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AMELIORATING EFFECT OF EARTHWORM ON SOIL METABOLISM IN FLY ASH AMENDED SOIL

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KEYWORDS

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ABSTRACT

Laboratory experiment was conducted to assess the relationship of the microbial population and the CO_2 evolution in presence and absence of earthworms (*Drawida willsi*) in three (5, 10 and 15%) different levels of coal fly ash amended soil. The bacterial population and CO_2 evolution showed a gradual rise reaching to maximum on 90th day at a time interval of 15 days, in 5% FA amendment in the presence of earthworms *i.e.* 31 ± 0.5 X 10^9 to 42.6 ± 2.8 X 10^9 cfu/g soil and 6.8 ± 0.2 to 9.0 ± 0.3 mg CO_2 / g soil / h respectively. Both the values decreased from 31 ± 0.52 X 10^9 to 22.7 ± 0.56 X 10^9 cfu/g soil and 6.75±0.2 to 6.32±0.2 mg CO_2/g soil/h in absence of earthworms. A positive significant correlation was observed between the two in both presence and absence of earthworms with r = 0.94716 (p≥0.01) and 0.850125 (p≥ 0.025) respectively. In 10 % amendment the value showed a variation from 21.7 ± 0.3 X 10^9 to 14.3 ± 1.83 X 10^9 cfu/g soil and 6.8±0.3 to 7.6± 0.2 mg CO_2/g soil/h in presence of *Drawida willsi* whereas in their absence the value ranged from 21.7±0.3 to 11.6± 0.7 10^9 cfu/g soil and 6.53±0.1 to 5.5 ± 0.03 CO_2/g soil/h. A negative correlation with r = -0.68516 (p ≤ 0.1) was observed with earthworm in 10% concentration of FA. A decrease was also observed in 5% amendment ie from 15.3 ± 1.1 X 10^9 to 7.63 ± 0.6 X 10^9 cfu/ g soil and 6.75 ± 0.2 to 6.0 ± 0.3 mg CO_2/g soil/hr with the earthworms and without them the value differed from 15.3±1.1 X 10^9 to 3.43 ± 0.75 X 10^9 cfu/g soil and 6.63 ± 0.01 to 4.2 ± 0.1 mg CO_2/g soil/h. The CO_2 release and bacterial population was comparatively higher in presence of earthworms than in their absence showing their ameliorating effect. With increase in concentration of FA the soil activity were hindered. Therefore, low dose of FA is optimum to be used in agriculture prospects.

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INTRODUCTION

Many industrial waste products are deposited in soils deliberately either as a beneficial amendment or for waste disposal, or inadvertently through spills, atmospheric deposition, inadequate waste management, etc. One such waste product with potentially beneficial application is fly ash, a solid waste, essentially inorganic by-product of fossil fuel power generation (Arthur *et al.,* 1984). Fly ash varies in chemical composition depending on the parent coal and the operating conditions of the furnace. In general, approximately 95 to 99% of fly ash consists of oxides of Si, Al, Fe and Ca, and about 0.5 to 3.5% consists of Na, P, K, and S (Adriano *et al.,* 1980). Fly ash consists of practically all the elements present in soil expect of organic carbon and nitrogen. Thus it was found that this material could be used as additive or amendment material in agriculture applications (Jeyanthi *et al.,* 2006).

Total ash production in India may reach to 117 million ton by the year 2012 (Vimal *et al.,* 2005) which may be disposed of in landfills, settling ponds, or by utilization in industrial processes. Land application of fly ash as an agricultural amendment is an alternative means of disposal which in turn helps in regeneration of wasteland. Numerous studies have demonstrated that coal ash can benefit soil and spoils as a conditioner, diluent, neutralizing agent, or micro-nutrient supplier (Fail and Wochok, 1977).

