

Influence of Microplastic on Reproductive Outputs of the Epigeic Earthworms, *Eisenia fetida* and *Eudrilus eugeniae*

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Abstract

The increasing use of plastics in the agricultural fields has significant adverse effects on non-target soil organisms like earthworms. The overuse of plastic commodities in industries and agriculture practices has irrevocable effect on the ecosystem. The occurrence of microplastics from various sources in the terrestrial ecosystem has recently become a hotspot of current research. Therefore, the present study investigates the impact of microplastic on the growth and reproduction of earthworms, *Eisenia fetida* and *Eudrilus eugeniae*. In the present work, both the epigeic earthworm species were exposed to the No Observed Effect Concentration (NOEC) of Poly vinyl chloride (PVC) along with the cattle manure as a control. The observations were made with respect to biomass, growth rate, sexual maturity and cocoon production of both earthworms over a period of 17 weeks. The results showed significant variations in all the reproductive outputs. Based on the present study, it can be concluded that, the microplastic is going to influence and affects the growth and reproduction of the earthworms, especially more to *Eudrilus eugeniae* than *Eisenia fetida*.

Key words: Epigeic earthworms, *Eisenia fetida*, *Eudrilus eugeniae*, PVC, Microplastic

Agricultural ecosystem, especially the horticultural sector is more contaminated with variety of pollutants particularly plastic pollutants, of which, microplastics (MPs) with < 5 mm makes the major contributions in the soil. There are many sources of microplastics enter in to the soil ecosystem through sewage sludge application, plastic mulching, littering, irrigation, street runoff, flooding and atmospheric input etc. [1]. Globally, 6300 million tons (Mt) of plastic products are produced, of which, more than 75% are deposited in landfills and other ecological segments [2]. The overuse of plastic commodities in industries and agriculture practices has irrevocable effect on the terrestrial soil ecosystem organisms, especially earthworms. The occurrence of microplastics from various sources in the terrestrial ecosystem has recently become a hotspot of current research. The majority of microplastics found in nature is non-biodegradable and remain stable for a long period. It is due to rapid growth in the industries and overuse in agricultural land, plastic production has amplified exponentially. However, the management of these plastic waste materials has a deficit thing due to the proliferation of microplastic pollution [3].

Earthworms are the dominant geophagous soil fauna contributing significantly in the soil formation and its fertility by their burrowing and feeding habitats. They also maintain the structure and function in the soil ecosystem. Earthworms have high growth and reproductive rate but they are vulnerable to toxic and harmful substances present in the soil. Therefore, earthworms have been widely used as a model animal in many eco-toxicological research studies [4-5]. Due to their close

interaction with soil constituents and sensitivity to environmental changes, earthworms serve as reliable bioindicators for assessing soil health and contamination levels.

Several ecotoxicological studies have investigated the toxic effects of different types of microplastics on earthworms, primarily focusing on oxidative stress, DNA damage, and histopathological alterations. Changes in antioxidant enzyme activities, such as superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST), have also been reported in earthworms [6-7].

To evaluate the effects of microplastic on soil macroinvertebrates such as earthworms, the present study was undertaken to assess the influence of microplastic on the growth and reproduction of the epigeic earthworms, *Eisenia fetida* and *Eudrilus eugeniae*.

MATERIALS AND METHODS

Collection and maintenance of earthworms

The epigeic earthworms, *Eisenia fetida* and *Eudrilus eugeniae* were collected from the stock culture maintained in the Vermitechnology laboratory, Department of Zoology, Karnatak University, Dharwad and maintained separately for experimental use.

Preparation of food for earthworms

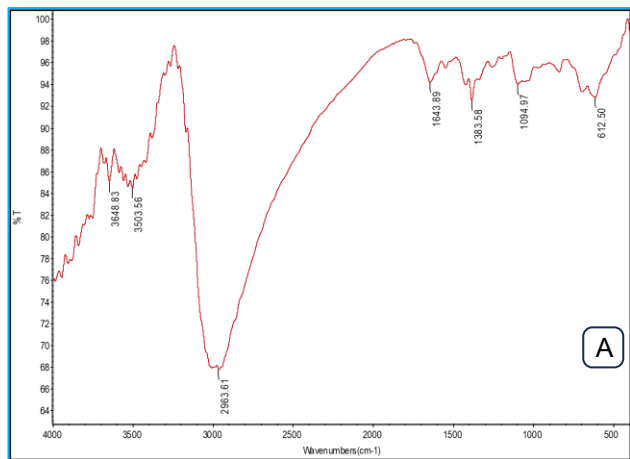
Locally available dried cattle manure was collected from the farmers, broken down into small pieces and kept moist (approximately 70–80% moisture) by regular sprinkling of tap

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water so as to initiate microbial activity (primary degradation) in the cattle manure. Soon after the primary degradation. This cattle manure as stock was used as feed material for earthworms for further experimental studies.

Collection and characterization of microplastics (MPs)

Polyvinyl Chloride (PVC) plastics with molecular formula $(C_2H_3Cl)_n$ (CAS No: 9002-86-2) was used for this



work. It is purchased from the Kemphasol and before using these plastic particles, they were verified by using Fourier Transform Infrared-(FTIR) (Fig 1A) Spectroscopy confirms these particles as PVC-MP (Polyvinyl chloride-microplastic) and the particle size and shapes were confirmed through Scanning Electron Microscopy-SEM [8]. The microplastics used for this experimental study was around 137 μm in size and roughly having spherical shape structure (Fig 1B).

