Int J Pharm Bio Sci 2014 July ; 5(3): (B) 449 - 455

Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

BIOREMEDIATION OF PAHS CONTAMINATED SOIL BY UTILIZING AN INDIGENOUS EARTHWORM SPECIES, *PERIONYX EXCAVATUS*

SUBASH, N* AND SASIKUMAR, C

P. G. & Research Department of Biotechnology, Nehru Memorial College (Autonomous), Puthanampatti - 621 007, Tiruchirappalli, Tamil Nadu. India.

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are insurmountable pollutant that contaminates the soil and occurs frequently. The restoring of contaminated soil fertility is pricey and time intense process. Earthworms accelerate the removal of PAHs from contaminated soil by feeding and get better the soil structure and aeration of the soil. The main aim of this research work is to utilize the earthworm *viz., Perionyx excavatus* for the taking away of PAHs from the soil and restoring the soil fertility. During this study, the mortality rate of *P. excavatus* was 2% which means *P. excavatus* can tolerate the PAHs compounds present in the experimental medium. The worm worked soil showed significant increase in total nitrogen, total phosphorus and total potassium and total magnesium than worm un-worked soil. It is obvious from the results that the earthworm *viz., Perionyx excavatus* may perhaps be used to remove the PAHs from soil and restore the soil fecundity through their excretory creation.

KEY WORDS: **Vermiremediation, PAHs, *Perionyx excavatus***

SUBASH, N
P. G. & Research Department of Biotechnology, Nehru Memorial College (Autonomous), Puthanampatti - 621 007, Tiruchirappalli, Tamil Nadu. India.

***Corresponding author**

INTRODUCTION

Vermiremediation is a low-cost, convenient technology for combating soil and land pollution. Earthworms in general are tolerant to many chemical contaminants in soil including heavy metals and organic pollutants and have been reported to bio-accumulate some of them in their tissues. Certain species of earthworms such as *Eisenia fetida, Aporrectodea tuberculata, Lumbricus terrestris, Lumbricus rubellus, Dendrobaena rubida, Dendrobaena veneta, Eiseniella tetraedra, Allobophora chlorotica,* and *P. excavatus* have been found to remove heavy metals, pesticides and lipophilic organic micropollutants like the polycyclic aromatic hydrocarbons (PAHs) from the soil[1,2]. are rich in NPK (nitrates, phosphates and potassium), micronutrients and beneficial soil microbes including the 'nitrogen fixers' and mycorrhizal fungus[3]. The developing nations rarely are farmland in free of persistent pesticides (e.g. aldrin, chlordane, dieldrin, endrin, heptachlor, mirex, toxaphene, etc.). According to National Environment Protection Council there are over 80,000 contaminated sites in Australia—30,000 in New South Wales, another 30,000 in Queensland, 10,000 in Victoria, 4,000 in South Australia, 4,000 in Western Australia, 1,000 in Northern Territory and 500 each in Tasmania and the Australian Capital Territory (ACT). There are 40,000 contaminated sites in the United States; 55,000 in just six European countries and 7,800 in New Zealand. However, some hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs), are more resistant to degradation and could, therefore, affect human health[4]. PAHs are ubiquitous, nonpolar, and highly hydrophobic and due to their affinity for fatty tissues tend to accumulate in food chains[5]. Although several hundred PAHs exist, most studies focus on a limited number of them, namely the 16 listed by the US Environmental Protection Agency and the European Community as pollutants[6, 7]. This suggests that adding earthworms to contaminated soil could increase the removal of the contaminant. The effects of earthworms on the removal of PAHs, which serve as a model for soil contaminated with petroleum or as a remediation test, have been reported by several authors[8, 9, 10, 11, 12 ,13].

Earthworms improve soil fertility

Vermiremediation aims to improve the soil fertility. Earthworms swallow large amount of soil every day, grind them in their gizzard and digest them in their intestine with aid of enzymes. Only 5–10% of the material ingested is absorbed into the body and the rest is excreted out in the form of fine mucus-coated granular aggregates called vermin castings, which are rich in nitrates, phosphates, potassium, micronutrients and beneficial soil microbes including the 'nitrogen fixers' and 'mycorrhizal fungus. The organic matter in the soil undergoes humification in the worm intestine in which the large organic particles are converted into a complex amorphous colloid containing 'phenolic' materials. About one-fourth of the organic matter is converted into humus. The colloidal humus acts as 'slow release fertilizer' in the soil[14]. The main objective of this paper is to review the use of earthworms in the remediation of PAH-contaminated soil. First, survival of earthworms in soil contaminated with PAHs will be discussed.

