

Vermiremediation and Mycoremediation of Polycyclic Aromatic Hydrocarbons in Soil and Sewage Sludge Mixture: A Comparative Study

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Abstract—Polycyclic aromatic hydrocarbons-contaminated soil and sewage sludge mixtures in microcosms were tested separately in triplicate with two types of biological agents, namely earthworms (*Lumbricus rubellus*) and spent fungal mycelia of *Pleurotus sajor-caju* (SPC) for 60 days. The results indicated that vermiremediation (VMR) and mycoremediation (MYC) showed degradation result of 99.99% PAHs i.e. Anth, Phen and BaP removal. Ten earthworms and 750 g of SPC depicted optimum results in nutrient element content among respective treatments. Treatment of MYC resulted a consistence increment of soil macronutrients except for TP and TKN (500 g treatment) compared to VMR. Hence, sixty days incubation of spent fungal mycelia of *Pleurotus sajor-caju* compost (SPC) is potential to degrade 99.99% of Anth, Phen and BaP but at the same time enrich the soil macronutrients i.e. TOC, TKN and TK.

Index Terms—Bioremediation, *lumbricus rubellus*, spent mushroom compost, vermitechnology.

I. INTRODUCTION

Polycyclic aromatic hydrocarbons or polynuclear aromatic hydrocarbons (PAHs) are chemical compounds made up of more than two fused aromatic rings in a linear or clustered arrangement, usually containing only carbon (C) and hydrogen (H) atoms, although nitrogen (N), sulphur (S) and oxygen (O) atoms may readily substitute in the benzene ring to form heterocyclic aromatic compounds [1]. PAHs originate from pyrogenic, petrogenic and natural sources; the former two are the major anthropogenic sources of PAHs in the environment [2]. Pyrogenic sources coming from incomplete combustion of organic matters such as fossil fuels and wood whereas petrogenic sources originated from crude oil and petroleum products [3]. Pyrogenic PAHs are released into the atmosphere as gasses and soot particles [4]. They are transported onto land through wet and dry deposition. Petrogenic PAHs are released through oil spills, leaking of engine and fuel oil, dumping of used crankcase oil and

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unburned fuel absorbed into street dust [4].

Research on the potential utilisation of earthworms has shown an ability to manage polluted land and even sewage sludge, which is termed as vermicomposting or vermistabilisation. In its basic form, this is a low-cost technology system that primarily uses earthworms in the processing or treatment of organic wastes. In this field, application of *Lumbricus rubellus* is scarce compare to *Eisenia foetida*. Apart from the role of earthworms in bioremediation, white-rot fungi also potential in producing extracellular enzymes, which degrade PAHs. Similar scenario reported for *Pleurotus sajor-caju*, which not commonly tested on its ability to degrade persistence organic pollutants.

Therefore, the objective of the study is to compare the potential of *L. rubellus* and spent *P. sajor-caju* compost (SPC) in polycyclic aromatic hydrocarbons (PAHs) viz. Anth, Phen and BaP biodegradation and the nutrient elements content in the soil and sewage sludge (SS) mixture after treatment.

II. MATERIALS AND METHODS

A. Preparation of Bioremediation Agents

The earthworms were obtained from a vermicomposting farm in Ijok, Selangor and reared in the Earthworms Reservoir, Institute of Biological Sciences (ISB), University of Malaya. Before the earthworm was selected as a bioremediation agent, vermiculture commenced and the worms were cultured in SS for one month; only clitellated earthworms were taken as the agent before being introduced to the treatment microcosms. SPC as a feed material (bulking agent) was mixed together with SS for culturing in a ratio of 20:80 (SS: SMC). Before the culture started, the mixed substrate was pre-composted for 21 days. This was to ensure that non-thermophilic and non-pathogenic conditions occurred when the earthworms introduced to the culturing substrates. The temperature was maintained at $27 \pm 1^\circ\text{C}$, pH was 7 ± 1 and moisture content was $70 \pm 10\%$. The treatment was monitored once a week by sprinkling distilled water using wash bottles (80–160 ml per microcosm) for moisture content maintenance, temperature stabilisation and pH control.

SPC was taken from a mushroom cultivation farm in Tanjung Sepat, Selangor, which produces more than a tonne per day. Six-month old *P. sajor-caju* grown on sawdust substrate in plastic bags was discarded after 4-5 harvests. These bags weighed ~600 g each, and were usually dumped or burnt in the farm.

Garden organic soils were purchased from the market with

4.2 kg per packet, as a mixture of soil and humus. Clay, silt and sand percentages were calculated. Sewage sludge (SS) was taken from Bunus STP in Titiwangsa, Kuala Lumpur. SS was collected in a large circular black plastic bucket (45ℓ) and non-biodegradable particles such as rubber band, plastics, metal pieces, glass pieces, chewing gum etc. were removed after the collection. PAHs, i.e. Anth, Phen and BaP, were purchased as follows:

- i. anthracene (Sigma-Aldrich C/N: A89200)
- ii. phenanthrene (Sigma-Aldrich C/N: 77472)
- iii. benzo(α)pyrene (Sigma-Aldrich C/N: B1760)

Microcosm; a plastic container with a size of 360mm × 280mm × 200mm (length × width × height) was designed with an aperture size of 250mm × 100mm on lid covered with net at the centre to prevent intervention from pests. This was to give aeration for aerobic species and microclimatic conditions for treatment [5], [6].

