JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Nature's Cleanup Crew: Exploring the contribution of *Perionyx excavatus* species for microplastic degradation

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Abstract
Microplastic pollution has become a pervasive environmental issue, with its detrimental effects on ecosystems and human health garnering increasing attention. This research delves into the potential of earthworm species as a natural solution for mitigating microplastic pollution. The epigeic species *Perionyx excavatus*, commonly referred to as the blue worm, were investigated. The study revealed that these earthworms could ingest LDPE microplastics present in soil, leading to their degradation within a relatively short span of 20-25 days, while exhibiting a remarkable survival rate with 0% mortality. Structural and morphological changes in the microplastics were detected through SEM images, accompanied by approximately 40% weight loss. Moreover, alterations in functional groups were observed via FTIR analysis. Despite their significant potential, the interaction of earthworm species with microplastics in soil ecosystems has been underexplored, prompting further investigation into their capacity to ingest, degrade, and redistribute microplastics within these environments.

Keyword: Bioremediation; Earthworm; Microplastic; Pollution

1.Introduction

Microplastic pollution has emerged as a significant environmental concern, posing threats to terrestrial and aquatic ecosystems worldwide (Kumari et al., 2022). These minute plastic particles, often measuring less than 5 millimeters in diameter, originate from various sources, including the degradation of larger plastic items, industrial processes, and the fragmentation of plastic debris (Caldwell et al., 2022). Despite their small size, microplastics have profound ecological consequences, impacting biodiversity, soil health, and human well-being (Chukwuemeka et al., 2024). The pervasive nature of microplastic pollution is underscored by its ubiquity in diverse environments, ranging from remote wilderness areas to densely populated urban centers (Talbot and Chang, 2022). Soil ecosystems, in particular, serve as a reservoir for microplastics, with accumulating evidence suggesting widespread contamination across agricultural, urban, and natural landscapes (Zhou et al., 2020). The deposition of microplastics in soil occurs through various pathways, including atmospheric deposition, runoff from contaminated surfaces, and the application of plastic-based mulches and amendments in agricultural practices (Campanale et al., 2022). Once introduced into soil environments, microplastics interact with soil biota and biogeochemical processes, influencing nutrient cycling, microbial communities, and plant-microbe interactions (Zhai et al., 2024).
Among soil organisms, earthworms occupy a central ecological role due to their profound influence on soil structure, nutrient dynamics, and organic matter decomposition. India is one of the nations with a mega diversity of earthworms (Narayanan et al., 2023). Earthworms play a crucial role in enhancing soil health by increasing microbial activity and facilitating nutrient cycling through their burrowing and feeding activities (Kiyasudeen et al., 2015). This heightened microbial activity, in turn, can expedite the breakdown of organic matter, including plastics (Amobonye et al., 2021). Additionally, earthworms are known to alter the physical

and chemical properties of soil (Asawalam and Johnson, 2007), which can have implications for the distribution and fate of microplastics. For instance, the burrows created by earthworms may serve as pathways for microplastics to migrate deeper into the soil profile, where different degradation processes may take place (Yu et al., 2019). These intricate interactions underscore the complexity of the relationship between earthworms and microplastics in soil ecosystems, emphasizing the necessity of adopting holistic approaches to address environmental challenges (Zeb et al., 2023).

These ecosystem engineers play a vital role in soil health and fertility through their burrowing activities, which enhance soil aeration and water infiltration, and their ingestion of organic matter, which facilitates nutrient cycling and soil enrichment (Le Bayon et al., 2021). Despite their ecological significance, the interaction between earthworms and microplastics remains poorly understood (Helmberger et al., 2020). While numerous studies have investigated the effects of microplastics on aquatic organisms, relatively few have focused on terrestrial species, particularly soil-dwelling organisms such as earthworms (Büks et al., 2020). Understanding how earthworms interact with microplastics is essential for elucidating their potential role in microplastic remediation and informing strategies for mitigating microplastic pollution in soil ecosystems (Thapliyal et al., 2024).

