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Screening the LDPE microplastic degradation potential of the epigeic earthworm species reported from the district Hamirpur of Himachal Pradesh

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Abstract

Microplastic pollution has emerged as an issue of global environmental concern, posing significant threats to ecosystem as well as human health. Earthworm species belong to phylum Annelida and are known to act as an essential tool for bioremediation. They act as vital components of soil ecosystems, play a crucial role in decomposition and soil health maintenance. They have potential to degrade organic and inorganic waste to much extent which help in compacting the soil pollution. This research investigates the microplastic degradation potential of epigeic earthworm species *Bimastos parvas*, *Perionyx excavatus* and *Eisenia fetida* respectively. It contributes to the broader understanding of the ecological roles of earthworm species in combating microplastic pollution and underscores the importance of considering soil fauna as a beneficial entity for bioremediation strategies with context to the waste management and soil health.

Keywords: Microplastic; Degradation; Earthworms; Soil; Epigeic species; Pollution; Ecosystem

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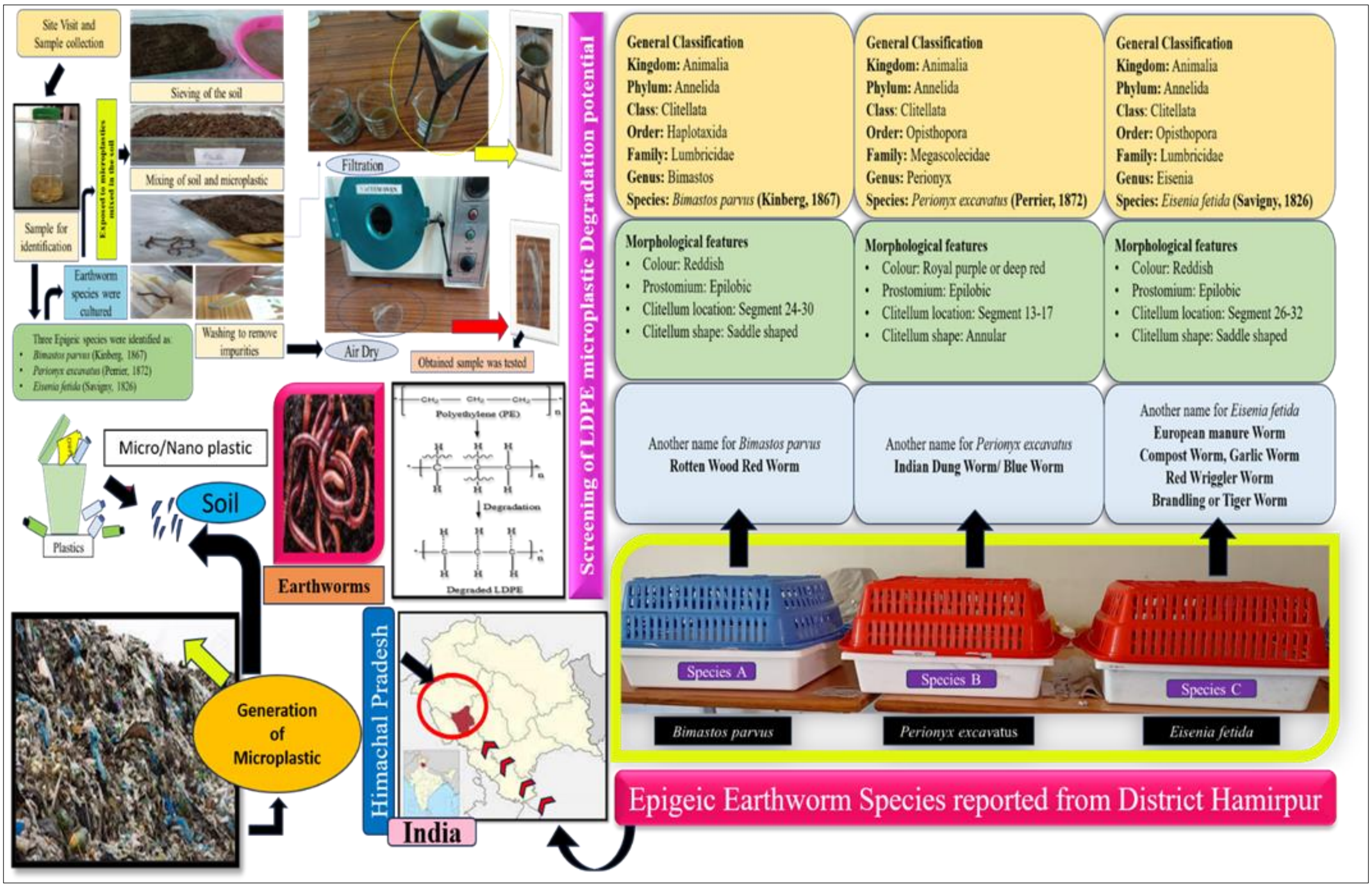
Graphical abstract