B. Experimental Design

The soil was primed in a microcosm with 1 kg for each replicate and it was applied to all treatments. The soil mixed with SS by manual turning was prepared with ratio of 10:1 (soil:SS) and PAHs were artificially inserted in the soil and SS mixture at each treatment by weight (Anth 500 mg kg⁻¹, Phen 100 mg kg⁻¹ and BaP 50 mg kg⁻¹) using an electronic balance.

The vermiremediation (VMR) process utilised earthworms *L. rubellus* species; three treatments were tested in the process: 10, 30 and 50 earthworms were introduced to the contaminated mixture. Culturing in the contaminated mixture tested the earthworms and only clitellated earthworms were selected from the vermiculture for the treatment process.

The mycoremediation (MYC) process involved three treatments: 250g, 500g and 750g of the spent *P. sajor-caju* compost (SPC) was introduced to the contaminated mixture. Each treatment was done in triplicate. The moisture content was maintained at 70 ± 10% using distilled water, temperature 27 ± 1°C, pH 7 ± 1 (these parameters applied to all treatments).

C. Laboratory Analysis

Approximately 50 g of soil mixture from all treatments was sampled for laboratory analysis after the allotted time set for all treatments. The PAHs were analysed using Gas Chromatography-Mass Spectrometry (GCMS). ASTM E 949 determined total Organic Carbon (TOC). Total Kjeldahl nitrogen (TKN) was estimated by ASTM E 778. ASTM D 5198 detected total Phosphorus (TP). Total Potassium (TK) was measured using ASTM E 926.

D. Statistical Analysis

Data were analysed for significant differences ($P < 0.05$) between treatments using one-way analysis of variance (ANOVA) for concentrations of Phen, Anth and BaP using SPSS for Windows version 16.0 (SPSS, Inc., Chicago, IL, USA).

III. RESULTS AND DISCUSSION

A. Vermiremediation (VMR)

Identical results were illustrated for Anth and Phen in all treatments and control at day 30 and 60 (<2.00 µg kg⁻¹); the same trend was seen for BaP (<5.00 µg kg⁻¹) (Table I). The results implied removal of 99.99% of PAHs compared to the day 0 concentrations viz. Anth 500 mg kg⁻¹, Phen 100 mg kg⁻¹ and BaP 50 mg kg⁻¹. A similar pattern of degradation was found for all treatments at day 30 and day 60, the PAHs i.e. Anth, Phen and BaP resulted in the removal of 99.99% during the analysis period. This finding is in agreement with [7], who recorded the removal of Anth, Phen and BaP in soil with the presence of earthworms i.e. *Eisenia foetida* with 51% removal for Anth, 100% for Phen and 47% for BaP.

The nutrient contents i.e. TKN, TP and TK of the soil, particularly TKN, at day 30 were comparatively higher than at day 60 (Table II; Table III). Among the day 30 results, the 10-earthworms treatment showed high nutrients content compared to other treatments. Statistical analysis of one-way ANOVA showed no significant differences in nutrient elements for all treatments ($P > 0.05$) at day 30, whilst at day 60 only TK showed a significant difference ($P < 0.05$; $df = 2$; $F = 6.814$). N might originate from the addition of N by the earthworm itself in the form of mucus, nitrogenous excretory substances, growth-stimulating hormones and enzymes [8]. The results of P and K content were probably due to the direct action of the earthworm gut enzymes and indirectly by stimulation of the microflora. Removal of PAHs is related to the nutrient content as the earthworms excrete nutrients such as N and P in their casts, as well as to the presence of microorganisms, which possibly accelerate the removal of PAHs [9], [10].

TABLE I: PAHs CONTENT IN TREATMENTS OF VERMIREMEDIATION AND MYCOREMEDIATION AT DAY 30 AND 60

Treatment	Anth (µg kg ⁻¹)	Phen (µg kg ⁻¹)	B(a)P (µg kg ⁻¹)
T10/250	ND (<2.00)	ND (<2.00)	ND (<5.00)
T30/500	ND (<2.00)	ND (<2.00)	ND (<5.00)
T50/750	ND (<2.00)	ND (<2.00)	ND (<5.00)

T: Treatment; ND: not detected; T10/250: 10 earthworms / 250 g of SPC; T30/500: 30 earthworms / 500 g of SPC; T50/750: 50 earthworms / 750 g of SPC.

