Chapter 2
Study of Total Microflora in Earthworm Burrow wall

“I spent whole afternoons in the dirt, making my patch of ground flawless. I even cleared the worms away; before I found out that all the tunnels they make give air, and probably other molecules I don’t know about yet, to the plants.”

Jane Hamilton, The Book of Ruth

Introduction

Microorganisms one of the major functional components of a terrestrial ecosystem, are diverse in their occurrence. Their total count, composition and their interaction with other soil organisms and higher plants determine their role in decomposition and their potential importance in soil. Several types of microorganisms, such as microalgae, bacteria, microfungi, protozoa and actinomycetes occur in the soil. The lives of earthworms and microbes are closely entwined. Earthworms derive their nutrition from fungi, bacteria, and possibly protozoa and nematodes, and in turn promote the activity of these organisms by shredding and increasing the surface area of organic matter and making it easily available. Earthworms may derive benefit from microorganisms when they have to survive on materials rich in cellulose or hemi cellulose.

Kale and Karmegam (2010) emphasize the existence of a mutualistic relation between earthworms and microorganisms. The level of the complex interrelationship of earthworms and microorganisms has been found to be in their digestive tract, castings, and burrow walls (Edwards and Arancon 2004). The colony forming units (CFUs) of bacteria and fungi in the casts of Pontoscolex corethrurus significantly deviated from the CFU found in adjacent soil. The correlation between the physicochemical parameters and microbial populations of the casts of P. corethrurus showed that the establishment of microbial population requires optimum moisture, organic carbon, and nitrogen content (Karmegam and Daniel 2000). Although earthworms derive their nutrition from microorganisms, many more microorganisms are present in their feaces or casts than in the organic matter that they consume. As organic matter passes through their intestines, it is fragmented and inoculated with microorganisms. Increased microbial activity facilitates the cycling of nutrients from organic matter and their conversion into forms readily taken up by plants. Earthworms also influence
other soil-inhabiting invertebrates by changing the amount and distribution of organic matter and microbial populations. Soil microorganisms are markedly concentrated at the surface of the burrow walls and within the adjacent 2mm of the surrounding soil. This microenvironment comprises less than 3 percent of the total soil volume but contains 5-25 percent of the whole soil microflora and is where some of the functional groups of microbes predominate (Lavelle and Spain 2001).

**Bacteria and actinomycetes**

Bacteria are the most frequent group of microorganisms present in the soil and they constitute approximately half of the total microbial biomass. One gram fertile soil may contain as many as $10^9$ bacteria. Bacteria occur in all types of soil, but their number decreases with the depth of the soil. There are many different species of bacteria, each with its own role in the soil environment. Some common soil bacteria are the species of *Pseudomonas, Arthrobacter, Achromobacter, Bacillus, Clostridium, Micrococcus, Flavobacterium, Chromobacterium* and *Mycobacterium*. One of the major benefits bacteria provide for plants is in making nutrients available to them. Some species release nitrogen, sulfur, phosphorus, and trace elements from organic matter. Others break down soil minerals, releasing potassium, phosphorus, magnesium, calcium, and iron. Still other species make and release plant growth hormones, which stimulate root growth. A few species of bacteria fix nitrogen in the roots of legumes, while others fix nitrogen independently of plant association. Bacteria are responsible for converting nitrogen from ammonium to nitrate and back again, depending on certain soil conditions. Other benefits to plants provided by various species of bacteria include increasing the solubility of nutrients, improving soil structure, fighting root diseases, and detoxifying soil. Bacteria are important in soils because they contribute to the carbon cycle by fixation and decomposition. Some bacteria are important decomposers and actinomycetes are particularly effective at breaking down tough substances such as cellulose and chitin.

Fifty five percent of the bacteria isolated from burrows were capable of fixing nitrogen and 16% of them were denitrifying with maximum populations at 20-40 cm depth in the soil (Bhatnagar 1975). Similarly, Parkin and Berry (1999), found elevated populations of nitrifying and denitrifying bacteria and increased rates of nitrification.
and denitrification in the drilosphere as compared to bulk soil. The excretion products that the earthworms secrete are nitrogen rich and are probably responsible for the elevated levels of nitrogen transformation in the drilosphere. Karsten and Drake (1995) found more anaerobes and cellobiose-utilizers in earthworm guts than in soil. Burrow walls of *Lumbricus terrestris* were dominated by *Cellulomonas* and *Promicromonospora*. They also had *Azotobacter*, *Streptomyces*, Myxobacteriales, and motile Gram-negative rods (mostly *Aquaspirillum*, *Alcaligenes* and *Enterobacter*) whereas * Bacillus* and *Streptomyces* prevailed in the control soil. Clegg et al., (1995) found that total bacterial counts in burrow and bulk soil were initially no different, but increased through time in casts and remained elevated compared with bulk soil. Also at greater depths the contrast between drilosphere and bulk soil and the relative contribution of burrow walls to the total soil microbial activity probably increase strongly (Stehouwer et al., 1993; Joergensen et al., 1998). Tiunov and Scheu (1999) in a study of three forest ecosystems in two seasons have shown that the volume of bacteria in the drilosphere increased in the lime forest soil (up to a factor of 5.5 in June, by a factor of 2.1 in October) and in the oak and beech forest soil by factors of 3.0±3.2 and 2.5±2.6, respectively. Large number of rod-shaped bacteria was observed in the lime forest in June which were less abundant in October. Also the bacterial volume in the beech forest decreased by about 70% from June to October. In casts of *Allolobophora terrestris*, as the casts aged, the number of actinomycetes and bacteria appeared to increase (Parle 1963).

Actinomycetes are threadlike bacteria that look like fungi. While not as numerous as bacteria, they too perform vital roles in the soil. Like the bacteria, they help decompose organic matter into humus, releasing nutrients. They also produce antibiotics to fight diseases of roots. Many of these same antibiotics are used to treat human diseases. Actinomycetes are responsible for the sweet, earthy smell noticed whenever a biologically active soil is tilled. They prefer neutral to alkaline soils; high oxygen requirement; prevalent in dry regions. They release carbon, nitrogen, and ammonia during decomposition of organic matter. They help form humus and associate with non-leguminous plants to fix nitrogen and make it available to other plants in the area. Some nitrogen may be unusable without the bacteria converting it to a form that can be used. A large number of actinomycetes are particularly abundant in the soil rich in decomposed organic materials. Species of *Streptomyces*,

Micromonospora and Nocardia are some common actinomycetes occurring in soils. Reports highlight the higher number of actinomycetes and Vibrio spp. found in earthworm casts as compared to bulk soil (Contreras 1980; Mariaglieti 1979).

Fungi
Studies on the fungal population from diverse vegetation sites have concluded that soil microfungi show ecological and geo climatic specificity with response to environmental parameters (Christensen et al., 1962; Gochenaur and Whittingham 1978; Christensen and Whittingham 1965; Christensen 1969). They are also important for immobilizing or retaining nutrients in the soils. Several micro fungi are present in the soil and they play significant role for the improvement of soil nutrients in neutral and alkaline soils. Hundreds of species of moulds inhabit the soil. The quality and quantity of organic materials present in the soil have a direct effect on the fungal population of the soil. The development of microfungi is especially favoured by soils having an acidic reaction and where the aerobic condition is likely to be present near the surface.

Fungi come in many different species, sizes and shapes in soil. Some species appear as threadlike colonies, while others are one-celled yeasts. Many fungi aid plants by breaking down organic matter or by releasing nutrients from soil minerals. They are generally quick to colonize larger pieces of organic matter and begin the decomposition process. Some fungi produce plant hormones, while others produce antibiotics including penicillin. There are species of fungi that trap harmful plant-parasitic nematodes. The mycorrhizae are fungi that live either on or in plant roots and act to extend the reach of root hairs into the soil. Mycorrhizae increase the uptake of water and nutrients, especially phosphorus. They are particularly important in degraded or less fertile soils. The fungi benefit by taking nutrients and carbohydrates from the plant roots they live in.

Fungi are important for immobilizing or retaining nutrients in the soil. Gange (1993) has reported the importance of earthworms in both the reduction and dispersal of soilborne animal and plant fungal pathogens and the spread of beneficial groups like the mycorrhizal fungi. Generally, a substantial part of the fungal community in
“lined” burrow walls were typical “litter” species, mainly *Trichoderma* and *Mucor* (Tiunov and Dobrovolskaya 2002). Tiwari and Mishra (1993) isolated 27 species of fungi from earthworm casts. A study of the burrow wall soil of *L. terrestris* in three forest ecosystems revealed, the fungal volume to be higher in the burrow walls of beech and oak forests where as the same was not seen in case of the lime forest (Tiunov and Scheu 1999). A seasonal increase in the fungal volume with a significantly higher volume in June than in October was also observed by these authors. Parle (1963) has shown that in older casts of *Allolobophora terrestris*, the numbers of yeasts and filamentous fungi increased. McLean *et al.*, (2006) has shown that the presence of earthworms decreased zygomycete species abundance probably due to disruption of fungal hyphae.

Invasive earthworms can negatively impact the fungal community in forests by changing nutrient cycling (Scheu and Parkinson 1994; Steinberg *et al.*, 1997). A study in the Canadian Rocky Mountains, conducted by Scheu and Parkinson (1994), demonstrated that within eight weeks of introduction, earthworms reduced the fungal content of the soil from an initial 55% to between 30 and 40%. Fungi that are most drastically affected by the actions of invasive earthworms are fast-growing fungal decomposer species such as *Mucor, Trichoderma* and *Fusarium* (Visser 1985).

Not much work in the aspect of microflora has yet been done in the earthworm burrow walls. Therefore, the present work aims to find out the occurrence, distribution, dominance and variation of soil bacteria, actinomycetes and fungi in this ecosystem.

**Materials and Methods**

**Generation of Soil Samples**

*Collection of Earthworms Species (Lampito mauritii and Pontoscolex corethrurus)*

A natural plot (garden) was identified for collection of earthworms. About one square meter plot was cleared off surface plant growth. One percent jaggery (unrefined brown sugar made from palm sap) solution was sprayed at the spot and allowed to remain for 48 hrs to attract the underlying earthworm population. The spot was dug and the earthworms were collected for use in the laboratory. *Lampito mauritii* was
identified by the dorsal colour which is grayish, brownish or yellowish with purplish tinge at the anterior end (Kale 1997). *Pontoscolex corethrurus* was identified by the calciferous glands (Julka 1988).

**Adaptation of Earthworms to Laboratory Conditions**

To adapt the earthworms to laboratory conditions they were maintained for two months in glass troughs having a layer of garden soil and a top layer of humus prepared by mixing garden soil and leaf litter for a period of two months.

**Experimental Set Up**

The experimental set up in the laboratory was prepared in chambers with a dimension of 30cm width x 40cm length and a depth of 10cm (Fig 1) in two sets for each species of earthworm. The sample material was generated in the laboratory by maintaining earthworms in these chambers. Three fourth of the chambers were filled with air dried garden soil and the top one fourth of the chamber was filled with humus. The chamber was moistened to maintain 60% water level before releasing ten earthworms into each set up.

![Laboratory set up for sample generation](image)

**Figure 1.1-** Laboratory set up for sample generation

**Sample Collection**

Soil sample was collected by destructive method with sterile spatula by carving the inside lining of the burrows. Sampling was carried out at 30 days and 45 days at two depths of 0-15cm and 15-30 cm. Control soil was collected away from the burrow walls, at both intervals and depths.

The samples collected were abbreviated as follows

<p>| Upper burrow wall soil (30 days) | UBWS (30d) |</p>
<table>
<thead>
<tr>
<th>Upper control soil (30 days)</th>
<th>UCS (30d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower burrow wall soil (30 days)</td>
<td>LBWS (30d)</td>
</tr>
<tr>
<td>Lower control soil (30 days)</td>
<td>LCS(30d)</td>
</tr>
<tr>
<td>Upper burrow wall soil (45 days)</td>
<td>UBWS (45d)</td>
</tr>
<tr>
<td>Upper control soil (45 days)</td>
<td>UCS (45d)</td>
</tr>
<tr>
<td>Lower burrow wall soil (45 days)</td>
<td>LBWS (45d)</td>
</tr>
<tr>
<td>Lower control soil (45 days)</td>
<td>LCS(45d)</td>
</tr>
</tbody>
</table>

A total of 8 soil samples (4 from burrow wall and 4 from control) were generated from each species of earthworm for each interval. Finally 16 samples, 8 each from the two species of earthworms were used for further analysis.

**Physical and chemical analysis of soil samples**

The soil was analyzed for the following physical characteristics

**pH**

The pH value, which is the measure of the hydrogen ion activity of the soil-water system, indicates whether the soil is acidic, neutral or alkaline in reaction. A glass electrode pH meter with calomel reference electrode was used for pH measurement.

Procedure

- 10 Grams of soil was taken in a 100ml beaker to which 40ml of distilled water was added.
- The suspension was stirred at regular intervals for 30 minutes and the pH recorded.

