ALTERATIONS IN CATALASE ACTIVITY AND LIPID PEROXIDATION IN THE EARTHWORM *Eudrilus eugeniae* (Kinberg) IN RESPONSE TO VARIABLE TEMPERATURE EXPOSURES

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CERTIFICATE-1

This is to certify that the thesis entitled **"ALTERATIONS IN CATALASE ACTIVITY AND LIPID PEROXIDATION IN THE EARTHWORM *Eudrilus eugeniae* (Kinberg) IN RESPONSE TO VARIABLE TEMPERATURE EXPOSURES"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Zoology** to the Orissa University of Agriculture and Technology is a faithful record of bonafide and original research work carried out by **Kumari Poornima Mohanta** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the course of investigation has been duly acknowledged.

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This is to certify that the thesis entitled "**ALTERATIONS IN CATALASE ACTIVITY AND LIPID PEROXIDATION IN THE EARTHWORM *Eudrilus eugeniae* (Kinberg) IN RESPONSE TO VARIABLE TEMPERATURE EXPOSURES"** submitted by **Kumari Poornima Mohanta** (Adm.no – 09 Zol/15) to College of Basic Science and Humanities, Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of Master of Science in Zoology has been approved by the Student's advisory committee and the external examiner.

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ABSTRACT

Temperature is one of the principal environmental agents which influence the survival and activity of animals. Surface dwelling soil animals including the earthworms are likely to be exposed to environmental temperature alterations which might affect their metabolism. Variation in tissue protein content, lipid peroxidation and activity of catalase have been considered as useful biomarkers of important ecological factors like temperature. This study presents the results of the effects of different temperature exposures for variable time durations on tissue protein, lipid peroxidation and catalase activity in the African night crawler *Eudrilus eugeniae* which is used in large scale for vermicomposting of organic waste. The study indicated significant variations in these biochemical parameters of the earthworm at different temperature and exposure periods of 15 min and 30 min durations. The highest protein (216.64 mg/ml) was observed at 35°C with 30 min exposure and the lowest (123.19 mg/ml) was observed at 4°C with 15 min exposure. Lipid peroxidation indicated its highest value (0.15 n mol/mg protein) at 4°C with 30 min exposure and the minimum (0.07 n mol/mg protein) at 35°C with 30 min exposure. The catalase activity was recorded to be maximum (0.11 U/mg protein) at protein at 30°C with 15 min exposure and minimum (0.02 U/mg protein) at 35°C with 15 min exposure. The study indicated that tissue protein, lipid peroxidation and catalase could be useful biomarkers to study the organismal impact of environmental temperature alterations due to climate change.

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ABBREVIATIONS USED

et.al	:	and others
mg	:	milligram
ml	:	millilitre
min	:	minutes
%	:	percentage
S.D.	:	Standard deviation
nm	:	nanometer
O.D.	:	Optical density
conc.	:	Concentration
Diff.	:	Difference
SDS	:	Sodium dodecyl sulphate
BHT	:	Butylated hydroxytoluene
TBA	:	Thiobarbituric acid
LPX	:	Lipid peroxidation
CAT	:	Catalase

Chapter-1

INTRODUCTION

It is now widely accepted that increased emission of carbon dioxide and other green house gases will produce a warming of at least 1°C by the end of 2050. The potential impacts of climate change on the soil biota have received considerably less attention than other ecosystem component and there are few prediction concerning climate change responses of soil invertebrate (Briones *et al*., 1997). Temperature is one of the principal environmental agents determining activity of biota and rates of decomposition processes in the soil (Swift *et al*., 1979). Temperature effects on a number of soil functions have been investigated, including activities of soil invertebrates (Byzova, 1973, 2007; Peterson and Luxton, 1982). It has been argued that increasing temperature may lead to rapid breakdown of organic matter which in turn might affect soil heterotrophic community (Tinker and Ineson, 1990; Sochlenius and Bastrom, 1999). However, Whitford (1992) reported that soil is thermally buffered and soil communities may be less sensitive to changes in atmospheric temperature than above ground fauna.

