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Effect of PH on caudal regeneration of earthworm, *Eisenia fetida*

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Abstract
The earthworms are very sensitive to the pH of soil. The thin, moist cuticle which is permeable and the openings such as mouth and dorsal pores make earthworms highly susceptible for changes in hydrogen ion concentration (pH). The groups of earthworms, *Eisenia fetida* were exposed to different pH concentrations like 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 for caudal regeneration and their regenerating efficiency were 42.85%, 55.55%, 62.50%, 70.00%, 66.66%, 50.00% and 42.85% respectively and the control group regenerating efficiency was 70.00% after 30 days. When worms exposed to low and high pH, showed decreased rate of caudal regeneration as compared to others.

Keywords: Earthworm, *Eisenia fetida*, Caudal regeneration, pH, *Environmental factors.*

Introduction

Earthworms are eucoelomate, vermiform, metamerically segmented animal belong to phylum Annelida (Verma and Prakash, 2020) [28]. The earthworms are very sensitive to the hydrogen–ion concentration, so it is not surprising that the pH of soil is sometimes a factor that limits the distribution, numbers and species of earthworms that live in any particular soil. The thin, moist cuticle which is permeable and the openings such as mouth and dorsal pores make earthworms highly susceptible for changes in hydrogen ion concentration (pH). Several workers have stated that most species of earthworms prefer soils with a pH of about 7.0 (Petrov, 1946) [22], *Lumbricus terrestris* occurs in soil with pH of 5.4 in Ohio, USA. (Olson, 1928) [21], *Allolobophora caliginosa* in soils with a pH of 5.4 – 5.2 in Denmark, although there were a few earthworms in soils with a pH below 4.3, except for one species, *Dendrobaena octaedra*, which seemed to be acid tolerant (Bornebusch, 1930) [4].

The natural behaviors of earthworms were disturbed due to pH level. The pH of soil indicates whether it is acidic (1.0 to 6.0), neutral (7.0), or alkaline (8.0 to 14.0). Low pH values are usually unfavorable for many species of earthworms and often cause a decline in species numbers, except for few acid tolerant species that can harbor acidic soils (Werner, *et al.*, 2005) [30]. Earthworms are known to take up many inorganic and organic soil contaminants. Availability of contaminants for uptake from the soil is controlled by soil characteristics. Earthworms that are used in vermicomposting are reported to be sensitive to pH of the organic waste (Singh, *et al.*, 2005) [27]. A pH range of 5.0 to 9.0 was reported to be optimal for worm growth (Singh, *et al*., 2005) [27]. The pH of the soil determines the rate at which nutrients are absorbed in the soil. Micronutrients tend to be unavailable to soil with high pH whereas macronutrients are not available at low pH (Ansari and Rajpersaud, 2012) [1]. Henceforth, it is necessary for the vermicompost to have a neutral pH for maximum absorption of nutrients. It has been demonstrated that earthworms are very sensitive to the hydrogen–ion concentration, so it is not surprising that the pH of soil is sometimes a factor that limits the distribution numbers and species of earthworms that live in any particular soil. Several workers have stated that most species of earthworms prefer soils with a pH of about 7.0.

In the annelids, neurosecretory centres, located in the brain or supraoesophageal ganglion, exert a controlling influence over important physiological processes. The experimental work has clearly shown that, in *Nereid* polychaetes, the supraoesophageal ganglion secretes hormones that are essential for wound-healing and regeneration (Durchon, 1956 [10]; Clark and Bonney, 1960 [7]; Hauenschild, l960 [15]; Herlant-Meewis and Van Damme, 1962) [17]. There is also evidence that the same is true of the *Nephtyidae* (Clark, 1959) [5]. The endocrinology of regeneration in *Nereis diversicolor* has been studied in detail by Clark and Rustan (1963) [8]. These authors have shown that a hormone necessary for the regeneration of segments begins to accumulate in the supraoesophageal ganglion within a few hours after amputation of the posterior end of the worm. The hormone content of the ganglion rises to a maximum on the third day and is gradually released into circulation on the fourth day. By the fifth day the ganglion again contains very little hormone. Intact worms also contain small quantity of the hormone which may therefore control normal as well as regenerative segment proliferation. Berrill (1952) [2] reports that similar hormones are secreted by the brains of Lumbricid oligochaetes. However, too high pH values also reduce earthworm activity (Werner, *et al*., 2005) [30].

In addition to the above literature, regeneration studies were also reported in the following earthworms such as *Eisenia andrei*, *Eisenia fetida*, *Lumbricus rubellus* (Blakemore, 1999a [3]; Gates, 1927 [14]; Blakemore, 1999 [3]; Sims and Gerard, 1985) [26], *Ptychodera flava* (Rychel and Swalla, 2008) [23]. But there was no report on regeneration studies in *Eisenia fetida*. Hence the study was taken this region an impact of soil pH on earthworm *Eisenia fetida*.

