In this study, there were two priority heavy metals i.e. arsenic (As) and lead (Pb). Selection of these two heavy metals is based on our previous study showing that the concentration of As and Pb were high in the contaminated sites. This study focused on terrestrial plants at that contaminated site.

MATERIALS AND METHOD

Plant and soil/sludge sample collection

Sampling has been carried out at the contaminated sludge farm (SF) and land farm (LF) in Malacca, Malaysia. Screening analysis of the sludge sample surrounding the plant's root zone was conducted (**Table 1**).

Dominant plants were sampled at random keeping a minimum of at least three true replicates. Plants and soil co-existing in the same place were collected together. The plants were identified, tagged and photographed before being taken to the laboratory. The plants sample were then placed in the polyethylene bag and labeled properly. Unidentified plants were sent to the herbarium in Universiti Kebangsaan Malaysia (UKM) for identification. The soil sludge samples were taken within the root area (rhizosphere) of the plant. The soil was sampled using soil corer at approximately 20 cm depth from soil surface and put in a glass bottle with Teflon cap. The percentages of family of plants were calculated based on the number of plants in the same family compared with the total number of plants families in the sampling area.

Calculation of Biological Accumulation Coefficient (BAC) values

BAC calculation was used in order to gauge ability the of plants to uptake metal from the substrate (*Bini et al. 1995*). The determination for BAC was based on the following equation:

$$BAC = \frac{\text{Concentration in plant tissue (mg kg}^{-1})}{\text{Concentration in soil (mg kg}^{-1})} \quad (1)$$

The results of the determination of BAC were matched with categories of plants (**Table 2**) to classify which plants are hyperaccumulator plants or otherwise.

Laboratory analysis

Sludge preparation

Sludge/soil samples were dried openly at room temperature for two weeks and were pounded using crucible

Table 1. Sludge characterization in area study.

No.	Character	Unit	Value
1	pH		3.1 – 4.3
2	Total solid	%	28.6 – 78.9
3	Oxidation reduction potential	mV	-283 to 224
4	Total organic carbon	% C	3.2 – 6.9
5	Total phenol	mg kg^{-1}	< 0.2
6	Sulphide	mg kg^{-1}	< 0.1
7	Cyanide	mg kg^{-1}	< 0.5
8	Oil and grease	mg kg^{-1}	15,000 – 53,800
9	Total nitrogen	mg kg^{-1}	2,360 – 7,470
10	Phosphate	mg kg^{-1}	< 0.05 – 0.41
11	Sulphate	mg kg^{-1}	2,100 – 9,180
12	Nitrate	mg kg^{-1}	0.34 – 5.14
13	Chloride	mg kg^{-1}	20 – 31
14	TPH	mg kg^{-1}	987 – 48,709
15	PAH	mg kg^{-1}	< 0.5
16	BTEX	mg kg^{-1}	< 0.2
17	Total aerobic count	CFU g^{-1}	2.7x106 – 7.8x106
18	Total anaerobic count	CFU g^{-1}	2.4x106 – 7.6x106
19	Arsenic	mg kg^{-1}	< 1
20	Lead	mg kg^{-1}	8 - 22

Table 2. Category of BAC values (*Bini et al. 1995*).

Category	Range
High accumulator plants	1 -10
Moderate accumulator plants	0.1 -1
Low accumulator plants	0.01 - 0.1
Non accumulator plants	< 0.01

pounder. Then the sample was sieved through <2 mm mesh and then <63 µm mesh for heavy metal determinations. Analysis of metal concentrations in the <63 µm sediment fraction is recommended because these particles are the most important sources of bioavailable metals in sediments (*Bat and Raffaelli 1999*).

Extraction of the Sludge for TPH Analysis

Extraction of the soil/sediment/sludge for TPH analysis was conducted using ultrasonic extraction method of *USEPA* (*2007*). Approximately 10 g of the soil sludge mixture sample was placed in a 250 mL Scotch bottle and was mixed with approximately 2-3 g sodium sulfate (Na_2SO_4). The purpose of adding Na_2SO_4 was to trap the water molecule. Water can affect the extraction process directly altering the accuracy of the TPH analysis.

