Original Research Article

Eisenia fetida Squirms Biased Bioremediation of Pyrene and Indeno(1, 2, 3–*cd*)Pyrene Soil and Petroleum Wastewater Contamination

ABSTRACT

Raw mean levels of pyrene (pyr) and indeno(1,2,3-cd)pyrene petroleum wastewater were 8.00±0.00 and 9.20±0.03 mg/L respectively. Petroleum wastewater impacted soil gave Pyr (8.00±0.01) and Ind (9.50±0.00) mg/kg. The biased and effective nature of *Eisenia fetid*a vermiaccumulated Pyr (6.94±0.02 mg/kg) and Ind (7.56±0.01 mg/kg), had vermiremoval efficiency of Pyr (86.75%) and Ind (82.17%). Thus, the treatment unit indicated Pyr (0.07±0.00 mg/kg) and Ind (0.00±0.00 mg/kg) for soil with petroleum wastewater sample while petroleum wastewater sample was Pyr (0.00±0.00 mg/L) and Ind (0.00±0.00) within 5 weeks detention period. Identifications and quantifications of the two polycyclic aromatic hydrocarbons (2PAHs) Pyr and Ind were carried out by GC/MD in accordance to manufacturer's operational specifications. *E. fetida* squirms were proven bioremediation tools, capable to remove or detoxify the two environmental health contaminants from the contaminated pilot ecosystem.

Keywords: Eisenia fetida, Biased bioremediation, Pyrene, Indeno(1,2,3–cd)Pyrene, Soil, Petroleum Wastewater. Environmental health contaminants.

1. INTRODUCTION

Environmentally friendly and cost effective are among the major advantages of bioremediation compared to both chemical and physical methods of remediation. A mechanism of bioremediation is to reduce, detoxify, degrade, mineralise or transform more toxic pollutants to a less toxic or non toxic. Environmental pollution has been on the rise in the past few decades due to increased human activities such as population explosion, unsafe agricultural practices, unplanned urbanization, deforestation, rapid industrialization,non-judicious use of energy reservoirs and other anthropogenic activities [1]. Among the pollutants that are of environmental and public health concerns due to their toxicities are: hydrocarbons, heavy metals, chemical fertilizer, nuclear wastes, pesticides, herbicides, insecticides and greenhouse gases [6].

According to the recommendations by the EU Scientific committee for food (SCF), the European Union (EU) and US Environmental Protection Agency (EPA), Pyrene (Pyr) and Indeno (1,2,3–*cd*) Pyrene (Ind) are among the toxic 17 Polycyclic Aromatic Hydrocarbons (17PAHs) also referred as persistent organic pollutants (POPs) [1] and [2]. However, POPs are toxic chemicals that adversely affect human health and the environment around the world. They persist for long periods of time in the environment, accumulate and pass from one species to the next through the food chain [3].

The effectiveness of bioremediation depends on many factors; including, the chemical nature and concentration of pollutants, the physicochemical characteristics of the environment, and their accessibility to existing microorganisms or soil macrofauna such as earthworms [4], [5] and [6]. In this study, the aim is to ascertain the bias nature of *Eisenia fetida* (earthworm) squirms in the context of bioremediation of toxic Pyr and Ind (2PAHs) present in soil impacted with petroleum wastewater.

2. MATERIALS AND METHODS

2.1 Study Site

Petroleum wastewater sample was obtained from Okopoka creek, (Port-Harcourt, River State) Niger-Delta Nigeria with the location coordinates of N04^0 46' 38'' E0070 03' 55''. Okpoka creek is one of the adjoining creeks off the upper Bonny River estuary in the Niger-Delta with evidences of petrogenic activity by artisanal refiners. It is also a major route for crude oil bunkering and other cargoes that

leads to the Port Harcourt quays, Federal ocean terminals, Onne, Port Harcourt Refinery Company Terminal Jetty and Okirika community.
Rich humus soil samples and *E. fetida* squirms were identified and collected from the vegetation of the Federal College of Forestry, Jos Nigeria, location coordinates $N09^0$ 50'31" $E008^0$ 53'55".
The study was performed in the green house of Federal College of Forestry, Jos Nigeria. All analytical preparations of the samples and analysis were performed at the Chemistry Postgraduate Research Laboratory, University of Jos, and Department of Petroleum Engineering, Rivers State University, Nigeria with the locations coordinates $N09^0$ 53'46.2" $E008^0$ 53'58.5" and $N04^0$ 47'41.42" $E006^0$ 58' 40.94'' respectively.

