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Influence of Various Concentrations of Detergent on Growth and Reproduction of Different Epigeic Earthworm Species

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Abstract: The present study was designed to find out the influence of various concentration of detergents on the growth and reproduction of different epigeic earthworm species (*Eudrilus eugeniae*, *Eisenia fetida*, *Perionyx excavatus*). The epigeic earthworm, *Eudrilus eugeniae* showed good growth rate at 1 g concentration of detergent as compared to other two species whereas rate of cocoon production was more at 0.1 g concentration of detergent but, *Eisenia fetida* showed better results in control than that of various other detergent concentrations and rate of cocoon production was also good at 0.1 g concentration. In case of *Perionyx excavates* 1 g concentration favoured for better biomass and growth rate, whereas sexual maturity was better in 0.1 g concentration of detergent and rate of cocoon production favoured in case of control-CM.

Keywords: Detergent, Earthworm, Growth rate, Reproduction, Epigeic, Cocoon, Biomass, Sexual maturity

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Introduction

Nowadays, detergent is part of our life commonly available in powder or in concentrated solution. These are surfactants which have good cleaning properties and do not form insoluble salts with hard water ions (IUPAC, 1997). Household surfactants are composed of various pollutants that significantly impact aquatic ecosystems (Bautista-Toledo *et al.*, 2018). Appreciable quantity of surfactant is present in the sewage due to effluent of domestic waste and surfactant-based industries.

The earthworms are important components of natural agriculture ecosystems, functioning in the biodegradation of large quantity of organic waste materials and comprises key link in the food chains (Edwards, 2013). They are found in a wide range of habitats throughout the world, having adapted too many different types of soil types as well as lakes and streams. Earthworms are considered as the primary test organisms for assessing soil contamination among terrestrial invertebrates. The earthworm population is

influenced by various factors such as soil type, temperature, moisture, pH, C:N ratio quality and quantity of organic waste, which maybe from plant residues and animal or human waste applied to land (Baker *et al.*, 1992). Soil texture can also influence the earthworm population because of its effects on the other soil properties, such as soil moisture relation, nutrients status and cation exchange capacity (Jégou *et al.*, 2001). All of such factors can have prominent influence on earthworm population.

A detergent is a surfactant or a mixture of surfactants with cleaning properties, including laundry and fresh water detergents (IUPAC, 1997). They are also used in pesticide formulation and for dispersing oil spills at sea. These detergents are poorly broken down in rivers as well as soils and may cause toxic effect to soil organisms (Light Owless, 2004). The unregulated release of detergents into aquatic environments and agricultural landmass can lead to diminished biological productivity and activity which potentially resulting in negative ecological and economic consequences. Hence, the present study was designed to find out the influence of various concentration of detergents on the growth and reproduction of different epigeic earthworm species (*Eudrilus eugeniae*, *Eisenia fetida*, *Perionyx excavatus*).

Materials and Methods

Collection of earthworms

Three different epigeic earthworms such as *Eudrilus eugeniae*, *Eisenia fetida* and *Perionyx excavatus* were collected from the stock culture maintained at vermitechnology laboratory in Department of Zoology, Karnatak University, Dharwad, India.

Preparation of food

The cattle manure was powdered and the detergent 'Surf-excel' in the concentrations 0.1g, 0.5g and 1g was selected and mixed in known weight with cattle manure. Sufficient water was added to maintain 70-80% moisture content and

kept for primary degradation for about one week so as to initiate the microbial action.

Inoculation of worms

One week aged five juveniles of all three epigeic earthworm species having similar weight were inoculated in the individual pot containing different concentrations of detergent (0.1 g, 0.5 g and 1 g) with sufficient amount of cattle manure (500 g) along with control-CM in triplicates and they were covered with mesh net. Moisture content (70-80%) and sufficient food was maintained throughout the experiment.

Investigations

Weekly observations were made with respect to biomass, sexual maturity and cocoon production. Growth rate was calculated by using biomass data as per formula given by Biradar *et al.* (1999):

$$\text{Growth rate (mg/day)} = \frac{(\text{wto} - \text{wti})}{N} \times 1000$$

Where, wto=weight of earthworm on the day of observation; wti=initial weight; N= number of days on the day of observation.

Statistical analysis

The statistical analysis such as significance variation was carried out by using ANOVA of SPSS software (21.0v).

Results

Biomass

Table 1 represents the data of biomass (g) of the epigeic earthworm, *Eudrilus eugeniae* cultured at different concentrations of detergent (0.1 g, 0.5 g and 1 g). The initially inoculated earthworm (IW) biomass was about 0.008-0.009 g. There was steady increase in the biomass, the maximum mean biomass of *E. eugeniae* was observed in 1 g detergent (0.886 ± 0.13 g) followed by 0.1 g detergent (0.841 ± 0.08 g), Control-CM (0.471 ± 0.1 g) and least in 0.5 g detergent (0.461 ± 0.1 g). No significant difference was observed (P=0.797) in the biomass of *E. eugeniae* among (Table 1) and between different concentration of detergents and Control-CM as shown in Table 2.

Table 1: Biomass (g) of the epigeic earthworm, *Eudrilus eugeniae* cultured at various concentrations of detergent (0.1 g, 0.5 g and 1 g) and control-CM with their significant difference among them with F and P-values. Values are Mean $\pm$ SE

S. No.	Weeks	Detergent			Control-CM	Significant difference among them with F and P- values
		0.1 g	0.5 g	1 g		
1	I W	0.009 $\pm$ 0.0002	0.008 $\pm$ 0.0001	0.009 $\pm$ 0.0001	0.009 $\pm$ 0.0004	F=0.341 P=0.797
2	1	0.022 $\pm$ 0.0001	0.041 $\pm$ 0.0002	0.022 $\pm$ 0.004	0.068 $\pm$ 0.0001	
3	2	0.054 $\pm$ 0.02	0.172 $\pm$ 0.08	0.067 $\pm$ 0.02	0.147 $\pm$ 0.07	
4	3	0.189 $\pm$ 0.00	0.210 $\pm$ 0.21	0.194 $\pm$ 0.12	0.214 $\pm$ 0.21	
5	4	0.285 $\pm$ 0.01	0.310 $\pm$ 0.31	0.317 $\pm$ 0.20	0.331 $\pm$ 0.33	
6	5	0.498 $\pm$ 0.001	0.409 $\pm$ 0.40	0.499 $\pm$ 0.29	0.311 $\pm$ 0.31	
7	6	0.613 $\pm$ 0.04	0.482 $\pm$ 0.48	0.565 $\pm$ 0.32	0.332 $\pm$ 0.33	
8	7	0.782 $\pm$ 0.15	0.548 $\pm$ 0.54	0.937 $\pm$ 0.50	0.529 $\pm$ 0.52	
9	8	0.808 $\pm$ 0.12	0.583 $\pm$ 0.58	0.954 $\pm$ 0.48	0.557 $\pm$ 0.55	
10	9	0.985 $\pm$ 0.15	0.589 $\pm$ 0.58	1.041 $\pm$ 0.53	0.640 $\pm$ 0.64	
11	10	1.076 $\pm$ 0.17	0.597 $\pm$ 0.59	1.158 $\pm$ 0.60	0.613 $\pm$ 0.61	
12	11	1.176 $\pm$ 0.20	0.610 $\pm$ 0.61	1.362 $\pm$ 0.71	0.613 $\pm$ 0.61	
13	12	1.178 $\pm$ 0.22	0.550 $\pm$ 0.55	1.372 $\pm$ 0.69	0.568 $\pm$ 0.56	
14	13	1.304 $\pm$ 0.20	0.632 $\pm$ 0.63	1.324 $\pm$ 0.66	0.649 $\pm$ 0.64	
15	14	1.505 $\pm$ 0.20	0.643 $\pm$ 0.64	1.411 $\pm$ 0.72	0.730 $\pm$ 0.73	
16	15	1.565 $\pm$ 0.22	0.657 $\pm$ 0.65	1.510 $\pm$ 0.77	0.755 $\pm$ 0.75	
17	16	1.565 $\pm$ 0.23	0.647 $\pm$ 0.64	1.593 $\pm$ 0.81	0.725 $\pm$ 0.72	
18	17	1.523 $\pm$ 0.20	0.608 $\pm$ 0.60	1.620 $\pm$ 0.81	0.681 $\pm$ 0.68	
	Mean $\pm$SE	0.841 $\pm$ 0.08	0.461$\pm$ 0.10	0.886 $\pm$ 0.13	0.471$\pm$ 0.10	