**Electrical conductivity**

Ions being the carrier of electricity, the electrical conductivity (EC) of the soil-water system rises according to the content of soluble salts in the soil, giving rise to more of ion pairs on dissociation as it happens in case of a dilute solution. Thus the measurement of EC can be directly related to the soluble salts concentration of the soil at any particular temperature. The apparatus for measuring electrical conductivity consists of an AC salt bridge and conductivity cell having electrodes coated with platinum black.

**Reagents**

Standard potassium chloride solution- 0.7456g of dry reagent grade potassium chloride dissolved in 1000ml double distilled water (at 25°C it gives an EC of 1.41mmhos/cm).
Procedure

- 10g of soil was taken in a 150ml erlenmeyer flask to which 40ml of distilled water was added.
- The suspension was allowed to stand for 30 minutes.
- The conductivity of the supernatant was determined with the help of the conductivity bridge.
- The measurement of EC was expressed in mmhos/cm

Chemical Analysis of Soil Samples included the following -

*Organic carbon*

The organic matter (humus) in the soil gets oxidized by chromic acid (potassium dichromate plus conc. sulphuric acid) utilizing the heat of dilution of sulphuric acid. The unreacted dichromate is determined by back titration with ferrous ammonium sulphate (redox titration).

**Reagents**

1N potassium dichromate, 0.5N ferrous ammonium sulphate (FAS), diphenylamine indicator, concentrated sulphuric acid containing 1.25 percent silver sulphate, 85%

**Ortho-phosphoric acid**

**Procedure**

- 1g of soil, ground and passed through 0.2mm sieve was placed at the bottom of a dry 500ml conical flask.
- 10ml 1 N K₂Cr₂O₇ was pipetted in and swirled a little and kept on asbestos sheet.
- 20ml of H₂SO₄ (containing 1.25% silver sulphate) was run in and swirled again 2-3 times.
- The flask was allowed to stand for 30 minutes and 200ml of distilled water was added.
- After incorporation of 10ml of 85% phosphoric acid and 1ml of diphenylamine indicator the contents were titrated with 0.5N ferrous ammonium sulphate solution.
- End point was blue-violet to green.
- Simultaneously a blank was run without soil.
Calculation

Organic carbon $\% = \frac{10 \cdot (B - T)}{B} \times 0.003 \times \frac{100}{\text{Wt. of soil}}$

Where $B =$ volume in ml of ferrous ammonium sulphate solution required for blank titration; and
$T =$ volume of ferrous ammonium sulphate solution required for soil sample.

**Phosphorus**

Phosphorus in soil occurs as orthophosphate in several forms and combinations and only a small fraction of the total amount present may be available to plants, which is of direct relevance in assessing the phosphorus fertility level. The available phosphorus content is readily determined by extraction with suitable reagents according to specified soil to solution ratio and time of shaking. In the filtered extract, phosphorus is estimated colorimetrically by adding ammonium molybdate and thereafter reducing the molybdenum-phosphate complex in acidic medium. The intensity of blue colour on reduction provides a measure for the concentration of phosphorus in the test solution.

**Reagents**

- **Olsen's reagent:** 0.5 molar sodium bicarbonate (pH 8.5) - 42g of NaHCO$_3$ in 1000ml distilled water. The pH is adjusted to 8.5 with small quantities of 10% NaOH.
- **Dickman and Bray's reagent** - 15g of ammonium molybdate is dissolved in 300ml of warm water (about 60°C), cooled and filtered. To this, 400ml of 10N HCl is added and made up to 1000ml.
- **Stannous chloride solution** - (40% stock) 10g of crystalline stannous chloride (LR) is dissolved in 25ml concentrated HCl by warming and stored in amber coloured bottle. Just before use 0.5ml is diluted to 66ml with distilled water.
- **100ppm stock solution of phosphorus (100µg/ml), Potassium dihydrogen orthophosphate** (dried in oven for 60°C for 1 hour and cooled) 0.439g in 500ml distilled water, 25ml of 7N H$_2$SO$_4$ is added and made up to 1000ml with distilled water.

**Procedure**

- To 0.25g of soil in 100ml conical flask a little of activated carbon (free of phosphorus) was added followed by 50ml of Olsen's reagent.
- A blank was run without soil with each set.
The flasks were shaken for 30 minutes on a shaker and the contents filtered immediately through Whatman No.1 filter paper.

5ml of the filtrate was pipetted into a 25ml volumetric flask and 5ml of Dickman and Bray's reagent was added drop by drop with constant shaking till the effervescence due to CO$_2$ evolution ceases.

The contents were diluted to about 22ml.

1 ml of diluted stannous chloride solution was added and the volume made up to the mark.

The intensity of the blue colour was measured using 660nm after 10 minutes.

For the standard curve different concentrations of phosphorus were made from the stock solution in 25ml volumetric flasks.

5ml of Olsen's reagent was added followed by 5ml of Dickman and Bray's reagent and 1 ml of diluted stannous chloride solution was added for colour development.

The volume was made up to the mark with distilled water.

Colorimeter reading was taken at 660nm after 10 minutes.

The curve was plotted taking OD on the y-axis and the amount of phosphorus in the x-axis and the concentration of phosphorus determined from the standard curve.

Calculation

Available P (Kg/ha) = R x \( \frac{\text{Total vol. of extract}}{\text{Vol. of sample}} \) x \( \frac{1}{\text{Wt. of soil}} \) x \( \frac{2.24 \times 10^6}{10^6} \)

Where R = µg P in the sample from the standard curve

Potassium

The estimation of available potassium was carried out with the help of flame photometer. The term available potassium incorporates both exchangeable and water soluble forms of the nutrient present in the soil. These are determined in the neutral normal ammonium acetate extract of soil. The ammonium ion provides a sharp and rapid separation from exchange complex while other cations bring about a gradual replacement of either lesser or greater amount of K which generally increases with the
period of contact. The most commonly employed extractants are the strong inorganic acids like 6N H$_2$SO$_4$, hot HCl and boiling 1N HNO$_3$.

Reagents
Neutral normal ammonium acetate- Equal volumes of 2N glacial acetic acid and 2N ammonium hydroxide are mixed, cooled and pH adjusted to 7.0 with acetic acid or ammonia.

Potassium chloride solution (1000µg/ml)- 1.908g of AR grade potassium chloride dried at 60°C for 1 hour is dissolved in 1000ml distilled water.

Procedure
- 5g of soil was shaken with 25ml neutral normal ammonium acetate for 5 minutes and filtered immediately through a dry Whatman No. 1 filter paper.
- Potassium concentration in the extract was determined in the flame photometer after setting and calibration of the instrument.
- From the stock solution, dilutions were prepared with ammonium acetate solution to give 10 to 40 ppm of potassium.
- The flame photometer was set to zero for the blank (ammonium acetate) and at 100 for 40 ppm potassium.
- The standard curve was obtained by plotting the readings against different concentrations (10, 15, 20, 25, 30, 40 ppm) of potassium.

Calculations
Available K (Kg/ha) = \( R \times \frac{\text{Total vol. of extract}}{\text{Vol. of sample}} \times \frac{2.24 \times 10^6}{10^6} \)

Where R= ppm of K in the extract from standard curve

Micronutrient analysis
Digestion of the soil sample
Reagents: Aqua regia [nitric acid (HNO$_3$)- hydrochloric acid (HCl) 1:3 V N]
Procedure
- 5g of well-mixed sample was kept in an oven for 2 hrs at 104°C and ground in a pestle and mortar to get a fine powder.
- 1-2g of fine powdered sample was taken in a Kjeldahl flask with 20ml of distilled water (metal free) and 10-15ml of Aqua regia.
The sample was digested on a sand bath in an exhaust chamber till the entire black residue turns colorless.

The digestion vessel was removed and cooled and contents transferred to a 100ml volumetric flask through Whatman filter paper No. 41.

The digestion vessel was rinsed with distilled water 2-3 times and the contents transferred through the same filter paper.

The filtrate was made up to 100ml in a standard volumetric flask. This solution was used for analysis of heavy metal by Atomic Absorption Spectrophotometer.

The micronutrients analysed include - zinc, iron, copper and manganese.

**Microbiological analysis of soil samples**

Soil samples collected were analyzed for total count of bacteria, fungi and actinomycetes. Selective media were used to isolate specific microorganisms.

**Total Count of Bacteria, Fungi and Actinomycetes**

*Preparation of serial dilutions*

One gram of soil was mixed with 10ml sterile distilled water to get a dilution of $10^{-1}$. This was vortexed well to disperse the soil in the water. 1 ml from $10^{-1}$ dilution was taken into 9ml sterile distilled water to get a dilution of $10^{-2}$. This was continued to get dilutions of $10^{-3}$, $10^{-4}$ and $10^{-5}$.

*Isolation of bacteria on Soil Extract Agar*

Soil extract agar contains the wide range of the amino acids and growth factors required by soil microorganisms. 1 ml of the dilution $10^{-5}$ was aseptically pour plated with sterile soil extract agar. The plates were incubated at 37°C for 24 hours. The total colony count in each plate was taken using a colony counter. The number of colony forming units (CFU) was calculated by multiplying with the dilution factor. An average of three plates was taken. The colony characteristics and the total number of each colony were noted. A pure culture of the representative colonies was made on soil extract agar slants for biochemical characterization.

*Isolation of Fungi on Martin’s Rose Bengal agar*
Aliquots of $10^{-3}$ were pour plated on Martins Rose Bengal agar. The plates were incubated at $28^\circ$C for 3-5 days. The total colony forming units were counted and multiplied by the dilution factor to get CFU/g counts. A pure culture of the representative colonies was made on Martins Rose Bengal agar slants for identification and characterization.

**Isolation of Actinomycetes on Actinomycetes agar**
Aliquots of $10^{-3}$ were pour plated on actinomycete agar. The plates were incubated at $28^\circ$C for 5-7 days. The total colony forming units were counted and multiplied by the dilution factor to get CFU/g counts. A pure culture of the representative colonies was made on actinomycetes agar slants for identification and characterization.

**Isolation of Bacteria on Selective Media**
Selective media or differential media support the growth of some organisms while specifically inhibiting the growth of others. These media expedite the rapid isolation and identification of particular microbe by preventing the growth of interfering organisms. Most selective media contain inhibitors that suppress the growth of contaminants. These media allow certain organisms to produce macroscopically distinct colonies, or characteristic zones around colonies, that are helpful in distinguishing these organisms from others in the sample. Once isolated, pure culture of the bacteria were preserved on respective media for further analysis.

**Eosin Methylene Blue Agar**
EMB agar is a differential-plating medium that can be used in the isolation and detection of the Enterobacteriaceae or related coliform bacilli from specimens with mixed bacteria. The eosine and methylene blue dyes inhibit Gram-positive and fastidious Gram-negative bacteria. They combine to form a precipitate at acid pH, thus also serving as indicators of acid production. Typical strong lactose-fermenting colonies, notably *E. coli*, produce colonies that are green- black with a metallic sheen. Weak fermenters, including *Klebsiella, Enterobacter, Serratia* and *Hafnia*, produce colonies within 24-48h. Non- lactose fermenters, including *Proteus, Salmonella* and *Shigella*, produce transparent colonies. Eosin methylene blue agar (Hi-media) was
prepared and sterilized. A pour plate of 1ml of $10^{-3}$ dilution was made. The plates mixed well and incubated at 30°C for 24 hours.

**MacConkey's Agar**

MacConkey’s agar is a differential-plating medium for the selection and recovery of the Enterobacteriaceae and related enteric Gram-negative bacteria. The bile salts and crystal violet inhibit the growth of Gram-positive bacteria and some fastidious Gram-negative bacteria. Lactose is the sole carbohydrate. Lactose-fermenting bacteria produce colonies that are of varying shades of red, owing to the conversion of the neutral red indicator dye from the production of mixed acids. Colonies of non-lactose fermenting bacteria appear colourless or transparent. Typical strong lactose fermenters, such as species of *Escherichia*, *Klebsiella* and *Enterobacter*, produce red colonies surrounded by a zone of precipitated bile. Slow or weak lactose fermenters, such as *Citrobacter*, *Providencia*, *Serratia*, and *Hafnia*, may appear colourless after 24h or slightly pink in 24-48h. Species of *Proteus*, *Edwardsiella*, *Salmonella* and *Shigella*, with rare exceptions, produce colourless or transparent colonies. MacConkey's Agar (Hi-media) was prepared and sterilized. A pour plate of 1ml of $10^{-3}$ dilution was made. The plates mixed well and incubated at 30°C for 24 hours.

**Baird Parker Agar**

Baird Parker media is a selective medium for the isolation of staphylococci. The colonies appear black owing to reduction of potassium tellurite to metallic tellurium. Baird Parker agar (Hi-media) was prepared and sterilized. A pour plate of 1ml of $10^{-3}$ dilution was made. The plates mixed well and incubated at 30°C for 24 hours.

**King’s B media for Pseudomonas**

King’s B a selective medium for isolation of *Pseudomonas* was prepared and sterilized. A pour plate of 1ml of $10^{-3}$ dilution was made. The plates mixed well and incubated at 30°C for 24 hours. The pure cultures of representative colonies were made on King’s B agar slants.