Soils are central to the sustainability of our ecosystem. Soil metabolism refers to the overall activities of the soil organisms involving biochemical processes of their metabolic activity which is computed by the measurement of CO_2 evolution, enzyme activities of the soil and essential nutrients like carbon and nitrogen (Wong and Wong, 1986). Microorganisms are the main source of enzymes in soils (Serdar, 2010). The most commonly used microbial activity indicators for soil health monitoring are microbial biomass, soil respiration and soil enzyme activity (Neilsen and Winding, 2002). The analysis of microbial communities is potentially a sensitive way of detecting changes in soil functioning and could therefore be employed to evaluate the effectiveness of soil protection policies. The best index of overall metabolic activity of soil microbial populations 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 (Hanley *et al.,* 2008). The inherent organic matter of soil plays an important role as a source of energy and nutrients for microorganisms thereby promoting microbial development (Smith and Paul, 1990). Soil respiration is an important aspect of soil quality and an indicator of soil fertility (Staben *et al.,* 1997). Therefore, the soil metabolism may be depicted by microbial activity and CO_2 evolution. Earthworms are the natural factories, which serve as bio-catalytic agents to enhance the soil fertility through physical, chemical and biological processes. Recycling of wastes using earthworms has become an important component of substantial agriculture, which has a multi-directional impact in terms of safe disposal of wastes preventing environmental pollution besides providing nutrient rich material. In this study experiments were carried out to relate indices of bacterial population, CO_2

evolution and dehydrogenase activity in graded levels of fly ash amended soil in both presence and absence of earthworms (*Drawida willsi*) in order to assess the utilization of fly ash in agriculture prospects and observing the ameliorating effect of the earthworms on soil metabolism.

MATERIALS AND METHODS

Laboratory experiment was conducted using fly ash (FA) collected from Patratu Thermal Power Plant. It was amended with the soil from the field within the Ranchi University campus in three different proportions i.e. 5, 10 and 15%. In another sets earthworms (*Drawida willsi*) collected from the campus were inoculated with the FA amended soil. The experimental setup was done using a quadruplicate plastic trays.

Estimation of microbial (bacterial) population

Dilution plate method (Waksman, 1922) was used to estimate the bacterial population for a period of 90days at an interval of 15days. The isolation of bacteria from soil samples was initiated by taking 1g of soil from each composite and transferring it to sterilized test tube for suspension in 9 mL of sterilized deionized water by shaking for 30 mins. 1 mL inoculant was taken from the aliquots of 1: 10^7 dilutions of the primary suspension (1 g soil in 10 ml distilled water). Each dilution was plated in Petri dishes containing Czapak Dox Agar (Thom and Raper, 1945) media(peptone -10g/L, NaCl-5g/L, beef extract- 10g/L, agar-15g/L, pH-7) for the bacterial culture. For each amendment three replicates of Petri plates were prepared. After 48 h incubation of the petri plates at ambient temperature of 37ºC, the bacterial colonies were counted.

Estimation of CO_2 Evolution

CO_2 evolution was measured by alkali absorption technique (Witcamp, 1966) by exposing the soil to 20 mL of 0.1 N KOH for 2 h. After incubation the KOH solution was removed and precipitated with saturated solution of $BaCl_2$ to form $BaCO_3$. The unspent KOH was titrated against an equivalent strength of HCl using phenopthalein indicator. CO_2 evolution was expressed as mg CO_2/g soil/h.

Estimation of dehydrogenase enzyme activity

The dehydrogenase activity of the sample soil was measured following Casida *et al.*(1964) by the amount of triphenyl formazan produced during the microbial reductions of 1% 2,3,5-triphenyl tetrazolium chloride(TTC). The incubation mixture contained 2 g fresh soil saturated with 2 mL of 1% TTC and 0.5 ml of 1% glucose in a screw cap test tube. The contents were mixed thoroughly in sealed test tubes and were incubated at 32°C for 24 h. Following incubation, the contents were stirred with 10 mL 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.

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 three graded levels of fly ash amended soil with and without inoculation.

