American-Eurasian J. Agric. & Environ. Sci., 14 (7): 610-614, 2014
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2014.14.07.12364

Phytoremediation Potential of *Impatiens balsamina* Towards Naphthalene Contaminated Soil in Different Parts of Plant

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Abstract: Organic wastes materials have been determined in soil samples for many years and may arise in the environment as consequences from natural sources and human activities. Hence, phytoremediation as a new alternative technology offer better remediation of contaminated sites in term of its cost effectiveness, aesthetic advantages and long term applicability. In this study, the existence of organic contamination levels in the *Impatiens balsamina* plant as well as in the soil sample was investigated. The accumulation of naphthalene in the parts of *Impatiens balsamina* plant and in the soil sample was analysed by gas chromatography with flame ionization detector (GC-FID). From the findings, the naphthalene concentration in the soil sample was reduced by 181.917%, while in the *Impatiens balsamina* stem was increased by 13.301 %, reflects that the *Impatiens balsamina* was able to tolerate and absorb contaminated from soil sample during one month of the treatment process. The naphthalene concentration in the *Impatiens balsamina* leaf and root was decreased by 46.867 % and 6.435 %, respectively as the phytoextraction occur in the plant. Thus, *Impatiens balsamina* could be used to remove naphthalene as the organic contaminant in the contaminated soil. It is hoped that the mechanism of phytoextraction in this ornamental plant could be understood well and phytoremediation technology involve of this species may be applied soon in Malaysia.

Key words: Phytoremediation · Phytoextraction · *Impatiens balsamina* · Polycyclic aromatic hydrocarbons (PAHs) · Naphthalene

INTRODUCTION

Hydrocarbons can be gases (e.g. methane and propane), liquids (e.g. hexane and benzene), waxes or low melting solids (e.g. paraffin wax and naphthalene), or polymers (e.g. polyethylene, polypropylene and polystyrene). There are three major categories of aromatic hydrocarbons of concern as contaminants in environmental soil which are: (i) polycyclic aromatic hydrocarbons (PAHs), (ii) heterocyclic aromatic hydrocarbons and (iii) alkyl PAHs [1]. Organic wastes materials such as naphthalene, toluene and aniline are also known as polycyclic aromatic hydrocarbons (PAHs) have been determined in soil samples for many years. According to Khan *et al*. [2], PAHs can arise in the environment from natural sources, oil and petroleum and combustion processes. Naphthalene was known by the average citizen as a moth repellent in the form of balls or flakes [3]. On top of that, naphthalene was being chosen from all of PAHs because of its availability in soil sample contaminate was higher compared with others as reported by Talib [4].

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In most developing countries of the world, the rapid expansion of industrial areas leading to uncontrolled processing and utilization of industrial products, agricultural wastes, vehicular emissions and improper disposal of liquid and solid wastes may cause soil to become contaminated with chemical hazards such as organic compounds and heavy metals as noted by Okieimen *et al*. [5]. These hazardous pollutants consist of a variety of organic compounds and heavy metals, which pose serious risks to human health [6]. Mathew *et al.* [7] stated that the industrial activity accelerates the pollution of biosphere, especially the soil. Nowadays, soil pollution is getting considerable public attention since the magnitude of this problem is growing rapidly. Phytoremediation technologies have been proposed as a cost effective, non-intrusive and environmentally friendly technology for the restoration of soils contaminated with PAHs [8].

Phytoremediation was an emerging technology, which should be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages and long term applicability [9]. This technology can be defined as the efficient use of plants to remove, detoxify or immobilized environmental contaminants in soils, waters or sediments through the natural, biological, chemical or physical activities and process of plants [10]. *Impatiens balsamina* that locally known as Garden Balsam are of Southeast Asian origin, from where dispersals to boreal Eurasia and North America, to central Asia and Eastern Europe via the Himalayas and to India and Africa have occurred [11]. It is found growing throughout tropical Africa, India, southwest Asia, southern China, Japan, as well as parts of Europe, Russia and North America. It has also been cultivated as an ornamental plant in many parts of the world including Malaysia [12].

Therefore, this study was conducted to help findings the ways to remediate the contaminated soil that suitable with the Malaysia condition. The ability of *Impatiens balsamina* tolerant to the organic contaminants and their ability to accumulate the organic contaminants would be determined.

MATERIALS AND METHODS

Soil and Plants Samples Preparation: About 200 g of the top soil (0-15 cm) was collected from Senaling, Kuala Pilah landfill, stored in aluminium-foil covered containers and then conveyed to the laboratory for further analysis. The soils samples were air-dried for 24 hours and milled by mortar and pastle. The fine powdered soils samples were stored in air-tight containers at room temperature for further analysis.