MATERIALS AND METHODS

Collection of Soil Samples

PAHs contaminated soil samples were collected from Automobile station at Thuraiyur. The sample was collected at a depth of 5cm from the surface of the soil. They were collected in sterile polythene bags and tightly packed and keep it for further use.

Collection of earthworms and Cow dung

The composting earthworm *i.e., P. excavatus* was collected from our college vermicomposting yard. The cow dung was also collected from nearby dairy yard of our college site.

Chemical

Fluorine (Hi media) was used as the sole carbon source for remediation of PAHs Polluted soil.

Experimental setup and preliminary vermiremediation study

PAHs contaminated soil was collected from the Automobile station at Thuraiyur. Total PAHs in site soil was greater than 11,820 mg/kg of soil [15]. The legislative standards for soil PAHs are only 100 mg/kg for industrial sites and 20 mg/kg for residential sites. Contaminated soil (1 kg) was collected and placed into trays to represent two different treatments. The first treatment of the first tray served as a control and no manipulation was done (1kg of fluorene contaminates soil + 1kg of cow dung + No worms was introduced). The second, third and fourth tray had approximately (1kg fluorene contaminated soil + 1kg of cow dung + 100 worms) added to the different trays. The Second treatment of the first tray served as a control and no manipulation was done (1kg of crude oil contaminates soil + 1kg of cow dung + No worms was introduced). The second, third and fourth tray had approximately (1kg crude oil contaminates soil + 1kg of cow dung + 100 worms) added to the different trays. The *Perionyx excavatus* varied in age and size. Moisture content of the two treatments was maintained between 70 and 80%. The test trays were incubated for 4 weeks at room temperature under thatched roof. Significant dilution of PAH compounds was expected and was taken into consideration while determining the impact of earthworm due to removal of PAH compounds.

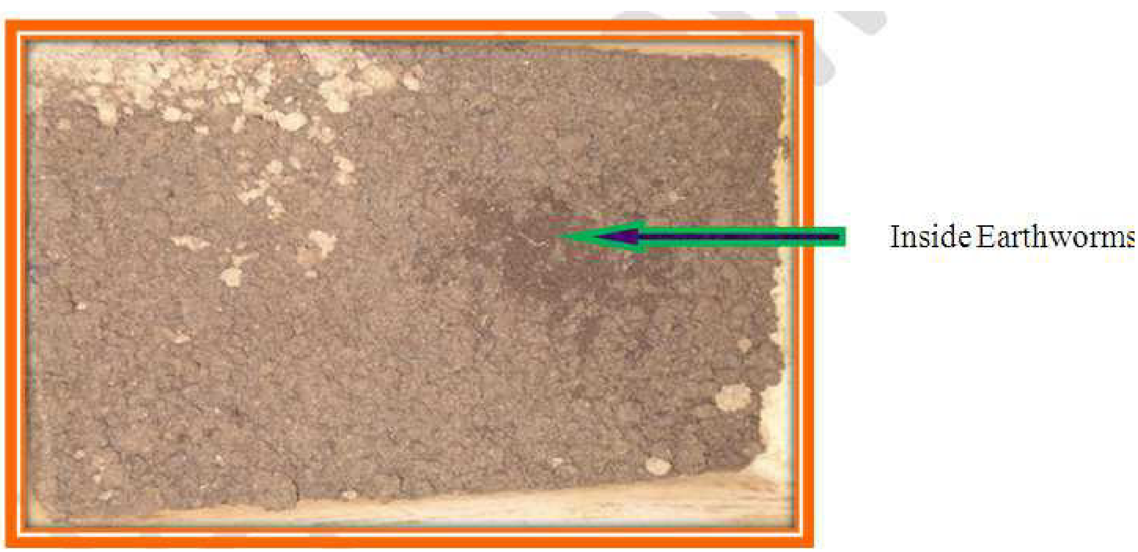

Figure. 1.
Earthworm inside the experimental trays

Laboratory Analysis

Just about 1 g of contaminated soil and vermicompost was analyzed for different physico – chemical parameters *viz.,* pH, electrical conductivity, moisture, organic carbon, total nitrogen, phosphorous, potassium, calcium, magnesium, sulphur, sodium and C: N ratio[16].