RESULTS AND DISCUSSION

The bacterial colonies were enumerated and represented as the number of colony forming units (cfu) per g of the soil sample in both presence and absence of earthworms (Figs. 1 and 2) and carbon dioxide evolution (Figs. 3 and 4) were studied over a period of 90 days in time interval of 15 days. Rate of carbon dioxide evolution, is a sensitive indicator for the assessment of soil and give a good insight into the complexities of the nutrient profile in soil and therefore was examined to study the effect of fly ash on the bacterial count. Along with them enzyme dehydrogenase which depicts the oxidative and reductive reactions in the soil plays an important role in soil metabolism. Maximum population was observed in 5% FA amended soil with earthworms. The value showed continuous increase from 31 ± 0.52 to 43.2 ± 1.15 cfu/ g soil till 75th day of experiment and then decreased to 42.6 ± 2.83 cfu/ g soil on the 90th day. This showed a highly significant correlation ($r = 0.947162$; $p \geq 0.01$) with CO_2 evolution in 5% amendment with earthworm with incremented value from 6.8 ± 0.2 to 9.0 ± 0.3 mg CO_2/ g soil/h. The two parameters here were highly correlated (Fig. 7) with $r^2 = 0.897116$. The effective activity was observed in the 5% amendment with higher microbial population and CO_2 evolution in presence of earthworms. Jala (2005) observed that soil mixed with 5% fly ash showed a consistent increase in CO_2 production which ranged from 72.6 to 79.7 and 74.5 to 80.9 mg CO_2/100 g soil/day from day 1 to day 4, implying the synergistic interaction between glucose and fly ash. With enhancement in the bacterial population continuous increase was seen in the dehydrogenase activity in 5% amendment up to 90 days from 1.8 ± 0.3 to 6.2 ± 0.2 μg formazan/g soil/h. Whereas in 10% an increase from 1.50 ± 0.01 to 5.5 ± 0 μg formazan/g soil/day was observed till 60days and then decreased to 4.6 ± 0.1 μg formazan/g soil/ day on 90th day. 15 % fly ash amended soil with earthworms showed an increase from 0.85 ± 0.3 to 4.8 ± 0.2 μg formazan/ g soil/day in 45 days and further an exceptional decrease to 1.0 ± 0.2 μg formazan/g soil/day on 90th day (Fig. 5). Two way ANOVA revealed that concentration of fly ash and time interval played a significant role on the enzyme activity($F = 7.0979$,df $= 6,2$; $p \leq 0.00$; $F = 5.7134$, df $= 6,2$, $p \leq 0.01$).

In 10% FA amendment with earthworm's presence the population varied from 21.7 ± 0.36 to 14.3 ± 0.4 cfu/g soil. But CO_2 evolution rate showed gradual rise from 6.8 ± 0.3 to 8.02 ± 0.001 mg CO_2/g soil/h up till 60th day of experiment and then showed decline to 7.6 ± 0.2 mg CO_2/ g soil/h on 90th day. A negative correlation with $r = -0.68516$; $p \leq 0.1$ was noticed in 10% amendment with the presence of earthworms and were related by the equation $y = 9.701628 - 0.11655x$ and $r^2 = 0.469446$ (Fig 8). In 15% amendment positive correlation was seen among them ($r = 0.621199$; $p \leq 0.1$) with decline in the population count from 15.33 ± 1.1 to 7.63 ± 0.6 cfu/ g soil and CO_2 increased from 6.75 ± 0.2 to 6.9 ± 0.3 mg CO_2/g soil/h till 30th day and thereby decreased to 6.0 ± 0.3 mg CO_2/g soil/h. The CO_2 evolution showed significant dependency on the population count as represented by the

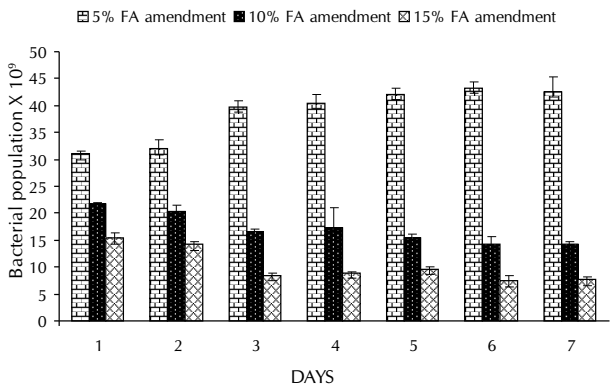

Figure 1: Bacterial population in three different concentration of FA in presence of earthworms over a period of 90 days