1. Introduction

Microplastics are defined as plastic particles less than five millimeters in size, have become a ubiquitous environmental pollutant posing a significant threat to terrestrial and aquatic ecosystems worldwide [1]. These minute plastic particles originate from various sources including the breakdown of larger plastic debris, microbeads in personal care products, and fibers from synthetic textiles [2]. Despite their small size, microplastics have been found to accumulate in soils, water bodies, and organisms, raising concerns about their ecological impacts and potential threats to human health [3]. The widespread distribution of microplastics in terrestrial ecosystems has raised concerns about their impacts on soil health, biodiversity, and ecosystem functioning [4].

Soil, being a vital component of terrestrial ecosystems, serves as a sink for microplastics, with studies indicating their presence in various soil types, ranging from agricultural lands to urban areas [5]. Once deposited in soils, microplastics can interact with soil organisms and undergo physical, chemical, and biological transformations, influencing their fate and environmental behavior [6]. In recent years, there has been a growing interest in understanding the role of soil organisms, particularly earthworms, in the degradation and dispersion of microplastics in terrestrial environments [7].

Earthworms belong to Phylum Annelida of Kingdom Animalia and are classified into three types epigamic, endogeic and anecic [8]. Earthworms are one of the most common segmented worms and they play a vital role in the functioning of the soil ecosystem. Their diversity and distribution fluctuate concerning edaphic factors [9]. Earthworms, as key soil-dwelling organisms, play crucial roles in soil ecosystem processes such as nutrient cycling, organic matter decomposition, and soil structure enhancement [10]. Their burrowing activities and feeding behaviors facilitate the mixing and turnover of soil particles, leading to the incorporation of organic matter into the soil profile [11].

Implementing pre-treatments on plastics prior to their interaction with earthworms, such as industrial composting, is widely advocated for industrial waste management. This practice sets the stage for favorable conditions conducive to plastic degradation. While it may diminish the tensile strength of plastic materials, rendering them more prone to breakdown, it presents a feasible approach to addressing plastic pollution. Earthworms have a remarkable ability to act like natural grinders, effectively resizing microplastics as they pass through their digestive tract, which features ample surface area ideal for microbial and enzymatic activities. Furthermore, they excel at breaking down complex minerals and organic matter, providing an environment for the microbial action, which could hasten the degradation of microplastics into simpler components, potentially shortening degradation times.

Consequently, earthworms are recognized as potential agents for the degradation and dispersion of microplastics in soils [12]. The interactions between earthworms and microplastics have garnered increasing attention in recent years, with studies exploring the ingestion, egestion, and degradation of microplastics by different earthworm species [13]. Epigeic earthworms, which inhabit the top layer of soil and primarily feed on organic matter deposited on the soil surface, have been of particular interest due to their potential interactions with microplastics present in soil and litter layers [14]. These earthworms are known to ingest soil particles as they feed, thereby inadvertently consuming microplastics present in the soil matrix [15].

The district of Hamirpur, situated in the state of Himachal Pradesh, India, stands out for its diverse landscapes, encompassing the Himalayan mountains, foothills, and plains [16]. Characterized by a blend of agricultural lands, forests, and human settlements, Hamirpur's ecological diversity is coupled with its proximity to urban centers and industrial activities, rendering it susceptible to environmental pollutants, including microplastics. In this region, epigeic earthworm species, which reside in the top layer of soil and feed on organic matter deposited on the soil surface, are prevalent [17]. Despite the ecological significance of earthworms and the potential implications of microplastic pollution in terrestrial ecosystems, limited research has been conducted to assess the microplastic degradation potential of epigeic earthworm species in regions like Hamirpur. Understanding the interactions between earthworms and microplastics in such contexts is essential for evaluating the capacity of soil organisms to mitigate microplastic pollution and maintain soil health [18].

This study aims to address the knowledge gap by investigating the microplastic degradation potential of epigeic earthworm species reported from the district of Hamirpur, Himachal Pradesh. It reveals the potential to elucidate the capacity of these earthworm species to ingest and degrade microplastics under controlled conditions. Understanding the role of epigeic earthworms in the degradation of microplastics is essential for assessing their potential contribution to mitigating microplastic pollution in terrestrial ecosystems.

2. Materials and Methods

2.1. Sampling

Epigeic earthworm species were collected from different regions of the Hamirpur district. Species 1 was procured from the Badsar region, Species 2 was procured from the Bhota whereas Species 3 from collected from the Bhoranj region.

