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AGING EFFECTS ON NUTRIENT DYNAMICS, BACTERIAL DENSITY AND ENZYME ACTIVITIES IN MIDDEN OF EARTHWORM *DRAWIDA CALEBI* (GATES)

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KEYWORDS

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ABSTRACT

Earthworm casts are stable structures characterized by higher nutrients contents, microbial biomass and activity than uningested material, thereby constituting hot spots of microbial driven processes such as nutrient release or nutrient immobilization and decomposition. Present paper dealt the changes produced by aging in the chemical and microbiological properties of casts of the earthworm *Drawidacalebi* (Gates). The results obtained showed that aging favored the release of microbial retained N, P, K and organic C content, and which was associated with the high phosphatase activity . In addition, we found an age dependent decrease in both microbial biomass activities after the 21st day of the observation. First of all microbial population increased up to 21st day and there after sharp declined was observed. The initial bacterial population (number/g soil), wet weight and dry weight (mg/g soil) were found to be $15.7 \pm 0.66X10^{9}$; $23.55 \pm 0.99X10^{-3}$ and $4.71 \pm 0.19X10^{-3}$ respectively, while the peak values for the same attributes were $20.0 \pm 0.85X10^{9}$, $30.0 \pm 1.28X10^{-3}$ and $6.0 \pm 0.25X10^{-3}$ respectively on 21st day of observation. This could indicate the existence of C limitation for microbial metabolism in casts but the enhanced cellulose activities suggest that new pools of labile C may be used by microbes during aging of casts. Aged casts showed reduced enzyme activities because of decreased moisture content, lower nutrient concentrations and decline in microbial activity.

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INTRODUCTION

Earthworms play a major role in soil nutrient dynamics by altering the soil physical, chemical and biological properties. Their casts, burrows and associated middens constitute a very favourable microenvironment for microbial activity (Hale *et al.,* 2005; Hale and Host, 2005). Soil microorganisms are responsible for decomposition of residual agrochemicals in soil (Higa, 1993), greater mineralization of Carbon (Daly and Stewart, 1999), more efficient release of nutrients from organic matter (Sangakkara and Weerasekera, 2001) and improved resistant to adverse weather (Higa, 1993) etc. In soil microorganisms by virtue of the exo-enzymatic activities are considered as primary decomposers playing key role in mineralization and demineralization process facilitating cycling of minerals in biosphere (Rodriquez *et al.,* 2011) resulting in fertility of the soil.

Soil enzymes produced by plants, animals and microorganisms play a crucial role in soil fertility. Soil worm casts have been shown to have enhanced microbial and enzymatic activities and micro and macro nutrients (Lavelle and Martin, 1992). Tomlin *et al.* (1995) reviewed that the stability of earthworm casts compared with bulk soil aggregates can be greater or similar depending on whether they are fresh or old. The stability of the casts is enhanced by bacteria in the soil increasing their secretion production of gums (McKenzie and Dexter, 1987; Haynes and Fraser, 1998) in passing through the gut or by the cementing effect of calcium (Lee and Foster, 1991). The increased stability of the cast and soil aggregates is the single most important soil property affecting soil erodibility (Horn *et al.,* 1998; Reichert and Norton, 1994) through the influence of particle detachment due to water drop impact, surface sealing and water infiltration. Bacterial population can influence carbon or mineral cycles and have the ability to colonize harsh environments. As there is paucity of knowledge about the effect of aging of cast of earthworm *Drawida calebi* on microbial population and enzyme activity. The present communication deals with the aging effect (0-42 day) of earthworm cast on nutrient dynamics, bacterial population and enzyme activity in experimental condition.

MATERIALS AND METHODS

Soil sample and earthworm collection

Drawida calebi, (Gates), earthworms were collected from different agro ecosystem sites in Ranchi, located between 21º58'N – 25º19, NL and 83º20'E- 88º4'EL and at a height of 629m above mean sea level (MSL) and study was carried out in laboratory by culturing the earthworms in plastic container under oxygenated and moist condition. The middens were collected from the plastic container and used for microbial and enzymatic study.