The analysis of the TPH was performed using Perkin Elmer Clarus 500 series Gas Chromatography with split less injection fitted with fused silica capillary column (30.0m x 0.32µm x 0.25µm) and mass spectrometry (MS) detection. The injector temperature was maintained at 320°C and the oven temperature was programmed at 400°C held for 3 min and ramped at 10°C min^{-1} to 320°C and held for 9 min. Helium was used as the carrier gas at a flow rate of 1.00 mL min^{-1}. The analyses time was 40 min.

About 2 µL of the extract was injected into the GC to obtain the chromatogram of the TPH. TPH quantification was done using five points calibration plot (1, 5, 10, 50 and 100 mgL^{-1}) of aliphatic hydrocarbon ranging from C8 to C40. The quantification for each carbon number was done by summarizing the area for each carbon number at the respective retention time and correlated with the area for the individual carbon number of the calibration standards. Headspace sampling coupled with gas chromatography (HS-GC) is a widely used technique for the analysis of beer throughout the world. HS-GC is typically used for quality control (QC), to identify problems or changes occurring in the brewing or fermentation process that affect the taste or quality of the final product (*Perkin Elmer 2005*).

Extraction of the soil and sediment for heavy metal (As and Pb) analysis

The EDTA (ethylene diamine tetraacetic acid) method based on *Quevauviller* (*1998*) and *Mahvi et al.* (*2005*) was used in soil/sediment extraction for heavy metal analysis: 50 mL of 0.05 M EDTA was used to extract 5 g sample. The mixture was then agitated using a shaker at 30 rpm for 1 hour at room temperature. Immediately after extraction, a portion was transferred to a centrifuge tube; run for 10 min at 3000 rpm. The sample was then filtered through filter paper. The filtrate was kept in a polyethylene bottle at 4°C temperature until analysis time. Prior to analysis, the sample has to be shaken for 5 minutes to homogenize the content. Samples were analyzed for As and Pb using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), Optima 7300DV Perkin Elmer (USA). All standard materials on heavy metal analysis are based on the sample using ICP multi-element standard solution XVI (Merck).

Plants

Plant Preparation

Prior to analysis, plant samples were carefully washed with tap water and thoroughly rinsed with deionized water to remove any soil particles attached to the plant surfaces. After washing, the samples were oven-dried at 60°C for 24 h. The dried tissues were weighed and ground into fine powder ready to use in analysis for heavy metals.

Extraction of the plants for heavy metals (As and Pb) analysis

Wet digestion as in *APHA* (*1992*) was used for the analysis in the laboratory. Fine powder of dried plants was placed in a 100 mL conical flask and added with 10mL of concentrated nitric acid (HNO_3, 69%) (Merck, Germany). The flask was covered over with glass cover overnight until the sample was fully digested and no bubbles were observed. Once the plant was digested, it was heated on a sand plate at 125°C for one hour. Distilled water was added to attain the wet digestion. After the digestion process was completed, the flask was cooled and 1 mL of hydrogen peroxide (H_2O_2) was added. It was then returned to the sand bath for continuous heating. It is advisable to continuously add 1 mL of H_2O_2 for every 4-8 mL of solution loss. This will continue until the digested samples were clear. The glass cover was then removed and the temperature was reduced to 80°C. The digestion was continued until all the samples dried. Once fully dried, the beaker was taken out to cool. On cooling, 2 mL of acid mixture 1:3 HNO_3 - HCl (conc. HCL, 37%) was added. Then the solution was filtered using Whatman filter paper No. 42. The samples were then ready for analysis. All analyses were carried out using an Optima 7300DV inductively coupled plasma-optical emission spectrometer (ICP-OES) (Perkin Elmer, USA).

Quality assurance/quality control (QA/QC)

Laboratory QA/QC was ensured where every batch of analyses was incorporated with calibrating standards, a test of both sample homogeneity and laboratory precision at an appropriate frequency. Good quality control was practiced throughout the analysis to avoid sample contamination and to reduce error. All the reagents used were of analytical grade and were used without further purification. Distilled water was used for the preparation of reagents. All of the glassware for TPH analysis was first washed with hexane, then rinsed with acetone and left to dry before rinsing with deionised water. Then all glassware was heated at 70°C for 24 h before used. Meanwhile, all glassware for heavy metal analysis were immersed in 20% HNO_3 overnight before being heated (70°C) for 24 h. In addition, all instruments involved in this analysis were calibrated before use. In order to maintain the greatest precision possible for all the analysis, TPH and heavy metals analysis procedures were done in triplicate for each sample.