The aim of this study is to explore the contribution of earthworm species to microplastic remediation in soil environments. *Perionyx excavatus*, an epigeic earthworm species, was studied to assess its efficacy in degrading LDPE microplastics, addressing environmental concerns. By investigating the interactions between earthworms and microplastic, we seek to elucidate the mechanisms underlying earthworm-mediated microplastic degradation, ingestion, and transport. Through a combination of laboratory experiments and field observations, we aim to assess the efficacy of earthworms as a natural cleanup crew for microplastic pollution and explore the potential implications for soil health and ecosystem functioning. The research aimed to elucidate the role of these earthworms in mitigating plastic pollution, offering insights into sustainable waste management strategies amid growing environmental challenges.

2.Material and Method

2.1 Sampling

Earthworms were collected from the sampling site Bhota (31.6098° N, 76.5676° E) of Hamirpur district of the Himachal Pradesh as shown in **Fig.1**.

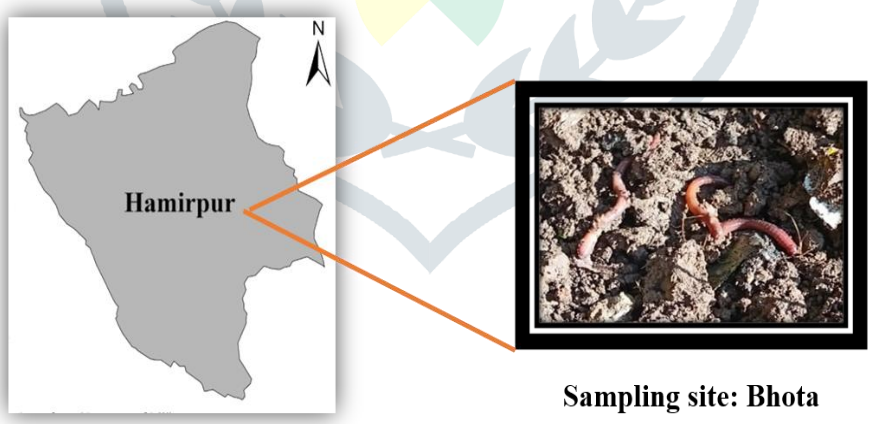

Figure 1: Sampling site

2.2 Identification

Taxonomic keys were used for the identification of collected earthworm species. identified as *Perionyx excavatus* (Perrier, 1872)

2.3 Culturing

Cultivating earthworms entails establishing a conducive habitat for their flourishing. The bedding should be sufficiently moist without being waterlogged, maintaining moisture levels and temperatures ideally between 60-80°F (15-27°C), which are preferred by the earthworms. Additionally, it's essential to consistently introduce food scraps and bedding materials to sustain the colony and deter overcrowding. The steps like sampling, identification and culturing have been represented in **Fig.2**.