Table 2: Significant difference between the biomass of the epigeic earthworm, *Eudrilus eugeniae* cultured at various concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM

Detergent	0.1 g	0.5 g	1 g	Control-CM
0.1 g	-	0.514	0.940	0.525
0.5 g	0.514	-	0.469	0.986
1 g	0.940	0.469	-	0.479
Control-CM	0.525	0.986	0.479	-

Table 3 represents the data of biomass (g) of the epigeic earthworm, *Eisenia fetida* cultured at different concentrations of detergent (0.1 g, 0.5 g, and 1 g) and Control-CM. The biomass of inoculated *E. fetida* (IW) was about 0.0008 g. Steady increase in the biomass was noticed and the maximum mean biomass was observed in 0.1 g detergent (0.539 $\pm$ 0.03 g) followed by Control-CM (0.495 $\pm$ 0.04 g), 0.5 g detergent (0.454 $\pm$ 0.05 g) and least in 1 g detergent (0.003 $\pm$ 0.00001 g). No significant difference (P=0.071) was noticed among (Table 3) and between different concentration of detergent and Control-CM except

between 0.1 g detergent and 1 g detergent (P = 0.022), 0.5 g detergent and 1 g detergent (P = 0.044), 1 g detergent and Control (P = 0.032) as shown in the Table 4.

Table 5 represents the data of biomass (g) of the epigeic earthworm, *Perionyx excavatus* cultured at different concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM. The biomass of inoculated *P. excavates* (IW) was 0.006 g. The trend of biomass increase was noticed with the maximum mean biomass of *P. excavates* was observed in 1 g detergent concentration (0.253 $\pm$

Table 3: Biomass (g) of the epigeic earthworm, *Eisenia fetida* cultured at various concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM with their significant difference among them with F and P-values. Values are in Mean $\pm$ SE

S. No.	Weeks	Detergent			Control-CM	Significant difference among them with F and P-values
		0.1 g	0.5 g	1 g		
1	IW	0.008 $\pm$ 0.0001	0.008 $\pm$ 0.0002	0.008 $\pm$ 0.0002	0.008 $\pm$ 0.00001	F= 3.459 P= 0.071
2	1	0.086 $\pm$ 0.0001	0.064 $\pm$ 0.01	0.041 $\pm$ 0.0001	0.092 $\pm$ 0.01	
3	2	0.217 $\pm$ 0.04	0.186 $\pm$ 0.05	0.010 $\pm$ 0.002	0.218 $\pm$ 0.07	
4	3	0.369 $\pm$ 0.07	0.320 $\pm$ 0.04	-	0.437 $\pm$ 0.06	
5	4	0.380 $\pm$ 0.01	0.298 $\pm$ 0.16	-	0.424 $\pm$ 0.05	
6	5	0.493 $\pm$ 0.03	0.377 $\pm$ 0.19	-	0.616 $\pm$ 0.06	
7	6	0.556 $\pm$ 0.00	0.446 $\pm$ 0.23	-	0.810 $\pm$ 0.10	
8	7	0.593 $\pm$ 0.03	0.489 $\pm$ 0.25	-	0.721 $\pm$ 0.05	
9	8	0.615 $\pm$ 0.04	0.506 $\pm$ 0.25	-	0.464 $\pm$ 0.23	
10	9	0.607 $\pm$ 0.06	0.530 $\pm$ 0.27	-	0.513 $\pm$ 0.25	
11	10	0.562 $\pm$ 0.06	0.547 $\pm$ 0.27	-	0.564 $\pm$ 0.28	
12	11	0.632 $\pm$ 0.08	0.599 $\pm$ 0.30	-	0.531 $\pm$ 0.26	
13	12	0.579 $\pm$ 0.07	0.594 $\pm$ 0.29	-	0.499 $\pm$ 0.25	
14	13	0.738 $\pm$ 0.05	0.576 $\pm$ 0.28	-	0.565 $\pm$ 0.28	
15	14	0.772 $\pm$ 0.06	0.618 $\pm$ 0.31	-	0.577 $\pm$ 0.28	
16	15	0.855 $\pm$ 0.09	0.654 $\pm$ 0.33	-	0.622 $\pm$ 0.31	
17	16	0.833 $\pm$ 0.10	0.675 $\pm$ 0.34	-	0.629 $\pm$ 0.31	
18	17	0.812 $\pm$ 0.08	0.679 $\pm$ 0.34	-	0.620 $\pm$ 0.31	
	Mean $\pm$ SE	0.539 $\pm$ 0.03	0.454 $\pm$ 0.05	0.003 $\pm$ 0.00001	0.495 $\pm$ 0.04	

Table 4: Significant difference between the biomass of the epigeic earthworm, *Eisenia fetida* cultured at various concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM

Detergent	0.1 g	0.5 g	1 g	Control-CM
0.1 g	-	0.666	0.022	0.824
0.5 g	0.666	-	0.044	0.833
1 g	0.022	0.044	-	0.032
Control-CM	0.824	0.833	0.032	-

0.02 g), followed by 0.1 g detergent (0.191 $\pm$ 0.01 g). The mean biomass was similar in case of 0.5 g detergent (0.189 $\pm$ 0.01 g) and Control-CM (0.189 $\pm$ 0.01 g). No significant difference (P=0.228) was observed among (Table 5) and between different concentration of detergent (Table 6).