RESULTS AND DISCUSSION

The plant screening studies showed that there were six families and 11 species that dominate in the Sludge Farm (SF). Graminae family known as the grass family was the most dominant. While for the Land Farm (LF) area, there were 10 species of plants from 9 different families identified. The plants at LF area were more diverse in variety as compared to the plants that grew at the Sludge Farm. This may reflect the environment at the LF, which is more similar to the natural environment rather than the SF which has been designed more as a treatment plant site.

The Gramineae family, commonly known as the grass family, formed the dominant plants at 35%, followed by Cyperaceae (18%), Leguminosae (legumes) (13%) and Sterculaceae (9%) (**Table 3**). The lesser dominant plants were Onagraceae (5%), Pteridophytes, Euphorbiaceae, Vitaceae, Melastomaceae and Malvaceae, all with 4%. Grasses and Legumes have high potential to remediate petroleum hydrocarbon (*Ndimele 2010*). Grass family have extensive, fibrous root systems. Grass root systems have the maximum root surface area (per m^3 of soil) of any plant type and may penetrate the soil to a depth of up to 3 m. They also exhibit an inherent genetic diversity, that may give them a competitive advantage in becoming established under unfavourable soil condition (**Figure 1**).

Table 3. Plants species sampled at the contaminated site in Malacca, Malaysia.

Family	Species
Gramineae	*Paspalum vaginatum* Sw
Gramineae	*Paspalum scrobiculatum* L. varbispicatum Hack
Gramineae	*Chloris barbata*(L.) Sw
Gramineae	*Eragrostis atrovirens* (Desf.) Trin. ex Steud
Vitaceae	*Cayratia trifolia* (L.) Domin
Malvaceae	*Melocia corchorifilia*
Cyperaceae	*Pycreus polystachyos* (Rottb.) Beauv
Euphorbiaceae	*Sebastiana chamaelea* (L.) M. A.
Gramineae	*Ischaemum timorense*Kunth
Pteridophyte	*Thelypteridace aeamphineuron* terminans (Hook) Holtlum
Cyperaceae	*Cyperus difformis* L.
Onagraceae	*Ludwigia octovalvis* (Jacq.) P. H. Raven
Cyperaceae	*Cyperus sphacelatus* Rottb.
Leguminosae	*Mimosa pigra* L.
Leguminosae	*Sennatora* (L.) Roxb.
Malvaceae	*Urena lobata* L.
Gramineae	*Echino chloacolona* (L.) Link.
Sterculiaceae	*Melochia corchorifolia* L
Leguminosae	*Vigna umbellata* (Thunb.) Ohwi & H. Ohashi
Melastomaceae	*Melastoma malabathricum* L.
Cyperaceae	*Cyperus imbricatus* Retz.

Screening of potential plants as phytoremediator for hydrocarbon

A plant must be able to germinate, survive and grow in the contaminated condition to be considered as a potential plant for phytoremediation (*Medina et al. 2003*). The plant

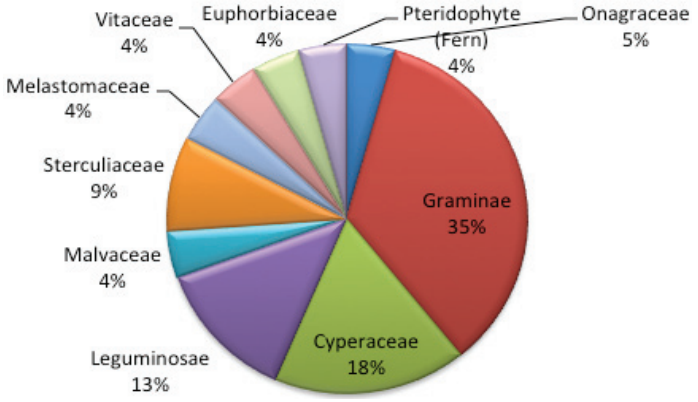

Figure 1. Percentage of plant families sampled.

is considered resistant to the contaminant when it is able to survive and reproduce under contaminated soil conditions. Resistance of the plants happens through the metabolic changes of the plant where the changes in the hormone production and mobilization occur affecting the rate-controlling enzymes thereby modifying substrate concentration and enzyme activity (*Hoffman and Parson 1991*).

