

RESEARCH REPORT

Evaluating the implications of moisture deprivation on certain biochemical parameters of the earthworm *Eudrilus eugeniae* with microbial population and exoenzyme activities of the organic substrate**CSK Mishra, S Samal*, A Rout, A Pattanayak, P Acharya***Department of Zoology, Odisha University of Agriculture and Technology, College of Basic Science and Humanities, Bhubaneswar-751003, India**This is an open access article published under the CC BY license**Accepted January 8, 2020***Abstract**

Reduction in moisture in the top soil and decomposing organic substrate is likely to influence the epigeic earthworms along with the microbial population and exoenzyme secretions. This study reports the results of the effects of consistent moisture reduction in semidecomposed organic substrate on the tissue protein, lipid peroxidation and catalase activity of the earthworm *Eudrilus eugeniae* along with pH, organic carbon reduction, bacterial-fungal population, activities of exoenzymes, amylase, cellulase, invertase over an experimental period of 22 days. Consistent depletion in tissue protein, increase in lipid peroxidation level and catalase activity was observed in the earthworm with moisture depletion. Catalase activity indicated significant negative correlation with substrate moisture. Significant differences in the carbon reduction, microbial population, exoenzyme activities in the substrate was observed with reduction of moisture and with respect to control. Significant positive correlation was observed between percent substrate moisture with microbial population and activities cellulase. It was concluded that desiccation of decomposing organics could enhance physiological stress on the earthworm and adversely impact the microbial population, exoenzyme secretions, consequently impairing mineralization.

Key Words: earthworm; exoenzyme; microbes; moisture deprivation; organic substrate**Introduction**

Abiotic factors such as moisture and temperature considerably influence the life of organisms (Acevedo-Whitehouse and Duffus, 2009; Singh *et al.*, 2014). Soil organisms too are likely to be influenced by variations in temperature and moisture. Rising global atmospheric temperature might result in deprivation of moisture from the surface soil thus adversely impacting major groups of biota. Moisture is expected to considerably influence physiological state of organisms due to its role as a suitable solvent for uptake of nutrients by microbes and plants and cutaneous respiration and excretion in certain soil fauna (Wall *et al.*, 2008; Blankinship *et al.*, 2011; Eisenhauer *et al.*, 2012). Water availability is a major determinant of the biotic community composition and functioning in soil. Climate change induced alterations of soil water

content could occur as a consequence of interacting changes to precipitation and temperature (Knapp *et al.*, 2008; Bell *et al.*, 2010). Below ground ecosystems are expected to be affected by soil moisture which could influence nutrient availability and decomposition rates (Trofymow *et al.*, 2002).

Earthworms, the major group of soil sub system have the tendency to live in moist soils (Berry and Jordan, 2001). Earthworms in natural habitats may be subjected to periods of drought and in agricultural lands are frequently turned out of the soil and exposed to surface conditions. In the vermicomposting process the moisture level might be reduced drastically during summer. With consistent substrate desiccation, the worms are likely to suffer from oxidative stress. Likewise, bacteria and fungi which play vital role in organic matter decomposition might be influenced considerably due to moisture variations. Microbes in soil produce various enzymes which are constantly being synthesized, accumulated, inactivated and decomposed in soil and their activities are useful for assessing the functional diversity of the microbial communities or organic mass turnover (Zhang *et al.*,

Corresponding author:

Suryasikha Samal

Department of Zoology

Odisha University of Agriculture and Technology

College of Basic Science and Humanities

Bhubaneswar-751003, India

E-mail: suryasikha.777@gmail.com

2009). Fluctuating physical parameters such as soil moisture could adversely impact microorganisms and their activities in soil and decomposing organic matter. Information is rare on the impact of depleting moisture in the substrate on microbes and epigeic earthworms which facilitate decomposition. The epigeic earthworm *Eudrilus eugeniae*, native to Africa is globally used as an efficient vermicomposting agent for processing the solid organic wastes. Therefore, the present study was intended to evaluate the effects of variable moisture levels in organic substrate on the tissue protein, lipid peroxidation and catalase activity of the earthworm with the microbial population and exoenzyme activities.