Growth rate

Table 7 represents the data of growth rate (mg/day) of the epigeic earthworm, *Eudrilus eugeniae* cultured at different concentrations of

detergent (0.1 g, 0.5 g and 1 g) and Control-CM. The growth rate was calculated by using the biomass of earthworms. Maximum growth rate among all was noticed with 1 g detergent (17.614 $\pm$ 9.21) on 11th week. The mean maximum growth rate was observed with 1 g detergent (12.600 $\pm$ 1.59) followed by 0.1 g detergent (11.830 $\pm$ 0.76), Control-CM (8.143 $\pm$ 1.60) and least in 0.5 g detergent (8.031 $\pm$ 0.64). There is no significant difference (P=0.923) observed in growth rate of

Table 5: Biomass (g) of the epigeic earthworm, *Perionyx excavates* cultured at various concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM with their significant difference among them with F and P-values. Values are in Mean $\pm$ SE

S. No.	Weeks	Detergent			Control (CM)	Significant difference among them with F and P-value
		0.1 g	0.5 g	1 g		
1	IW	0.006 $\pm$ 0.0001	0.006 $\pm$ 0.0001	0.006 $\pm$ 0.0002	0.006 $\pm$ 0.0001	F=1.785 P=0.228
2	1	0.017 $\pm$ 0.0001	0.015 $\pm$ 0.0002	0.052 $\pm$ 0.03	0.019 $\pm$ 0.0002	
3	2	0.049 $\pm$ 0.01	0.062 $\pm$ 0.01	0.027 $\pm$ 0.00	0.043 $\pm$ 0.01	
4	3	0.098 $\pm$ 0.02	0.074 $\pm$ 0.03	0.085 $\pm$ 0.03	0.079 $\pm$ 0.00	
5	4	0.116 $\pm$ 0.02	0.090 $\pm$ 0.03	0.107 $\pm$ 0.05	0.092 $\pm$ 0.01	
6	5	0.123 $\pm$ 0.02	0.211 $\pm$ 0.07	0.159 $\pm$ 0.04	0.119 $\pm$ 0.02	
7	6	0.175 $\pm$ 0.02	0.191 $\pm$ 0.07	0.291 $\pm$ 0.08	0.178 $\pm$ 0.02	
8	7	0.177 $\pm$ 0.02	0.136 $\pm$ 0.00	0.262 $\pm$ 0.06	0.167 $\pm$ 0.03	
9	8	0.184 $\pm$ 0.03	0.146 $\pm$ 0.01	0.258 $\pm$ 0.08	0.168 $\pm$ 0.02	
10	9	0.211 $\pm$ 0.03	0.163 $\pm$ 0.01	0.288 $\pm$ 0.06	0.190 $\pm$ 0.01	
11	10	0.219 $\pm$ 0.03	0.186 $\pm$ 0.00	0.311 $\pm$ 0.05	0.203 $\pm$ 0.01	
12	11	0.243 $\pm$ 0.01	0.205 $\pm$ 0.00	0.338 $\pm$ 0.04	0.236 $\pm$ 0.03	
13	12	0.243 $\pm$ 0.00	0.213 $\pm$ 0.00	0.334 $\pm$ 0.02	0.227 $\pm$ 0.02	
14	13	0.274 $\pm$ 0.01	0.261 $\pm$ 0.01	0.319 $\pm$ 0.06	0.276 $\pm$ 0.03	
15	14	0.320 $\pm$ 0.03	0.332 $\pm$ 0.03	0.422 $\pm$ 0.10	0.342 $\pm$ 0.03	
16	15	0.334 $\pm$ 0.04	0.364 $\pm$ 0.01	0.444 $\pm$ 0.07	0.349 $\pm$ 0.04	
17	16	0.337 $\pm$ 0.06	0.368 $\pm$ 0.02	0.448 $\pm$ 0.09	0.358 $\pm$ 0.03	
18	17	0.321 $\pm$ 0.08	0.376 $\pm$ 0.03	0.404 $\pm$ 0.05	0.354 $\pm$ 0.03	
	Mean $\pm$ SE	0.191 $\pm$ 0.01	0.189$\pm$ 0.01	0.253$\pm$ 0.02	0.189$\pm$ 0.01	

Table 6: Significant difference between the biomass of the epigeic earthworm, *Perionyx excavates* cultured at cattle manure with various concentrations of detergent (0.1 g, 0.5 g and 1 g)

Detergent	0.1 g	0.5 g	1 g	Control-CM
0.1 g	-	0.807	0.115	0.944
0.5 g	0.807	-	0.078	0.862
1 g	0.115	0.078	-	0.103
Control-CM	0.944	0.862	0.103	-

E. eugeniae among (Table 7) and between different concentrations of detergent (Table 8).

Table 9 represents the data of growth rate (mg/day) of the epigeic earthworm, *Eisenia fetida* cultured at different concentrations of detergent

(0.1 g, 0.5 g and 1 g) and Control-CM. Maximum growth rate (20.420 $\pm$ 3.29) was observed in Control-CM on 3rd week. The mean maximum growth rate of *E. fetida* was observed in Control-CM (9.932 $\pm$ 0.96) followed by 0.1 g detergent

Table 7: Growth rate (g) of the epigeic earthworm, *Eudrilus eugeniae* cultured at various concentration of detergent (0.1 g, 0.5 g and 1 g) and Control-CM with their significant difference among them with F and P-values. Values are in Mean $\pm$ SE

S. No.	Weeks	Detergent			Control (CM)	Significant difference among them with F and P-value
		0.1 g	0.5 g	1 g		
1	IW	-	-	-	-	F=0.156 P=0.923
2	1	1.980 $\pm$ 0.35	4.638 $\pm$ 0.56	1.838 $\pm$ 0.39	8.495 $\pm$ 1.09	
3	2	3.271 $\pm$ 1.52	11.847 $\pm$ 5.92	4.152 $\pm$ 2.09	10.090 $\pm$ 5.35	
4	3	8.606 $\pm$ 0.34	9.892 $\pm$ 9.89	8.977 $\pm$ 5.90	10.061 $\pm$ 10.06	
5	4	9.877 $\pm$ 0.44	10.964 $\pm$ 10.96	11.122 $\pm$ 7.22	11.736 $\pm$ 11.73	
6	5	13.987 $\pm$ 0.25	11.605 $\pm$ 11.60	14.095 $\pm$ 8.45	8.808 $\pm$ 8.80	
7	6	14.380 $\pm$ 0.97	11.403 $\pm$ 11.40	13.306 $\pm$ 7.70	9.420 $\pm$ 9.42	
8	7	15.785 $\pm$ 3.26	11.121 $\pm$ 11.12	18.992 $\pm$ 10.22	10.734 $\pm$ 10.73	
9	8	14.273 $\pm$ 2.29	10.365 $\pm$ 10.36	16.938 $\pm$ 8.67	9.890 $\pm$ 9.89	
10	9	15.491 $\pm$ 2.24	9.311 $\pm$ 9.31	16.423 $\pm$ 8.46	10.121 $\pm$ 10.12	
11	10	15.255 $\pm$ 2.50	8.487 $\pm$ 8.48	16.455 $\pm$ 8.57	8.713 $\pm$ 8.71	
12	11	15.160 $\pm$ 2.65	7.888 $\pm$ 7.88	17.614 $\pm$ 9.21	7.927 $\pm$ 7.92	
13	12	13.922 $\pm$ 2.72	6.511 $\pm$ 6.51	16.260 $\pm$ 8.23	6.729 $\pm$ 6.72	
14	13	14.239 $\pm$ 2.20	6.920 $\pm$ 6.92	14.486 $\pm$ 7.30	7.106 $\pm$ 7.10	
15	14	15.265 $\pm$ 2.11	6.534 $\pm$ 6.53	14.340 $\pm$ 7.39	7.421 $\pm$ 7.42	
16	15	14.826 $\pm$ 2.15	6.229 $\pm$ 6.22	14.910 $\pm$ 7.33	7.164 $\pm$ 7.16	
17	16	13.900 $\pm$ 2.09	5.755 $\pm$ 5.75	13.910 $\pm$ 7.12	6.453 $\pm$ 6.45	
18	17	12.726 $\pm$ 1.75	5.085 $\pm$ 5.08	13.565 $\pm$ 6.84	5.703 $\pm$ 5.70	
	Mean $\pm$ SE	11.830 $\pm$ 0.76	8.031 $\pm$ 1.64	12.600$\pm$ 1.59	8.143 $\pm$ 1.60	

