

## Enumeration and characterization of microorganisms in raw coir pith and coir pith dumped soil

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**Abstract** Coir pith is being considered as the reject generated during the extraction of coir fibre from coconut husks. It is a light weight and fluffy material with dusts and bits of fibres. The coir pith has many undesirable components and hence it does not carry any value addition. It needs to be degraded for the effective utilization for further activities. Microbes have the ability to decompose coir pith. Coir pith itself possesses its own native microbes. Hence in the present study, raw coir pith and the soil dumped with coir pith in the dumping yards along the vicinity of the coir fibre factories were subjected for the enumeration and characterization of microorganisms. The coir pith for the study was collected from two locations in Cuddalore District. The results exhibited the presence of huge quantity of microbes in the raw coir pith than the coir pith dumped soil. Furthermore, the percentage occurrence of bacteria was significantly higher when compared with that of the fungi and actinomycetes. Totally 12 species of bacteria, six species of fungi and three species of actinomycetes were recorded. The results showed significant variation in the quantity and quality of the microbes.

### Introduction

Coir pith, generally called as coco-peat, is a non-fibrous cottony material [1] and it contains large quantities of lignocellulose [2]. It is dumped in huge piles on roadside and along the vicinity of the industries. Because of its high lignin, tannin and cellulose content and slow degradation in natural environment it leads to environmental pollution [3]. Microorganisms live in every part of the biosphere including soil, hot springs, on the ocean floor, high in the atmosphere and deep inside rocks within the Earth's crust. Microorganisms are crucial to nutrient recycling in ecosystems as they act as decomposers. Soil is made up of five major groups of microbial population including bacteria, actinomycetes, fungi, algae and protozoa, and among these groups, bacteria are the most abundant group [4] and the most important microbes for decomposing any waste.

Biological processes play a major role in the removal of contaminants and they take advantage of the astonishing catabolic versatility of microorganisms to degrade or convert such compounds [5]. In such case, microbial biodegradation of the wastes is generally considered to be a safe, effective and environmental friendly process [6]. In the present study, the microbial population inhabiting in raw coir pith and coir pith dumped soil collected from two locations were enumerated quantitatively and qualitatively.

### Materials and Methods:

#### Collection of coir pith

The coir pith required for the investigation was collected from Coir society industry and Palani murugan coir fibre industry, Tsunami Nagar of Cuddalore district Tamil Nadu, India, which is about 1km away from Cuddalore Town and 50kms away from the University campus. Specifically, the Palanimurugan coir industry is located near the seashore of around 300 meters. These coir industries supply high quality coir fibres to produce coir ropes for the home workers in

and around Cuddalore Town and they have high hillocks of coir pith in the vicinity of the industries. While collecting the coir pith from the mounds, the surface layer of the mound to a depth of 15 cms was scrapped and discarded. The fresh coir pith required for the work was then collected aseptically and was transported to the laboratory in polythene bags for further studies.

### **Quantitative analysis of microbes**

In order to carry out quantitative analyses of microbes, the total microbial populations (bacteria, fungi and actinomycetes) were determined in the abovementioned samples by the following methods.

### **Determination of total microbial populations**

One gram of sample was diluted in 100 ml of sterile saline in a sterile conical flask. The flask containing the substrate was shaken thoroughly in a Vortex mixture for 5 seconds. Three sets of Nutrient Agar (NA) and MacConkey Agar (MA) plates for bacterial growth [7], three sets of Sabouraud Dextrose Agar (SDA) and Rose Bengal Agar (RBA) plates for fungal growth [8], and three sets of Actinomycetes Agar (AA) plates for actinomycetes growth [8] were used as substrates. The substrate inoculum in 0.01ml was spread on the surface of these media to estimate the number of bacterial, fungal and actinomycetes colonies. The bacterial plates were incubated at 37°C for 18-24 hours, 25°C to 37°C for 5-7 days of incubation for fungal growth and 25°C to 35°C for 10-12 days of incubation for actinomycetes. The different microbial colonies developing on the plates were estimated by counting. The number of colony forming unit (CFU) on the surface of the media was counted and expressed as  $\text{CFU} \times 10^5 \text{ g}^{-1}$ , according to the method described by Baron et al., [9].

