

Bioengineered Approach for *Ex situ* Vermiection of Acenaphthylene, Benzo (E)Pyrene and Benzo (Ghi) Perylene Soil Contamination

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Authors' contributions

This work was carried out in collaboration among all authors. Author CF conceptualised the study, performed the data analysis, performed the analytical quality (AQ) and wrote the first draft of the manuscript. Authors SJS and DAD supervised the research and manuscript. Author HACN managed the literature searches and samples analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2021/v40i3231556

Editor(s):

(1) Dr. Chien-Jen Wang, National University of Tainan, China.

Reviewers:

(1) Nazanin Khakipor, Islamic Azad University, Iran.

(2) Ouedraogo Wendlassida, University Norbert Zongo, Burkina Faso.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/73292>

Original Research Article

Received 05 July 2021
Accepted 11 September 2021
Published 06 November 2021

ABSTRACT

The *ex situ* study of vermiection of Acenaphthylene (AcPY), Benzo(e)pyrene (BeP) and Benzo(ghi)perylene (BP) from constructed vermiculture containing petroleum contaminated soil (8.00±0.01, 9.80±0.00 and 5.02±0.00 mg/kg respectively) and vermiection (AcPY, 1.05±0.00, BeP, 2.01±0.00 and BP, 1.73±0.00 mg/kg) by *Esenia fetida* squirms with mean vermiremoval efficiency of 100% while vermiconversions were AcPY, 86.88, BeP, 79.49 and BP, 65.54%. The identification and quantification of the 3 polycyclic aromatic hydrocarbons (3PAHs) were performed by GS/MD in accordance with analytical procedure of US. EPA 8270; 625. The bioengineered approach by *E. fetida* squirms in the vermiculture proved effective to detoxify and remove the persistent organic pollutants of the 3PAHs.

Keywords: Bioengineered approach; ex situ; vermiculture; acenaphthylene; Benzo(e)pyrene, Benzo(ghi)perylene; soil; vermiculture.

1. INTRODUCTION

In the advent of industrialisation and civilisation, life has certainly become easy and human living conditions have drastically improved vastly. However, it has also brought with it the increasing of environmental pollution which has become a serious cause of concern and existential threat for life on earth. Soils where plants are grown for food, recreation, pharmaceuticals, medicine, maintaining earth's atmosphere, energy and among others, for the survival of man has been ironically polluted by man's anthropogenic activities at different levels. Also the choice of practice to remediate the polluted environment is colossal.

But bioremediation has proven to be environmentally acceptable, sustainable, viable and cost-effective treatment of soil contaminations [1] and [2]. There have been strong indications that similar results can be expected in petroleum and petroleum products contaminated sites. Hydrocarbons are generally quite biodegradable and oil-degrading microorganisms are ubiquitous, but the priority pollutants or contaminants of polycyclic aromatic hydrocarbons (PAHs) are limited. Besides, they are mostly referred to as harmful persistent organic pollutants (POPs) because of their persistence in the environment, not easily biodegradable while the high-molecular-weight (HMW) PAHs are even more detrimental to the environment and human health. Acenaphthylene (AcPY), Benzo(e)pyrene (BeP) and Benzo(ghi)perylene (BP) are three, five and six fused benzene rings in various structural configurations, their benzene ring arrangements have a wide diversity of physical, chemical and toxicological characteristics commonly present in the petroleum and petroleum products [3] and [4]. However, PAHs containing up to four rings are referred to as light PAHs and those that contain more than four rings are heavy PAHs. Heavy PAHs are more stable and more toxic than the light PAHs [4] and [5].

Nonetheless, vermiculture is an earthworm based biotechnological approach or strategy to remove contaminants from water or soil. The process water is remediated and soil is improved with rich nutrients for healthy growth microbes, plants and their (earthworms) survival [5] and [6]. Vermiculture could also be referred to as

vermiculture or vermiculture because the process mostly involves biostimulation; the actions generally improve environmental condition of sites and thereafter influence the activities of microorganisms.

Lately the globe has witnessed rapid growth in the awareness of PAHs in petroleum products, food, air, soil, wastewater and sewage. Also assortment of bioremediation and biodegradation processes are now emerging to transform PAHs to less hazardous or nonhazardous compounds.

Thus, the pilot study in the greenhouse is aimed to investigate and assess the bioengineering approach of *Eisenia fetida* earthworms to remove or degrade the 3 PAHs (AcPY, BeP and BP) that are also POPs present in the sample soil.