Soil constitutes a major storage for heat, acting as a reservoir of energy during the day and source of heat to the surface at night (Geiger *et al*., 2003). The temperature of soil depends on the ratio of energy absorbed to that lost from the soil. It fluctuates annually and daily, affected mainly by variation in air temperature and solar radiation (Wu and Nofziger, 1999). Soil temperature is one of the important factors that influence soil properties and biochemical processes (Buchas, 2001). It also influences interspheric processes of gas exchange between the atmosphere and soil (Lehnert, 2013). It has been reported that temperature of the soil could alter the rate of organic matter decomposition and the mineralization process in soil in addition to water retention.

It has been proposed that the soil temperature range of 10°C-28°C influence soil metabolism by enhancing extracellular enzyme activity that degrade polymeric organic matter in soil (Conant *et al*., 2008). An increase in microbial intake of soluble substrate and

respiration rate with increase in soil temperature has been reported (Yan and Hangwen, 2014). It has further been observed that soil temperature below the freezing point decreases mineralisation by inhibiting microbial activity (Kaiser *et al*., 2007).

Soil temperature beyond the optimal range of 10°C - 24°C increase the rate of metabolism in soil macro-fauna requiring them to either feed more or burn their own fat stores (Conant *et al*., 2008). Majority of soil macro organisms succumb to high soil temperature which proves unfavourable for their survival.

ECOLOGICAL IMPORTANCE OF EARTHWORMS

Earthworms have been considered as an important group of soil meso fauna in terms of biomass and activity. These animals have often been designated as ecosystem engineers (Lavelle, 1988). Earthworms are important components of soil subsystem because of their significant role in organic matter decomposition and interaction with soil microorganisms. It has been reported that earthworm activities increase total soil microbial biomass. Depending on the species, earthworms possess varying temperature optima and tolerances as well as different strategies for coping with low temperatures (Lee, 1985; Holmstrup and Zachariassen, 1996).

Soil temperature is likely to influence the life and activities of earthworm. In Indian situations, earthworm operate actively between 0°C - 30°C and their number decrease when soil temperature is below 4°C or 25°C subject to availability of moisture (Dash, 2012). With occurrence of very low and high temperature, surface dwelling epigeic and anecic earthworms usually migrate deeper into the soil, coil into slime ball and reduce metabolic activities to the minimum and undergo hibernation and diapause. Prolonged exposure to extreme temperature might bring heavy mortality in these earthworms. Epigeic earthworms in subtropical conditions usually prefer soil temperature of 15°C - 25°C in field conditions.

However, in laboratory culture worms can withstand temperature up to 40°C with adequate available moisture in soil. Life cycle of the worms is likely to be influenced by soil temperature, moisture and food availability. The incubation period of cocoons in different species of earthworms has been reported to be different in various geographical regions of world due to wide variations in soil temperature. It has been observed that cocoons laid by *Lampito mauritii,* and *Octochaetona surensis* in August to October exhibit an incubation period of 28 -30 days. The incubation period for *Darwida wilsi* is 14-18 days (Dash and Senapati, 1980). The European worms show longer incubation period of 60 to 210 days (Satchell, 1967; Rundgren, 1977). Temperature affected incubation of cocoons of *Allolobophora chlorotica,* 36 days in 20°C, 50 days in 15°C and 112 days in 10°C in European conditions. It is therefore expected that epigeic earthworms could indicate altered metabolism in response to temperature variations.

LIPID PEROXIDATION (LPX)

Lipid peroxidation is considered as the main molecular mechanism involved in the oxidative damage to cell structures and in the toxicity process that lead to cell death. It is a complex process known to occur in both plants and animals. It is the oxidative degradation of lipids. It is the process in which free radicals steal electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. The reaction consists of three major steps: initiation, propagation and termination. Initiation is the step in which a fatty acid radical is produced. The most notable initiators in living cells are reactive oxygen species (ROS), such as OH and COOH which combines with a hydrogen atom to make water and a fatty acid radical. In propagation, the fatty acid radical is not a very stable molecule, so it reacts readily with molecular oxygen, thereby creating a peroxyl- fatty acid radical. This radical is also an unstable species that reacts with another free fatty acid, producing a different fatty acid radical and a lipid

