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Tolerance of Earthworms in Soil Contaminated with Polycyclic Aromatic Hydrocarbon

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ABSTRACT: Pyrene is a very resistant polycyclic aromatic hydrocarbon (PAH) with four benzene rings that survives in the environment. This study was aimed at investigating the tolerance of earthworms in soil contaminated with pyrene. The studies were performed by employing earthworms gathered from shady regions adjacent to sewage ponds as pyrene degraders to eradicate pyrene from the soil. Numerous factors affecting pyrene degradation efficiency were explored, including the effect of contaminant concentration, earthworm and soil ration, and soil condition. The highest pyrene removal (31.2%) was shown by earthworms in the condition of soil mixed with cow dung. Pyrene decomposition was inhibited during soil sterilization due to the absence of soil microorganisms and indigenous pyrene-degrading bacteria. Nonetheless, earthworms are suitable for use as pyrene degraders in contaminated soil.

KEYWORDS: Resistence; polycylic aromatic hydrocarbons; earthworms; contaminated soil.

1. Introduction

The economic development of a country is highly dependent on several aspects of development, involving the processing and production of natural resources such as coal and petroleum. Several processes and products of natural resources are given negative side effects, especially those that produce polycyclic aromatic hydrocarbons (PAHs) as by-products. These PAHs have more than one benzene ring and are high molecular weight compounds that are widely distributed [1]. In coal tar and fossil fuel, they are ubiquitous natural constituents. The main sources of contamination of PAHs mostly come from human activities such as automobile exhaust, forest burning, and incomplete combustion of fossil fuels. PAHs as priority pollutants have been listed and differentiated into 16 types by the United States Environmental Protection Agency (US-EPA) based on their characteristics as PAHs, whereas some of them are considered as human carcinogens [2]. Efforts to study the degradation of PAHs began a few decades ago. Nevertheless, the efficiency of degradation increases gradually when the molecular weight of PAHs increases. Thus, to develop degradation approaches by several types of degradation techniques or methods is still being carried out to date. Several physical-chemical methods are used for organic pollutant removal, such as adsorption, biodegradation, composting, and nanotechnology [3–7].

Some conventional methods have limitations, such as high maintenance and operational costs, long treatment duration, large space operation, and other tedious requirements that hinder further development and adoption. Therefore, there are a number of biodegradation methods that have emerged that specify a solution for the degradation of PAHs. In recent years, various types of biomaterials or organisms have been applied for biodegradation, including bacteria, fungi, and plants [8-10]. Earthworm is another option for PAH degraders. In the past, earthworms have been extensively reported as soil engineers due to their ability to stimulate the accessibility of oxygen in the soil. Research has shown the prospective of earthworms' ability to adapt and survive in contaminated soil with high concentrations of PAHs. Besides, their ability to digest PAHs into simpler molecules [11,12]. Investigation by Dabke reported that earthworms promoted and enhanced microbial activities, when added into contaminated soil by creating good conditions for bacteria and consequently eliminating adverse pollutants such as phenolic compounds from the soil [13]. In addition, in soil contaminated with petroleum hydrocarbons, *Eisenia fetida* was able to survive without constraints and accelerate the degradation of crude oil in the soil [11]. Therefore, it can be considered that earthworms not only degrade pollutants, but also stimulate and synergise with other microorganisms in the removal of soil pollutants. Analytical results reported by Asgharnia et al. further showed that earthworms enhance potential soil quality, increase microbial activity and cause an increase in phenanthrene bioavailability, allowing higher efficiency microbial degradation [14]. Nevertheless, there is a need to select a suitable biomaterial or organism for PAH degradation to avoid the production of unwanted toxic and fatal by-products during the degradation process. In this research, earthworms were selected for pyrene degradation. Although the use of earthworms has been extensively studied in recent years, a stable and consistent degradation strategy has not been well developed. Hence, this study was focused on establishing a suitable biodegradation approach.

2. Materials and Methods

2.1. Materials, earthworm and growth condition

The pyrene was purchased from TCI (Tokyo, Japan). The Thin Layer Chromatography (TLC) silica gel 60 F254 aluminum sheets and Silica 60 (0.2-0.5mm) were supplied by Merck (Germany). Other solvents were supplied by Sigma-Aldrich (USA). Earthworm and soil sampling were undertaken on three farms adjacent to sewage ponds in the region of Johor Bahru, Malaysia. The soils were slightly acidic silty clay loams. Twenty adult earthworms were handsorted in the same area as the soil cores. Earthworms were maintained in a damp, shaded, moist environment. Meanwhile, 5 g of earthworms (4-5 earthworms) were added to the culture mix and weighed to equal 200 g of soil. Another alternative to soil was to use gathered cow dung from the farm. Subsequently, 200 g of cow dung was added to 5 g of earthworms. The moisture content was maintained at 60 %, and compost was used to feed the earthworms. The data of growth and degradation was recorded at 7, 14, and 30 days of incubation.