Materials and methods

Experimental set up

For both microbial and earthworm studies, two different experimental setups were prepared. For control (C) and experimental (E) setups, rectangular plastic pots (30 cm x 30 cm) were taken in triplicates. Each pot was filled with 4 kg air dried and powdered semi-decomposed cattle dung as substrate material. The water holding capacity of the substrate was measured gravimetrically. The maximum water holding capacity of the organic substrate was calculated to be 30 % which was taken as control. The substrate moisture level in both C and E were made up to 30 % level by adding distilled water. This moisture level was maintained in C throughout the experimental period of 22 days. In the experimental set, no water was added further, thus allowing moisture loss from the substrate over time. The percent moisture of the substrate in C and E sets were measured with a portable moisture meter (OMEGA-HSM50). The experiment was conducted at room temperature (Maximum 28 ± 2.3 °C and minimum 21 ± 3.2 °C) and there was no significant variation in average temperature during the experimental period. The earthworms (*Eudrilus eugeniae*) were obtained from the vermiculture unit of Quality Control Laboratory of the Government of Odisha and were acclimatized in laboratory condition for 7 days.

After acclimatization, 30 adult worms of approximately equal weight were inoculated in to each of the control and experimental pots. Both substrate (3 samples from each replicate) and earthworm (3 samples from each pot) were collected at random on day 1 and subsequently at 2 day interval for analysis.

Biochemical analysis of earthworm tissue

The gut contents of the worms were cleaned before biochemical analysis by keeping the worms submerged in distilled water kept in glass beaker for 15 min. The animals were rinsed well in clean distilled water and then sacrificed for obtaining the body wall tissue. The tissue samples were homogenized and centrifuged with potassium phosphate buffer (0.05 M, pH-7.4) at 4 °C and 10,000 rpm for 10 min using a table top refrigerated centrifuge (REMI, India). The supernatant was collected and maintained in a

chilled condition for further analysis. Protein was estimated using Folin and Ciocalteu's Phenol method (Lowry *et al.*, 1951). The amount of protein was determined at 700 nm with UV-VIS spectrophotometer (Systronics, India) taking bovine serum albumin (BSA) as standard. Lipid peroxidation (LPX) was measured in terms of malondialdehyde (MDA) at 532 nm as per Ohkawa *et al.* (1979). Catalase assay was done as per Cohen *et al.* (1970) taking both phosphate buffer and hydrogen peroxide. The absorbance was measured at 242 nm.

Evaluation of substrate chemical parameters, microbial load and enzyme activities

The substrate pH was measured using digital pH meter. Organic carbon (OC) was determined titrometrically as per Walkley and Black method (1934). Bacterial and fungal populations were estimated by serial dilution and spread plate method taking nutrient agar and potato dextrose agar as nutrient media respectively (Parkinson *et al.*, 1971). Three carbohydrate exoenzymes amylase, cellulase and invertase were assayed by spectrophotometric method (Ross and Robert, 1970) taking starch, carboxymethyl cellulose and sucrose as substrates respectively.

The data were checked for normality prior to further analysis and were found to be normally distributed. One way ANOVA was applied along with post hoc using SPSS software (IBM® SPSS® Statistics, ver. 25) to determine the significance levels at $\alpha = 0.05, 0.01, 0.001$. The significance in variation was checked between both the control and experimental sets. Correlation analysis was done between percent substrate moisture and biological parameters.

Results

Biochemical studies

Consistent reduction in tissue protein level was observed in the earthworm in the experimental set with respect to control over the incubation period. The highest percent change in protein content was 70.72 % on day 13. The level of protein got reduced by 47.4 % on day 22 with respect to day 1 (Fig 1a). A significant variation ($F = 4.62, p = 0.001$) in the percent tissue protein was observed between control and experimental sets. LPX level in experimental worms increased from day 1 to day 22. The highest percent change in LPX level (194.21 %) was noticed on day 22 with respect to control (Fig 1b). A significant variation ($F = 4.33, p = 0.021$) was observed in the LPX level of the animal between control and experimental sets. The percent change in catalase activity in the experimental worms showed an increasing trend up to day 16 with the maximum increase on this day (604.7 %). On day 22, the activity increased up to 337.05 % with respect to control (Fig1 c). The variation in percent change in catalase activity between control and experimental sets was found to be significant ($F = 8.1, p = 0.001$). Correlation analysis indicated significant negative correlation between percent substrate moisture and catalase activity ($r = -0.66, p < 0.05$).