The plant species *P. vaginatum*, *P. scrobiculatum*, *C. barbata*, *E. atrovirens*, *C. trifolia*, *P. polystachyos*, *I. timorense* were able to survive in the soil sludge with were significantly high concentration of TPH (**Table 4**). *C. trifolia* and *E. atrovirens* showed the ability to grow at the highest TPH contaminated condition ranging from 224 – 177,595 and 264 – 7,268 mg kg^{-1}, respectively. Based on our previous study (*Idris et al. 2014*), the highest percentage degradation of TPH by *P. vaginatum*, *P. scrobiculatum*, *E. atrovirens* and *C. trifolia* were 91.9, 74.0, 68.9 and 62.9%, respectively under greenhouse condition.

Most of the plants that can survive in a contaminated site with high concentrations of hydrocarbon were Gramineae (*P. vaginatum, P. scrobiculatum, C. barbata* and *I. timorense*). Gramineae have a fibrous rooting system with large surface area (*Kaimi et al. 2007*). Most of the plants growing in the contaminated site have fibrous rooting system (**Table 4**). Fibrous roots provide a larger surface than taproots for colonization by soil microorganisms (*Anderson et al. 1993*). They also allow a close interaction between the rhizosphere microbial community and the contaminant (*Schwab and Banks 1994*). This supports that plants with a fibrous rooting system can improve microbial activity for cleanup petroleum hydrocarbon-contamination (*Aprill and Sims 1990*).

Hydrocarbon toxicity is due to its volatility and hydrophobicity (*Kaimi et al. 2006*). Volatile hydrocarbons, primarily small and lightweighthydrocarbons, can easily move through cell membranes, thus causing toxic effects (*Adam and Duncan 2002*). On the other hand, the hydrophobicity in oil-contaminated soils prevents water infiltration and aeration that are required for the growth and development of plant roots (*Kirk et al. 2005*). Therefore, observations showed that some plants only present and grow in low concentration of TPH.

Leguminosae such as *M. pigra, S. tora* and *V. umbellata* have a symbiotic relationship with nitrogen-fixing bacteria.

Table 4. Concentration of TPH in soil sludge surrounding plant roots at SF and LF.

Plant species	Location	Rooting system identified	TPH concentration (mg kg^{-1}) in soil grown with the representative plant
P. vaginatum	SF	**Fibrous**	**171 – 3,341**
P. scrobiculatum	SF	**Fibrous**	**43 – 2,156**
C. barbata	SF	**Fibrous**	**314 – 1,423**
E. atrovirens	SF	**Fibrous**	**264 – 7,268**
M. corchorifilia	SF	Fibrous	160 – 416
C. trifolia	SF	**Fibrous**	**224 – 177,595**
P. polystachyos	SF	**Fibrous**	**771 – 5,197**
S. chamaelea	SF	Fibrous	773
I. timorense	SF	**Fibrous**	**97 – 1,044**
T. amphineuron	SF	Rhizome	295
C. difformis	SF	Fibrous	152
L. octavalvis	LF	Fibrous	127 – 162
C. sphacelatus	LF	Fibrous	33 – 115
M. pigra	LF	Tap	17 – 111
S. tora	LF	Fibrous	44 – 348
U. labota	LF	Tap	46 – 207
E. colona	LF	Fibrous	148
C. imbricatus	LF	Fibrous	309
M. malabathricum	LF	Tap	0 - 58
V. umbellata	LF	Tap	168

This symbiotic relationship suggests that leguminosae could grow well in petroleum–contaminated soil in which the C/N ratio tends to be high (*Adam and Duncan 2003*) and could therefore be effective in phytoremediation. Based on *Ismail* (*2014*), Legumes plant, Pigeon pea (*Cajanus cajan*) and Hyacinth bean (*Lablab purpureus*), have potentials for phytoremediation and could be important tools in reclaiming soil with low levels of spent engine oil contamination.