2.3. Batch studies

The design of the experiment: Prior to exposure, each earthworm was rinsed with tap water, gently dried on filter paper, weighed, and placed in individual Petri plates for 48 hours to allow the gut to empty. Then, earthworms were individually placed in the exposure microcosm (day 0) using a size-class approach to ensure that each treatment had a similar mean earthworm weight. For 7, 14, and 21 days, four to five earthworms were exposed to either pyrene-contaminated soil or control soil, with an initial control group (unexposed worms) for both populations at day 0. Earthworms that decompose pyrene were classified morphologically and macroscopically. The exterior morphology of the earthworms was researched and recorded in greater detail. Three soil samples were created for the earthworm habitat: sterilized dirt, unsterilized soil, and soil mixed with cow dung. The concentration of pyrene was varied to 10, 50, and 100 mg per 200 g of soil while maintaining an earthworm to soil ratio of 1:1, 1:2, 1:4, and 1:8. The soil's moisture content was maintained at 60%.

2.4. Pyrene extraction and purification

After removing the earthworm from the soil, wet filter paper was made and stored for 24 hours. Earthworms were frozen at 0°C and dried with liquid nitrogen. 5 g of sodium sulfate (Na_2SO_4) was added to the 5 g of earthworms and mixed. Meanwhile, 20 mL of ethyl acetate was added and stored overnight for earthworm extraction. The identical method was repeated twice. Meanwhile, pyrene was extracted from soil using the soxhlet extraction process. Prior to placing the thimble in the soxhlet extractor, a total of 50 g of dirt was added to the soxhlet thimble. Dichloromethane was utilized as the solvent extraction and was added to a 150 mL distillation flask. In a round flask, anti-bumping granules were applied. The extraction was heated to the boiling point (40°C) for 24 hours with dichloromethane. After numerous extraction cycles, the chemicals were concentrated in the distillation flask. Following that, 1-2 mL of concentrated extracts were collected using a rotary evaporator. Purified concentrated crude extracts were purified using column chromatography. Hexane was diluted into 5 g of packed silica gel in the column. The crude extract was concentrated to 1-2 mL using a rotary evaporator and then eluted with 150 mL of hexane. Toluene was then added to the concentrated purified extract up to a final volume of 10 mL prior to injection into gas chromatography.

3. Results and Discussion

3.1. Morphology and survival of earthworm

Earthworms range in length from 6 to 12 cm, have a diameter of 0.3 to 0.6 cm, and contain between 80 and 120 segments, depending on the size of the individual worm. Mucus is excreted by earthworms when they travel through the soil or on the floor. Earthworms are extremely resilient and can survive for up to three weeks without nourishment. They are able to live and thrive in contaminated environments and exhibit a high tolerance for PAHs. Earthworm survival rates dropped as the pollutant concentration increased (Fig. 1). After 21 days of incubation, earthworms reproduced at a rate of greater than 100% in the control condition, but their weight fell in the other conditions. Earthworms have been shown to bioaccumulate a variety of contaminants, including organic pollutants, heavy metals), and nanoparticles. They are able to get chemicals from pore water through their skin and soil [15,16].

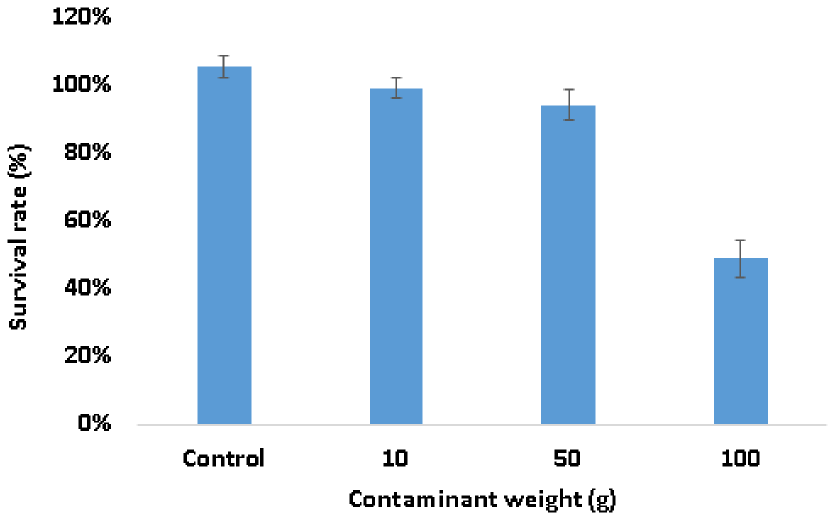

Figure 1. Survival of earthworm in different concetration of contaminant.