peroxide, or a cyclic peroxide if it had reacted with itself. This cycle continues, as the new fatty acid radical reacts in the same way. When a radical reacts with a non- radical, it always produces another radical so the process is called a 'chain reaction mechanism'. In termination, the free radical chain reaction propagates until two free radicals conjugate with each other to terminate the chain. The reaction can also terminate in the presence of a chain-breaking anti- oxidant such as vitamin E (α-tocopherol) (Halliwell and Gutteridge, 1984). Lipid peroxidation cause a decrease in membrane fluidity and in the barrier functions of the membranes. The many products of lipid peroxidation such as hydroperoxides or their aldehyde derivatives inhibit protein synthesis, blood macrophage actions and alter chemotactic signals and enzyme activity (Fridovich and Porter, 1981). This process is however referred to as lipid peroxidation and can be determined from the end product called malondialdehyde or MDA (Paulina *et al*., 2011).

CATALASE (CAT)

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyses the decomposition of hydrogen peroxide to water and oxygen. It protects the cell from oxidative damage by reactive oxygen species (ROS). It is one of the highest turnover numbers of all enzymes. One catalase molecule can convert millions of hydrogen peroxides molecules to water and oxygen in each second. In 1900, Oscar Loew was the first to give it the name 'catalase'. Catalase activity is a useful enzyme released during physiological stress to scavenge reactive oxygen species.

PROTEIN

Proteins are required in relatively large amounts by animals to sustain life and it is largely obtained from the food. Proteins are considered as building blocks of cells and muscle tissue in animals and are stored in body to defend pathogen and heal or repair injured tissue.

Chapter-2

REVIEW OF LITERATURE

Although elaborate studies on the effect of variable temperature ranges on soil invertebrate metabolisms are extremely limited, information is available on the combined effect of xenobiotics with temperature variation on soil organisms especially earthworm species. Spurgeon *et al*., (1997) conducted an experiment to study the influence of temperature on the toxicity of Zinc to the earthworm *Eisenia fetida.* They have observed that the worms exposed to sub-lethal concentration of Zinc indicated significantly higher accumulation of heavy metal with increase in soil temperature. They therefore concluded that Zinc burden in earthworm tissue was dependent on both concentration of heavy metal and temperature indicating that rise in soil temperature induces higher Zinc absorption and toxicity level in this earthworm species. Saint- Denis *et al*., (1998) have worked on four antioxidant enzymes – catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione-S-transferase (GST) in earthworm *Eisenia fetida* exposed to different concentration of hydrogen peroxide (H_2O_2). They have reported a significant relation between stress inducing hydrogen peroxide and the enzyme activities. Effects of a low temperature range of 5°C - 15°C on mitochondrial membrane enzymes of the earthworm *Lumbricus terrestris* has been studied by Crockett *et al*., (2001). They are of the opinion that *Lumbricus terrestris* is able to maintain its normal metabolism at reduced temperature with little or no change in enzyme action. Naddafi *et al*., (2004) studied the effects of variable temperatures on the growth and ecological function of the earthworm *Eisenia fetida* and reported a maximum growth rate at 25°C relative to higher and lower soil temperatures. Ravi Kiran and Aruna (2010) have investigated the levels of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) along with lipid peroxidation in earthworm *Eudrilus eugeniae.* They have found age related variation in these parameters in the earthworm species. Tripathi *et al*., (2011) investigated the variation in the tissue protein content and activities of malate dehydrogenase and lactate dehydrogenase of three eco-

physiologically different earthworm species namely *Metaphire posthuma*, *Perionyx sansibaricus* and *Lampito mauritii* exposed to a variable temperature range of 12°C to 44°C. They have reported that an increase in protein content upto 28°C and subsequent significant decrease upto 42°C. They have proposed that higher and lower temperature exposure could hamper protein synthesis in earthworms. They have also observed a wide variation in enzyme activities in these three species of worms. The earthworm *Lumbricus terrestris* acclimated at 2°C and 6°C above their habitat temperature (10°C) had respectively 15 and 40% higher rate of respiration than those at the control temperature (Khan *et al*., 2012). However, at 20-30°C a decrease in the respiration rate by 85% was observed. They have proposed that earthworm was under physiological stress at lower and higher temperature than that the habitat temperature. Effects of the pesticide Malathion and Furadon on lipid peroxidation and reduced glutathione in pre-clitellar, clitellar and post-clitellar regions of the earthworm *Eudrilus eugeniae* have been investigated (Parida and Mohanta, 2013; Parida *et al*., 2014).