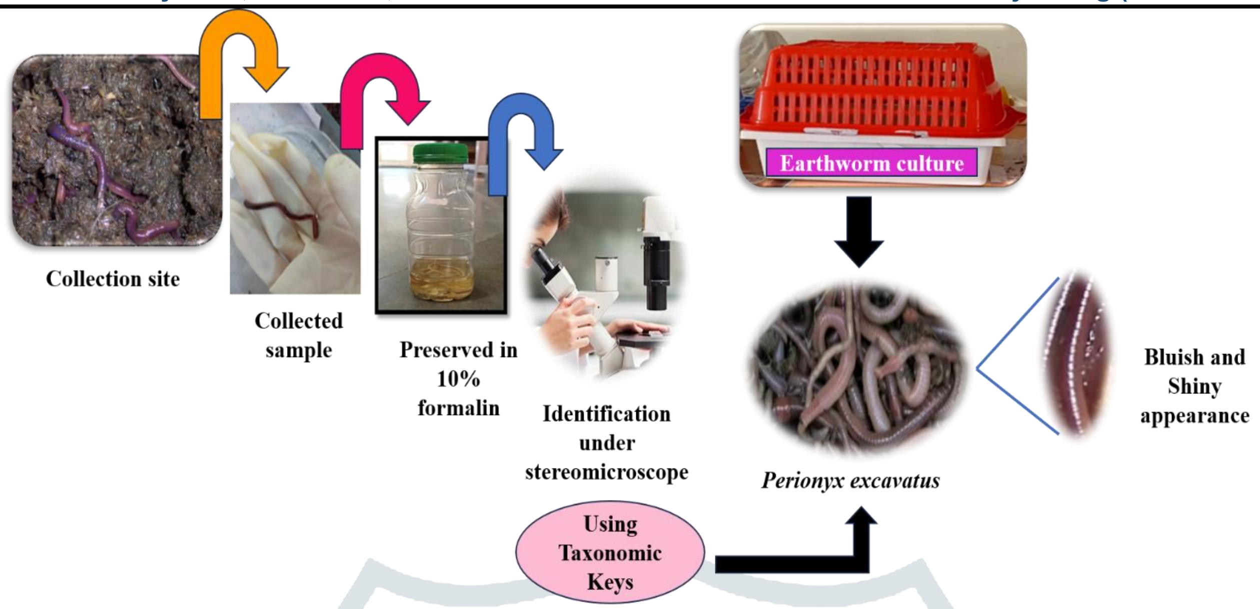

Figure 2: Representation of sampling, identification and culturing

2.4 Experimentation

Different concentrations of low-density polyethylene microplastics, as detailed in **Table 1**, were added to each tray. Earthworms from the culture trays were introduced into each experimental tray containing varying concentration microplastics. These earthworms were exposed to the microplastic concentrations for a duration of 21 days, after which observations were recorded upon completion of the experimental trial. The process that was followed in experimental setup has been represented in the **Fig.3**.

2.4.1 Soil Preparation

The soil used in the experiment underwent thorough sieving to eliminate any pebbles before being dried and weighed. The soil was properly sterilized and air dried in order to remove undesired entities.

2.4.2 Mixing of LDPE microplastic into the soil

Varying microplastic concentrations were mixed into soil present in the experimental trays. Setup A was kept as a control and no earthworm species were added into it whereas 0.5 and 0.7% microplastic concentration was mixed into Setup B and Setup C respectively.

2.4.3 Introduction of *Perionyx excavatus* into the experimental tray

The cultured *Perionyx excavatus* species were then added into the experimental tray containing prepared soil. Along with that the moisture and pH was also maintained at optimum level. The species were made to feed on microplastics for 21 days.

Table 1: Composition of the experimental tray

Experimental tray	Composition of the experimental tray		
	Soil	LDPE Concentration	Number of earthworm species (n)
A (Control)	500gm	0.5 mg	0
B	500gm	0.5 mg	5
C	500gm	0.7 mg	5

2.4.4 Isolation of microplastic from the earthworm species

After 21 days, the earthworm species were removed from the soil. With the help of tissue paper, they were made to vomit and microplastic was collected from their castings and secretions. Even on dissection, microplastic presence was observed inside the earthworm gut depicting their ingestion.

2.4.5 Isolation of microplastic from the soil

The soil used in the experiment was taken in a beaker and distilled water was mixed into it. It was stirred and left undisturbed for 1-2 hours. Soil settled down whereas the light weighted and insoluble microplastic stated floating on the top. It was then filtered by using Whatman filter paper.

2.4.6 Test sample

After the experiment, all the obtained microplastic sample was washed using ethanol in order to remove the impurities and then it was dried in vacuum oven. Further the sample was taken and subjected to microscopic analysis.

Figure 3: Experimental Setup

3.Result and Discussion

Characterization of the obtained microplastic sample was done to check the degradation and comparison was made with the pure microplastic which has not been subjected for the experiment.

3.1 Weight loss

The initial and final weight of the microplastic showed variation and thereby weight loss was reported. Weight loss percentage was calculated for each experimental tray, by using the given formula.

$$\text{Weight Loss Percentage} = \frac{(\text{Initial Weight} - \text{Final Weight})}{\text{Initial Weight}} \times 100\%$$

The weight loss percentage in LDPE microplastic in case of *Perionyx excavatus* earthworm species has been represented in **Table 2**.

