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Effect of Industrial and Hospital Effluents on Lipase Content of the Coelomic Fluid of *Eiseniafetida*

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ABSTRACT: Lipases are enzymes that catalyze the hydrolysis of triacylglycerol to free fatty acids and glycerol. In earthworms, many enzymes are present which help in the bioremediation of contaminated soils. The present study is aimed at revealing the effects of non-treated various industrial and hospital effluents on the biochemistry of coelomic fluid in *Eisenia fetida*. In this study, we analyzed the lipase content of the coelomic fluid of earthworm subjected to different dilutions of industrial and hospital effluents at different time intervals using a commercially available kit. The study reveals that non-treated various industrial and hospital effluents adversely affect the lipase content of the coelomic fluid of earthworm and that this adversity is directly correlated with the duration for which earthworms are exposed.

KEYWORDS: *Eiseniafetida,* Coelomic fluid, lipase.

I. INTRODUCTION

Earthworms belong tothe class Oligochaeta ofphylum Annelida. Evolved in the late Precambrian period (1), earthworms are nocturnal, soft-bodied, saprotrophic invertebrates of the agroecosystem and also one of the major microfaunae of the soil biota. The importance ofearthworms in an ecosystem is well established. Their presence in soil indicates soil health. (2,3) The body of an earthworm is metamerically segmented, both externally and internally, which allows the organism to have flexibility. Earthworms are the first group of animals to have a complete digestive system and closed circulatory system with hemoglobin. The immune system of the earthworms comprises both humoral and cellular defense mechanisms. Coelomic fluid, which is a milky white alkaline liquid present in the body cavity accounts for many physiological processes including locomotion, nutrition, excretion, detoxification of tissues, heavy metal accumulation and protects the internal organs from external shocks and injuries. Coelomocytes present in the coelomic fluid varies in their morphology, population, cytochemistry, and functions according to their source of origin as well as the age of the organism. (4,5,6). Coelomocytes have been shown to exhibit phagocytic activities and secrete various enzymes which help the organism in metabolizing toxic substances.(7,8,9). The ability of earthworms to decompose waste materials is proving to be an economical and environmentally preferred technology over conventional composting techniques. Earthworm diversity has been studied for developing bioindicators of soil health associated with pollution, pesticides, and heavy metal degradation. The body of earthworm function as biofilters and hence can purify and disinfect and detoxify industrial wastewater (10) Earthwormshave been shown to decompose a wide variety of organic wastes ranging from municipal to industrial wastes. (11,12,13,14)However, untreated toxic chemical wastes released by various industries directly into soil adversely affect the local population of earthworms. Different enzymes like cellulase and chitinase (15) lipase and protein (16) are present in earthworms.Ross and Caim(1982)(17) reported the presence of enzymes like cellulase, protease, amylase, lipase in the casts of earthworms which decompose organic materials present in the soil.Lipases are enzymes that breakdown triglycerides and hence form fatty acids and glycerol (18). They also hydrolyze the ester bonds of substates insoluble in water. The present study aims at studying the effects of various industrial wastes on the activity and secretion of Lipase in the coelomic fluid of earthworm *EiseniaFetida.*

II. MATERIALS AND METHODS

Collection of Industrial effluents and Hospital Waste: -

Industrial effluents were collected from paper, stone, and textile industries located at RIICO industrial area, Sanganer, Jaipur, and hospital wastewater were collected from (Santokba Durlabhji Memorial Hospital) SDMH Hospital Jaipur. The wastewater collected was sprinkled on different cultures of earthworm to maintain moisture.

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| Volume 4, Issue 5, May 2021 |

COLLECTION AND CULTURE OF EARTHWORMS: -

Earthworms were collected from the Department of Soil Science and Agricultural Chemistry (S.K Rajasthan Agricultural University). Agricultural Research Station, Durgapura, Jaipur. They were reared and maintained in earthen pots containing soil and cow dung mixture in a 2:1 ratio under laboratory conditions.Optimum temperature of 30°c-35°c and 80-90% moisture of the culture was maintained by constantly sprinkling water in the culture pots. The collected specimens were identified as per the specifications in the key by Julka (1988) (19).

