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REMOVAL OF PATHOGENIC BACTERIA DURING VERMICOMPOSTING OF FLORAL WASTES

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ABSTRACT:

During vermicomposting of organic waste, interactions between epigeic earthworms and the detrital microbial population result in a decrease in the abundance of various potentially hazardous species. Direct pathogen identification in waterways and wastes, such as *Salmonella* and *Shigella,* is difficult and time-consuming. In the present study, we carried out experiments to test the effect of the earthworm *Eisenia fetida* on total coliform numbers and pathogens in floral wastes. During process various parameters viz. temperature, pH and moisture content were measured. Study showed that different parameters changed periodically and became constant at the end of process. We found that after passing through the earthworms' stomachs, the coliform population was reduced by 98 percent, implying that digestive processes in the gut of *E. fetida* are the key reasons behind the drop in total coliforms seen in the low dose vermin-reactors. Total coliform counts decreased independently of bacterial biomass, indicating that earthworms have a distinct negative effect on coliforms. The study also showed the complete removal of pathogens *Salmonella* and *Shigella* and significant increase in *Pseudomonas* sp. in vermicompost as compared to control without worms. The study suggests that the population of bacterial group is more affected by the passage through the gut of *E. fetida.* The study also suggests that the physical factors were reached to their optimum values which were proper to eliminate pathogens.

KEYWORDS: Floral waste, vermicomposting, earthworms, pathogenic bacteria, physical parameters

INTRODUCTION:

Composting is the manipulation of a biological process called decomposition, in which raw organic materials including manure, leaves, grass clippings, food wastes, and municipal biosolids are turned into soil-like humic compounds. When organic material comes into touch with soil, it undergoes a controlled natural breakdown process. Food, paper, wastewater sludge, and garden wastes, for example, are amenable to biodegradation because they include large amounts of diverse organic substrates such as sugars (as sucrose), lipids, proteins, hemicelluloses, celluloses, and lignins. (Gray et al., 1971a; Rhyner et al., 1995; Eklind et al., 1997). Nearly 700 million tones of organic waste are generated annually in India, including leaves, husks, sawdust, steam bark, flowers, and other materials, which are either burned or dumped on land (Bhiday, 1994). It is critical to use this waste material in the productive process for both economic and environmental reasons. India is a religiously diverse and religiously tolerant country. Throughout India's history, religion has played a significant role in the country's culture. The great majority of Indians identify with a religion. Devotees present flowers and other items to their God while recovering the blessed during worship. Shri Mahakaleshwer, Harsidhi Mata, Chitaman Ganesh, Kal Bhairav, and Chardham are just a few of the numerous notable temples in Ujjain. Devotees travel from far and wide to present flowers to the Gods and Goddesses. Flowers and

other nirmalyas are tossed as waste in open locations after being offered, where they decompose aerobically and anaerobically, causing foul odour and pollution, and therefore creating an unhealthy environment. Anaerobic waste composting is expensive to manage, while aerobic waste composting takes longer. Vermicomposting looks to be the most cost-effective, biotechnologically sound technique, in which earthworms and soil microbes decompose waste materials in 45-60 days, transforming them to valuable manure rich in plant nutrients. The interactions between epigeic earthworms and the detrital microbial population during vermicomposting of organic waste result in a decrease in the abundance of several potentially harmful microorganisms. Direct Pathogen identification such as *Salmonella, Shigella* in waterways and wastes is challenging and time consuming (Monroy et al., 2009). As a result, coliforms bacteria such as *Citrobacter, Klebsiella, E.coli*, *Enterobacter* etc. are ulitized as indicators to determine whether pathogens are present. Compost made from faeces, sewage sludge and plant wastes may contain pathogens that come into contact as manures may provide a health risk to humans, and therefore this has to be pathogens free. Thus, in the present study, we carried out experiments to test the effect of the earthworm *Eisenia fetida* on total coliform numbers and pathogens in floral wastes.