Material and methods

The experiment was conducted to see the effect of different pH concentrations on regeneration and their regenerating efficiency of earthworms, *Eisenia fetida*. The soil collected from garden and used for experiment. The soil used for this experiment was taken from the chemical–free area. The pH were increased from 4.0 to 7.0 using Hydrochloric acid (HCl) and increased from 8.0 to 10.0 using Sodium hydroxide (NaOH). Different pH concentrations acidic, neutral and alkaline in nature like 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 stock solution were made. Initially first experimental group was maintained in 250 ml beaker and 30 ml stock solution for group (1) 4.0, (2) 5.0, (3) 6.0, (4) 7.0, (5) 8.0, (6) 9.0 and (7) 10.0 pH concentrations pH thoroughly mixed with 200 gm wet garden soil and the beaker was filled and group (9) was served as control. Acclimatized 10 earthworms looking healthy and having approximately equal size and weight were selected. Usually 10 caudal segments amputated of each earthworm using fine sterilized scissor under dissecting microscope and inserted in beaker the day of the experiment. For amputation, the earthworms used were not anaesthetized. Same procedure followed as per survivality experiment. Record of progress in each beaker was thus maintained. All the experimental beakers were kept in a laboratory and provide natural conditions. The pH and moisture level was maintained throughout the experimental period by periodic sprinkling of adequate quantity of tap water. The experiment was carried out till 30 days. After 30 days to observed the effect of pH concentrations on regeneration and their regenerating efficiency, development of new segments and physiology of earthworms, *Eisenia fetida*. The counting of regenerated segments is easy due to their vascularized state, transparency and dimensions. Such counting, however, is possible up to a period of 30 days, after which new segments assume normal dimensions and colour. For calculating the number of segments regenerated the earthworms were lightly anaesthetized (because of their extreme agility) in 0.5% ethanol (V/V) in tap water and were held on paraffin tray. Dissecting microscope and hand lens were used to confirm body structures that could not easily be seen with naked eye during the identification process.

The percentage of regenerating efficiency was calculated using the given formula.

$$\% \text{ of Regenerating efficiency} = \frac{\text{Number of worms regenerating more than 50\% caudal segments}}{\text{Number of regenerants}} \times 100$$

Observation and result

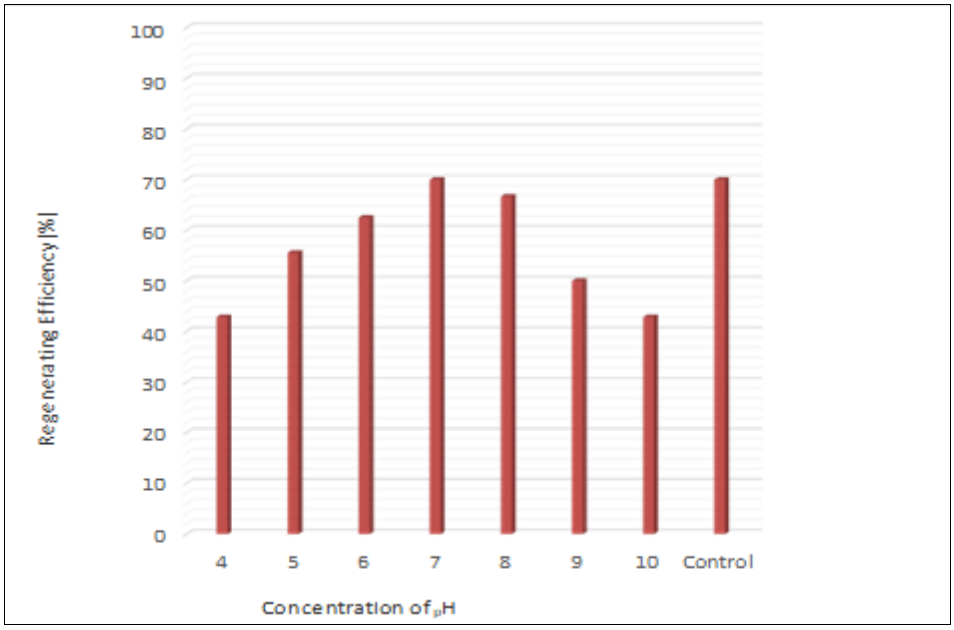
The present work on pH and their effects on adult earthworm, *Eisenia fetida* were studied. Differences between control and chemicals infected earthworms are compared, and conclusion is drawn. During the experimental period earthworms showed progressive signs and symptoms mentioned. If the pH conditions change they are coming outside of the soil they showed some changes such as coiling, curling and excessive mucous secretion with fast movements. Swelling of the clitellum was also seen after amputated the posterior segments. Extrusion of coelomic fluids resulting in bloody lesions within 48 hours of exposure was observed. Earthworms showed degenerative changes at the posterior part. Disappearance of metameric segmentations and loss of pigmentations were observed. It was observed that in all the earthworms, the regenerating parts clearly found. The control earthworms showed excellent burrowing movements and exhibited no extra ordinary behavior. This suggests that pH conditions is associated with behavior and physiology. It showed inhibitory effects on survivality and regeneration in earthworms. This work aims to assess the effects of pH on earthworms, *Eisenia fetida* for caudal regeneration and their regenerating efficiency.