Screening of potential plants as hyperaccumulator for heavy metals (As and Pb)

Overall, the concentration of heavy metals in soils vary across areas where different plant species were grown (**Table 5**). The maximum and minimum values of Pb in soil are 152.3 mg kg^{-1} for *E. atrovirens* and 7.3 mg kg^{-1}. for *S. tora*, respectively. The soil where *E. atrovirens* and *C. barbata* could grow contains Pb concentration of more than 100 mg kg^{-1}.

For As, the highest concentration in soils was 99.2 mg kg^{-1} at which *V. umbellata* was found growing. Soils containing As concentration of 8.4 mg kg^{-1} showed the presence of *C. barbata* in the area. Almost all of the soils contained As concentration below 100 mg kg^{-1}.

The natural existence of metal in soil is usually less than 100 mg kg^{-1} (*Alloway 1995*), but metals also co-exist with other minerals, which could add to their enormous presence. The following discussion showed that although the concentration of metals is high in soils, it did not indicate that the plants could accumulate high concentration of metals in the plant parts (**Table 6**).

Table 5. Sludge characterization in area study.

Plant species	Concentration of heavy metals in soil (mg kg^{-1})	
	As	Pb
P. vaginatum	66.2	88.7
P. scrobiculatum	33.0	91.6
C. barbata	8.4	105.7
E. atrovirens	8.7	152.3
M. corchorifilia	8.7	82.8
C. trifolia	9.5	72.7
P. polystachyos	10.2	51.9
S. chamaelea	11.4	24.8
I. timorense	11.8	53.7
T. amphineuron	12.3	8.0
C. difformis	12.8	29.5
L. octavalvis	15.0	10.5
C. sphacelatus	17.0	11.6
M. pigra	45.5	9.8
S. tora	53.5	7.3
U. labota	54.7	9.6
E. colona	59.4	8.4
C. imbricatus	61.5	7.9
M. malabathricum	65.6	7.5
Lemna sp.	68.1	9.9
V. umbellata	99.2	8.1

The concentration of As in the stem and leaf was not detectable from the ICP-OES analysis. The species of *L. octovalvis* seemed to accumulate high concentration of As in the roots at 25.8 mg kg^{-1} as compared to the other plants. Four plants species were indicated to accumulate no detectible levels of As in the root. These were *M. malabathricum, V. umbellata, T. amphineuron* and *I. timorense*.

The concentration of As accumulated in whole plants ranged from 0 – 26 mg kg^{-1}, while some plants did not contain any detectable levels of As such as *I. timorense* and *T. amphineuron*. However *L. octovalvis* seemed to be the plant that was able to accumulate As at 25.8 mg kg^{-1}. This was followed by *U. lobata* (23.1 mg kg^{-1}), *C. imbricatus* (21.9 mg kg^{-1}) and *C. sphacelatus* (19.9 mg kg^{-1}). Based on a previous study by *Titah et al.* (*2015*), the As uptake and accumulation could reach up to 528.5 ± 68.3 mg kg^{-1} in leaves of *L. octovalvis* after 42 days of exposure at As concentration initial of 39 mg kg^{-1} under greenhouse condition. This suggest that the effectiveness of Asphytoremediation increase with time exposure.