Figure. 2.
Worm worked and worm un-worked soil collected from experimental trays

Statistical Analysis
Paired sample *t*-test was also performed to evaluate significant difference between composting and vermicomposting for different chemical parameters deliberates.

RESULTS AND DISCUSSION

Propensity of composition of PAHs contaminated soil and cow dung (50:50) and its bioconversion into vermicompost by *P. excavatus* is presented in Table 1. The mean number of days required for bio-conversion was 49 (Thuraiyur oil contaminated soil), 28 (control), 19 (Fluorene contaminated soil) and 28 (Control). The average weight of vermicompost obtained after vermicomposting of PAHs contaminated soil was 1595g (Thuraiyur oil contaminated soil), 1275g (Control), 1430g (Fluorene contaminated soil) and 1385g (Control). The mean percent conversion of vermicompost was 80% (Thuraiyur oil contaminated soil) 64% (Control) 72% (Fluorene contaminated soil) and 69% (Control). (Table 1).

Table 1
Propensity of composition of PAHs contaminated soil and cow dung (50:50) and its bioconversion into vermicompost by P. excavates.

Particulars	Thuraiyur oil contaminated soil	Control	Fluorene contaminated soil	Control
Weight of PAHs contaminated soil (g)	1000	1000	1000	1000
Weight of cured cow dung (g)	1000	1000	1000	1000
Total weight mixture (g)	2000	2000	2000	2000
Number of adult worms introduced	50	-	50	-
Total weight of compost/vermicompost obtained (g)	1595	1275	1430	1385
Percent conversion of vermicompost	80	64	72	69
Number of days taken for conversion	14	28	19	28
Total number of adult earthworms observed	49	-	38	-

Each experiment was conducted in triplicates.

The pH of the vermicompost is near neutral value (6.43±0.41). Statistically, the vermicompost and compost did not show any significant difference. The worm worked substrate (vermicompost) had decreased electrical conductivity than worm un-worked substrate (Compost). The electrical conductivity did not show any significant difference between vermicompost and compost. Vermicompost samples did not show any significant difference inmoisture than control (p<0.001). Comparatively, vermicomposting process caused more reduction in Organic carbon than normal compost. However statistically, vermicomposting process showed significant difference for organic carbon than compost (p<0.001). A significant augment was noticed in Total Nitrogen content of the vermicompost than control. Iincrease in TN was significantly higher (*t*-test; *P* < 0.001 for all) in vermicomposted material than that of composted material.The Total Phosphorus content was significantly higher in vermicompost than compost (p<0.001). 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[17]. 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 micro flora. 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 [18]. Statistically vermicomposted materials showed significant difference for Total Potassium than composted material (p<0.001) as per paired sample T test. The concentration of Total Calcium and Total Magnesium was significantly higher in vermicompost when compared to control (p<0.001). But the total calcium did not any significant difference between vermicompost and compost. The difference between vermicomposted and composted material for Total Sodium was statistically significant (p<0.001). The total sulphur was significantly increased when compared to control. But the total sulphur did not show any significant difference between vermicompost and control (p<0.001).

Table 2
The magnitude of physico – chemical parameters of vermicompost prepared from PAHs contaminated soil by utilizing P. excavates.

S.No.	Parameters	Thuraiyur oil contaminated soil Mean ± S.D.	Control Mean ± S.D.	Fluorene contaminated soil Mean ± S.D.	Control Mean ± S.D.
1	pH	6.02 ± 0.02 NS	5.20 ± 0.30	6.43 ± 0.41	5.02 ± 0.02
2	Electrical conductivity (dSm^{-1})	0.70 ± 0.05	1.03 ± 0.04	0.81 ± 0.02 NS	0.62 ± 0.01
3	Moisture (%)	19.50 ± 0.10 NS	11.03 ± 0.03	25.5 ± 0.04 NS	10.23 ± 0.05
4	Organic carbon (%)	21.15 ± 0.01	38.02 ± 0.02	29.4 ± 0.44	36.24 ± 0.03
5	Total Nitrogen (%)	0.95 ± 0.03	0.52 ± 0.01	1.02 ± 0.02	0.64 ± 0.01
6	Total phosphorous (%)	0.93 ± 0.01	0.42 ± 0.01	0.65 ± 0.04	0.36 ± 0.02
7	Total potassium (%)	1.02 ± 0.02	0.12 ± 0.01	1.25 ± 0.05	0.23 ± 0.02
8	Total calcium (%)	2.33 ± 0.02 NS	1.02 ± 0.02	2.91 ± 0.02	1.35 ± 0.01
9	Total magnesium (%)	1.05 ± 0.03	0.97 ± 0.02	1.24 ± 0.03	1.02 ± 0.02
10	Total sulphur (%)	0.25 ± 0.04	0.13 ± 0.03	0.39 ± 0.07 NS	0.11 ± 0.03
11	Total sodium (%)	0.73 ± 0.02	0.43 ± 0.02	1.14 ± 0.11 NS	0.87 ± 0.02
12	C:N Ratio	21:1	71:1	28:1	55:1