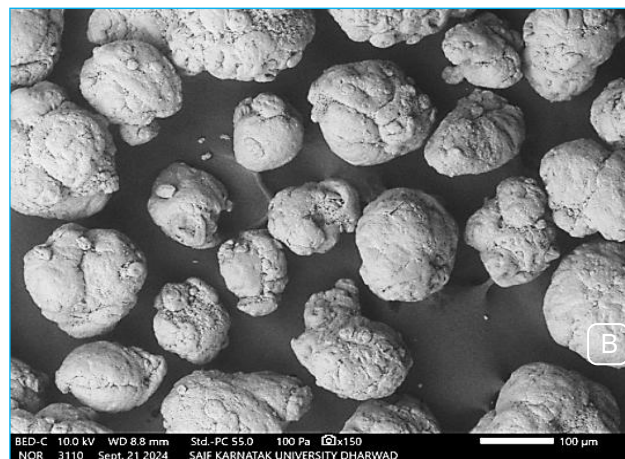


Fig 1 (A) FTIR spectroscopy of PVC microplastic pellets (B) SEM- The typical surface morphology of the PVC microplastic particles

Experimental design

The growth and reproductive assay were performed as per the OECD (Organization of Economic Co-operation and Development) guidelines 222 [9] for stabilizing the concentration grade of the microplastic for this particular experiment.

For this study, NOEC (No Observed Effect Concentration) value of about 4g of PVC microplastic was standardized and used in this experiment. The 4g PVC-MP was mixed with the 100g of cattle manure (weight/weight) as a treated group and pure cattle manure (100g) was used as a Control, free of PVC-MP. All the experimental set up were maintained in triplicates to maintain accuracy of the data.

Incubation and hatching of cocoon

Three fresh cocoons of both *E. fetida* and *E. eugeniae* earthworms were collected from the stock culture maintained in the laboratory to evaluate the incubation period, percent hatching, number of hatchlings/cocoons. All the experimental set up were maintained with 70-80% of moisture content and regular observations were made with respect to incubation period, percent hatching, number of hatchlings/cocoon and weight of freshly emerged juveniles at the time of hatching.

Growth and reproduction of earthworms

Five freshly emerged one-week aged juveniles of both *E. fetida* and *E. eugeniae* species having approximately similar weight were collected from the incubation and hatching experiment. They were cultured in the earthen pots of size 11cm×17cm height (triplicates) and initial weight of all the juveniles of both the species were noted. The experimental pots were maintained in the uncontrolled laboratory conditions for up to 17 weeks with proper moisture content of 70-80% and sufficient food was maintained throughout the experimental period. Weekly observations were made with respect to increase in worm biomass, growth rate, attainment of sexual maturity and cocoon production. The growth rate was calculated based on the biomass of worm as per the following formula given by Biradar *et. al.* [10].

$$\text{Growth rate worm}^{-1} \frac{\text{weight of worm on the day of observation} - \text{Initial weight}}{\text{Number of days on the day of observation}} \times 100 \text{ day}^{-1} \text{ mg} =$$

Statistical analysis

The statistical analysis of the experimental data was done through ANOVA by using SPSS 23.0 software. All the data were presented in the form of Mean \pm SE and the significance level was measured at $P \leq 0.05$.

RESULTS AND DISCUSSION

Data related to reproductive outputs such as incubation period, percent hatching, number of hatchlings/cocoons, weight of freshly emerged juveniles, biomass, growth rate, sexual maturity and cocoon production of the epigeic earthworms, *Eisenia fetida* and *Eudrilus eugeniae* cultured in treated microplastic (MP) and controlled cattle manure (CM) were all represented in (Table 1-5, Graphs 1-11) along with significant F and P values.

The data depicted in (Table 1, Graph 1-4) represents the incubation period, percent hatching, number of hatchlings/cocoon and weight of freshly emerged juveniles by the cocoons of the epigeic earthworms, *Eisenia fetida* and *Eudrilus eugeniae* respectively. The lowest incubation period (24.00 ± 0.577) was seen in Control-CM as compared to Treated-MP (31.66 ± 0.881) where as 100% hatching rate was observed in both Treated-MP and the Control-CM. More number of hatchlings/cocoon (2.66 ± 0.33) was witnessed in Control-CM as compared to Treated-MP (1.66 ± 0.33) but weight of the freshly emerged juveniles in both Treated-MP (0.0013 ± 0.00015) and Control-CM (0.0013 ± 0.00006) shows almost similar weights (Table 1). No significant difference was noticed among the groups expect inoculation period (Table 1).

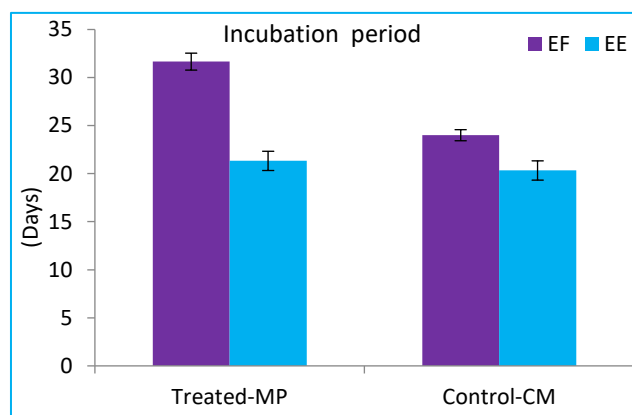
In *Eudrilus eugeniae* also Control-CM showed lowest incubation period (20.33 ± 0.333) than that of Treated-MP (21.33 ± 10.879) but 100% cocoon hatchings were seen only in Control-CM whereas 55.55% hatchings were seen in treated-