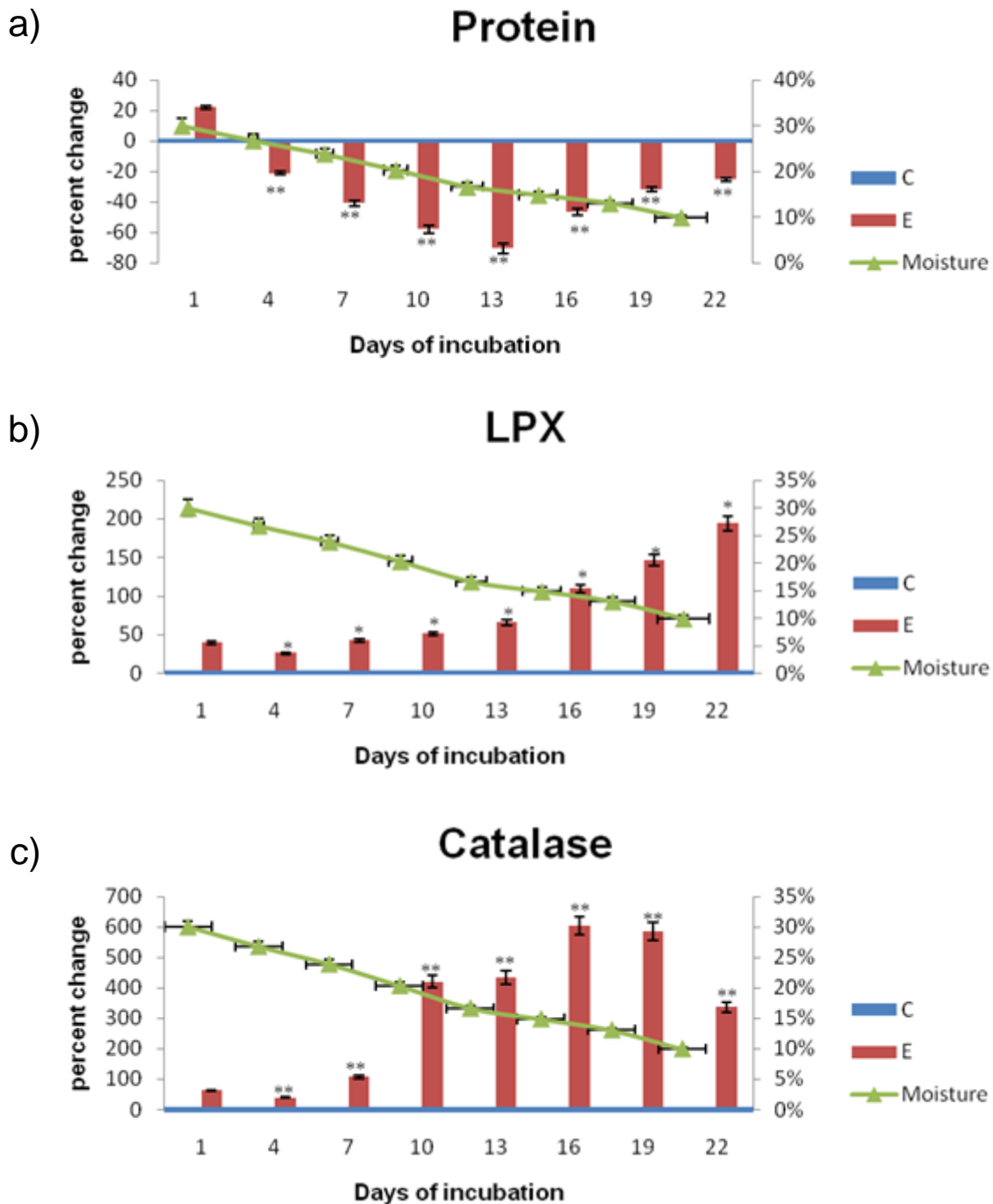


Fig. 1 Mean \pm SD indicates percent changes in tissue protein, LPX level and catalase activity in *Eudrilus eugeniae* with respect to control and substrate moisture. “**” indicates significant variation at $p < 0.05$ and “***” indicates at $p < 0.01$ between control and experimental sets, $n = 9$, C-control, E-experimental

Soil physico-chemical and microbiological study

The percent moisture and values of the substrate chemical parameters at different days of measurement have been presented in Table 1. The percent moisture in the experimental set decreased from the $30 \pm 1.1\%$ on day 1 to $9.93 \pm 0.9\%$ on day 22 indicating a reduction of 66.9 % over the experimental period. The variation in percent

moisture over the days of incubation was found to be significant ($F = 17.9$, $p = 0.0008$). The mean substrate pH on day 1 was found to be 6.73 ± 0.12 in control and 7.05 ± 0.01 in experimental set. A minor variation in the pH value was observed over the experimental period. The substrate pH was 7.20 ± 0.01 in control and 7.41 ± 0.02 in experimental set on the day 22.

