ORIGINAL RESEARCH PAPER

Volume - 15 | Issue - 06 | June - 2026 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ESTIMATION OF GLYCOGEN AND MORPHOMETRIC STUDY OF THE COMMON INDIAN EARTHWORM, *Pheretima posthuma*

Zoology

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ABSTRACT

The study was conducted to analyse the morphological variations and physiological energy reserves (glycogen) of *Pheretima posthuma* collected from local agricultural land around the university. The morphometric data included total body length(mm), total number of segments, wet weight(g) and maximum body width(mm). It was found that body length and maximum body width were strongly correlated which formed the basis of identification in a sample of twenty specimen of *P. posthuma.* The mean of the body length was found to be 88.5 mm and body weight was 1.81 g which shows a weak to moderate positive correlation between body length and wet weight which signified that with increase in length the weight of the worm also increases. Biochemical quantification of glycogen was also performed using Phenol-Sulphuric acid method. A mean glycogen concentration of 1.35 mg/g was obtained. In the course of research, it was observed that highest glycogen accumulation was seen in clitellar/post-clitellar region where chloragogen cells were densely distributed that act as glycogen reserves present in the intestinal walls forming the typhlosole. There was a significantly high reserves of glycogen in mature worms than juveniles indicating their increased need for coping with environmental stress or drought conditions. These findings consisting of physiological data of *P.posthuma* form a baseline for future ecotoxicological studies and vermicomposting research.

KEYWORDS

P. posthuma, Glycogen, Morphometrics, Clitellar region,Pre- Clitellar Post-Clitellar

INTRODUCTION

Earthworm serves as a vital bio-indicator for detecting soil pollution in toxicological and environmental science and research. Their skin is highly permeable, making them sensitive to heavy metals and other pesticides and insecticides. They provide data on movement of chemicals through food chain by concentrating toxins within their tissues (**Suthar, 2008**). A biomarker can be defined as a "biochemical, cellular, physiological, or behavioural variations that can be measured in towel or body fluid samples or at position of whole organisms, to give substantiation of exposure and/or goods from one or other pollutants" (**Depledge, 1994**). Earthworms serve as biomarker tools in the field of soil monitoring and assessment as an early warning of adverse ecological goods (**Rodriguez-Castellanos** and **Sanchez-Hernandez**, **2007**). Earthworms produce a specific lumbrokinase enzyme which plays important role in cancer treatment and fibrinolysis (**Tang et al., 2000**; **Li et al., 2008**). This particular enzyme lumbrokinase is used in the manufacture of medicines because of remedial eventuality (**Kim et al., 1998**). A healthy soil is indicated by a high population of insects, spiders, bacteria, viruses, fungi, and other organisms, as well as healthy earthworm population (**Khunteta** and **Singh**, **2016**). Earthworms are therefore biological markers of soil quality (**Lachnicht** and **Hendrix**, **2001**).Morphological differences (clitellum, genital pores, setae arrangement) are crucial but substantially visible in mature worms. Identification keys are frequently point-specific, leading to taxonomic complexity (**Ahmed et al., 2021**). Earthworms play a great part in pedogenesis by causing soil bioturbation (**Cunha**, **2016**). They maintain soil health and carry out a major part in soil ecology and agronomy (**Feller et al., 2003**).

MATERIALS AND METHODS

Sample Collection

For the morphometric analysis of common Indian earthworm *Pheretima posthuma*, earthworm sample were collected from Krishi Vigyan kendra where vermicomposting is being practised The collection included both mature and juvenile individuals. Then Storing the collected samples, Blotting, Narcotization/Anesthetia, Measuring weight, Fixation, Preservation, Morphometric Analysis and Statistical Analysis were done For estimation of glycogen content of *pheretima* posthuma, thephenol-sulfuric acid method was used.

RESULTS

MORPHOMETRIC STUDY OF THE COMMON INDIAN EARTHWORM *Pheretima posthuma*

Here in this morphometric analysis was conducted by considering parameters like body length, total number of segments, maximum body width and wet weight. The arrangement or condition of setae was observed using stereomicroscope and compound microscope.. Clitellum was observed and its location was marked. It was lightly pigmented, distinct and bulged part occupying the 14th, 15th, and 16th segment in case of *P.posthuma* with maximum width in comparison to the rest part of the body. It was observed that the samples exhibited perichaetine arrangement of setae which serves as the hall mark of genus *Pheretima* forming a continuous ring around the circumference of each segment. They are S-shaped, chitinous structures embedded in the body wall and help in locomotion.

TABLE 1: THE TABLE REPRESENTS THE LIST OF MORPHOMETRIC DATA OF 20 SPECIMENS OF THE COMMON INDIAN EARTHWORM *Pheretima posthuma.*