MP. The number of hatchlings/cocoons was more in Control-CM (2.666 ± 0.33) as compared to Treated-MP (0.333 ± 0.19). The mean weight of the freshly emerged juvenile was also more

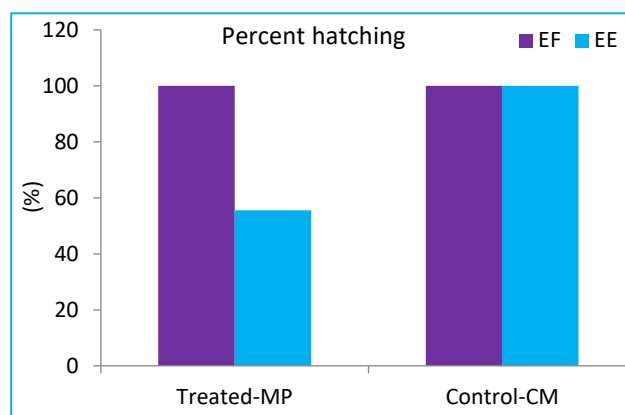
in Control-CM (0.0097 ± 0.0008) as compared to Treated-MP (0.0043 ± 0.002) and no significant difference was noticed in all parameters expect the hatchlings/cocoon (Table 1).

Table 1 Data of Incubation period, percent hatching, number of hatchlings/cocoon and weight of freshly emerged juveniles of the epigeic earthworms, *E. fetida* and *E. eugeniae*. Data are in Mean \pm SE with F and P values

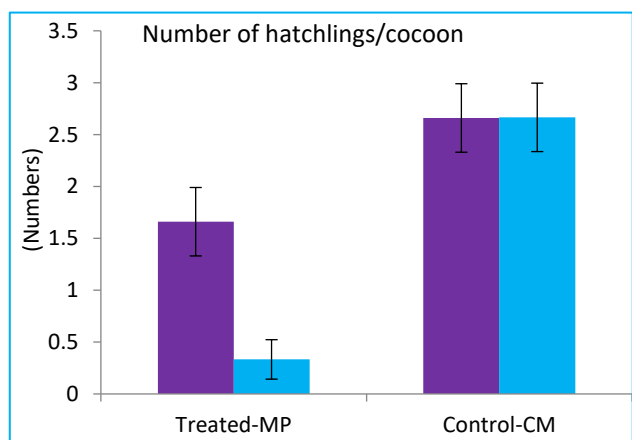
Parameters and species	Incubation period (days)		Percent hatching (%)		Number of hatchings/cocoons		Weight of freshly emerged juveniles (mg)	
	EF	EE	EF	EE	EF	EE	EF	EE
Treated-MP	31.66 \pm 0.881	21.33 \pm 0.87972	100	55.55	1.66 \pm 0.33	0.333 \pm 0.19	1.3 \pm 0.15	4.3 \pm 2.0
Control-CM	24.00 \pm 0.577	20.33 \pm 0.333	100	100	2.66 \pm 0.33	2.666 \pm 0.33	1.3 \pm 0.06	9.7 \pm 0.8
F- Value	52.900	0.009	-	-	4.500	18.000	0.000	5.120
P- Value	0.002	0.930	-	-	0.101	0.013	1.000	0.086



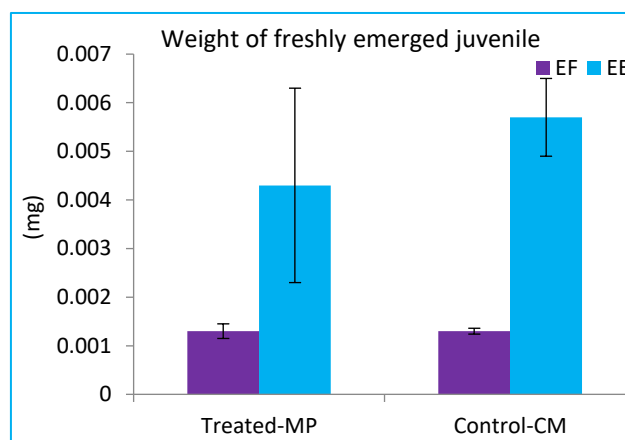
Graph 1 Incubation period of *E. fetida* and *E. eugeniae* cultured in Treated-MP and Control-CM



Graph 2 Percent hatching (%) of *E. fetida* and *E. eugeniae* cultured in Treated-MP and Control-CM



Graph 3 Number of hatchlings per cocoon of *E. fetida* and *E. eugeniae* cultured in Treated-MP and Control-CM



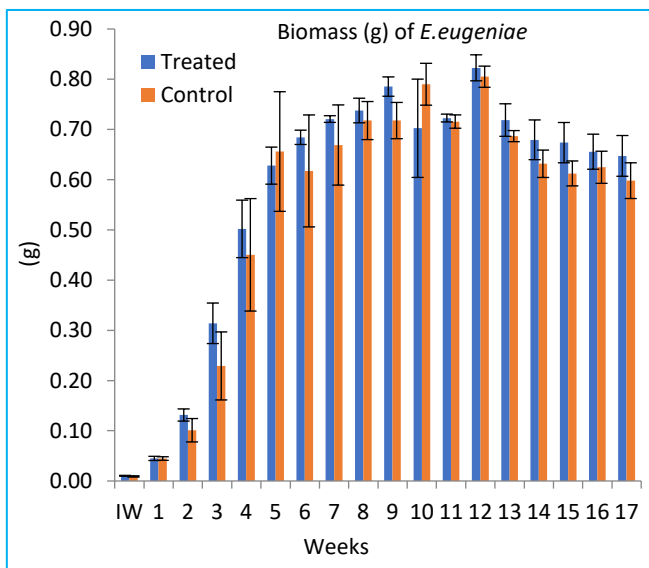
Graph 4 Weight of freshly emerged juveniles (mg) of *E. fetida* and *E. eugeniae* cultured in Treated-MP and Control-CM