III. EXPERIMENT DESIGN

To study the effect of toxic wastes from industrial and hospital sources, earthworms were subjected to different concentrations of toxic effluents for different time duration. Toxic wastes collected from stone, paper, and textile industries and hospitals sprinkled on earthworm cultures were divided into 5 groups. In each group, 35 earthworms were selected for experiments.

GROUP I- (control) drained with only normal water
GROUP II- drained with paper industry effluent
GROUP III- drained with stone industry effluent
GROUP IV- drained with textile industry effluent
GROUP V- drained with hospital waste

Earthworms of each group except the control group were subjected to 30%, 50% and 70% concentrated toxic effluents for 15, 30, and 45 days each. (Fig 1)

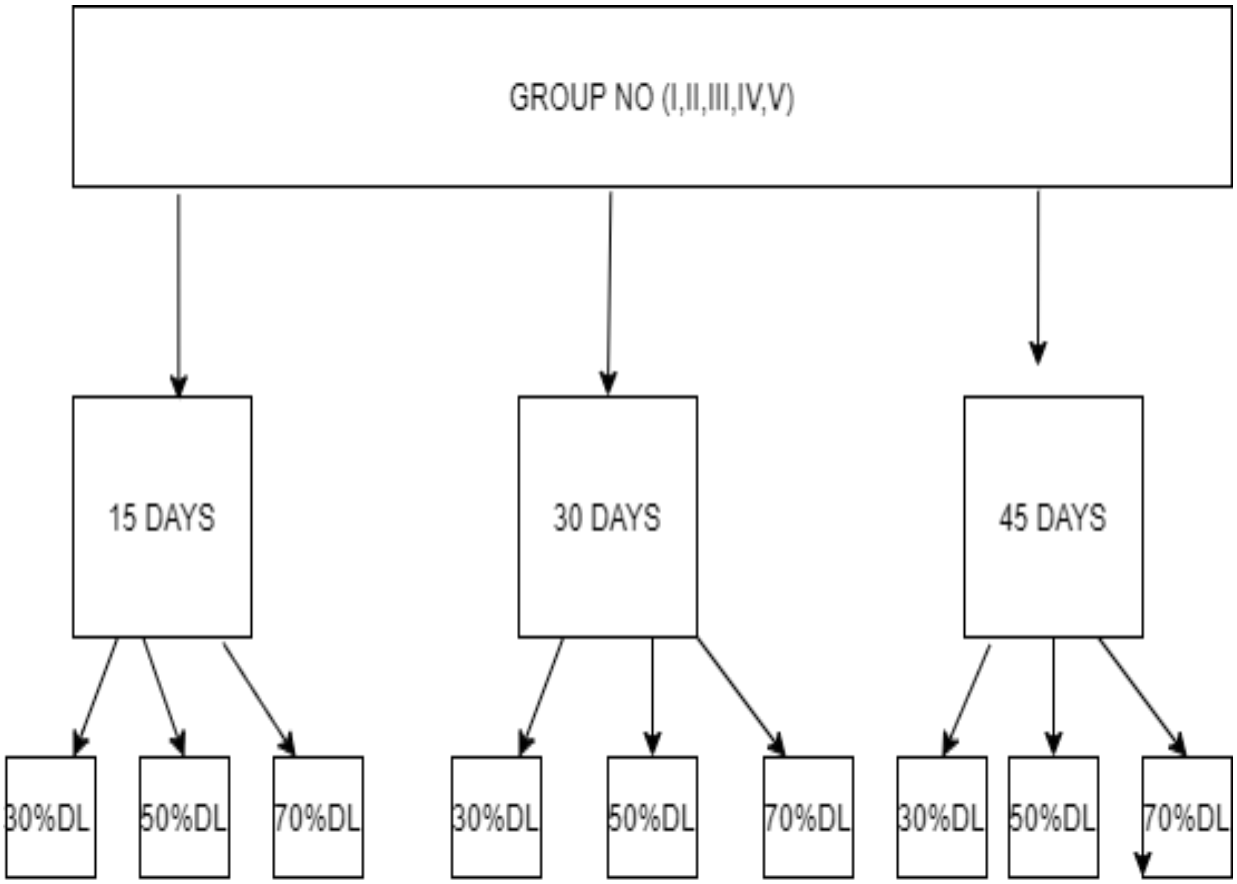

Fig :1 The experimental design

EXTRACTION AND COLLECTION OF COELOMIC FLUID: -

Earthworms from each experimental culture were placed on a cotton mesh and were washed with a gentle flow of distilled water to remove the soil particles. They were then dried on filter paper. The weight of animals was recorded

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| Volume 4, Issue 5, May 2021 |

before and after the experiments. The number of viable worms was recorded. Coelomic fluid was extracted usingthe following three methods,as described by Soumya et al,2017(20) :

Cold Shock Method: In this method earthworms were exposed to cold shock by placing them in Petri plates filled with ice cubes and the fluid released was collected in a clean dry test tube. Around 1.5 to 2 ml of coelomic fluid was collected.

Heat Shock Method:A different set of earthwormswere exposed to hot water (55-60°C), Through this method, 0.5-0.25ml of coelomic fluid wasyielded.

Electric Shock Method. A different set of earthworms were subjected to a mild electric shock of about 5 volts for 30 minutes. Around 2 ml of coelomic fluid was yielded.
Collected coelomic fluid was centrifuged at 3000 rpm for 15 minutes to sediment larger particles and other debris. The supernatant was carefully removed, collected in sterile Eppendorf tubes, and stored at -20 C for further analysis.

Colorimetric analysis of coelomic fluid: -