Table 8: Significant difference between the growth rate of epigeic earthworm, *Eudrilus eugeniae* cultured at cattle manure with various concentrations of detergent (0.1 g, 0.5 g and 1 g)

Detergent	0.1 g	0.5 g	1 g	Control-CM
0.1 g	-	0.670	0.931	0.679
0.5 g	0.670	-	0.610	0.990
1 g	0.931	0.610	-	0.618
Control-CM	0.679	0.990	0.618	-

(9.746 $\pm$ 0.58), 0.5 g detergent (8.173 $\pm$ 0.87) and least in 1 g detergent (0.294 $\pm$ 0.16). Significant difference was noticed in growth rate (P=0.022) of worms among (Table 9) and between different concentrations of detergent except between 0.1 g detergent and 0.5 g detergent (P=0.576), 0.1 detergent and Control-CM (P=0.947) (Table10).

Table 11 represents the data of growth rate

(g/day) of the epigeic earthworm, *Perionyx excavates* cultured at different concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM. Maximum growth rate (8.217 $\pm$ 0.66) was noticed with 1 g detergent on 6th week. The mean maximum growth rate of *P. excavates* was observed in 1 g detergent (4.138 $\pm$ 0.38) followed by 0.1 g detergent (2.994 $\pm$ 0.17), Control-CM (2.855 $\pm$ 0.14) and least in 0.5 g

Table 9: Growth rate (g) of the epigeic earthworm, *Eisenia fetida* cultured at various concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM with their significant difference among them with F and P-values. Values are in Mean $\pm$ SE.

S. No.	Weeks	Detergent			Control (CM)	Significant difference among them with F and P-value
		0.1 g	0.5 g	1 g		
1	IW	-	-	-	-	F=5.714 P=0.022
2	1	11.123 $\pm$ 0.53	8.000 $\pm$ 2.44	4.942 $\pm$ 1.34	12.085 $\pm$ 2.69	
3	2	14.947 $\pm$ 3.46	12.709 $\pm$ 3.59	0.352 $\pm$ 0.20	15.033 $\pm$ 5.35	
4	3	17.203 $\pm$ 3.45	14.853 $\pm$ 2.36	0.000 $\pm$ 0.00	20.420 $\pm$ 3.29	
5	4	12.345 $\pm$ 1.03	10.450 $\pm$ 5.65	0.000 $\pm$ 0.00	14.861 $\pm$ 1.99	
6	5	13.850 $\pm$ 0.89	10.632 $\pm$ 5.63	0.000 $\pm$ 0.00	17.369 $\pm$ 1.77	
7	6	13.057 $\pm$ 0.17	10.495 $\pm$ 5.55	0.000 $\pm$ 0.00	19.096 $\pm$ 2.50	
8	7	11.928 $\pm$ 0.64	9.877 $\pm$ 5.11	0.000 $\pm$ 0.00	14.555 $\pm$ 1.05	
9	8	10.833 $\pm$ 0.88	8.949 $\pm$ 4.55	0.000 $\pm$ 0.00	8.192 $\pm$ 4.13	
10	9	9.499 $\pm$ 1.05	8.338 $\pm$ 4.26	0.000 $\pm$ 0.00	8.059 $\pm$ 4.07	
11	10	7.914 $\pm$ 0.96	7.736 $\pm$ 3.90	0.000 $\pm$ 0.00	7.982 $\pm$ 3.99	
12	11	8.105 $\pm$ 1.09	7.711 $\pm$ 3.89	0.000 $\pm$ 0.00	6.831 $\pm$ 3.42	
13	12	6.597 $\pm$ 0.77	7.014 $\pm$ 3.50	0.000 $\pm$ 0.00	5.882 $\pm$ 2.96	
14	13	8.046 $\pm$ 0.65	6.278 $\pm$ 3.15	0.000 $\pm$ 0.00	6.150 $\pm$ 3.09	
15	14	7.797 $\pm$ 0.71	6.257 $\pm$ 3.15	0.000 $\pm$ 0.00	5.840 $\pm$ 2.92	
16	15	8.065 $\pm$ 0.94	6.179 $\pm$ 3.12	0.000 $\pm$ 0.00	5.873 $\pm$ 2.94	
17	16	7.363 $\pm$ 0.91	5.986 $\pm$ 3.01	0.000 $\pm$ 0.00	5.574 $\pm$ 2.79	
18	17	6.752 $\pm$ 0.74	5.659 $\pm$ 2.83	0.000 $\pm$ 0.00	4.970 $\pm$ 2.50	
	Mean $\pm$ SE	9.746 $\pm$ 0.58	8.173 $\pm$ 0.87	0.294 $\pm$ 0.16	9.932 $\pm$ 0.96	

Table 10: Significant difference between the growth rate of epigeic earthworm, *Eisenia fetida* cultured at cattle manure with various concentrations of detergent (0.1 g, 0.5 g and 1 g)

Detergent	0.1 g	0.5 g	1 g	Control-CM
0.1 g	-	0.576	0.008	0.947
0.5 g	0.576	-	0.019	0.533
1 g	0.008	0.019	-	0.007
Control-CM	0.947	0.533	0.007	-

detergent (2.794 $\pm$ 0.19). There is no significant difference (P=0.215) observed in growth rate of worms among (Table 11) and in between different concentration of detergent (Table 12).

Sexual maturity

Table 13 represents per cent sexual maturity of the epigeic earthworm, *Eudrilus eugeniae* cultured at different concentrations of detergent (0.1 g, 0.5

g and 1 g) and Control-CM. The first sexual maturity was witnessed in 0.5 g detergent and Control-CM on 4th week (20.00 $\pm$ 20.00 and 13.33 $\pm$ 13.33, respectively) whereas late maturity was observed on 5th week with 13.33 $\pm$ 13.33 in 1 g detergent and on 6th week with 33.33 $\pm$ 6.66 in 0.1 g detergent. 100% sexual maturity was observed between 8th week to 17th week in 0.1 g whereas in case of Control-CM and 0.5 g detergent it was

Table 11: Growth rate (g) of the epigeic earthworm, *Perionyx excavates* cultured at various concentration of detergent (0.1g, 0.5g and 1g) and Control-CM with their significant difference among them with F and P-values. Values are in Mean $\pm$ SE