Data presented in (Table 2, Graph 5-6) shows the data of the biomass of *Eisenia fetida* and *Eudrilus eugeniae* cultured in Treated-MP (microplastic) and Control-CM (control medium) for a period of 17 weeks. The initially inoculated juvenile weight of *Eisenia fetida* in Treated-MP and Control-CM was 0.010 ± 0.0008 and 0.0091 ± 0.001 respectively. The biomass was varied throughout experimental period (17 weeks) and there is a sudden increase in biomass from 3-4 week in both the Treated-MP and Control-CM. The mean worm biomass of *Eisenia fetida* Treated-MP and the Control-CM was 0.5656 ± 0.0351 and 0.5375 ± 0.0362 respectively. Highest worm biomass was noticed during 12th week in Treated-MP and Control-CM is 0.8227 ± 0.02 and 0.8050 ± 0.0210 respectively. No significant difference ($P \leq 0.534$) was seen among these two samples (Table 2).

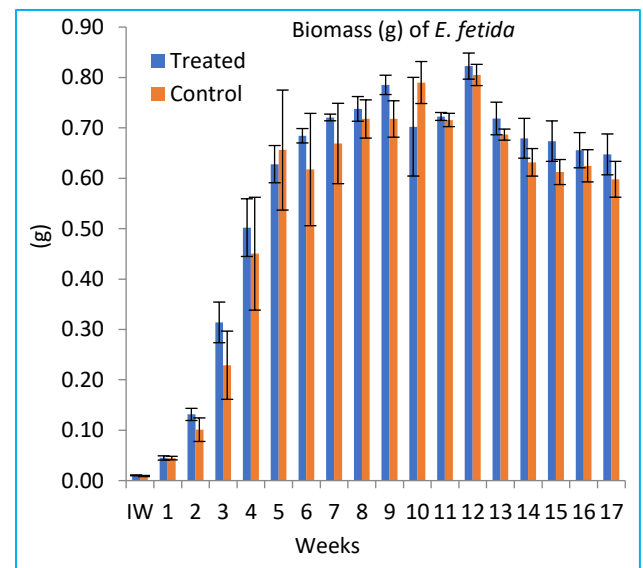
There is a gradual increase in the worm biomass of *Eudrilus eugeniae* was also seen in both the samples throughout the experiment up to 17th weeks. This may be attributed to the increase in microbial load, which progressively enhances the feeding habitat of the worms. The initially inoculated worm biomass was 0.0097 ± 0.001 and 0.011 ± 0.001 in treated-MP and Control CM respectively. The mean worm biomass of *E. eugeniae* was more in Control-CM (0.8742 ± 0.0853) than that of Treated-MP (0.5253 ± 0.0659) and highest biomass was seen during last week i.e. on 17th week. Significant difference ($P \leq 0.001$) was seen in biomass of *Eudrilus eugeniae* among these two samples (Table 2). The observed variation in biomass between treated and control samples indicates that substrate composition and treatment conditions play a crucial role in influencing the growth performance of *Eudrilus eugeniae*.

Table 2 Biomass (g) of *E. fetida* and *E. eugeniae* cultured in Treated-MP and Control-CM and their F and P values at $P \leq 0.05$.
Data are in Mean \pm SE

Species and samples	Weeks	<i>Eisenia fetida</i>		<i>Eudrilus eugeniae</i>	
		Treated-MP	Control-CM	Treated-MP	Control-CM
IW		0.010 \pm 0.000	0.009 \pm 0.001	0.009 \pm 0.001	0.011 \pm 0.001
1		0.044 \pm 0.004	0.044 \pm 0.003	0.082 \pm 0.064	0.022 \pm 0.002
2		0.131 \pm 0.012	0.101 \pm 0.023	0.050 \pm 0.007	0.046 \pm 0.009
3		0.314 \pm 0.040	0.229 \pm 0.067	0.052 \pm 0.004	0.082 \pm 0.002
4		0.502 \pm 0.057	0.450 \pm 1.111	0.109 \pm 0.014	0.223 \pm 0.062
5		0.628 \pm 0.036	0.606 \pm 1.119	0.157 \pm 0.025	0.417 \pm 0.102
6		0.684 \pm 0.014	0.617 \pm 1.111	0.171 \pm 0.039	0.558 \pm 0.045
7		0.720 \pm 0.006	0.669 \pm 0.079	0.158 \pm 0.041	0.697 \pm 0.057
8		0.737 \pm 0.024	0.717 \pm 0.037	0.209 \pm 0.054	0.897 \pm 0.061
9		0.785 \pm 0.019	0.717 \pm 0.036	0.301 \pm 0.067	1.026 \pm 0.044
10		0.702 \pm 0.097	0.790 \pm 0.041	0.536 \pm 0.097	1.210 \pm 0.038
11		0.722 \pm 0.007	0.705 \pm 0.013	0.881 \pm 0.111	1.220 \pm 0.113
12		0.827 \pm 0.025	0.805 \pm 0.021	0.915 \pm 0.091	1.209 \pm 0.136
13		0.718 \pm 0.035	0.686 \pm 0.011	1.096 \pm 0.072	1.472 \pm 0.093
14		0.679 \pm 0.039	0.631 \pm 0.027	1.056 \pm 0.055	1.561 \pm 0.102
15		0.673 \pm 0.040	0.612 \pm 0.024	1.102 \pm 0.071	1.610 \pm 0.094
16		0.557 \pm 0.034	0.624 \pm 0.032	1.229 \pm 0.082	1.721 \pm 0.045
17		0.647 \pm 0.040	0.598 \pm 0.035	1.337 \pm 0.075	1.743 \pm 0.055
Mean \pm SE		0.565 \pm 0.035	0.537 \pm 0.036	0.525 \pm 0.065	0.874 \pm 0.085
Significance F and P Value		F= 0.462 P=0.534		F=83.223 P=0.001	



