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Studies on Microbial Population Density and Soil Respiration in Cropland Soil and Middens of Earthworm *Linnogaster pusillus*, Stephenson

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Abstract: The present paper dealt with the relationship of the microbial population and the CO_2 evolution in soil and midden of earthworm *Linnogaster pusillus*, Stephenson. A trend of decrease in bacterial population and CO_2 evolution was recorded up to 42 days of observation in both the samples. The initial bacterial population (number/g soil) was found to be 36.3±1.504 X10^9 and 51.1±1.350 X10^9 in soil and midden respectively and thereafter a declining pattern was noticed. Maximum value of CO_2 evolution (mg CO_2/kg soil/h) was 6.23± 0.98 and 8.20±1.72 observed in soil and midden respectively. A positive significant correlation was observed between the two in both soil and midden of earthworms with r^2=0.9524 ($p \leq 0.001$) and 0.9856 ($p \leq 0.01$) respectively. The CO_2 release and bacterial population was comparatively higher in midden of earthworm than in cropland soil.

Key words: *Linnogaster pusillus* · Midden · CO_2 Evolution · Microbial Activity

INTRODUCTION

Soil biotas are thought to harbor a large part of the world biodiversity that include organisms from microscopic microflora to micro fauna. The microbes are the important element of soil environment as they participate in the degradation of organic matter and make the nutrient available to other soil organisms. Soil microbes are responsible for sequestration of green house gases especially methane. They oxidize atmospheric methane and are responsible for an estimated 5-10% of its total removal from atmosphere [1]. In agricultural soils the earthworms are recognized as soil ecosystem engineers. They form micro-habitats where a large number of physical, chemical and microbial changes occur because the structures are the outcome of an intestinal transit in earthworm cast and midden [2]. Protection of the soil habitat is the first step towards sustainable management of its biological properties that determine long-term quality and productivity.

Soil metabolism refers to the overall activities of the soil organisms involving biochemical process of their metabolic activity which is computed by the CO_2 evolution and enzyme activity of the soil [3]. Microorganisms are the main source of enzymes in soil [4]. The best index of overall metabolic activity of soil microbial population is CO_2 evolution or soil respiration, which can be rapidly determined. The measurement of soil CO_2 respiration is a means to gauge biological soil fertility [5]. Soil respiration is an important aspect of soil quality and an indication of soil fertility [6]. Therefore, soil metabolism may be depicted by microbial activity and CO_2 evolution. Earthworms are the natural soil engineers, which serve as bio catalytic agent to enhance the soil fertility through physical, chemical and biological process. Structure known as earthworm midden formed at the soil surface by the feeding and casting activities of earthworm, contributes significantly to soil heterogeneity. Midden acts as a focus for soil micro fauna and encourages microbial activity [7]. In this study, experiments were carried out to relate indices of bacterial population and CO_2 evolution in cropland soil and midden of earthworm *Linnogaster pusillus*, Stephenson in order to assess the utilization of earthworms in agricultural prospect.

MATERIALS AND METHODS

Soil Collection and Bacterial Isolation: Soil was collected from agro-ecosystem of Ranchi, Jharkhand and was kept in two separate plastic containers under moist condition. One container was normal soil without earthworm and

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other was culture of the earthworms *Linnogaster pusillus*, Stephenson in plastic trays. The middens were collected from the plastic trays and used for microbial study. Bacterial cultures were done from both soil and midden samples by dilution plate method [8]. The isolation of bacteria from soil samples was initiated by taking 1g of soil from both groups and was diluted with 9 mL of sterilized deionized water till 10^{-7} dilution. One mL inoculums of the primary suspension was taken for bacterial culture in a Petri plate (diameter = 100mm) containing Czapek Dox agar media, (peptone - 10g/L, beef extract – 10g/L, agar – 15g/L NaCl- 5g/L, pH- 7.2) and inoculated at 37°C for 48 h [9]. After that colony counts were continued at every interval of 7 days till 42nd day.

Estimation of CO_2 Evolution: CO_2 evolution was measured by alkali absorption technique [10]. CO_2 evolution was expressed as mg CO_2/kg soil/h.

Statistical Analysis: The data were subjected to the analysis of variance (ANOVA). Correlation and linear regression analysis were also done to assess the strength or weakness of the relationship between the microbial population and CO_2 evolved or soil respiration in the cropland soil and midden of earthworm *Linnogaster pusillus*.