S. No.	Weeks	Detergent			Control (CM)	Significant difference among them with F and P-value
		0.1 g	0.5 g	1 g		
1	IW	-	-	-	-	F=1.856 P=0.215
2	1	1.285 $\pm$ 0.11	1.399 $\pm$ 0.44	6.890 $\pm$ 5.18	1.761 $\pm$ 0.75	
3	2	3.100 $\pm$ 1.26	4.066 $\pm$ 0.88	1.504 $\pm$ 0.44	2.584 $\pm$ 1.05	
4	3	4.415 $\pm$ 1.07	3.276 $\pm$ 1.47	3.777 $\pm$ 1.53	3.460 $\pm$ 0.33	
5	4	3.926 $\pm$ 0.99	3.032 $\pm$ 1.29	3.623 $\pm$ 1.86	3.038 $\pm$ 0.49	
6	5	3.340 $\pm$ 0.78	2.820 $\pm$ 1.35	4.392 $\pm$ 1.30	3.213 $\pm$ 0.58	
7	6	4.021 $\pm$ 0.51	4.424 $\pm$ 1.86	8.217 $\pm$ 0.66	4.090 $\pm$ 0.53	
8	7	3.490 $\pm$ 0.52	2.660 $\pm$ 0.19	5.230 $\pm$ 1.41	3.277 $\pm$ 0.76	
9	8	3.182 $\pm$ 0.55	2.513 $\pm$ 0.31	4.501 $\pm$ 1.48	2.883 $\pm$ 0.43	
10	9	3.254 $\pm$ 0.50	2.493 $\pm$ 0.16	4.479 $\pm$ 1.04	2.982 $\pm$ 0.34	
11	10	3.044 $\pm$ 0.47	2.576 $\pm$ 0.11	4.364 $\pm$ 0.76	2.801 $\pm$ 0.25	
12	11	3.080 $\pm$ 0.22	2.594 $\pm$ 0.08	4.314 $\pm$ 0.54	2.982 $\pm$ 0.40	
13	12	2.889 $\pm$ 0.10	2.467 $\pm$ 0.06	3.906 $\pm$ 0.31	2.622 $\pm$ 0.26	
14	13	2.940 $\pm$ 0.18	2.811 $\pm$ 0.14	3.581 $\pm$ 0.83	2.956 $\pm$ 0.35	
15	14	3.206 $\pm$ 0.37	3.403 $\pm$ 0.32	4.249 $\pm$ 1.08	3.428 $\pm$ 0.32	
16	15	3.130 $\pm$ 0.43	3.418 $\pm$ 0.17	4.171 $\pm$ 0.75	3.264 $\pm$ 0.38	
17	16	2.952 $\pm$ 0.55	3.234 $\pm$ 0.18	3.946 $\pm$ 0.82	3.140 $\pm$ 0.35	
18	17	2.645 $\pm$ 0.71	3.110 $\pm$ 0.25	3.347 $\pm$ 0.44	2.918 $\pm$ 0.29	
	Mean $\pm$ SE	2.994 $\pm$ 0.17	2.794 $\pm$ 0.19	4.138 $\pm$ 0.38	2.855 $\pm$ 0.14	

Table 12: Significant difference between the growth rate of the epigeic earthworm, *Perionyx excavates* cultured at cattle manure with various concentrations of detergent (0.1 g, 0.5 g and 1 g)

Detergent	0.1 g	0.5 g	1 g	Control-CM
0.1 g	-	0.769	0.120	0.838
0.5 g	0.769	-	0.075	0.928
1 g	0.120	0.075	-	0.087
Control-CM	0.838	0.928	0.087	-

33.33% from 6th week and 7th week, respectively until end of the experiment. Similarly, in 1 g detergent 66.66% maturity was shown from 9th week till 17th week. No significant difference was noticed (P= 0.516) among (Table 13) and between different concentrations of detergent (Table 14).

Table 15 represents the per cent of sexual maturity of the epigeic earthworm, *Eisenia fetida*

cultured at different concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM. First sexual maturity was observed on 4th week in 0.1 g detergent, 0.5 g detergent and in Control-CM, with 53.33 $\pm$ 6.66, 6.66 $\pm$ 6.66 and 46.66 $\pm$ 17.63, respectively, whereas no sexual maturity was noticed in 1 g detergent as all worms did not survive. 100% sexual maturity was seen on 8th and

Table 13: Sexual maturity (%) of the epigeic earthworm, *Eudrilus eugeniae* cultured at various concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM with their significant difference among them with F and P-values. Values are in Mean $\pm$ SE

S. No.	Weeks	Detergent			Control (CM)	Significant difference among them with F and P-value
		0.1 g	0.5 g	1 g		
1	IW	-	-	-	-	F=0.826 P=0.516
2	1	-	-	-	-	
3	2	-	-	-	-	
4	3	-	-	-	-	
5	4	-	20.00 $\pm$ 20.00	-	13.33 $\pm$ 13.33	
6	5	-	20.00 $\pm$ 20.00	13.33 $\pm$ 13.33	20.00 $\pm$ 20.00	
7	6	33.33 $\pm$ 6.66	20.00 $\pm$ 20.00	26.66 $\pm$ 26.66	33.33 $\pm$ 33.33	
8	7	80.00 $\pm$ 11.54	33.33 $\pm$ 33.33	46.66 $\pm$ 29.05	33.33 $\pm$ 33.33	
9	8	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	53.33 $\pm$ 29.05	33.33 $\pm$ 33.33	
10	9	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
11	10	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
12	11	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
13	12	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
14	13	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
15	14	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
16	15	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
17	16	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
18	17	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
	Mean $\pm$ SE	61.85 $\pm$ 6.39	23.70 $\pm$ 5.62	41.11 $\pm$ 6.52	24.07 $\pm$ 5.72	

Table 14: Significant difference between the sexual maturity of epigeic earthworm, *Eudrilus eugeniae* cultured at cattle manure with various concentrations of detergent (0.1 g, 0.5 g and 1 g)

Detergent	0.1 g	0.5 g	1 g	Control-CM
0.1 g	-	0.211	0.481	0.215
0.5 g	0.211	-	0.552	0.990
1 g	0.481	0.552	-	0.561
Control-CM	0.215	0.990	0.561	-

7th week in 0.1 g detergent and Control-CM, respectively, whereas it was 66.66% on 8th week in 0.5 g detergent. There is a significant difference (P= 0.005) observed among (Table 15) and between different concentrations of detergent except between 0.1 g detergent and 0.5 g detergent (P=0.106), 0.1 g detergent and Control-CM (P=0.982), 0.5 g detergent and Control-CM (P=0.110) (Table 16).