Each experiment was done in triplicates. NS- Non significance
The mean and standard deviation values were obtained from three individual observations;
****The difference between control and vermicompost was statistically significant (p<0.01 level by Paired sample T test).***

The difference between vermicomposted and composted materials for C:N ratio was significant (t test p<0.001). It is clear that earthworms can accelerate the removal of PAHs from soil. They affect the soil in different ways, *e.g.* mixing and aeration, improving soil structure and increasing microbial activity thereby stimulating the removal of PAHs [19,20,21,22, 23]. The addition of earthworms to soil can stimulate degradation of hydrocarbons in different ways. Indirectly, the secretion of mucus by the earthworm might stimulate the growth of hydrocarbon degrading microorganisms. Furthermore, beneficial microbes, such as nitrogen-fixing bacteria, might have accumulated in the worm casts. At the same time, earthworm burrowing improves aeration, mixes the soil and increases the soil surface area for microbial interactions, can inhibit soil-borne pathogens, and neutralize pH [24, 25].

CONCLUSION

Vermiremediation may prove a very cost-effective and environmentally sustainable way to remedy polluted soils and contaminated sites within just few weeks or months, and also vermiremediation leads to significant improvement of soil fertility. During the vermiremediation process of soil, the population of earthworm's increases significantly benefits the soil in quite a lot of ways. Further studies required to test the capability of *P. excavatus* on-site environmental circumstance.

ACKNOWLEDGEMENT

The Authors thanks to the Management and Principal of our college for the facilities extended for work done this research. We thank the PG & Research Department of Biotechnology and Department of Zoology Staff members of Nehru Memorial College (Autonomous) for their help and Special thank to Dr. P. Neelanarayanan Associate professor, Department of Zoology Nehru Memorial College (Autonomous) Puthanampatti – 621007 for encouraging and support and Mrs. J. Viji, Research scholar for helping to Physicochemical analysis studies. We wish to thanks to Mr. S. Ramachandran, Mrs. Kamatchi and Mrs. A. Uma for the various way help during this study.

REFERENCES

1. Contreras-Ramos Silvia, M., Alvarez-Bernal, Dioselina and Dendooven, Luc. 2006. Eisenia fetida increased removal of polycyclic aromatic hydrocarbons (PAHs) from soil. *Environ Pollut* ,141:396–401.
2. Contreras-Ramos S, M., Alvarez-Bernal, D and Dendooven, L. 2008. Removal of polycyclic aromatic hydrocarbons from soil amended with biosolid or vermicompost in the presence of earthworms (Eisenia fetida). *Soil Biol. Biochem*, 40: 1954–1959.
3. Chaoui, H. I., Zibilske, L. M and Ohno, T. 2003. Effects of earthworms casts and compost on soil microbial activity and plant nutrient availability. Soil Biol Biochem 35: 295–302.
4. Cerniglia, C. 1993. Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3: 351–368.
5. Van der Oost, R., Beyer, J and Vermeulen, N.P.E. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13:57–149.
6. Samanta, S.K., Chakraborti, A.K and Jain, R.K. 1999. Degradation of phenanthrene by different bacterial: Evidence for novel transformation sequences involving the formation of 1-naphtol. Appl. Microbiol. Biotechnol. 53: 98–107.
7. Puglisi, E., Cappa, F., Fragoulis, G., Trevisan, M and Del Re, A.A.M. 2007. Bioavailability and degradation of