Coelomic fluid extracted from each batch of earthworm culture was analyzed for Lipase enzyme activity using Infinite lab kits and the results werecalorimetricallyanalyzed at 578nm.

IV. RESULT AND DISCUSSION

An earthworm is the first terrestrial invaded organism and serving in soil with millions of micro-organisms. The social, economic, and environmental importance of earthworms has been realized fora long time. Because of this,it was chosen as an experimental animal. In the present study remarkable decrease in the body weights of the experimental animals was observed in comparison to the body weights of the control animals. (Table 1) It was observed that in Group II and Group III 30% of animals were viable, while in Group IVabsolute mortality was observed in all the dilutions. The experimental animals showed an increase in lipase activity in all the dilutions and also even after 45 days of exposure to industrial effluent (Table 2,3,4,5). Our results are in accordance with the findings of Girdhar and Indira (1997) (21).
All animals exhibit biochemical responses against environmental stress. This is regarded as early warning signals of pollution of the environment. Industrial wastewater has a high content of lead (Pb) and cadmium (Cd). (22,23). When the earthworms are exposed to toxic levels of Pb and Cd, the lipid content decreases as demand for lipids increases under stress conditions. (24,). Amudha et al in 2002 (25) reported dairy effluent-induced alterations in the protein-carbohydrate and lipid metabolism of freshwater fish Oreochromis mossambicus. Similarly, Shukla and Kumar in 2004(26) reported a decrease in lipid content after earthworms were exposed to pesticides endosulfan, and cypermethrin. To overcome this stress energy is required, which is obtained by breaking down of lipids into free fatty acids by lipase enzyme. Hence increase in lipase enzyme occurs when the animal is exposed to wastewater in different dilutions. The free fatty acids undergo β oxidation to form Acetyl CoA which enters the TCA cycle and causes the release of energy. Thus, it is evident that industrial effluents pollute the water bodies as well as the soil of the surrounding areas, largely affecting the fauna of the area.