Very little information is available on the variation in tissue protein, lipid peroxidation and catalase activity of earthworm *Eudrilus eugeniae* in response to variation in environmental temperature. Therefore, the present project was under taken with the following major objectives.

OBJECTIVES:-

1. To quantify tissue protein of the earthworm with exposure to different temperatures and duration of time.
2. To study lipid peroxidation and catalase activity in the earthworm tissue in response to temperature variations.

Chapter-3

MATERIALS AND METHODS

EXPERIMENTAL SET UP

Earthworm *Eudrilus eugeniae* was procured from the vermiculture unit of Quality Control Laboratory located at Sahid Nagar, Bhubaneswar. The worms were acclimatised in earthen pot (40cm×40cm) inside the laboratory for three days. Twelve 250ml glass beakers with 100ml distilled water were placed at 4 different temperature ranges. The control set was kept at room temperature of 30°C and experimental sets were kept at 35°C, 40°C (in an incubator) and 4°C (in refrigerator) for 15 minutes. Each set was taken in triplicate. After 15 minutes 120 clitellated earthworms of identical size and weight were sampled from the pot and transferred into the beakers, each beaker containing 10 earthworms. The worms were exposed to different temperature for 15 minutes. The same procedure was repeated for exposure of worms to various temperature ranges for 30 minutes. The experiments were done during the month of January 2017.

SAMPLE PREPARATION

Earthworms were washed in distilled water. The gut content was removed by using scissor, foreceps and needle and then it was weighed. The tissue was homogenised with phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 10 min using a table top cooling centrifuge (REMI). Supernatant was collected and stored in -20°C in deep freezer (Celfrost) for further use.

BIOCHEMICAL ANALYSIS

PROTEIN

Protein estimation was done as per Lowry *et al*, (1951). Reaction mixture was prepared by adding three solutions- solution A (Sodium carbonate, sodium hydroxide) solution B (Copper sulphate) and solution C (Sodium potassium tartrate). Reaction mixture was added to

sample. After 20 min gap, Folin and Ciocalteu's Phenol Reagent was added to it. After 20 min reading was taken at 700nm in UV-VIS Spectrophotometer (Systronics). The O.D. values which were obtained were converted to concentration of released protein in terms of mg/ml as follows:

Conc. of protein (mg/ml) = [(Sample conc. × Sample O.D.) /BSA O.D.] × Dilution factor

LIPID PEROXIDATION

Lipid peroxidation was determined as per Ohkawa *et al*, (1979). Reaction mixture was prepared by adding 8.1% Sodium dodecyl sulphate (SDS), 20% Acetic acid, Butylated hydroxytoluene (BHT), Thiobarbituric acid (TBA), added to sample and boiled for 45 minutes. It was cooled upto normal temperature and then centrifuged at 10,000 rpm for 15 min using a table top cooling centrifuge (REMI). Reading was taken at 532 nm in UV-VIS Spectrophotometer (Systronics). Value was expressed as n mol/mg protein.