Meanwhile, *Impatiens balsamina* plant was harvested and wrapped in aluminium foil after one month being treated with contaminated soils and transported to the laboratory for further analysis. The plant was differentiated into three main parts of plant that were leaves, root and stem and air-dried for about 3-4 days. Samples were air-dried at ambient temperature and stored in paper and sealed with polyethylene bags in darkness. The dried plant materials were milled and sieved. The fine powdered plant was stored in air-tight containers at room temperature for analysis purposed.

Some precautions were taken during sampling to avoid external contaminations such as wearing special glove and stainless steel tools used.

Soils and Plants Sample Extraction

Soxhlet Extraction (EPA method 3540c): About 100g of soil sample that has been air-dried for 48 hours were placed into the soxhlet extraction thimble with set up of 120 ml solvent hexane. The soxhlet extraction were set to 60°C and run in 4 hours. After 4 hours, the solvent in the reflux tube should be colorless otherwise reflux will be extended for another 1 hour. If the solvent in the reflux tube still coloured then the solvent, thimble and extracted soil were discarded and the extraction procedure was repeated using a smaller quantity of sample.

Meanwhile, the collected plant samples that included of stems, leaves, flowers and roots, were dried in a ventilated oven at 80°C for 24 hours before milled to a fine powder by a set of mortar and pastel. The fine powdered plant was mixed with 70 ml of hexane (99% purity) added for extraction at temperature of 40°C for 30 minutes. The sample then was filtered using filter paper prior to other treatment. Both of the extracts from two different techniques were concentrated in vacuum up to 5-10 ml which the rotavapor water baths were set to 69 °C with about 60 rpm.

Identification of naphthalene by Gas Chromatography: The organics contaminated soil and plant extracts were analysed for organic materials using gas chromatography with flame ionization detector (GC-FID). The GC was equipped with fused-silica capillary column (50mL × 0.25mm I.D. × 0.25 µL F.T.) coated with cross-linked 5% phenylmethylsilicone, was attached to the injection port. Hydrogen was used as carrier gas. The operating conditions of the GC-FID were split less mode,

inlet temperature (270°C), detector (300°C), gas flow (2 mL/min), linear velocity (33.986 cm/sec) and pressure (3.5psi) at 50°C. The oven temperature was programmed from 50°C for 1minute, increased to 100°C (10°C/minute), then increased to 250°C (60°C/minute) and held for 1 minute. The concentrations of organic materials were measured as the area of total ion chromatogram from one retention time to another. Extraction samples (1µL of liquid sample) were injected into the carrier gas flow by the aid of a syringe introduced to the GC column.

Statistical Analysis: Statistical analysis was done by comparing the chromatogram of sample with the Chromatogram of standard in term of retention time, peak area and calibration curve. Minitab software was used to calculate sample mean and standard deviation while one-way ANOVA analysis to compare the significant means between the three part of plant treatment.

RESULTS AND DISCUSSIONS

The retention time of naphthalene in soils and plants samples were compared with reference chromatogram for the standards solution shows in Fig. 1. For unknown compound peak with retention times within limits the range of 7.510-7.570 minutes [13] to that of the standard naphthalene, the peak was considered as peak of naphthalene.

Based on that Table 1, contaminant level of the polluted soil was decreased in concentration after the treatment was done which was from 20.445 ppm to-16.748 ppm. Thus, it shows that there was removal of naphthalene concentration in the polluted soil (181.9%) which was assumed had been absorbed into the *Impatiens balsamina* plant parts. Meanwhile, for the plant sample, the sample was divided into three different parts which were leaves, stems and roots to study which parts directly participated in phytoremediation process. Fortunately, among three parts of the plants, naphthalene concentration in stem was increased by about 13.301% which is from 185.801 ppm to 210.514 ppm. Therefore, it can be assumed that absorption of naphthalene occur from the polluted soil into the plant fibre which was stored in the stems of the plant [14].

In addition, study by Hopkins and Huner [15] reported that nutrients and other compounds whether metals or non-metal compounds were absorbed and transported through the xylem from the root through the stem to the aerial organs and specifically happened in the xylem fibres of the xylem, the stem was act as the intermediate centre or temporary storage before the compound transported into the specific organs. The increased concentration of the naphthalene in the stem indicated that the absorbed naphthalene from the polluted soil was temporary stored in the xylem of the stem. Mechanism of phytoremediation removed contaminants from soil by translocates the contaminants into plant tissues which is xylem fibre of the stem [14].