**Identification of bacteria**
Gram staining of the bacteria was done for initial identification of the isolates. Biochemical tests were carried out to identify the bacteria further. Bergey’s Manual of Determinative Bacteriology (Hensyl 1994) was used as reference for identification of bacterial isolates.

A few Hi media biochemical test kits were used for identification of bacteria. These tests are based on the principle of pH change and substrate utilization. On incubation, organisms undergo metabolic changes which are indicated as a colour change in the media that can be either interpreted visually or after addition of the reagent.

**KB001 HiIMViC Biochemical Test Kit**

KB001 is a comprehensive test system that can be used for differentiation of Gram-negative Enterobacteriaceae species. Organisms belonging to Enterobacteriaceae are Gram-negative, oxidase negative and nitrate positive rods. The organisms that can be identified with this system include species of *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella* and *Proteus* among others.

**Principle**

Each HiIMViC kit is a standardized colorimetric identification system utilizing four conventional biochemical tests (Indole, Methyl red, Voges Proskaeur's and Citrate utilization) and eight carbohydrate utilization tests (Glucose, Adonitol, Arabinose, Lactose, Sorbitol, Mannitol, Rhamnose and Sucrose).

**KB004 Hi Staph Identification Kit**

KB004 is a biochemical test kit for identification and differentiation of genus *Staphylococcus*. About 43 species of staphylococci can be identified with this system.

**Principle**

KB004 is a standardized, colorimetric identification system utilizing twelve conventional biochemical tests which include Voges-Prokauer's test, Alkaline Phosphatase test, ONPG, Urease, Arginine utilization, Mannitol, Sucrose, Lactose, Arabinose, Raffinose, Trehalose and Maltose.

**KB002 Hi Assorted Biochemical Test Kit (for Gram-negative rods)**
KB002 is a test system that can be used for identification of Gram-negative rods. The organisms that can be identified with this system include species of *Aeromonas*, *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Proteus* and *Pseudomonas* among others.

**Principle**

Is similar to the HiIMViC kit utilizing seven conventional biochemical tests (Citrate utilization, Lysin and Ornithine decarboxylase, Urease, Phenylalanine Deamination, Nitrate Reduction, H₂S production) and five carbohydrate utilization tests (Glucose, Adonitol, Lactose, Arabinose and Sorbitol).

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**KB009 HiCarbohydrate Kit**

KB009 is a comprehensive test system that can be used to study the biochemical profile of a wide variety of organisms.

**Principle**

Each Hi Carbohydrate kit is a standardized colorimetric identification system utilizing thirty five carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On incubation organisms undergo metabolic changes which are indicated by a spontaneous colour change in the media.

**Procedure**

- Pure culture of the isolates were made on nutrient agar
- A single well isolated colony was inoculated in 5ml Nutrient broth and incubated at 37°C for 4-6 hours until the inoculum turbidity reached ≥ 0.10 D at 620nm. Alternatively a homogeneous suspension was made in 2-3 ml sterile saline. The density of the suspension was adjusted to 0.10 D at 620nm
- The kit is opened aseptically and the sealing tape peeled off.
- Each well is inoculated with 50 μL of the above inoculum by surface inoculation method.
- Alternatively the strip was inoculated by stabbing each individual well with a loopful of inoculum.
- The strips were incubated at 35 – 37°C for 18 - 24 hours.

**Interpretation**
Results were interpreted by the colour change in the strips and standards given in the result interpretation chart. In cases where reagents had to be added the same was done before interpretation.

**Identification of Bacteria using standard biochemical tests**

The pure cultures obtained from each of the media were characterized using biochemical tests that include

**Arginine hydrolysis test**

Amino acid serves as the building blocks of proteins and as precursors of many other important biomolecules like purines, pyrimidines etc in many higher animals. Many bacteria have been seen to be carrying out the biosynthesis of amino acids than their degradation particularly during rapid growth. However, some bacteria can degrade amino acids, particularly if they represent the sole source of carbon. The arginine dehydrolase enzyme brings about hydrolysis of arginine. In the laboratory, the organisms are inoculated to arginine agar. The presence of arginine dehydrolase is given by alkaline reaction, often in four days of incubation. Phenol red is the indicator (pH=6.8-8.0), which changes the colour of the medium from yellow to red.

**Procedure**

- Arginine agar was prepared and 3.5ml of it was dispensed into the tubes and sterilized.
- The tubes were cooled and inoculated with the organism.
- About 1 cm of sterile, liquid paraffin was added on the top of the agar to provide an anaerobic condition.

**Interpretation**

The positive result was given by the change in colour from red to pink. A negative result is indicated by no colour change.

**Catalase test**

Catalase is an enzyme that decomposes hydrogen peroxide into water and oxygen. Excluding the streptococci, most aerobic and facultative bacteria possess catalase
activity. Hydrogen peroxide forms as one of the oxidative end products of aerobic carbohydrate metabolism. If allowed to accumulate, it is lethal to bacterial cells.

Procedure

- With an inoculating needle or a wooden applicator stick, a colony was transferred to the surface of a glass slide.
- A drop of 3% hydrogen peroxide is added and bubble formation was observed.

Interpretation

The rapid and sustained appearance of bubbles or effervescence constitutes a positive test. Because some bacteria possess enzymes other than catalase that can decompose hydrogen peroxide, a few tiny bubbles forming after 20 to 30 seconds is not considered a positive test.

**Carbohydrate fermentation**

Fermentative degradation of various carbohydrates such as glucose, sucrose, and cellulose by microorganisms, under anaerobic condition is carried out in a fermentation tube. A fermentation tube is a culture tube that contains a Durham’s tube for the detection of gas production, as an end product of metabolism. The indicator in the medium is red at neutral and turns yellow at acidic conditions.

Procedure

- The sugar fermentation broths were inoculated with the test organism and incubated for 24 hours at 35°C.

Interpretation

Change in colour of indicator phenol red from red to yellow indicates a positive test for acid and air bubble in the Durham’s tube indicates a positive test for gas production.

**Citrate utilization**

Some bacteria can obtain energy in a manner other than by the fermentation of carbohydrates by using citrate as the sole carbon source. The measurement of this characteristic is important in the identification of many members of the Enterobacteriaceae. The utilization of citrate by a test bacterium is detected in citrate
medium by the production of alkaline by-products. The medium includes sodium citrate, an anion, as the sole source of carbon, and ammonium phosphate as the sole source of nitrogen. Bacteria that can use citrate can also extract nitrogen from the ammonium salt, with the production of ammonium (NH₄⁺), leading to alkalinization of the medium from conversion of the ammonia to ammonium hydroxide. Bromothymol blue which is yellow below pH 6.0 and blue above pH 7.6 is the indicator.

Procedure
- The test organism was inoculated as a single streak on the slant surface of the citrate agar tube.
- The tubes were incubated at 35°C for 24 to 48 hours.

Interpretation
A positive test is represented by the development of deep blue colour within 24 to 48 hours, indicating that the test organism has been able to utilize the citrate contained in the medium, with the production of alkaline products.

Gelatin hydrolysis
Gelatin is a protein produced by hydrolysis of collagen. It dissolves in warm water (50°C) and exists as a liquid above 25°C, and gels when cooled below 25°C. Hydrolysis of gelatin is brought about by microorganisms capable of producing a proteolytic exoenzyme known as gelatinase, which acts to hydrolyse this protein to amino acids. Hydrolysis of gelatin in the laboratory can be demonstrated by growing microorganisms in nutrient gelatin.

Procedure
- The gelatin tubes were inoculated with the test organism and incubated at 37°C for 24-48 hours.
- The incubated tubes were placed in the refrigerator at 4°C for 15 minutes.

Interpretation
Gelatin inoculated tubes that remain liquefied on refrigeration have organisms that produce gelatinase and show positive test for gelatin hydrolysis. Those tubes that remain solid on refrigeration demonstrate negative reaction for gelatin hydrolysis.

Indole production
Indole, a benzyle pyrrole, is one of the metabolic degradation products of the amino acid tryptophan. Bacteria that possess the enzyme tryptophanase are capable of hydrolyzing and deaminating tryptophan with the production of indole, pyruvic acid and ammonia. The indole test is based on the formation of a red complex when indole reacts with the aldehyde group of p-dimethylaminobenzaldehyde. This is the active chemical in Kovac’s reagent used for the detection of indole. A medium rich in tryptophan is used.

Procedure
- Tryptophan broth was inoculated with the test organism and incubated at 37°C for 18 to 24 hours.
- At the end of this, 15 drops of Kovac's reagent was added down the inner wall of the tube.

Interpretation
The development of bright red colour at the interface of the reagent and the broth within seconds after adding the reagent is indicative of the presence of indole and is a positive test.

*Methyl red test*
Methyl red is a pH indicator with a range between 6.0 (yellow) and 4.4 (red). The pH at which methyl red detects acid is considerably lower than the pH for other indicators used in bacteriologic culture media. Since many species of the Enterobacteriaceae may produce sufficient quantities of strong acids that can be detected by methyl red indicator during the initial phases of incubation, only organisms that can maintain this low pH after prolonged incubation (48 to 72 hours), overcoming the pH-buffering system of the medium, can be called methyl red positive.

Procedure
- MRVP broth was inoculated with a pure culture of the test organism.
- This was incubated at 35°C for 48 to 72 hours (not less than 48 hours).
- At the end of this time, 5 drops of methyl red reagent was added directly to the broth.

Interpretation
The development of stable red colour in the surface of the medium indicates sufficient acid production to lower the pH to 4.4 and constitutes a positive test. An intermediate orange colour between yellow and red does not indicate a positive test.

**Nitrate reduction**

Organisms demonstrating nitrate reduction have the capability of extracting oxygen from nitrates to form nitrites and other reduction products. The presence of nitrites in the test medium is detected by the addition of α- naphthylamine and sulphanilic acid, with the formation of a red diazonium dye, p-sulfobenzene-azo α-naphthylamine.

**Procedure**

- The nitrate medium was inoculated with a loopful of the test organism and incubated at 30°C for 24 to 48 hours.
- After incubation 1 ml of α- naphthylamine and sulphanilic acid was added.

**Interpretation**

The development of a red colour within 30 seconds after adding the test reagents indicates the presence of nitrates and represents a positive test.

**Oxidative-fermentative test (Hugh and Leifson)**

Saccharolytic microorganisms degrade glucose either fermentatively or oxidatively. A sensitive oxidative-fermentation medium of Hugh and Leifson is required for their detection of mixed acids. The lower protein/carbohydrate ratio in the medium reduces the formation of alkaline amines that can neutralize the small quantities of weak acids that may form oxidative metabolism. The relatively larger amount of carbohydrate serves to increase the amount of acid that can potentially be formed. The semisolid consistency of the agar permits acids that form on the surface of the agar to permeate throughout the medium, making interpretation of the pH shift of the indicator easier to visualize. Motility can also be observed in the medium.

**Procedure**

- Two tubes were used for the OF test, each inoculated with the test organism, using a straight needle, stabbing the medium three to four times halfway to the bottom of the tube.
- One tube of each pair was covered with a 1-cm layer of sterile mineral oil or melted paraffin, leaving the other tube open to the air.
- Both the tubes were incubated at 35°C and examined daily for several days.

**Interpretation**

Acid production was detected in the medium by the appearance of a yellow colour. In the case of oxidative organisms, colour production may be first noted near the surface of the medium. The reaction patterns are as follows:

<table>
<thead>
<tr>
<th>Open Tube</th>
<th>Covered Tube</th>
<th>Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid (Yellow)</td>
<td>Alkaline (Green)</td>
<td>Oxidative</td>
</tr>
<tr>
<td>Acid (Yellow)</td>
<td>Acid (Yellow)</td>
<td>Fermentative</td>
</tr>
<tr>
<td>Alkaline</td>
<td>Alkaline (Green)</td>
<td>Nonsaccharolytic</td>
</tr>
</tbody>
</table>

**Oxidase test**

Cytochromes are haeme containing catalytic proteins, which are tightly bound to the plasma membrane in prokaryotic cells. There are different kinds of cytochrome, of which cytochrome c is more abundant in nature. This does not react directly with oxygen, but the reduced form is oxidized by cytochrome oxidase with which it is closely associated. In this test, an oxidisable amine namely tetramethyl paraphenylene diaminohydrochloride is used to detect cytochrome c and hence the oxidase enzyme.

**Procedure**

- One or two drops of oxidase reagent was placed on a 6cm square, piece of Whatman filter paper.
- A small amount of the colony of the test organism was transferred using a glass rod on to the soaked filter paper and was observed for purple colour development.

**Interpretation**

A positive test was indicated by the development of purple colour in 5-10 seconds.

**Starch hydrolysis test**

Amylase is an exoenzyme that hydrolyses starch, a polysaccharide into maltose a disaccharide and some monosaccharides such as glucose. The ability to degrade starch is used as a criterion for the determination of amylase production by a microbe. Starch in the presence of iodine produces a dark-blue colouration of the medium, and a
yellow zone around the colony if starch is used by the organism, in an otherwise blue medium indicating amylolytic activity.