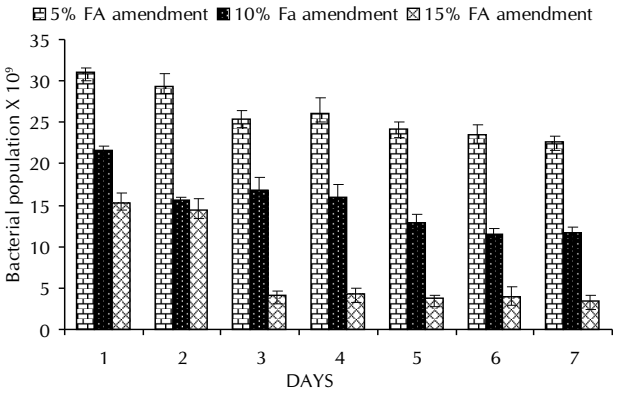

Figure 2: Bacterial population in three different concentration of FA in absence of earthworms over a period of 90 days

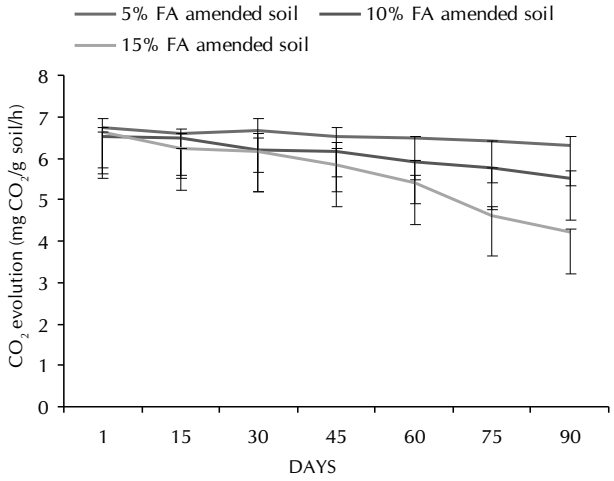

Figure 3: CO_2 evolution in three different concentration of FA in the presence of earthworms

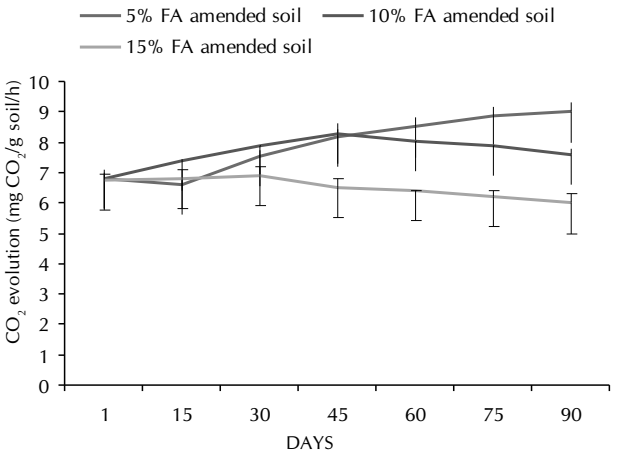

Figure 4: CO_2 evolution in three different concentration of FA in absence of earthworms