phenanthrene in compost amended soil. Chemosphere 67: 548–556.
8. Ma, W.C., Immerzeel, J and Bodt, J. 1995. Earthworms and food interactions on bioaccumulation and disappearance in soil of polycyclic aromatic hydrocarbons: studies on phenanthrene and fluoranthene. Ecotoxicol. Environ. Saf. 32: 226–232.
9. Eijsackers, H., van Gestel, C., De Jonge, S., Muijs, B and Slijkerman, D. 2001. Polycyclic Aromatic hydrocarbons-polluted dredged peat sediments and earthworms: a mutual Interference. Ecotoxicology 10: 35–50.
10. Contreras-Ramos S. M., Alvarez-Bernal, D and Dendooven, L. 2008. Removal of polycyclic aromatic hydrocarbons from soil amended with biosolid or vermicompost in the presence of earthworms (Eisenia fetida). *Soil Biol. Biochem*, 40:1954–1959.
11. Geissen, V., Gomez-Rivera, P., Lwanga, E., Mendoza, R., Narcias, A.T and Marcias, E.B. 2008. Using earthworms to test the efficiency of remediation of oil-polluted soil in tropical Mexico. Ecotoxicol. Environ. Saf. 71: 638–642.
12. Hickman, Z.A., Swindell, A.L., Allan, I.J., Rhodes, A.H., Hare, R., Semple, K.T and Reid, B.J. 2008. Assessing biodegradation potential of PAHs in complex multicontaminant matrices. Environ. Pollut. 156: 1041–1045.
13. Tejada, M and Masciandaro, G. 2011. Application of organic wastes on a benzo(a)pyrene polluted soil. Response of soil biochemical properties and role of Eisenia fetida. Ecotoxicol. Environ. Saf. 74: 668–674.
14. Butt, K. R.1999. Inoculation of earthworms into reclaimed soils: the UK experience. J Land Degrad Dev 10:565–575
15. Ryan, D. 2006. Vermiremediation and other bioremediation options for PAHs contaminated soil; Project report submitted for the partial fulfillment of the degree of Bachelor of Environmental Engineering, School of Engineering, Griffith University, Brisbane (Supervisor: Dr. Rajiv Sinha)
16. Tandon, H. 2005. Methods of analysis of soils, plants, water, fertilizers and organic manures. *Fertilizer Development and Consultation Organization*. *Pamposh Enclave*, New Delhi, India pp: 224
17. Tripathi, G and Bhardwaj, P. 2004. “Comparative studies on biomass production, life cycles and composting efficiency of *Eisenia foetida* (Savigny) and *Lampito mauritii* (Kinberg),” *Bioresource Technology* vol. 92: 275-278.
18. Schaefer, M., Petersen, S. O and Filser, J. 2005. “Effects of *Lumbricus terrestris*, *Allolobophora chlorotica* and *Eisenia foetida* on microbial community dynamics in oil-contaminated soil,” *Soil Biology & Biochemistry*, vol. 37: 2065-2076,
19. Martin, A., Cortez, J., Barois, I and Lavelle, P. 1987. Les mucus intestinaux de ver deterre moteur de leurs interactions avec la microflore. Rev. *Ecol. Biol. Sol*. 24; 549–558.
20. Safawat, H.S.H and Weaver, R.W. 2002. Earthworms survival in oil contaminated soil. *Plant Soil* 240 : 127–132.
21. Singleton, D.R., Hendrix, P.F., Coleman, D.C and Whitman, W.B. 2003. Identification of Uncultured bacteria tightly associated with the intestine of the earthworm Lumbricus rubellus (Lumbricidae; Oligochaeta). Soil Biol. *Biochem*. 35: 1547–1555.
22. Drake, H.L and Horn, M.A. 2007. As the worm turns: the earthworm gut as a transient habitat for soil microbial biomes. Annu. Rev. *Microbiol*. 61:169–189.
23. Lapied, E., Nahmani, J and Rousseau, G.X. 2009. Influence of texture and amendments on soil properties and earthworm communities. *Appl. Soil Ecol*. 43: 241–249.
24. Edwards, C.A and Bohlen, C.J. 1996. Biology and Ecology of Earthworms. Chapman & Hall, London.
25. Schaefer, M and Filser, J. 2007. The influence of earthworms and organic additives on the biodegradation of oil contaminated soil. *Appl. Soil Ecol*. 36: 53–62.