Table 1 Mean \pm SD of substrate moisture (%), pH, OC (%) at different days of incubation (n=9). “*” indicates significant variation at $p < 0.05$, “***” at $p < 0.01$ and “****” at $p < 0.001$ between control and experimental sets

		Moisture (%)						
	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16	Day 19	Day 22
C	30.08 \pm 1.3	30.01 \pm 0.7	30.07 \pm 0.5	30.02 \pm 1.2	30.02 \pm 0.7	30.03 \pm 1.3	30.02 \pm 1.6	30.01 \pm 0.9
E	30.01 \pm 1.1	***26.7 \pm 0.4	***23.86 \pm 1.3	***20.30 \pm 1.8	***16.63 \pm 2.3	***14.86 \pm 1.9	***13.05 \pm 0.8	***9.93 \pm 0.9

		pH						
	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16	Day 19	Day 22
C	6.73 \pm 0.12	7.56 \pm 0.04	7.54 \pm 0.02	7.46 \pm 0.06	7.46 \pm 0.07	7.63 \pm 0.27	7.26 \pm 0.03	7.20 \pm 0.01
E	*7.50 \pm 0.01	*7.27 \pm 0.02	*7.38 \pm 0.18	7.32 \pm 0.02	7.32 \pm 0.11	**9.01 \pm 0.21	*7.61 \pm 0.02	7.4 \pm 0.02

		OC (%)						
	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16	Day 19	Day 22
C	3.25 \pm 0.06	3.19 \pm 0.12	3.16 \pm 0.18	3.08 \pm 0.24	2.40 \pm 0.54	2.41 \pm 0.14	2.38 \pm 0.07	2.34 \pm 0.30
E	3.29 \pm 0.01	3.12 \pm 0.17	*3.01 \pm 0.69	*2.78 \pm 0.12	2.3 \pm 0.23	2.47 \pm 0.25	*2.51 \pm 0.14	*2.67 \pm 0.24

A significant variation ($F = 4.8$, $p = 0.041$) in the OC (%) was observed over the days of the experiment. The maximum reduction in the OC was 9.7 % on day 10. It increased after day 16 and reached 14.1 % on day 22. In the control set the % OC reduction in the substrate was 28 % where as in the experimental it was 18.84 %. The noticeably lower % OC reduction in experimental set is likely due to significantly lower substrate moisture and microbial activity.

The mean bacterial population on day 1 was found to be 34.67×10^5 CFU/g in control and 48.33×10^5 CFU/g in experimental samples. A variation in the bacterial population was observed over the days of incubation. The bacterial population decreased to 19.33×10^5 CFU/g and 11×10^5 CFU/g soil in control and experimental sample respectively on the day 22. The bacterial population increased by 65.3 % up to day 10. The maximum reduction (43 %) of the bacterial population in experimental set with respect to control was observed on day 22 (Fig 2a). The fungal populations on day 1 was found to be 92×10^5 CFU/g and 99×10^5 CFU/g soil in control and experimental sets respectively which declined to 10×10^5 CFU/g soil in control and 8×10^5 CFU/g soil in experimental sets on day 22. The maximum increment in fungal count (63.77 %) was recorded on day 10 in the experimental set with respect to control. The fungal population indicated a reduction

of 18.7 % in the experimental set on the day 22 (Fig 2a). Significant difference ($F = 6.8$, $p = 0.02$) in the bacterial population of control and experimental sets was observed over the days of incubation. However, the variation in fungal population was not significant. Percent substrate moisture indicated significant positive correlation with both bacterial ($r=0.9$, $p < 0.01$) and fungal ($r = 0.8$, $p < 0.01$) populations.

The maximum increase in amylase activity (96.5 %) in experimental sample with respect to control was noticed on day 10. The enzyme activity increased up to day 13 and subsequently decreased. The maximum reduction in activity (31.3 %) was observed on day 19 with 13 % moisture level (Fig 2b). Cellulase activity showed an initial increment with the maximum enzyme activity (28 %) on day 10 after which the activity declined. Maximum reduction in enzyme activity (47.3 %) was observed on day 19 (Fig 2b). This enzyme activity indicated significant variation between control and experimental sets ($F = 5.66$, $p = 0.032$). Invertase activity too showed an identical trend. The enzyme activity increased by 38.4 % on day 10 with respect to control and then showed a reduction. Minimum activity of this enzyme (27.5 %) was recorded on day 22 (Fig 2b). Significant positive correlation was observed between substrate moisture with cellulase ($r = 0.6$, $p < 0.05$) and invertase ($r = 0.61$, $p < 0.05$) activity.