Bacterial culture and isolation

Dilution plate method (Parkinson *et al.,* 1971) was used for estimating the bacterial population in midden. The isolation of bacteria from midden samples was initiated by taking 1g of sample and was diluted with 9mL of sterilized deionized water till

10^{-7} dilution. 1mL inoculums of the primary suspension was taken for bacteria culture in a petriplate (diameter = 100mm) containing CzapekDox agar (Thom and Raper, 1945) media (peptone - 10g/L, beef extract – 10g/L, agar – 15g/L NaCl- 5g/L, pH- 7.2) and were inoculated at 37°C for 48h. After that colony count were continued at every interval of 7 days till 42nd day. For each experiment, three replicates of petri dishes were incubated. The mean fresh weight of a bacterium cell was taken as 1.5 X 10^{-12}g (Toth and Hammer, 1977). This value, when multiplied with the number of bacterial colony gave the fresh weight of bacteria. Assuming 80% of bacterial cell to be water (Clark and Paul, 1970) dry weight of bacterial biomass was calculated (Satpathy *et al.,* 1982). Student's test was done to determine the significance of change in population and biomass.

Physico - chemical estimation of midden

Standard methods were followed to estimate the organic carbon (Walkley and Black, 1934), nitrogen content (Kjeldahl and Jackson method, 1973), potassium and phosphorus content of soil and midden was measured according to method described by Misra (1973) and pH was measured by pH meter.

Estimation of enzyme activity

Dehydrogenase activity

The dehydrogenase activity of themidden sample was measured following Casida *et al.* (1964) by the amount of triphenylformazan produced during the microbial reductions of 1% 2,3,5-triphenyl tetrazolium chloride (TTC). The incubation mixture contained 2g fresh soil saturated with 2mL of 1% TTC and 0.5mL of 1% glucose in a screw cap test tube. The contents were mixed thoroughly in sealed test tubes and were incubated at 32ºC for 24h. Following incubation, the contents were stirred with 10mL methanol and the resulting slurry was washed into Buchner funnel (Whatman 30). The absorbance of the resulting filtrate was read at 485 nm using methanol as blank. The dehydrogenase activity was expressed in μg formazan/g soil/h.

Cellulose activity

Cellulose activity in soil was measured using 3, 5 dinitosalicylic acid (Ross, 1965). 3g soil was mixed with 0.2mL toluene in flask and 6mL of Sorenson's buffer along with 6mL of substrate solution was added. After shaking they were placed in the incubator at 30ºC. In control flask water was added instead of substrate and was centrifuged. Colour was developed by adding 3, 5 dinitrosalycilic acid solution to 1mL of supernatant. The reducing sugar forms a pink color, read at 540nm. The cellulose activity was expressed in μg glucose g^{-1} $soilh^{-1}$.

Urease activity

Urease activity in soil was estimated as per Tabatabai and Bremner (1972) except that NH_3 released in incubation was determined colorimetrically by an indophenol reaction (Kaplan, 1969). Urease activity was expressed in μg $NH_3g^{-1}soilh^{-1}$.

Phosphatase activity

Phosphatase activity in soil was measured according to Kramer and Yardei (1959). 1g of sample with 4mL of 0.25 M toluene and 1mL of p-nitrophenyl phosphate solution was added in the flask. The contents were mixed thoroughly in sealed flask and were incubated at 37ºC. After 1h, 1mL of 0.5 M $CaCl_2$ and 4mL of 0.5 M NaOH was added and soil suspension was filtered throughfilter paper (Whatman No. 12). The filtrate was transferred into colorimetric tube and the intensity was measured at 400nm.The phosphatise activity was measured in μg phenol g^{-1} $soilh^{-1}$.

RESULTS AND DISCUSSION

Physico- chemical properties of earthworm midden have been presented in Table 1. The pH of the midden was observed that 7.2 which was suitable for microbial growth. Initially organic C (mg C/g) was 8.21± 0.96 which gradually decreased to 7.32± 0.83. On the first day of observation nitrogen content in midden was 0.72 ± 0.09 mg N/g which increased to 0.76 ± 0.06 and declined to 0.65 ± 0.05. Phosphate and potassium content was observed as 3.57 ± 0.62 g P/m^2, 16.2 ± 0.71g K/m^2 respectively. A large no. of nutrient (N, P, K) are easily assimilable by plant in fresh cast depositions (Bhadauria and Ramakrisnan, 1989). Most of these nutrients are derived from earthworm urine and mucus (Barois and Lavelle, 1986).