Graph 5 Biomass (g) of *E. fetida* cultured in treated-MP and control-CM



Graph-6 Biomass (g) of *E. eugeniae* cultured in treated-MP and control-CM

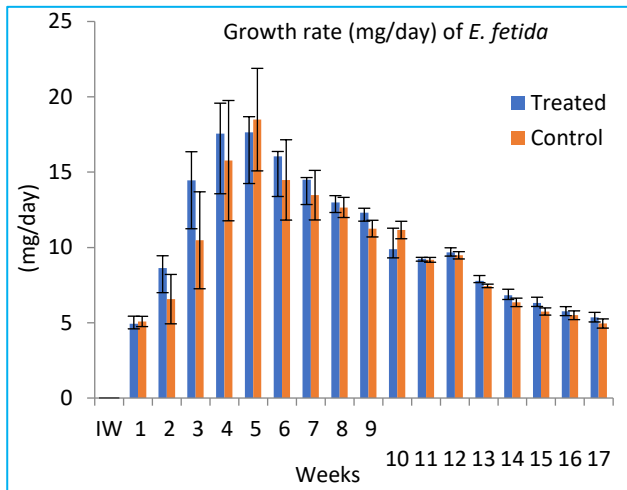
The growth rate (worm/day/mg) was calculated by using the biomass of the worm using the formula as given by Biradar *et al.* [10]. The data depicted in (Table 3, Graph 7-8) represents the growth rate of the *Eisenia fetida* and *Eudrilus eugeniae* cultured in Treated-MP and Control-CM. There is a gradual increase in the growth rate of *Eisenia fetida* in both the samples up to 5th week, but there is a decrease from 6th week onwards till 17th week. The maximum growth rate was seen in Treated-MP (17.645 ± 1.033) and in the control-CM (18.483 ± 3.404) on 5th week. The maximum mean growth rate (GR) of *Eisenia fetida* was 9.994 ± 0.669 and 9.3344 ± 0.6974 in Treated-MP and Control-CM respectively. There is no significance difference ($P \leq 0.576$) was seen in growth rate of *Eisenia fetida*

among these two groups (Table 3). Once these earthworms attain sexual maturity, they begin to allocate more energy to reproduction rather than feeding. Therefore, after the 4th or 5th week onwards, a gradual decrease in the growth rate of the worms is observed.

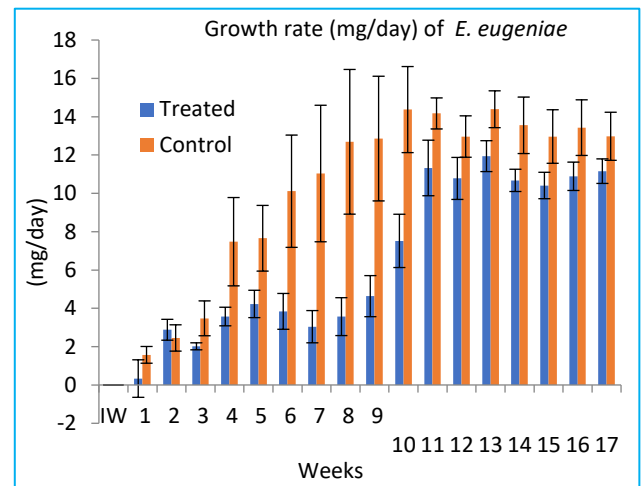
There is a slight variation in the growth rate of *Eudrilus eugeniae* in both the samples. The maximum growth rate was observed on 13th week in Treated-MP (11.94 ± 0.806) followed by Control-CM (14.388 ± 0.960). The mean growth rate of *E. eugeniae* was more in Control-CM (9.896 ± 0.7580) followed by Treated-MP (6.822 ± 0.6982). No significance difference ($P \leq 0.114$) was noticed in growth rate of *Eudrilus eugeniae* among these two samples (Table 3).

Table 3 Growth rate of worm (day⁻¹ mg) of *E. fetida* and *E. eugeniae* cultured in Treated-MP and Control-CM and their F and P values at P≤0.05. Data are in Mean ± SE