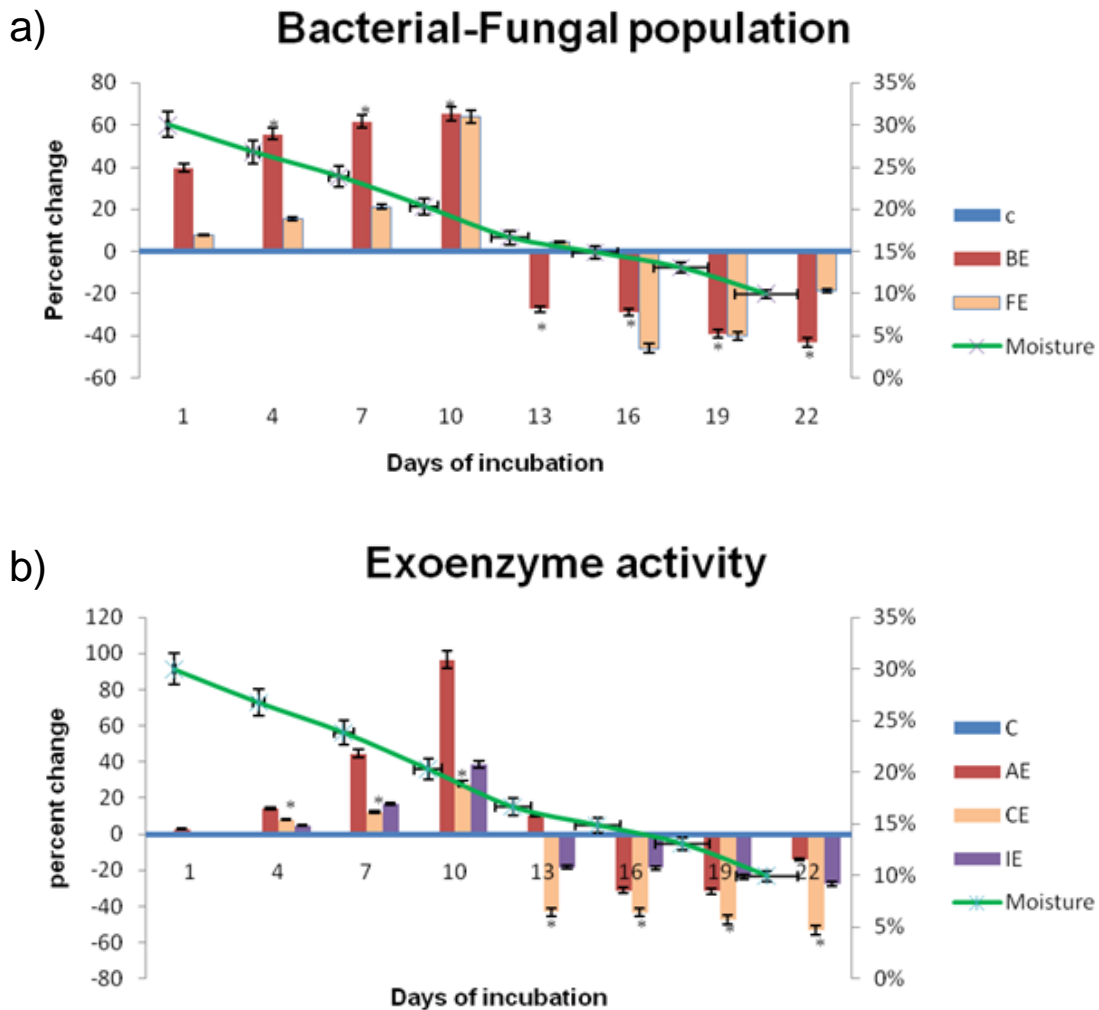


Fig. 2 Mean \pm SD indicates percent change in the Bacterial-Fungal populations and activities of amylase, cellulase and invertase in the substrate with respect to control and substrate moisture. C-control, E-experimental, BE- bacterial experimental, FE- fungal experimental, AE- amylase experimental, CE- cellulase experimental, IE- invertase experimental. “**” indicates significant variation at $p < 0.05$, $n = 9$

Discussion

Studies on the effects of soil abiotic factors on the biomolecules in earthworms are limited (Tripathi *et al.*, 2011; Mishra *et al.*, 2018 a). Bilalis *et al.* (2013) studied the effects of aluminium and moisture levels on the tissue protein content of the earthworm *Octodrilus complanatus*. They have reported that although tissue protein content decreased with aluminium accumulation, it did not indicate much variation with moisture levels of 100 % and 60 %. Mishra *et al.* (2018a) reported that low and high temperature exposures could significantly reduce tissue protein level in *E. eugeniae*. Mishra *et al.* (2019) also observed non significant variation in tissue protein of the earthworm *E eugeniae* exposed to low intensity colour lights over a period of 21 days. In the present study, a consistent decrease in the protein level in the earthworm with reduction in soil moisture is consistent with these earlier reports.