Procedure

- The starch agar medium was melted and poured aseptically into sterile petriplates and allowed to solidify.
- The test organism was streaked on to the plate and incubated at 30°C for 24-48 hours.
- The plate was flooded with iodine solution for 30 seconds and the excess iodine was poured off.

Interpretation

A positive test for starch hydrolysis is shown by the clear zone around the growth of the organism and blue colouration away from the growth of the organism. A negative test is blue colouration throughout the medium.

**Triple sugar iron test**

Triple sugar iron (TSI) agar medium contains three sugars; glucose (0.1%), lactose (1%) and sucrose (1%). It also contains high content of nitrogenous compounds. Ferrous sulphate is also incorporated to test hydrogen sulphide production as a result of reduction of sulphur containing compounds in the medium. Phenol red is the pH indicator used, which is yellow in acidic conditions and pink in alkaline conditions. Glucose in the media is in small amounts and when exhausted; microorganisms utilize the alternate sources of carbon and energy. If they utilize sucrose or lactose or both, acidic end products are formed. If they cannot utilize sugars, they use tryptone or peptone, as a result of which alkaline end products are formed.

Procedure

- TSI agar slants were streaked with the test organisms on the slant and the stabbed on the butt.
- The tubes were incubated at 37°C for 24 to 48 hours.

Interpretation

Results were read based on the colour change in the slant and butt as follows.

<table>
<thead>
<tr>
<th>RESULT-SLANT/BUTT</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red/Red</td>
<td>Glucose fermented</td>
</tr>
<tr>
<td>Pink/Yellow</td>
<td>Glucose fermented, Tryptone and Peptone oxidized</td>
</tr>
<tr>
<td>Yellow/yellow</td>
<td>All three sugars fermented</td>
</tr>
<tr>
<td>Black butt or slant</td>
<td>H₂S produced</td>
</tr>
<tr>
<td>---------------------</td>
<td>--------------</td>
</tr>
</tbody>
</table>

**Urease test**

Urease is an enzyme possessed by many species of microorganisms that can hydrolyse urea. The ammonia reacts in solution to form ammonium carbonate, resulting in alkanilization and an increase in the pH of the medium.

**Procedure**

- The surface of the agar slant was streaked with the test organism and incubated for 37°C for 18 to 24 hours.

**Interpretation**

Rapid urea splitters: red colour throughout the medium

Slow urea splitters: red colour initially in slant only, gradually converting the entire tube.

**Voges-proskauer test**

Pyruvic acid, the pivotal compound formed in the fermentative degradation of glucose, is further metabolized through various metabolic pathways, depending on the enzyme systems possessed by different bacteria. One such pathway results in the production of acetoin (acetyl methyl carbinol), a neutral-reacting end product. Organisms such as members of *Klebsiella- Enterobacter- Hafnia- Serratia* group produce acetoin as the chief end product of glucose metabolism and form smaller quantities of mixed acids. In the presence of atmospheric oxygen and 40% potassium hydroxide, acetoin is converted to diacetyl and α- naphthol serves as a catalyst to bring out a red complex.

**Procedure**

- MRVP broth was inoculated with a pure culture of the test organism.
- This was incubated for 48 hours at 35°C.
- At the end of this time 0.6ml of 5% α- naphthol, followed by 0.2ml of 40% KOH was added.
- The tube was shaken gently to expose the medium to atmospheric oxygen and allowed to remain undisturbed for 10-15 minutes.

**Interpretation**
A positive test was represented by the development of a red colour 15 minutes or more after the addition of the reagents, indicating the presence of diacetyl, the oxidation product of acetoin.

**Identification of Fungi**

The fungi isolated were observed by lactophenol wet mount.

The following manuals were used as reference for identification of fungi

- The ‘Illustrated Genera of Imperfect Fungi’ Barnett and Barry (1972)
- ‘Aspergillus and related telomorphs from Taiwan’, Tzean et al., 1990

The fungi were authenticated by the ‘National Center for Fungal Taxonomy, New Delhi.

**Identification of Actinomycetes**

Actinomycetes isolated were identified by slide cultures, Gram staining and biochemical methods. The manual ‘The Biology of Actinomycetes’ by Goodfellow and Cross (1983) was used as reference for identification.

**Results**

**Physical and chemical analysis of soil samples**

pH of all soil samples were found to be normal, in the range of 7.6- 7.9 and did not change drastically in any of the trials. Electrical conductivity (EC) which is the measurement of the soluble salts concentration in the soil at a given temperature was found to be higher in earthworm worked soil (Table 2.1). *Lampito mauritii* worked 45 days sample showed the maximum value of 0.2 mmhos/Cm. The EC increased in 45 days sample of both species of earthworms but well within normal range.

Organic carbon percentage increased in the 45 days soil sample worked by *P. corethrurus* (0.75%) compared to non earthworm worked soil (0.66%). In the 30 days samples it was 0.52% which was lesser than control. In *L. mauritii* both 30 days (0.85%) and 45 days (0.75%) showed an increase in the organic carbon percentage compared to non earthworm worked soils.
The average phosphorous was lower than normal values with no change being seen in
the soil sample with *P. corethrurus* compared to control. In *L. mauritii* worked soils
an increase in the 45 days sample was seen though it was less than the control. The
average potassium in all samples tested was higher than normal values. The
earthworm worked soils showed an increase in the potassium with 45 days sample of
both species especially *P. corethrurus* showing an increase.

Among the micronutrients, all samples showed higher concentration in the earthworm
worked soil compared to control. In both species of earthworms the 45 days sample
showed an increase in zinc and copper compared to 30 days soil. Iron did not show
any change in *L. mauritii* worked soil and was similar to the control in both 30 and 45
days samples whereas there was an increase in the 30 days sample of *P. corethrurus*
which showed a decrease in the 45 days sample. A similar result was also seen in the
presence of manganese where it was higher in the samples compared to the control.

**Microbiological analysis of soil samples**
During collection of burrow wall soil by destructive method, an average of 8 adults
and 6 juveniles were retrieved in the sets with *P. corethrurus*. In the sets with *L.
mauritii* an average of 9 adults and 8 juveniles were retrieved. *Lampito mauritti* was
found to be more active compared to *P. corethrurus*.

**Total Count of Fungi**
Fungal count in burrow wall and control soils of *L.mauritii* was found to be higher in
45 days compared to 30 days trials (Table 2.2). A significant decrease was seen in the
lower burrow wall soil (3.47 ± 0.05 x 10⁵ CFU/g) compared to lower control soil (7.22
± 0.63x 10⁵ CFU/g) in 30 days trials. Similar result were also seen in the 45 days
lower burrow wall soil where a significant decrease in the total fungal count of 6.55 ±
0.67 x 10⁵ CFU/g was observed compared to control (8.79 ± 0.97 x 10⁵ CFU/g). In the
upper burrow wall soil of 30 and 45 days trials no significant change was seen
compared to control.
In *P. corethrurus* the burrow wall soil showed a significant increase in the fungal count of both 30 and 45 day trials. The lower burrow wall showed a total fungal count of 5.39 ± 0.3 x10^6 and lower control soil 4.71± 0.02 x 10^6 CFU/g (Table 2.3). A total fungal count of 3.49± 0.46 x10^6 CFU/g was observed in upper burrow wall soil and in the upper control soil a total fungal count of 2.65± 0.35 x10^6 CFU/g was observed.

**Total Count of Bacteria**

The total count of bacteria in *L. mauritii* showed a significant decrease in the lower burrow wall in the 30 day sample (0.28 ± 0.06 x 10^5 CFU/g) compared to control (1.78 ± 0.26 x 10^5 CFU/g) (Table 2.2). In all other trials an increase in the total bacterial count was observed in the burrow wall soil. In the 30 days upper burrow wall soil (1.32 ± 0.21 x 10^5 CFU/g) the increase was not statistically significant compared to control (1.24 ± 0.18 x 10^5 CFU/g). In 45 days trials both the upper and lower burrow wall soil showed a significant increase with the upper burrow wall soil showing a total count of 6.9 ± 0.88 x10^5 CFU/g, and control with 5.0 ± 0.62 x10^5 CFU/g. The lower burrow wall soil had a total bacterial count of 3.7± 0.65 x 10^5 CFU/g compared to control with 0.4 ± 0.13 x 10^5 CFU/g.

It was interesting to note that the bacterial count in *P. corethrurus* was the inverse of that seen in *L. mauritii*. A significant decrease in the upper burrow wall soils in both trials. In 30 days lower burrow wall soil where though there was an increase it was not very significant and the 45 days lower burrow wall a decrease was seen that was not significant (Table 2.3). In the upper burrow wall soil the bacterial total count was observed to be 1.56 ± 0.19x 10^6 CFU/g whereas in the upper control it was 4.4 ± 0.37x 10^6 CFU/g. The 30 days lower burrow wall and control soil showed 3.80 ± 0.14 x 10^6 CFU/g and 3.5 ± 0.47x 10^6 CFU/g respectively. In the 45 days lower burrow wall soil the total bacteria count was 0.75 ± 0.03 x 10^6 CFU/g and in the control it was 1.022 ± 0.16 x10^6 CFU/g, whereas the upper burrow wall soil showed a total count of 0.56 ± 0.08 x10^6 CFU/g and in the control it was 1.54 ± 0.13 x10^6 CFU/g.

**Total Count of Actinomycetes**

In both species of earthworms studied the 30 days lower burrow wall and 45 days upper burrow wall soil showed a significant increase in the total actinomycete count.
compared to respective control (Figure 2.2). In the 30 days upper burrow wall soil of *P. corethrurus* a significant increase was seen whereas in *L. mauritii* a significant decrease was seen. In 45 day trials in the lower burrow wall soil a significant increase was seen in *L. mauritii* burrow wall soil compared to control, but though a decrease was seen in the burrow wall soil of *P. corethrurus* it was not significant. In *L. mauritii* 30 days lower burrow wall and control soil showed a total count of 5.0 ± 0.45 x 10^3 CFU/g and 2.0 ± 0.26 x 10^3 CFU/g respectively whereas the upper burrow wall and control soil showed 1.0 ± 0.05 x 10^3 CFU/g and 5.0 ± 0.02 x 10^3 CFU/g respectively (Table 2.2). The 45 days lower and upper burrow wall soil showed a significantly higher total count of 9 ± 0.08 x 10^3 CFU/g and 8 ± 0.33 x 10^3 CFU/g respectively. The 45 days control soil showed a significant decrease in the total count with the lower burrow wall showing 6 ± 0.58 x 10^3 CFU/g and 1 ± 0.04 x 10^3 CFU/g respectively (Table 2.3). In *P. corethrurus* the 30 days upper and lower burrow wall soil showed a total count of 9 ± 0.29 x10^3 CFU/g and 4 ± 0.17 x10^3 CFU/g while the lower and upper control showed 8 ± 0.30 x 10^3 CFU/g and 3 ± 0.26 x 10^3 CFU/g respectively. In the 45 days trials the lower and upper burrow wall soil showed a total count of 2.2 ± 0.21 x10^3 CFU/g and 6.4 ± 0.43 x 10^3 CFU/g respectively and lower and upper control soil showed 4 ± 0.18 x10^3 CFU/g and 3.6 ± 0.07 x 10^3 CFU/g respectively.

**Isolation of Gram negative bacteria on selective media**

Selective media like MacConkey’s and Eosin methylene blue agar were used to isolate Gram- negative bacteria. The results are tabulated in Table 2.4. On MacConkey’s medium in both species of earthworms studied, total count of Gram negative bacteria were found to decrease significantly in lower burrow wall soils compared to control in 30 days trials where as in the upper burrow wall soils there was a significant increase. In the 30 day trials of *L. mauritii* a total count of 2.8 ± 0.2 x 10^4 CFU/g and 1.03 ± 0.09 x 10^4 CFU/g were seen in the lower and upper burrow wall soil respectively while a total of 6.0 ± 0.21 x 10^4 CFU/g and 0.8 ± 0.15 x 10^4 CFU/g were observed in the lower and upper control soils respectively. In the 45 day trials the lower and upper burrow wall soil showed a total of 1.16 ± 0.14 x 10^4 CFU/g and 0.24 ± 0.04 x 10^4 CFU/g respectively whereas the lower and upper control soil showed a total of 0.27 ± 0.05 x 10^4 CFU/g and 0.86 ± 0.1 x 10^4 CFU/g respectively. In
45 day trials different results to the above were obtained in both species of earthworms. In *L. mauritii* there was a significant increase in the total count in the burrow wall soil while a significant decrease was seen in the upper burrow wall soil. In *P. corethrurus* a significant decrease and increase was seen in the lower burrow wall soil and upper burrow wall soil respectively compared to control. Gram-negative bacteria were not isolated from lower burrow wall soil but the upper burrow wall soil showed a count of $80 \pm 7.5 \times 10^3$ CFU/g. The lower and upper control soil showed a count of $7.6 \pm 0.64 \times 10^3$ CFU/g and $0.9 \pm 0.15 \times 10^3$ CFU/g respectively.