### **Qualitative analysis of microbes**

For the qualitative analysis of microbes, the sample was collected from the initial substrate. Species of fungus, bacteria and actinomycetes isolation from test samples were done according to the method described by Mackie and McCartney [10].

### **Isolation of bacteria**

The bacteria were isolated from the culture medium by using the stain Gram's Iodine and Safranin. After staining process, the bacterial cell on the slide was photographed under the Nikon microscope. Biochemical characterization of bacteria isolates were determined by Cappuccino and Sherman [11].

### **Isolation of fungi**

Fungal isolates were examined microscopically and macroscopically using the needle mouth technique. Their identification was performed according to the protocol of Cheesbrough [12].

### **Isolation of actinomycetes**

Morphological features of the actinomycetes were studied by inserting sterile cover slips in the actinomycetes agar medium at an angle of 45°C. Spore suspension of the actinomycetes was inoculated at the intersection of the medium and cover slip. The cover slips were observed under light microscope after incubation for one week at 37°C. Biochemical characterization of actinomycetes was determined by Cappuccino and Sherman [11].

## **Results**

The microbial populations were quantified both in raw coir pith and coir pith dumped soil in two different places. The results showed the existence of huge quantity of microbes in both the raw coir pith samples and significantly lesser quantity in soils. Among the microorganisms, bacteria was high and actinomycetes was low. The study indicates the significance of coir pith character which

has notable microbial content (Table 1). Comparatively sea shore sample has relatively low content of microbial populations. The ANOVA,  $P < 0.05$  showed that there is significant difference in the mean count among the locations. Table 2 exhibits the percentage of microorganisms present in the coir pith and coir pith dumped soil in the two locations.

**Table 1. The mean counts of microorganisms isolated from the two different locations (Mean $\pm$  SD) (Six replicates):**

| Samples   | CFU/g | of | Bacteria ( $\times 10^5$ ) | Fungi ( $\times 10^3$ ) | Actinomycetes ( $\times 10^4$ ) |
|-----------|-------|----|----------------------------|-------------------------|---------------------------------|
| SO        |       |    | 15.61 $\pm$ 1.89*          | 6.12 $\pm$ 0.87*        | 3.86 $\pm$ 1.53*                |
| SY (Soil) |       |    | 9.56 $\pm$ 1.24*           | 4.32 $\pm$ 0.98*        | 2.61 $\pm$ 1.34*                |
| SS        |       |    | 13.44 $\pm$ 1.78*          | 4.83 $\pm$ 1.67 *       | 3.15 $\pm$ 1.65 *               |
| SX (Soil) |       |    | 8.34 $\pm$ 1.46*           | 2.74 $\pm$ 1.65*        | 1.98 $\pm$ 0.75*                |

\*Mean values are significant at 5% level SNK (ANOVA)

SO- Society office coir pith

SY- Soil sample from society office

SS- Palanimurugan coir industry coir pith

SX- Soil Sample from palanimurugan industry

**Table 2. Percentage occurrence of microorganisms**

| Samples   | % of occurrence among microbes |       |               | % of occurrence among samples |       |               |
|-----------|--------------------------------|-------|---------------|-------------------------------|-------|---------------|
|           | Bacteria                       | Fungi | Actinomycetes | Bacteria                      | Fungi | Actinomycetes |
| SO        | 61.00                          | 23.92 | 15.08         | 33.24                         | 33.98 | 33.27         |
| SY (Soil) | 57.97                          | 26.19 | 15.82         | 20.36                         | 23.98 | 22.50         |
| SS        | 65.82                          | 23.65 | 15.42         | 28.64                         | 26.83 | 27.16         |
| SX (Soil) | 63.85                          | 20.98 | 15.16         | 17.76                         | 15.21 | 17.07         |