MATERIAL & METHODS:

a) Collection of floral waste: The experiment was carried out with *Eisenia f*etida commonly known as red wriggler worms mainly used in vermicomposting process were reared in reactor, floral waste. The earthworms *Eisenia fetida* were purchased from the vermicompost manufacturing unit. The cattle dung was collected locally from randomly selected cattle houses of municipal territory of the study area, Ujjain. The floral waste used in this experiment was collected from Chamunda Mata and Harsiddhi Mata temple.

b) Preparation of vermicomposting bed: Collected floral wastes were chopped into small pieces. The chopped wastes were mixed with cattle dung in following ratio 50% Floral waste (FW): 50 % Cattle Dung (CD) [1.0 Kg Floral waste +1.0 Kg Cattle dung] in cylindrical plastic bins (*h* = 32cm). Conversion of floral waste into compost, 50% proportion was proved to be good (Shouche et al., 2014). The composition was prepared in three sets. The mixture was subjected to precomposting for 10 days. The windrow compost method was used. Bins were sprinkled with distilled water after turning it upside down to maintain high moisture content. After 10 days twenty earthworms (*E. foetida*) were added in two composting bins (Singh, et al, 2004) labeled as VC. The third compost bed without earthworms was kept as control labeled as C.

c) Determination of physical parameters of during the entire process:

i) **Measurement of moisture content:** 5 gm sample were taken, then kept in incubator for 24 hrs at 70°C and then weight of the dry mixture was taken and moisture content were estimated in all the mixture (Shouche et al., 2011). After addition of earthworms moisture control was maintained via sprinkling of water so as to keep earthworms alive as less moisture leads to death of worms.

ii) **Measurement of temperature:** Temperature was measured with the help of Mercury thermometer at the depth of 10 cm from three different sites and their mean value was taken in centigrade (Shouche et al., 2011).

iii) **Measurement of pH:** It was taken with the help of pH meter (Shouche et al., 2011).

d) Isolation of bacteria from compost sample:

1. Sample preparation: 10 gms of sample from both control and vermicompost or castings of

earthworms was collected from the in a sterilized petridish and transported to the laboratory for isolating and identifying the bacterial species in the vermicompost. Sampling during pre-composting period was also done. One gram was taken separately in a sterilized conical flask containing 100 ml of sterile distilled water and mixed well. One ml of diluted sample was taken and poured into 9 ml of sterile distilled water and then the sample serially diluted (Selvi et al., 2015) for culturing.

2. Isolation of bacteria from sample and control: For isolating gram negative bacteria from vermicompost, HiChrome media obtained from HiMedia, a chromogenic agar mainly used for selective isolation of coliform bacteria such as *E. coli, Klebsiella, Citrobacter,* and pathogens such as *Shigella, Salmonella.* Spread plate method was followed for isolation in which the serial diluted samples were poured on respective petriplates containing the HiChrome agar media and then incubated at 37°C for 24 hours in a bacteriological incubator (Selvi et al., 2015). After incubation the appeared bacterial colonies were subjected to sub culturing for characterization.

e) Characterization and identification of bacteria: The bacterial strains isolated were characterized first on the basis of their morphological characteristics as per HiChrome technical data and counting was followed by sub culturing for purification of colonies. In HiChrome agar the blue colonies indicates *E.coli,* light pink as Kliebsiella, dark pink as *Citrobacter* and *Enterobacter,* white colonies as *Salomonella* and *Shigella.* The purified colonies were further identified via microscopic and biochemical testing according to Mackie and McCartney (1989). **f) Statistical analysis:**

The estimated microbial populations were expressed as the mean ± SD. The purpose to determine any significant differences among the parameters analyzed in compost with worms and without worms during stabilization process.

RESULTS AND DISCUSSIONS:

Variation in the moisture content:

Results of variation in moisture content during composting are given in Table 1. At the beginning of composting the moisture contents were 80% in both control and vermicompost and then reached to 60-70% in both the reactors. Moisture content irregularly fluctuates with increasing period of composting (Fig. 2).

Variation in temperature

Another significant observation was that temperature was generally increased in earlier period of composting i.e. within 2-3 days when it increased from initially 25°C to 45-60°C during pre-composting and then reduced in later period i.e. after 35-40 days to 25°C in VC and 28°C in control (Fig. 1).

Variation in pH

In initial stage pH value was 8.01 in both the mixtures. The pH values of the composting mixtures decreased to 5-6 in VC and 4-5 in control and then started increasing gradually and reached to 6-7 in VC and 7.5-7.6 in control at the 60th day of the process (Fig 3).

Our above findings were similar to Shouche et al. (2011) and almost near to the optimum values necessary for stabilization vermicomposting process. At initial stage of stabilization process various pathogens which are *Salmonella, Shigella, E.coli.* etc. were found to be dominant but during the subsequent days there level decreased significantly (Table 1). The microbial population of *Salmonella* decreased from 19x10^3±0.5 to 9x10^3±0.5 cfu/g at 30th day and completely removal at the end of the process in vermicompost and 20x10^3±0.5 to 2x10^3±0.5 cfu/g in control (Table 1, Fig 4).