Table 17 represents the percent sexual maturity of the epigeic earthworm, *Perionyx excavates* cultured at different concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM. First sexual maturity was noticed on 4th week in all the concentrations of detergent (Table 17) and Control-CM. 100% sexual maturity was observed on 8th week in the concentration of 0.1 g detergent, 0.5 g detergent and control-CM except in 1 g

Table 13: Sexual maturity (%) of the epigeic earthworm, *Eudrilus eugeniae* cultured at various concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM with their significant difference among them with F and P-values. Values are in Mean $\pm$ SE

S. No.	Weeks	Detergent			Control (CM)	Significant difference among them with F and P-value
		0.1 g	0.5 g	1 g		
1	IW	-	-	-	-	F=0.826 P=0.516
2	1	-	-	-	-	
3	2	-	-	-	-	
4	3	-	-	-	-	
5	4	-	20.00 $\pm$ 20.00	-	13.33 $\pm$ 13.33	
6	5	-	20.00 $\pm$ 20.00	13.33 $\pm$ 13.33	20.00 $\pm$ 20.00	
7	6	33.33 $\pm$ 6.66	20.00 $\pm$ 20.00	26.66 $\pm$ 26.66	33.33 $\pm$ 33.33	
8	7	80.00 $\pm$ 11.54	33.33 $\pm$ 33.33	46.66 $\pm$ 29.05	33.33 $\pm$ 33.33	
9	8	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	53.33 $\pm$ 29.05	33.33 $\pm$ 33.33	
10	9	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
11	10	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
12	11	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
13	12	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
14	13	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
15	14	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
16	15	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
17	16	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
18	17	100.00 $\pm$ 0.00	33.33 $\pm$ 33.33	66.66 $\pm$ 33.33	33.33 $\pm$ 33.33	
	Mean $\pm$ SE	61.85 $\pm$ 6.39	23.70 $\pm$ 5.62	41.11 $\pm$ 6.52	24.07 $\pm$ 5.72	

Table 14: Significant difference between the sexual maturity of epigeic earthworm, *Eudrilus eugeniae* cultured at cattle manure with various concentrations of detergent (0.1 g, 0.5 g and 1 g)

Detergent	0.1g	0.5g	1g	Control-CM
0.1 g	-	0.211	0.481	0.215
0.5 g	0.211	-	0.552	0.990
1 g	0.481	0.552	-	0.561
Control-CM	0.215	0.990	0.561	-

detergent where 100% maturity noticed on 7th week. No significant difference was observed (P=0.422) among (Table17) and between different concentration of detergent (Table18).

Cocoon production

Table19 represents the data of cocoon production of the epigeic earthworm, *Eudrilus eugeniae* cultured at different concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM. Cocoons

were first observed in 0.5 g detergent concentration on 5th week whereas in 1 g detergent and Control-CM it was observed on 6th week but in 0.1 g detergent, it was observed late on 7th week. Cumulative cocoon number for 17 weeks were 13.08 $\pm$ 9.79, 10.297 $\pm$ 10.29, 10.19 $\pm$ 8.69 and 12.429 $\pm$ 12.42 in 0.1 g detergent, 0.5 g detergent, 1 g detergent and Control-CM, respectively. The mean cocoon rate was maximum

Table 15: Sexual maturity (%) of the epigeic earthworm, *Eisenia fetida* cultured at various concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM with their significant difference among them with F and P-values. Values are in Mean $\pm$ SE

S. No.	Weeks	Detergent			Control (CM)	Significant difference among them with F and P-value
		0.1 g	0.5 g	1 g		
1	IW	-	-	-	-	F=9.436 P=0.005
2	1	-	-	-	-	
3	2	-	-	-	-	
4	3	-	-	-	-	
5	4	53.33 $\pm$ 6.66	6.66 $\pm$ 6.66	-	46.66 $\pm$ 17.63	
6	5	73.33 $\pm$ 13.33	20.00 $\pm$ 20.00	-	73.33 $\pm$ 17.63	
7	6	80.00 $\pm$ 11.54	33.33 $\pm$ 17.63	-	73.33 $\pm$ 17.63	
8	7	93.33 $\pm$ 6.66	60.00 $\pm$ 30.55	-	100.00 $\pm$ 0.00	
9	8	100.00 $\pm$ 0.00	66.66 $\pm$ 33.33	-	100.00 $\pm$ 0.00	
10	9	100.00 $\pm$ 0.00	66.66 $\pm$ 33.33	-	100.00 $\pm$ 0.00	
11	10	100.00 $\pm$ 0.00	66.66 $\pm$ 33.33	-	100.00 $\pm$ 0.00	
12	11	100.00 $\pm$ 0.00	66.66 $\pm$ 33.33	-	100.00 $\pm$ 0.00	
13	12	100.00 $\pm$ 0.00	66.66 $\pm$ 33.33	-	100.00 $\pm$ 0.00	
14	13	100.00 $\pm$ 0.00	66.66 $\pm$ 33.33	-	100.00 $\pm$ 0.00	
15	14	100.00 $\pm$ 0.00	66.66 $\pm$ 33.33	-	100.00 $\pm$ 0.00	
16	15	100.00 $\pm$ 0.00	66.66 $\pm$ 33.33	-	100.00 $\pm$ 0.00	
17	16	100.00 $\pm$ 0.00	66.66 $\pm$ 33.33	-	100.00 $\pm$ 0.00	
18	17	100.00 $\pm$ 0.00	66.66 $\pm$ 33.33	-	100.00 $\pm$ 0.00	
	Mean $\pm$ SE	72.222 $\pm$ 5.63	43.703 $\pm$ 6.54	-	71.851 $\pm$ 5.78	

Table 16: Significant difference between the sexual maturity of the epigeic earthworm, *Eisenia fetida* cultured at cattle manure with various concentrations of detergent (0.1g, 0.5g and 1g)

Detergent	0.1 g	0.5 g	1 g	Control-CM
0.1 g	-	0.106	0.002	0.982
0.5 g	0.106	-	0.024	0.110
1 g	0.002	0.024	-	0.002
Control-CM	0.982	0.110	0.002	-

in 0.1 g detergent followed by Control-CM, 1g detergent and least in 0.5 g detergent (Table19). No significant difference (P=0.995) was noticed among (Table 19) and between different concentrations of detergent (Table 20).

Table 21 represents the cocoon production of the epigeic earthworm, *Eisenia fetida* cultured at different concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM. Cocoons were first

observed in 0.1 g detergent, 0.5 g detergent and Control-CM on 5th week, whereas in 1 g detergent concentration no cocoons were found. Cumulative cocoon number for 17 weeks were 46.06 $\pm$ 8.95, 17.927 $\pm$ 11.12, and 29.729 $\pm$ 16.35 in 0.1 g detergent, 0.5 g detergent, and Control-CM, respectively. The mean cocoon rate was maximum in 0.1 g detergent followed by Control-CM and 0.5 g detergent. Significant difference (P= 0.045) was

Table 17: Sexual maturity (%) of the epigeic earthworm, *Perionyx excavates* cultured at various concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM with their significant difference among them with F and P-values. Values are in Mean $\pm$ SE

S. No.	Weeks	Detergent			Control (CM)	Significant difference among them with F and P-value
		0.1 g	0.5 g	1 g		
1	IW	-	-	-	-	F=1.049 P=0.422
2	1	-	-	-	-	
3	2	-	-	-	-	
4	3	-	-	-	-	
5	4	66.66 $\pm$ 17.63	33.33 $\pm$ 13.33	6.66 $\pm$ 6.66	33.33 $\pm$ 13.33	
6	5	73.33 $\pm$ 13.33	33.33 $\pm$ 13.33	40.00 $\pm$ 30.55	46.66 $\pm$ 13.33	
7	6	73.33 $\pm$ 13.33	46.66 $\pm$ 13.33	66.66 $\pm$ 33.33	46.66 $\pm$ 13.33	
8	7	93.33 $\pm$ 6.66	86.66 $\pm$ 6.66	100.00 $\pm$ 0.00	93.33 $\pm$ 6.66	
9	8	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	
10	9	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	
11	10	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	
12	11	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	
13	12	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	
14	13	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	
15	14	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	
16	15	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	
17	16	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	
18	17	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	
	Mean $\pm$ SE	72.59 $\pm$ 5.65	66.66 $\pm$ 5.88	67.40 $\pm$ 6.35	67.77 $\pm$ 5.84	

Table 18: Significant difference between the sexual maturity of the epigeic earthworm, *Perionyx excavates* cultured at cattle manure with various concentrations of detergent (0.1 g, 0.5 g and 1 g)

Detergent	0.1 g	0.5 g	1 g	Control-CM
0.1 g	-	0.512	0.195	0.234
0.5 g	0.512	-	0.871	0.774
1 g	0.195	0.871	-	0.901
Control-CM	0.234	0.774	0.901	-

observed among (Table 21) and only between 0.1 g detergent and 1 g detergent (0.009), 1 g detergent and Control-CM (0.056) (Table 22).