RESULTS AND DISCUSSION

Bacterial population in soil in the beginning was 36.3±1.504 X 10^9 and in midden was 51.1±1.35 X 10^9. Thereafter a sharp decline in bacterial population was observed. The change in population was found to be significant ($p \leq 0.001$). In soil, bacterial population gradually decreased to 32.8 ± 2.80 X10^9, 24.5± 2.44 X 10^9, 21.4± 2.55 X 10^9, 18.9± 1.66 X 10^9, 13.3± 1.171 X 10^9 and 7.9± 0.907 X 10^9 on 7th, 14th, 21st, 28th, 35th and 42nd day respectively. On 7th day of observation bacterial population in midden was 44.9± 2.41 X 10^9 which was decreased to 12.7± 0.750 X 10^9 (Fig. 1). The percentage decrease in bacterial population over initial population in cropland soil was recorded as 9.64, 32.50, 41.04, 47.93, 63.36 and 78.23% on 7th, 14th 21st, 28th, 35th and 42nd day of observation. While the initial percentage decrease in bacterial population of midden was 12.13%, which was more pronounced on last day of observation i.e. 75.14% over the initial population (Table 1). Percentage change in bacterial population in between soil and midden was 40.77% which has been gradually increased till up to the 60.78% on the 42nd day that was more pronounced difference in bacterial population in soil and midden in which bacterial population was always higher in midden. Bacterial population has been reported higher in midden compared to the standardized soils ingested by the earthworm [11].

Soil respiration and bacterial population were gradually decreased. Soil respiration or CO_2 evolution (mg CO_2/kg soil/h) in soil and midden were maximum at 1st day 6.23 ± 0.98 and 8.20± 1.72 respectively. CO_2 evolution rate in midden gradually decreased from 7.92±0.99 to 3.62±0.31 on 42nd day of experiment, due to the lower moisture and nutrient content. Bacterial population showed a highly significant correlation (r^2= 0.9524, $p \leq 0.001$) with CO_2 evolution in soil with incremented value from 6.23 ± 0.98 to 3.02 ± 0.22 mg CO_2/kg soil/h (Fig. 2). The two parameters were highly correlated in midden (Fig. 3), with r^2= 0.9856. The effective activity was observed with higher bacterial population and CO_2 evolution in midden of earthworm.

In the present investigation a significant positive correlation (r^2= 0.9856, $p< 0.01$) of bacterial population and CO_2 evolution was found. The CO_2 evolution showed significant dependency on the population count as represented by the equation $y= 0.67826 + 0.164166x$ (Fig. 2). One way ANOVA showed that bacterial population plays a significant role in CO_2 evolution in the midden of earthworm ($F= 9.92$, $df= 6$, $p \leq 0.001$). Respiration is probably the process that is most closely associated with life [12]. Soil respiration is attributed to a wide range of microorganisms. Soil fauna also makes a

Table 1: Percentage change between the bacterial populations of cropland soil and midden of earthworm *Linnogaster pusillus*

Days of observation	Non ingested soil	Earthworm midden	% change
0	36.3±1.504 X10^9	51.1±1.350 X10^9	+ 40.77 %
7	32.8±2.809 X10^9(-9.64)	44.9±2.417 X10^9(-12.13)	+36.89%
14	24.5±2.441 X10^9(-32.50)	27.8±1.02 X10^9(-36.39)	+33.19%
21	21.4±2.553 X10^9(-41.04)	31.1±0.650 X10^9(-45.59)	+29.90%
28	18.9±1.665 X10^9(-47.93)	26.3±0.80 X10^9(-47.94)	+40.7%
35	13.3±1.171 X10^9(-63.36)	24.3±0.750 X10^9(-61.64)	+47.36%
42	7.9±0.907 X10^9(-78.23)	12.7±0.750 X10^9(-75.14)	+60.78%

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

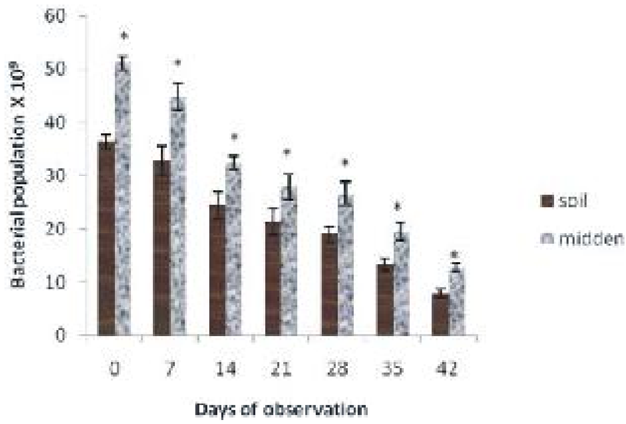

Fig. 1: Bacterial population in soil and midden over a period of 42 days
* represent the significant difference at 1% level; n=3