Similarly *Shigella, Kleibsiella, Citrobacter* count decreased from 106x10^3±0.5 to 0x10^3±0.5 cfu/g, 15x10^3±0.5 to 0x10^3±0.5 cfu/g, 35x10^3±0.5 to

$0x10^3 \pm 0.5$ cfu/g respectively in vermistabilization and $107x10^3 \pm 0.5$ cfu/g to $66x10^3 \pm 0.5$ cfu/g, $14x10^3 \pm 0.5$ to $5x10^3 \pm 0.5$ cfu/g, $38x10^3 \pm 0.5$ to $9x10^3 \pm 0.5$ cfu/g in control respectively (Table 1, Fig 4). The *E.coli* count however was not visible in 10^3 dilutions but visible in 10^2 dilutions and removed at the end of the process $4x10^2 \pm 0.5$ to $0x10^2 \pm 0.5$ in VC and $5x10^2 \pm 0.5$ to $0x10^2 \pm 0.5$ in C (Table 1, Fig 4). During the experiment however population of bacteria occurs which produce green fluorescens, which on further testing was found to be *Pseudomonas* sp. significantly increased from $4x10^3 \pm 0.5$ to $46x10^3 \pm 0.5$ cfu/g in VC but this is not the case in control where count is between $5x10^2 \pm 0.5$ to $6x10^2 \pm 0.5$ cfu/g in C (Table 1, Fig 4). This shows that in gut of earthworms some species of *Pseudomonas* flourishes and at the end no other coliforms appears in VC suggests that *Pseudomonas* may produces some antagonistic substances which can inhibit the growth of *Salmonella, Shigella, E.coli. Kliebsiella* (Ganeshan and Kumar, 2007). Our findings of the present study were similar to the experimental results of Kumar and Shweta, (2011) where microbiomics of gut and castings proved reduction of *Salmonella, Shigella, E.coli and Flexibacter* in Rice straw, sugar cane and vegetable wastes mixed with cow dung. Kumar and Sekaran (2005) also reported removal of *Salmonella* and *E.coli* in 35 days in municipal solid wastes by *Lampito mauriti*. Bajsa et al., (2003) reported the removal of fecal coliforms, *Salmonella*, enteric viruses, helminthes ova from sewage and sludge appear to be much more rapid when they are processed by *E.foetida*. Safawat (2002) also confirmed human pathogen reduction in biosolids, vermicomposted by earthworms. According to Edwards and Bohen (1996b), microflora passage through the gut may selectively decrease the number of certain microbes such as *Shigella* and *E.coli*. The removal of pathogens may be due to release of antibiotics by earthworms responsible to kill pathogenic organisms inhabit and render it virtually sterile (Sinha et al., 2009a). The worms also release the coelomic fluids that may have antibacterial properties which may destroy the pathogens in reactors (Pierre et al., 1982). However absolute removal of pathogens is difficult to achieve, some may survives even after the whole process of waste degradation. The study also suggests that the physical factors were reached to their optimum values which were proper to eliminate pathogens.

CONCLUSION:

From the above study it can be concluded that the physical parameters were effective for the elimination of fecal coliforms and pathogens. The earthworms also release the coelomic fluids that may have antibacterial properties which may destroy the pathogens in reactors. The presence of *Pseudomonas* sp. in high number in vermicompost also have some role in complete removal of *E. coli* and other pathogenic bacteria at the end of the degradation process. Thus, vermicomposting is an effective waste management tool for temple waste on one hand and also remove pathogens from compost made from faeces, sewage sludge and plant wastes, floral.

Future aspects:

The study showed that vermicomposting not only can provide you valuable rich eco-friendly manure with nutrients and but also a habitat to explore new antagonistic compounds that can be extracted and identified to use against antibiotic resistance organisms. The technique can be widely used mainly for the wastes left in open, near water bodies, municipal solid wastes and hospital wastes which carry pathogens of public health concern in large quantities.