LPX amount (n mol/mg protein) = (LPX O.D. / Protein amount required conc.) × 1000

CATALASE

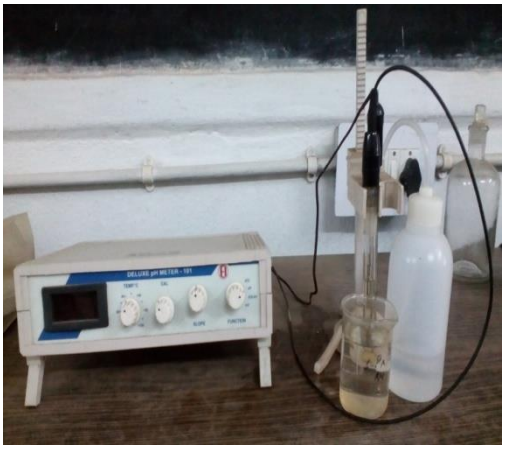
Catalase was assayed as per Cohen *et al*, (1970). Sample was added to reaction mixture containing phosphate buffer and hydrogen peroxide. Reading was taken at 242 nm in UV-VIS Spectrophotometer (Systronics). Value was expressed as U/mg protein.

CAT activity (U/mg protein) = (Diff. in CAT O.D. / Protein amount required conc.) × 1000

STATISTICAL ANALYSIS

All data were expressed as Mean ± Standard deviation (SD). Statistical analysis of data through one way ANOVA was done by M- STAT- C Software (Michigan State University, USA).

Incubator



pH meter

Cooling centrifuge

Deep freezer

Refrigerator

Eudrilus eugeniae

Fig-1 Equipments used for the experiment and the experimental animal

Chapter-4

RESULTS AND DISCUSSION

RESULTS

PROTEIN

Results of biochemical analysis are mentioned in Table 1 and statistical analysis results of data are depicted in Table 2 and Table 3. A wide variation in the amount of tissue protein of the earthworm exposed to different temperatures was observed with both 15 min and 30 min exposure periods (Fig 2). In 15 min exposure, the highest protein 183.61 mg/ml was observed at 35°C and the lowest of 123.19 mg/ml at 4°C. Interestingly, tissue protein at the highest temperature at 40°C was 136.69 mg/ml which is higher than that at 4°C. The worms exposed to the optimal room temperature of 30°C indicated 173.12 mg/ml tissue protein. The variation in tissue protein at 15 min exposure (Table 2) was found to be statistically significant ($p<0.05$, $F = 3.58$). In 30 min exposure, the highest protein value of 216.64 mg/ml was observed at 35°C and the lowest of 136.75 mg/ml was at 4°C. The variation in the protein content (Table 3) between different temperature exposure was found to be statistically significant ($p< 0.05$, $F = 3.31$).

LIPID PEROXIDATION

Lipid peroxidation (LPX) indicated minor variation between different temperature exposures and durations (Fig 3). In 15 min exposure period, the minimum lipid peroxidation of 0.09 n mol/mg protein was recorded at 40°C and maximum of 0.11 n mol/mg protein was recorded at 4°C. There was little difference in the lipid peroxidation between optimal temperature (30°C) and that at 35°C. Statistical analysis did not show significant variation in lipid peroxidation values between temperature exposures (Table 4). In 30 min exposure period, the highest lipid peroxidation value of 0.15 n mol/mg protein was recorded at 4°C and

lowest of 0.07 n mol/mg protein at 35°C. Significant variation ($p<0.05$, $F = 10.85$) was observed in lipid peroxidation values between different temperature exposures (Table 5).

CATALASE

In 15 min exposure, catalase activity indicated at highest value of 0.11 U/mg protein at the optimal temperature of 30°C (Fig 4). The minimum value of 0.02 U/mg protein was obtained at 35°C followed by 0.05 U/mg protein at both 40°C and 4°C. Statistical analysis indicated significant variation ($p<0.05$, $F = 30.44$) in the catalase activity between different temperature exposure (Table 6). In 30 min exposure, the maximum catalase activity was observed at the optimal temperature of 30°C and the minimum value of 0.03 U/mg protein at 40°C (Fig 4). Significant variation ($p<0.05$, $F = 19.45$) in the catalase activity between different temperature exposures was also observed (Table 7).