TABLE II: NUTRIENT ELEMENTS CONTENT IN VMR ON DAY 30

Treatment	TOC	TKN	TP	TK
10 earthworms	0.50 ± 0.22	0.69 ± 0.03	0.16 ± 0.02	0.44 ± 0.05
30 earthworms	0.20 ± 0.03	0.58 ± 0.04	0.16 ± 0.01	0.46 ± 0.02
50 earthworms	0.40 ± 0.09	0.61 ± 0.07	0.13 ± 0.02	0.29 ± 0.03
Value are means and standard error (mean ± S.E.M; n = 3)				

TABLE III: NUTRIENT ELEMENTS CONTENT IN VMR ON DAY 60

Treatment	TOC	TKN	TP	TK
10 earthworms	0.57 ± 0.03	0.49 ± 0.04	0.19 ± 0.01	0.57 ± 0.04
30 earthworms	0.27 ± 0.12	0.38 ± 0.07	0.14 ± 0.03	0.44 ± 0.10
50 earthworms	0.43 ± 0.23	0.42 ± 0.04	0.21 ± 0.03	0.38 ± 0.03
Value are means and standard error (mean ± S.E.M; n = 3)				

B. Mycoremediation (MYC)

Similar degradation results to vermiremediation were obtained in mycoremediation (Table I). The removal of Anth, Phen and BaP by 99.99% within 30 days was highest compared to reported work by [11], *Trametes versicolor* was

able to remove PAHs in sterile soil by 50% after 10 weeks of treatment and *Irpex lacteus* removed PAHs by 27% after the same duration. The degradation of the PAHs was due to the enzymatic actions produced by the remnant mycelia of *P. sajor-caju* utilised in this treatment even though this requires further analysis.

Nutrient content recorded the increment on day 60 compared to day 30 except for TKN in 500 g of SPC and all treatments in TP (Table IV; Table V). The highest content of TOC and TK is in 750 g treatment at day 60 compared to day 30. Whereas, for TKN and TP content the highest in 250 g (day 60) and 250 g (day 30) treatments. Statistical analysis of one-way ANOVA showed that no significant differences were seen for nutrient elements in all treatments ($P > 0.05$) at both day 30 and day 60. The increment of TOC indicates the availability of the carbon supplements in order to sustain microbial community to degrade PAHs. Thus, additional carbon supplies are not required in this study because results shown dramatic degradation of PAHs within the incubation period and it was without extra carbon supplement. Increment of TKN postulates that biostimulation of electron acceptor primarily used for microbial cellular growth (NH_4^+ or NO_3^-) is not require to assist indigenous microbial assimilation. Decrease in TP on day 60 might be due to consumption of the indigenous microbial communities for cellular growth to enhance the PAHs degradation in the microcosms. Increment of TK was the effect of mineralisation resulted from enhanced microbial and enzymes' activities as part of the degradation of PAHs.

TABLE IV: NUTRIENT ELEMENTS CONTENT IN MYC ON DAY 30

Treatment	TOC	TKN	TP	TK
250 g	0.40 ± 0.06	0.33 ± 0.05	0.18 ± 0.03	0.42 ± 0.10
500 g	0.27 ± 0.17	0.45 ± 0.06	0.15 ± 0.01	0.35 ± 0.02
750 g	0.53 ± 0.38	0.33 ± 0.05	0.16 ± 0.05	0.38 ± 0.04

Value are means and standard error (mean ± S.E.M; n=3)

TABLE V: NUTRIENT ELEMENTS CONTENT IN MYC ON DAY 60

Treatment	TOC	TKN	TP	TK
250 g	0.83 ± 0.15	0.50 ± 0.13	0.13 ± 0.03	0.48 ± 0.04
500 g	2.53 ± 0.87	0.26 ± 0.05	0.14 ± 0.01	0.46 ± 0.04
750 g	0.87 ± 0.09	0.41 ± 0.10	0.13 ± 0.01	0.50 ± 0.04

Value are means and standard error (mean ± S.E.M; n=3)

C. Comparison between VMR and MYC

There is no difference in the effects of PAHs degradation in soil and SS mixture since both of the treatments resulted similar content of PAHs after the incubation period. Nutrient elements content has shown a comparable result between the two bioremediation techniques. VMR only showed increment in all treatments for TOC on day 60 compared to day 30. The other soil macronutrients in VMR reflected inconsistency of soil nutrients content between treatments. On the other hand, MYC showed consistency increment of soil nutrient content between day 30 and 60 except for TP and TKN (500 g treatment). These results indicate *L. rubellus* as macroinvertebrate consumed much of the nutrients available to meet its dietary uptake. Whereas in MYC, spent fungal mycelia of *P. sajor-caju* incorporates with indigenous microbes community to degrade PAHs and enriching the soil macronutrients' content.

IV. CONCLUSION

Vermiremediation utilising *L. rubellus* and mycoremediation using spent *P. sajor-caju* compost has proved its potential to degrade polycyclic aromatic hydrocarbons i.e. Anth, Phen and BaP in 30 days of incubation. These treatments can be further tested in pilot scale or *in-situ* in order to study its ability at on-site environmental contamination.

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