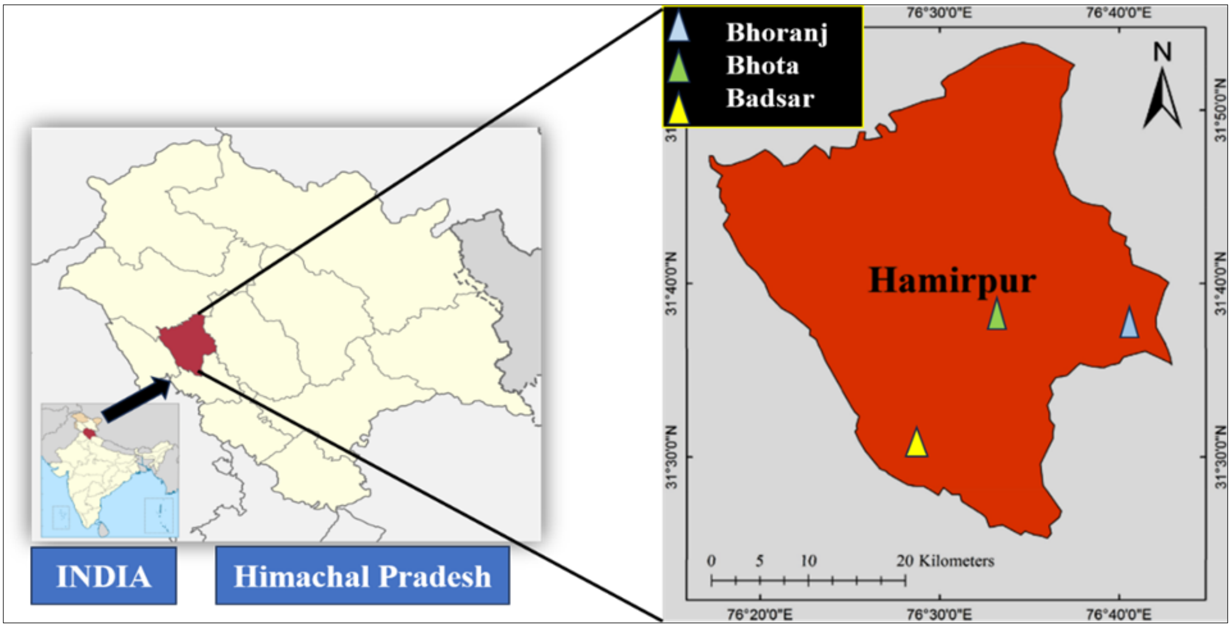


Figure 1 Earthworm sampling sites from Hamirpur district of Himachal Pradesh

2.2. Identification

Taxonomic keys were used for the identification of collected earthworm species. The Species 1 was identified as *Bimastos parvas* (Kinberg 1867), Species 2 was identified as *Perionyx excavatus* (Perrier, 1872) whereas Species 3 was identified as *Eisenia fetida* (Savigny 1826).