TABLE 1: Mean ± S.D. of protein, LPX (MDA) and catalase activity at different temperature and time exposures

Parameter	Temperature						
	30°C (Control)	4°C		35°C		40°C	
		15 min	30 min	15 min	30 min	15 min	30 min
Protein (mg/ml)	173.12 ± 2.06	123.19 ± 0.01	136.75 ± 0.02	183.61 ± 3.03	216.64 ± 4.01	136.69 ± 0.02	187.08 ± 3.60
MDA (n mol/mg protein)	0.103 ± 0.02	0.111 ± 0.04	0.157 ± 0.03	0.101 ± 0.02	0.078 ± 0.02	0.096 ± 0.01	0.082 ± 0.02
Catalase (U/mg protein)	0.111 ± 0.02	0.051 ± 0.01	0.055 ± 0.01	0.028 ± 0.01	0.040 ± 0.02	0.055 ± 0.01	0.039 ± 0.01

TABLE 2: ANOVA test result of protein (mg/ml) in earthworm *Eudrilus eugeniae* with 15 min exposure at different temperatures

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	17438.1	3	5812.693	3.584847	0.028472	3.008787
Within Groups	38915.1	24	1621.462			
Total	56353.2	27				

TABLE 3: ANOVA test result of protein (mg/ml) in earthworm *Eudrilus eugeniae* with 30 min exposure at different temperatures

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	23097.7259	3	7699.242	3.312635	0.037053	3.008787
Within Groups	55780.9113	24	2324.205			
Total	78878.6372	27				

TABLE 4: ANOVA test result of lipid peroxidation (n mol/mg protein) in earthworm *Eudrilus eugeniae* with 15 min exposure at different temperatures

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000854	3	0.000285	0.279883	0.839362	3.008787
Within Groups	0.024423	24	0.001018			
Total	0.025277	27				

TABLE 5: ANOVA test result of lipid peroxidation (n mol/mg protein) in earthworm *Eudrilus eugeniae* with 30 min exposure at different temperatures

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.027323995	3	0.009108	10.8577863	0.000107	3.008787
Within Groups	0.020132277	24	0.000839			
Total	0.047456273	27				

TABLE 6: ANOVA test result of catalase activity (U/mg protein) in earthworm *Eudrilus eugeniae* with 15 min exposure at different temperatures

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.026323	3	0.008774	30.44116	2.39E-08	3.00878657
Within Groups	0.006918	24	0.000288			
Total	0.033241	27				

TABLE 7: ANOVA test result of catalase activity (U/mg protein) in earthworm *Eudrilus eugeniae* with 30 min exposure at different temperatures

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0244848	3	0.008162	19.45509	1.29057E-06	3.008786572
Within Groups	0.01006823	24	0.00042			
Total	0.03455303	27				

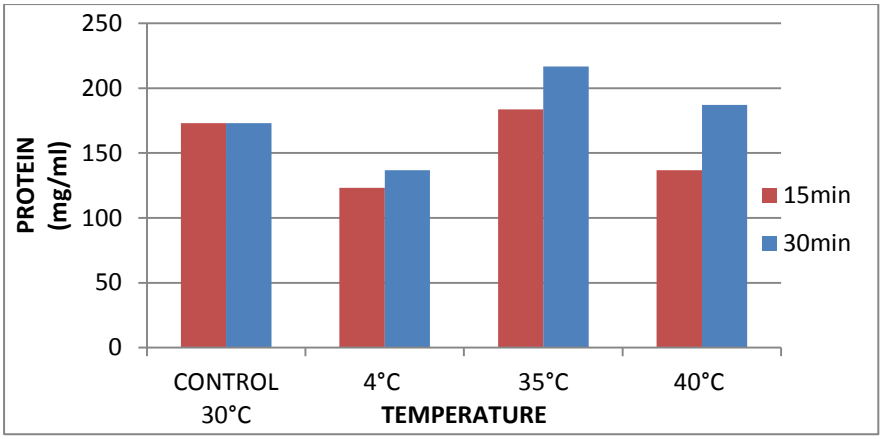


Fig 2 Protein (mg/ml) in earthworm *Eudrilus eugeniae* with different temperature exposures