Six plants were found to accumulate Pb higher than 1.0 mg kg^{-1} in the leaf. These plants were *P. polystachyos, S. chamaelea, M. corchorifolia, C.trifolia, C. barbata* and *P. vaginatum* with concentrations of 3.5, 3.3, 2.4, 1.8, 1.1, and 1.0 mg kg^{-1}, respectively (**Table 6**). Plants that accumulated Pb in the stem were *M. malabathricum* (11.4 mg kg^{-1}), *C. barbata* (2.2 mg kg^{-1}), *P. polystachyos* (0.6 mg kg^{-1}), *C. trifolia* (0.3 mg kg^{-1}), *L. octovalvis* (0.3 mg kg^{-1}) and *M. pigra* (0.3 mg kg^{-1}). Pb was also detected in all plants roots except *T. amphineuron* or commonly known as fern. The highest Pb was detected in the root of *L. octovalvis*, 24.9 mg kg^{-1}., while other plants accumulated Pb below 10.0 mg kg^{-1}. in their rooting system. The accumulation of Pb in the root system could imply that Pb is not easily translocated to other plant parts especially to the leaves, hence accumulation remained in the rooting system. Many plants retain Pb in their roots via absorption and precipitation with only minimal transport to the aerial part of the plant due to the bioavailibiy of Pb (*Paz-Alberto and Sigua 2013*). Pb, an important environmental pollutant, is highly immobile in soils. Pb is known to be molecularly sticky since it readily forms a precipitate within the soil matrix. It has low aqueous solubility, and, in many cases, is not readily bioavailable. It was observed that almost all plants

Table 6. Accumulation of Pb and As in plant parts.

Species	Pb (mg kg^{-1})				As(mg kg^{-1})			
	Leaf	**Stem**	**Root**	**Total**	**Leaf**	**Stem**	**Root**	**Total**
P. vaginatum	1.0	n.d	4.6	5.6	n.d	n.d	16.7	16.7
P. scrobiculatum	n.d	n.d	5.6	5.6	n.d	n.d	12.9	12.9
C. barbata	1.1	2.2	2.0	5.2	n.d	n.d	0.1	0.1
E. atrovirens	0.8	n.d	4.7	5.5	n.d	n.d	1.9	1.9
M. corchorifilia	2.4	n.d	6.4	8.8	n.d	n.d	9.7	9.7
C. trifolia	1.8	0.3	5.4	7.5	n.d	n.d	1.3	1.3
P. polystachyos	3.5	0.6	3.1	7.3	n.d	n.d	2.0	2.0
S. chamaelea	3.3	n.d	6.1	9.4	n.d	n.d	1.3	1.3
I. timorense	n.d	n.d	0.2	0.2	n.d	n.d	n.d	0.0
T. amphineuron	0.2	n.d	0.0	0.2	n.d	n.d	n.d	0.0
C. difformis	0.7	n.d	6.6	7.3	n.d	n.d	3.0	3.0
L. octavalvis	n.d	0.3	24.9	25.2	n.d	n.d	25.8	25.8
C. sphacelatus	n.d	n.d	3.5	3.5	n.d	n.d	19.9	19.9
M. pigra	0.5	0.3	3.0	3.7	n.d	n.d	6.6	6.6
S. tora	0.2	n.d	4.5	4.8	n.d	n.d	15.0	15.0
U. labota	0.6	n.d	1.5	2.1	n.d	n.d	23.1	23.1
E. colona	0.5	n.d	1.9	2.3	n.d	n.d	8.6	8.6
M. corchorifolia	0.1	n.d	1.7	1.8	n.d	n.d	14.2	14.2
C. imbricatus	0.1	n.d	1.7	1.8	n.d	n.d	21.9	21.9
M. malabathricum	n.d	11.4	2.5	13.9	n.d	n.d	n.d	0.0
V. umbellata	0.3	n.d	1.7	2.0	n.d	n.d	n.d	0.0

n.d is defined as not detected

accumulated heavy metals (As and Pb) in roots. There are less Pb and As detected in stem and leaf part of the plants.

According to *Stephen et al.* (*2002*), there were five processes involved in the absorption of heavy metals from the soil i.e; mobility and absorption of heavy metals from the soil; storage and separation in the roots; the transfer process from xylem and transportation; distribution of heavy metals through transportation by xylem; and the distribution of xylem to the leaf shoots and its specificities in the leaf cell storage. Based on this argument, further research which would be carried out in the next phase would be looking at the physiology of the plant after being exposed to the heavy metals. These values would be used to run the toxicological testing for selected plants in the next phase.