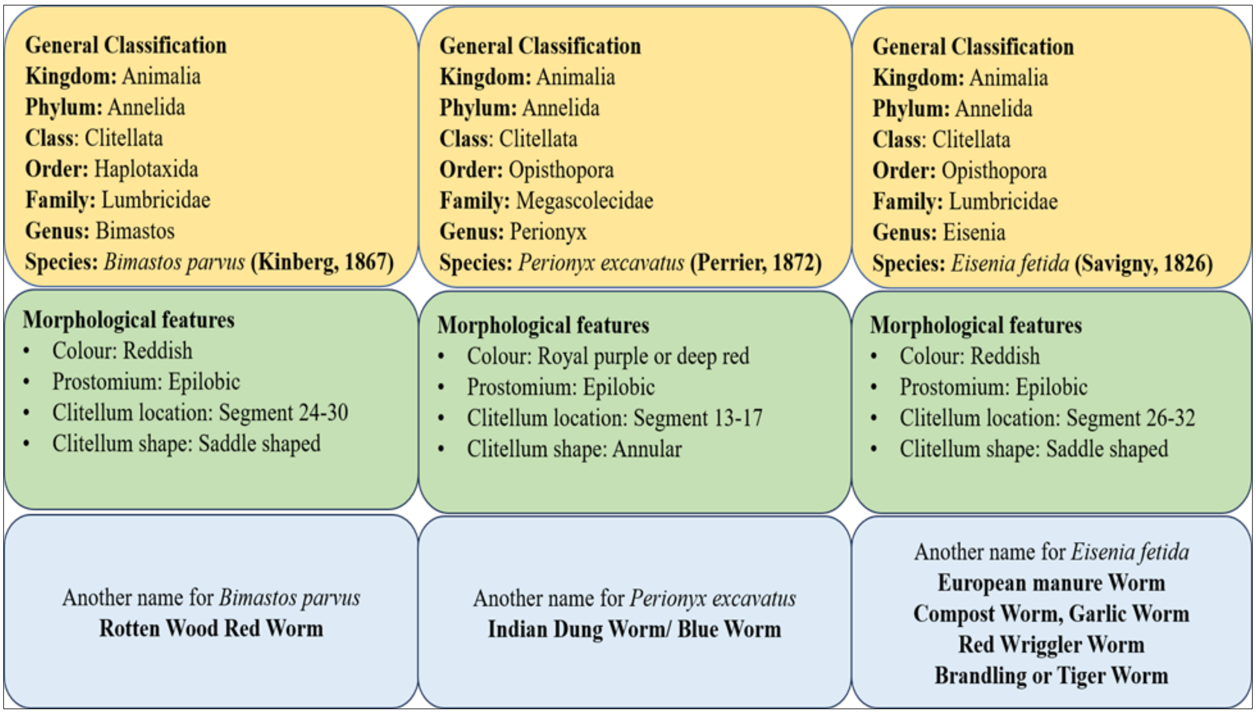


Figure 2 General classification and morphological features of epigeic earthworm species

2.3. Culturing

The epigeic species of earthworm were cultured in the trays. The conditions such as pH, moisture and temperature were maintained. Cow dung and banana peels were used as a feed to earthworm cultures from time to time. The culture trays have been represented in the **Figure 3**. All the three epigeic species were cultured separately in order to avoid mixing.

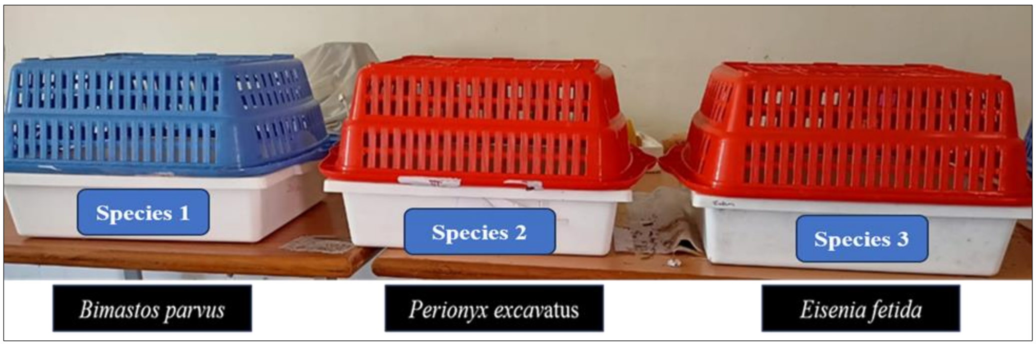


Figure 3 Culturing of different epigeic species under normal conditions inside the laboratory

2.4. Experiment

Fresh soil was procured from the garden and pebbles were removed from it. It was sieved, dried and then sterilized in a vacuum oven in order to remove the impurities or undesirable entities. After that different microplastic concentrations were mixed into it. Different epigeic earthworm species namely Sp1, Sp2 and Sp3 were exposed to the soil containing microplastics for about 21 days. After 21 days, the worms were taken out and were made to vomit on the tissue paper. The secretions were containing microplastic detecting the ingestion of microplastic in case of all three epigeic earthworm species.

The leftover soil was mixed into the distilled water and stirred properly. It was left undisturbed for about 30 minutes. After that, the insoluble microplastic was found floating on the surface. It was then filtered by using whatman filter paper. Likewise, microplastic was isolated, washed with the ethanol for removing impurities. The final obtained degraded samples were dried and tested. The steps followed during experiment can be depicted from the **Figure 4**.