Table 1: Phyoremediation of naphthalene before and after the treatment (ppm)

Parameter	[Naphtalene] (ppm) before	[Naphtalene] (ppm) after	Absorption %
Soil	20.445	-16.748	-181.917
Leaf	217.254	115.433	-46.867
Stem	185.801	210.514	13.301
Root	581.773	544.338	-6.435

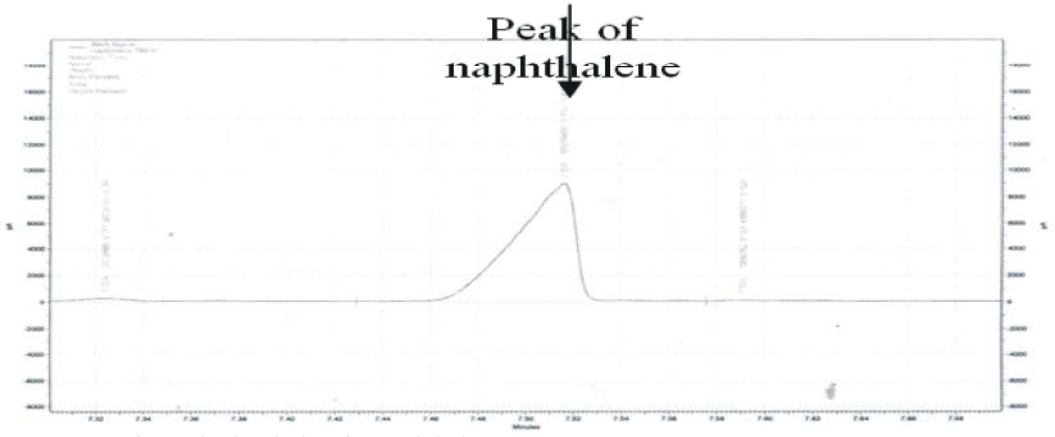

Fig. 1: Chromatogram of Standard Solution for naphthalene

However, the concentration of naphthalene in leaf also shows some reduction with percentage absorption was -46.867 % (217.254 ppm to 115.433 ppm). It was followed by reduction in concentration of naphthalene in root which is of -6.435 % (from 581.773 ppm to 544.338 ppm). This indicated that, there was no naphthalene absorption occurred in the root of the plant. As reported by Hopkins and Huner [15] mineral nutrients acquired by plant are uptake through roots and leaves, associations with mycorrhizal fungi. The roots were act as passage for transportation of the naphthalene to the stem of the plant [16]. Therefore, the naphthalene were absorbed by the root and transported and stored to the stem before being transfer to the specific organs such as leaf.

According to Beck *et al*., 1997 [17] contaminants can be transferred to air via plant transpiration. It may also entail the diffusion of contaminants from the stems or other plant parts that the contaminant travels through before reaching the leaves. Phytovolatilization occur with contaminants present in soil, sediments, or water and has been found to occur with volatile organic compounds, including trichloroethene, as well as inorganic chemicals that have volatile forms, such as selenium, mercury and arsenic [18].

As mentioned by previous studied by Htwe [19], it also was found that plant hormone that derived from the naphthalene which was naphthalene-1- acetic acid (NAA) which act as a root inducing, auxin type compound that was sometimes used in media, especially to promote callus growth and encourages the root development of the plants. There also have different sensitivities of NAA to the roots, buds and stems of plant. Roots were much more sensitive than stems while buds exhibited a sensitivity intermediate between roots and stems [14]. This explained why naphthalene was found more in roots followed by leaf and stems of the plant before the treatment.

One-way ANOVA was done to compare the mean between ability to absorb contamination by three part of plant which is leaves, stems and roots. From the ANOVA test, there was a significant different between the mean of the three part of plant where the p-value was (p= 0.013), where the different shows that all those three part absorbed different amount of pollution depended on the ability of the structure of the plant parts to absorb the contaminants. Further study can be done on others organic contaminant such as phenanthrene, benzene, fluorine, acenaphthene, anthracene and fluoranthene. This study can evaluate the ability of *Impatiens balsamina* to absorb different kind of organic contaminants other than naphtalene.

CONCLUSION

Based on the result findings, it can be concluded that *Impatiens balsamina* plant was able to tolerate in the contaminated soil. It was shown that the naphthalene concentration in the *Impatiens balsamina* stem was increased by 13.301 % but not in leaf and root which were decreased to 46.867 % and 6.435 % respectively. This shows that the *Impatiens balsamina* was able to tolerate and absorb contamination from soil and stored them in the stem tissue.

AKNOWLEDGEMENT

The authors would like to thank Ministry of Malaysia Education and Universiti Teknologi MARA for providing the fund for this research. This research was supported by Research Acculturation Grant Scheme [600-RMI/RAGS 5/3 (17/2012)] and Excellent Fund [600-RMU/ST/DANA 5/3/Dst (5/2012)]

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