Table 23 represents cocoon production of the epigeic earthworm, *Perionyx excavatus* cultured at different concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM. Cocoons were first observed in 0.1 g detergent and in Control-CM on

5th week, whereas in case of 0.5 g detergent, it was noticed on 8th week, and it was seen late on 9th week in 1 g detergent. Cumulative cocoon number for 17 weeks were 20.195 $\pm$ 6.19, 32.932 $\pm$ 11.4, 1.936 $\pm$ 1.93, 20.229 $\pm$ 8.58 in 0.1 detergent, 0.5 detergent, 1 g detergent and Control-CM, respectively. The mean cocoon rate was maximum in 0.5 g detergent followed by Control-CM and 0.1

Table 19: Cocoon production (worm/week) of the epigeic earthworm, *Eudrilus eugeniae* cultured at various concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM with their significant difference among them with F and P-values. Values are in Mean $\pm$ SE

S. No.	Weeks	Detergent			Control (CM)	Significant difference among them with F and P- value
		0.1 g	0.5 g	1 g		
1	IW	-	-	-	-	F=0.024 P=0.995
2	1	-	-	-	-	
3	2	-	-	-	-	
4	3	-	-	-	-	
5	4	-	-	-	-	
6	5	-	0.733 $\pm$ 0.73	-	-	
7	6	-	0.533 $\pm$ 0.53	0.433 $\pm$ 0.44	1.000 $\pm$ 1.00	
8	7	1.000 $\pm$ 0.76	0.400 $\pm$ 0.40	1.220 $\pm$ 1.22	0.333 $\pm$ 0.33	
9	8	2.500 $\pm$ 2.02	1.000 $\pm$ 1.00	1.110 $\pm$ 1.11	2.776 $\pm$ 2.77	
10	9	1.833 $\pm$ 1.58	2.000 $\pm$ 2.00	0.220 $\pm$ 0.22	1.776 $\pm$ 1.77	
11	10	1.416 $\pm$ 0.96	0.333 $\pm$ 0.33	0.663 $\pm$ 0.38	0.776 $\pm$ 0.77	
12	11	1.583 $\pm$ 0.93	0.800 $\pm$ 0.80	1.666 $\pm$ 1.20	0.553 $\pm$ 0.55	
13	12	0.250 $\pm$ 0.25	0.466 $\pm$ 0.46	0.220 $\pm$ 0.22	0.443 $\pm$ 0.44	
14	13	0.916 $\pm$ 0.68	1.433 $\pm$ 1.43	0.666 $\pm$ 0.66	0.000 $\pm$ 0.00	
15	14	0.583 $\pm$ 0.36	0.533 $\pm$ 0.53	1.220 $\pm$ 1.22	1.666 $\pm$ 1.66	
16	15	1.500 $\pm$ 0.76	1.000 $\pm$ 1.00	0.886 $\pm$ 0.58	1.110 $\pm$ 1.11	
17	16	0.416 $\pm$ 0.41	1.066 $\pm$ 1.06	0.776 $\pm$ 0.77	0.886 $\pm$ 0.88	
18	17	1.083 $\pm$ 1.08	0.000 $\pm$ 0.00	1.110 $\pm$ 0.67	1.110 $\pm$ 1.11	
	CCN* for 17 weeks	13.08 $\pm$ 9.79	10.297 $\pm$ 10.29	10.19 $\pm$ 8.69	12.429 $\pm$ 12.42	
	$\bar{X}$ cocoon rate (worm/week) $\pm$ SE	1.189 $\pm$ 0.89	0.792 $\pm$ 0.79	0.849 $\pm$ 0.72	1.035 $\pm$ 1.03	

CCN*=Cumulative Cocoon Number

Table 20: Significant difference between the cocoon production of the epigeic earthworm, *Eudrilus eugeniae* cultured at cattle manure with various concentration of detergent (0.1 g, 0.5 g and 1 g)

Detergent	0.1 g	0.5 g	1 g	Control-CM
0.1 g	-	0.841	0.836	0.963
0.5 g	0.841	-	0.994	0.878
1 g	0.836	0.994	-	0.873
Control-CM	0.963	0.878	0.873	-

g detergent and least in 1 g detergent. Significant difference (P=0.000) was observed among (Table 23) and between different concentrations of detergent except between 0.1 g detergent and Control-CM (0.366) (Table 24).

Discussion

In the present study there is a variation in the

biomass, growth rate sexual maturity and cocoon production in different epigeic earthworm species (*Eudrilus eugeniae*, *Eisenia fetida* and *Perionyx excavatus*). This may be due to intrinsic property of each earthworm species to adjust with various concentrations of detergent, influence of chemical substances present in the detergent, availability of moisture content, variation in temperature and

Table 21: Cocoon production (worm/week) of the epigeic earthworm, *Eisenia fetida* cultured at various concentration of detergent (0.1 g, 0.5 g and 1 g) and Control-CM with their significant difference among them with F and P-values. Values are in Mean $\pm$ SE

S. No.	Weeks	Detergent			Control (CM)	Significant difference among them with F and P-value
		0.1 g	0.5 g	1 g		
1	IW	-	-	-	-	F=4.239 P=0.045
2	1	-	-	-	-	
3	2	-	-	-	-	
4	3	-	-	-	-	
5	4	-	-	-	-	
6	5	0.633 $\pm$ 0.18	0.133 $\pm$ 0.13	-	1.533 $\pm$ 0.92	
7	6	1.116 $\pm$ 0.25	0.333 $\pm$ 0.33	-	1.733 $\pm$ 0.87	
8	7	2.600 $\pm$ 0.41	1.216 $\pm$ 0.69	-	3.000 $\pm$ 1.50	
9	8	7.566 $\pm$ 1.41	1.433 $\pm$ 1.19	-	5.266 $\pm$ 2.64	
10	9	5.183 $\pm$ 1.22	0.866 $\pm$ 0.86	-	2.733 $\pm$ 1.39	
11	10	3.233 $\pm$ 0.14	1.383 $\pm$ 0.71	-	1.533 $\pm$ 0.76	
12	11	2.766 $\pm$ 0.78	3.550 $\pm$ 1.88	-	1.400 $\pm$ 0.70	
13	12	1.900 $\pm$ 0.20	0.700 $\pm$ 0.47	-	0.533 $\pm$ 0.26	
14	13	1.683 $\pm$ 0.30	1.800 $\pm$ 0.91	-	1.066 $\pm$ 0.63	
15	14	4.700 $\pm$ 1.58	1.250 $\pm$ 0.90	-	2.666 $\pm$ 2.18	
16	15	4.150 $\pm$ 1.34	2.843 $\pm$ 1.52	-	2.333 $\pm$ 1.16	
17	16	6.033 $\pm$ 0.97	1.753 $\pm$ 0.87	-	3.600 $\pm$ 2.07	
18	17	4.500 $\pm$ 0.17	0.667 $\pm$ 0.66	-	2.333 $\pm$ 1.27	
	CCN* for 17 weeks	46.06 $\pm$ 8.95	17.927 $\pm$ 11.12	-	29.729 $\pm$ 16.35	
	$\bar{X}$ cocoon rate (worm/week) $\pm$ SE	3.54 $\pm$ 0.68	1.379 $\pm$ 0.85	-	2.286 $\pm$ 1.25	