The groups of earthworms, *Eisenia fetida* were exposed to different pH concentrations like 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 for caudal regeneration and their regenerating efficiency were 42.85%, 55.55%, 62.50%, 70.00%,66.66%, 50.00% and 42.85% respectively and the control group regenerating efficiency was 70.00% after 30 days (Table–1 and Graph–1). The earthworms, *Eisenia fetida* were 100% survival and their regenerating efficiency was more at 7.0 to 8.0 pH and the control group compared with experimental groups for regenerating efficiency. It has been demonstrated that earthworms are very sensitive to the hydrogen ion concentration of aqueous solutions.

Table 1: Effect of pH on the caudal regeneration in earthworm, Eisenia fetida.

	Concentration of pH							
	4.0	**5.0**	**6.0**	**7.0**	**8.0**	**9.0**	**10.0**	**Control**
Group	1	2	3	4	5	6	7	8
Number of worms used	10	10	10	10	10	10	10	10
Number of worms survival after 30 days	08	10	10	10	10	09	08	10
Number of worms displaying regeneration	07	09	08	10	09	08	07	10
Number of worms displaying non vascular pygidium	01	01	02	00	00	01	01	00
Number of worms displaying vascular pygidium	00	00	00	00	01	00	00	00
Number of worms regeneration segments.								
10 Segments amputated from each worm. 1	00	00	00	00	00	00	00	00
2	01	01	01	01	01	01	01	00
3	02	01	01	01	01	01	02	01
4	01	02	01	01	01	02	01	02
5	01	01	01	02	01	01	01	02

6	01	01	01	01	01	01	01	02
7	01	01	01	01	01	01	01	01
8	00	01	01	01	01	01	00	01
9	00	01	01	01	01	00	00	01
10	00	00	00	01	01	00	00	00
Regenerating Efficiency	42.85%	55.55%	62.50%	70.00%	66.66%	50.00%	42.85%	70.00%



Graph–1: The chart has shown the rate of caudal regeneration in different pH.

Discussion

Edwards and Bohlen (1996) [12, 13] cited soil pH as a limiting factor on earthworm distribution. Most of the earthworms are neutrophilic, preferring a pH of 6.0–7.0 and the species diversity is drastically reduced at pH > 7.0 except for tolerant species, which may be due to the fact that soil with pH considerably higher than 7.0 are mostly semiarid or arid and are unfavorable for earthworms (Sathianarayanan and Khan, 2006) [25]. Satchell (1955) [24] described that *"Bimastos eiseni, Dendrobaena octaedra* and *Dendrobaena rubida* were acid tolerant species, and *Allolobophora caliginosa*, *Allolobophora nocturna*, *Allolobophora longa* were acid intolerant" (Singh, *et al.*, 2005) [27]. Soil moisture, pH, organic carbon and organic nitrogen play important role in the distribution of earthworms (Decaens, *et al.*, 2003) [9]. Soil pH in this study varied from acidic to alkaline (4.0 to 10.0), (Table – 1). Chaudhuri and Bhattacharjee (1999) [5] reported earthworms are mostly distributed in a pH range of slightly acid to moderate alkaline and pH values recorded. The optimum operating conditions at 7.0 to 8.0 pH good for survival and regenerating, the lost segment of earthworms, *Eisenia fetida*. Tians, *et al.*, (2000) [28] studied of based on increasing in the number of earthworm (*Hyperiodrilus africanus, Eudrilus eugenia*) in pH 5 the change in pH was considered as an effective factor in the process of earthworm regeneration, the observation has been shown the significant reduction of regeneration in alkaline soil. Similarly, correlated result of Jamshidi, *et al.*, (2014) [18] reported that the 5.0 of pH in the soil were defined as an optimum condition for *Aporrectodea caliginosa* reproducing the lost segment. However, several earthworm species are known to live in soils with much less pH values than 6.2 (Edwards, 1988 [11]; Werner, *et al*., 2005) [30]. Nana-Osei, *et al.*, (2008) [20] reported with near-neutral pH (6.3 to 7.8) to the earthworm. The recent findings by Musaida *et al.*, (2012) [19] reported the optimum operating conditions were pH content ranged from 5.5 to 7.7 for the vermicomposting. Earthworms are not comfortable in an acidic environment, therefore cannot perform at their greatest potential in such a harmful environment (Hauxwell, *et al*., 1992) [16].

Conclusions

1. Acid and alkaline both are not favourable for regeneration.
2. Adverse effect of acid and alkaline.
3. Optimum pH neutral is best for regeneration.

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