On eosin methylene blue agar lactose fermenting colonies were isolated only from *L. mauritii* samples. A significant decrease in the total count was seen in the lower burrow wall soil of 30 $(9.6 \pm 0.36 \times 10^4$ CFU/g) and 45 days $(0.9 \pm 0.14 \times 10^4$ CFU/g) trials in *L. mauritii* whereas there was a significant increase in the total count of upper 30 days $(1.3 \pm 0.29 \times 10^4$ CFU/g) and upper burrow wall soils of 45 days $(0.44 \pm 0.05 \times 10^4$ CFU/g) though the increase was not significant in the 45 day trials (Table 2.4). In *P. corethrurus* no significant difference was seen in the upper $(3.3 \pm 0.54 \times 10^3$ CFU/g) and lower $(1.8 \pm 0.27 \times 10^3$ CFU/g) samples in 30 day trials whereas in the 45 day trials upper burrow wall $(7 \pm 2 \times 10^4$ CFU/g) soil showed a significant increase compared to control $(4.5 \pm 0.49 \times 10^3$ CFU/g). In the lower burrow wall soil of 45 day trials no organisms were isolated both in Mac Conkey’s and EMB agar. On Baird Parker agar no Gram positive colonies were isolated from samples of both species of earthworms studied.

The total count of *Pseudomonas* (Table 2.4) on Kings B medium in 30 $(58 \times 10^6$ CFU/g) and 45 $(36.67 \times 10^6$ CFU/g) day trials significantly increased in the upper burrow wall soil in *L. mauritii* compared to control where as it decreased significantly in the lower burrow wall soil (30 days- $13.33 \times 10^6$ CFU/g; 45 days $10.33 \times 10^6$ CFU/g). In *P. corethrurus* a significant increase was seen only in the 30 days $(36.67 \times 10^6$ CFU/g) upper burrow soil and a decrease was seen in the lower burrow wall soils though it was not significant.

*Comparison of total count of fungi and bacteria*
On comparing the total count of fungi and bacteria in the two species studied, it was found that the total counts were higher in the set up with *P. corethrurus* (Figure 2.1). Total count of both bacteria and fungi in 30 day trials of *P. corethrurus* were higher in the lower burrow wall soil compared to lower control. In *L. mauritii* interestingly an inverse result was observed where the total bacterial and fungal counts decreased in the lower burrow wall soil compared to control. In *P. corethrurus* in the 30 day trials the upper and lower burrow wall soil showed an increase in total fungal count, but decrease in the bacterial count compared to control. In both bacterial and fungal count there was no significant difference seen in the burrow wall soil of *L. mauritii*.

In the 45 days trials the total fungal count in *P. corethrurus* was higher in both in the upper and lower burrow wall soil compared to control whereas bacteria showed a decrease in the lower and upper burrow wall soil. In case of *L. mauritii*, similar to in 30 days trials, the fungal count decreased in the burrow wall soil compared to control and the bacterial count increased in both upper and lower burrow wall soil.

**Identification of Fungi**

A total of 55 fungi were isolated and identified in this study. In the 30 days trials number of fungi were found to be higher in burrow wall soil of *P. corethrurus* compared to the burrow wall soil of *L. mauritii* whereas in the 45 days trials the burrow wall soil showed a higher number in *L. mauritii* (Figure 2.3). A total of 18 fungal isolates were observed in the lower burrow wall soil of *P. corethrurus* and 15 from the *L. mauritii*. In upper burrow wall soil 15 fungi were isolated from *P. corethrurus* when a total of 11 fungi were isolated from *L. mauritii*. In the 45 days trials the lower burrow wall soil showed a fungal number of 21 whereas the upper burrow wall soil had 18 species of fungi. In *P. corethrurus* 16 and 14 fungi were isolated from the upper and lower burrow wall soil respectively. When compared to control the upper burrow wall soil showed an increase in the fungal number whereas in the lower burrow wall soil there was no change in *L. mauritii*. In *P. corethrurus* the upper burrow wall soil showed an increase in the fungal number compared to control whereas the lower burrow wall showed a decrease in the fungal number compared to control.

*Fungi isolated from 30 days burrow wall and control soil of P. corethrurus*
A total of 21 fungi were isolated from the burrow wall and control soil of 30 day trials of *P. corethrurus*. Among the fungi isolated 6 fungi *viz.* *Aspergillus albus*, *Penicillium citrinum*, *Rhizopus oryzae*, *Trichoderma virens*, *Trichoderma viride* and *Penicillium purpurascence* were isolated only from the lower and upper burrow wall soil (Figure 2.4). It is significant here that *Trichoderma* species are important biocontrol agents. *Curvularia lunata var.aeria* was isolated from only lower burrow wall soil. Seven fungi *viz.* *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium pallidoroseum*, *Mucor heimalis*, *Mucor racemosus* and *Saccharomyces cerevisiae* were isolated from both lower and upper burrow wall and control soil. *Phoma glomerata* was isolated only from the upper and lower control soil and *Trichoderma harzianum* only from upper control soil. *Aspergillus oryzae*, *Cladosporium shaerospermum* and *Fusarium solani* were isolated from lower and upper burrow wall soil and also from upper control soil. *Cladosporium cladosporioides* was isolated from lower burrow wall soil and from lower and upper control soil. *Fusarium chlamydosporium* was isolated only from upper control soil. *Verticillium chlamydosporum* was isolated only from upper burrow wall soil (Figure 2.5). Six fungi *viz.* *A. alternata*, *A. flavus*, *A. niger*, *C. cladosporioides*, *C. shaerospermum* and *Fusarium oxysporum* were isolated from both upper and lower burrow wall and control soil. Seven fungi *viz.* *Acremonium chrysosporum*, *A. periciun*, *A. roseum*, *Chaetomium indicum*, *F. chlamydosporium*, *F. solani*, *Nigrospora oryzae* and *Rhizopus oryzae* were isolated from only lower and upper control soils. *C. lunata var.aeria* and *M. heimalis* were isolated only from upper control soils.

**Fungi isolated from 45 days burrow wall and control soil of *P. corethrurus***

A total of 27 fungi were isolated from the burrow wall and control soil of 45 day trials of *P. corethrurus*. Seven fungi *viz.* *A. albus*, *A. oryzae*, *Cunninghamella elegans* *Geotrichum candidum*, *Paecilomyces varioti*, *P. citrinum*, *S. cerevisiae* were isolated only from the lower and upper burrow wall soil. *P. lilacinus*, *T. virens* and *Volutella sclerotia* were isolated only from the lower burrow wall soil. *Verticillium chlamydosporum* was isolated only from upper burrow wall soil (Figure 2.5). Six fungi *viz.* *A. alternata*, *A. flavus*, *A. niger*, *C. cladosporioides*, *C. shaerospermum* and *Fusarium oxysporum* were isolated from both upper and lower burrow wall and control soil. Seven fungi *viz.* *A. periciun*, *A. roseum*, *Chaetomium indicum*, *F. chlamydosporium*, *F. solani*, *Nigrospora oryzae* and *Rhizopus oryzae* were isolated from only lower and upper control soils. *C. lunata var.aeria* and *M. heimalis* were isolated only from upper control soils.

**Fungi isolated from 30 days burrow wall and control soil of *L. mauritii***

A total of 30 fungi were isolated from the burrow wall and control soil of 30 day trials of *L. mauritii*. *Acremonium kiliense*, *Neurospora crassa*, *Penicillium chrysogenum*,
Talaromyces flavus, T. virens and Verticillium dahaliae were isolated only from lower burrow wall soil whereas Epicoccum purpurascence and Nigrospora oryzae were isolated from only the upper burrow wall soil samples (Figure 2.6). Paecilomyces lilacinus and P. varioti were isolated from both upper and lower burrow wall soil samples and not from control just like seen in 45 days trials of P. corethrurus. Four fungi viz., A. alternata, C. cladosporioides, F. oxysporum and F. solani were isolated from both burrow wall and control soil. A. fusidiodes, Humicola grisea, Sordaria fumicola and Steptomyces griseus were isolated from lower control soil only. Byssochlamus niveus, Fusarium equesetii, Penicillium citrinum, P. italicum and Trichothecium roseum were isolated only from upper control soil. Aspergillus flavus and Phoma glomerata were isolated from both lower and upper control soils. Aspergillus oryzae was isolated from lower burrow wall and upper control soil. Curvularia clavata could be isolated from upper burrow wall soil and control. Fusarium pallidoroseum occurred in all samples except lower burrow wall soil and Mucor cymosus in all samples except upper burrow wall soil. Rhizopus nigricans was isolated in all samples except lower control soil.

**Fungi isolated from 45 days burrow wall and control soil of L.mauritii**

A total of 33 fungi were isolated from burrow wall and control soil of 45 day trials of L. mauritii. Byssochlamus niveus, Cunninghamaella echinulata, P. citrinum and R. nigricans were isolated only from lower burrow wall soils where as Circinella sp., Penicillium chrysogenum were isolated only from upper burrow wall soil. Acremonium fusidiodes and Verticillium dahaliae were isolated from both the upper and lower burrow wall soil (Figure 2.7). Altenaria alternata A. flavus, A. niger, F. oxysporum and F. solani were isolated from all samples studied. Cladosporium cladosporioides, F.pallidoroseum, M. cymosus and T. virens were isolated only from lower burrow wall and control soil. Chaetomium indicum, Drechslera hawiensis, Fusarium equesetii, Nigrospora oryzae, Periconia byssoides and Trichothecium roseum were isolated from upper burrow wall and control soil. Curvularia clavata and E. purpurascence were isolated from only upper control soil where as Humicola grisea was isolated only from lower control soil. Aspergillus oryzae and Penicillium italicum were found in upper and lower burrow wall soil and upper control soil whereas Phoma glomerata and Paecilomyces varioti were found in lower and upper
control and lower burrow wall soil. *Phoma exigua* and *T. viride* were isolated from lower burrow wall soil and upper control soil while *Sordaria fumicola* was isolated from only the lower control soil.

**Dominant fungal species**

A total of 55 species of fungi belonging to 29 genera were isolated in this study. Thirty eight species of fungi were isolated from both the burrow wall and control soil of *L. mauritii* and 34 species from the burrow wall and control soil of *P. corethrurus*. Among this 6 species were isolated that were specific only to the burrow wall of *P. corethrurus* viz. *A. albus*, *C. elegans*, *G. candidum*, *P. purpurascence*, *V. chlamydosporum* and *V. sclerotii* whereas 7 species were specific to the burrow wall of *L. mauritii* viz., *A. kiliense*, *Cunninghamella echinulata*, *Circinella Sp.*, *N. crassa*, *P. chrysogenum*, *Talaromyces flavus*, and *V. dahaliea* (Table 2.5). *Alternaria alternata* was found to be present in all samples studied. It was seen that the percentage of *A. alternata* decreased in the 45 lower burrow wall soil but increased in the upper burrow wall soil both in *P. corethrurus* and *L. mauritii*. It was significant that *Paecilomyces lilacinus* and *P. varioti* was isolated from the burrow wall soil of both species of earthworms studied. since these are species that are known to have detrimental effect on nematode cysts. *Acremonium chrysosporum*, *A. pericinum*, *A. roseum* and *Trichoderma harzianum* were unique to the control soil of *P. corethrurus* and *Sordaria fumicola* and *Streptomyces griseus* were specific to the control soil of *L. mauritii*.

Five species of *Acremonium* were isolated in this study *A. fusiodides*, *A. kiliense*, *A. chrysosporum*, *A. pericinum* and *A. roseum*; of which *A. fusiodides*, *A. kiliense* were isolated from the burrow wall soil of *L. mauritii* and the other three only from control soil of *P. corethrurus*. Four species of *Aspergillus* viz., *A. albus*, *A. flavus*, *A. niger* and *A. oryzae* were isolated in this study; where *A. albus* was isolated only from the burrow wall soil that too from *P. corethrurus*, the others were isolated from all samples. *Chaetomium indicum* seems to have increased in percentage in the burrow wall soil compared to control in *L. mauritii* and absent in burrow wall soil of *P. corethrurus* though it was isolated in the control soil. *Drechslera hawiensis* and *Periconia byssoides* showed a decrease in the percentage in the burrow wall soil.
compared to control soil in *L. mauritii*. The dominant and specific fungi in each trial are depicted in Table 2.5 and 2.6.