The percentage occurrence of bacteria was high in all the samples extensively and actinomycetes were low in all the samples. When the percentage occurrence of microbes was calculated, the coir pith from coir pith society showed the maximum occurrence of microbes. Table 3 reveals the different types (12 bacteria (table 4), 6 fungi and 3 actinomycetes) of microorganisms namely bacteria, fungi and actinomycetes that dwell in raw coir pith and coir pith dumped soil. In coir society coir pith sample, ten species of bacteria, six species of fungi and three species of actinomycetes were recorded. Eight species of bacteria, three species of fungi and two species of actinomycetes were notified in soil sample from the coir society. Palanimurugan coir industry coir pith sample exhibited nine species of bacteria, four species of fungi and three species of actinomycetes. Among the twelve species of microbes recorded in Palanimurugan coir industry soil sample, seven species were bacteria, three species fungi and two species were actinomycetes.

**Table 3. The list of microbes present in the of raw coir pith and coir pith dumped soil from the two different locations**

| Sampling Locations | Bacteria   | Fungi  | Actinomycetes  |
|--------------------|--|--|--|
| Coir Society (SO)  | <i>Escherichia coli</i><br><i>Xanthomonas sp</i><br><i>Methylobacterium</i><br><i>Acinetobacter sp</i><br><i>Vibrio sp.</i><br><i>Enterobacter aerogenes</i><br><i>Pseudomonas fluorescens</i><br><i>Pseudomonas aeruginosa</i><br><i>Bacillus subtilis</i><br><i>Proteus vulgaris</i> | <i>Aspergillus flavus</i><br><i>A.nidulans,</i><br><i>A.niger,</i><br><i>A.terreus,</i><br><i>Penicillium citrinum</i><br><i>Fusarium oxysporum,</i> | <i>Streptomyces albus,</i><br><i>Nocardia caviae</i><br><i>Nocardia asteroides</i> |

|  |  |   |   |
|--|--|---|---|
| <b>Coir Society (SX)<br/>(Soil)</b>                    | <i>Escherichia coli</i><br><i>Xanthomonas sp</i><br><i>Acinetobacter sp</i><br><i>Enterobacter aerogenes</i><br><i>Pseudomonas fluorescens</i><br><i>Bacillus subtilis</i><br><i>Bacillus cereus</i><br><i>Klebsiella pneumonia</i>                              | <i>Aspergillus flavus</i><br><i>A.nidulans</i> ,<br><i>A.terreus</i>  | <i>Streptomyces albus</i> ,<br><i>Nocardia caviae</i>                               |
| <b>Palanimurugan<br/>coir industry (SS)<br/>(CP)</b>   | <i>Escherichia coli</i><br><i>Xanthomonas sp</i><br><i>Methylobacterium</i><br><i>Acinetobacter sp</i><br><i>Enterobacter aerogenes</i><br><i>Pseudomonas fluorescens</i><br><i>Pseudomonas aeruginosa</i><br><i>Bacillus subtilis</i><br><i>Bacillus cereus</i> | <i>Aspergillus nidulans</i> ,<br><i>A.terreus</i> ,<br><i>Fusarium oxysporum</i> ,<br><i>Penicillium citrinum</i> | <i>Streptomyces albus</i> ,<br><i>Nocardia caviae</i><br><i>Nocardia asteroides</i> |
| <b>Palanimurugan<br/>coir industry (SY)<br/>(Soil)</b> | <i>Escherichia coli</i><br><i>Xanthomonas sp</i><br><i>Acinetobacter sp</i><br><i>Enterobacter aerogenes</i><br><i>Methylobacterium</i><br><i>Pseudomonas fluorescens</i><br><i>Bacillus subtilis</i>  | <i>Aspergillus flavus</i><br><i>A.terreus</i> ,<br><i>Penicillium citrinum</i>                                    | <i>Streptomyces albus</i> ,<br><i>Nocardia asteroides</i>                           |