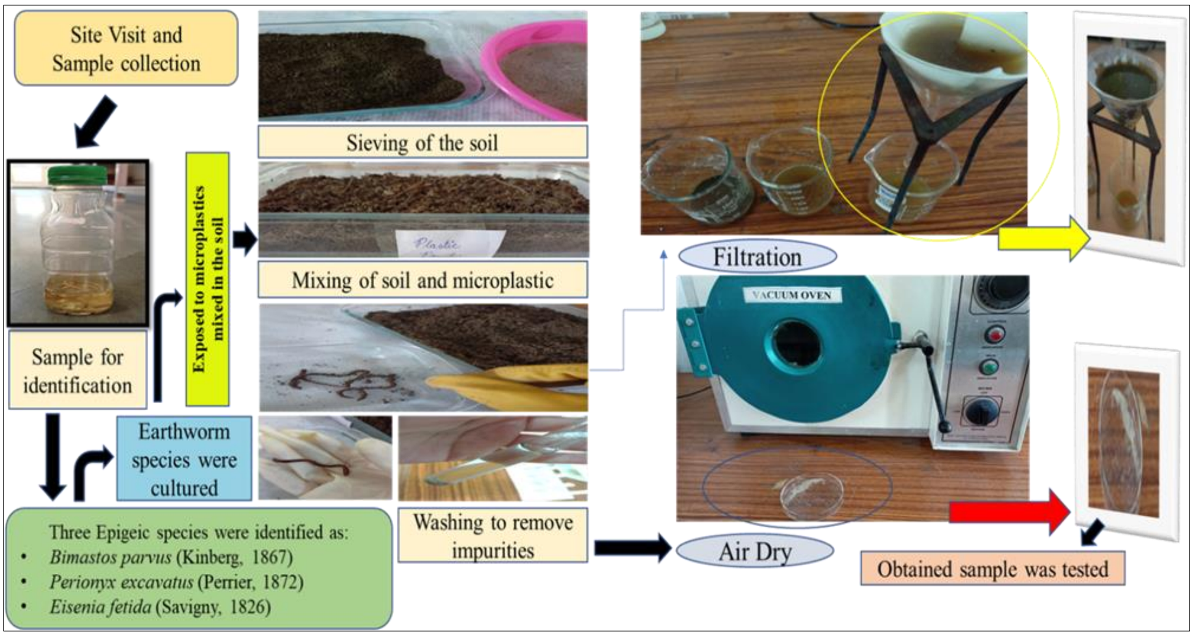


Figure 4 The experimental setup

3. Result and Discussion

3.1. Weight loss

A comparative analysis was done to find out the degradation potential of different epigeic earthworm species. Reduction in the weight of microplastic sample was observed when the species were subjected to the different microplastic concentrations mixed in the soil. Weight loss percentage observed was 10%. 40% and 50% for Species 1, 2 and 3 respectively. However, the standard deviation calculated was maximum i.e. 23.57% in case of Species 3 followed by 18.86% in case of Species 2 and 4.74% recorded for Species 1 as shown in the **Table 3, 2 and 1.** This comparative analysis of the weight loss percentage depicted the degradation efficiency of epigeic earthworm species. The difference between the initial and final weight of LDPE microplastic clearly depicts the weight loss indicating degradation.

Table 1 The weight loss percentage in LDPE was calculated in case of *Bimastos parvas* 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 A1 (Soil+ microplastic)	0.5	0.5	0%		44.4889		
2.	Setup B1 (Soil+ microplastic+ *Bimastos parvas*)	0.5	0.45	10%	6.67%	11.0089		
3.	Setup C1 (Soil+ microplastic+ *Bimastos parvas*)	0.7	0.63	10%		11.0089	22.5022	4.74%

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 A2 (Soil+ microplastic)	0.5	0.5	0%	26.67%	711.4889	355.769	18.86%
2.	Setup B2 (Soil+ microplastic+ *Perionyx excavatus*)	0.5	0.30	40%		177.4889		
3.	Setup C2 (Soil+ microplastic+ *Perionyx excavatus*)	0.7	0.42	40%		177.4889		

Table 3 The weight loss percentage in LDPE was calculated in case of *Eisenia fetida* 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 A3 (Soil+ microplastic)	0.5	0.5	0%	33.33%	1110.8889	555.444	23.57%
2.	Setup B3 (Soil+ microplastic+ *Eisenia fetida*)	0.5	0.25	50%		277.2221		
3.	Setup C3 (Soil+ microplastic+ *Eisenia fetida*)	0.7	0.35	50%		277.2221		

Species	Degradation Potential (Weight loss %age)
Bimastos parvas	10%
Perionyx excavatus	40%
Eisenia fetida	50%

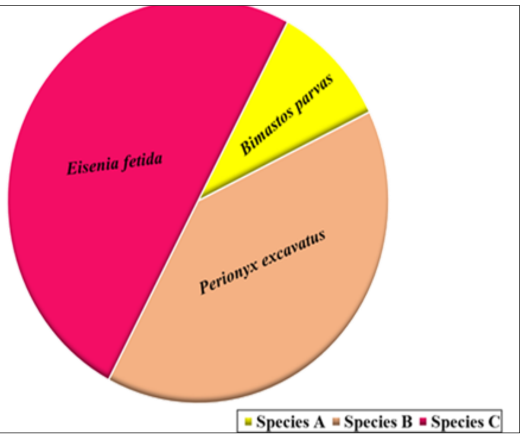


Figure 5 Comparison between microplastic degradation potential of epigenic earthworm species