Table 1: Phisico- chemical parameters of grassland midden of *Drawida calebi*

Days→ Parameters ↓	0	7	14	21	28	35	42
pH	7.31±0.37	7.42±0.48	7.48±0.49	7.52±0.24	7.21±0.52	7.11±0.62	6.92±0.57
Org. C(mg C/g)	7.83±0.96	7.95±0.98	8.12±0.89	8.21±0.87	7.63±0.99	7.52±0.82	7.32±0.83
Nitrogen (mg N/g)	0.72±0.09	0.74±0.12	0.75±0.14	0.76±0.06	0.69±0.07	0.68±0.08	0.65±0.05
Phosphorus (g P/hec.)	3.92±0.65	3.81±0.72	3.72±0.69	3.51±0.78	3.45±0.81	3.38±0.69	3.21±0.56
Potassium (g K/hec.)	17.5±1.25	17.1±1.28	16.3±1.13	16.0±1.19	15.9±1.24	15.7±1.09	15.3±1.08

Table 2: Bacterial population (number/g midden), wet wt. and dry wt. (Biomass) as mg/g soil in different age of earthworm midden

Days of observation	Bacterial population in midden	Wet weight of bacterial population in midden	Biomass
0	15.7 ±0.665X10^9	23.55±0.998 X10^{-3}	4.71±0.199 X10^{-3}
7	17.13 ±0.832 X10^9(+9.10)	25.69±1.248 X10^{-3}(+9.10)	5.13±0.249X10^{-3}(+9.10)
14	18.7 ±0.808 X10^9(+19.10)	28.05±1.212 X10^{-3}(+19.10)	5.61±0.242X10^{-3}(+19.10)
21	20.0 ±0.854 X10^9(+27.38)	30.0±1.281 X10^{-3}(+27.38)	6.0±0.256X10^{-3}(+27.38)
28	11.4 ±0.96 X10^9(-27.38)	17.1±1.441 X10^{-3}(-27.38)	3.42±0.288 X10^{-3}(-27.38)
35	6.63 ±0.85 X10^9(-57.77)	9.94±1.275 X10^{-3}(-57.77)	1.98±0.255X10^{-3}(-57.77)
42	4.26 ±0.305 X10^9(-72.86)	6.39±0.458 X10^{-3}(-72.86)	1.27±0.091 X10^{-3}(-72.86)

Values in parenthesis are percentage increase (+) or decrease (-) over initial value

In addition to this mixing effect, mucus production associated with water excretion in the earthworm gut is known to enhance the activity of microorganisms (Barois, 1987). This is followed by the production of organic matter. So fresh middens show high nutrient contents (Table 1) over longer period of time, this enhance microbial activity decreases when the cast dry and aggregation is then reported physically protect SOM against mineralization thus C mineralization rate decreases and mineralization of SOM from casts may be blocked for several months (Martin, 1991; Lavelle and Martin, 1992). EWs are known also to increase N mineralization, through direct and indirect effect on the microbial community. N mineralization by microflora is also quite increase in the EWs gut and continues for several homes in fresh cast (Blair *et al.*, 1997; Bossuyt *et al.*, 2005). This result thus highlights the important effects that EWs have on C and N cycling processes in agro- ecosystem.

In quantitative analysis, bacterial population, wet weight and biomass were observes and have been presented in Table 2. The bacterial population in earthworm midden of *Drawida calebi,* in the beginning was $15.7 \pm 0.66 \times 10^9$ which gradually increases to $17.13 \pm 0.83 \times 10^9$ and $18.7 \pm 0.808 \times 10^9$ and reaching at its maxima as $10.0 \pm 0.85 \times 10^9$ on 7th, 14th and 21st day respectively. There after a sharp decline in bacterial population was observed. The percentage increase in bacterial population over initial population was recorded as 9.1%, 19.1%, 27.38% on 7th, 14th and 21st day while the decrease was more pronounced as 27.38%, 57.77% and 72.86% over initial population on 28th, 35th and 42th day respectively. The change in population was found to be significant at $p < 0.001$. Impact of aging of earthworm midden in context of bacterial population has been reported by Kumari *et al.,* 2009. Brown *et al*. (2000) emphasize the importance of temporal and spatial scale when evaluating the effect of earthworm on the soil profile, suggesting that fresh earthworm midden behaves differently than aged midden. The changed behavior of fresh and old earthworm midden may primarily be due to various in bacterial population as the stability of midden increases with age at least for 3 weeks due to product of secretion by bacterial population.

The wet weight (mg/g soil) of bacterial population increased by 9.1% ($23.55 \pm 0.99 \times 10^{-3}$ to $25.69 \pm 1.24 \times 10^{-3}$) on 7th day (Table 2). After 21st day a sharp decline was recorded by 27.38%, 57.77% and 72.86% on 28th, 35th and 42nd day. A similar trend of rise and fall in biomass (mg/g soil) was observed. The maximum biomass was recorded on 21st day as $30.0 \pm 0.45 \times 10^{-3}$mg/g soil on last day of observation. The population of bacteria in earthworm midden increased with the aging of the casts (Kumari *et al.,* 2009, 2011). Some physical properties and microbial activity of the casts of the earthworm *Aporrectodea caliginosa* have been investigated by Piekarz and and Lipiec (2001) and reported that 20 day old cast is more stable than fresh cast. An increase in microbial population and biomass has been recorded with aging of earthworm middens up to 21st days which is in agreement with above finding.