**Table 4. Biochemical characterization of bacterial isolates**

| S. No. | Bacterial isolates             | Gram Stain | Motility | Indole | Methyl red test | Voges-Proskauer | Citrate | Triple Sugar iron | Catalase | Oxidase | Urease |
|--------|--------------------------------|------------|----------|--------|-----------------|-----------------|---------|-------------------|----------|---------|--------|
| 1      | <i>Escherichia coli</i>        | -          | +        | +      | +               | -               | -       | +                 | +        | -       | -      |
| 2      | <i>Xanthomonas sp</i>          | -          | +        | -      | -               | -               | +       | +                 | +        | -       | -      |
| 3      | <i>Methylobacterium</i>        | -          | +        | -      | -               | -               | +       | +                 | +        | -       | +      |
| 4      | <i>Acinetobacter sp</i>        | -          | -        | -      | -               | -               | +       | -                 | +        | -       | -      |
| 5      | <i>Vibrio sp.</i>              | -          | +        | +      | +               | +               | +       | +                 | +        | +       | -      |
| 6      | <i>Enterobacter aerogenes</i>  | -          | +        | -      | -               | +               | +       | +                 | +        | -       | -      |
| 7      | <i>Proteus vulgaris</i>        | -          | +        | +      | +               | -               | -       | +                 | +        | -       | +      |
| 8      | <i>Klebsiella pneumonia</i>    | -          | -        | -      | -               | +               | +       | +                 | +        | -       | -      |
| 9      | <i>Pseudomonas fluorescens</i> | -          | +        | -      | -               | -               | +       | -                 | +        | +       | -      |
| 10     | <i>Pseudomonas aeruginosa</i>  | -          | +        | -      | -               | -               | +       | -                 | -        | +       | +      |
| 11     | <i>Bacillus subtilis</i>       | +          | -        | -      | -               | -               | -       | +                 | +        | +       | -      |
| 12     | <i>Bacillus cereus</i>         | +          | -        | -      | -               | -               | -       | +                 | +        | +       | -      |

## Discussion

The results of the present study fall in line with the works of Adesemoye *et al.* [13] and Ezeronye and Ubalua [14]. Bartha and Atlas [15] reported that when natural environments are contaminated with pollutants the indigenous microbial communalities are likely to contain microbial populations of different taxonomic characteristics which are capable of degrading the contaminating waste. They are autochthonous microbial communities and have the potential of waste degradation [16]. Gram negative bacteria were dominant in the genera of the bacteria isolated from both the samples from both the places. This was in accordance with the results of Daboor [17] which reported the prevalence of microbes in fish farm soil. Among all the groups, the highest countable microorganisms were present in coir pith sample from coir society and low in soil sample from Palanimurugan coir industry. It is presumed that, higher salinity could have been the major attribute for the low quantity and quality of microorganisms. Similar results have been documented by several authors in different wastes and waste waters [18; 19; 20].

## Conclusion

From the present investigation, it is well evident that coir pith has unique property to accommodate microorganisms significantly. The coir pith dumped soil has lesser amount of microbial population because their properties may be less congenial than the coir pith for the effective survival of microbes. Moreover, the coir pith dumped soil might have been rich in the native toxic elements present originally in coir pith because the leachate from the coir pith during monsoon seasons would have contaminated the soil with abundance of tannin, lignin, cellulose and pectic substances and thus creating an unconducive environment for the natural growth of microbes in the coir pith dumped soil. Whereas the microbes inhabiting the matrix of coir pith have the characteristic property of degrading the above toxic constituents as a function of time. This microbial degradation in due course may enhance the microbial population which could be reflected in the suitability of subjecting coir pith as an effective substrate for the growth of vegetable crops, ornamental plants and forest nurseries at large. Further studies may be required to study the micro and macronutrients status in coir pith in order to design and evolve a better culture medium from coir pith.

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