2.2 Soil Sampling

Organically rich humus soil of was collected from the study site into clean dried plastic vessels and transported at room temperature to the greenhouse for bioremediation process.
Soil samples from well labelled different vessels (control and treatment) were carefully separated (after detention period of 5 weeks) from the earthworms squirms and air dried to constant weight, ground homogenously with clean dried 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 Pyr and Ind.

2.3 Petroleum Wastewater Sampling

Petroleum wastewater samples were collected from the study site using the grab samples method. Discrete grab samples were taken using a clean dried glass cup and amber bottles with Teflon lined caps. Collections of samples were obtained at different depths (2, 5 and 10cm) of a selected location at the study site. However, collected samples were amalgamated to obtain 60,000cm^3 of representative sample. The homogenised and representative sample was filtered and preserved (acidified) on the site with 120 cm^3 concentrated H_2SO_4 to bring the pH to ≤ 2. The sample was refrigerated at ≤ 4^0C and extracted within 14days of collection for the analyses of the 2PAHs (Pyr and Ind).
After 5 weeks of detention period, petroleum wastewater samples of 500 cm^3 were collected from experimental vessels for the analysis of Pyr and Ind.

2.4 *E. fetida* (earthworm) Sampling

The adult *E. fetida* squirms were specifically 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 bioremediation 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 4^0C. 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 characterisation Pyr and Ind.

2.5 Preparation of Bioremediation Vessel (Pilot Ecosystem)

The organically rich humus soil sample with humidity (40-45%), temperature (20-30^0C), moisture content (45-50%) and pH (6.5-7.0) was weighed (500 g) and impacted with 1500 cm^3 petroleum wastewater sample into a clean plastic vessel with a suitable dimension. In the treatment unit earthworm squirms were introduced and covered with a mesh size net of 0.6 mm and old newspapers to prevent escape, to reduce illumination (earthworms are sensitive to light) and maintain condition. The control unit was repeated with same condition of the treatment unit but soil sample of 500g was not impacted with petroleum.

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

The samples of soils and earthworms of 1g each from the control and treatment groups 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 till the organic layers were clearly separated from the aqueous phases. The organic (extract) layers were collected and stored in different labelled amber bottles with Teflon-lined caps and refrigerated at 4^0C.

2.7 Extraction of Petroleum Wastewater Sample

The liquid-liquid extraction of the filtered representative sample was subjected to separatory funnel extraction procedure. Sample of 500cm^3 was individually extracted in 1000cm^3 glass separatory funnel fitted with glass stopper. Acetone and n-hexane of 125 cm^3 (1:1, v/v) were added to the sample and was shaken on a reciprocating mechanical shaker at 120 oscillations per minute for 4hours. The mixture of sample was poured into separatory funnel and allowed to stand till the organic layer was separated clearly from the aqueous phase. The organic (extract) layer was collected and stored in labelled amber bottle with Teflon-lined caps and refrigerated at 4^0C.

2.8 Analysis of Pyr and Ind

The representative extracts (samples) of soils, earthworms and petroleum wastewater were characterised for the toxic Pyr and Ind polycyclic aromatic hydrocarbons (2PAHs) using Gas chromatography mass spectrometry detection (GC-MSD) Agilent Technologies 7890A in accordance to the manufacture's operational specifications.

2.8.1 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.

Obtained data were represented in mean values with their standard deviations.

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

2.8.2 Data analysis

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

3. RESULTS AND DISCUSSION

3.1 Results

The results of Pyr and Ind contents in soil, petroleum wastewater and *E. fetida* samples were shown in Table 1. The control Pyr mean values of soil without petroleum wastewater and *E. fetida* accumulation were 0.01±0.00 mg/kg and N.D while Ind values were N.D and N.D respectively. The raw Pyr mean values of petroleum wastewater, soil with petroleum wastewater and *E. fetida* accumulation samples gave 8.00±0.00 mg/L, 8.00±0.01 mg/kg and 6.94±0.02 mg/kg respectively, while Ind indicted 9.20±0.03 mg/L, 9.50±0.00 mg/L and 7.56±0.01 mg/kg respectively. Treatment unit represented the Pyr mean values of petroleum wastewater (0.00±0.00 mg/L), soil with petroleum wastewater (0.07±0.00 mg/kg), vermiremoval (86.75%) efficiency while Ind values had 0.00±0.00 mg/L, 0.00±0.00 mg/kg and 82.17% respectively.