Enzyme activities in earthworm midden have been presented in Fig. 1. Maximum dehydrogenase activity (μg formazan/g soil/h) was observed 8.49 ± 0.62 on 21st day thereafter they

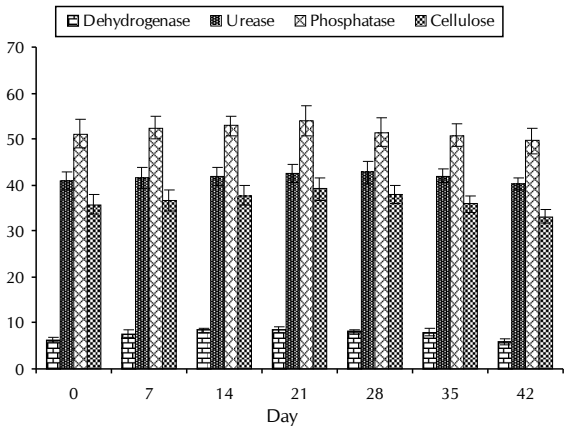

Figure 1: Enzymatic activity of earthworm midden in different age
Dehydrogenase (μg formazan/g soil/h), Urease (μg $NH_3g^{-1}soilh^{-1}$), Phosphatase (μg phenol g^{-1} $soilh^{-1}$.) and Cellulose (μg glucose g^{-1} $soilh^{-1}$)

declined. Dehydrogenase activity is widely used in evaluating the metabolic activity of soil microorganisms (Trevors, 1984; Pascual, 2002). Dehydrogenase enzymes play a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors (Dick and Tabatabai, 1993). Urease activity (μg $NH_3g^{-1}soilh^{-1}$) varies from 40.12 ± 1.32 to 42.72 ± 2.36. On the 1st day of observation Phosphatase activity (μg phenol g^{-1} $soilh^{-1}$) was 51.16 ± 3.21 which increased to 53.89 ± 3.32 on 21st day and thereafter they declined to 49.67 ± 2.81 on 42nd day. Initial cellulose activity in midden was 35.72 ± 3.02 on 21st day and thereafter they sharply declined to 33.18 ± 1.92 on last day of observation.Bityutskii *et al.,* 2005 described the species composition and the activity of enzyme in coprolites and excreta of earthworms (*L. terrestris, A. caliginosa* and *E. fetida*). Parthasarathi and Ranganathan (2000) reported that cellulose, invertase, protease and phosphatase activities in fresh cast 15 and 30 day old casts of *Lampito mauritii* and *Eudriluse ugeniae* decreased considerably with cast age. Soil cast, middens have been shown to have enhanced microbial and enzyme activities and micro and macro nutrients (Lavelle and Martin, 1992). Higher activities of cellulose, urease, phosphatase and dehydrogenase in the wormcasts have been reported (Edwards and Bohlen, 1996; Sharpley and Syers, 1976). Bonmati *et al.* (1985) observed that soil phosphatase activities were more marked, probably reflecting substantial greater microbial group due to the presence of easily decomposable organic compounds. A great variety of enzymes are produced by soil microorganisms, during their metabolism (Acosta-Martinez and Tabatabai, 2000). Soil phosphatase hydrolyse phosphate and make it available to plants. Thus phosphatase activity measurement provides an index of potential availability of phosphatase in soil (Mansell *et al.,* 1981). The increased amount of inorganic P released during cast deposition was related to and proceded by increased microbial and phosphatase activity (Sharpley and Syers, 1976). Enhanced phosphate content in the soil and presumed casta of *Lampito mauritii* and *Eudriluse ugeniae* has been reported (Parthasarathi and Ranganathan, 1999). In the case of *Drawida calebi,* the enzyme activities initially increased and thereafter they declined due to age of midden. It is supposed that the contribution of earthworms to the formation of soil humic acids depends on

the ability of particular species to decompose organic matter and induce polyphenol oxidase activity.Enhancement of the activities of these enzymes could be ascribed to the nutrient rich substrate, active microbial population and optimal moisture conditions. Aged midden showed reduced enzyme activities because of decreased moisture content, lower nutrient concentrations and a decline in microbial activity.

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