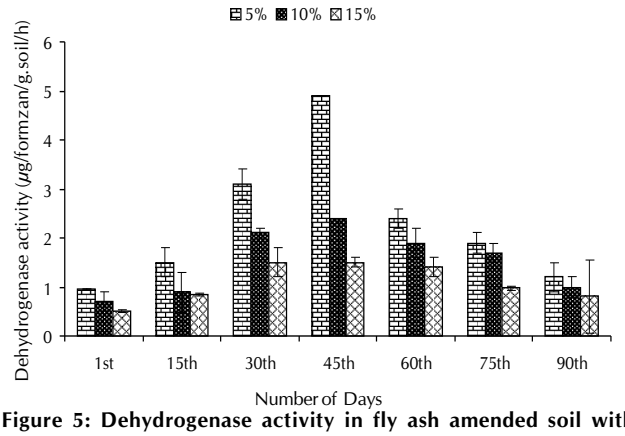

Figure 5: Dehydrogenase activity in fly ash amended soil with earthworms

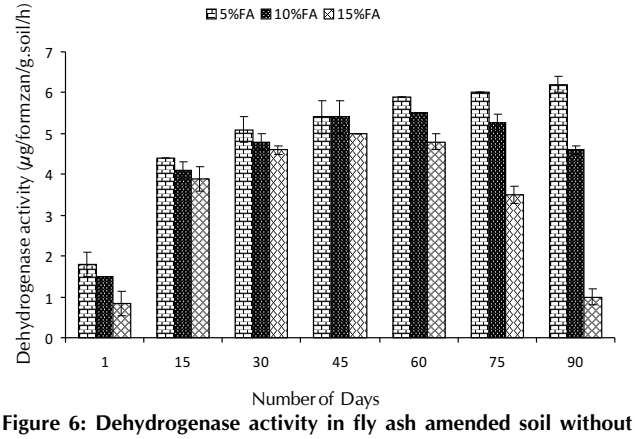

Figure 6: Dehydrogenase activity in fly ash amended soil without earthworms

equation y = 5.853633 + 0.064067x (Fig 9). Two way analysis showed that concentration played significant role in CO_2 evolution in the presence of earthworms (f = 9.2338, df = 6, 2; p≤0.001; F = 0.91495, df = 6,2; p≤0.5). Thereby, the increased FA dose hindered the activity.

In the absence of *Drawida willsi*, the bacterial population showed a gradual decrease from 31 ± 0.52 to 22.7 ± 0.56 cfu/g soil, 21.7 ± 0.36 to 11.6 ± 0.75cfu/g soil and 15.33 ± 1.1 to 3.43 ± 0.75 cfu/g soil respectively in 5, 10 and 15% FA amendment. A decreasing trend was also observed in CO_2 evolution rate in the three experimental proportion of FA amended soil. It varied from 6.75 ± 0.2 to 6.32 ± 0.2, 6.53 ± 0.1

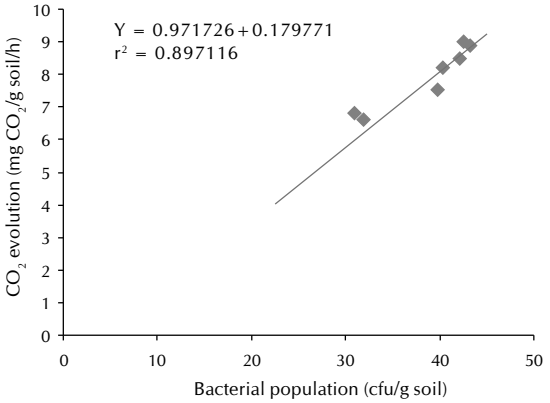

Figure 7: Linear regression between bacterial population and CO_2 evolution in 5% FA amended soil with earthworms

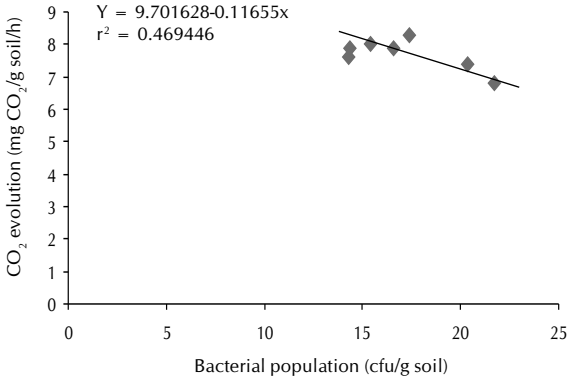

Figure 8: Linear regression between bacterial population and CO_2 evolution in 10% FA amended soil with earthworms

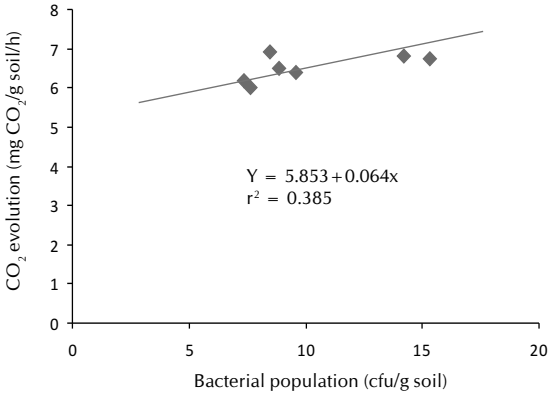

Figure 9: Linear regression between bacterial population and CO_2 evolution in 15% FA amended soil with earthworms