3.2. Effect of contaminant concentration

Pyrene was added at a dosage of 10–100 mg per 200 g of soil to containers containing 200 g of soil. As a control, 5 g of earthworms were introduced to uncontaminated water as a control. Meanwhile, 5 g of earthworms were put on polluted soil and cultured for 21 days. In Figure 2, the effect of contaminant concentration on the weight of earthworms is depicted. After 21 days of incubation, earthworms gained weight in the control condition but lost weight as the pollutant concentration increased. The earthworm's weight was reduced to 2.51 g in soil contaminated with 100 mg/L pyrene. It took 14 days for 10 mg of pyrene to grow in the lab, but the weight dropped after 14 days.

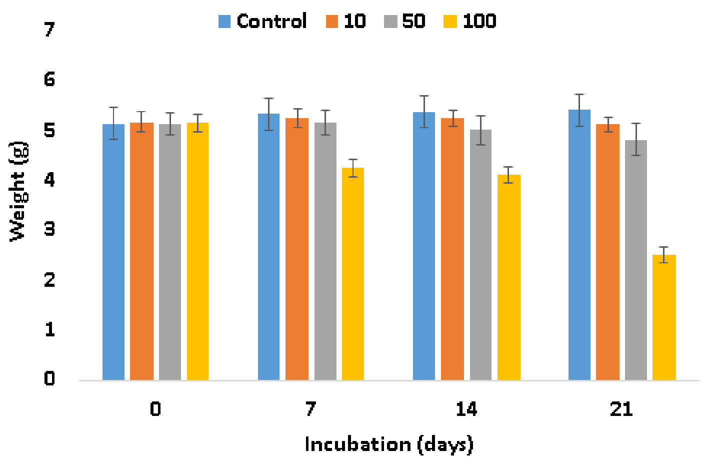

Figure 2. Effect of contaminant concentration on the weight of earthworms.

This occurs as a result of the earthworm adapting to a potentially detrimental condition or environment (the presence of pyrene) at the start of the study. Earthworm growth was suppressed in soil contaminated with 100 mg. Thus, soils with a higher initial pyrene concentration were found to be toxic to earthworms. Contaminants in the soil disrupt critical physiological activities of earthworms, including survival, feeding, immunity, growth, and reproduction, and these impacts vary according to the matrix, exposure period, and the types and levels of pollutants present in the environment [17,18].

3.3. Effect of earthworm and soil ratio

Earthworms were weighed at a weight of 5 g and placed in containers containing dirt in ratios of 1:1, 1:2, 1:4, and 1:8. A consistent moisture content of 60 was maintained in containers containing worms and put in a shady region. Figure 3 depicts the weight of earthworms. The weight of earthworms was decreased to 3.47 g, 4.92 g, and 4.82 g in soil with a ratio of 1:2, 1:3, and 1:4, respectively, but a ratio of 1:1 indicated that the earthworm was dead following incubation. The worm struggled to adjust to its new home and attempted to climb out of the flask. This occurs as a result of the absence of congestion, the absence of movement restrictions, and the worms' comfort in bigger and more crowded environments (Fig. 3).

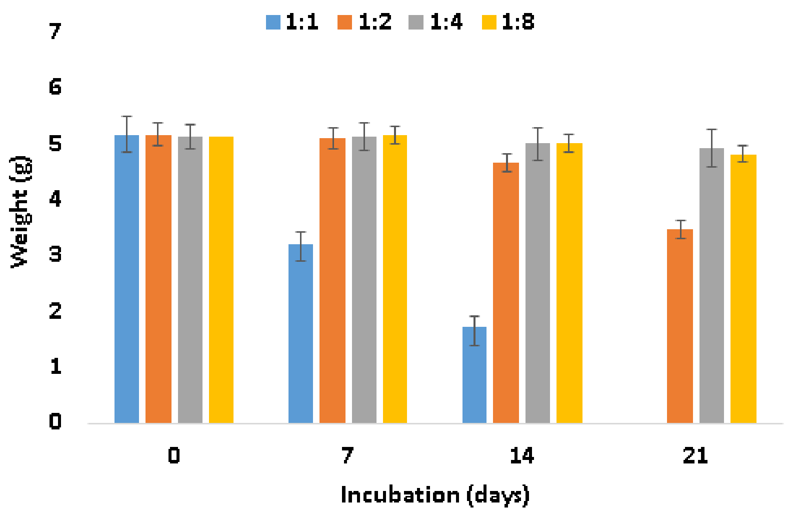

Figure 3. Effect of earthworm and soil ratio on the weight of earthworms.