High LPX level in *E eugeniae* has been observed with exposure to different abiotic factors

such as temperature and light (Mishra *et al.*, 2018a; Mishra *et al.*, 2019). Reports are also available on the chemical stress induced LPX levels in earthworms (Samal *et al.*, 2017; Nayak *et al.*, 2018; Samal *et al.*, 2019). Liu *et al* (2010) had observed increase in the LPX level in the earthworm *Eisenia fetida* after exposure to rising concentrations of 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta- γ -2-benzopyran. The consistent increase in the LPX level in *E eugeniae* with reducing substrate moisture in the present study corroborates these earlier results and indicates that drier substrate could cause cellular damage with enhanced lipid peroxidation in the earthworm.

Catalase counters the reactive oxygen species produced in organisms so as to minimize oxidative damage to cells. With increased stress conditions due to desiccation of substrate it is expected that the catalase activity would increase in the animal as has been observed in the present study. It has been reported earlier that with increasing temperature and decreasing moisture, the catalase activity was

induced in different organisms (Khessiba *et al.*, 2005; Mishra *et al.*, 2018a). However, Hackenberger *et al.* (2018) observed reduction in catalase activity of *E. fetida* in soil treated with different concentrations of propiconazole and chlorantranilipole at low moisture level. Our results more or less are in agreement with the results of some of these earlier workers and with the hypothesis that enhanced lipid peroxidation due to environmental stress would enhance catalase activity.

Zhang and Wienhold (2002) reported that under aerobic conditions, there was no significant alteration in soil pH with an increase in moisture level in agricultural soil. However, with an increase in moisture from 100 g/kg to 400 g/kg, the soil pH increased marginally from 6.2 to 6.6. In the present study, the marginal decline in pH of the substrate in the experimental set might be due to accumulation of organic acids due to reduced decomposition of organics in a moisture deprived environment. Dan *et al.* (2016) during their studies on the effects of temperature and moisture on soil organic matter decomposition observed consistent rise in the rate of decomposition and carbon reduction with increase in soil moisture. Mishra *et al.* (2018a, b) observed variation in the reduction of organic carbon content exposed to low intensity lights and temperature. With higher temperature and lower moisture levels the decomposition process slowed down. The results of the present study are in partial agreement with the findings of Dan *et al.* (2016) indicating that lower substrate moisture level could adversely impact organic carbon degradation and decomposition because of reduced microbial population.

Barros *et al.* (1995) and Schnürer *et al.* (1982) had reported positive correlation between soil moisture and microbial growth. Stark and Firestone (1995) observed a decline in nitrifying bacterial activity at low soil water content. However, Chen *et al.* (2007) reported a non significant difference in the soil microbial biomass between 60 % and 80 % moisture levels. They reported that fungi in soil might adversely respond to over moisture supersaturation. Baldrian *et al.* (2010) observed significant difference in microbial biomass in dry and wet patches in forest soil.

More recently Borowik and Wyszowska (2016) noticed an optimal soil moisture level of 20 % for maximal development of organotrophic bacteria and 40 % for *Azotobacter* and *Actinomyces* in agricultural soil of Poland indicating that different bacteria have different moisture requirement in the same habitat. It has been reported that moisture in soil plays vital role in sustaining the growth and activity of bacteria, fungi and other microorganism (Ojeda *et al.*, 2013; Borowik and Wyszowska, 2016). Majority of the above findings corroborate the results obtained in the present study which has shown significant reduction in bacterial and fungal population with depletion of moisture in the substrate.

A reduced moisture level is likely to adversely impact microbial population and exoenzyme activities in the organic substrate. Baldrian *et al.* (2010) have reported significant positive correlation

between soil moisture and the activities of exoenzymes laccase, Mn-peroxidase, endo-1,4- β -glucanase, endo-1,4- β -xylanase, cellobiohydrolase, acid phosphatase along with few other enzymes in hardwood forest ecosystem. Steinweg *et al.* (2012) proposed that low moisture level can strongly limit *in situ* enzyme activity in soils, negating any positive effect of warming. However, contradictory result was obtained by Geisseler *et al.* (2011) who studied the combined effects of soil moisture potential and plant residue addition and concluded that, residue quality and quantity modulate exoenzyme activities and moisture plays a relatively minor role. The findings on exoenzyme activities in the present study are in agreement with majority of the above reports and it is apparent that reduction of substrate moisture will slow down microbial growth, enzyme activities and consequently the decomposition of organics.