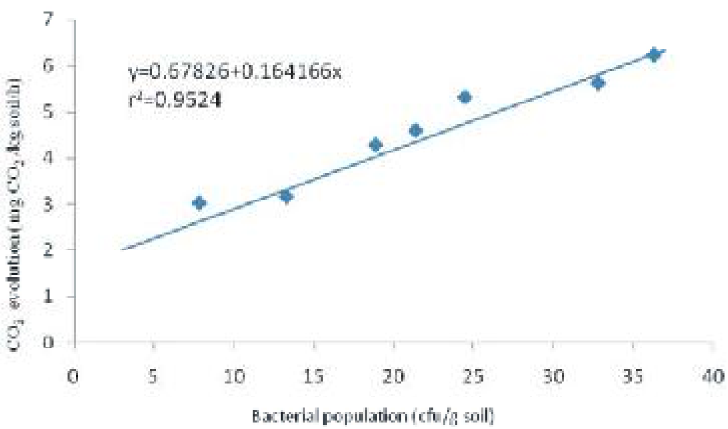

Fig. 2: Linear regression between bacterial population and CO_2 evolution in cropland soil

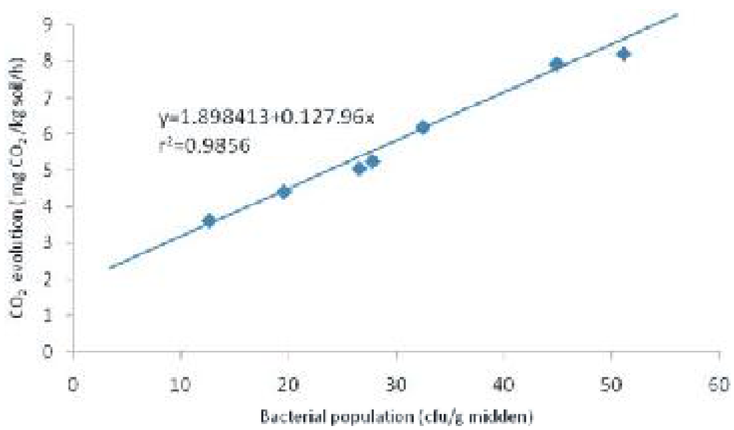

Fig. 3: Linear regression between bacterial population and CO_2 evolution in midden of earthworm *Linnogaster pusillus*

significant contribution (about 10%). But plant roots also contribute between 12 and 30% to the total release of CO_2 through respiration in field [12]. Hence, field based methods give the total respiration of all organisms, whereas laboratory methods give only the microbial respiration. Measurement of CO_2 evolution was taken as an index of the metabolic activities of soil organisms and it helps in assessing the organic input into the system. The energy flow and rate of mineralization comparatively the parameters of soil metabolism were incremented by the incorporation of earthworm than in their absence [13-15].

The enhancement in the population with earthworm is due to the ameliorating effect of the earthworm. Earthworm activity may raise N_2O emissions from agro ecosystems. Rather than emitting N_2O themselves, earthworms are thought to enhance soil microbial activity (nitrification, denitrification) by changing physiochemical properties, excreting mucus and increasing carbon available by enhancing microbial activities earthworm's aid in nutrient cycling processes and in soil structure development [16]. They have b een considered beneficial animals which can significantly influence soil structure and soil fertility [17].

CO_2 evolution was higher in midden than soils where there were significant variations in CO_2 evolution due to temperature, moisture and nutrients. There was a difference in response of microbial respiration between soil and midden. Specific respiration in midden was significantly decreased with age, which is in agreement with the finding of Tiunov and Scheu [18]. In this experiments, significant positive correlation (r^2= 0.9856) for bacterial population and CO_2 evolution was found. And also significant positive correlation between bacterial population and CO_2 evolution has been reported by many workers [19, 20].

The microbial community of soil may be profoundly affected by the presence of earthworms. Studies of various earthworm species have generally shown an increase in microbial activity [11, 21-28]. In contrast other studies have found earthworm reduced the microbial activity were of the opinion that selective feeding of earthworms on materials with higher microbial counts accounted for the population increase in midden produced [29-36]. Earthworm strongly enhanced microbial activity measured as CO_2 production in midden, which indicates that there are hot spots for soil microbial dynamics and increasing habitat heterogeneity for soil microorganism and increase soil fertility which is important for sustainable development [37]. The earthworms can specifically affect soil fertility that may be of great importance to increase sustainable land use in naturally degraded ecosystems as well as agro ecosystems. Proper earthworm management may sustain crop yields whilst fertilizer inputs could be reduced. Moreover, our data strongly support the fact that the impact of this earthworm species *Linnogaster pusillus*, Stephenson, in the soil is restricted to their middens and increasing soil heterogeneity.

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