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ACETYLCHOLINE ESTERASE ACTIVITY IN EARTHWORM EUDRILUS EUGENIAE EXPOSED TO LOW DENSITY POLYETHYLENE MICROPLASTICS CONTAMINATED SOIL

Zoology

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ABSTRACT

Microplastic made of low-density polyethylene poses an environmental hazard because to its haphazard disposal and slow rate of degradation in landfills. The importance of evaluating the effects of pollution on soil ecosystems has increased, and interest in using invertebrates as sentinel creatures has increased as well. The necessity for a deeper comprehension of earthworm biomarker reactions has been emphasized in recent papers. This study looked at the earthworm acetylcholinesterase (AChE) activity of Eudrilus eugeniae following exposure to varying amounts of LDPE microplastic-contaminated soil (0, 50, 200, 350, 500, and 650 mg) combined with nanoparticles and naturally occurring microorganisms. The results obtained in the avoidance assay indicated a lack of preference for either soil type; however, upon moving, the earthworms lost surface mucus, resulting in burns and lesions on their bodies, which were reflected in the increase in AChE enzyme levels, which was not directly related to microplastic ingestion, but rather likely acts as an external physical stress agent. The results of the ingestion bioassay showed that microplastic was present in the earthworm segments, with a higher number of particles in the hindgut. The Eudrilus eugeniae did not distinguish microplastics from soil particles, and given the high exposure concentrations, microplastics produced physical lesions on the mucous memebranes of earthworms showed to be a suitable for bioremediation of LDPE contaminated soil.

KEYWORDS

LDPE microplastics, Contaminated soil, Eudrilus eugeniae, Acetylcholinesterase.

INTRODUCTION

Concerns over plastic and low density polyethylene (LDPE) microplastics entering soil and possibly affecting soil biota biodiversity through commercial fertilizer use have grown. The overall amount of LDPE microplastics present can have a significant effect on the bulk density, microbial activity and water-holding capacity of the soil. The biophysical system and soil biota are also impacted by these LDPE microplastics. Thus, removing LDPE microplastics from the planet is essential to preserving healthy soil. Thus, plastic pollution in the soil, especially with regard to LDPE microplastics, has a substantial effect on human health and the food chain.

In the field of ecology and the environment, LDPE microplastics rank as the second most important scientific issue, according to Barnes *et al.* (2009). LDPE Microplastics are extensively distributed across aquatic environments, according to a number of studies (Derraik, 2002). Many studies have shown that LDPE microplastics may accumulate in the body and cause harm to a wide range of animals, especially those that live in water (Gambardella *et al.,* 2017; Anbumani and Kakkar, 2018). However, terrestrial ecosystems have received a great deal less attention regarding LDPE microplastics than aquatic ones. According to Horton *et al.* (2017), plastic contamination in terrestrial ecosystems may be four to twenty-three times higher than that in seas, with land being the primary source of LDPE microplastic pollution in aquatic settings.

Reports on the state of microplastic pollution in the terrestrial ecosystem have also been published. Prior research has demonstrated the presence of microplastics in a broad range of soil types, including industrial, coastal, and farming soils (Fuller and Gautam, 2016). Additionally, people could ingest plastic particles by the food chain, which could be detrimental to their health. Thus, studies on the effects of microplastics on terrestrial animals can contribute to our knowledge of the toxicity of plastic particles in the environment and support the need for environmental management.

In many species, acetylcholine esterase acts as a biomarker of neurotoxicity by breaking down acetylcholine to counteract the neurotoxic effects of contaminants. Earthworms are vital to terrestrial ecosystems and are a valuable biomarker for toxicological studies. It was unknown how earthworms were affected by microplastics in terms of oxidative stress and neurotoxicity. To investigate the ecological toxicity of microplastics, earthworms must be chosen. The purpose of this study was to examine the neurotoxicity response, as shown by AChE activity, in earthworms (*Eudrilus eugeniae*) following exposure to LDPE microplastics containing nanoparticles and native bacteria. For this reason, LDPE was utilized as a representative in this investigation.

MATERIALS AND METHODS

Low density polyethylene microplastics

Utilizing sterile spatulas, low-density polyethylene (LDPE) microplastics were gathered from several plastic waste disposal sites and landfills in Gobichettipalayam, Erode District, Tamil Nadu, India, and preserved in polythene bags with appropriate labeling for each sample. A laser particle size analyzer was used to measure the particular size distribution, with a range of 100 µm to 200 µm. For the studies, the LDPE particles had been dried overnight at 40°C after being cleaned twice with 70% ethanol for disinfectants and ultrapure water to eliminate contaminants (Kathiresan, 2003).