Name of sample	Total no of body segments	Mean Length (in mm)	Weight in grams(g)	Mean Diameter (in mm)		
				Pre-C	C	Po-C
Worm 1	115	87.49	2.15	2.99	3.28	2.46
Worm 2	106	88.66	1.84	2.56	3.09	2.12
Worm 3	118	118.02	1.75	3.34	3.61	3.29
Worm 4	112	108.04	2.34	2.78	3.73	2.69
Worm 5	105	92.37	2.05	2.80	2.82	2.75
Worm 6	110	89.60	1.82	3.22	3.26	2.87
Worm 7	108	90.27	1.68	3.26	3.68	3.15
Worm 8	102	104.35	2.22	2.78	3.11	2.79
Worm 9	107	96.20	2.10	2.86	3.09	2.77
Worm 10	116	113.19	2.60	3.17	3.27	2.70
Worm 11	103	69.01	1.92	2.16	2.25	1.56
Worm 12	117	75.05	0.82	1.63	1.80	1.49
Worm 13	114	81.63	0.85	1.87	2.03	1.62
Worm 14	109	80.25	1.12	1.66	1.70	1.58
Worm 15	116	68.14	1.25	1.51	1.71	1.48
Worm 16	118	74.61	0.96	1.82	2.07	1.80
Worm 17	108	78.09	2.88	1.78	1.90	1.72
Worm 18	114	81.27	1.98	1.89	1.94	1.62
Worm 19	105	87.56	2.30	3.18	3.39	3.06
Worm 20	113	86.06	1.55	2.64	2.80	2.25

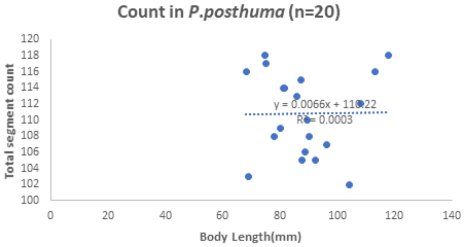

Fig 1: Correlation b/w length vs. total segment count.

 PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

It depicts the absence of any correlation between the body length and the total segment count. The R^2 value of 0.0003 shows that the independent variable(length) does not predict the dependent variable (segment count). This suggests that the number of segments is fixed or determined initially during emergence from cocoon and is not added with increasing age. Increase in the width of the segments occur in *Pheretima posthuma*.

GLYCOGEN CONTENT IN *Pheretima posthuma*

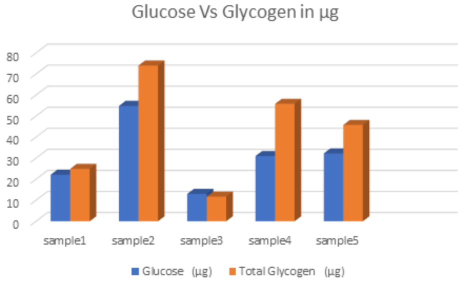

Fig-2: Level of Glucose Vs Glycogen in µg/g tissue

Standard deviation measures the amount of variation/dispersion in the morphometric data. The S.D. value of 0.81 shows a fairly high degree of variation between individuals.

The range represented the distance between the smallest and largest specimen in the test. The glycogen content was calculated from different body segments and it was found that the posterior end of the earthworm body had very low glycogen. The post-clitellar region containing the typhlosole had rich glycogen reserves. The typhlosole bears the intestine which is lined by chloragogen cells which store huge reserves of glycogen functioning as the liver in earthworms. Glycogen serves to be the primary source of energy that is to be used by invertebrates during the period of starvation. This study puts forward a baseline of the morphometric characters and physiological profile of the common Indian earthworms *Pheretima posthuma*.

DISCUSSION

Khan et al., (2024) conducted research on "Morphological based identification of earthworm species from Poonch division Azad Kashmir Pakistan." He stated that earthworms provide significant economic and environmental benefits, making their accurate identification crucial. This study aims to taxonomically classify earthworm species based on their morphological traits. Earthworms were collected through simple random sampling from eighteen locations in the Poonch division of Pakistan, between April and October 2019, using digging and hand sorting methods. The specimens were preserved in a 10% formaldehyde solution and examined with a stereomicroscope. Identification was conducted using keys from Julka (2008) and Chih-Han (2016). The research findings indicated that a total of eleven earthworm species, categorised into two families and six genera, were identified. Additionally, a significant statistical correlation (Turkey test, $P<0.05$) was found among the morphological variables, including length, weight, and total segment count.

Here in this morphometric analysis was conducted by considering parameters like body length, total number of segments, maximum body width and wet weight. The arrangement or condition of setae was observed using stereomicroscope and compound microscope.. Clitellum was observed and its location was marked. It was lightly pigmented, distinct and bulged part occupying the 14^{th}, 15^{th}, and 16^{th} segment in case of *P.posthuma* with maximum width in comparison to the rest part of the body. It was observed that the samples exhibited perichaetine arrangement of setae which serves as the hall mark of genus *Pheretima* forming a continuous ring around the circumference of each segment. They are S-shaped, chitinous structures embedded in the body wall and help in locomotion.

CONCLUSION

These findings shows that the growth in *P.posthuma* is basically characterised by the expansion and elongation of the body segments rather than addition of new segments or metameres upon attaining maturity. The glycogen content was estimated using a standard curve of glucose. The R^2 value presented a high degree of linearity within the tested range. The calculated mean glycogen content (mg/g) of common Indian earthworm *Pheretima posthuma* was found to be 1.36 ± 0.81 mg / g of wet tissue (Mean ± SD, n=4). The observed variance of 0.652 and standard deviation depict a significant physiological range in carbohydrate storage among the specimens. This may be the result of individual metabolic rate or different feeding patterns before collection.

The glycogen content was calculated from different body segments and it was found that the posterior end of the earthworm body had very low glycogen. The post-clitellar region containing the typhlosole had rich glycogen reserves. The typhlosole bears the intestine which is lined by chloragogen cells which store huge reserves of glycogen functioning as the liver in earthworms. Glycogen serves to be the primary source of energy that is to be used by invertebrates during the period of starvation. This study puts forward a baseline of the morphometric characters and physiological profile of the common Indian earthworms *Pheretima posthuma*.

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