CCN*=Cumulative Cocoon Number

Table 22: Significant difference between the cocoon production of the epigeic earthworm, *Eisenia fetida* cultured at cattle manure with various concentration of detergent (0.1 g, 0.5 g and 1 g)

Detergent	0.1 g	0.5 g	1 g	Control-CM
0.1 g	-	0.068	0.009	0.256
0.5 g	0.068	-	0.216	0.402
1 g	0.009	0.216	-	0.056
Control-CM	0.256	0.402	0.056	-

pH. It may also be due to the presence of various concentrations of detergents, feeding preferences varied with different species and the odour of the detergent may directly or indirectly effect on various life activities of these species as earthworms are hyperosmic-organisms.

Epigeic earthworms always prefer non-toxic partially degraded organic waste for their better growth and reproduction, but presence of

detergent, which is chemically toxic in nature may affected the earthworm's growth, maturity, reproduction and more importantly their survivability.

The chemical components, which might affect the microbiota of the organic waste that may also influence on the growth and reproduction of earthworms. This might be influenced on microbial composition of the gut of these worms

Table 23: Cocoon production (worm/week) of the epigeic earthworm, *Perionyx excavates* cultured at various concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM with their significant difference among them with F and P-values. Values are in Mean $\pm$ SE

S. No.	Weeks	Detergent			Control (CM)	Significant difference among them with F and P-value
		0.1 g	0.5 g	1 g		
1	IW	-	-	-	-	F=23.457 P=0.000
2	1	-	-	-	-	
3	2	-	-	-	-	
4	3	-	-	-	-	
5	4	-	-	-	-	
6	5	0.416 $\pm$ 0.41	-	-	0.200 $\pm$ 0.20	
7	6	0.800 $\pm$ 0.41	-	-	0.266 $\pm$ 0.26	
8	7	1.433 $\pm$ 0.47	-	-	0.800 $\pm$ 0.40	
9	8	0.800 $\pm$ 0.30	1.033 $\pm$ 0.26	-	0.333 $\pm$ 0.33	
10	9	1.683 $\pm$ 0.56	8.086 $\pm$ 3.08	0.276 $\pm$ 0.27	1.866 $\pm$ 0.65	
11	10	2.683 $\pm$ 0.75	5.770 $\pm$ 2.21	0.610 $\pm$ 0.61	1.466 $\pm$ 0.46	
12	11	1.000 $\pm$ 0.50	2.526 $\pm$ 0.40	-	1.000 $\pm$ 0.52	
13	12	2.116 $\pm$ 0.99	1.276 $\pm$ 0.68	-	1.666 $\pm$ 0.35	
14	13	1.083 $\pm$ 0.14	0.886 $\pm$ 0.58	0.110 $\pm$ 0.11	0.200 $\pm$ 0.20	
15	14	1.266 $\pm$ 0.26	0.666 $\pm$ 0.66	-	1.466 $\pm$ 0.17	
16	15	0.733 $\pm$ 0.35	1.700 $\pm$ 0.43	0.110 $\pm$ 0.11	1.000 $\pm$ 0.72	
17	16	2.866 $\pm$ 0.69	5.003 $\pm$ 1.84	0.220 $\pm$ 0.22	3.966 $\pm$ 1.27	
18	17	3.316 $\pm$ 0.36	5.986 $\pm$ 1.26	0.610 $\pm$ 0.61	6.000 $\pm$ 3.05	
	CCN* for 17 weeks	20.195 $\pm$ 6.19	32.932 $\pm$ 11.4	1.936 $\pm$ 1.93	20.229 $\pm$ 8.58	
	$\bar{X}$ cocoon rate(worm/week) $\pm$ SE	1.553 $\pm$ 0.47	3.293 $\pm$ 1.14	0.21 $\pm$ 0.21	1.556 $\pm$ 0.66	

CCN*=Cumulative Cocoon Number

Table 24: Significant difference between the cocoon production of the epigeic earthworm, *Perionyx excavates* cultured at with various concentrations of detergent (0.1 g, 0.5 g and 1 g) and Control-CM

Detergent	0.1 g	0.5 g	1 g	Control-CM
0.1 g	-	0.009	0.001	0.366
0.5 g	0.009	-	0.000	0.002
1 g	0.001	0.000	-	0.004
Control-CM	0.366	0.002	0.004	-

which also affected the worm sexual maturity (Suthar, 2007). The feeding preferences by earthworms also varied due to variation in quality of the feeding materials (Pulikeshi *et al.*, 2003).

The nutrient abundance in the feed or food quality is directly associated with the growth, time taken for attainment of sexual maturity and cocoon production (Dominguez *et al.*, 2000).

Amala *et al.* (2019) reported that decreased concentration of detergent (0.1 g) favours normal growth and reproduction of earthworms. Various studies are also available, which states that lower the concentration of toxic substances and better the biology of worm. The effect of detergent was majorly observed in *Eisenia fetida*, where the survivability of these worms were less as compared to *Eudrilus eugeniae*, whereas *Perionyx excavatus* showed better result as compared to other two species. This indicates that toxic substances impact on survivability of the worms (Celine *et al.*, 2013).

Surf detergent has also impacted on the growth of bacterial population in the sea water as reported by Effendi *et al.* (2017). In case of *Perionyx excavatus* and *Eudrilus eugeniae*, the worms showed good biomass at higher concentration of detergent (1 g), this might be due to the presence of detergent metabolites in the earthworms (Chad *et al.*, 2008). Bayu *et al.* (2023) reported that earthworms show great promise in removing monitored compounds from sewage sludge.

Conclusion

Based on the results obtained, it is concluded that lower concentration of detergent favours for better growth and reproduction of these earthworms. Among all three species, *Perionyx excavatus* showed better results followed by *Eudrilus eugeniae* and *Eisenia fetida*. The 0.1 g concentration of detergent favoured the growth and reproduction of epigeic earthworms. Hence, lower concentration of detergent is not much harmful and shows negligible effect on the earthworms. The detergent discharged in the soil gets accumulated in lower concentration and slightly affecting the worms, if the concentration increases, the normal function of these earthworms may be affected. Overall, the detergent is toxic to soil ecosystem and also soil organisms, if the concentration is in high quality.

Ethical Statement

The authors confirm that all ethical issues have

been dealt with the research ethics guidelines provided by the Department of Zoology, Karnatak University, Dharwad 580003, Karnataka, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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