Collection And Culture Of Earthworm *Eudrilus Eugeniae*

These earthworms were collected from vermicomposting unit, Nasiyanur, Erode District, Tamil Nadu, India, and collected worms were cultured and further identified using Manual of earthworm identification (Julka, 2001).

Preparation Of Vermibed

A mixture of loam soil, cow dung and partially decomposed leaves in the first vermin bed while in the second bed was a mixture of cow dung, partially decomposed rice straw and rice hull and shredded moist newspapers. The succeeding layers earthworms were reared in garden soil and garden waste in a vermibeds of dimension 4 x 2 x 4.4 feet (length x breadth x height) sufficient for vermiculture with a controlled optimal moisture content of 60 to 70% by adequate watering and

maintained temperature between 26ºC and 28ºC. Nylon net was used to cover the bed to prevent the entry of predators. The earthworms *E. eugeniae* were stored in cement container and two to three weeks aged cow dung (CD) was used as feed substrate to culture the earthworms and sixty five to seventy five per cent of moisture content of the culture tanks were frequently maintained by sprinkling of water. Further, all the worms were well adapted to laboratory conditions before inoculating into the different experimental treatments.

Inoculation of earthworm

Immediately after the addition of additives, earthworms were sorted out from the holding containers, washed with clean water and 25 healthy and matured earthworms *Eudrilus eugeniae* measured, weighed and inoculated into the experimental pots. The setup was placed inside the laboratory under dark and sprinkled with water to maintain that the substrate moisture level which was maintained around 65-75% and checked morning and evening on a daily basis for 60 days. The experimental pots were supplemented with 1.250 L of molasses, 2 L of mineral medium and 750 ml of enriched microorganisms along with plastic waste contaminated soil (Senthilkumar, 2016).

Enzymatic Analysis

Earthworm Sample Collection

The test species are gathered by following specific procedure (Owa *et al.,* 2013). The test species are anaesthetized (10 mL of 95% ethanol to 90 mL of Ringer's solution) and kept in a prechilled mortar and pestle. An ice cold 50mM Tris-sucrose buffer (1:9, w/v, pH 7.5) is added to the mortar and ground well. The homogenate is taken and centrifuged at 3000 rpm at 4°C for 15 min. The supernatant is carefully pipetted out and be used for the desired analytic purpose.

Acetylcholinesterase (ache) Activity

The acetylcholinesterase (AChE) activity is measured by the procedures propounded by Dauberschmidt *et al.* (1997). The principle behind this modified method is the acetylthiocholine iodide on hydrolysis by AChE, yellow byproduct is synthesized, it leads the reaction of acetylthiocholine along with DTNB in adequate milieu. The rate of yellow product is correspondent to the rate of acetylcholinesterase activity which is measured by spectrophotometrical method at 410 nm.

Statistical Analysis

Two-way analyses of variance (ANOVA) (SPSS - Version No.10) are used for computing the level of significance and the difference between various samples with respect to physico-chemical and biochemical parameters.

RESULTS AND DISCUSSION

Acetyl Cholinesterase (ache) Activity

Acetyl cholinesterase activity in the vermicompost of *E. eugeniae* during the period of 60th days was determined. The acetyl cholinesterase activity was increased in all treatments (T1, T2, T3 and T4) from LDPE contaminated soil with nanoparticles and indigenous microorganisms. Initially, the acetyl cholinesterase activity was low (16.38±1.16 µg mL^{-1}). The acetyl cholinesterase activity on 60th day increased to T1 (18.81±1.25 µg mL^{-1}); T2 (19.94±1.06 µg mL^{-1}); T3 (27.79±1.91 µg mL^{-1}) and T4 (38.24±1.67 µg mL^{-1}) in 350mg of LDPE. It was comparatively high when compared to initial (Table 1). No earthworm death was observed during the treatments. Statistically significantly higher levels of AChE were found at 50, 200 and 350 mg/kg LDPE on days 30 and 60 compared with higher concentrations (500 and 650 mg/kg LDPE), and more egested microplastics were recorded on day 60 compared with that on day 30 at the same concentration of 50, 200 and 350 mg/kg LDPE, which indicated microplastics might be accumulated in the bodies of the earthworms.