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Table 1: Enumeration of coliforms and pathogenic bacteria in vermicompost during stabilization of waste

Reactors	0 day (Precomposting period)	3 days (Precomposting period)	30 days	60 days
Salmonellla sp.				
VC	$19x10^3±0.5$	$17x10^3±0.8$	$9x10^3±0.5$	$0x10^3±0.5$
C	$20x10^3±0.5$	$16x10^3±0.5$	$9x10^3±0.5$	$2x10^3±0.5$
Shigella sp.				
VC	$106x10^3±1.2$	$104x10^3±0.8$	$89x10^3±0.8$	$0x10^3±0.5$
C	$107x10^3±0.5$	$103x10^3±0.5$	$98x10^3±0.9$	$66x10^3±0.5$
Kleibsiella sp.				
VC	$15x10^3±0.5$	$9x10^3±0.5$	$5x10^3±0.5$	$0x10^3±0.5$
C	$14x10^3±0.5$	$9x10^3±0.5$	$7x10^3±0.5$	$5x10^3±0.5$
Citrobacter sp.				
VC	$35x10^3±0.5$	$20x10^3±0.5$	$7x10^3±0.8$	$0x10^3±0.5$
C	$38x10^3±0.5$	$21x10^3±0.5$	$19x10^3±0.5$	$9x10^3±0.9$
Pseudomonas sp.				
VC	$4x10^3±0.5$	$6x10^3±0.5$	$34x10^3±0.9$	$46x10^3±0.5$
C	$5x10^3±0.5$	$6x10^3±0.5$	$7x10^3±0.5$	$6x10^3±0.5$
E.coli				
VC	$4x10^2±0.5$	$0x10^2±0.5$	$0x10^2±0.5$	$0x10^2±0.5$
C	$5x10^2±0.5$	$1x10^2±0.5$	$0x10^2±0.5$	$0x10^2±0.5$

All values are mean and standard deviation of readings and expressed in cfu g^{-1}