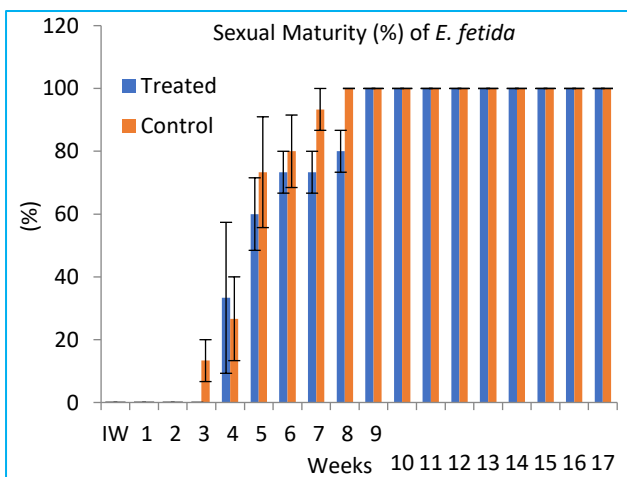
Species and samples	<i>Eisenia fetida</i>		<i>Eudrilus eugeniae</i>	
	Treated-MP	Control-CM	Treated-MP	Control-CM
IW	0.000 ± 0.000	0.0000± 0.000	0.000 ± 0.000	0.000 ± 0.000
1	4.933 ± 0.503	5.0857± 0.340	0.333 ± 1.571	1.571 ± 0.436
2	8.638 ± 0.808	6.5667± 1.640	2.881 ± 0.544	2.452 ± 0.685
3	14.457 ± 1.895	10.473 ± 3.217	2.015 ± 0.183	3.476 ± 0.908
4	17.557 ± 2.026	15.759 ± 3.993	3.571 ± 0.486	7.476 ± 2.305
5	17.645 ± 1.033	18.483 ± 3.404	4.228 ± 0.713	7.657 ± 1.713
6	16.046 ± 0.324	14.482 ± 2.665	3.841 ± 0.934	10.111 ± 2.930
7	14.495 ± 0.143	13.468 ± 1.646	3.040 ± 0.841	11.034 ± 3.562
8	12.986 ± 0.450	12.653 ± 0.668	3.565 ± 0.988	12.690 ± 3.775
9	12.300 ± 0.301	11.247 ± 0.555	4.634 ± 1.072	12.857 ± 3.254
10	9.884 ± 1.393	11.156 ± 0.579	7.519 ± 1.390	14.371 ± 2.245
11	9.250 ± 0.093	9.176 ± 0.160	11.324 ± 1.450	14.168 ± 0.810
12	9.669 ± 0.303	9.475 ± 0.243	10.777 ± 1.096	12.964 ± 1.079
13	7.783 ± 0.350	7.446 ± 0.118	11.941 ± 0.806	14.388 ± 0.960
14	6.825 ± 0.397	6.353 ± 0.281	10.676 ± 0.580	13.551 ± 1.474
15	6.316 ± 0.376	5.745 ± 0.236	10.409 ± 0.689	12.965 ± 1.396
16	5.761 ± 0.307	5.496 ± 0.288	10.889 ± 0.740	13.428 ± 1.451
17	5.352 ± 0.335	4.949 ± 0.303	11.156 ± 0.640	12.977 ± 1.254
Mean ± SE	9.994 ± 0.669	9.334 ± 0.697	6.822 ± 0.698	9.896 ± 0.758
Significance F and P Value	F = 0.370 P = 0.576		F = 4.073 P = 0.114	



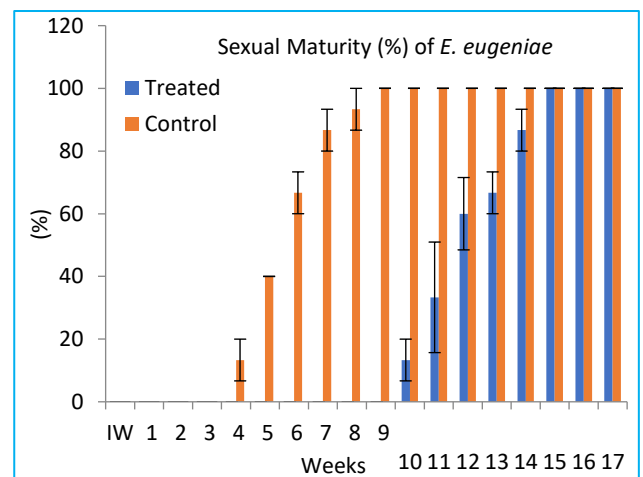
Graph 7 Growth rate of worm (mg/day) of *E. fetida* cultured in treated-MP and control-CM



Graph 8 Growth rate of worm (mg/day) of *E. eugeniae* cultured in treated-MP and control-CM



Graph 9 Percent sexual maturity of *E. fetida* cultured in treated-MP and control-CM



Graph 10 Percent sexual maturity of *E. eugeniae* cultured in treated-MP and control-CM

The data on the attainment of sexual maturity of the epigeic earthworms, *E. fetida* and *E. eugeniae* were shown in the (Table 4, Graph 9-10). The early sexual maturity of *E. fetida* was noticed on 3rd week in the Control-CM and on the 4th week in Treated-MP. 100% sexual maturity was noticed on 8th week in Control-CM followed by Treated-MP on 9th week. No significance difference ($P \leq 0.331$) was seen in sexual maturity of *E. fetida* among these two groups (Table 4).

The early sexual maturity of *E. eugeniae* was noticed in the Control-CM on 4rd week and it was late in Treated-MP on 10th week. The time taken for sexual maturity was more in Treated-MP as compared to the Control-CM. The 100% sexual maturity was noticed on 9th week and 15th week in Control-CM and Treated-MP respectively. Significance difference ($P \leq 0.001$) in sexual maturity of *E. eugeniae* was shown among these two groups (Table 4).