3.2. FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful analytical technique used to identify chemical compounds based on their absorption of infrared radiation. It provides valuable information about the functional groups present in a sample. Interpreting an FTIR spectrum involves identifying characteristic peaks corresponding to specific functional groups. For example, the presence of a sharp peak around 1700 cm^{-1} indicates the presence of a carbonyl group (C=O), while peaks in the region of 2800-3000 cm^{-1} are associated with C-H stretching vibrations. By comparing the spectrum of an unknown sample to reference spectra or databases, we can determine the chemical composition and structure of the polymer. The change in FTIR peaks was observed when different epigeic earthworm species were subjected to the microplastic concentration mixed in the soil. In addition, some wrinkles, cracks rough and fractured flaky surface textures were seen on the surface of degraded LDPE microplastic. From range 2500-2000 cm^{-1}, peaks were seen in case of pure microplastic sample but no peaks were observed in case of degraded samples as CH_2 functional group have broken due to degradation process as shown in the **Table 4.** Moreover, C=O stretching between range 2000-1500 cm^{-1} showed more number of peaks if compared to other ranges in case of all the degraded samples obtained after exposure to the Sp 1, Sp 2 and Sp 3 respectively. The appearance of carbonyl group formation and the simultaneous decrease in native bonds in the FTIR spectrum of Low-density polyethylene microplastic indicate that the polymer has undergone degradation [19].

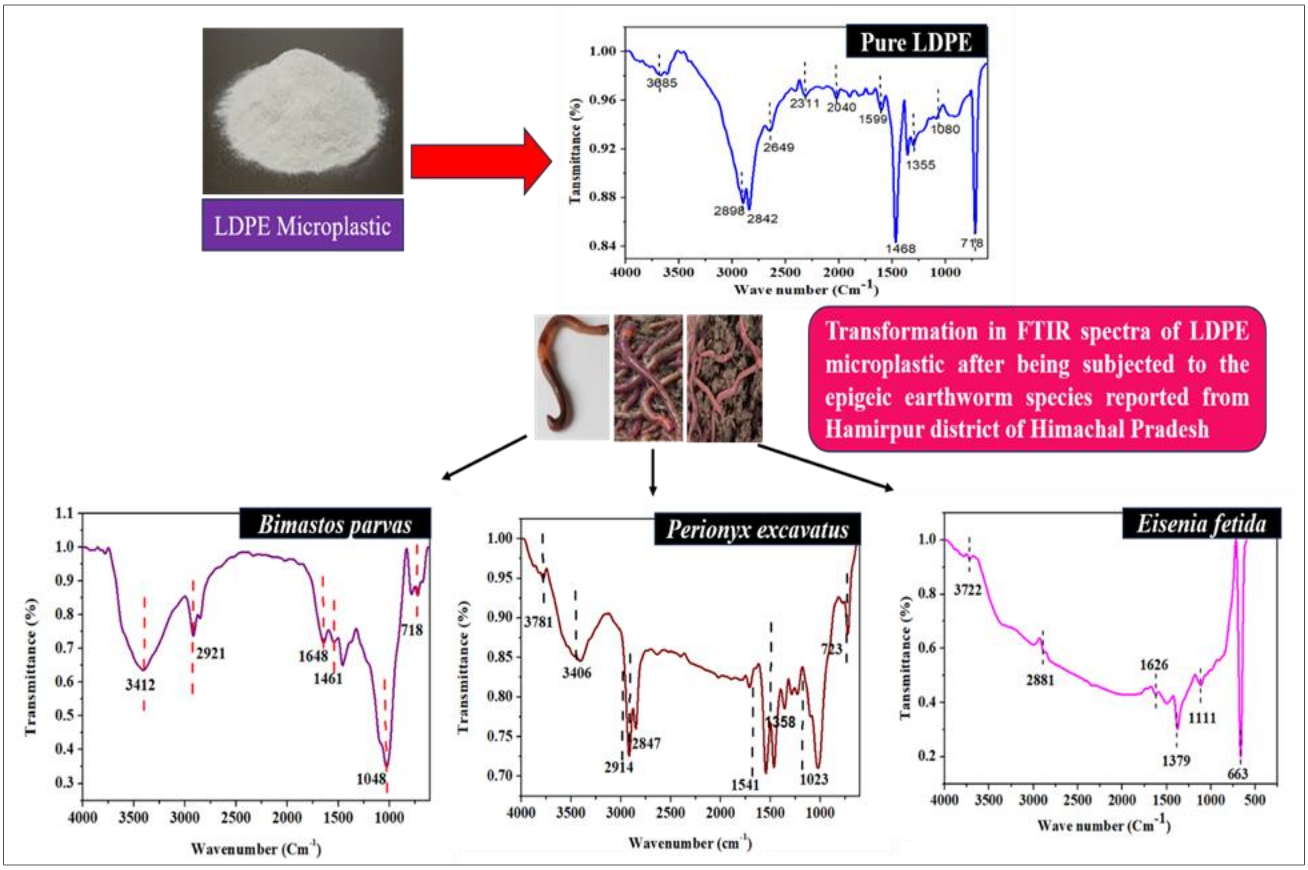


Figure 6 Comparison of FTIR analysis of LDPE microplastic degradation by the epigeic earthworm species reported from Hamirpur district of Himachal Pradesh