Acetylcholinesterase (AChE) is considered as the main cholinesterase in earthworms (Rault *et al.,* 2007; Lionetto *et al.,* 2012). In few earthworm species, its activity is reported and biochemically characterized. It is a significant enzyme that play crucial role in the transmission mechanism of nervous system. The neurotransmission takes place at cholinergic synapses by rapid hydrolysis of acetylcholine (neurotransmitter) to choline and acetate (Caselli *et al.,* 2006). Table 1 showed that significantly higher activity of AChE was observed up to 350 mg/kg of LDPE compared with the initial day ($p<0.05$). At 50, 200, 350 mg/kg LDPE, the AChE activity increased with increasing exposure time compared with the initial, and stimulated significantly on day 60. The AChE activity of the 500 and 650 mg/kg LDPE decreased at 30 days when compared with 350 mg/kg LDPE. Present results indicated that AChE activity was enhanced at lower concentrations (50 and 200 mg/kg LDPE) and stimulated up to 350 mg/kg LDPE. But, an enhanced enzyme activity was observed at all concentrations in *Lampito mauritii* exposure to urea (Samal *et al.,* 2017) thus agreeing with our findings.

CONCLUSION

The study showed that LDPE microplastics can inhibit neurotoxicity response to *Eudrilus eugeniae* up to 350 mg/kg LDPE concentrations meanwhile increasing concentrations (500 and 650 mg/kg LDPE) stimulate the neurotoxicity response after exposure 60 days. This result illustrates that microplastics up to 350 mg may have reduced the adverse biochemical effects on earthworms by nanoparticles and indigenous bacteria, which provides an enhancement of soil fertility. LDPE degrading bacteria may exist in the intestinal tract of *E. eugeniae* to degrade LDPE into smaller particle sizes.

Table 1 Acetyl Cholinesterase (ache) Activity Of The Earthworm Grown In Different Concentrations Of Ldpe Contaminated Soil In Various Treatments (t1 To T4) For A Period Of 60 Days (each Value Represents An Average Performance (± Sd) Of Four Replica)

Treatments	Experimental days	Acetyl cholinesterase (AChE)(µg m^{-1}) in Different concentrations of low-density polyethylene						P Value
		0mg	50mg	200mg	350mg	500mg	650mg	
T1 (Earthworm)	0 day	16.38±1.16	16.38±1.16	16.38±1.16	16.38±1.16	16.38±1.16	16.38±1.16	
	30th day	17.42±1.24	17.87±1.76	18.20±1.16	18.54±1.66	18.16±1.31	18.00±1.09	0.000001*
	60th day	18.18±1.28	18.25±1.98	18.64±1.86	18.81±1.25	18.40±1.33	18.16±1.71	
T2 (Microbes+ Earthworm)	0 day	16.38±1.16	16.38±1.16	16.38±1.16	16.38±1.16	16.38±1.16	16.38±1.16	
	30th day	18.40±1.72	18.63±1.65	19.08±1.88	19.65±1.45	19.31±1.16	19.09±1.16	0.000001*
	60th day	19.02±1.00	19.23±1.89	19.40±1.24	19.94±1.06	19.66±1.25	19.21±1.99	
T3 (Nanoparticles+ Earthworm)	0 day	16.38±1.16	16.38±1.16	16.38±1.16	16.38±1.16	16.38±1.16	16.38±1.16	
	30th day	19.74±1.36	19.96±1.43	20.07±1.18	20.14±0.96	20.05±1.56	19.91±1.03	0.000001*
	60th day	20.95±1.75	22.19±1.34	25.66±1.69	27.79±1.91	26.42±1.94	25.05±1.29	
T4 (Microbes+ Nanoparticles+ Earthworm)	0 day	16.38±1.16	16.38±1.16	16.38±1.16	16.38±1.16	16.38±1.16	16.38±1.16	
	30th day	20.96±1.89	21.16±1.81	21.67±1.69	21.95±1.25	20.24±1.20	20.00±1.76	0.000001*
	60th day	31.10±1.24	34.51±1.87	36.97±1.98	38.24±1.67	37.91±1.81	35.01±1.21	

Two-way ANOVA of acetyl cholinesterase activity

* Significance ($P< 0.05$)

** Insignificance ($P>0.05$)

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