The highest total concentrations of Pb in whole plants were found in *L. octovalvis* (25.2 mg kg^{-1}) and *M. malabathricum* (13.9 mg kg^{-1}). The lowest concentrations were found in *I. timorense* (0.2 mg kg^{-1}) and *T. amphineuron* (0.2 mg kg^{-1}). Overall, most of the plants accumulated Pb in the range of 1.0 – 10.0 mg kg^{-1}. Despite the high presence of Pb in the soil at 152.3 mg kg^{-1}, the plant that grew in high Pb concentration of soil did not accumulate similar levels of high Pb concentration as shown in *E. atrovirens*. This could indicate that the genotypic role of the plant may have an influence over the plant.

The BAC values (**Table 7**) indicate that some plants can tolerate certain levels of toxicants such as heavy metals in the environment where it grows. These values are calculated using Eqn. (1). To specify the hyperaccumulator plants, the BAC values as categorized by *Bini et al.* (*1995*) were followed as in **Table 1**. BAC is a parameter used to characterize the accumulation of heavy metals by plants in relation to the bioavailibilty of the metals in the soil (*Nagaraju and Karimulla 2002*). According to *Bini et al.* (*1995*), plants with BAC values ranging from 1-10 could be considered as high accumulator plants (hyper-accumulator plants).

Based on the calculated BAC value (**Table 7**), high BAC values for As where indicated in *M. corchorifolia, L. octovalvis, P. vaginatum* and *C. sphacelatus* with 1.1, 1.7, 1.1, and 1.2, respectively. High BAC values for Pb were indicated in *L. octovalvis* and *M. malabathricum* with 2.4 and 1.4, respectively (**Table 7**). The higher values of BAC from these samples show that these plants are potential hyperaccumulator plants for Pb and As. Thus, according to this assumption, we managed to select and identify which plants have the most potential to be hyperaccumulator plants. Although *M. corchorifolia* and *L. octovalvis* showed high BAC values, its accumulation were found only in the rooting system, especially for As. This is an indication that in the exposure test and the design of the physiological experiments, soil texture, structure and the bulk density of the soil must be taken

into consideration. Soil bulk density is a basic soil property influenced by some soil physical and chemical properties. Bulk density of a soil is a dynamic property that varies with the soil structural conditions influenced by the amount of organic matter in soils, their texture, constituent minerals and porosity (*Chaudhari et al. 2013*).

Table 7. BAC value of screened plants.

Species name	BAC	
	Arsenic (As)	Lead (Pb)
P. vaginatum	**1.1*****	0.4**
P. scrobiculatum	0.9**	0.3**
C. barbata	0.01*	0.1
E. atrovirens	0.2**	0.04*
M. corchorifilia	**1.1*****	0.1**
C. trifolia	0.1**	0.1**
P. polystachyos	0.2**	0.1**
S. chamaelea	0.1**	0.4**
I. timorense	0.0	0.003
T. amphineuron	0.0	0.03*
C. difformis	0.2**	0.2**
L. octavalvis	**1.7*****	**2.4*****
C. sphacelatus	**1.2*****	0.3**
M. pigra	0.1**	0.4**
S. tora	0.3**	0.7**
U. labota	0.4**	0.2**
E. colona	0.1**	0.3**
M. corchorifolia	0.2**	0.2**
C. imbricatus	0.3**	0.2**
M. malabathricum	0.0	**1.4*****
V. umbellata	0.0	0.2**

Legend :
*** High accumulator
** Moderate accumulator
* Low accumulator
Non accumulator

CONCLUSIONS

The *C. trifolia* and *E. atrovirens* were found to be the potential plants for the phytoremediation of TPH contaminated soil, as they are able to grow in soil with highTPH concentration. There are seven plants (*P. vaginatum, P. scrobiculatum, E. atrovirens, C. barbata, I. timorense, C. trifolia* and *P. polystachyos*) that could potentially be selected for TPH phytoremediation in contaminatedsites. The plants were chosen due to the high degree of survival in contaminated sites (SF and LF in Malacca). The computation of BAC values provided a basis in choosing potential plants and pursuing further heavy metal uptake and toxicity testing to determine the most potential phytoremediator plants. The selected plants for heavy metal phytoremediation are *M. corchorifolia, L. octovalvis, P. vaginatum, C. sphacelatus* and *M. malabathricum*. As a conclusion, most of the plants found at the petroleum contaminated site in Malacca, Malaysia showed good potential for hydrocarbon degradation and heavy metal uptake.

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