**Identification of Bacteria**

Fourteen species of bacteria were isolated in this study using soil extract agar (Table 2.7). In *P. corethrurus* 3 species viz. species *Acinetobacter, Serratia* and *Staphylococcus* were unique to the 30 day burrow wall and *Bacillus* spp. PC 3 to the control soil. *Bacillus sphaericus* dominated in the 30 days burrow wall soil in *P. corethrurus* and *B. sterothermophilus* in the 45 days upper burrow wall soil (Table 2.10). *Bacillus pasteurianum* and *Bacillus* PC 1 dominated in the 30 and 45 days control soil. *Agrobacterium* spp. was isolated only from the burrow wall of *L. mauritii* and Staphylococcus and Bacillus sps PC 3 from the control soil. Eight species viz., *Aeromonas* spp., *B. pasteurianum*, *B. sphaericus*, *B. sterothermophilus* *B. subtilis*, Clostridium, *Bacillus* spp. PC 1, *Bacillus* spp.PC 2 and *Pseudomonas* spp. were isolated from the burrow wall and control soil for *P. corethrurus*. *Acinetobacter* spp. *B. sterothermophilus*, *B. subtilis*, *Micrococcus* spp. *Proteus* spp. *Pseudomonas* spp. *Serratia* spp. and *Escherichia* spp. were isolated from the burrow wall and control soil of *L. mauritii*. *B. subtilis* and *B. sterothermophilus* dominated in the burrow wall and control soil. *Micrococcus* was isolated only from *L. mauritii* soils and *B. sphaericus*, *Bacillus* spp. PC 1, *Bacillus* spp. PC 2, *Bacillus* spp. PC 3 and *Pseudomonas* only from *P. corethrurus* soils. *Staphylococcus* was isolated only from the burrow wall soils of *P. corethrurus* and control soil of *L. mauritii*. Table 2.9 shows the dominant bacteria in all trials.

Gram negative bacteria isolated from *L. mauritii* included species of *Aeromonas, Agrobacterium, Escherichia, Serratia, Proteus* and *Pseudomonas* (Table 2.8). The percentage of *Proteus* sps. increased in the 30 days lower burrow wall soil and decreased in the upper burrow wall soil. It was not isolated from the 45 days trials. *Escherichia* sps. was isolated from all samples except upper burrow wall soil. There was a decrease in the percentage in the burrow wall soil compared to control soil. The percentage of *Pseudomonas* significantly increased in the burrow wall soil of both 30 and 45 day trials. *Agrobacterium* sps. was isolated only from the burrow wall soil of both 30 and 45 day trials. *Aeromonas* spp. and *Serratia* sps were both isolated from
the 30 and 45 day trials. From the burrow wall soil of *P. corethrurus* only two species of Gram negative bacteria were isolated *viz.* *Aeromonas* and *Pseudomonas* species.

**Identification of Actinomycetes**

Ten species of actinomycetes were isolated in this study (Table 2.7). There was no unique actinomycete species isolated in the burrow wall of *P. corethrurus* whereas Ac4 was unique to control soil. Four species *viz.*, *Micromonospora*, *Streptomyces*, *Actinoplanes* and *Streptovercillium* were isolated both in the burrow wall and control soil of *P. corethrurus*. Species of *Streptomyces*, *Micromonospora* and *Actinoplanes* dominated in the burrow wall and control soils. Eight species *viz.* *Micromonospora*, *Geodermatophilus*, *Streptomyces*, *Actinoplanes*, *Nocardia*, *Dactylosporangium*, Ac 1 and *Streptovercillium* were isolated from the burrow wall of *L. mauritii* and Ac2 and *Microbispora* only from the control soils (Table 2.11). From *P. corethrurus* burrow wall soils *Micromonospora*, *Streptomyces*, *Actinoplanes* and *Streptovercillium* were isolated where as *Microbispora* was isolated only from the control soil. It was observed that *Microbispora* was isolated only from the control soils of both species of earthworms studied. *Geodermatophilus* was isolated from only the burrow wall of *L. mauritii* and *Dactylosporangium*, *Nocardia*, Ac 1 and 2 were not isolated from the soils of *P. corethrurus*. Table 2.10 shows the dominant actinomycetes isolated from all trials.

**Discussion**

Earthworms are recognized for their important role in the improvement of physical and chemical characteristics of soil, and thus increasing its fertility (Aina 1984; Edwards and Bohlen 1996; Abdul Rida and Bouché 1997). Earthworms strongly affect soil organic carbon cycling. The term ‘soil organic carbon’ (SOC) refers to the C occurring in the soil in soil organic matter (SOM). SOC is important for all three aspects of soil fertility, namely chemical, physical and biological fertility. Several studies have shown that earthworm activity may contribute to the stabilization of SOC through enhanced long-term protection by soil aggregates (Fonte *et al.*, 2007; Pulleman *et al.*, 2005; Scheu and Wolters 1991; Tiunov and Scheu 2000). It is significant here that in the earthworm worked soils of both species of earthworms studied the organic carbon increased. Carbon stocks in earthworm burrows strongly
depended on the earthworm activity which maintains continuous C input into the burrows. Axel et al., (2008) have shown that earthworm activity does not substantially increase subsoil C stocks but burrows serve as fast ways for fresh C transport into deep soil horizons. Studies by Weihua and Yin (2007) have shown that earthworms not only increased the content of organic C and total N in the soil, but also decreased the values of C/N in the soil and leaf litters. Additions of anecic earthworms significantly increased average soil organic carbon content from 16.1 to 17.9 g C kg$^{-1}$ for the 0–10 cm soil, and from 12.4 to 14.7 g kg$^{-1}$ at 10–20 cm depth (Shuster et al., 2001).

Soil electrical conductivity (EC) is a measurement that correlates with soil properties that affect crop productivity, including soil texture, cation exchange capacity (CEC), drainage conditions, organic matter level, salinity, and subsoil characteristics. EC is viewed as the quantity of available nutrients in the soil since only nutrients that are dissolved in the soil water is ‘available’ for crops. Where EC levels are high less fertilizer is needed. The fact that earthworm soils showed an increase in the EC especially higher in 45 days trials substantiates that earthworms can improve the availability of nutrients in the soil as their period of stay increases. This study also shows an increase in the organic carbon percentage in the earthworm worked soils.

The present study shows an increase in the potassium levels in earthworm worked soils compared to control. This correlates with studies using Aporrectodea caliginosa that have inferred that earthworms increase the availability of K by shifting the equilibrium among the forms of K from relatively unavailable forms to more available forms in the soil chosen for the study (Basker et al., 1992). Their results also indicated that the exchangeable K content increased significantly due to earthworm activity.

These studies also show an increase in micronutrients in earthworm worked soils. Some micronutrients, such as zinc and boron, are more available in the excrement of earthworms through chelation of the micronutrients. Studies by Asawalam and Johnson (2007) indicate that the contents of nitrogen and phosphorus were increased in soils modified by earthworms. In the same study with regard to micronutrient concentration, earthworm casts contained higher concentrations of copper (Cu),
molybdenum (Mo), and manganese (Mn) than the other soil materials. A similar result is also seen in this study which substantiates that soil reworked by earthworms appear to have high potential for agronomic use. In a study of the physico-chemical characteristics of casts of Perionyx ceylanensis, electrical conductivity, moisture content, total nitrogen, total phosphorus, total potassium, calcium, copper, iron and zinc were found to be higher than that of control while organic carbon and C/N ratio observed in the vermicast showed decrease over control (Prakash et al., 2008).

Earthworm burrow wall creates a favorable microhabitat for the soil microflora. Earthworm burrow walls and other zoogenous soil structures harbour distinctive communities of soil animals, e.g. protozoa, nematodes and microarthropods, which presumably control microbial activity in these microhabitats (Hamilton and Stillman 1989; Anderson and Bohlen 1998; Maraun et al., 1999; Tiunov and Kuznetsova 2000; Tiunov et al., 2001a). High microbial biomass and activity in earthworm burrow walls were recorded in a range of field and laboratory studies (Tiunov and Scheu 1999). Effect of earthworms on the microbial community depends, in part, on the timing of the measurement. Some effects of earthworms may become apparent only after an extended period of time because changes that affect microbial community composition and trophic interactions, such as diffusion of nutrients beyond the burrow walls and development of pore structure in the burrow walls, may occur gradually. There have been contrasting effects on microbial biomass, with microbial biomass increasing, decreasing, or showing no net change relative to soil unaffected by earthworms (Brown 1995). Loquet (1977) found that casts and gallery walls had a greater microbial activity than the surrounding soil. Tiunov and Dobrovolskaya (2002) observed that both bacterial and fungal communities differed significantly from the control soil. Idowu et al. (2006) in a related study also reported that the total aerobic and anaerobic counts of microflora were higher in casts than in surrounding soil. It has also been reported that the biomass of fungi in the earthworm species is higher in Lumbricus terrestris casts than in the surrounding soil (Tiunov and Kuznetsova 2000). Devliegher and Verstraete (1997) reported that fungal growth was neither stimulated nor reduced by the earthworms. The present study using two different species showed a very interesting information wherein the increase or decrease of microbial populations were associated with the species of earthworm and thus it exhibited species specificity with regard to this association. Experiments with
burrow walls of *Lumbricus terrestris* in forest soils show 2.5 times larger microbial biomass compared to surrounding soil. Earthworms are known to line their burrows with cast material (Lavelle 1988) and fungi have been observed to grow in casts for up to 15 days following deposition (Parle 1963). Moreover reports show that hyphal growth may contribute to increased stability of earthworm cast (Parle 1963; Haynes and Fraser 1998). There was an indication in the present investigation that in *L. mauritii* fungal hyphae were lesser than bacteria whereas in *P. corethrurus* the fungal counts were significantly higher than bacteria in the burrow wall soil. Also the total fungal count in *P. corethrurus* burrow wall increased compared to their respective controls. This probably explains the greater stability of the casts of *P. corethrurus*. Another study by Polyanskaya and Tiunov (1996) in *Lumbricus terrestris* showed that fungal hyphae were less dense and bacteria were more abundant in the drilosphere than bulk soil. The colony forming units (CFUs) of bacteria and fungi in the casts of *Pontoscolex corethrurus* significantly deviated from the CFU found in adjacent soil (Karmegam and Daniel 2000).

The total bacterial and actinomycetes count increased in the burrow wall soil except in the 30 days lower sample in *L. mauritii*. In *P. corethrurus* the bacterial count decreased in all burrow wall soils except in the 30 days upper burrow wall soil and actinomycetes showed a total increase in all burrow wall soil studied except the 45 days lower burrow wall soil where it was lower than in the control soil. Clegg et al. (1995) found that total bacterial counts in burrow and bulk soil did not differ initially, but increased through time and remained elevated in burrow wall soil. The present study shows that the total fungal count decreased in the burrow wall of *L. mauritii* and increased in the burrow wall of *P. corethrurus*, whereas the inverse was true with regard to bacteria. The total count of bacteria increased in the burrow wall of *L. mauritii* and decreased in the burrow wall of *P. corethrurus*. The actinomycete count increased in the 30 days burrow wall soil and decreased in the 45 days trails compared to controls in *P. corethrurus*. In *L. mauritii* a significant decrease was seen in the 30 days and increase in the 45 days earthworm burrow wall soils.

Out of 55 fungal species isolated from this study 7 species *viz*. *Acremonium kiliense*, *Circinella Sp.*, *Cunninghamella echinulata*, *Neurospora crassa*, *Penicillium chrysogenum*, *Talaromyces flavus* and *Verticillium dahliae* were found to be specific
to *L. mauritti* burrow wall soil. *Aspergillus albus*, *Cunninghamella elegans*, *Geotrichum candidum*, *Penicillium purpurascence*, *Verticillium chlamydosporum* and *Volutella sclerotia* were isolated only from the burrow wall soil of *P. corethrurus*. Tiwari and Mishra (1993) recorded a total of 27 species in study of casts. In a study of the microfungal community of *Lumbricus terrestris* middens forest 90 fungal taxa were isolated among which the casts were found to contain 61 taxa (Orazova, *et al.*, 2003). Both bacterial and fungal communities differed significantly from the control soil with a substantial part of the fungal community in “lined” burrow walls showing typical “litter” species, mainly *Trichoderma* and *Mucor* (Tiunov and Dobrovolskaya 2002). In the same study a total of 60 fungal taxa were isolated. Four species of *Trichoderma* that were isolated in the present study *viz.* *T. virens*, *T. viride*, *T. harzianum* and *T. roseum*. All of these isolates increased in the burrow wall soil compared to control soil. *T. harzianum* was not isolated from the burrow wall soil. The isolated 3 *Mucor* species *viz.* *M. cymosus* *M. heimalis* and *M. racemosus*, decreased in the burrow wall compared to control. The abundance of *Trichoderma* spp. and *Mucor* spp. was directly related to their abundance in litter in a study using *L. terrestris* (Tiunov and Dobrovolskaya 2002). *Paecilomyces lilacinus* and *P. variotii* were the only species that were isolated in the burrow wall soil of both species of earthworms studied, though *P. variotii* was also isolated from the control soil of *L. mauritti* 30 day trials. *Paecilomyces lilacinus* is reported to infect eggs and cysts of nematodes (Carneiro 1992) and may be significant in the burrow walls. *Penicillium citrinum*, *P. purpurascence* and *Rhizopus oryzae* was found to be stimulated in the burrow wall of *P. corethrurus*. *Acremonium chrysosporum*, *A. pericinum*, *A. roseum*, *F. chlamydosporium*, *Humicola grisea*, *Sordaria fimicola*, *S. griseus* and *T. harzianum* were isolated only from the control or bulk soil of earthworms studied indicating that there has been suppression of these fungi in burrow wall. Orazova *et al.* (2003) have shown that the cast community was dominated by *Trichosporiella hyalina*, *Humicola grisea*, and *Monilia* spp. They also showed that the abundance of *Trichoderma koningii*, *Paecilomyces carneus* and *Cylindrocarpon destructans*, *Alternaria alternata* and *C. herbarum* strongly reduced in the casts. Similar trend was observed with regard to the abundance of *A. alternate*, but with not much difference in the burrow wall soil and control in this study. Karmegam and Daniel (2000) isolated 14 different species of fungi belonging to the genera *Aspergillus*, *Chaetomium*, *Cladosporium*, *Cunninghamamella*, *Fusarium*, *Mucor*, *Penicillium* and
Rhizopus from the casts of *P. corethrurus*. It must be noted here that in *L. mauritii* dominant fungi did not differ much between burrow wall and control soil whereas in *P. corethrurus* there was a difference.