TABLE 1: Effect of Industrial Effluents on the Body Weight of experimental animal *Eisenia fetida*

INDUSTRY	MEAN BODY WEIGHT (gms)	
	INITIAL BODY WEIGHT	FINAL BODY WEIGHT
CONTROLS	13.0	12.8
PAPER INDUSTRY	10.8	4.6
STONE INDUSTRY	9.76	3.2
TEXTILE INDUSTRY	12.5	2.4
HOSPITAL WASTE	11.9	5.7
Number of Earthworms in each group =35		

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| Volume 4, Issue 5, May 2021 |

Table 2: Lipase enzyme content in the coelomic fluid of *Eisenia fetida* after exposure to effluents from Paper Industry

No of Days	Control					Paper Industry				
	Pure water	10% DL	30% DL	50% DL	70% DL	Pure water	10% DL	30%DL	50% DL	70% DL
15	30	1.2	3	5.2	7.4	17	1.8	5.2	8.9	10.2
30	30	1.2	3	5.2	7.4	14	1.5	1.2	7.5	10.2
45	30	1.2	3	5.2	7.4	11	1.4	3.8	5.9	8.1

Number of Earthworms in each group =35
UNIT µ/L

Table 3: Lipase enzyme content in the coelomic fluid of *Eisenia fetida* after exposure to effluents from Stone Industry

No of Days	Control					Stone Industry				
	Pure water	10% DL	30% DL	50% DL	70% DL	Pure water	10% DL	30%DL	50% DL	70% DL
15	30	1.2	3	5.2	7.4	19	2	6	10.1	13.6
30	30	1.2	3	5.2	7.4	12	1	3.2	6.2	8.9
45	30	1.2	3	5.2	7.4	19	2.2	5.9	10.1	14.6

Table 4: Lipase enzyme content in the coelomic fluid of *Eisenia fetida* after exposure to effluents from Textile Industry

No of Days	Control					Textile Industry				
	Pure water	10% DL	30% DL	50% DL	70% DL	Pure water	10% DL	30%DL	50% DL	70% DL
15	30	1.2	3	5.2	7.4	00	00	00	00	00
30	30	1.2	3	5.2	7.4	00	00	00	00	00
45	30	1.2	3	5.2	7.4	00	00	00	00	00

Number of Earthworms in each group =35,100 % mortality was observed in all dilutions.
UNIT µ/L

| ISSN: 2582-7219 | www.ijmrset.com | Impact Factor: 4.988

| Volume 4, Issue 5, May 2021 |

Table 5: Lipase enzyme content in the coelomic fluid of *Eisenia fetida* after exposure to effluents from Hospitals

No of Days	Control					Hospital Waste				
	Pure water	10% DL	30% DL	50% DL	70% DL	Pure water	10% DL	30%DL	50% DL	70% DL
15	30	1.2	3	5.2	7.4	16	1.7	3.8	6.2	8.2
30	30	1.2	3	5.2	7.4	23	2.6	7.0	12.6	16.7
45	30	1.2	3	5.2	7.4	8.3	1.0	2.8	4.8	6.5

V. CONCLUSION AND FUTURE WORK

Several organic and inorganic chemicals are used in the textile and stone and paper industries. The industrial effluent and Hospital wastewater are filled with toxic chemicals, this wastewater becomes part of the water ecology of the surrounding cultivable land, of which earthworms form a major component. They bioaccumulate these chemicals and also bio-transform them. Thus, earthworms can be used as useful bioindicators to protect the environment.
Further research is required to eradicate the damage caused by these effluents and also what techniques can be developed to alter the damage caused by the industrial effluents, to make the soil healthy again. Also, it is the need of the hour to educate people about the harmful and hazardous effects of these chemicals on the environment and human life.

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| Volume 4, Issue 5, May 2021 |

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