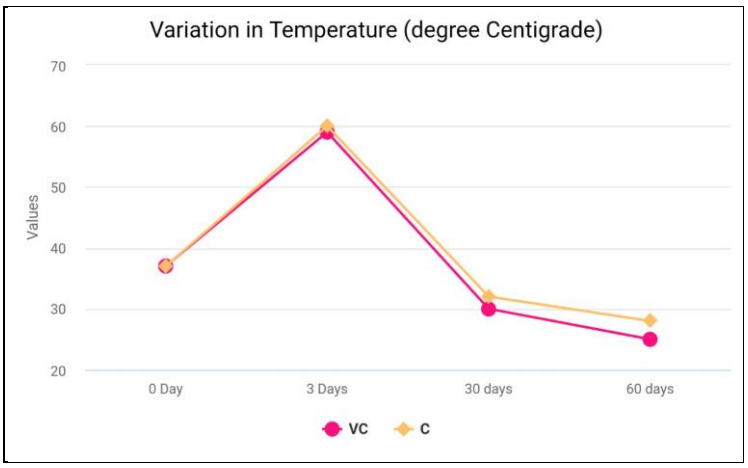

Fig. 1. Variation in Temperature

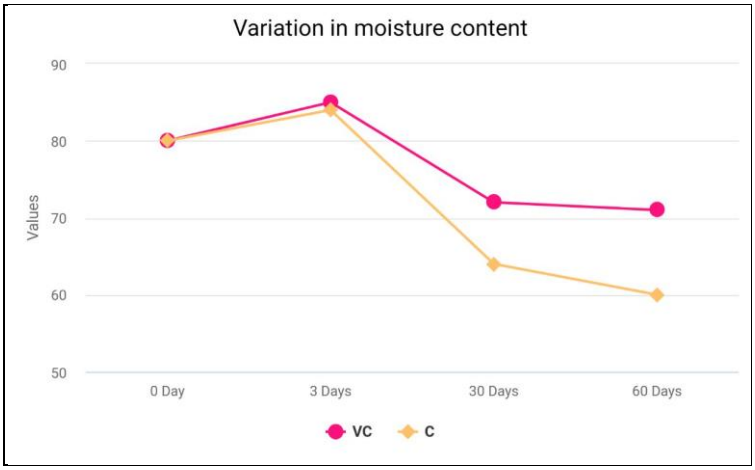

Fig. 2. Variation in moisture content

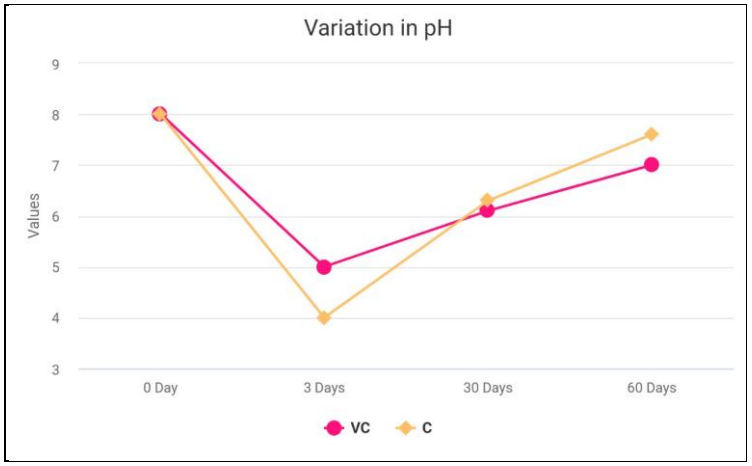

Fig. 3. Variation in pH

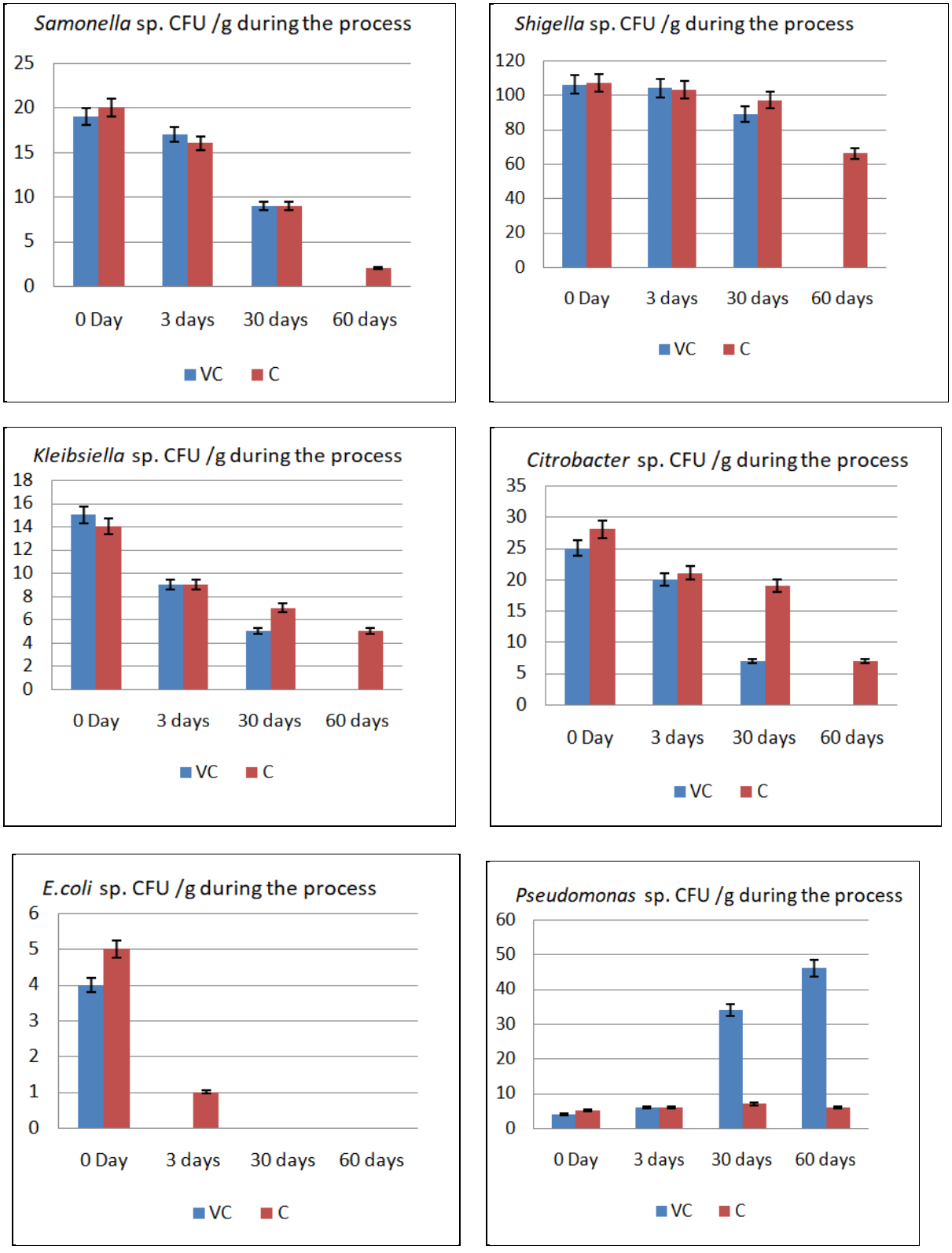

Fig.4 showing CFU count of bacteria during the entire process of vermicomposting: *Salmonella*, Shigella, *Klebsiella*, *Citrobacter*, *E.coli*, *Pseudomonas*