Table 2: The weight loss percentage in LDPE was calculated in case of *Perionyx excavatus* earthworm species

S. No.	Experimental trays subjected with different microplastic concentration	Initial weight of microplastic mixed in soil (W_1)	Final weight of microplastic obtained after the experiment (W_2)	Weight Loss Percentage ($\frac{W1-W2}{W1} \times$ 100)	Mean	Squared difference	Mean of squared differences	Standard Deviation
1.	Setup A (Soil+ microplastic)	0.5	0.5	0%		711.4889		
2.	Setup B (Soil+							

	microplastic + *Perionyx excavatus*)	0.5	0.30	40%	26.67%	177.4889	355.769	18.86%
3.	Setup C (Soil+ microplastic + *Perionyx excavatus*)	0.7	0.42	40%		177.4889		

3.3 FTIR analysis

FTIR (Fourier Transform Infrared Spectroscopy) is useful technique for studying microplastic degradation. It detects changes in the chemical structure of plastics that have occurred due to degradation. As microplastics degrade, the intensity of certain absorption bands may change or new bands associated with degradation byproducts appear. Setup A shows no degradation and no change in the graph whereas in case of Setup B and Setup C the FTIR graph conformation shows alterations that have occurred due to degradation by *Perionyx excavatus* species of earthworm as shown in the **Fig.4**.

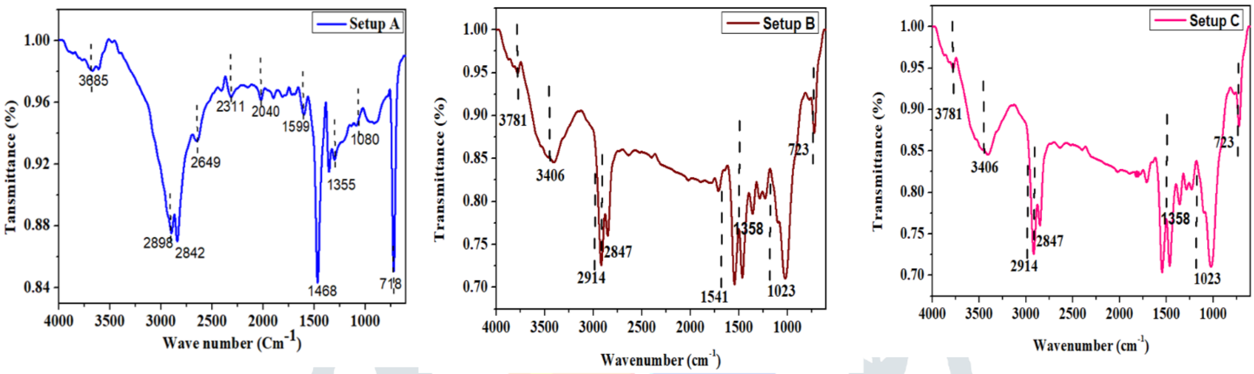

Figure 4: FTIR analysis of microplastic sample

3.4 SEM analysis

Scanning electron microscopy provides valuable insights about the physical changes occurring in LDPE microplastics due to earthworm degradation, helping in understanding the environmental fate of plastic pollution. Rough surface having cracks and deteriorated morphology was detected in the microplastic sample obtained in case of Setup B and Setup C whereas no such change was detected in case of microplastic in case of Setup A where earthworms were absent. This transformation in the morphology of the microplastic is evidence for degradation conformation as shown in the **Fig.5**.