Surface feeding soil fauna like *E. eugeniae* are likely to be affected by moisture depletion from top soil in the natural habitat due to rising atmospheric temperature as a result of climate change. Even during vermicomposting of organic substrate, these worms are likely to suffer from physiological stress in a desiccated environment which is likely to adversely influence their ecological functions. Moisture deprivation in soil and organic substrate could reduce the population of microbes and their exoenzyme secretions which is likely to seriously impair the decomposition and mineralization process, vital for nutrient release in to the soil. We therefore conclude that an optimal soil and substrate moisture level is essential to facilitate microbial activity and reduce physiological stress on the earthworms.

References

- Acevedo-Whitehouse K, Duffus AL. Effects of environmental change on wildlife health. *Philos. Trans. Royal Soc. B: Bio Sci.* 364: 3429-3438, 2009.
- Baldrian P, Merhautová V, Petránková M, Cajthaml T, Šnajdr J. Distribution of microbial biomass and activity of extracellular enzymes in a hardwood forest soil reflect soil moisture content. *Appl Soil Ecol.* 46: 177-182, 2010.
- Barros N, Gomez-Orellana I, Feijóo S, Balsa R. The effect of soil moisture on soil microbial activity studied by microcalorimetry. *Thermochim. Acta.* 249: 161-168, 1995.
- Bell JE, Sherry R, Luo Y. Changes in soil water dynamics due to variation in precipitation and temperature: An ecohydrological analysis in a tall grass prairie. *Water Resour Res.* 46: 1-11, 2010.
- Berry EC, Jordan D. Temperature and soil moisture content effects on the growth of *Lumbricus terrestris* (Oligochaeta: Lumbricidae) under laboratory conditions. *Soil Biol Biochem.* 33: 133-136, 2001.
- Bilalis D, Tzortzi I, Vavoulidou E, Karkanis A, Emmanouel N, Efthimiadou A, *et al.* Effects of aluminum and moisture levels on aluminum bioaccumulation and protein content in the earthworm *Octodrilus complanatus*. *J. Soil Sci. Plant Nutr.* 13: 845-854, 2013.