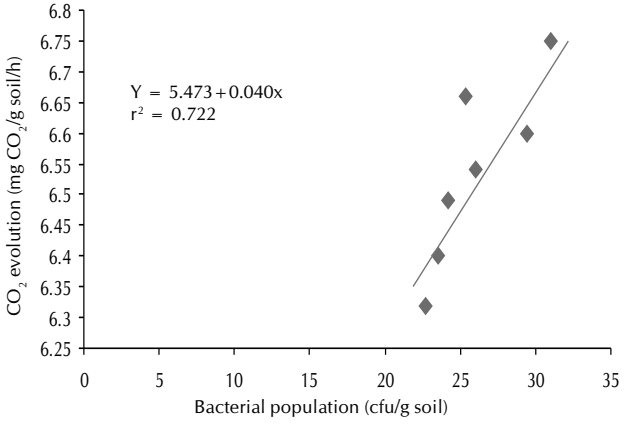

Figure 10: Linear regression between bacterial population and CO_2 evolution in 5% FA amended soil without earthworms

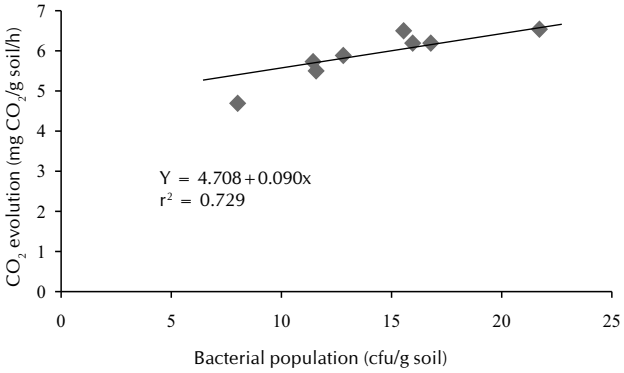

Figure 11: Linear regression between bacterial population and CO_2 evolution in 10% FA amended soil without earthworms

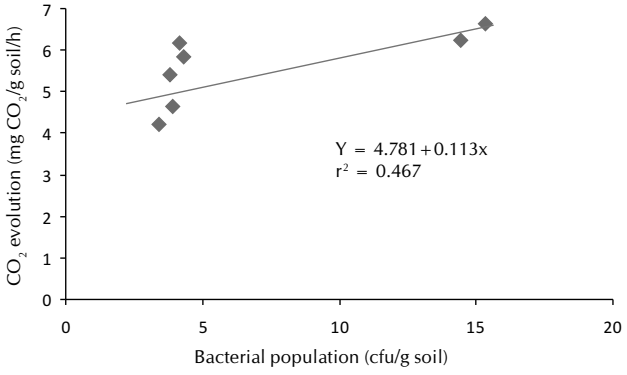

Figure 12: Linear regression between bacterial population and CO_2 evolution in 15% FA amended soil without earthworms

to 5.5±0.3 and 6.63± 0.01 to 4.2±0.1 mg CO_2/g soil/h respectively in 5, 10 and 15% FA amendment. Significant positive correlation was found among them (r= 0.850125; p≥ 0.025, r= 0.854046; p≥0.025 and r= 0.684098; p≥0.1). The relatedness among them were shown by these equations(y = 5.473396 + 0.040871x; y=4.708236+0.09075 x and y=4.781121+0.113919x) (Figs. 10, 11 and 12). In the absence of earthworms both concentration and time interval showed significant effect on CO_2 evolution with F= 4.4037, df= 6,2 p≤0,001; F=10.49934, df=6,2; p≤0.0001 and also on dehydrogenase activity(F=5.8581, df= 6,2, p≤0.001; F= 8.2551,df=6,2; p≤0.001). Enzyme activity was observed to increase gradually up to 45th day from 0.96±0.001 to 4.9±0, 0.7±2 to 2.4±0

and $0.5\pm$ to 1.5 ± 0.1 μg formazan/g soil/h in 5, 10 and 15% fly ash amended soil without earthworm respectively and then showed a sharp decline to 1.2 ± 0.3, 1.0 ± 0.2 and 0.81 ± 0.75 μg formazan/g soil/h on 90th day. (Fig. 6).Measurement of CO_2 evolution is 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 (Macfadyen, 1970; Wanner, 1970 and Witkamp, 1971) The results were in accordance with the work done by Sharada and Sanjat (2003**)** who demonstrated little or no inhibition of soil respiration and enzyme activities up to 2.5% fly ash amendment. With further addition of fly ash, all the activities were significantly decreased. Further it's observed that with earthworm inoculation there was comparatively higher dehydrogenase activity. From the result obtained it is reflected that co-application of fly ash and earthworms at lower doses can stimulates soil biological activity. Dehydrogenase activity is widely used in evaluating the metabolic activity of soil micro-organisms (Trevors, 1984, Pascual, 1996).