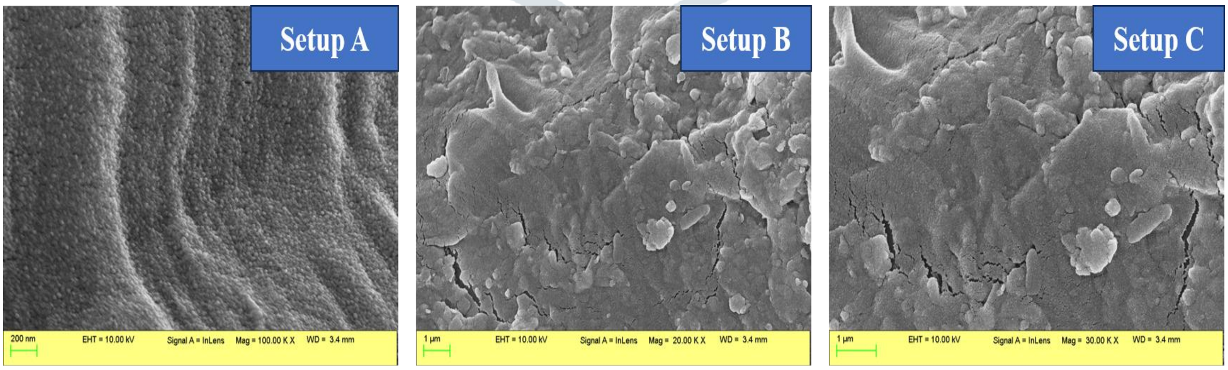

Figure 5: SEM images of Setup A, B and C

Perionyx excavatus species of earthworm grow better between 75% to 80% moisture levels (Halatt et al., 1992). The earthworm species is rich with nutrients and its gut is capable for the colonization of facultative anaerobic bacteria that tends to establish a relationship with its adjacent terrestrial environment (Edward and Arancon, 2022). The earthworm species also have potential role in pollutant detoxification enabling biodegradation, absorption, transformation and bioaccumulation (Zeb et al., 2020). In current study it was observed that *Perionyx excavatus* has potential for microplastic degradation. The comparison can be made between Setup A, B and C. The difference in the initial and final weight of microplastic gives out the weight loss percentage. Weight decreased in case of Setup B and C which were subjected to the *P. excavatus* earthworm species for about 21 days. Alterations were detected in case functional groups and peaks analyzed from FTIR which shows that degradation process has taken place (Rajandas et al., 2012). The characteristic absorption bands in the infrared spectrum showed variation in different setups as shown in the **Fig.4**. The Low-density polyethylene constitutes of hydrogen and carbon bonding. The breakage in the LDPE bonds such as C-C and formation of carbonyl groups leads to decrease in the molecular weight of the polymer which makes it capable of degradation (Sen and Raut, 2015). Indication of aldehyde and carbonyl stretching via FTIR was also depicted that confirms the degradation process (Campanale et al., 2023). Such changes in the functional groups lead to change in the surface morphology of the microplastic which was depicted through the SEM images as shown in the **Fig.5**. Understanding of morphological changes complements chemical analyses and environmental assessments, contributing to a more holistic evaluation of earthworm-mediated degradation processes and their implications for plastic pollution remediation (Sanchez-Hernandez et al., 2020). Therefore, the present research underscores the potential of the earthworm species *Perionyx excavatus* in degrading LDPE microplastics, advocating for a nuanced approach to leveraging their abilities in waste management practices.

4. Conclusion

The current study underscores the important role of earthworms as a natural mechanism for microplastic remediation in soil ecosystems. Integrating *Perionyx excavatus* species of earthworm into sustainable soil management practices offers a promising avenue for combating this pervasive environmental challenge and promoting ecosystem resilience in the face of anthropogenic pressures. This species exhibits the capability to degrade LDPE microplastics, evidenced by a 40% weight reduction, changes in functional groups via FTIR analysis, and morphological alterations confirmed through Scanning Electron Microscopy. By enhancing our understanding of earthworm-microplastic interactions, we can develop targeted strategies to harness their remediation potential and mitigate microplastic pollution effectively.

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Credit authorship contribution statement

All authors have contributed equally.

Declaration of competing interest

There are no competing interests or conflicts between the authors.

Acknowledgment

The authors acknowledge the facilities and support provided by the Career Point University, Hamirpur.