Table 4 Percent sexual maturity of *E. fetida* and *E. eugeniae* cultured in Treated-MP and Control-CM and their F and P values at $P \leq 0.05$. Data are in Mean \pm SE

Species and samples	Weeks	<i>Eisenia fetida</i>		<i>Eudrilus eugeniae</i>	
		Treated-MP	Control-CM	Treated-MP	Control-CM
IW		-	-	-	-
1		-	-	-	-
2		-	-	-	-
3		-	13.33% \pm 6.66	-	-
4		33.33% \pm 24.03	26.66% \pm 13.33	-	13.33% \pm 6.66
5		60.00% \pm 11.54	73.33% \pm 17.63	-	40.00% \pm 0.00
6		73.33% \pm 6.66	80.00% \pm 11.54	-	66.66% \pm 6.66
7		73.33% \pm 6.66	93.33% \pm 6.66	-	86.66% \pm 6.66
8		80.00% \pm 11.54	100% \pm 0.00	-	93.33% \pm 6.66
9		100% \pm 0.00	100% \pm 0.00	-	100% \pm 0.00
10		100% \pm 0.00	100% \pm 0.00	13.33% \pm 6.66	100% \pm 0.00
11		100% \pm 0.00	100% \pm 0.00	33.33% \pm 17.63	100% \pm 0.00
12		100% \pm 0.00	100% \pm 0.00	60.00% \pm 11.54	100% \pm 0.00
13		100% \pm 0.00	100% \pm 0.00	66.66% \pm 6.66	100% \pm 0.00
14		100% \pm 0.00	100% \pm 0.00	86.66% \pm 6.66	100% \pm 0.00
15		100% \pm 0.00	100% \pm 0.00	100% \pm 0.00	100% \pm 0.00
16		100% \pm 0.00	100% \pm 0.00	100% \pm 0.00	100% \pm 0.00
17		100% \pm 0.00	100% \pm 0.00	100% \pm 0.00	100% \pm 0.00
Mean \pm SE		-	-	-	-
Significance F and P Value		F = 1.221 P = 0.331		F = 63.095 P = 0.001	

The data of cocoon production of *Eisenia fetida* and *Eudrilus eugeniae* in Treated-MP and Control-CM were shown in (Table 5, Graph 11-12). Soon after attainment of sexual maturity, worms started producing cocoons. The cocoons were first noticed on 4th week in both the samples of *Eisenia fetida*. The highest number cocoon production (worm/week) was seen on 11th week in both Treated-MP (4.088 \pm 0.311) and in Control-CM (3.5833 \pm 0.416). The cumulative cocoon number (CCN) for 17 weeks was 33.327 \pm 5.181 and 40.779 \pm 6.240 in Treated-MP and Control-CM respectively. No significance

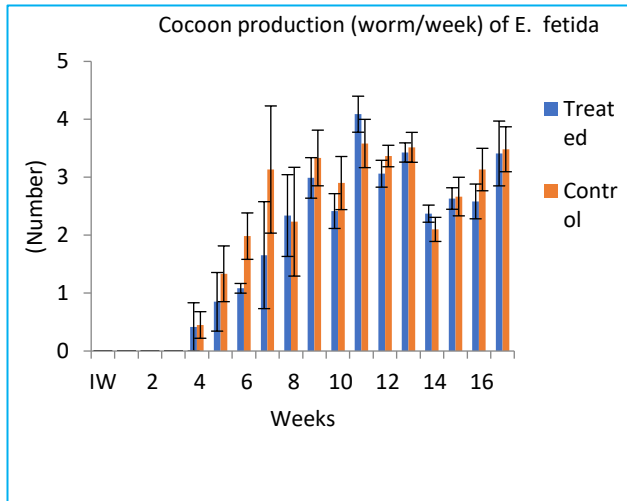
difference ($P \leq 0.476$) was observed among these two samples (Table 5).

In case of *E. eugeniae*, first cocoons were noticed in control-CM on 5th week followed by Treated-MP on 11th week. The mean cocoon rate (worm/week) was more in control-CM (1.846 \pm 0.4166) as compared to Treated-MP (0.914 \pm 0.253). The cumulative cocoon number (CCN) was 6.399 \pm 1.772 and 23.998 \pm 5.416 in Treated-MP and Control-CM respectively. Here, significance difference ($P \leq 0.015$) was noticed in cocoon rate of *E. eugeniae* among these two samples (Table 5).

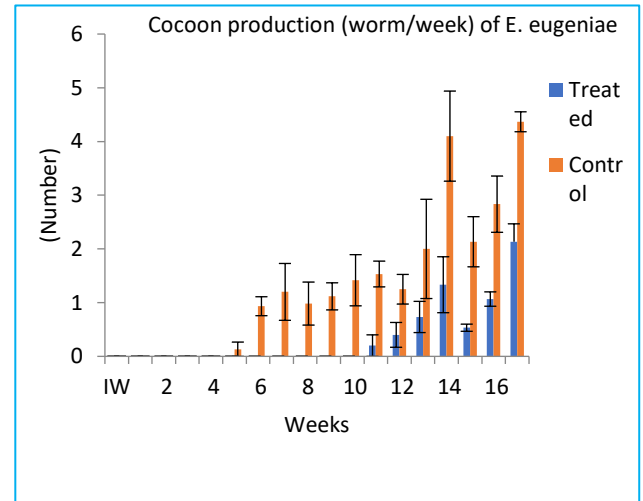
Table 5 Cocoon production of *E. fetida* and *E. eugeniae* cultured in Treated-MP and Control-CM and their F and P values at $P \leq 0.05$. Data are in Mean \pm SE