- Blankinship JC, Niklaus PA, Hungate BA. A meta-analysis of responses of soil biota to global change. *Oecologia*. 65: 553-565, 2011.
- Borowik A, Wyszowska J. Soil moisture as a factor affecting the microbiological and biochemical activity of soil. *Plant Soil Environ*. 62: 250-255, 2016.
- Chen MM, Zhu YG, Su YH, Chen BD, Fu BJ, Marschner P. Effects of soil moisture and plant interactions on the soil microbial community structure. *Eur. J. Soil Biol*. 43: 31-38, 2007.
- Cohen G, Dembiec D, Marcus J. Measurement of catalase activity in tissue extracts. *Anal. Biochem*. 34: 30-38, 1970.
- Dan WA, Nianpeng HE, Qing WA, Yuliang LÜ, Qiufeng WA, Zhiwei XU, *et al*. Effects of temperature and moisture on soil organic matter decomposition along elevation gradients on the Changbai Mountains, Northeast China. *Pedosphere*. 26: 399-407, 2016.
- Eisenhauer N, Cesarz S, Koller R, Worm K, Reich PB. Global change belowground: impacts of elevated CO₂, nitrogen, and summer drought on soil food webs and biodiversity. *Global Change Biol*. 18: 435-447, 2012.
- Geisseler D, Horwath WR, Scow KM. Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. *Pedobiologia*. 54: 71-78, 2011.
- Hackenberger DK, Palijan G, Lončarić Ž, Glavaš OJ, Hackenberger BK. Influence of soil temperature and moisture on biochemical biomarkers in earthworm and microbial activity after exposure to propiconazole and chlorantraniliprole. *Ecotox Environ Safe*. 148: 480-489, 2018.
- Khessiba A, Roméo M, Aïssa P. Effects of some environmental parameters on catalase activity measured in the mussel (*Mytilus galloprovincialis*) exposed to lindane. *Environ Pollut*. 133: 275-281, 2005.
- Knapp AK, Beier C, Briske DD, Classen AT, Luo Y, Reichstein M, *et al*. Consequences of more extreme precipitation regimes for terrestrial ecosystems. *Bioscience*. 58: 811-821, 2008.
- Liu Y, Zhou Q, Xie X, Lin D, Dong L. Oxidative stress and DNA damage in the earthworm *Eisenia fetida* induced by toluene, ethylbenzene and xylene. *Ecotoxicol*. 19: 1551-1559, 2010.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. 193: 265-275, 1951.
- Mishra CSK, Nayak S, Samal S. Low intensity light effects on survivability, biomass, tissue protein and enzyme activities of the earthworm *Eudrilus eugeniae* (Kinberg). *ISJ-Invert Surviv J*. 4: 8-14, 2019.
- Mishra CSK, Parida SS, Mohanta KP, Samal S. Thermal stress induced alterations in tissue protein, lipid peroxidation and activities of lactate dehydrogenase, acetylcholinesterase and catalase in the earthworm *Eudrilus eugeniae* (Kinberg). *Curr. J. Appl. Sci. Techn*. 27: 1-8, 2018a.
- Mishra CSK, Priyadarsini D, Samal S. Microbial Population Dynamics and Exoenzyme Activities in Semidecomposed Organic Substrate Exposed to Low Intensity Colour Lights during Vermicomposting. *Int. J. Curr. Microbiol. App. Sci*, 7: 1027-1032, 2018b.
- Nayak S, Mishra CSK, Guru BC, Samal S. Histological anomalies and alterations in enzyme activities of the earthworm *Glyphidrilus tuberosus* exposed to high concentrations of phosphogypsum. *Environ. Monit. Assess*. 190: 529, 2018.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem*. 95: 351-358, 197.
- Ojeda G, Patrício J, Navajas H, Comellas L, Alcañiz JM, Ortiz O, *et al*. Effects of nonylphenols on soil microbial activity and water retention. *Appl Soil Ecol*. 64: 77-83, 2013.
- Parkinson D, Gray TR, Williams ST. Methods to study ecology of soil microorganisms. IBP Handbook No. 19, Blackwell Scientific Publ. Oxford. PP. 116, 1971.
- Ross DJ, Roberts HS. Enzyme activities and oxygen uptakes of soils under pasture in temperature and rainfall sequences. *J. Soil Sci*. 21: 368-381, 1970.
- Samal S, Mishra CSK, Sahoo S. Setal-epidermal, muscular and enzymatic anomalies induced by certain agrochemicals in the earthworm *Eudrilus eugeniae* (Kinberg). *Environ Sci Pollut Res*. 26: 8039-8049, 2019.
- Samal S, Sahoo S, Mishra CSK. Morpho-histological and enzymatic alterations in earthworms *Drawida willsi* and *Lampito mauritii* exposed to urea, phosphogypsum and paper mill sludge. *Chem. Ecol*. 33: 762-776, 2017.
- Schnürer J, Clarholm M, Rosswall T. Microorganisms: In: Rosswall, T (ed) Progress Report 1981. Ecology of arable land. The role of organisms in nitrogen cycling. Swedish University Agr Sci, Uppsala, 77-88, 1982.
- Singh G, Sharma R, Kaur A. Impact of environmental factors on production and reproduction in domestic animals. *North-East Vet*. 14: 23-24, 2014.
- Stark JM, Firestone MK. Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Appl. Environ. Microbiol*. 61: 218-221, 1995.
- Steinweg JM, Dukes JS, Wallenstein MD. Modeling the effects of temperature and moisture on soil enzyme activity: linking laboratory assays to continuous field data. *Soil Biol Biochem*. 55: 85-92, 2012.
- Tripathi G, Kachhawa N, Dabi I, Bandooni N. Temperature-dependent alterations in metabolic enzymes and proteins of three ecophysiologicaly different species of earthworms. *Braz Arch Biol Technol*. 54: 769-776, 2011.
- Trofymow JA, Moore TR, Titus B, Prescott C, Morrison I, Siltanen M, *et al*. Rates of litter decomposition over 6 years in Canadian forests: influence of litter quality and climate. *Can J For Res*. 32: 789-804, 2002.
- Walkley A, Black IA. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the

- chromic acid titration method. *Soil Sci.* 37: 29-38, 1934.
- Wall DH, Bradford MA, ST. John MG, Trofymow JA, Behan-Pelletier VA, Bignell DE, *et al.* Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. *Global Change Biol.* 14: 2661-2677, 2008.
- Zhang R, Wienhold BJ. The effect of soil moisture on mineral nitrogen, soil electrical conductivity, and pH. *Nutr Cycl Agroecosys.* 63: 251-254, 2002.
- Zhang YL, Sun CX, Chen LJ, Duan ZH. Catalytic potential of soil hydrolases in northeast China under different soil moisture conditions. *J. Soil Sci. Plant Nutr.* 9: 116-124, 2009.