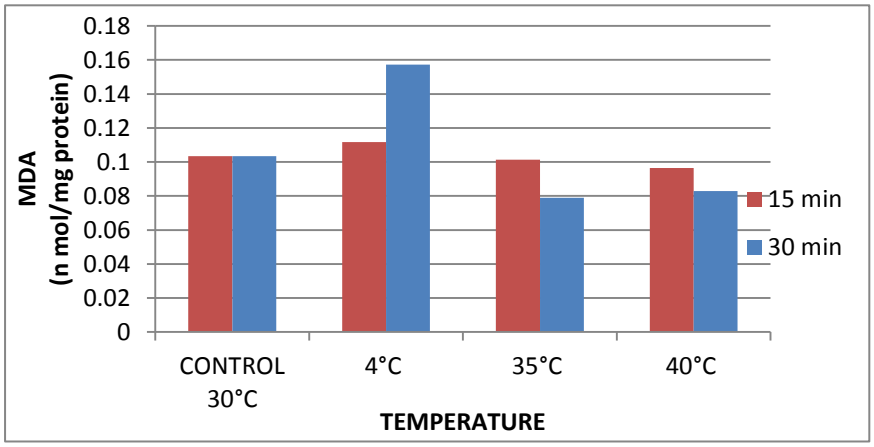


Fig 3 MDA (n mol/mg protein) in earthworm *Eudrilus eugeniae* with different temperature exposures

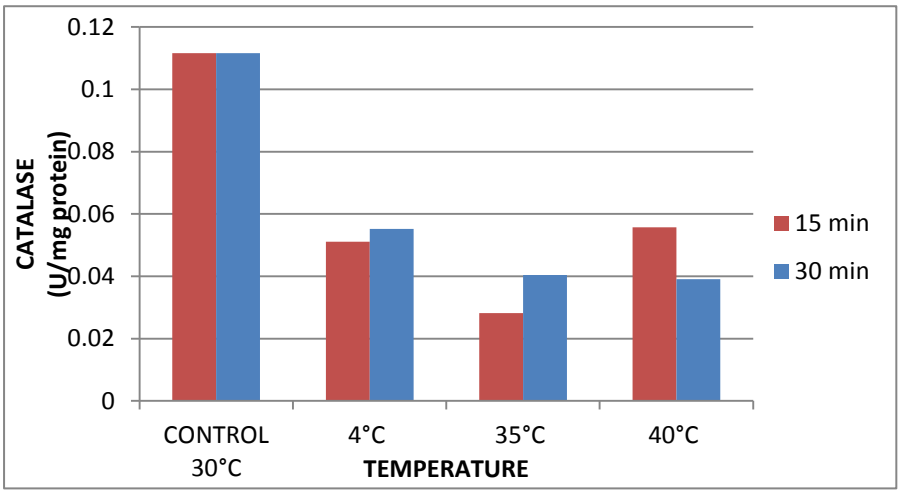


Fig 4 Catalase activity (U/mg protein) in earthworm *Eudrilus eugeniae* with different temperature exposures

DISCUSSION

PROTEIN

Tripathi *et al*., (2011) have reported a significant variation in the tissue protein of the earthworms *Metaphire posthuma, Perionyx sansbaricus* and*Lampito mauritii* which were exposed to a temperature ranges from 12°C to 44°C. They have observed an increase in protein from 12°C to 20°C and subsequent decrease upto 44°C. Parida and Mohanta (2013) reported consistent increase of tissue protein in the earthworm *Eudrilus eugeniae* upto 72 hrs of exposure to the sublethal dose of pesticide Furadon. The protein content thereafter decreased. They have proposed that the physiological stress due to this pesticide resulted in an increment in the synthesis of stress protein in the earthworm resulting in higher protein values. Mosleh *et al*., (2003) have reported that the treatment of herbicide Isoproturon at high concentration significantly reduced the protein content in the mature earthworm *Lumbricus terrestris*. Although the variation in protein was not significantly different over time of exposure, variation between concentrations was significant. Earlier Ismail *et al*., (1997) had observed reduction in protein content in *Aporrectodea calliginosa* in response to chlorfluazuron. These authors were of the opinion that depletion of worm protein was one of the primary toxic effects of the pesticide. In the present study, the highest tissue protein was observed at 35°C exposure which is 5°C higher than the optimal temperature indicating that a marginal increment in temperature could induce higher protein synthesis. It was further observed that worms exposed to 40°C temperature and 4°C indicated low tissue protein content which implies that very high and low temperature ranges adversely impact tissue protein synthesis and accumulation in this earthworm species. The results are in agreement with the observations reported earlier in other species of earthworms.