2. MATERIALS AND METHODS

2.1 Study Site

The soil samples and *Eisenia fetida* earthworms were identified and collected from the vegetation of the Federal College of Forestry, Jos Nigeria. The Ex situ study was also performed in the greenhouse of Federal College of Forestry, Jos Nigeria with location coordinates N09°50'31" E008°53'55". Also, samples analytical preparations were executed at the Chemistry Postgraduate Research Laboratory, University of Jos, Nigeria with the location coordinates N09°53'46.2" E008°53'58.5" and Department of Petroleum Engineering, Rivers State University, Nigeria.

2.2 Soil Collection and Sampling

Collection and sampling of soil was conducted under International Atomic Energy Agency, IAEA-TECDOC-1415 of soil sampling and collection for environmental contaminants as well as USEPA method 5035 as recommended for site investigators assessing the extent of volatile organic compound in soil [7]. The earthworm earthworms were collected and sampled under the protocol for assessing ecotoxicity of chemical substances of earthworms OECD 207 [8] and [9].

The rich humus soil was collected from the study site into plastic vessels and transported at a

room temperature to the greenhouse for vermiculture preparation.

Soils from the different vermiculture vessels were carefully separated (after detention period of 5 weeks) from the earthworms and air dried to constant weight, ground homogenously with clean mortar, sieved through a 2 mm sieve to remove debris and stones while stored in transparent polythene bags at room temperature for the characterisation of AcPY, BeP and BP.

2.3 Acenaphthylene, Benzo(e)pyrene and Benzo(ghi)perylene Contaminated Soil Sampling

Petroleum-contaminated surface water sample of 500 cm³ was impacted with the constructed vermiculture and mixed homogenously while 5 weeks detention period was observed.

2.4 *Eisenia fetida* Collection and Sampling

The adult and juvenile *Eisenia fetida* squirms were identified and collected from study site into clean plastic vessel and transported at a room temperature to the greenhouse. After 5 weeks of detention period for the vermiculture processes, the earthworm squirms were harvested and washed with tap water to remove soil and any dirt from the body surface then rinsed with deionised water and gut-voided by placing the squirms in glass lined with wet Whatman No.1 filter papers for 24 hours. The earthworms were thereafter washed again with deionised water and kept in a freezer at 4⁰C. The dead earthworm squirms were air dried and

ground with laboratory mortar into fine powder. The dried powdered earthworms' sample was kept in transparent plastic bag and stored in a cool dark room for the AcPY, BeP and BP analysis.

2.5 Preparation of Bioengineered Vermiculture

Organically rich humus soil of 1000g was weighed into a clean plastic vessel with a suitable dimension while earthworm bed consist of old newspapers was made at the pit of the container. The humidity, temperature, moisture content and pH of the humus soil sample were maintained at 40-45%, 20-30⁰C, 45-50% and 6.5-7.0 respectively. *Eisenia fetida* squirms were introduced and covered with old newspapers to reduce or prevent illumination (earthworms are sensitive to light) and maintain the vermiculture's condition.

2.6 Extractions of Soil and *E. fetida* Samples to be Analysed

Soils and earthworms samples of 1g each from the control and experimental groups of vermiculture vessels were differently sorted and homogenously mixed with 30cm³ of acetone and hexane (1:1, v/v) by a mechanical shaker at 120 oscillations per minute for 4hours. The mixtures of each sample were poured into different separatory funnels and allowed to stand for a couple of minutes for the organic layers to separate clearly from the aqueous phases. The organic (extract) layers were collected and stored in different well labelled amber bottles with Teflon-lined caps and refrigerated at 4⁰C.

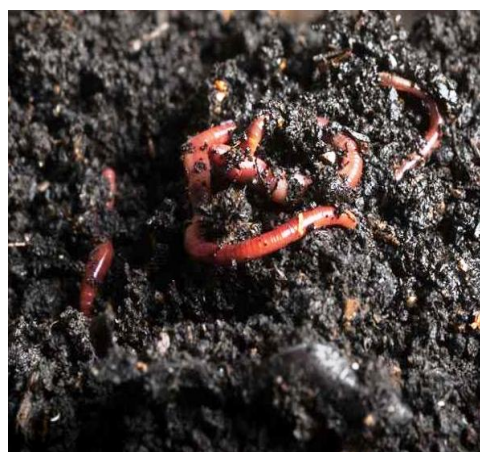


Fig. 1. Vermiculture (Field work)

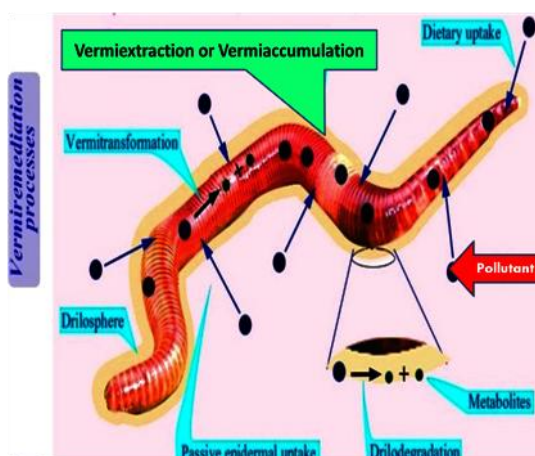


Fig. 2. Schematic Vermiextraction Strategy [9]