Table 1. *E. fetida* Biased Bioremediation of Pyr and Ind Impacted Soil of Petroleum wastewater

2PAHs (Priority Constituents)	Control unit		Raw unit		Treatment unit			
	Soil without Petroleum wastewater contents (mg/kg)	*E. fetida* Vermiaccu-mulation (mg/kg)	Soil with Petroleum wastewater contents (mg/kg)	Petroleum wastewater sample (mg/L) (y)	*E. fetida* Vermiaccu-mulation (mg/kg) (x)	Soil with petroleum wastewater (mg/kg)	Petroleum Wastewater sample (mg/L)	Vermiremoval efficiency (%) (e)
Pyr	$0.01^{a} \pm 0.00^{b}$	N.D	$8.00^{a} \pm 0.01^{b}$	$8.00^{a} \pm 0.00^{b}$	$6.94^{a} \pm 0.02^{b}$	$0.07^{a} \pm 0.00^{b}$	$0.00^{a} \pm 0.00^{b}$	86.75
Ind	N.D	N.D	$9.50^{a} \pm 0.00^{b}$	$9.20^{a} \pm 0.03^{b}$	$7.56^{a} \pm 0.01^{b}$	$0.00^{a} \pm 0.00^{b}$	$0.00^{a} \pm 0.00^{b}$	82.17

N.D = no detection, n = 3, a ± b = mean ± standard deviation, e = (x/y)100%

3.2 Discussion

The levels of Pyr and Ind observed in the study, revealed toxicity of petroleum wastewater impacted soil. Recent research has confirmed that polycyclic aromatic hydrocarbons (PAHs) are recalcitrant compounds, some of which are known carcinogens, mutagens and tetratogens are often found in high residual soil concentrations at industrial sites [4] and [5]. However, hydrocarbon spills in the form of petroleum and petroleum products both on land and in water have been a problem since discovery of petroleum as a fuel source. They have devastating effects on the biota of an environment. Petroleum spills and petroleum waste discharged into the sea from refineries, factories or shipping contain poisonous compounds that constitutes potential danger to plants and animals. The poisons can pass through the food web of an area and may eventually be eaten by humans [5] and [6].

Despite the involvement of many factors in biological remediation, the presence of *E. fetida* squirms was glare to the contaminated soil with Pyr and Ind in the present research. Noticeably, survivals, production of cocoons and juvenile productions of *E. fetida* were visible while bioremediation was concurrently taken place. The mean vermiaccumulation of the two persistence organic pollutants (POP) read Pyr (6.94±0.02 mg/kg) and Ind (7.56±0.01 mg/kg). However, vermiaccumulation is the bioengineering method in which earthworms take up contaminants and store them in their bodies in order to convert them to useful products such as soil nutrients appearing as cocoons or vermicasts. The vermiremoval efficiency of Pyr (86.75%) and Ind (82.17%) proved the efficaciousness or the biased nature of *E. fetida* to remove or detoxify the environmental health contaminated soil despite the possible presence of soil microorganisms that have natural ability to biodegrade toxic substances. Therefore, the mean values from the treatment unit indicated detoxification of Pyr and Ind from the soil with petroleum wastewater sample also from the petroleum wastewater sample.

These contaminants pose variety of health and environmental hazards, humans are harmed by contact or via exposure to contaminated soil/land, air, surface water and ground water. When contaminated soils are not properly managed, humans and wildlife can be exposed to these contaminants through inhalation, ingestion, or dermal contact [7], [8] and [9]. Pyr can cause eye irritation, birth defects, decrease in body weight, skin irritation, leukemia, heart malformations, childhood asthma and nausea, vomiting, diarrhea and confusion [10]. Also, Ind has the ability to bind to cellular proteins and DNA (deoxyribonucleic acid) resulting biochemical disruption and cell damage lead to mutations, developmental malformation, tumors and cancer [8] and [10].

4. Conclusion

The biased bioremediation of *E. fetida* squirms removed the toxicities of Pyr and Ind in the soil and petroleum wastewater contamination despite possibility of the presence of soil microorganisms for degradation or remediation. The obtained results were evident that *E. fetida* have high biological potency to tolerate, clean up and convert organic pollutants to harmless and nutritive products.

The high effectiveness of *E. fetida* to bioremediate the environmental health contaminants is cost effective compared with other remediation techniques. This study is a natural process and did not produce toxic by-products rather it detoxified hazardous substances instead of mere transferred contaminants from one environmental medium to another. The applicability of *E. fetida* squirms had summarily signified complete mineralisation and degradation of the contaminants (Pyr and Ind) in the petroleum wastewater sample and Ind in the petroleum wastewater impacted soil sample. However, *E. fetida* squirms could optimise the environmental conditions and enhance nutrients to stimulate or accelerate the activities of bioremediation by the production of numerous nutritious vermicasts.

COMPETING INTERESTS DISCLAIMER

Authors have declared that no competing interests exist. 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.

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