Table 4 Comparison between the number of peaks obtained through FTIR

General range and functional groups present in LDPE microplastic		Number of Peaks			
Range	**Functional Groups**	**Pure sample**	**Species 1**	**Species 2**	**Species 3**
4000-3500	Free OH OH stretching	4	0	2	1
3500-3000	-OH groups	0	1	1	0
3000-2500	Asymmetric and Symmetric C-H stretch	3	1	2	1
2500-2000	CH_2	4	0	0	0
2000-1500	C=O and carbonyl group	6	2	2	2
1500-1000	C-H link and CO stretching	6	1	1	1
1000-500	CH_2 splitting	2	1	1	1

3.3. SEM analysis

SEM helps in analyzing the changes that have taken place in the surface morphology of microplastics as they undergo degradation. This includes changes in surface roughness, the formation of cracks, pits, or other structural alterations [20]. It also helps in comparing the morphology of different degraded microplastic samples as shown in the **Figure 7**. The figure shows a comparative analysis between the microplastic samples when they were subjected to different epigeic earthworm species such as *Bimastos parvas* (Kinberg 1867), *Perionyx excavatus* (Perrier 1872) and *Eisenia fetida* (Savigny 1826) respectively.

In case of Sp 1, Sp 2 and Sp 3, structural deformations can be easily recognized whereas no such deformations are present in pure LDPE microplastic sample which clearly marks that degradation has taken place. However, the degradation potential varies with the type of species. Sp 1 is showing some deformations whereas a lot of structural deformations along with rough surface can be depicted in case of Sp 3 followed by Sp 2.