In a study by Tiunov and Dobrovolskaya (2002) the burrow wall of *Lumbricus terrestris* was found to be dominated by *Cellulomonas* and *Promicromonospora*, whereas *Bacillus* and *Streptomyces* prevailed in the control soil. Dominance of *Bacillus* species in both the species of earthworms was observed in the present investigation. *Acinetobacter* and *Serratia* and *Staphylococcus* were specific to the burrow wall of *P. corethrurus*. Different species of *Bacillus* were dominant in the burrow wall and control soil of *L. mauritii* and *P. corethrurus*. *Bacillus sphaericus* dominated in the 30 days burrow wall soil in *P. corethrurus* and *B. sterothermophilus* in the 45 days burrow wall soil. *Bacillus pasteurianum* and *Bacillus PC 1* dominated in the 30 and 45 days control soil. *B. subtilis* and *B. sterothermophilus* dominated in the burrow wall and control soil of *L. mauritii*. The diversity of bacteria were higher in *P. corethrurus* compared to *L. mauritii*. Only 5 species were isolated from *L. mauritii* whereas 12 species were isolated from *P. corethrurus*. Micrococcus was specific to *L. mauritii* soils. Species of *Micrococcus*, *Bacillus*, *Vibrio*, *E. coli* and *Pseudomonas* were isolated from earthworm intestine in a study by Khambata and Bhat (1957). Fifteen bacterial species belonging to 10 genera including *Staphylococcus aureus*, *Bacillus* spp., *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Clostridium* spp., *Spirocheata* spp., *Azotobacter* spp., *Micrococcus lylae*, *Acinetobacter* spp., *Halobacterium* spp. were isolated from the casts of the earthworm *Libyodrillus violaceous* (Idowu et al., 2006).

Gram negative bacteria were found to be more diverse in *L. mauritii* burrow wall soil with 6 isolates. Only 2 species were isolated from *P. corethrurus* burrow wall soils. Tiunov and Dobrovolskaya (2002) isolated Gram-negative rods mostly *Aquaspirillum*, *Alcaligenes* and *Enterobacter* from burrow wall soil which were not detected in the control soil. *Aeromonas*, *Agrobacterium*, *Escherichia*, *Serratia*, *Proteus* and *Pseudomonas* were isolated from *L. mauritii* burrow wall soil. The percentage of *Proteus* spp. increased in the 30 days lower burrow wall soil and decreased in the upper burrow wall soil. *Proteus* spp. was not isolated from the 45 days trials. There was a decrease in the percentage *Escherichia* spp. in the burrow
wall soil compared to control soil. It was significant to note that the percentage of *Pseudomonas* significantly increased in the burrow wall soil and *Agrobacterium* spp. was isolated only from the burrow wall soil of both 30 and 45 day trials. Being plant growth promoting rhizobacteria (PGPRs) they could be involved in enhancing plant growth. Among the 2 Gram negative bacteria isolated from *P. corethrurus* burrow wall soil *viz.* *Aeromonas* and *Pseudomonas* species, *Aeromonas* was isolated only from the burrow wall of 30 days sample. Though the percentage of *Pseudomonas* increased in some cases, it was not significant.

According to Tiunov and Scheu (1999) fungal volume did not show any increase in contrast to bacterial volume in the drilosphere and the fungal-to-bacterial ratio decreased strongly in the burrow walls. In the present investigation the total count of both bacteria and fungi in 30 day trials of *P. corethrurus* were higher in the lower burrow wall soil compared to control whereas in *L. mauritii* the total bacterial and fungal counts decreased in the lower burrow wall soil compared to control. In *P. corethrurus* when the fungal count increased in the burrow wall soil the bacterial count decreased in 30 days upper and 45 days upper and lower burrow wall soil. Loquet *et al.*, (1977) reported the most pronounced increases in microbial activity in the drilosphere at a depth of 20±40 cm. Reports suggests that at greater depths the contrast between drilosphere and bulk soil and the relative contribution of burrow walls to the total soil microbial activity probably increase strongly (Stehouwer *et al.*, 1993; Joergensen *et al.*, 1998). A similar trend was observed in the present study.

Earthworm- associated microhabitats may contribute to the maintenance of soil faunal diversity. The present study reinforces the general concept that the burrow wall soil tends to be more microbiologically active than surrounding soil and might be a specialized microhabitat for enhanced microbial activity in soil. The relatively small soil volume of earthworm burrow walls that sustain large and active microbial communities play an important role in the soil system by regulating microbial mediated chemical processes.
Figure 2.1: Comparison of Total fungi and bacteria from earthworm burrow wall of *Pontoscolex corethrurus* and *Lampito mauritii* and control soil

Legend: LBWS- Lower Burrow Wall Soil; LCS- Lower Control Soil; UBWS- Upper Burrow Wall Soil; UCS- Upper Control Soil
Figure 2.2: Comparison of Total actinomycetes from earthworm burrow wall of *Pontoscolex corethrurus* and *Lampito mauritii* and control soil

Legend: LBWS- Lower Burrow Wall Soil; LCS- Lower Control Soil; UBWS- Upper Burrow Wall Soil; UCS- Upper Control Soil

Figure 2.3: Total number of fungi isolated from earthworm burrow wall of *Pontoscolex corethrurus* and *Lampito mauritii* and control soil

Legend: LBWS- Lower Burrow Wall Soil; LCS- Lower Control Soil; UBWS- Upper Burrow Wall Soil; UCS- Upper Control Soil
Plate 2

Set up before addition of humus

Set up after addition of humus

Introduction of earthworms into setup

Burrows on setup

Burrow walls in setup

Stages during collection of burrow wall and control soil samples
Plate 4
Table 2.1: Physical and Chemical characteristics of earthworm worked soils of *Lampito mauritii* and *Pontoscolex corethrurus* and non earthworm worked soil

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>E.C (mmhos/Cm 25°C)</th>
<th>Organic carbon %</th>
<th>Av. Phosph (Kg/ac)</th>
<th>Av. potash (Kg/ac)</th>
<th>Zn</th>
<th>Fe</th>
<th>Cu</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (non earthworm worked soil)</td>
<td>7.8</td>
<td>0.08</td>
<td>0.66</td>
<td>5</td>
<td>150</td>
<td>1.5</td>
<td>17</td>
<td>2.4</td>
<td>28.1</td>
</tr>
<tr>
<td><em>L. mauritii</em> (30 days)</td>
<td>7.7</td>
<td>0.12</td>
<td>0.85</td>
<td>3</td>
<td>224</td>
<td>3.4</td>
<td>18</td>
<td>2.6</td>
<td>37.4</td>
</tr>
<tr>
<td><em>L. mauritii</em> (45 days)</td>
<td>7.9</td>
<td>0.2</td>
<td>0.75</td>
<td>4</td>
<td>240</td>
<td>3.8</td>
<td>18</td>
<td>2.7</td>
<td>36.9</td>
</tr>
<tr>
<td><em>P. corethrurus</em> (30 days)</td>
<td>7.6</td>
<td>0.15</td>
<td>0.52</td>
<td>5</td>
<td>168</td>
<td>2.2</td>
<td>20</td>
<td>2.3</td>
<td>35.7</td>
</tr>
<tr>
<td><em>P. corethrurus</em> (45 days)</td>
<td>7.9</td>
<td>0.17</td>
<td>0.85</td>
<td>5</td>
<td>380</td>
<td>3.8</td>
<td>14</td>
<td>2.8</td>
<td>33.4</td>
</tr>
</tbody>
</table>
### Table 2.2: Total microflora from burrow wall soil of *Lampito mauritii* and control soils

<table>
<thead>
<tr>
<th></th>
<th>30 days (CFU/g)</th>
<th>45 days (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LBWS</td>
<td>LCS</td>
</tr>
<tr>
<td><strong>Fungi</strong> (x 10^5)</td>
<td></td>
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<tr>
<td></td>
<td>3.47 b ± 0.05</td>
<td>7.22 a ± 0.63</td>
</tr>
<tr>
<td><strong>Bacteria</strong> (x 10^5)</td>
<td></td>
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<tr>
<td></td>
<td>0.28 c ± 0.06</td>
<td>1.78 a ± 0.26</td>
</tr>
<tr>
<td><strong>Actinomycetes</strong> (x 10^3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0 a ± 0.22</td>
<td>2.0 b ± 0.23</td>
</tr>
</tbody>
</table>

Means with same superscript in each column do not differ significantly at P<0.05 level by Duncan’s Multiple Range Test (DMRT).

Legend: LBWS- Lower Burrow Wall Soil; LCS- Lower Control Soil; UBWS- Upper Burrow Wall Soil; UCS- Upper Control Soil

### Table 2.3: Total microflora from burrow wall soil of *Pontoscolex corethrurus* and control soils

<table>
<thead>
<tr>
<th></th>
<th>30 days (CFU/g)</th>
<th>45 days (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LBWS</td>
<td>LCS</td>
</tr>
<tr>
<td><strong>Fungi</strong> (x 10^6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.39 a ± 0.3</td>
<td>4.71 b ± 0.02</td>
</tr>
<tr>
<td><strong>Bacteria</strong> (x 10^6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.80 ab ± 0.14</td>
<td>3.5 b ± 0.47</td>
</tr>
<tr>
<td><strong>Actinomycetes</strong> (x 10^3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 a ± 0.29</td>
<td>8 b ± 0.30</td>
</tr>
</tbody>
</table>

Means with same superscript in each column do not differ significantly at P<0.05 level by Duncan’s Multiple Range Test (DMRT).

Legend: LBWS- Lower Burrow Wall Soil; LCS- Lower Control Soil; UBWS- Upper Burrow Wall Soil; UCS- Upper Control Soil
Table 2.4: Bacteria isolated from earthworm burrow wall soil of *Lampito mauritii* and *Pontoscolex corethrurus* and control soil on Mac Conkey’s, Eosin Methylene Blue Agar and Kings B media

<table>
<thead>
<tr>
<th></th>
<th>30days (CFU/g)</th>
<th>45 days (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LBWS</td>
<td>LCS</td>
</tr>
<tr>
<td><em>L. mauritii</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mac Conkey</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(x 10^4)</td>
<td>2.8^b ± 0.2</td>
<td>6.0^a ± 0.21</td>
</tr>
<tr>
<td>EMB (x 10^4)</td>
<td>9.6^b ± 0.36</td>
<td>12^a ± 0.60</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(x 10^6)</td>
<td>13.33^a</td>
<td>28.67^b</td>
</tr>
<tr>
<td><em>P. corethrurus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mac Conkey</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(x 10^3)</td>
<td>0.1^b ± 0.2</td>
<td>5.8^a ± 0.61</td>
</tr>
<tr>
<td>EMB (x 10^3)</td>
<td>1.8^b ± 0.27</td>
<td>2.3^b ± 0.4</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(x 10^6)</td>
<td>0.40^b</td>
<td>3.80^b</td>
</tr>
</tbody>
</table>

Means with same superscript in each column do not differ significantly at $P<0.05$ level by Duncan’s Multiple Range Test (DMRT).