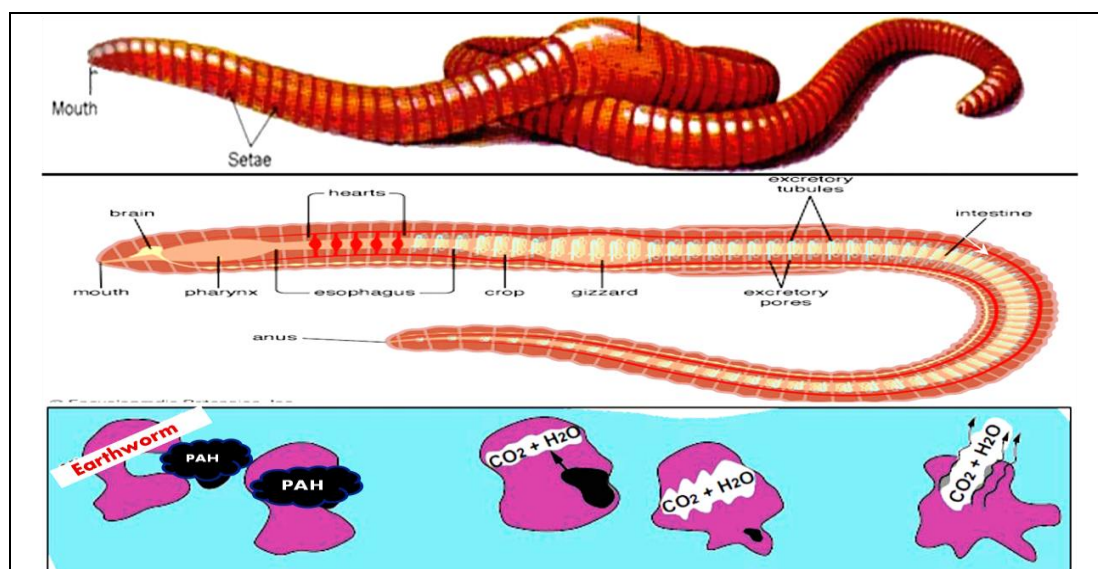


Fig. 3. Schematic Vermiextraction, Vermiaccumulation, Vermidegradation and Vermitransformation of Polycyclic Aromatic Hydrocarbons [10]

2.6.1 Analysis of AcPY, BeP and BP

The representative extracts (samples) of soils and earthworms from control and treatment groups were characterised prioritising the AcPY, BeP and BP polycyclic aromatic hydrocarbons using Gas chromatography mass spectrometry detection (GC-MSD) Agilent Technologies 7890A in adherence to the manufacture's operational specifications.

2.6.2 Analytical quality

The quality control measures in analysis procedure were taken to confirm the accuracy of the analytical data. In order to establish the quality assurance and quality control of the results; collected samples were analytically pooled together to give various representative samples for the analysis.

All reagents used were of analytical grade and certified standard solutions.

2.6.3 Data analysis

Mean concentrations of sample results were analysed using statistical programme for social sciences.

3. RESULTS AND DISCUSSION

3.1 Results

The results of the representative samples were categorically shown in Table 1. The 3 PAHs

(AcPY, BeP and BP) were present in the soil sample with 8.00 ± 0.01 , 9.80 ± 0.00 and 5.02 ± 0.00 mg/kg respectively when impacted with petroleum-contaminated wastewater at the experimental unit. The control unit had N.D or no concentrations in the vermiculture containing the soil and earthworm samples. Vermiextraction of the AcPY, BeP and BP evidently took place by sustaining the vermiaccumulation of 1.05 ± 0.00 , 2.01 ± 0.00 and 1.73 ± 0.00 mg/kg respectively within the 5 weeks detention (experimental) period.

The vermiconversion occurred into possible different metabolites of AcPY, BeP and BP with percentages of 86.88, 79.49 and 65.54% respectively; however, with visible evidences of excreted rich nutrients vermicasts in their multiple numbers, burrowing movements, burrow creations, soil digestions and viscid slippery secretion.

3.2 Discussion

The presence of AcPY, BeP and BP indicate environmental and human health toxicity in the impacted soil with petroleum-contaminated surface water. Human exposure of these 3PAHs through food chain, skin in contact with contaminated soil or products like heavy oils, coal tar, roofing tar or creosote could have chronic effects such as breathing problems, asthma, cataracts, decreased immune function, kidney and lungs damage [11] and [12].