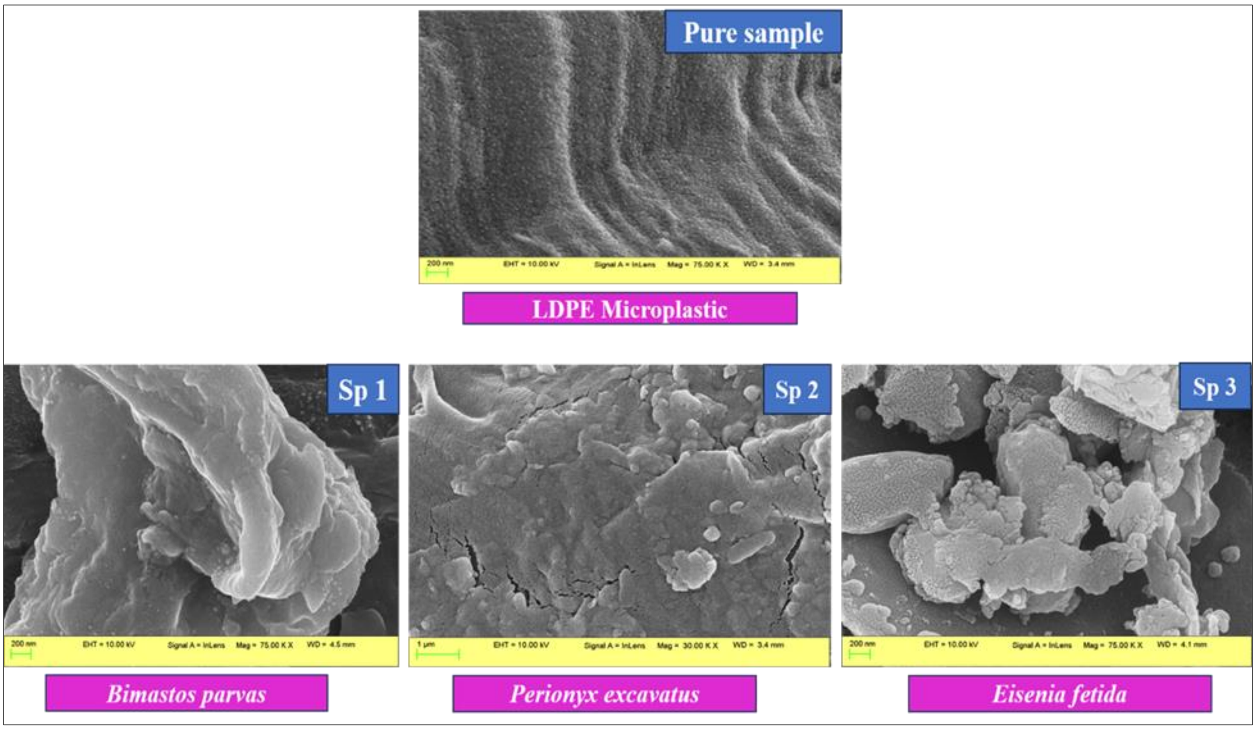


Figure 7 Comparison of SEM analysis of LDPE microplastic degradation by the epigeic earthworm species

Earthworms have shown significant promise as potential allies in the management of ecosystem services [21], making them a viable option for aiding in the breakdown of plastics within soil ecosystems. However, to effectively harness earthworms for plastic degradation, it is essential to understand their roles in this process, as well as the challenges and limitations involved. While some efforts have been made earlier to utilize vermicompost and earthworms in degrading plastic films and microplastics, further investigation into the potential use of earthworms specifically for microplastic degradation was still lacking [12]. Therefore, this present study represents the inaugural effort to screen epigeic earthworm species reported from Hamirpur district of Himachal Pradesh, India in order to evaluate their potential for microplastic degradation. Moreover, the presence of earthworms in soil increases the microbial population [22].

LDPE is one of the major sources of environmental pollution since large quantities of this plastic are accumulated in the environment. The comparative analysis of LDPE microplastic degradation potential among epigeic species documented in Hamirpur district indicates that *Eisenia fetida* (Savigny 1826) can degrade up to 50% of the microplastics, while *Perionyx excavatus* (Perrier 1872) contributes 40%, and *Bimastos parvas* (Kinberg 1867) exhibits a 10% degradation rate as depicted from the Figure 5. *Eisenia fetida* (Savigny 1826) shows maximum degradation among epigeic species due to its ability to tolerate wide range of temperature. This species also has good growth and breeding rate as compared to other species. It is easily found in many land-use systems whereas *Bimastos parvas* (Kinberg 1867), is found in restricted habitats generally the shady areas and pine stand.

Degradation process is depicted from the roughness in the morphology of microplastic sample after it is subjected to earthworms for degradation [23]. Moreover, the formation of voids or cavities within the polymer has been depicted as a result of bond cleavage occurred due to degradation [24]. With context to this, the observations about morphological changes have been clearly shown in the SEM images given in Figure 7.

Similarly, the change in the functional groups as mentioned in the Figure 6 and related peak variations depicted in the Table 4 clearly depicts the chemical and mechanical changes in the microplastic structure which have been occurred as a consequence of the degradation process inside the earthworm gut.

One previous study found that the presence of microplastics did not impact the mortality of earthworms. Additionally, microplastics increased the phosphate content by 30% and decreased cadmium traces by 80%. This research delved into the potential for bioremediation of contaminated dewatered anaerobically digested sewage sludge by reducing both microplastics (MPs) and pathogens, with results indicating that none of the microplastic treatments caused significant lethal effects on the earthworms [25]. Their finding co-relates with our finding depicting that microplastic treatment for earthworms is not lethal if it is in limited concentration. Optimum microplastic concentration was used and results were noted where negligible mortality was observed. It goes similar with the findings that showed that 7% concentration of LDPE microplastic was not harmful to *L. terrestris* earthworm species when exposed for 60 days [26].

Thus, the experimental observations along with the analytical techniques elucidates the capability and efficiency of the epigeic earthworm species to degrade LDPE microplastic. This output helps in combating soil contamination and pollution by paving a way for the eco-friendly solution with context to the waste remediation.

4. Conclusion

The current study aims to address this knowledge gap by investigating the microplastic degradation potential of epigeic earthworm species reported from the district of Hamirpur, Himachal Pradesh. By combining laboratory experiments and field surveys, we seek to elucidate the mechanisms underlying the ingestion, retention, and degradation of microplastics by epigeic earthworms in soils. Additionally, this research aims to assess the spatial distribution and abundance of microplastics in soils inhabited by these earthworm species across different land-use types. Overall, this research endeavors to contribute to the growing body of knowledge on microplastic pollution and soil ecology by shedding light on the role of epigeic earthworms in mediating the fate of microplastics in terrestrial ecosystems. The findings of this study have implications for environmental management and conservation practices aimed at mitigating the impacts of microplastic pollution on soil ecosystems in regions like Hamirpur and beyond.

Compliance with ethical standards

Acknowledgement

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Author contributions

All authors contributed equally.

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