Legend: LBWS- Lower Burrow Wall Soil; LCS- Lower Control Soil; UBWS- Upper Burrow Wall Soil; UCS- Upper Control Soil
Figure 2.4: Percentage of fungi isolated from 30 days burrow wall soil of *Pontoscolex corethrurus* and control soil
Figure 2.5: Percentage of fungi isolated from 45 days burrow wall of *Pontoscolex corethrurus* and control soil
Figure 2.6: Percentage of fungi isolated from 30 days burrow wall of *Lampito mauritii* and control soil

Legend: LBWS- Lower Burrow Wall Soil; LCS- Lower Control Soil; UBWS- Upper Burrow Wall Soil; UCS- Upper Control Soil
Figure 2.7: Percentage of fungi isolated from 45 days burrow wall of *Lampito mauritii* and control soil
Table 2.5: Dominant fungi from burrow wall of *Pontoscolex corethrurus* and *Lampito mauritii* and control soil at 30 and 45 days intervals

<table>
<thead>
<tr>
<th></th>
<th><em>P. corethrurus</em></th>
<th></th>
<th></th>
<th><em>L. mauritii</em></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UBWS 30 days</td>
<td>UCS 30 days</td>
<td>LBWS 30 days</td>
<td>UCS 45 days</td>
<td>LBWS 45 days</td>
<td>LCS 45 days</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Cladosporium cladosporioide s</td>
<td>Alternaria alternate</td>
<td>Phoma glomerata</td>
<td>Aspergillus flavus</td>
<td>Acremonium chrysosporum</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>Cladosporium cladosporioide s</td>
<td>Fusarium pallidoroseum</td>
<td>Cladosporium shaerospermu m</td>
<td>Saccharomyce s cerevisiae</td>
<td>Penicillium citrinum</td>
<td>Aspergillus niger</td>
<td>Verticillium chlamydosporu m</td>
</tr>
<tr>
<td>Mucor heimalis</td>
<td>Mucor racemosus</td>
<td>Mucor racemosus</td>
<td>Saccharomyce s cerevisiae</td>
<td>Fusarium oxysporum</td>
<td>Nigrospora oryzae</td>
<td>Saccharomyce s cerevisiae</td>
</tr>
<tr>
<td>Saccharomyce s cerevisiae</td>
<td>Phoma glomerata</td>
<td>Saccharomyce s cerevisiae</td>
<td>Volutella sclerotiiella</td>
<td>Saccharomyce s cerevisiae</td>
<td>Rhizopus oryzae</td>
<td>Saccharomyce s cerevisiae</td>
</tr>
<tr>
<td></td>
<td>Saccharomyce s cerevisiae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Alternaria alternata</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alternaria alternata</td>
<td></td>
<td></td>
<td>Alternaria alternata</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alternaria alterna te</td>
<td></td>
<td></td>
<td>Alternaria alterna te</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cladosporium cladosporioide s</td>
<td>Fusarium oxysporum</td>
<td>Fusarium oxysporum</td>
<td>Fusarium solani</td>
<td>Aspergillus niger</td>
<td>Fusarium oxysporum</td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>Fusarium solani</td>
<td>Fusarium solani</td>
<td>Rhizopus nigricans</td>
<td>Fusarium solani</td>
<td>Fusarium oxysporum</td>
<td>Fusarium oxysporum</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sordaria fimicola</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fusarium solani</td>
</tr>
</tbody>
</table>
### Table 2.6: Fungal species common to burrow wall soil of *Pontoscolex* corethrurus, *Lampito* mauritii and control soil

<table>
<thead>
<tr>
<th>Alternaria alternata</th>
<th>Fusarium oxysporum</th>
<th>Phoma glomerata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus flavus</td>
<td>Fusarium pallidoroseum</td>
<td>Penicillium citrinum</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Fusarium solani</td>
<td>Trichoderma virens</td>
</tr>
<tr>
<td>Aspergillus oryzae</td>
<td>Nigrospora oryzae</td>
<td>Trichoderma viride</td>
</tr>
<tr>
<td>Chaetomium indicum</td>
<td>Paecilomyces lilacinus</td>
<td>Trichothecium roseum</td>
</tr>
<tr>
<td>Cladosporium cladosporioides</td>
<td>Paecilomyces varioti</td>
<td></td>
</tr>
</tbody>
</table>

**P. corethrurus**

<table>
<thead>
<tr>
<th>Only from burrow wall soil</th>
<th>Only from control soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus albus</td>
<td>Acremonium chrysosporum</td>
</tr>
<tr>
<td>Cunninghamella elegans</td>
<td>Acremonium pericinum</td>
</tr>
<tr>
<td>Geotrichum candidum</td>
<td>Acremonium roseum</td>
</tr>
<tr>
<td>Penicillium purpurascence</td>
<td>Fusarium chlamydosporium</td>
</tr>
<tr>
<td>Verticillium chlamydosporum</td>
<td>Trichoderma harzianum</td>
</tr>
<tr>
<td>Volutella sclerotioria</td>
<td>Rhizopus nigricans</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**L. mauritii**

<table>
<thead>
<tr>
<th>Only from burrow wall soil</th>
<th>Only from control soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acremonium kiliense</td>
<td>Humicola grisea</td>
</tr>
<tr>
<td>Circenella sps.</td>
<td>Sordaria fumicola</td>
</tr>
<tr>
<td>Cunninghamella echinulata</td>
<td>Streptomyces griseus</td>
</tr>
<tr>
<td>Neurospora crassa</td>
<td></td>
</tr>
<tr>
<td>Penicillium purpurascence</td>
<td></td>
</tr>
<tr>
<td>Verticillium chlamydosporum</td>
<td></td>
</tr>
</tbody>
</table>

**Fungi common to both burrow wall soil and control soil**

<table>
<thead>
<tr>
<th>Fungi common to both burrow wall soil and control soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladosporium shaerospermum</td>
</tr>
<tr>
<td>Curvularia lunata var.aeria</td>
</tr>
<tr>
<td>Mucor heimalis</td>
</tr>
<tr>
<td>Mucor racemosus</td>
</tr>
<tr>
<td>Rhizopus oryzae</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>Acremonium fusidiodes</td>
</tr>
<tr>
<td>Byssochlamus niveus</td>
</tr>
<tr>
<td>Curvularia clavata</td>
</tr>
<tr>
<td>Drechslera hawiensis</td>
</tr>
<tr>
<td>Epicoccum purpurascence</td>
</tr>
<tr>
<td>Fusarium equesetii</td>
</tr>
<tr>
<td>Mucor cymosus</td>
</tr>
<tr>
<td>Periconiabyssoides</td>
</tr>
<tr>
<td>Phoma exigua and Penicillium italicum</td>
</tr>
</tbody>
</table>

Legend: LBWS- Lower Burrow Wall Soil; LCS- Lower Control Soil; UBWS- Upper Burrow Wall Soil; UCS- Upper Control Soil
Table 2.7: Bacterial and actinomycetes species isolated from burrow wall soil of *Pontoscolex corethrurus*, *Lampito mauritii* and control soil

<table>
<thead>
<tr>
<th></th>
<th><em>P. corethrurus</em></th>
<th></th>
<th><em>L. mauritii</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Only from burrow wall soil</td>
<td>Only from control soil</td>
<td>Only from burrow wall soil</td>
<td>Only from control soil</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Actinomycetes</td>
<td>Bacteria</td>
<td>Actinomycetes</td>
<td>Bacteria</td>
</tr>
<tr>
<td><em>Serratia</em> spp.</td>
<td></td>
<td></td>
<td>Actinoplanes</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp.</td>
<td></td>
<td></td>
<td>Nocardia</td>
<td></td>
</tr>
</tbody>
</table>

**Bacteria common to both burrow wall soil and control soil**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Actinomycetes</th>
<th>Bacteria</th>
<th>Actinomycetes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas</em> spp.</td>
<td><em>Micromonospora</em></td>
<td><em>Acinetobacter</em> spp.</td>
<td><em>Micromonospora</em></td>
</tr>
<tr>
<td><em>B. pasteurianum</em></td>
<td><em>Streptomyces</em></td>
<td><em>B. sterothermophilus</em></td>
<td><em>Streptomyces</em></td>
</tr>
<tr>
<td><em>B. sphaericus</em></td>
<td><em>Actinoplanes</em></td>
<td><em>B. subtilis</em></td>
<td><em>Dactylosporangium</em></td>
</tr>
<tr>
<td><em>B. sterothermophilus</em></td>
<td><em>Streptoverticillium</em> sp.</td>
<td><em>Micrococcus</em> spp.</td>
<td>Ac 1</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td><em>Proteus</em> spp.</td>
<td><em>Pseudomonas</em> spp.</td>
<td></td>
</tr>
<tr>
<td><em>Clostridium</em></td>
<td><em>Pseudomonas</em> spp.</td>
<td><em>Serratia</em> spp.</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus</em> spp. PC 1</td>
<td></td>
<td><em>Escherichia</em> spp.</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus</em> spp. PC 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: LBWS- Lower Burrow Wall Soil; LCS- Lower Control Soil; UBWS- Upper Burrow Wall Soil; UCS- Upper Control Soil
Table 2.8: Gram negative Bacteria from burrow wall of *Pontoscolex corethrurus* and *Lampito mauritii* and control soil at 30 and 45 days intervals

<table>
<thead>
<tr>
<th>Earthworm species</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. corethrurus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L. mauritii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 days (%)</td>
<td>45 days (%)</td>
<td>30 days (%)</td>
<td>45 days (%)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sl. No.</td>
<td>LBWS</td>
<td>LCS</td>
<td>UBWS</td>
<td>UCS</td>
<td>LBWS</td>
<td>LCS</td>
<td>UBWS</td>
<td>UCS</td>
<td>LBWS</td>
<td>LCS</td>
<td>UBWS</td>
<td>UCS</td>
<td>LBWS</td>
<td>LCS</td>
</tr>
<tr>
<td>2</td>
<td>Aeromonas sps</td>
<td>5.3</td>
<td>70.4</td>
<td>23.3</td>
<td>0</td>
<td>0</td>
<td>88.4</td>
<td>0</td>
<td>11.2</td>
<td>0</td>
<td>0.17</td>
<td>0.86</td>
<td>4.63</td>
<td>8.0</td>
</tr>
<tr>
<td>3</td>
<td>Serratia sps.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Eschericia sps.</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>22.6</td>
<td>33.4</td>
</tr>
<tr>
<td>5</td>
<td>Psuedomonas sps.</td>
<td>94.7</td>
<td>29.6</td>
<td>76.7</td>
<td>100</td>
<td>100</td>
<td>11.6</td>
<td>100</td>
<td>88.8</td>
<td>0</td>
<td>0</td>
<td>13.9</td>
<td>7.3</td>
<td>28.98</td>
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<td>6</td>
<td>Agrobacterium sps.</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>86.1</td>
<td>0</td>
<td>52.59</td>
</tr>
<tr>
<td>7</td>
<td>Proteus sps.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>77.4</td>
<td>66.6</td>
<td>0</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total Percentage</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Legend: LBWS- Lower Burrow Wall Soil; LCS- Lower Control Soil; UBWS- Upper Burrow Wall Soil; UCS- Upper Control Soil
Table 2.9: Dominant Bacteria from burrow wall of *Pontoscolex corethrurus and Lampito mauritii* and control soil at 30 and 45 days intervals

<table>
<thead>
<tr>
<th>P. corethrurus</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>UBWS 30 days</td>
<td>UCS 30 days</td>
<td>LBWS 30 days</td>
<td>LCS 30 days</td>
<td>UBWS 45 days</td>
<td>UCS 45 days</td>
<td>LBWS 45 days</td>
</tr>
<tr>
<td>B. sphaericus</td>
<td>B. pasteurianum</td>
<td>B. sphaericus</td>
<td>B. pasteurianum</td>
<td>B. sterothermophilus</td>
<td>Bacillus spp. PC 1</td>
<td>Clostridium</td>
</tr>
<tr>
<td>B. sterothermophilus</td>
<td></td>
<td></td>
<td>B. subtilis</td>
<td></td>
<td></td>
<td>Bacillus spp. PC 1</td>
</tr>
<tr>
<td>L. mauritii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UBWS 30 days</td>
<td>UCS 30 days</td>
<td>LBWS 30 days</td>
<td>LCS 30 days</td>
<td>UBWS 45 days</td>
<td>UCS 45 days</td>
<td>LBWS 45 days</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>Acinetobacter spp.</td>
<td>B. sterothermophilus</td>
<td>B. subtilis</td>
<td>B. subtilis</td>
<td>B. subtilis</td>
<td>B. subtilis</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>B. subtilis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Micrococcus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Clostridium</td>
</tr>
</tbody>
</table>

Legend: LBWS- Lower Burrow Wall Soil; LCS- Lower Control Soil; UBWS- Upper Burrow Wall Soil; UCS- Upper Control Soil

Table 2.10: Dominant Actinomycetes from burrow wall of *Pontoscolex corethrurus and Lampito mauritii* and control soil at 30 and 45 days intervals

<table>
<thead>
<tr>
<th>P. corethrurus</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>UBWS 30 days</td>
<td>UCS 30 days</td>
<td>LBWS 30 days</td>
<td>LCS 30 days</td>
<td>UBWS 45 days</td>
<td>UCS 45 days</td>
<td>LBWS 45 days</td>
</tr>
<tr>
<td>Streptoverticillium</td>
<td>Streptoverticillium</td>
<td>Micromonospora</td>
<td>Actinoplanes</td>
<td>Actinoplanes</td>
<td>Streptomyces</td>
<td>Streptomyces</td>
</tr>
<tr>
<td>L. mauritii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UBWS 30 days</td>
<td>UCS 30 days</td>
<td>LBWS 30 days</td>
<td>LCS 30 days</td>
<td>UBWS 45 days</td>
<td>UCS 45 days</td>
<td>LBWS 45 days</td>
</tr>
<tr>
<td>Geodermatophilus</td>
<td>Ac 1</td>
<td>Ac 1</td>
<td>Ac 2</td>
<td>Streptoverticillium</td>
<td>Streptomyces</td>
<td>Streptomyces</td>
</tr>
<tr>
<td>Micromonospora</td>
<td>Micromonospora</td>
<td>Micromonospora</td>
<td>Dactylosporangium</td>
<td>Micromonospora</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: LBWS- Lower Burrow Wall Soil; LCS- Lower Control Soil; UBWS- Upper Burrow Wall Soil; UCS- Upper Control Soil