Comparatively the parameters of soil metabolism were incremented by the incorporation of earthworms than in their absence. It was observed that with increase in FA proportion the bacterial population CO_2 evolution and enzyme activity declined. Minimum population was noticed in 15% amendment in the absence of earthworms. It made clear that the higher FA concentration hindered the soil metabolic activity. Total bacteria, actinomycetes, fungal counts as well as enzyme activities in the soil decreased with increased ash content (Arvind *et al.,* 2010). Further it was also found that inclusions of earthworms in the waste materials resulted in many fold increase in the concentration of total viable bacteria count (Jeyanthi *et al.,* 2010). The enhancement in the population with earthworm was due to the ameliorating effect of the earthworms. 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 and nitrifier denitrification) by changing physico-chemical properties, excreting mucus, and increasing available carbon (Lubbers *et al.,* 2010). By enhancing microbial activities earthworms aid in nutrient cycling processes and in soil structure development. They have been considered beneficial animals which can significantly influence soil structure and fertility (Lee, 1985). Implying the utility of fly ash as a soil ameliorant which would not have any adverse effect on soil respiration since it provides nutrients to the micro-organisms for carrying out various metabolic activities (Wong and Wong, 1986). But the present result doesn't go with it. Optimum concentration of fly ash plays an important role for enhancing the activity. Bacterial counts in soil decreased with increase ash content was also reported by Pitchel and Hayes (1990). Significant stimulation of soil respiration and microbial activities were observed up to 5% fly ash amendment when the soils contained earthworms. This may be due to increased microbial activity induced by substrates that are produced by the earthworms. Co-application of fly ash and earthworms at lower doses can thus be considered to stimulate soil biological activity and thereby improve nutrient cycling in acidic soil (Pati and Sahu, 2004). The flush of CO_2 observed in the soil is not thoroughly understood but may result from (1) nonliving soil organic matter becoming more susceptible to microbial attack, which induces the rapid mineralization of C from exposed aggregates (Adu and Oades, 1978) and (2) the contribution of cellular lysing from water-induced osmotic shock to an easily mineralizable C pool that is consumed by the surviving soil microbes (Halverson *et al.,* 2000). Significant positive correlation has been reported by many workers between bacterial population and CO_2 evolution (Dkhar and Mishra, 1987; Pandey *et al.,* 2010). Skujins (1973) and Casida (1977) reported close correlations of dehydrogenase activity with CO_2 release and O_2 uptake, respectively, but there was no correlation with microbial number because the dehydrogenase activity depends on the total metabolic activity and soil microorganisms. 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). This activity is a measure of microbial metabolism and thus of the oxidative microbial activity in soils. Soil enzymes are extracellular secretions by living soil organisms. Therefore, any alteration in the life and function of these organisms alters soil enzymatic activity irrespective of their source of production such as bacteria, fungi or even earthworms (Cervelli *et al.,* 1975). It has the potential to predict the soil fertility (Moore and Russell, 1972) 5% amended soil showed the maximum fertility with highest enzyme activity which was further enhanced by addition of earthworms. Fly ash can promote soil microbial activity and mixing with an organic substrate enhances its benefits, which assumes importance owing to eco-friendly disposal of fly ash. A consistency was observed in the rate of carbon dioxide evolution in soil plus fly ash mixtures over a given time period and with increasing fly ash percentage.

Lower proportion of fly ash in soil proved to be optimum for the soil metabolic activities. The application of wastes to soil as a recycling option can only be sustained if there are demonstrable 'ecological benefits' which is usually justified in terms of elevated organic carbon and its effect on soil conditions and stimulation of microbial activity and nutrient supply and this is sustainable only if threshold levels of pollutants does not exceed. The earthworms acted throughout as a basic ameliorant enhancing the overall activity in the fly ash amended soil further retarding the harmfulness of the solid waste.

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