Table 1. Vermiection of AcPY, BeP and BP soil contamination

3PAHs (Priority Constituents)	Vermiculture (mg/kg)					Vermiremoval Efficiency in the Soil (%) (a)	Vermiconversion (metabolites) by Earthworms % (a)
	Soil			Earthworms			
	Control unit without petroleum contents (a)	Experimental unit with petroleum contents	Vermiaccumulation at the experimental unit (a)	Control unit without petroleum contents (a)	Vermiaccumulation at the experimental unit (a)		
AcPY (3rings)	0.00	8.00±0.01	0.00	N.D	1.05±0.00	100.00	86.88
BeP (5rings)	0.00	9.80±0.00	N.D	N.D	2.01±0.00	100.00	79.49
BP (6rings)	N.D	5.02±0.00	N.D	N.D	1.73±0.00	100.00	65.54

N.D = no detection, n = 3, a = mean, a ± b = mean ± standard deviation

However, BeP and BP have been found to be carcinogenic, mutagenic and genotoxic in experimental animals and humans after inhalation or intratracheal ingestion [13]. Also main sources of emission for the 3PAHs in China, India, USA and Global are biofuel (66.4, 92.5, 9.1 & 56.7%), wild fire (0, 0, 3.3 & 17.0%), consumer product use (0.9, 0.6, 35.1 & 6.9%) and petroleum refining (1.0, insignificant, 9.5 & 2.4%) [14].

The occurrence of vermiremediation in this study validates the vermiremediation of the toxic AcPY, BeP and BP in the contaminated soil with evidence of the nutritious vermicast productions during the bioengineering processes of *Eisenia fetida*. The potentials of earthworms can be employed in bioremediation strategies to promote biodegradation of organic and inorganic contaminants in an environmental friendly approach. This is because of their biological, chemical and physical activities ranging from burrowing, production of casts both surface and below ground, their internal gut and processes, their surface in contact with the soil, other biological, chemical and physical interactions, in addition to the associated soil microorganisms [15], [12] and [16]. Earthworms have the ability to aerate soils, improve their nutritional status and fertility, which are variables known to limit bioremediation [17] and [18].

The results of vermiculture obtained at the experimental unit suggested that earthworms are key organisms in environmental toxicology to remediate or detoxify persistent organic pollutants present in soils. Interest in earthworm ecotoxicology can be traced back to the inception, ring-testing and international standardisation of the acute earthworm toxicity test [15]. Earthworms are referred as unheralded soldiers of mankind; this was one of the earliest references of the wonder works of earthworms [11]. Their role as earth waste managers is well documented by vermiculture scientists however recently, research in the use of earthworms is tending towards its role in remediation of soil pollutants, development of medicine, as feeds in fisheries and dairy including its use as raw materials in rubber, soap and detergent industries [6] and [5]. Acute toxicity test was designed to be included in the risk assessment framework for newly registered chemicals and pesticides and the earthworm *Eisenia fetida* was identified as a model organism for assessing the effects of chemicals on terrestrial environment

[11], [5] and [15]. On the other hand, earthworms are hardy organisms capable of surviving highly toxic environment like being the only survival soil fauna after the 1976 Seveso chemical plant explosion in Italy [12]. Data obtained also showed that the vermiremediation of the 3PAHs in the soil was 100% efficient with the suggested vermiconversion of the contaminants to various metabolites states (gaseous, liquids and solids) as illustrated in figure 3. Earthworms also retard the binding of organic contaminants to soils, release previously soil-bound contaminants to different metabolites for easy and subsequent degradation, promote and disperse organic contaminant degrading microorganisms [10] and [16].

4. CONCLUSION

The bioengineering approach of *E. fetida* squirms vermiremediated the toxicities of AcPY, BeP and BP from the vermiculture employing the processes of vermiculture and vermiconversion yielding a mean vermiremediation efficiency of 100%. However, the ex situ vermiremediation of 3PAHs was lubricated by the released cutaneous mucus of *E. fetida* squirms to facilitate movement and protect themselves from being encased by soil particles, sequentially increasing the availability of nutrient contents, soil enzymes and fertility. *E. fetida* shows to be promising for vermiremediation use, on the basis of its bioengineering efficiency and its ability to tolerate AcPY, BeP and BP soil contamination. Thus vermiremediation can be considered as double-edged technology which plays a major role in sustainable development. The method is simple, effective, convenient, and noiseless. It saves water, energy, landfills and helps rebuild soil.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

ACKNOWLEDGEMENT

We wish to appreciate the Tertiary Education Trust Fund and the Office of Research and Development, University of Jos, Nigeria for the

wonderful privilege and opportunity to further our research endeavours.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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