Species and samples	Weeks	<i>Eisenia fetida</i>		<i>Eudrilus eugeniae</i>	
		Treated-MP	Control-CM	Treated-MP	Control-CM
IW		-	-	-	-
1		-	-	-	-
2		-	-	-	-
3		-	-	-	-
4		0.416 \pm 0.416	0.450 \pm 0.229	-	-
5		0.850 \pm 0.505	1.333 \pm 0.480	-	0.133 \pm 0.133
6		1.083 \pm 0.083	1.983 \pm 0.400	-	0.933 \pm 0.176
7		1.655 \pm 0.923	3.133 \pm 1.097	-	1.200 \pm 0.529
8		2.338 \pm 0.705	2.233 \pm 0.938	-	0.983 \pm 0.400
9		2.988 \pm 0.349	3.333 \pm 0.480	-	1.116 \pm 0.252
10		2.416 \pm 0.300	2.900 \pm 0.458	-	1.416 \pm 0.476
11		4.088 \pm 0.311	3.583 \pm 0.416	0.200 \pm 0.200	1.533 \pm 0.240
12		3.061 \pm 0.231	3.366 \pm 0.185	0.400 \pm 0.230	1.250 \pm 0.275
13		3.427 \pm 0.165	3.516 \pm 0.258	0.733 \pm 0.290	2.000 \pm 0.923

14	2.372 ± 0.147	2.100 ± 0.208	1.333 ± 0.520	4.100 ± 0.838
15	2.633 ± 0.185	2.666 ± 0.333	0.533 ± 0.066	2.133 ± 0.466
16	2.583 ± 0.300	3.133 ± 0.366	1.066 ± 0.133	2.833 ± 0.523
17	3.411 ± 0.559	3.483 ± 0.387	2.133 ± 0.333	4.366 ± 0.185
CCN for 17 Weeks	33.322 ± 5.186	40.779 ± 6.240	6.399 ± 1.772	23.998 ± 5.41
Mean cocoon rate (Worm/week) ± SE	2.380 ± 0.370	2.912 ± 0.445	0.914 ± 0.253	1.846 ± 0.416
Significance F and P Value	F = 1.221 P = 0.331		F = 63.095 P = 0.001	



Graph 11 Rate of cocoon production (worm/week) of *E. fetida* cultured in treated-MP and control-CM



Graph 12 Rate of cocoon production (worm/week) of *E. eugeniae* cultured in treated-MP and control-CM

Recently, microplastics plays a significant attention from the researches point of view due to the concern about environment. Microplastics found in various domains such as beaches [11], agricultural land [12], biosolids [13] etc. Earthworms are soil macro invertebrates, ideal model organisms for investigating the effect of microplastics on land and also testing the possibility of degrading the microplastics through their gut enzymes.

The mechanism and adverse effects of PVC microplastic toxicity level can be tested /examined by taking No Observed Effect Concentration (NOEC) as it was not fully understood because of a lack of data from the previous researchers. But this study reveals that, exposure of microplastic was slightly influenced on the reproduction of the epigeic earthworm, *E. eugeniae* as compared to *E. fetida*. Here, the concentration of microplastic will be the dominant factor, which is going to influence on the life activities of earthworms rather than simply plastic material [14-15].

In the present study, there is a slight variation in the incubation period, number of hatchlings/cocoon and the weight of the hatchlings in two different experimental groups (Treated-MP and Control-CM). These variations may be due to the quality of food, C: N ratio in the culture media, moisture content etc. as witnessed by many researchers, like Reinecke *et al.* [16], Chaudhari *et al.* [17] and Pulikeshi *et al.* [18].

There is a variation in the biomass, growth rate and reproduction of the epigeic earthworms, *E. fetida* and *E. eugeniae* in the present study. These variations may be due to the intrinsic property of worms, preferential feeding habits, particle size, texture and chemical composition of the food and the environmental conditions in which earthworms were cultured [19-23]. These variations may be attributed to the accumulation of PVC microplastics in the intestinal gut of earthworms, which can induce gut irritation and immune-related stress. Such physiological disturbances may

subsequently impair the feeding behavior and nutrient assimilation, leading to reduced growth rate and developmental performance in earthworms [24-25]. The impact of microplastic on reproduction of earthworms was also noticed and reported by Rodriguez-Seijo *et al.* [26] and this variation possibly due to the effect of microplastic on earthworm reproductive outputs [27]. Ju *et al.* [28] also reported that the particle size and type of microplastics also affect the growth and reproduction of earthworms.

Therefore, the present study addresses an important knowledge gap and adds to the limited available information on by evaluating the No Observed Effect Concentration (NOEC) of PVC microplastics in *Eisenia fetida* and *Eudrilus eugeniae*.

CONCLUSION

The present study focused on the research advances in the field of PVC microplastic and its effect on the growth and reproduction of the epigeic earthworms, *E. fetida* and *E. eugeniae*. The results revealed that, the ingestion of microplastics (MPs) can alter the reproductive outputs such as incubation period, percent hatching, number of hatchlings/cocoons, weight of freshly emerged juveniles, growth and reproduction of the worms. Based on the present study it can be concluded that, the microplastic is going to influence and affects the growth and reproduction of the earthworms, especially *E. eugeniae* as compared to *E. fetida* even though it is NOEC. Although the present investigation provides valuable insights into the effects of PVC microplastics on the growth and physiological responses of earthworms, further studies are required to comprehensively understand the physico-chemical parameters in the vermicompost and plastic-induced molecular level stress indicators such as oxidative stress enzymes and gene expression profiling of the mechanistic pathways involved in plastic-induced effects. Future research

should focus on different polymer types such as polyethylene, polypropylene, polystyrene, and polyethylene terephthalate are recommended, as variations in polymer chemistry, additives and degradation behavior may influence their biological impacts.

Declaration of competing interests

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

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