LIPID PEROXIDATION

Lipid peroxides resulting from hydroxyl radical attack on the important polyunsaturated fatty acid serve as marker of oxidative stress. The damage from this oxidative stress in fatty acid rich structures such as cell membrane results in cellular and tissue damage (Paulina *et al*., (2011). Eseigbe *et al*., (2013) during their study on the effect of hydrocarbon induced stress on the earthworm *Eudrilus eugeniae* observed significantly enhanced malondialdehyde (MDA) level in the worm relative to untreated worms. Ravi Kiran and Aruna (2010) observed a wide variation in lipid peroxidation in terms of malondialdehyde (MDA) level with increasing age of earthworm *Eudrilus eugeniae*. They have proposed that MDA levels increase the free radicals which might be influenced by age and stress. Parida and Mohanta (2013) and Parida *et al*., (2014) observed high lipid peroxidation in *Eudrilus eugeniae* exposed to the pesticide Furadon and Malathion. They have proposed that exposure to stress inducing agents enhance the MDA level in the earthworm with higher lipid peroxidation. In the present study, in our studies the highest lipid peroxidation has been observed at 4°C and low lipid peroxidation in the range of 35°C to 40°C. It is therefore apparent that the earthworm suffers the maximum oxidative damage due to free radical at very low temperature of 4°C. However, with rise in temperature upto certain threshold, the lipid peroxidation may be neutralised by enhanced activity of antioxidant enzymes in the earthworm which results in lower lipid peroxidation.

CATALASE

Catalase is a useful enzyme released in animals under physiological stress to scavenge reactive oxygen species. The prevention of oxidation is an essential process in all aerobic organisms as decreased antioxidant protection might lead to cytotoxicity, mutagenicity or carcinogenicity (Mates, 2000). The catalase activity in the earthworm was found to be

maximum at the optimal temperature of 30°C and lower catalase activity than the optimal was recorded at higher and lower temperatures. Thus, indicating that the enzyme activity is sensitive to environmental temperature fluctuations. Saint – Denis *et al*., (1998) observed an increased catalase activity with hydrogen peroxide concentration which acts as physiological stress. They have also reported that the enzyme activity increased with temperature from 10°C onwards and a maximum at 30°C and subsequently declined. Ravi Kiran and Aruna (2010) have reported wide variation in catalase activity in this earthworm species at different developmental stages and exposed to various stress conditions. Venkadapathi *et al*., (2016) observed that enhanced level of catalase activity in earthworms *Eudrilus eugeniae, Perionyx ceylanensis* and *Perionyx excavates* exposed to above optimal concentration of the pesticide Carbayl and lead in soil. The present results are in agreement with those of these earlier authors and indicate that the activity of catalase in *Eudrilus eugeniae* increases consistently from low to an optimal temperature of 30°C. Further rise in temperature possibly inhibit this enzyme activity and hence increase the probability of oxidative damage due to reactive oxygen species.

Chapter-5

SUMMARY AND CONCLUSION

Eudrilus eugeniae is an extremely important epigeic earthworm widely used for vermicomposting of organic waste. These worms are also presently found in agricultural fields in tropical and sub-tropical countries. Because of their high reproductive rate, surface dwelling habit and enhanced efficiency of feeding, these worms play significant role in maintaining soil nutrient status. Environmental temperature variations due to the process of global warming is likely to affect these worms adversely thus influencing their normal ecological function. Biochemical stress indicators such as protein, LPX and Catalase activity could be useful markers to study the organismal impact of climate change. An elaborate study on long term impact of temperature variation on the growth, reproduction and metabolism of this earthworm species is desirable to understand the impact of climate change on below ground organisms in general and earthworms in particular.

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