3.4. Effect soil condition

Three different conditions of soil were utilized to cultivate the worms: sterilized dirt, unsterilized soil, and soil mixed with cow dung. Each medium was created with 100 mg of pyrene per 200g of soil. The results of pyrene elimination in soil by earthworms after 7, 14, and 21 days are shown in Figure 4. At a rate of 23.2 percent, pyrene was removed from unsterilized soil at a rate of 23.2 percent after 21 days of incubation, whereas 31.2 percent and 23.2 percent of pyrene were degraded in unsterilized soil and soil mixed with cow dung, respectively. Pyrene decomposition was inhibited during soil sterilization due to the absence of soil microorganisms and indigenous pyrene-degrading bacteria. Symbiotic relationships between bacteria and enzymes produced by earthworm bodies may play a critical role in the metabolism of pyrene but better breakdown takes more time. Earthworms enhance biological activity and substrate conditioning to act as a catalyst for the degradation process [19,20]. Earthworms accelerate pyrene breakdown in unsterilized soil by encouraging microbial activity and growth through the secretion of readily degradable carbon [21]. Earthworms boosted the bioavailability of pyrene and the breakdown of pollutants in unsterilized soil by rearranging soil particles and creating aerobic conditions in the soil through constant mixing. PAHs breakdown rate on its own or by stimulating bacteria or microorganisms already present in the soil by increasing aeration in polluted soil. Similar studies have been conducted to determine the effect of adding earthworms to the PAHs elimination procedure. In this regard, the degradation rate has significantly increased as compared to when only indigenous microorganisms are used [22]. When earthworms burrow and crawl in the soil, they enhance the soil structure by contracting their bodies. This provides the oxygen that microorganisms require to degrade the contaminants.

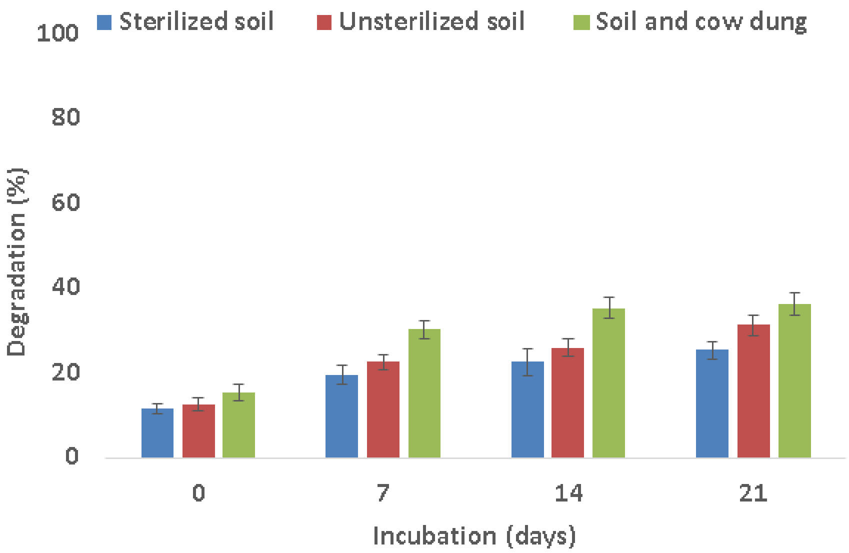

Figure 4. Effect of soil condition on the weight of earthworms.

4. Conclusion

Earthworms are extremely resilient and can survive for up to three weeks without nourishment. They are able to live and thrive in contaminated environments and exhibit a high tolerance for PAHs. The weight of earthworms was lowered to 3.47 g, 4.92 g, and 4.82 g in soil with a ratio of 1:2, 1:3, and 1:4. Pyrene decomposition was inhibited during soil sterilization due to the absence of soil microorganisms and indigenous pyrene-degrading bacteria. This research provides a framework for the exploration of earthworm as a PAH degrader. Furthermore, this method can be used in petroleum spillage cleanup under extreme conditions. As a whole, this study presents a potential environmental benefit and low-cost operations.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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